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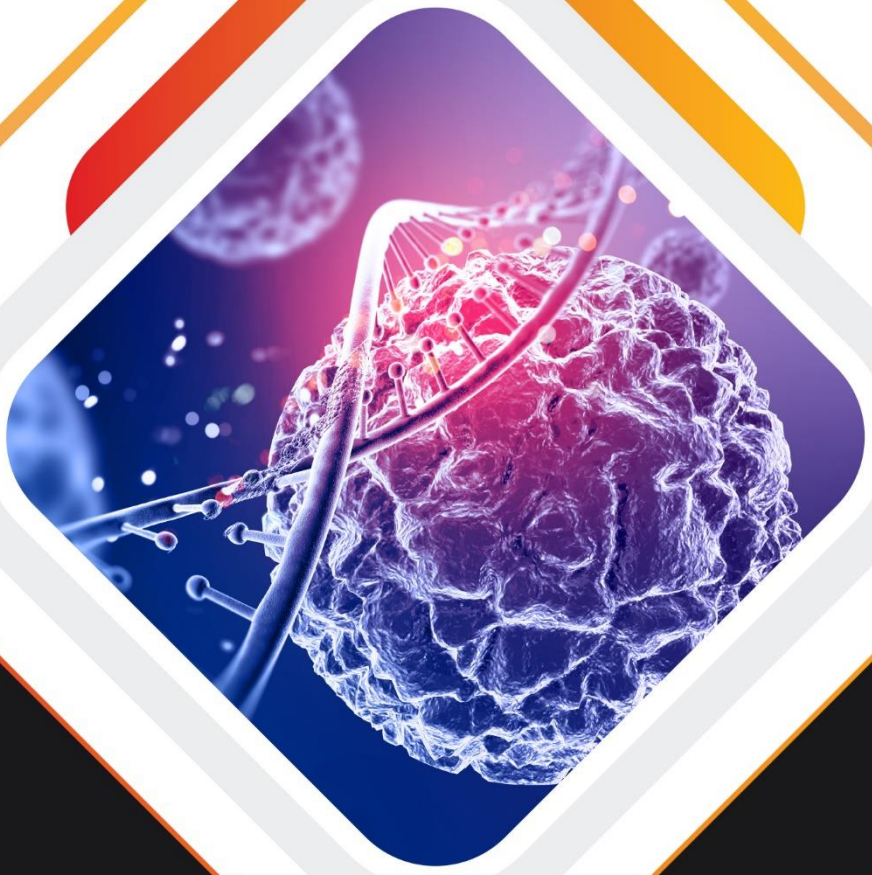

ABSTRACT BOOK

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ABSTRACT BOOK

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2022

15th NATIONAL – 1st INTERNATIONAL CONGRESS OF HISTOLOGY AND EMBRYOLOGY
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WELCOME TO NICHE 2022

Dear Members of the Turkish Histology and Embryology Family,

The 15th National – 1st International Congress of Histology and Embryology (NICHE 2022) will be held online by the Turkish Histology and Embryology Society between 26-28 May 2022. It is our great pleasure to invite you to our congress.

Despite our extreme efforts as the 16th Term Administrative Board of the Turkish Histology and Embryology Society to hold this congress face-to-face, our congress, will be online due to the progression of COVID- 19 pandemic and economic situations in Turkey.

In addition to the conferences of valuable researchers who have a word in their fields, our congress, where the results of current research will be shared, and experts from abroad will be invited, will be held with your valuable participation. We are excited to meet you at our congress even on the screen.

At the 15th National – 1st International Congress of Histology and Embryology, we will be very closely involved in developmental biology, molecular biology and cell biology, stem cells, cellular therapies, regenerative and reconstructive medicine, assisted reproduction techniques, tumor biology, biomedical engineering, biotechnology, bioinformatics and neuroscience. It is aimed to create a scientific environment with multidisciplinary contributions on current issues of interest.

I invite you to the 15th National – 1st International Congress of Histology and Embryology, which we are sure will be enriched scientifically with your participation and the knowledge and experience you will share.

With my best regards.

Prof. Dr. Gamze TANRIOVER

President of Congress

CONTENTS

15th NATIONAL – 1st INTERNATIONAL CONGRESS OF HISTOLOGY AND EMBRYOLOGY

Oral Presentation

EVALUATION OF THE ZINC-INDUCED RESPONSES OF ADIPOCYTE-DERIVED MESENCHYMAL STEM CELLS (AD-MSCS) IN AN EXPERIMENTAL PARKINSON'S DISEASE MODEL

Sendegul YILDIRIM İLDEM[1], Ayse OZKAN[2], Osman SINEN[3], Ethem Taner GOKSU[4], Mustafa Gokhan ERTOSUN[5], Gamze TANRIOVER[6]

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Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by losing dopaminergic neurons (DN). The neuroinflamatory cytokine and chemokine responses that occur in PD activate M2 microglia. This activation helps anti-inflammatory mediators inhibit neurodegeneration in the substantia nigra pars compacta (SNpc) area thus, neuroprotection is started. Adipose-derived mesenchymal stem cells (AD-MSCs) are known in the literature to secrete neuroprotective factors to prevent neuronal damage caused by trauma and nerve diseases. It is known that zinc (Zn) tries to prevent by blocking NF-kβ in case of inflammation. This effect of zinc also suppresses microglial activation and helps neuronal survival.

Objectives: Our study aims to investigate whether Zn affects the activities of AD-MSCs in the experimental PD model. For this purpose, the effect of Zn on locomotor activity by behavioral analysis and anti-inflammatory cytokine responses by immunohistochemical analysis was examined.

Materials-methods: 8-10 weeks old male C57BL/6 mice were randomly divided into six groups (n=6): Control, Zn, PD, PD+Zn, PD+AD-MSC, PD+AD-MSC+Zn. MPTP toxin (20 mg/kg) was dissolved in saline, and intraperitoneally injected to experimental groups for two days with 12h intervals. 3rd day, AD-MSC was given to the right lateral ventricle of the PD+AD-MSC and PD+AD-MSC+Zn groups by stereotaxic surgery. Then, $ZnSO_4H_2O$ was administered intraperitoneally for 4 days at 2mg/kg. Eight days after starting the experiment, the motor activities of mice were evaluated. Effects on DNs in SNPc were determined by TH and BDNF immunohistochemistry. MCP-1, TGF-β, IL-10 immunohistochemistry was also performed for anti-inflammatory responses in DNs.

Results: Our results showed that locomotor activity was lower in Group-PD (p<0.0001) than Group-C. AD-MSC and Zn administration has improved this impairment. MPTP caused a decrease in TH immuno-expression in DNs in Group-PD (p<0.0001). However, an immunoreaction was found to be more intense in PD+Zn (p=0.0323), PD+AD-MSC (p=0.0167) and PD+AD-MSC+Zn (p=0.0004) groups. MCP-1, TGF-β, and IL-10 immunoexpressions increased in the PD+Zn (p<0.05), PD+AD-MSC (p<0.0001), and PD+AD-MSC+Zn

(*p*<0.0001) groups compared to the Group-PD. It was observed that BDNF expression decreased in the Group-PD compared to the Control (*p*<0.0001). On the contrary, BDNF expression was more clearly observed in the PD+AD-MSC (*p*<0.0001), and PD+AD-MSC+Zn (*p*<0.0001) groups.

Conclusion: In our study, it has been shown that the administration of Zn alone and in combination with AD-MSCs reduces the neuronal damage that occurs in the PD model. In addition, anti-inflammatory responses that occur with the effect of Zn and AD-MSCs may have a neuroprotective effect in the disease.

This project was supported by Akdeniz University Research Foundation (TSA-2022-5710).

Keywords: Parkinson's disease, AD-MSC, Zn, Dopaminergic neuron, Anti-inflammation

Oral Presentation

ANALYSIS OF THE MRNA EXPRESSION OF C-TYPE NATRIURETIC PEPTIDE-3 AND NATRIURETIC PEPTIDE RESEPTOR-B IN ANGIOGENESIS OF CHICK CHORIOALLANTOIC MEMBRANE: A DESCRIPTIVE STUDY

Ekin EFE[1], Attila DAĞDEVİREN[1], Fevziye Figen KAYMAZ[2], Ahmet Çevik TUFAN[3]

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Introduction: C-type natriuretic peptide (CNP) has been shown to have autocrine and paracrine effects on the cardiovascular system as the secretion of endothelial cells, cardiomyocytes and vascular smooth muscle cells. In recent years, the involvement of CNP in the angiogenesis process has also been investigated widely. The chick chorioallantoic membrane (CAM) is a widely used model to follow the developmental processes in angiogenesis studies. This descriptive study aims to describe the putative involvement of CNP-3, the chick homolog of human CNP, and its receptor natriuretic peptide receptor (NPR)-B besides known angiogenic factors and their receptors, i.e., VEGFA/VEGFR-2 (vascular endothelial growth factor-A and its receptor-2) and FGF-2/FGFR-2 (fibroblast growth factor and its receptor), in angiogenesis of the CAM.

Material-Method: Fertilized chicken eggs ("ATAK-S" strain) were obtained from Republic of Turkey Ministry of Agriculture and Forestry, Poultry Research Institute and were incubated at 37.5 ºC in an egg incubator. Totally, 90 fertilized eggs were used for this study. The Ethical Review Committee of the Başkent University, School of Medicine, Ankara, Turkey, approved the procedures used in this study (Approval document number: DA20/14). CAM samples were collected between developmental days E7 and E20 for light microscopic and transmission electron microscopic analyses. Expression of CNP-3/NPR-B, VEGF-A/VEGR-2, FGF-2/FGFR-2 mRNA in these CAM samples were also studied between E7 and E20 (excluding E11, E14, E18) using semi-quantative reverse-transcriptase polymerase chain reaction (RT-PCR).

Results: Light microscopy and electron microscopy analyzes showed that, vascular organization was mostly within the chorionic mesenchyme as endothelial tube-like structures on E7-E8 days. On E12 and later, advanced blood vessels were observed within the mesenchyme. VEGF-A and FGF-2 expression were observed in the early stages of CAM development (E7-E9) with a peak at E8. These molecules showed a second peak at around E16. Co-expression of VEGF-A, FGF-2 and CNP-3 were seen at E16-E20. VEGFR-2 expression was observed between E7-E12, whereas expression of FGFR-2 showed its first peak between E7-E9 and its second peak between E16-20. On the other hand, NPR-B expression was observed between E7-E20 with its highest level at E16.

Conclusion: In conclusion, the results revealed that CNP-3 may have a role in vascular organization via its NPR-B receptor in the later stages, i.e., E16-E20, of CAM development.

Keywords: CNP; CNP-3; NPR-B; angiogenesis; chorioallantoic membrane; VEGF-A; FGF-2

This study was supported by a Başkent University Grant: DA20/14.

Oral Presentation

EVALUATION OF THE AQUAPORIN MOLECULES CHARACTERIZATION IN THE SPERM CELLS OF MEN FROM DIFFERENT AGED

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Introductıon: Male infertility is considered as the inability of a man to satisfy his reproductive aims through regular unprotected sexual intercourse, which accounts for about 30% of infertility cases. This situation has recently become an increasingly important public health matter.

Objectıves: Male infertility rises for many reasons, along with age; therefore, we aimed to research the characterization of aquaporin-3, 7, and 8 in human sperm belonging to different age groups.

Materıals & Methods: This study was conducted on sperm samples of men aged over 18 years. A total of 60 men were included in the study and divided into three age groups: group 1, age 18 - 25 years (n = 20); group 2, age 26 - 35 years (n = 20); and group 3, age ≥ 35 years (n = 20). Sperm ejaculates obtained from each participant were used for spermiogram tests, smear preparations for Kruger strict morphology analysis, and immunohistochemistry.

Results: We observed no statistically significant differences in terms of macroscopic and microscopic sperm testing. The immunostaining score of aquaporin-3 was the lowest in group 1 and increased in group 3 and group 2, respectively ($p < 0.05$). Aquaporin-8 immunostaining only increased in group 2 ($p < 0.05$). Aquaporin-7 immunostaining scores were not different between the groups ($p > 0.05$). When the immunostaining scores of aquaporin molecules were compared with each other, aquaporin-7 was statistically significantly increased compared with the others ($p < 0.05$).

Conclusıons: According to the results, it can be stated that aquaporin-3 and aquaporin-8 molecules were more expressed at age 26 to 35 years, and aquaporin-7 was densely expressed from age 18 to 25 years. If the characterization of these molecules is adversely affected, male infertility may eventually emerge. We recommend further advanced-level studies on this subject.

Keywords: aquaporin molecules; immunostaining; male infertility; sperm

Oral Presentation

EFFECT OF BORIC ACID ON GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) STIMULATED PERIPHERAL BLOOD ACCORDING TO THEIR HEMATOPOIETIC COLONY FORMING CAPACITY

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Object: The ex vivo generation of human blood cells on a large scale from hematopoietic stem cells (HSCs) has been considered as a potential method to overcome blood supply shortages. The blood supply is largely dependent on donations. Unfortunately, collecting the clinical-grade blood products has become a challenging mission due to accelerated population aging, which not only increases the need for blood transfusions but also decreases the number of healthy donors. Blood or bone marrow transplants most commonly are used to treat blood cancers or other kinds of blood diseases. Boric acid (BA) is an inorganic substance that have antiseptic, antifungal and antibacterial properties. In recent years studies show that boric acid increases the cell proliferation. In this study we conducted to compare effect of boric acid on colony forming capacity of G-CSF stimulated peripheral blood of healthy donors of pediatric patients using healthy donors HSCs.

Material&Method: 11 healthy donors of pediatric patients (G-CSF stimulated peripheral blood) undergoing allogenic transplantation were included in the study. Each donor received 10µg/kg per day of G-CSF as a single injection for 3 consecutive days. Colony forming capacity of hematopoietic stem cells evaluated with Colony Forming Unit (CFU)-Assay within semi-solid agar culture medium (MethoCult) that contains BA in following concentrations; 0.5µg/ml, 1 µg/ml, 5 µg/ml and 10 µg/ml. In addition control group was created without boric acid. After 14-18 days of culture CFU-Assay was evaluated according to CFU-Assay scoring procedures. CFU or Burst-forming units (BFU) are classified according to the type and number of mature cells produced in each colony as follows: CFU-E (colony-forming unit-erythroid), BFU-E (burst-forming unit-erythroid), CFU-GM (colony-forming unit-granulocyte, macrophage), CFU-GEMM (colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte).

Results: According to CFU assay scoring procedures, BFU-E, CFU-GM and CFU-GEMM colony capacity of stimulated peripheral blood at 5 µg/ml BA concentration control was increased and significantly different than control group. After 10 µg/ml concentration, number of all type of colonies showed decrease compare to control.

Conclusion: The ex vivo-generated blood cells could be an alternative blood source for traditional transfusion and transplantation procedures in the clinic. Boric acid may be used as a inducing cell proliferation agent to improve cell dose (especially for underweight donors) and to obtain a greater cell concentration in G-CSF stimulated peripheral blood allow smaller harvest volumes.

Keywords: Hematopoietic stem cells, CFU-Assay, Boric acid

Oral Presentation

OVEREXPRESSION OF DUAL-SPECIFICITY PHOSPHATASES 4 AND 13 ATTENUATES TRANSFORMING GROWTH FACTOR BETA 1-INDUCED MIGRATION AND DRUG RESISTANCE IN A549 CELLS IN VITRO

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Introduction; Transforming growth factor-beta (TGFβ) proteins induce an epithelial-mesenchymal transition (EMT) programme that is associated with increased invasive and drug-resistant phenotype of carcinoma cells. In addition to the canonical pathway involving SMAD proteins, the mitogen-activated kinase pathway (MAPK) via extracellular signal-regulated kinases (ERK1/2) is also involved in promoting and maintaining a mesenchymal phenotype by tumor cells following TGFβ signal activation. As dual-specificity phosphatases (DUSPs) regulate ERK1/2 activity by dephosphorylation, we aimed to examine DUSPs` expression upon TGFβ stimulation and whether DUSPs play a role in the EMT and related phenotypes promoted by TGFβ1 in A549 cells.

Material-Method: For this purpose, EMT was induced in A549 cells (non-small cell lung cancer cell line, NSCLC), with 5 ng/ml concentration of recombinant TGFβ1 protein for 48 h. EMT confirmed with phenotypical changes, real-time qPCR, western blot (WB), and immunofluorescence (IF). MAPK protein (ERK1/2, JNK and p38) levels were analyzed with WB. DUSP expression levels were determined by real-time qPCR, and WB in control and TGFβ1-treated cells. Ectopic plasmid transfection was applied to DUSPs with decreased expression levels, either individually or in different binary combinations. DUSP4/13 combination was able to attenuate TGFβ1's suppressive effect on E-cadherin. Cells were first transfected with either DUSP4/13 as combination, or with empty vector as control and treated with TGFβ1, then DUSP4/13 overexpression effect on EMT (analyzed with real-time qPCR and WB), ERK1/2 phosphorylation (analyzed with WB), F-actin staining (analyzed with fluorescence staining), invasion, migration (in Boyden chambers) and cell viability (cells were treated with 2.5 µg/ml cisplatin, 5 µg/ml gemcitabine and 10 µM paclitaxel for 48 h) were examined.

Results: We found that TGFβ1 stimulation led to marked chages in several DUSPs proteins, including significant decreases in DUSP4 and DUSP13 expressions. We then showed that the ectopic co-expression of DUSP4/13 suppresses TGFβ1-induced ERK1/2 phosphorylation and protein levels of Snail and Slug proteins. We then demonstrated that DUSP4/13 co-expression partially inhibited TGFβ1-promoted migration, invasion, and chemoresistance in A549 cells.

Conclusion: Collectively, our study, is first to suggest a cooperation between DUSP4 and DUSP13 in suppressing TGFβ1-induced ERK1/2 phosphorylation and attenuating migration/invasion and drug resistance in an EMT model in vitro.

Oral Presentation

EFFECT OF CRYOPRESERVATION ON MONKEY SPERMATOGONIAL STEM CELL SURVIVAL

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Introduction: Cancer treatments may induce loss of germ cells that may result in infertility. While survival rate of pediatric cancer patients (PCPs) has increased significantly, fertility preservation is a major concern after cure. Therefore, isolation of SSCs from cryopreserved testicular tissue pieces (TTPs) or testicular cell suspensions (TCSs) can be a suitable cell source to restore spermatogenesis for PCPs. Cryopreservation techniques to preserve the functionality of spermatogonial stem cells (SSCs) are very critical step for PCPs. Cryopreservation techiniques are still at experimental stage and their efficiency depends on the survival and functional capacity of SSCs.

Material and method: We investigated freezing effect on survival and function of SSCs from monkey TTPs and TCSs. We froze TTPs or TCSs from rhesus macaques using controlled slow freezing (CSF) or uncontrolled slow freezing (USF). TTPs were frozen using CSF or USF with conditions: 5% of Dimethyl Sulfoxide (DMSO)-5% Serum Substitute Supplement (SSS) or 10% DMSO-30% SSS. TCSs were frozen by CSF in 10% DMSO-15% SSS. Fresh cell suspensions were evaluated as controls. We determined the total and viable number of cells per gram of tissue; the live cell recovery rate compared with fresh cell suspensions; the number of UTF1(+) cells per gram of tissue; and xenotransplant colony numbers per 10^5 cells transplanted or per gram tissue.

Results and Conclusion: As expected, total and live cell number reduced after cryopreservation ($p<0.05$). Although total, live, and UTF1(+) cell number were highest in the CSF 5% DMSO-5% SSS, there was no significant difference between experimental groups. In contrast, the median number of colonies/10^5 cells was higher in the CSF 5% DMSO-5% SSS group than USF 5% DMSO-5% SSS, USF 10% DMSO-30% SSS and CSF-cell suspensions ($p<0.05$) and the median number of colonies per gram of tissue was higher than all other experimental groups ($p<0.05$). In conclusion, CSF 5% DMSO-5% SSS is better than the other experimental groups to protect functionality of SSCs. Optimization of freezing methods is a crucial step to enable therapeutic use of SSCs to restore fertility in cancer survivors. These results will help inform best practices for freezing patient tissues.

Oral Presentation

HİSTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION OF THE EFFECT OF LICORICE ON INFECTED BURN WOUNDS

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Introduction: Burn wounds (BW) on skin occur as a result of heat damage leading to protein denaturation in cells. Infection is the main cause of morbidity and mortality in BWs. In topical BW therapy, Silver sulfadiazine (SSD) was introduced as the gold standard having antibacterial properties. Long-term use of SSD is not recommended due to delayed healing and side effects such as nephrotoxicity and leukopenia. The use of herbal medicines in the treatment of BWs has been investigated in different studies, considering their toxicities as well as therapeutic properties. Licorice root extract (Glycyrrhiza glabra) is a medicinal herb being used in treatment of different dideases traditionally. It has anti-inflammatory, anti-bacterial, antioxidant, anti-viral properties.

Purpose: Because of the reasons that infections delay healing and increase morbidity and mortality as well as increase in resistance to antibiotics, we aimed to examine the effect of licorice on BWs infected with Staphylococcus aureus, an endogenous gram-positive bacterium found in the skin flora; and Enterococcus faecalis, a gram-negative bacterium from the endogenous intestinal flora.

Material-Method: A BW was created by putting a metal plate with a diameter of 2 cm, heated in water at 100 °C, on the backs of 32 male Wistar rats. Rats were divided into 4 groups: Control group (saline applied without bacterial inoculation for 7 days), Staphylococcus+Licorice group (inoculated with Staphylococcus aureus and Licorice applied after saline), Enterococcus+Licorice group (inoculated with Enterococcus faecalis), Licorice group (licorice applied without bacterial inoculation). Skin specimens were prepared for light microscopic evaluations by H&E, Masson's trichrome and TGF-ß1 immunohistochemical stain.

Results: Control group revealed the highest collagenization score whereas it took the lowest TGF-β1 expression score. Inflammation and TGF-β1 expression were increased in Enterococcus+Licorice group and granulation tissue formation was increased in Staphylococcus+Licorice group compared to control group. Vascularization was increased in Enterococcus+Licorice group compared to Staphylococcus+Licorice group.

Conclusion: Our results show that prevention of infection in BWS is the most important step in treatment. Uninfected BW took the lowest inflammation score, moreover the highest collagenization score. Granulation tissue deposition and TGF-Beta1 expression were increased with licorice application to infected or uninfected

BWs, indicating a faster healing. It would be beneficial to examine the effect of Licorice on BWs infected with different infectious agents or with different degrees of burns, with longer-term treatments.

Keywords: Burn, Licorice, TGF-ß1

Oral Presentation

EFFECT OF LINAGLIPTIN AND INSULIN COMBINED THERAPY ON UNFOLDED PROTEIN RESPONSE IN TYPE 1 DIABETIC MOUSE HEART

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Introduction: Diabetes mellitus is a global metabolic disease that is estimated to affect about 400 million people by 2030. Cardiovascular complications are the most common cause of morbidity and mortality in patients with diabetes. Various factors, including oxidative stress, and overload of protein synthesis, lead to endoplasmic reticulum dysfunction, called Endoplasmic Reticulum Stress (ERS), and activate a complex signaling network called unfolded protein response (UPR). ERS plays a critical role in the pathogenesis of diabetes mellitus and its associated cardiovascular complications. ERS has been found to contribute to myocardial apoptosis in animal models diabetes. Recent studies suggest that ER stress may be mediated by increased oxidative stress in diabetic cardiomyopathy.

Aim: In this study, we aimed to investigate the effects of Linagliptin, known to inhibit the activity of Dipeptidyl peptidase-4 (DPP-4), on ERS and associated apoptotic UPR signal proteins in diabetic mouse heart.

Materials and Methods: The effect of linagliptin on ERS related apoptotic proteins in the heart were evaluated by comparing it with the ERS inhibitor Tauroursadeoxycholic acid (TUDCA). 6 groups created randomized and using 6 weeks old 72 Balb/C female mice, 12 mice in each group: 1) Control, 2) Diabetes + Insulin, 3) Diabetes + Linagliptin, 4) Diabetes + Linagliptin + Insulin, 5) Diabetes + TUDCA, 6) Diabetes + TUDCA + Insulin. DPP-4 activity was measured by ELISA method to confirm the effectiveness of linagliptin in blood serum samples from mice. Histopathological evaluation was done. Immunohistochemistry method were implemented to demonstrate expression levels of apoptosis-inducing proteins (anti-ATF4, anti-ATF6(N), anti-active caspase 12, anti-active caspase 3 and anti-phospho JNK) in mice heart. The expression levels of Grp78 and pro-apoptotic Ddit3 genes, which are markers of ER stress, were examined by qRT-PCR method. In addition, malondialdehyde (MDA) and Nox1 activities which are the important markers of oxidative stress-related tissue damage, measurement was performed in the heart by ELISA method.

Results: ATF4, ATF6, p-JNK, Caspase 3, and Caspase 12 expressions were significantly decreased in Linagliptin and Linagliptin + Insulin groups. In addition, while the expressions of GRP 78 and Chop genes, MDA, and NOX markers increased in the insulin group, they decreased significantly in the TUDCA and Linagliptin groups.

Conclusion: Our results show the combined use of Linagliptin, and Insulin has therapeutic effects of at the molecular level by suppressing the expression of ERS and UPR-related apoptotic and oxidative stress molecules in cardiac tissues of diabetic mice.

Oral Presentation

INVESTIGATION OF THE EFFECTS OF SILYMARIN AND VITAMIN C ON KIDNEY DAMAGE AND AQUAPORIN-2 DOWNREGULATION IN LITHIUM-INDUCED NEPHROGENIC DIABETES INSIPIDUS IN MALE RATS

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In this study, it was aimed to investigate the protective effects of Silymarin (SIL) and Vitamin C (Vit C) on damaged kidney tissues of rats with experimental Nephrogenic Diabetes Insipidus (NDI). The 16-week-old male rats used in the study were divided into eight groups. Control group (CNT), Silymarin (SIL), Vitamin C (Vit C), and Silymarin+Vitamin C (SIL+Vit C) groups were fed with standard feed and Lithium (LIT), Lithium+Silymarin (LIT+SIL), Lithium+Vitamin C (LIT+Vit C), Lithium+Silymarin+Vitamin C (LIT+SIL+Vit C) groups were fed with added 80 mmol LiCl/kg feed for 28 days. The rats were treated with SIL (100 mg/kg, p.o), Vit C (200mg/kg, p.o.) or SIL+Vit C (100 mg/kg, p.o, 200 mg/kg, p.o respectively).The urine and blood samples taken from the groups; osmolality, sodium, potassium, urea, BUN and creatinine values were measured. Kidney tissues used to determine the levels of AQP2, ROS, GSH, SOD, MDA, histopathological and immunohistochemical evaluation. LIT and LIT+Vit C group's kidney tissues were examined histologically, the damage score was found to be higher and AQP2 amount and immunopositivity were decreased significantly compared to the CNT group. Serum osmolality and urine osmolality, sodium, potassium, urea, BUN, and creatinine values between the groups were compared, the values in the LIT and LIT+Vit C groups were found lower significantly. GSH and SOD levels were higher and ROS and MDA levels were lower significantly in LIT+SIL and LIT+SIL+Vit C groups.

As a result, it was determined that SIL application significantly reduced the negative effects of LIT-induced NDI on the kidney, while Vit C did not have a positive effect.

Keywords: Lithium, Nephrogenic Diabetes Insipidus, AQP2, Silymarin, Vitamin C

Oral Presentation

THE ROLE OF CYTOKINES IN THE EFFECT OF CHRONOLOGICAL AGE ON OVARIAN RESERVE; COMPARISON OF CYTOKINE CONCENTRATIONS IN FOLLICULAR FLUID AND OVARIAN RESERVES IN IVF CYCLES OF WOMEN OVER AND UNDER 35 YEARS OF AGE

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Introduction: Unlike men, women lose their fertility with aging. The reason is the decrease in the number and quality of oocytes in the ovaries and the decrease in reproductive hormones. Fertility declines with chronological age in both natural and induced cycles, so optimal fertility is considered to be between 20 and 30 years of age. Although it is known for certain that aging decreases the oocyte reserve, there are not many studies on the molecular mechanism involved in this process. When aging and age-related diseases are examined, it has been shown that cytokines have an irregular distribution.

Aim: In our study, we aimed to investigate the role of cytokines in ovarian reserve, which decreases with aging. Comparing women over and under 35 years of age, we investigated which cytokine and at what concentration ovarian reserve was affected by aging in this process. In addition, we evaluated the role of cytokines in ovarian reserve adequacy by evaluating ovarian reserve-related cytokines in the young patient group.

Materyal Metod: This prospective randomized study was performed with follicle fluids obtained from patients who underwent oocyte pick-up (OPU). 86 patients, aged between 22 and 44, who were diagnosed with primary or secondary infertility, who were decided on ICSI/ET due to various infertility reasons were included in the study. The patients included in the study were divided into 2 groups as under 35 years old and over 35 years old. Each group was divided into 2 subgroups as those with low and normal ovarian reserve. Thus, 4 groups were formed. Concentrations of IL-17F, IL-21, INF-alpha2 cytokines were measured by ELISA analysis. Correlation between patients' reproductive hormones, AMH (anti-mullerian hormone), antral follicle count (AFC), fertilization rates and cytokine concentrations were evaluated.

Results: We showed that IL-17F, IL-21 and INF-alpha2 levels in the follicular fluid change with aging ($p<0.05$). In addition, IL-17F level was found to be lower in patients with low ovarian reserve, regardless of age ($p<0.05$). We found that this decrease correlated with AMH and AFC ($p<0.05$).

Conclusion: These findings suggest that IL-17F decrease in follicular fluid may adversely affect ovarian reserve, regardless of age. By interfering with the cytokine IL-17F, which we have shown in our study to have a role in the pathogenesis of low ovarian reserve, a new treatment method can be developed and may provide an idea for diagnostic tests.

Keywords: Follicular fluid; cytokine; aging; ovarian reserve

Oral Presentation

EVALUATION OF SEMEN PARAMETERS AND SPERM MORPHOLOGY IN INDIVIDUALS WITH DIFFERENT BODY MASS INDEX (BMI)

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Introduction: at least one year of unprotected and frequent sexual intercourse is required for a pair to be considered infertile (WHO, 2010). Pregnancy may only be accomplished via the use of assisted reproductive technologies by couples who are suffering from infertility, which affects about 15% of the population. Obesity is on the rise all across the world. Obesity may be measured with a simple alternative metric: BMI (body mass index). Male infertility has been linked to an increase in the prevalence of obesity.

Purpose: The research linking BMI to sperm parameters is still ambiguous. In this study, male infertility clinic patients' BMI was compared to their semen characteristics to see whether there was any correlation.

Material-methods: Study participants were separated into three groups according to their BMI: normal weight (18.5-24.99 kg/m2), overweight (25-29.9 kg/m2), and obese (more than 30 kilograms/square meter) Each of the three BMI groups were assessed for semen parameters (volume, concentration, total semen count, morphology, and motility). In addition, oxidative stress values TOS, TAS were determined along with using the terminal uridine nick-end labeling (TUNEL) test to determine DNA fragmentation, whereas sperm chromatin condensation was analyzed with aniline blue.

Results: In the obese group, semen volume, total concentration, total motility, and motility at different rates were shown to be reduced. All parameters were not statistically significant in the reductions, although several were. Although the overweight and obese categories were statistically indistinguishable from one other. This study found that overweight and obese men had abnormally shaped and stained cells, as well as larger percentages of TUNEL-positive cells than normal men. There was a statistically significant difference between the two groups. TAS readings in the Normal group were actually greater than they were in the other groups. Results from the Normal, Overweight, and Obese categories did not demonstrate any statistically significant differences either. Although the TOS values in the obese group were higher than in the normal, overweight, and obese groups statistically, there was no discernible difference among the TOS findings.

Conclusion: In this study, the researchers discovered a correlation between obesity and the quality of sperm, morphology and chromatin structure, DNA integrity, and the antioxidant/oxidant balance of the sperm cells. As a result, an elevated BMI might have an adverse effect on healthy sperm.

Keywords: Infertility, body mass index, Obesity.

Oral Presentation

THE EFFECTS OF QUERCETIN, ROSMARINIC ACİD AND QUERCETIN & ROSMARINIC ACID COMBINATION ON METHOTREXATE INDUCED TESTICULAR INJURY

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The testes have a critical role in male fertility with their spermatogenesis and steroidogenesis functions. However, because the testes are organs where numerous mitosis and meiosis take place, they are sensitive to external influences, especially drug side effects. Methotrexate, which is frequently used in the treatment of many malignancies such as head and neck cancers, breast cancer, leukemia, lymphoma, as well as common inflammatory diseases such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus, dermatomyositis and Crohn's disease, is one of the leading drugs that have toxic effects on the testes. The detection of an important role of methotrexate-induced oxidative stress in these negative effects, led the researchers to antioxidant substances.

Quercetin, found in many plants such as onions, green tea, apples, strawberries, etc., is a powerful antioxidant that prevents oxidative damage and cell death by scavenging oxygen radicals. It has stimulant effects on sperm quality and reproductive organs and is used as an alternative medicine in the treatment of male infertility. Rosmarinic acid, a natural polyphenolic compound, commonly found in plants that we use in our daily meals such as rosemary, thyme, sage, and mint. It is used to increase sperm quality and fertility. Also it has many remarkable features such as anti-oxidant, anti-inflammatory, antimutagen, antibacterial, antiviral, etc.

In our study, 40 Sprague Dawley adult male rats were randomly divided into 5 equal groups. No application was made to the control group during the 7-day experiment. A single dose of 30 mg/kg methotrexate was administered intraperitoneally to the MTX, MTX+QT, MTX+RA and MTX+QT+RA groups on the 5th day of the experiment. During the experimental period; MTX+QT group received 30 mg/kg quercetin, MTX+RA group recieved 30 mg/kg rosmarinic acid, MTX+QT+RA group received 15 mg/kg quercetin and 15 mg/kg rosmarinic acid daily by gavage. By using weighing results and tissue samples; body and testis mass analysis, sperm analysis, blood testosterone and tissue MDA, tissue SOD, tissue GPx, tissue CAT measurements, hematoxylin&eosin, Masson trichrome, periodic acid Schiff and TUNEL stainings were performed.

The results showed that methotrexate caused physiological, histopathological, immunohistochemical and biochemical damage to the testis, and that 3 different antioxidant treatments made mostly partial and sometimes complete corrections in almost all of the deteriorated parameters. It was observed that the treatment groups were superior to each other in terms of success in some parameters.

Oral Presentation

SETD3-DEPENDENT GENE EXPRESSION CHANGES DURING ENDODERM DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Introduction: Mouse embryonic stem cells (mESCs) are pluripotent cells that have self renewal capability. They can differentiate into all three primary germ layers: mesoderm, endoderm, and ectoderm during embryonic development. The embryonic development is controlled via spatiotemporal regulation of gene expression changes. The collaborative effects of Wnt, Nodal, BMP, and FGF signaling pathways help form the primitive streak, and the subsequent definitive endoderm layer in the gastrulating embryo. Deactivation of core pluripotency network, and activation of germ layer specific transcription networks are required for this process. This is precisely achieved by chromatin-based regulation. SETD3 is a SET-domain containing methyltransferase that targets both histone and nonhistone proteins. An shRNA screen identified SETD3 as a key factor for mesendoderm commitment of mESCs. Setd3 knock-out mESC (setd3Δ) cannot upregulate pioneer transcription factors that initiate mesendoderm differentiation *in vitro*.

Purpose: In this project, we aimed to determine SETD3-dependent gene expression changes during the endoderm differentiation of mESCs.

Method: We performed time-course endoderm differentiation experiments and employed RNA-sequencing (RNA-seq) to identify differentially expressed genes (DEGs) in the absence of SETD3. Further bioinformatic analyses of the RNA-seq data and the obtained DEGs yielded candidate genes for validation. The expression levels of selected DEGs were validated via qRT-PCR. Time-course endoderm differentiation experiments were repeated with ectopic SETD3 expressing mESCs to investigate whether or not the changes in the expression of the selected DEGs were resulted from the absence of SETD3.

Results: Pathway enrichment analyses of the DEGs among wild type (WT) and setd3Δ cells, as well as the gene sets obtained from Short Time-series Expression Miner (STEM) analysis on each day of differentiation suggested that the expression of genes in Wnt, Activin, and BMP signaling pathways was affected in the absence of SETD3. Additionally, the core pluripotency network was affected when SETD3 was absent as suggested by transcription factor enrichment analysis via ChEA3. The close examination of the selected DEGs from the suggested pathways and networks via qRT-PCR analysis showed that the mESCs cannot downregulate the pluripotency network, and properly regulate the networks governing the endoderm differentiation in the absence of SETD3. Re-expression of SETD3 in setd3Δ cells was sufficient to rescue the defective phenotype.

Conclusion: Our results indicate a role for SETD3 in timely downregulation of the pluripotent state, and response to key signaling pathways which leads to the delayed and defective differentiation in its absence.

Oral Presentation

CONCURRENT ADMINISTRATION OF ZNO, CHITOSAN AND EXTRA VIRGIN OLIVE OIL SUPRESSS THE NASOPHARINGEAL CANCER CELL LINE

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Introduction: Patients with head and neck malignancies may apply to the clinic with concomitant nutritional deficiencies, mostly due to local effects of the tumor causing pain or dysphagia originating from organic obstruction. Besides prognostic factors, nutritional status has been reported to have significant effects on treatment morbidity and overall prognosis in patients with head and neck cancer. Emphasis is placed on the importance of extensive nutritional support with a multi-disciplinary approach in the treatment of head and neck cancers, and it is recommended that patients be supported with multiple nutritional supplements that will prevent the survival of cancer cells.

Purpose: The study was designed to investigate the antineoplastic effects of ZnO, Chitosan and Extra Virgin Olive Oil (EVOO) and their different combinations, on the Detroit 562 cell lines and to compare their activities at the cell culture level.

Material-Method: ZnO, Chitosan and EVOO and binary and ternary mixtures of these compounds were administered to Detroit 562 cell cultures, in a concentration range of 0.01-10%, then the suppression of the Detroit 562 cell lines were observed through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fluorescence microscopy (DAPI, PI, DioC6 staining were used), spectrophotometric evaluation of cell viability and transmission electron microscopic examination.

Result: Based on the MTT cell viability assay, we found that different combinations of ZnO, Chitosan and EVOO reduced cell viability in a dose-dependent manner in Detroit 562 cell lines. Although exposure to low doses of ZnO, Chitosan and EVOO significantly increased cell viability and mitochondrial membrane potential, dramatic decreases in cell viability were obtained with increasing dose starting from 1% concentration, except for EVOO+chitosan, which showed the weakest effect among all combinations. EVOO+ZnO and EVOO+ ZnO+chitosan showed the best cell viability suppression for both 24 and 48 hours in Detroit 562 cell lines when used at 10%. Moreover, TEM analysis showed that treatments caused some ultrastructural changes in terms of ER dilatations, lipid depositions, myelin shapes, autopagic vacuoles, and pseudopodia morphology. Interestingly, the chitosan group showed a significant reduction in mitochondria diameter compared to all other groups except ZnO.

Conclusion: Studies that explain and clarify the mechanisms involved in the anti-cancer activity of some effective nutrients and their combinations, can bring invaluable data to the field of chemotherapy in the fight against nasopharyngeal cancer.

Oral Presentation

THE EFFECT OF INFLAMMATION AND POST-INFLAMMATORY ADMINISTRATION OF N-ACETYLCYSTEINE ON LAG3 AND TLR2 RECEPTORS ON H4 CELLS IN THE CELLULAR MODEL OF PARKINSON'S DISEASE

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Introduction: Parkinson's disease is a complex, multifactorial neurodegenerative disease that has prevalence of 1% over the age of 55. Pathological hallmarks of Parkinson's disease include the loss of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of inclusion bodies that contain predominantly alpha-synuclein (α-syn) and ubiquitinated proteins, called Lewy bodies. Although the formation of α-syn occurs intracellular, it can also be translocalized to extracellular space and then taken into the other cells via receptor mediated endocytosis. TLR2 is a well-known receptor which detects extracellular α-syn and modulates the uptake of the protein by other cells. Uptaken α-syn is known to trigger expression and secretion of inflammatory cytokines such as TNF-α, IL-1β, IL-2 and IL-6 and induce neuroinflammation, apoptosis and mitophagy that results in cellular death. N-Acetylcysteine (NAC), an anti-inflammatory and anti-carcinogenic drug, has gained some focus to circumvent the detrimental effects of neuroinflammatory response.

Purpose: In this study, we hypothesized that, NAC treatment will result in anti-inflammatory response that restrict *in vitro* apoptotic and neuroinflammatory response by modulating transcription and expression of TLR2 and LAG3 receptors.

Material-Method: In order to test this hypothesis, WT α-syn overexpressing human neuroglioma (H4) cell line was treated with TNF-α to induce inflammation and NAC, as anti-inflammatory drug to recover from deleterious effects of TNF-α induced inflammation and apoptosis. α-syn protein transcription and expression were validated by q-PCR and Western Blot, respectively. Cell viability was measured by Toxilight assay, and apoptosis was evaluated by Western Blot and TUNEL methods. Alterations in Lag3 and TLR2 receptor levels evaluated by immunofluorescent labeling, Western blot and q-PCR methods.

Results: We observed that TNF-α not only increases the inflammation but also increases both endogenous and over-expressed α-syn levels. After inflammation is initiated, NAC treatment diminishes inflammation mediated toxicity and cell death by altering transcription and expression of TLR2 and LAG3 receptors.

Conclusion: This study demonstrates NAC to be a promising candidate to recover from neuroinflammation that occurs in Parkinson's Disease. Further studies are needed to elucidate molecular mechanisms underlying the disease, molecular pathways related to neuroinflammation and to develop possible new therapeutic approaches to slow the clinical progression of Parkinson's disease.

Keywords: Parkinson's Disease, Neuroinflammation, Alpha-synuclein (α-syn), N-Acetylcysteine (NAC), Apoptosis

Oral Presentation

HISTOPATHOLOGICAL ANALYSIS OF THE POTENTIAL PROTECTIVE AND/OR THERAPEUTIC EFFECT OF C-TYPE NATRIURETIC PEPTIDE IN THE PROCESS OF OSTEOARTHRITIS IN C57BL/6 MOUSE MODEL

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Introduction: The primary pathology in osteoarthritis (OA) is the inflammatory damage and degradation of articular cartilage resulting in joint failure. Therapeutic effects of systemic CNP analogs has been demonstrated in achondroplasia, the most common cause of dwarfism due to the delay in endochondral development of epiphyseal plates of long bones. Induction with CNP analogs results in proliferation of epiphyseal chondrocytes and increased matrix proteoglycan synthesis. The epiphyseal plate and the articular cartilage originate from the same tissue developmentally suggesting that CNP analogs may also have protective and/or therapeutic effects on progressive cartilage damage in OA.

Purpose: The aim of this study was to histopathologically analyze the effects of CNP on progressive articular cartilage damage in OA using the spontaneous primary OA C57Bl/6 mouse model *in vivo*.

Material-Method: A synthetic CNP analog, P-CNP37/BMN111 was obtained from BioMarin Pharmaceutical Inc. (CA., USA). Study groups were established as the sham control, 600 µg/kg, 400 µg/kg, 200 µg/kg, 100 µg/kg, 50 µg/kg, and 25 µg/kg dose groups in 9 month-old, i.e., showing onset of OA, and 16 month-old, i.e., showing established OA, C57BI/6 mice, respectively (n=6 in each group). The peptide was administered subcutaneously every day for 3 or 6 months. Knee joints were taken from the paraffin blocks and hematoxylin-eosin, safranin-o fast green and alcian blue stainings were performed. Articular cartilages on the femoral condylar and tibial plateau surfaces were evaluated and scored according to OARSI criteria, using at least 15 sections for each knee joint, and a comparative analysis was performed between groups.

Results: The protective effect of P-CNP37/BMN111 on articular cartilage of 9 month old C57BI/6 mice was observed in 600, 400, and 200 µg/kg dose groups after 6 months of treatment in comparison to sham group. However, the agent used showed nephrotoxic effects at these high doses. For the same treatment regime, results indicated statistically significant scores in terms of protected articular cartilages without any side effects in 100, 50, and 25 µg/kg doses (Mean Score±SEM: 0,0267±0,0125, 0,163±0,0286, 0,278±0,0633, respectively) in comparison to sham controls (Mean Score ± SEM: 1.960±0.180; $p \leq 0.001$). The therapeutic effect of treated articular cartilages has been observed without any side effects in 100, 50, and 25 µg/kg doses (Mean Score±SEM: 0,653±0,155; 0,700±0,172; 0,388±0,112, respectively) in comparison to sham controls (Mean Score ± SEM: 2.236±0.385; $p \leq 0.001$).

Conclusion: In conclusion, P-CNP37/BMN111 has both, protective and therapeutic effects on OA at 100, 50, and 25 µg/kg doses in C57Bl/6 mouse model.

This study was supported by the SAGEM Grant: 2017/SAGEM/002.

Oral Presentation

THE REGENERATIVE CAPABILITY OF HERBAL AGENTS ON THE INJURED PERIPHERAL NERVE: A STEREOLOGICAL AND FUNCTIONAL STUDY IN A RAT MODEL

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Introduction: In the last decades, peripheral nerve injury has gained most researchers' attention. It is deemed a worldwide public health problem that results in significant disabilities. Therefore, developing novel strategies to improve recovery is important. Herbal medicine has shown a great history of treating several diseases since ancient times.

Purpose: This study was conducted to evaluate the effect of AD (*Adansonia digitata*), EVOO (extra virgin olive oil) and EOOB (*Ocimum basilicum* essential oil) on the sciatic nerve following the crush model of injury.

Material and methods: The 64 male *Wistar* rats were divided into eight groups (n=8); Cont (Control), Inj (Injury), Inj+AD, Injury+ EVOO, Inj+EOOB, AD, EVOO and EOOB groups. The rats of injured groups were received crush surgery on the right sciatic nerve. The rats were treated orally for ten days postoperative; as the Inj+AD rats were received AD (400mg/kg/day), the Inj+EVOO received EVOO (2ml/kg/day), as well the Inj+EOOB were treated with EOOB (1500 mg/kg/day), while the rats of AD, EVOO and EOOB groups were received the same dose that mentioned above for the same duration respectively. The study duration was 21 days. The stereological and functional assessments were employed.

Results: The stereological results showed that the increasing myelin sheath thickness significantly differed between Inj+AD and Inj (p=0.01) groups. Regarding electromyography evaluation, amplitude results showed a highly significant increase was found among Inj+EVOO and Inj groups (p=0.00). On the other hand, we observed highly significant decreases between Inj+AD and Inj, Inj+EVOO and Inj groups (p=0.00) in terms of latency value.

Conclusion: AD improved the recovery in terms of myelin sheath thickness and latency. Whereas, EVOO showed excellent regenerative capacity regarding amplitude and latency. Thus, AD and EVOO could be used as good herbal agents in the peripheral neuroregeneration field of medicine.

Oral Presentation

THERAPEUTIC AND NEUROPROTECTIVE EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON OXIDATIVE STRESS AND NEUROINFLAMMATION IN AN EXPERIMENTAL MODEL OF ACUTE AND CHRONIC EPILEPSY

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Objectives: Epilepsy is a serious neurological disease that causes social, psychological and economic problems all over the world. In our study, it was aimed to investigate the effects of transcranial Direct Current Stimulation (tDCS) application on neuroinflammation in rats with acute and chronic temporal lobe epilepsy (TLE) model.

Material-Method: 50 male Wistar rats weighing 250-300 g, 10 rats in each group, were divided into three groups as Control, Epilepsy and Epilepsy+tDCS groups. The acute TLE model was created by administering a single dose of 60 mg/kg Pentylenetetrazole to the subjects. To the epilepsy+tDCS group, 30 min. on the 1st and 2nd days of the experiment. 1 mA anodal tDCS stimulus was given. In the chronic TLE model, the subjects were administered 60 mg/kg i.p. PTZ on the first day. Then, 35 mg/kg i.p. PTZ was injected into the groups every other day until the epilepsy pattern occurred. In the epilepsy+tDCS group, 30 min. on days without PTZ injection (2nd, 4th, 6th, 8th, etc.). 1 mA anodal tDCS was given. Locomotor activity was evaluated behaviorally by learning and memory experiments. nNOS and GFAP expressions and SOD, MDA, IL-1β and TNF-α levels were measured in the hippocampus region of the brain. Statistical analyzes were performed with the One Way ANOVA test.

Results: In the behavioral experiments data, a significant decrease was observed in the acute and chronic Epilepsy groups compared to the Control group, while a significant increase was observed in the acute and chronic Epilepsy+tDCS groups compared to the Epilepsy group($p<0.05$). Compared to the control group, GFAP and nNOS expressions were increased in epilepsy groups, while tDAS stimulation and GFAP and nNOS expressions were decreased in acute and chronic epilepsy+tDAS groups. A significant decrease was observed in SOD levels in acute and chronic epilepsy groups compared to the control group, while significant increases were observed in MDA, IL-1β and TNF-α levels($p<0.05$). There was an increase in SOD levels in the treatment groups compared to the epilepsy groups, while decreases in MDA, IL-1β and TNF-α levels were observed. However, these changes were statistically significant only in the chronic epilepsy+tDCS group.

Conclusions: In our study, it has been shown that tDCS administration in acute and chronic epilepsy has therapeutic and neuroprotective effects on oxidative stress and neuroinflammation and has a reducing effect on neuroinflation. Our project was supported by Coordination Unit of Scientific Research Projects of Hitit University (Project number: TIP19001.21.003).

Keywords: Epilepsy, learning and memory, neuroinflammation, PTZ, Tdcs

Oral Presentation

EVALUATION OF B-CAROTENE, CINNAMON AND GARLIC IN PROMOTING REGENERATION OF PERIPHERAL NERVE FOLLOWING CRUSH INJURY

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Introduction: Peripheral nerve injury (PNI) represents one of the common complicated problems experienced by many people all over the world. It affects the patient's physical activities, in addition to its impacts on social and psychological consequences. Furthermore, it affects the economic life of the patients.

Purpose: This study was aimed to evaluate the possible effects of b-carotene (BC), garlic (Gl) and cinnamon (Cinn) on peripheral nerve regeneration following crush injury of the right sciatic nerve in the rat model.

Materials and methods: Sixty-four *Wistar albino* male rats (weighting between 260 ± 30g and age with 12 weeks) were divided into eight groups (n=8). No surgery was performed, or treatment was given to the control (Cont) group or control positive groups that receive treatment with BC, Cinn and Gl at the dose of 100 mg/kg/day, 100 mg/kg/day, and 90 mg/kg/day respectively. In the injury group (Inj) or injury-treated groups, the sciatic nerve was subjected to crush by applying 50 Newton for 60 seconds (sec). In the Inj+BC group, the animals have received 100 mg/kg/day BC. In the Inj+Cinn group, the rats were treated by100 mg/kg/day Cinn. In the Inj+Gl group, the rats were given 90 mg/kg/day Gl. Each treated group receive the tested substance 24 hours after surgery using oral gavage for 28 days. The stereological analysis, histological and functional examinations were done to assess the sciatic nerve in each group.

Result: Stereological analysis revealed that no statistical differences were seen in points of the total number of myelinated axons, the myelin sheath thickness and axonal area in all treated groups compared to the Inj group ($p > 0.05$). Based on sciatic functional index (SFI) evaluation, only Inj+Gl ($p = 0.015$) showed significant difference in increasing way compared to the Inj group.

Conclusion: Based on stereological analysis administration of BC, Cinn and Gl had no effects on regeneration of axonal fibres after sciatic nerve crush injury, whereas functional analysis showed that Gl had beneficial motor improvements.

Oral Presentation

INVESTIGATION OF LNCRNA PART1 SNP (RS8176070) IN TURKEY KNEE OSTEOARTHRITIS PATIENT POPULATION BY DNA SEQUENCE ANALYSIS

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Introduction: It has recently been reported that long non-coding RNA (lncRNA) PART1 expression was detected in cartilage tissues and chondrocytes. It has been suggested that PART1 promoted osteoarthritis (OA) progression by regulating miR-373-3p/SOX4 axis. Thus, recent literature supports the involvement of lncRNA PART1 in OA pathogenesis.

Purpose: The aim of this study was to investigate the putative relationship of lncRNA PART1 SNP (rs8176070) polymorphism and OA in Turkish patients.

Material-Method: By obtaining the permission of the ethics committee for our study, the study group consisting of 102 primary OA patients and 81 healthy individuals were included in our study. Physical and radiological examinations were performed by obtaining the voluntary consent of all participants. Individuals with knee OA were determined according to the Kellgren-Lawrence scale (grade 1-4) and Western Ontario and McMaster Universities Index (WOMAC) surveys were conducted. 5 ml of peripheral venous blood was taken from each participant and genomic DNA isolations were made with commercial kits. Spectrophotometric absorbance of purified genomic DNA was measured and concentrations were calculated and used for standard polymerase chain reaction (PCR) as 1 microgram (µg). Primers for the PART1 DNA sequence have been designed and the PCR conditions have been optimized. As a result of the PCR reaction, the product of 508 base pairs (bp) was obtained. By the Restriction fragment length polymorphism (RFLP) method, BseYI performed the enzyme cut process according to the recognition sequence (5'-C▼CCAGC-3 ') in the region of the restriction enzyme SNP (rs8176070) polymorphism. DNA sequence analyzes of all individuals in our study has been carried out.

Findings: The median age of the subjects included in the study was 60.5 years (MP: 53.8-67.0) in the OA group, and 46.0 years (MP: 40.5-51.0) in the control group. It was observed that 75.5% (n = 77) of the OA group and 55.6% (n = 45) of the control group were women. When the two groups were compared, it was determined that the mean age and women gender of the OA group patients were significantly higher than the control group ($p < 0.05$). Similarly, Body Mass Index (BMI) and WOMAC score were higher in the patient group compared to the control group ($p \leq 0.001$). The wild type genotype (CC) is encoded as "bb", heterozygous polymorphic genotype (CN) "Bb" and homozygous polymorphic genotype (NN) "BB". When the genotype distribution in the OA and control groups was examined, 13.8% (n = 14) of the OA group and 13.6% (n = 11) of the control group were homozygous polymorphic, 43.1% (n = 44) of the OA group and 45.7% (n = 37) of the control group were heterozygous polymorphic, and 43.1% (n = 44) of the OA group and 40.7% (n = 33) of the control group were detected as having the wild type genotype. As a result, odds ratios were not found to be statistically significant

for BB or Bb genotypes (p> 0.05). Allele distributions in all groups were similar (p = 0.875). Statistical significance level was accepted as p <0.05 in this study.

Conclusion: In the literature, some of the existing population studies that analyzed the possible relationship between SNP (rs8176070) and OA described a positive relationship, while some others found negative results. In our study, it was shown that there was no significant relationship between the lncRNA PART1 SNP (rs8176070) and knee OA in Turkish patients. Further population studies analyzing the relationship between SNP (rs8176070) and OA are needed.

Keywords: LncRNA Part1, Osteoarthritis, PCR, DNA Sequence.

Oral Presentation

DEXPANTHENOL INHIBITED CELL APOPTOSIS TO ATTENUATE SEPSIS-INDUCED ACUTE KIDNEY INJURY VIA AUTOPHAGY ACTIVATION

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Introduction: Acute kidney injury (AKI) is one of the most common complications of sepsis and is characterized by an abrupt decrease in renal function. The decline in renal function that occurs in AKI results from a cellular injury that precipitates functional and structural changes in the kidney. Experimental evidence supports a pathogenic role of apoptosis in AKI. Proximal tubule cells are particularly highly susceptible to apoptosis, and damage at this site causes organ failure. On the other hand, recent studies have confirmed that autophagy exerts protective effects against AKI. This situation suggests that autophagy and its associated pathways can be potential targets and therapeutic interventions for AKI. Dexpanthenol (Dxp) has cellular repair ability, plays an important role in cellular defense mechanisms against pathological processes. Purpose: This experimental study was conducted to investigate the potential activity of Dxp on cellular repair ability against septic AKI using immunohistochemical, and histopathological parameters in rats. Material-Method: The sepsis model was successfully established in rats by cecal ligation and puncture (CLP). Rats were divided into four groups of eight: sham group (SH), Dxp group injected intraperitoneally Dxp (500 mg/kg), CLP performed group, CLP+Dxp group injected Dxp (500 mg/kg) after CLP. Kidney tissue specimens were harvested for histopathological and immunohistochemical analysis. Histopathologically, kidney tissues were evaluated semiquantitatively in terms of degenerative changes in tubule cells and for evaluation, ten randomly selected areas were graded as follows according to the degree of histological changes; 0: no change, 1: mild, 2: moderate, 3: severe change. Immunohistochemically, the severity and prevalence of immunoreactivity of apoptosis-related protein (cleaved caspase-3), and autophagy-related proteins (beclin-1, and MAP LC3β) in tubule cells were evaluated. For immunohistochemical evaluation, H score was calculated by multiplying the prevalence of the staining with the staining intensity. According to the prevalence of the immunoreactivity, the sections were graded as 1 = 0-25 % staining; 2 = 26-50 % staining; 3 = 51-75 % staining; 4 = 76-100 % staining in the total area examined. According to the immunoreactivity intensity, the sections were graded as follows: 0 = no staining; 1 = weak staining; 2 = mild staining; 3 = strong staining. Data were compared with the Kruskal-Wallis H test. The results were considered statistically significant at $p<0.05$. Results and Conclusion: The control and Dxp groups showed a normal histological appearance except for slight changes. It is determined that a significant increase in the histopathological changes, and caspase-3 and beclin-1 immunoreactivity in the CLP group. On the other hand, Dxp markedly reduced caspase-3 immunoreactivity and tubular damage while increasing the immunoreactivity of autophagy-related proteins. Results of this study support that Dxp can prevent septic AKI and its protective effect is mediated by promoting autophagy to inhibit renal tubular epithelial cell apoptosis.

Keywords: Apoptosis, autophagy, kidney, sepsis

Oral Presentation

HISTOMORPHOLOGICAL EVALUATION OF DIFFERENT WOUND CARE AGENTS LOADED ON NANOFIBERS PRODUCED BY ELECTROSPIN METHOD IN TERMS OF ANGIOGENESIS

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Introduction: The term "wound" refers to the temporary or permanent loss of biological and physiological characteristics of body structures due to the tissues' normal anatomical structure, integrity, and function deteriorating for a variety of reasons. Various mediators participate in the wound healing process, including parenchymal cells, extracellular matrix elements, and blood cells. Additionally, it is well established that certain substances such as hyaluronic acid (HA), epidermal growth factor (EGF), collagen, and boron promote wound healing.

Aim: In this study, we synthesized wound care dressings containing HA, EGF, collagen, and boron using the electrospinning method, a technique used to synthesize biomedical products for various medical applications, including tissue engineering scaffolds, bone products, and nerve regeneration products. This study aimed to compare the effects of these wound care products on wound healing in animal experiments.

Material-Method: Thirty adult Wistar albino rats were used in this study. Five groups were established: control group (saline dressing), group 1 (HA), group 2 (EGF), group 3 (collagen), and group 4 (boron). On the backs of the rats, a circular full-thickness skin defect with a diameter of 1.5 cm was created. The nanofibers used in the study were synthesized from biocompatible polymers and Calixarene compounds, a significant class of supramolecular chemistry. Nanometer-thin wound dressings were formed using the electrospin technique, and while these covers were being formed, collagen, EGF, boron-based molecules, and hyaluronic acids were loaded into the nanofibre. Every other day, the rats with wounds were dressed with nanofiber dressings. Macroscopically and histologically, wound healing was examined. Tissue sections previously evaluated with H&E were stained with Masson Trichrome for the overall assessment, anti-VEGF for vascularization assessment, and anti-Ki-67 for proliferation assessment.

Results: When tissues were examined, it was discovered that all nanofiber dressings significantly accelerated wound healing when compared to the control group. The EGF group achieved the best results in both macroscopic and histological evaluations. It was determined that the wounds in the EGF group healed almost completely. Additionally, while the EGF group exhibited the greatest vascularization and proliferation, the collagen group exhibited the least.

Conclusion: The study demonstrated that we can create our own wound dressings that accelerate wound healing using nanotechnology. Further studies are needed for the widespread use and mass production of nanofiber wound dressings, which have significant potential in wound care.

Oral Presentation

THE EFFECT OF TGF-BETA1, TGF-BETA2, AND FGF2 GROWTH FACTORS ON THE CHONDROGENESİS OF MESENCHYMAL STEM CELLS

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Introduction: Cartilage tissue has a limited capacity for regeneration and repair. However, cell-based therapies represent a promising treatment strategy for cartilage repair. Especially mesenchymal stem cells are one of the most attractive candidates for cartilage regeneration. Compared to other mesenchymal stem cell sources, Adipose-Derived Stem Cells (ADSCs) can be obtained easily and with sufficient quality. Growth factors are important molecules that affect cell proliferation, differentiation, and maturation in the chondrogenic process. To date, there are no studies in the literature that indicate the effects of combinations of TGFβ1, TGFβ2, and FGF2 growth factors on the chondrogenesis process of adipose-derived mesenchymal stem cells.

Objective: TGFβ1, TGFβ2, and FGF2 growth factors are highly effective in the *in vitro* chondrogenesis of mesenchymal stem cells. The aim of this study was to investigate the effects of specific days and combinations of TGFβ1, TGFβ2 and FGF2 growth factors on the chondrogenesis process of ADSCs in both monolayer and 3D pellet culture.

Material-Method: ADSCs were cultured in monolayer culture system for 3 days as group I-ITS, group II-ITS, group III-FGF2+TGFβ1 and group IV- FGF2+TGFβ1. After 3 days, the medium was changed, and monolayer culture system was continued for 3 days as group I-ITS, group II-TGFβ2, group III-ITS and group IV- TGFβ2. ADSCs were treated in 3D pellet culture system for 3 days, as group I-ITS, group II-ITS, group III-FGF2+TGFβ1, and group IV-FGF2+TGFβ1. After 3 days, the medium was changed and this time groups were formed as group I-ITS, group II-TGFβ2, group III-ITS, and group IV- TGFβ2. After 6 days, all groups of the 3D pellet culture system were cultured with a chondrogenic medium for 21 days. Alcian blue staining and Dimethylmethylene Blue (DMMB) analysis were established on the 6th day of monolayer culture systems. In 3D pellet culture systems, we performed Alcian blue, Safranin O, hematoxylin-eosin (H&E) stainings and DMMB analysis besides were determined COL2A1(Tip II collagen), ACAN (Aggrecan), and Tip I collagen expressions by immunohistochemistry method.

Results: In both monolayer culture and 3D pellet culture systems, which are models of ADSCs chondrogenesis, the combined use of TGFβ1, TGFβ2 and FGF2 had a positive effect on the formation of the cartilage matrix.

Conclusion: The application of TGFβ1, TGFβ2 and FGF2 of certain durations, and combinations to ADSCs culture system positively supports the *in vitro* chondrogenesis model.

Keywords: ADSCs, TGFβ1, TGFβ2, FGF2 *in vitro* chondrogenesis.

Poster Presentation

INVESTIGATION OF THE EFFECT OF NEW DESIGNED POROUS TITANIUM DENTAL IMPLANTS ON OSTEOINTEGRATION AND BONE FORMATION

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Production and use of dental implants are crucial for bone regeneration in tooth loss. Various modifications are applied to the surfaces of Titanium (Ti) implants to achieve faster new bone formation compared to conventional implants. It was assumed that if the effect of original porous Ti dental implants, designed by our group, whose virtual and real environment biomechanical tests have been previously completed, on strong osteointegration and new bone formation in the implant-bone interface could be evaluated in vivo animal model, the new porous implant might be economically mass-produced in our country. The original dental implants with 2 different surface properties produced within the scope of the project; middle one third porous and total porous dental implants were evaluated in vivo comparing with the commercial implant currently used in the market. Total porous Ti implant provided osteointegration in the tibia similar to the conventional implant. Total porous implant could be frequently used as a dental and orthopedic implant due to its easy and short-term production and high adaptability when compared to conventional implants.

Keywords: Dental, Titanium (Ti) implant, Bone, Osteointegration

Oral Presentation

PRODUCTION OF A 3-DIMENSIONAL ARTIFICIAL STOMACH MODEL WITH ALONG MUCUS LAYER

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Introduction: Since the animal studies were generally incompatible to humans, and human studies are limited due to ethical reasons, 3D culture models and artificial tissue paving the way for as replacements of conventional analyses methods. Artificial stomach tissue studies in this regard are highly limited in number.

Aim: In our study, we aimed to create an artificial gastric mucosa and mucus with tissue scaffolds and 3D culture.

Material-Method: Tissue scaffold was prepared by mixing Baseink™ and 7% GelMA polymers. Human fibroblasts and mesenchymal stem cells were added as 2 million cells per ml of hydrogel. Gels were then crosslinked under UV light to form solid scaffolds. Samples in wells were washed and human gastric epithelial cancer (AGS) cells were seeded on top. Samples were then cultured for 48 hours with DMEM/F12 culture media removed along with non-attached cells. In order to mimic cloudy mucus, various combinations of starch, xanthan gum, and bovine serum albumin (BSA) were used to provide gel-like and viscous consistency of gastric mucus. DNA from banana fruits were isolated and added as supporting polymer. Sodium bicarbonate was added to the samples to alkalize the medium. The media in the culture wells were withdrawn, and artificial mucus was added on top of the cells. No mucus was added to the control wells. Wells were covered with 1 ml of fresh media. In order to mimic stomach acid, 30% HCl solution was added. 2D cultures of AGS cells and wells without mucus were used as controls. Samples were cultured for 6-18 hours for analysis. At the end of the experiment, the live samples were examined under a fluorescent microscope and photographed.

Results: HCl caused drastic drop in media pH which was also observed via phenol red color change. Microscopic analyses showed that surface of the samples had intrusions and protrusions which were similar to gastric pits and were covered with epithelial AGS cells, mimicking stomach surface. Deeper layers of the artificial tissues had fibroblastic cells mimicking the connective tissue. Samples without mucus were found to be highly damaged by HCl while mucus covered samples were intact.

Conclusion: We have demonstrated that our model can mimic stomach tissue on cellular level. Further studies can be performed on the samples to create disease models such as gastric infections or ulcers. We believe our model can also be a step forward to future artificial organ production studies.

Oral Presentation

CIRCADIAN RHYTHM CHANGES ARE ASSOCIATED WITH DNA METHYLATING ENZYMES AND GLOBAL DNA METHYLATION IN MATERNAL OVARY

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Introduction: DNA methylation is one of the epigenetic mechanisms that play an important role in oogenesis, spermatogenesis and embryo development. This mechanism includes the maintenance and de novo methylation which are catalyzed by DNA methyltransferase (DNMT) enzymes. Recent data indicates a remarkable plasticity on chromatin states and dynamics in the genome during the circadian cycle. Regulation of the circadian clock is also subject to epigenetic influence. However, the potential effects of circadian rhythm changes on DNA methylation in the maternal ovarian tissues during pregnancy and lactation need to be further investigated.

Purpose: This study aims to investigate whether circadian rhythm changes differentially affect the DNA methylating enzymes and global DNA methylation in maternal ovary during pregnancy and lactation periods.

Material-Method: Pregnant rats were kept at control (C) (12 hours light/12 hours dark), short day (SD; 8 hours light/16 hours dark) and long day (LD; 16 hours light/8 hours dark) conditions during pregnancy (P) and lactation (L) periods. The experimental groups were subcategorized as C-P+L, C-P, SD-P, SD-L, SD-P+L, LD-P, LD-L, LD-P+L (n=6 rats/group) depending on the circadian condition. Ovarian tissues were taken and expressions of DNMT1, DNMT3A, DNMT3B and 5-methylcytosine (5-mC) proteins in ovarian follicles (primordial, primary, secondary, preantral, antral) were evaluated by immunohistochemistry. The staining intensity (0: no staining, 1: weak, 2: moderate, 3: strong) and stained area percentage (1 for <50%; 2 for ≥50%) were multiplied to establish a weighted score. The control and experimental groups were compared against each other and typically ≤ 0.05 were considered statistically significant.

Results: DNMT1, DNMT3A, DNMT3B and 5-mC expressions were primarily localized in granulosa cells of primordial, primary, secondary, pre-antral and antral follicles. DNMT1 and DNMT3A expressions were found to be significantly increased in primary, secondary and preantral follicles in the LD-P, LD-L and LD-P+L groups ($p<0.05$). On the other hand, there was an increased DNMT3B expression only in secondary and preantral follicles in the SD-P, SD-P+L, LD-P, LD-P+L groups ($p<0.05$). In line with this, global DNA methylation level by 5mC staining was significantly increased in primary, secondary, preantral and antral follicles in the LD-P, LD-L and LD-P+L groups ($p=<0.001$).

Conclusion: This study demonstrates that circadian rhythm changes, particularly long day conditions, may differentially affect DNA methylating enzymes and global DNA methylation in maternal ovary. Since altered DNA methylation may cause aberrant oocyte and follicular development, further studies are needed to elucidate the molecular basis of this association.

Oral Presentation

GALANTAMINE AND WEDELOLACTONE COMBINED TREATMENT SUPPRESSES LPS-INDUCED INFLAMMATORY RESPONSES AND NLRP3 ACTIVATION IN N9 MICROGLIA CELLS

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Introduction: NLRP3 (NLR Family Pyrin Domain Containing 3) inflammasome has been implicated in a neurodegenerative diseases. Inhibition of NLRP3 inflammasome activation can suppress neuroinflammation, as a result of which the progression of neurodegenerative diseases can be slowed down. Therefore, we planned an appropriate treatment strategy to prevent neurodegeneration can inhibit neuroinflammation caused by microglial activation. Purpose: The aim of this study was to investigate the anti-inflammatory effects of galantamine and wedelolactone on NLRP3 inflammasome activation in LPS (lipopolysaccharide)-activated microglial activation. In addition, we investigated the potential mechanisms underlying the pharmacological effects of galantamine and wedelolactone by assessing the response of NF-κB (Nuclear factor kappa B) signaling pathway and NLRP3 inflammasome activation.

Material-Method: In this study, N9 microglial cells were treated with LPS and ATP (Adenosine triphosphate) induced NLRP3 inflammasome activition. N9 microglial cells were pretreated with galantamine for 24 h, then treated with LPS for 4 h and wedelolactone 30 min and finally ATP for 1 h. A qRT-PCR and immunostaining was subsequently performed to measure the levels of IL-1β (Interleukin-1β), caspase-1, NLRP3 and NF-κB.

Results: It was found that galantamine and wedelolactone combined treatment protected microglial cells upon LPS-induced cell death. Galantamine and wedelolactone treatment inhibited the expression of NF-κB. The levels of NLRP3, caspase-1, as well as the secretion IL-1β were decreased by combined treatment.

Conclusion: Our results indicated that galantamine and wedelolactone combined treatment could inhibit inflammatory cytokine production and NLRP3 inflammasome activation in microglia, the underlying mechanism of which may be related to NF-κB signaling pathway; and should be considered as a therapeutic strategy for neuroinflammatory diseases.

Keywords: Galantamine, Wedelolactone, Lipopolysaccharide, Microglia, NLRP3 inflammasome, NF-κB

Oral Presentation

INVESTIGATION OF 3,4-METHYLENEDIOXY-B-NITROSTYRENE TREATMENT OF PANOPTOSIS IN AN EXPERIMENTAL RENAL ISCHEMIA-REPERFUSION INJURY MODEL

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Background; Renal transplantation is seen as the best treatment method for end-stage kidney diseases. However, renal ischemia reperfusion (RIR) injury occurring during transplantation is associated with mortality and morbidity. Despite improvements in treatment, mortality and morbidity rates associated with RIR injury still remain high. For this reason, there is still a need for new treatment targets and target-specific treatment approaches. A recent pathway for programmed cell death, called PANoptosis is controlled by a cytoplasmic multimeric protein complex called the PANoptosome. The PANoptosome can activate three parallel key modes of programmed cell death, pyroptosis, apoptosis and necroptosis. However, when each of the pyroptosis, apoptosis and necroptosis causing PANoptosis is examined separately, it is seen that they may be associated with ischemia and reperfusion injury. Inhibition or blockade on these pathways is predicted to reduce programmed cell death and RIR damage. Therefore, the use of specific agents targeting PANoptosis and PANoptosome may be the most effective way to reduce RIR damage.

Objective; in this study, it was aimed to investigate the kidney protective efficacy of 3,4-methylenedioxy-B-nitrostyrene (MSN), a potent inhibitor of NLRP3, which is involved in PANoptosis and PANoptosome, against RIR injury in the experimental ischemia-reperfusion injury model.

Methods; Thirty two female Wistar rats were divided into four groups: control group, sham group, DMSO solution group and MSN treatment group (each n = 8). The rats in the MSN group (Group 1) were given MSN 20mg/kg intraperitoneally 30 minutes before the start of reperfusion. The rats in the Sham group (Group 3) were not given any agent, only RIR was applied. DMSO solution was given intraperitoneally 30 minutes before the start of reperfusion to the rats in solution group (Group 4). The rats in the control group (Group 2) were not given any intraperitoneal agents and RIR was not created, and only anesthesia was administered. At the end of the experiments, kidneys of rats underwent immunohistochemical caspase 3 (apoptotic marker), MLKL (necroptotic marker) and gasdermine (pyroptotic marker)) and ultrastructural examinations.

Results; Pretreatment with MSN supplementation decreased renal tubular and glomerular damage. More importantly, we observed a significant decreased in the incidence necroptosis, apoptosis and pyroptosis in MSN treatment group. In conclusion, PANoptosis plays an important role in renal tubular and glomerular injury and the inhibition of NLRP3 which is involved in PANoptosis by MSN is a promising therapeutic strategy for protection against renal injury in kidney diseases.

Keywords: PANoptosis, PANoptosome, renal ischemia reperfusion

Oral Presentation

THE ROLE OF A438079 IN THE PREVENTION OF LPS-INDUCED P2X7 RECEPTOR-MEDIATED LIVER INJURY IN RATS

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Introduction-Objective: Introduction-Objective: The acute liver injury occurs after intraperitoneal administration of lipopolysaccharide (LPS) and triggers the activation of the immune response. P2X7 is an inflammation factor expressed in hepatocytes, modulates cytokine formation in macrophages, and directly affects cell death mechanisms in the hepatic parenchyma. A438079 is an inhibitor of the P2X7 receptor, and its activity in hepatotoxicity has been reported. For this reason, it aimed to investigate the hepatoprotective role of A438079 via mechanisms of the P2X7 receptor antagonist in the experimental liver damage induced by LPS.

Material-Method: To test the anti-inflammatory effects of A438079, the experimental model of inflammation was performed with an 8mg/kg dose of LPS (i.p), and then A438079 (i.p.) was administrated at a dose of 15mg/kg in the rats. Six hours later, all rats were anesthetized with sevoflurane, and the blood collecting from the hearts of rats, the liver tissues were removed for histologic and biochemical analysis. In the biochemical analysises, serum AST and ALT enzyme concentrations and tissue MDA, GSH level, and SOD activity were determined for all groups. The liver tissues were examined histopathologically, and relative expression levels of P2x7, Nf-kB-p65, IL-6, Bcl-2, and Caspase-3 were determined in the liver tissues of each rats group by western blot analysis. All data were analyzed with using One way Anova (Duncan) tes

Results: In the results, the serum AST and ALT concentrations, tissue GSH level, and SOD activity dramatically decreased in the LPS groups compared to the control and A438079 groups. However, these parameters were increased significantly in the A438079+LPS group ($p<0.05$). In histologic analysis, severe sinusoidal dilatation, necrotic hepatocytes, and inflammatory cell infiltration were observed in the LPS group, and these damages were relatively reduced in the A438079+LPS group. In the relative protein expression levels, P2x7, Nf-kB-p65, IL-6, and Caspase-3 were significantly higher in the LPS group than in other groups ($p<0.05$). On the other hand, these protein expressions were considerably higher in the A438079+LPS group than in the LPS group. Also, Bcl-2 protein expression was significantly lower in the LPS group and higher in the A438079+LPS group than in other groups ($p<0.05$).

Conclusion: Thus, the protective effect of A438079 against LPS induced hepatotoxicity in rats can be explained by its inhibition effect on P2X7 receptor-mediated inflammation and cell death pathways.

Keywords: LPS, A438079, P2X7, inflammation, cell death, rat liver.

Oral Presentation

THE ANTIAPOPTOTIC AND ANGIOGENIC EFFECTS OF ARONIA MELANOCARPA AGAINST CYCLOPHOSPHAMIDE SIDE EFFECTS ON THE UTERUS IN RATS

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Introduction: Cyclophosphamide is an alkylating chemotherapeutic agent, and it has an extensive use for adjuvant or neoadjuvant chemotherapy.Despite its catastrophic side effects on the reproductive organs are still not preventable today (1).Using natural antioxidants in order to minimize the side effects of chemotherapeutic agents such as CYC has increased recently.Aronia melanocarpa (A. melanocarpa) which has antitumor, anti-inflammatory, and antioxidant properties, reduces free radicals, affects telomere length, regulates the immune system, increases apoptosis in cancerous cells (2).The effects of A. melanocarpa treatment to increase ovarian reserve in premature ovarian failure (3) and polycystic ovarian syndrome (4) show that it has estrogenic effects, and its effects on the uterus are not yet known.

Objective: To evaluate the impact of systemic cyclophosphamide (CTX) treatment on the rat uterus and investigate the potential antiapoptotic and angiogenic effects of antioxidant and anthocyanin preparation A. melanocarpa against cyclophosphamide side effects.

Materyal-Metod: A 30 healthy adult female Wistar albino rats were used in this study. Rats with healthy estrous cycles were included in the experiment. Rats were randomly divided into three groups to determine the effects of A. melanocarpa against CTX side effects on the uterus(n=10 in each group); Group 1 was the control group(sham-operated), Group 2 was the CTX group, Group 3 was CTX(single dose, 100 mg/kg, i.p.) + Aronia(15 times, 200mg/kg, oral gavage) group. Vaginal smear samples were taken to evaluate model control and the effects of Aronia.After the animals were sacrificed and their uteruses were removed, they were weighed and evaluated histomorphologically and immunohistochemically.

Results: In vaginal cytological examination, A. melanocarpa treatment re-regulated the estrus stages that were disrupted in CTX. No statistically significant difference was observed between the groups in terms of uterine weights($p>0,05$). In the histopathological examination, vascularization decreased in the endometrium and interstitium in the CTX group;it was observed that the number of glandular glands decreased and the observed glandular glands had dysplasic morphology(Figure 1E).The number of glandular glands and vascularization were significantly increased in the uterus following treatment with aronia melanocarpa(Figure 1F). In the model in which apoptosis and avascularization were created in rat uterus with systemic CTX administration, Aronia melanocarpa increased VEGF; decreased histological damage and caspas-3 and caspas-9 immunohistochemically in the rat uterus.

Conclusion: Cytotoxic effects of natural alkylating chemotherapeutic agents like cyclophosphamide on the uterus can be prevented by A. melanocarpa. Further studies are needed to evaluate the effects of A. melanocarpa on estrogen receptors and estradiol levels.

Oral Presentation

THE EFFECTS OF ENDOMETRIAL SCRATCH ON THE ENDOMETRIUM IN RECURRENT IMPLANTATION FAILURE

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Purpose: The success of assisted reproduction techniques in patients with recurrent implantation failure (RIF) depends on the harmonious interaction of the endometrium and blastocyst. In women with RIF, evaluation of endometrial factors comes to the fore since pregnancy does not occur even though good quality embryos are transfered. Although there are many published studies showing that endometrial injury caused by endometrial biopsy with the help of a pipelle increases endometrial receptivity, the exact mechanism of this improvement is unknown. This study aimed to determine the effects of endometrial injury on endometrial receptivity by using histological and immunohistochemical methods.

Materials and Methods: In this study, 19 patients diagnosed with RIF underwent diagnostic hysteroscopy between days 19 and 21 of the menstrual cycle, and the collected endometrial tissue specimens were prepared in accordance with light and electron microscopic tissue preparation methods. Immunohistochemical examinations were performed for endometrial receptivity markers of LIF, integrin αvβ3, and estrogen alpha receptor. In the same patient group, the endometrial sampling materials taken in the next cycle between days 19 and 21 for the investigation of response to treatment were also evaluated for endometrial receptivity markers. The results were compared with those of the endometrial receptivity reference group composed of women who did not have any endometrial pathology and who could conceive without treatment.

Results: While cells rich in microvilli were diffuse and cells with pinopods and vesicles were scattered in the surface epithelium of the patients with RIF before the injury, the cells with pinopods and vesicles were observed to be the predominant cells of the epithelium after the injury. The endometrial surface epithelium of infertile patients were observed to contain patches of pseudostratified epithelium in some areas, whereas the endometrium was lined by a single layer of columnar epithelium after the injury, similar to the control group. The ultrastructural findings noted in the epithelium of infertile women turned into features similar to the endometrium of fertile women after the injury. Immunohistochemical examinations revealed that LIF, integrin αvβ3, and estrogen alpha receptor were expressed in the surface epithelium, glandular epithelium, and stromal cells. The immunoreactivity of LIF were increased by injury, however immunoreactivity of estrogen alpha receptor and integrin αvβ3 showed no significant difference with injury.

Conclusion: It is believed that endometrial injury to infertile women with RIF improves the endometrial receptivity by improving histopathological and immunohistochemical findings.

Keywords: Endometrial injury, recurrent implantation failure, LIF, integrin αvβ3, estrogen alpha receptor

Oral Presentation

INVESTIGATION THE EFFECTS OF INSULIN ON THE OVARIAN FOLLICLES IN RATS WITH DIABETES AT LIGHT AND ELECTRON MICROSCOPIC LEVEL

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Introduction: Diabetes is a metabolic disorder characterized with chronic hyperglycemia. Insulin injections are used in patients with diabetes. Menstruel irregularity, hypogonadism and early menopause are reported in women with diabetes. Hyperandrogenism derived polycystic ovaries due to insulin therapy are observed in clinical investigations. However, there has been limited research evaluating the effects of insulin on ovaries in diabetic women. In this study, investigation of the ovarian structural changes in a diabetic rat model and the effects of insulin therapy in diabetes were aimed.

Material-Method: Wistar albino female adolescent rats (n=24) were divided into 3 groups; in control group only 0.1 M citrate buffer was injected, in diabetes group single dose of 60 mg/kg i.p. streptozotocin was injected, and in insulin treatment group diabetes was induced with single dose of 60 mg/kg i.p. streptozotocin and then, 2 IU insulin was injected in the morning and evening throughout 4 weeks. Blood glucose concentrations were measured 72 hours after streptozotocin injection. Rats with blood glucose levels ≥250 mg/dl were included in the study. At the end of 4 weeks, blood samples were taken for biochemical analyzes. One ovary of each rat was prepared for electron microscopic and the other was prepared for light microscopic examination, and evaluated with JEOL-JEM 1400 TEM and BX53 light microscope.

Results: There were no significant difference in serum estradiol and testosterone hormone concentrations between the three groups, but androstenedione was increased in diabetic and insulin groups compared to the control group. Androstenedione increase in insulin group was significant. Primordial and primary follicles were decreased, follicular atresia was increased and cystic-like follicles were developed in the ovaries of the diabetes group. In the insulin treatment group, primary follicles were increased, follicular atresia was decreased and cystic-like follicles were more common than the diabetes group. In electron microscopic evaluations, apoptosis in the primordial follicle oocytes, paraptosis characterized with endoplasmic reticulum enlargement, mitochondrial swelling, cytoplasmic vacuolization and enlargement of perinuclear cisternae in granulosa cells of developing follicles in the diabetes group were seen. In addition, in the cystic-like follicles in the diabetes and insulin groups, ovaries revealed apoptotic bodies between the granulosa cells and early luteinisation signs of granulosa cells characterized with increased agranular endoplasmic reticulum cisternae, lipid droplets and mitochondria with tubular cristae in their cytoplasm.

Conclusion: As a consequence, it is thought that, these morphological changes play a role in the development of cystic follicles, and result in infertility.

Keywords: Diabetes, Follicle Development, Insulin, Ovary

Oral Presentation

EVALUATION OF GASTRIC CHANGES IN SARS-COV-2-INFECTED BALB/C MICE BY LIGHT MICROSCOPY

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Introduction: COVID-19 disease is an infectious disease that presents clinical outcomes at varying degrees and can result in death by affecting multiple systems. The direct tissue damage and the mechanisms of that in the gastrointestinal system should be explained.

Purpose: This study explores viral effects on each type of gastric cells of SARS-CoV-2 infected Balb/c mice due to gastric symptoms of unknown cause in COVID-19 patients. The presence of the virus in the acidic pH range is unclarified in the literature, this will be revealed. In addition, it is aimed to clarify the dose-dependent toxic effects of inactivated virus vaccine on the stomach.

Material & Method: Two doses of intraperitoneal newly developed inactivated virus vaccine injections, SARS-CoV-2 inhalations and sacrifications of five groups of adult Balb/c mice: control (n=5), infected (n=5), vaccinated with low dose (n=4), vaccinated with high dose (n=6), and vaccinated with low dose and subsequently infected (n=6) were performed at BSL-III conditions. Sections from paraffin bloks of stomachs went into cell types determination, viral detection and morphological, inflammatory and apoptotic examinations.

Results: Glandular part of the infected mice exhibited mild hypoxic damages and were immunpositive for viral spike protein. Neutrophils resided under the epithelium focaly. IL-1ß reactivity was intenser in the nonglandular to the glandular transition. The morphology of the low-dose vaccine group and the control group were similar. High-dose vaccine group demonstrated nonspecific alterations.

Conclusion: The viral proteins existed in the stomach. Yet, we observed a non-severe pathology in the stomachs of infected mice. The inflammatory responses were not present in the control or low dose vaccinated mice. This proves the difference between the effect of the viral infection and the vaccine. Stomach-related symptoms should not be expected in the short term of appropriate doses of inactivated vaccines.

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Oral Presentation

ROLE OF SUMOYLATION MECHANISM IN PERI-IMPLANTATION PERIOD MOUSE UTERUS

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Introduction: The events in uterus during peri-implantation period are critical for healthy progression and termination of pregnancy. These include development of embryo, acquisition of uterine receptivity, implantation and decidualization. SUMO (small ubiquitin-like modifier) proteins take role in post-translational modification which is very important for organism. SUMO-1 is found in cells in a conjugate form with proteins and usually localized in the nuclear membrane and nucleolus.

Purpose: In this study, we aimed to determine whether sumo members take role in implantation and decidualization. For this we evaluated the localization and expressions of SUMO-1 (small ubiquitin-like modifier), SENP1 (Sentrin-specific protease 1) and UBC9 (SUMO-conjugating enzyme UBC9), which are members of sumoylation mechanism in peri-implantation mouse uteri and implantation sites.

Material&Methods: One male and two female Balb/C mice were kept in same cage overnight, following day female mice with vaginal plaque were accepted on the first day of pregnancy. Uteri and implantation sites of mice on days 1, 4, 5, 6 and 8 of pregnancy were obtained. Expressions and localizations of SUMO-1, SENP1 and UBC9 were determined by Western blot and immunohistochemistry, respectively and were analysed via İmage J. Estrogen and progesterone levels were measured by ELISA.

Results: SUMO-1, SENP1 and UBC9 were expressed at different densities in the uteri and implantation sites of different days of pregnancy. According to our immunohistochemistry and Western blot results SUMO1 was at the highest level in the luminal epithelium, glandular epithelium, and stroma of implantation sites of the 5th day of pregnancy ($p<0,0001$). SENP1 was highest in the stroma of the uteri on the 4th day of pregnancy, and in the luminal and glandular epithelium of implantation sites of the 5th day of pregnancy ($p<0,0001$). UBC9 was highest in the stroma of implantation sites of the 5th day of pregnancy. According to our Western blot results, SUMO1 and SENP1 were statistically significantly decreased on the 8th day of pregnancy. UBC9 was higher on day 8 of pregnancy than SUMO1 and SENP1. According to ELISA results the level of estrogen was highest on the 4th day of pregnancy, and the level of progesterone was at highest on the 8th day of pregnancy.

Conclusion: SUMO1, SENP1, and UBC9 have spatial and temporal expression in uteri and implantation sites on different days of pregnancy. According to our findings, sumoylation mechanism may play a role in pregnancy and decidualization processes in mice.

Keywords: mouse, implantation, SUMO1, SENP1, UBC9

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Oral Presentation

THE POTENTIAL ROLE OF SIRTUIN 1 IN EMBRYO IMPLANTATION AND DECIDUALIZATION

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Introduction: Embryo implantation and decidualization are required for a successful pregnancy in mammals. Successful implantation requires "cross-talk" between the competent blastocyst and receptive uterus. Decidual cells provide growth factors and cytokines for embryo and regulate trophoblast invasion. Sirtuin 1 (SIRT1) plays an essential role in cellular processes such as cell cycle regulation, deoxyribonucleic acid (DNA) repair, aging, oxidative stress, regulation of gene expression, apoptosis, cell survival, inflammation and immune response.

Aim: In this study, the main goal was to determine if SIRT1 has a role in embryo implantation and decidualization in mice.

Material&Methods: Six to eight-weeks-old female and twelve-weeks-old male Balb/C mice were used. Two or three female mice were kept with male mice overnight for mating, and the vaginal plug was checked the following morning. Female mice with a vaginal plug were admitted on the 1st day of pregnancy. Uteri and implantation sites of mice were collected on the 1st (D1), 4th (D4), 5th (D5), 6th (D6) and 8th (D8) days of pregnancy. Also, inter implantation sites were collected on days 5 and 6 of pregnancy. Localization and expressions of SIRT1 were determined by immunohistochemistry and Western blot, respectively.

Results: SIRT1 was expressed at different intensities in the luminal epithelium, glandular epithelium, and stroma. Based on histological scoring, SIRT1 expression in the luminal epithelium gradually increased from D1 to D5 group. SIRT1 expression in the luminal epithelium was highest in inter-implantation sites of D5 group ($p<0.0001$) and lowest in D6 group ($p<0.0001$). In the glandular epithelium, SIRT1 expression was statistically significantly decreased in D4 group compared to all groups. SIRT1 expression in the stroma was significantly higher in D8 than D6 group ($p<0.0001$); however, there was no significant differences between D5 and D8 groups. According to Western blot analysis, SIRT1 expression was highest in D4 group. This expression was statistically significantly higher than D1 uterus ($p<0.0001$), implantation sites of D5 and D6 ($p<0.0001$) and inter implantation sites of D5 and D6 groups ($p<0.0001$).

Conclusion: Our findings suggest that SIRT1 may have role in embryo implantation and decidualization. We believe that our study may help understand the roles of SIRT1 in the peri-implantation period and elucidate the underlying causes of various pathologies such as implantation failure, pregnancy loss.

Keywords: SIRT1, mouse, uterus, implantation, decidualization

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Oral Presentation

EFFECTS OF PLACENTA-DERIVED MESENCHYMAL STEM CELLS CONDITIONED MEDIUM IN OVARIAN DAMAGE

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Objectives: Fertility preservation is an emerging discipline and has a significant clinical value in the care of cancer survivors. Radiation therapy, which is one of the cancer treatment methods, is defined as a factor accelerating ovarian aging by reducing and eliminating the number of primordial follicles in the ovarian follicle pool. Gonadotoxicity is an adverse effect of pelvic radiation therapy and ionized radiation has been reported to cause ovarian damage directly. The aim of this study is to determine the effect of mesenchymal stem cell conditioned medium isolated from human placenta amnion membrane (hAMSCs-CM) on ovarian damage caused by whole body irradiation through ER stress and apoptosis mechanisms.

Methods: 7 Gy whole body irradiation was used to create a ovarian damage model and serum-free hAMSCs-CM were given from the tail vein of rats. Follicle count was done from the ovaries. Expressions of GRP78, CHOP, IRE1, caspase 12, caspase 9, caspase 3 were determined through immunohistochemistry. TUNEL was performed. Levels of serum FSH, LH, E2, AMH and oxidative stress marker 8-ohdg were determined.

Results: We observed that the number of primordial, primary, secondary and graafian follicles decreased significantly and the number of atretic follicles increased in the irradiated group. After hAMSCs-CM transplantation, the number of atretic follicles decreased significantly while the number of other follicles increased. It has been determined that ionizing radiation targets ER and the immunoreactivities of ER stress markers GRP78, IRE1α and CHOP increased in the ovary. ER stress markers expressions decreased after hAMSCs-CM administration. The immunoreactivities of ER-related caspase-12, caspase-9 and caspase-3 increased in the irradiated group. When the Tunnel test was performed, it was seen that the radiation led to the death of the cells. After hAMSCs-CM, it was determined that the immunoreactivity of caspase-12, caspase-9, caspase-3 and the number of tunnel positive cells, which are the markers of apoptosis, decreased. After irradiation, while serum AMH and E2 decreased, FSH and LH increased. When hAMSCs-CM administered, AMH and E2 increased however FSH and LH decreased.

Conclusion: According to the results, amnion membrane-derived mesenchymal stem cell conditioned medium can play a therapeutic role in ionizing radiation-induced ovarian damage by reducing endoplasmic reticulum stress and apoptosis.

Keywords: Amnion membrane, Mesenchymal Stem Cell, Conditioned Medium, Radiation, Ovarian Damage, Endoplasmic Reticulum Stress, Apoptosis

Oral Presentation

THE HISTONE METHYLTRANSFERASES SETD1B AND SETDB1 SIGNIFICANTLY INCREASE IN THE AGED MOUSE TESTES

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Introduction: Spermatogenesis is a strictly regulated process during which mitosis, meiosis, and spermiogenesis events take place. These events are largely regulated by the epigenetic mechanisms such as histone methylation. Histone methylation is briefly defined as methylation of lysine or arginine residues of histone variants in nucleosomes. Functional and knockout studies revealed that the histone methyltransferases SETD1B and SETDB1 play key roles in regulating expression of the genes required for properly maintaining the spermatogenic events. As several spermatogenesis-related disturbances such as decreased sperm counts and spermatogenic arrests appear along with biological aging, molecular background of these changes remains elusive.

Purpose: In this study, we aimed to determine the spatiotemporal distributions and relative levels of the SETD1B and SETDB1 proteins in the postnatal mouse testes from early to aged periods.

Material-Method: Five groups were created from Balb/C male mice as following: early (1- and 2-week-old, n=4), prepubertal (3- and 4-week-old; n=4), pubertal (5- and 6-week-old, n=4), postpubertal (16-, 18-, and 20-week-old, n=6) and aged (48-, 50-, and 52-week-old, n=6). Obtained testes from these mice were fixed with immersing in Bouin's solution at +4 ºC overnight and then embedded in paraffin after applying routine tissue procedures. After performing immunohistochemistry for the SETD1B and SETDB1 proteins on the paraffin sections, we analyzed their immunostaining profiles with ImageJ software program. The data were analyzed using one-way ANOVA for determining statistical significance ($P<0.05$).

Results: In all groups, SETD1B protein was mainly localized in the nuclei of the cells found in the seminiferous tubules and intertubular area (Figure 1A). In the seminiferous tubules, in addition to weak cytoplasmic immunoexpression, SETD1B was strongly resided in the nuclei of the spermatogenic cells including spermatogonia, spermatocytes, round spermatids, and Sertoli cells. The relative SETD1B protein levels gradually increased from early to aged groups (Figure 1B; $P<0.05$). Similarly, the SETDB1 protein was more intensively localized in the nuclei of the cells found in the seminiferous tubules including spermatogonia, spermatocytes, round spermatids, and in the intertubular area (Figure 2A). There was a prominent cytoplasmic immunoreaction in the elongating spermatids of some seminiferous tubules in the aged group. Relative SETDB1 at low levels in the early and prepubertal groups ($P<0.001$), and progressively enhanced toward the aged group (Figure 1B; $P<0.001$).

Conclusion: Current findings suggest that increase of the SETD1B and SETDB1 levels in the aged groups may contribute attenuating adverse effects of aging, which should be examined in the future studies.

Keywords: Testis, spermatogenesis, biological aging, histone methyltransferases, SETD1B, SETDB1

Oral Presentation

DETERMINATION OF THE ROLE OF UBIQUITIN PROTEASOME SYSTEM (UPS) PROTEINS ON HUMAN SPERM CAPACITATION

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Introduction: The ubiquitin proteasome system (UPS) has a role in many biological processes such as antigen processing, cell cycle, apoptosis, cellular defense, cell signaling and transcription (1,2). In addition to these important intracellular mechanisms, it has been found in recent years that this system also plays a role in the function of many important steps in the reproductive system such as fertilization, capacitation, acrosomal reaction and ZP binding. However, the functions of p97/VCP(Valosin containing protein), PAWP(Postacrosomal sheath WW domain binding protein) and Nedl2(NEDD4-like ubiquitin ligase 2) proteins, which are thought to be related to sperm capacitation and fertilization known to work at UPS, have not yet been clarified.

Purpose: We aimed to investigate whether some proteins (having a role in UPS and especially having a relationship with proteasomes) have roles in sperm capacitation and, if so, to determine in which process or step they performed these functions.

Materials and Metod: Human sperm collection was performedunder the quidance of Approved Human Reserach Ethics Committee. Ejaculates with high motility an concentartion were used, especially preferred mens have new baby. Spermatozoa were washed with gradient methods and then were used only motile sperms. With using capacitation and Non-Capacitation mediums were created 9 groups. Group 1: NCM Group 2: 0.hour Group 3: 5.h Group 4: 18.h Group 5: 10 Mm DBEQ Group 6: 100 Mm DBEQ Group 7: 10 Mm 26S Inhibitors Group 8: 10 Mm 26S Inhibitors Group 9: DMSO. All groups were performenced by Immunofluorescence, Western Blot, Image Based Flow Cytometry and Flow Cytometry.

Results: VCP showed localization at entire sperm tail and equatorial region at human spermatozoa. But after capacitation VCP signal was at anterior head and entire sperm tail. It is noteworthy that localization of VCP/p97 changed after capacitation. NEDL2 showed localization at entire sperm tail especially at mid-piece. NEDL2 localization did not changed after capacitation but intensity of NEDL2 decreased after capacitation. When we used Ubiquitin Proteasome Inhibitors Coctail (MG132 + Epoximicin + CLBL) during capacitation experiments, VCP intensity decreased and NEDL2 increased compare to the 18h. capacitation experiments without inhibitors.

Conclusion: Our results support that the role of VCP/p97 might be a link between capacitation and acrosome reaction and NEDL2 plays a role in capacitation process The changes of VCP and NEDL2 when proteasomal inhibitors used that NEDL2 is a proteasomal subsrat similar to VCP and VCP and NEDL2 interact with sperm proteasomes during the capacitation experiments.

Oral Presentation

THE EFFECTS OF DIFFERENT PROPOLIS DOSES ON CIGARETTE SMOKE EXPOSURE IN RAT TESTIS AND SPERMATOZOON FUNCTION AND MORPHOLOGY

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Introduction: Chemical substances in cigarettes smoke cause reproductive system toxicity by causing pathological changes on testicular tissue and sperm parameters. Some studies indicate that oxidative stress induced apoptosis is responsible for these changes. Pathological changes that cause damage on the testicular tissue creates a decrease in sperm concentration and an increase in number of abnormal sperm. Propolis (bee gum) is the final resin-like product of bees. Propolis has anti-inflammatory, anti-bacterial, anti-viral effects. It also has anti-oxidant effects on many tissues that protect them from oxidative stress. In literature it has been shown that propolis reduces the damage which is caused by toxic agents on the testicular tissue and sperm.

Purpose: The aim of this study is to examine the protective effect of different propolis doses on testicular tissue and sperm exposed to cigarette smoke by evaluating seminiferous tubules stained with H&E, caspase-3 immunlabelling and epididymal sperm concentration, motility and morphology.

Material&Methods: 40 Wistar type male rats were divided into 4 groups as C: Control group, S: Cigarette smoke group, S+P20: cigarette smoke+20 mg/kg propolis, S+P50: cigarette smoke+50 mg/kg propolis daily for 30 days (exposed to smoke of 6 cigarettes/day). Testicular tissue sections were stained with H&E for the evalution of Johnsen's score. The sperm concentration and motility were assessed. Sperm morphology were evaluated for abnormal sperms and head, mid piece and tail anomalies. For apoptotic assessment, seminiferous tubules were labeled with caspase-3 antibody and scored between (0: none 1-2: mild 3: intense). Statistical analysis was performed with SPSS 22.0 statistical software. One way ANOVA test was used for comparison of groups. Dunnet post hoc test was used. $p<0.05$ was considered statistically significant.

Results: Total sperm concentration was decreased in groups S, S+P20, S+P50 in comparison with group C ($p<0.001$). Sperm motility was found similar between group C and S while a prominent decrease was observed in groups S+P20 and S+P50 ($p<0.001$). Abnormal sperm concentration slightly increase in S group compared to C and the difference is not statisticaly significant whereas both in the S+P20 and S+P50 groups the abnormal sperm concentration were prominently decreased compared to C and S ($p<0.001$). The concentration of

spermatozoa with total defects in S+P20 and S+P50 were significantly lower than the C and S groups, while the multiple anomaly index (MAI) of S+P50 group was higher than other groups. The sperm deformity index (SDI) of the S+P20 and S+P50 groups were lower than C and S ($p<0.001$). Testicular seminiferous tubules Johnsen scores showed that there were no significant difference between the all groups ($p>0.05$). There were no caspase-3 labelling in C group while intense staining was observed in S group and the caspase-3 intensity in S+P20 and S+P50 groups was decreased.

Conclusion: In conclusion when used with cigarette smoke, we suggest that high dose propolis may cause toxic effects on sperm function by increasing MAI that leads to reduce sperm motility. Although the immunohistochemical caspase 3 labelling in our study revealed that propolis may have a protective affect on testis by reducing cell apoptosis, further studies are needed for the establishment of ideal propolis dose that may have a beneficial activity without any deleterious effects on spermatozoon function and morphology.

Oral Presentation

CYANIDIN-3-O-GLUCOSIDE INHIBITS CELL PROLIFERATION AND REGULATES ONCOMIR EXPRESSIONS IN HEP3B HEPATOMA CELLS

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Introduction: Hepatocellular carcinoma (HCC) is a primary liver malignancy which usually appears on a condition of chronic liver disease. HCC is included in the most common types of liver cancer. microRNAs (miRNAs) are non-coding RNAs that can regulate gene expression. Dysregulation of oncomiR has been implicated in tumor initiation, progression and cell proliferation in HCC. Anthocyanin (cyanidin-3-O-glucoside, C3OG) is a naturally occurring purple colored phenolic compound in fruits and plants. Their chemical structure confer anti-apoptotic and anti-inflammatory activities. Anthocyanins have been the target of many studies due to their mitigating effect in some diseases such as diabetes, cancer and cardiovascular and neurological pathologies.

Purpose: The purpose of this study is to reveal the effect of C3OG on the proliferation of Hep3B hepatocellular carcinoma cells and the changes in oncomiR expressions in vitro depending on dose and time.

Material-Method: Hep3B hepatocellular carcinoma cells were cultured in DMEM medium supplemented with 10% FBS, 20 units/ml penicillin, and 20 µg/ml streptomycin and maintained at 37 °C in a humidified incubator with 95% air and 5% CO. The effects of C3OG on cell viability were determined by XTT method depending on time and dose. Total RNA was isolated with Trizol reagent and then miRNA cDNA was synthesized. Expression changes of hsa-miR-223-3p, hsa-miR-224-5p, hsa-miR-21-5p oncomiRNAs in control and dose groups were evaluated by Real-Time PCR method, using U6 as housekeeping miRNA.

Results: Cell viability of the Hep3B cell line decreased with increasing C3OG dose. The IC50 value of C3OG in Hep3B cells was 56.4 µl at 72nd hours. hsa-miR-223-3p, hsa-miR-224-5p hsa-miR-21-5p expression changed in C3OG treated Hep3B cells. According to results, C3OG inhibits the proliferation and regulates oncomiR expressions in Hep3B cells.

Conclusion: In conclusion, these findings have provided insight into the effects of C3OG on HCC for further molecular biological studies.

Oral Presentation

INHIBITION OF MAMMALIAN TARGET OF RAPAMYCIN (MTOR) IN TCAM-2 SEMINOMA CELL LINE

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Introduction: Testicular cancer is the most common solid organ malignancy in young men and remains the leading cause of cancer deaths. Seminoma is the germ cell tumor of the testis and TCam-2 cell line is the first seminoma-derived cell line that retains many of the characteristic traits of seminoma. Mammalian target of rapamycin (mTOR) is a key serine/threonine kinase playing a crucial roles in cell cycle, protein synthesis, energy metabolism, cell proliferation, growth, differentiation, and apoptosis. Previously, we showed mTOR signal pathway proteins in seminoma of human testis. Due to its diverse roles and it is generally dysregulated in cancer, mTOR pathway is an important target in cancer therapy. However, the studies on mTOR activity in seminoma are very limited.

Aim: In this study, we hypothesized that rapamycin treatment may be beneficial in testicular cancer treatment. Therefore, our aim was to investigate the expression pattern of mTOR signaling proteins in the TCAM-2 cell line after rapamycin treatment.

Material-Method: TCam-2 cells were cultured and treated with different concentrations of rapamycin (0, 4, 20, 100, 500 and 1000 nM) for 48 h and 72 h. Afterwards; mTOR, p-mTOR, P70S6K, p-P70S6K and proliferating cell nuclear antigen (PCNA) expression levels were analyzed by western blot. Migration was performed by wound healing assay. The apoptosis rates were evaluated with Annexin V/PI by flow cytometry.

Results: We detected that 1000 nM rapamycin significantly decreased mTOR activity ($p<0.05$) after 48 h treatment period. In addition, P70S6K acitivity significantly decreased in groups at all rapamycin concentrations ($p<0.05$). mTOR pathway activity were significantly decreased at 100 and 1000 nM rapamycin-treated groups ($p<0.05$) after 72 h treatment period. Moreover, P70S6K activity decreased at all the treatment groups ($p<0.05$). While there was a tend to decrease in the PCNA expression at 48 h in a dose-dependent manner, this decrease was not significant. On the other hand, PCNA expression decreased nearly significantly at 1000 nM rapamycin treatment after 72 h ($p=0.06$). 1000 nM rapamycin significantly decreased the migration rate ($p<0.05$) at the end of 72 h. Moreover, all doses of rapamycin slightly but significantly increase in apoptosis was observed ($p<0.05$) at 72 h treatment group.

Conclusion: In conclusion, our study suggests that the rapamycin treatment at higher doses (1000 nM) may inhibit the proliferation of TCam-2 seminoma cells by affecting migration via mTOR inhibition. We aim that the findings obtained as a result of our study will contribute to the development of new treatment approaches for seminoma patients in the future and in the process of restoring testicular functions and preserving fertility.

Keywords: TCam-2, Seminoma, mTOR, Rapamycin

Oral Presentation

PROTECTIVE EFFECTS OF THE ALLYL-DISULFIDE AND S-ALLYL-L-CYSTEINE ON CISPLATIN-INDUCED KIDNEY DAMAGE IN RATS

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Objectives: Cisplatin is an effective anticancer drug used in various cancer types, especially in gynecological cancers. However its adverse effect on kidneys (nephrotoxicity) limited its eligibility for optimal clinical use. Garlic (Allium sativum) is a well known medicinal plant, which is proven to have beneficial effects, both in its processed and unprocessed form, of its various organosulfur compounds. In the present study, we tried to investigate the protective effects and possible mechanisms of the garlic's organosulfur compounds called Allyl-Disulfide (ADS) and S-Allyl-L-Cysteine (SAC) on Cisplatin-induced kidney damage in rats.

Materials and Methods: The study was performed using 32 female Sprague Dawley rats and was randomly divided into 4 groups: healthy rats used as the normal control group, Cisplatin (5 mg/kg body weight Cisplatin-induced kidney damage) group, and two Cisplatin-induced kidney damage groups treated orally with either Allyl-Disulfide (100 mg/kg body weight) or S-Allyl-L-Cysteine (100 mg/kg body weight) for 14 days. Rats were kept in a controlled environment with an average temperature of 23±2°C and constant photoperiodic conditions with food and water ad libitum. Rats were sacrificed on the 15th day of the experiment. Kidney tissues were taken and routine histological process was performed and embedded in paraffin. Sections of 5 µm thickness were cut, then stained with Hematoxylin-Eosin (H-E), Periodic Acid Schiff (PAS), and Masson's Trichrome (TRI) stainings and examined under a light microscope.

Results: As a result, morphological abnormalities in the kidneys such as; disruption of the basement membrane integrity, enlargement of the Bowman's capsule size, tubular dilatation, and inflammation were observed in the Cisplatin group. Histopathological damages caused by Cisplatin in the kidney were attenuated in both treatment groups. These findings show that organosulfur compounds of garlic called Allyl-Disulfide and S-Allyl-L-Cysteine have strong protective effects. Among these treatment approaches Allyl-Disulfide exhibited a better protective effect against the nephrotoxic effects of Cisplatin.

Conclusion: In conclusion, our study demonstrates the potential benefits of organosulfur compounds of garlic and might shed light on the nephrotoxic effects of chemotherapeutic agents in various studies.

Oral Presentation

MESENCHYMAL STEM CELLS INDUCE IN VITRO SPERMATOGENESIS ON INDIRECT AIR-LIQUID INTERPHASE PLATFORM

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Introduction: Chemo-radiotherapy applications result in permanent infertility in half of male pediatric cancer survivors. Spermatogonial stem cells (SSC) constitute the only option for fertility preservation since the spermatogenesis is not initiated yet[1,2]. The rationale of the study is that bone marrow derived mesenchymal stem cells (BM-MSC) have similar embryonic origin and gene expression profile with the Sertoli cells[3] responsible for the self-renewal, maintenance, proliferation and differentiation of SSCs. We hypothesized that an air-liquid interphase (ALI) co-culture system based on syngeneic BM-MSC could support *in vitro* spermatogenesis.

Purpose: The aim of this study is to evaluate the inductive effect of BM-MSCs on *in vitro* spermatogenesis and cellular viability of neonatal C57BL/6 mice testes on ALI set up.

Material-Method: The animal experiments were approved by Hacettepe University Animal Experiments Local Ethical Board (#52338575-96). Isolated mouse BM-MSCs were characterized at passage 3, and the indirect ALI set up was constituted with transwell inserts. The dissected and decapsulated testicular strips were cultured for 7 to 42 days. Then, single cell suspension from testicular tissue was prepared; differentiating spermatogonia and cellular viability were evaluated by using c-Kit and Ki-67 antibodies, respectively, and quantitated by using flow cytometric analysis (ACEA, Novocyte, Novoexpress). Quantification of ID4(+) SSCs, Scp3(+) spermatocytes, Acr(+) round spermatids and Ki67(+) proliferative cells has also done by immunohistochemistry (Leica, LASv3). Since the data was normally distributed, one-way ANOVA was used in statistical analysis.

Results: The percentage of c-Kit labeled and germ cell viability in co-culture group was higher than the control at day 42 (p=0.047, 0.001, respectively). The number of ID4(+) SSCs, Scp3(+) spermatocytes, Acr(+) round spermatids and Ki(+) proliferative germ cells on days 7-42 (p=0.001). The results demonstrated that the new BM-MSC contributed co-culture system led to maintenance of germ cell pool and differentiation for 42 days *in vitro*.

Conclusion: Addition of BM-MSCs to ALI co-culture system provide a promising tool for a personalized cellular therapy platform for preservation of fertility in childhood cancer survivors by initiation of spermatogenesis in immature testis strips in vitro.

Keywords: Male infertility, spermatogonial stem cell, mesenchymal stem cell, germ cell, *in vitro* spermatogenesis

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Oral Presentation

EFFECTS OF GLUTAMATE AGONIST AND ANTAGONIST ON PROLIFERATION IN HUMAN GLIOBLASTOMA T98G CELLS

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Introduction: Glioblastoma (GB) represents the most common primary brain tumor, with complex biology and poor prognosis. Although many improvements were achieved in cancer biology, for almost all patients with GB, life expectancy remains within nearly 18 months. The glutamate receptor system is the main excitatory network of the central nervous system. It is composed of three sub-families, two ligand-gated ion channels (ionotropic receptors), the N-methyl-D-aspartate (NMDA) receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate) receptors. Although, in some cancer models (such as breast, lung, cervix) that express NMDA, NMDA receptor antagonists MK-801, Kainic acid and kainic acid receptor antagonists CNQX were shown to possess anti-proliferative and anti-invasive effects, there is no study showing the anti-proliferation effect in GB.

Purpose: Aim of this study was to investigate the anti-proliferative effects of NMDA or/and Kainic acid and the uncompetitive NMDA receptor antagonist MK-801 or/and kainic acid receptor antagonist CNQX on cell proliferation in T98G GB cells.

Material-Method: Firstly, the anti-proliferative activity of NMDA, MK-801, kainic acid and CNQX were tested in human glioblastoma cell line T98G cells by trypan blue and WST-1 assays. The inhibitory concentration at which 50% of the cells die was identified (IC50). The results were expressed as a percentage of the control. The absorbance of the untreated control cells was set to 100%, and the absorbance of treated cells was taken as a percentage of survival.

Results: The IC50 of NMDA, MK-801, kainic acid and CNQX for T98G cells were 1000 uM. Effective doses of agents that killed T98G cells did not produce a lethal one in HUVEC cells. When T98G cells were treated with 1000 uM NMDA and its antagonist MK-801 at 72 h, the reduction in proliferation was determined to be 91.8 and 90.02 % respectively, compared to an untreated culture (p= 0.012). When T98G cells were treated with 1000 uM kainic acid and its receptor antagonist CNQX at 72 h, the reduction in proliferation was determined to be 80.4 and 82.4%, compared to an untreated culture (p= 0.001).

Conclusion: We suggest that NMDA, MK-801, Kainic Acid and CNQX might be used for in vivo studies and future medical drug studies. But further studies and validations are needed.

Oral Presentation

ALLOGENEIC BONE MARROW MESENCHYMAL STEM CELL-DERIVED EXOSOMES AMELIORATE HYPOXIC ACUTE TUBULAR INJURY IN HUMAN PROXIMAL TUBULE-ON-A-CHIP WITHIN A PRECISE TREATMENT WINDOW

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Introduction: Mesenchymal stem cells (MSCs) and MSC exosomes (MSC-Exos) are promising therapeutic possibilities for ischemic acute kidney injury (AKI)[1], but their safety and efficacy are still debatable on proximal tubules (PT) at the cellular level. Recently microfluidic kidney-on-a-chip systems present smart platforms mimicking tubular microphysiopathological environment to assess theragnostic tools before translation to clinics. A gravity-driven membraneless microfluidic-based 3D culture platform may reproduce acute hypoxic PT injury and real-time assess the therapeutic potency of human bone marrow-derived MSC exosomes (hBMMSC-Exos) as cellular therapeutics.

Purpose: We aimed to isolate, characterize and quantitatively analyze the efficacy of hBMMSC-Exos with a real-time proliferation assay (RTCA) and assess the potency in terms of tubular epithelial permeability, epithelial polarity, expression of injury-specific genes and proliferation on the novel microfluidic acute hypoxic PT injury platform.

Material-Method: hBMMSC-Exos were isolated, characterized. RTCA determined the effective dose and treatment window on acute hypoxic PT injury. 2-lane 3D gravity-driven microfluidic platform was set to mimic PT *in vitro*. ZO-1, acetylated α-tubulin immunolabelling, permeability index assessed structural; cell proliferation by WST-1, BNIP3, HO-1, HIF1A1 expression by qRT-PCR measured functional integrity of PT.

Results: hBMMSC-Exos induced PT proliferation with ED50 of 172,582 µg/ml at 26th hour. Hypoxia significantly decreased ZO-1, increased permeability index, decreased cell proliferation rate, increased BNIP3, HO-1 and decreased HIF1A1 on 24-48 hours in the microfluidic platform. hBMMSC-Exos reinforced polarity by 1.72-fold increase in ZO-1 ($p=0.0121$), restored permeability by 20/45-fold against 20/155 kDa dextran ($p=0.0004$ and $p<0.0001$, respectively), increased epithelial proliferation 3-fold ($p<0.0001$) and decreased BNIP3 expression by 0.62-fold compared to control.

Conclusion: The real-time potency assay and 3D gravity-driven microfluidic acute hypoxic PT injury platform precisely demonstrated therapeutic performance window of allogeneic hBMMSC-Exos on ischemic AKI providing molecular, structural and functional cellular data. The novel standardized, non-invasive 2-step system validates cell-based personalized theragnostic tools in a real-time physiological microenvironment prior to safe and efficient clinical usage in nephrology.

Keywords: AKI, Proximal Tubule, Exosome, Microfluidic, Kidney-on-a-chip

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Oral Presentation

EFFECT OF TGF-B1 ON VDAC1 AND AMYLOID BETA-42 EXPRESSION IN CEREBELLUM AND TEMPORAL LOBE IN EXPERIMENTAL ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease is a cognitive, neuroinflammatory and neurodegenerative disease that is responsible for a large part of cognitive disorders. Although the pathological changes related to this disease are not fully known, the most important pathological changes are extracellular plaque deposits of the amyloid beta (Aβ) proteins and the accumulation of intracellular hyperphosphorylated tau proteins. VDAC1 (Voltage Dependent Anion Channel-1) is an other multifunctional protein which plays a role in AD progression, expressed in mitochondria and plasma membrane of cells. Transforming growth factor beta-1 (TGF-β1), an anti-inflammatory cytokine, has been reported to have neuroprotective effect in the treatment of neurodegenerative diseases. While positive effects of TGF-β1 treatment on neuroinflammation were seen, studies about the effects of this cytokine on amyloid beta 42 (Aβ-42) and VDAC1 expression in the cerebellum and temporal lobe are limited in the literature. For this purpose, in this study, it was aimed to examine the effect of TGF-β1 on the expression of amyloid beta-42 and VDAC1 in the cerebellum and temporal lobe of the brain in an experimental Alzheimer's-like model.

Materials and Methods: 28 Adult Swiss-Albino mice were divided into four groups as control, sham, experimental and treatment groups. Unlike the control group, sham group received physiological saline intraperitoneally. Experimental group received Scopolamine Hydrobromide intraperitoneally and treatment group received TGF-β1 intraperitoneally in addition to Scopolamine Hydrobromide administration. At the end of the 28th day, the mice were sacrificed and the cerebellum and temporal lobe tissues were analysed by light microscopic, immunohistochemical and electron microscopic methods.

Results: Immunohistochemically, in the cerebellum tissues of the subjects in experimental group; significant increase was detected in Aβ-42 expression in perineuronal area when compared to control, sham and treatment groups. Moreover, VDAC-1 expression were shown to increase in the cerebellum and temporal lobe. In electron microscopic examination, increased chromatin condensation in the nuclei of neurons at the cerebellum and temporal lobe and also organelle destruction in the cytoplasm, especially mitochondrial damage were noted in Scopolamine-treated mice. After the TGF-β1 treatment, decrease in Aβ-42 expression was determined, as well as decrease the VDAC1 expression were observed. In addition, ultrastructural changes were rarely encountered.

Conclusion: It was concluded that TGF-B1 can prevent Aβ accumulation and reduce mitochondrial damage in an experimental Alzheimer's-like model by down-regulating Aβ-42 and VDAC1 expression.

Keywords: Alzhemer's disease, Amyloid beta 42,VDAC1, TGF-β1

Oral Presentation

THE POTENTIAL EFFECT OF REPEATED SUPEROVULATION ON THE TERT LEVELS IN THE MOUSE OOCYTES

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Introduction: Superovulation (also known as ovarian stimulation) is a widely used protocol in assisted reproductive technology (ART) centers and experimental animal studies to obtain more oocytes. However, many studies have revealed that superovulation can lead to adverse effects such as decreased oocyte and embryo quality, delayed embryonic and fetal development, and reduced blastocyst formation and implantation rates. As it is known, telomeres located at the ends of linear chromosomes play a key role in protecting genome integrity. Shortening of telomeres results in inappropriate chromosome segregation and aneuploidy, which are also observed in the oocytes obtained by superovulation protocol. In normal conditions, shortened telomeres are elongated by the enzyme telomerase, which is composed of catalytic TERT and RNA template *Terc* subunits.

Purpose: In this study, we aimed to examine the effect of single or repeated superovulation protocols on the subcellular TERT distribution and its relative levels in the mouse germinal vesicle (GV) oocytes.

Material-method: Four groups were created from Balb/C female mice as follows: control (C; n=5), superovulated once (S1; n=2), superovulated three times with one week intervals (S3; n=2) and five times with one week intervals (S5; n=5). Superovulation was performed by injecting mice intraperitoneally with 8 IU of pregnant mare's serum gonadotropin (PMSG). At least three GV oocytes from each mouse were stained for the TERT protein using immunofluorescence method. The relative TERT protein levels were measured by ImageJ software program and obtained data were analyzed using student's *t*-test among groups. $P<0.05$ is considered statistically significant.

Results: We found that TERT protein is localized strongly in nuclear and moderately cytoplasmic regions of the GV oocytes in all groups (Figure 1). Although no difference was detected in the total and nuclear area analyses of the TERT protein among groups, the TERT level in the cytoplasmic region of S1 group was higher than that of control group ($P<0.05$) (Figure 2).

Conclusions: We continue analyzing the TERT levels in the GV oocytes by collecting more. Additionally, the same analyses will be performed on the metaphase II oocytes. The limited data shows that one time superovulation seems to affect the cytoplasmic level of the TERT protein. This finding suggests that cytoplasmic accumulation of TERT protein due to superovulation with PMSG may affect the telomere lengthening process in the GV oocytes, which should be evaluated in futures studies.

Keywords: Superovulation, oocyte, telomerase, TERT, telomere

Oral Presentation

INVESTIGATION OF THE EFFECTS OF IMATINIB USE ON THE OVARY IN THE EARLY PRENATAL PERIOD

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Introduction: For many years, imatinib using to treat chronic myeloid leukemia. Unexpected pregnancy consists in young women during this treatment and causes exposure during the embryonic period.

Aim: We designed this study to examine the effects of early embryonal exposure on female reproductive health in adult life to result in unexpected pregnancies during in-clinic imatinib treatment.

Material-Method: In our study, 12 Sprague Dawley female rats were divided randomly into four groups; Female rats determined to be in the estrus stage were mated with male rats. Groups; control group (no treatment was administered), Ima-20 (20 mg/kg), Ima-40 (40 mg/kg), Ima-60 (60 mg/kg). After a post-coital vaginal wash in the female rats was accepted on embryonic day 1 (E1) and single doses were administered 20mg/kg, 40mg/kg, and 60mg/kg imatinib to the groups E1-E8 days. The offspring of female rats were sacrificed on the 80th postnatal day. Hematoxylin and eosin (H&E) staining and The terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) method apply to the ovary. Histopathological analyzes and apoptotic cells were examined. We measured Thiobarbituric acid reactive substances (TBARS) and Total Thiol (TT) levels in the testes tissues.

Results: We observed ovarian tissues of the control group to have normal histological structures. Histopathological findings were observed in drug groups; apoptotic cells were demonstrated. Compared to the control group, medullary edema increased in the ima-40 ($p=0.022$) and ima-60 groups ($p=0.001$); the inflammation score increased in all groups ($p<0.05$). Determined significant increase in Ima-20 ($p=0.021$) and Ima-40 ($p=0.007$) groups in degenerative follicle scoring. Compared with the control group; increase in TBARS levels in the Ima-20 and Ima-40 groups ($p<0.05$). In addition, TT levels increased in all groups ($p<0.05$). When the drug groups were compared with each other, it was determined that the TT levels of the Ima-60 group decreased compared to the Ima-20 and Ima-40 groups ($p<0.05$).

Conclusion: Determined that imatinib-induced exposure in the embryonic period caused histopathological degenerative findings in the ovaries and oxidative stress occurred. Oxidative stress caused by the drug increased the Ima-20 and Ima-40 groups. Recommended supporting this change between drug groups by investigating different studies and examining different methods.

Keywords: Early, imatinib, ovary, prenatal rat.

Oral Presentation

THE EFFECTS OF THE USE OF BOUIN AND FORMALDEHYDE FIXATIVES IN TESTICULAR TISSUE ON HISTOPATHOLOGICAL EVALUATION IN ROUTINE AND IMMUNOHISTOCHEMICAL STAINING

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Aim: This study aims to compare the results of histopathological and immunohistochemical findings of formaldehyde and Bouin solutions used in testis fixation.

Methods: Testicular tissue of adult male rats was used in our study. One-half of the testicular tissues were fixed in a solution containing 10% formaldehyde and the other half was fixed in Bouin solution and examined in two groups. The sections in both groups were stained with hematoxylin and eosin (H&E), Masson Goldner, and anti-caspase-3. The use for analysis histopathological evaluation and immuno-positive grade methods.

Results: Examined H&E staining testis tissues in the Bouin's group, and observed that the seminiferous tubule structure and integrity between germ cells and cell borders were well preserved. Determined that the chromatin structures of primary spermatocyte and spermatid cells were visible in Bouin-fixed tissues. The interstitial area, vascular structures, and cell integrity were evaluated as morphologically normal. In the group with testicular tissues fixed with formaldehyde, germ cells in the seminiferous tubule and chromatin structures of the cells in the interstitial area were observed, and the integrity of the interstitial area was normal. However, when compared to the Bouin group, the cells were stained eosinophilically and were weaker in terms of the integrity of the germ cells in the tubule. Masson Goldner's stained with testicular sections of the formaldehyde group, observed that the germ cells and Leydig cells stained in a paler color, but the connective tissue cells were stained bright red. When the anti-caspase 3 stained sections were examined, we determined that positive cells were prominent in the germ cells in the sections in the formaldehyde group compared to the Bouin group.

Conclusion: Both fixators have various advantages in different staining such as immunohistochemical positivity density, visibility of chromatin structure, preservation of cell-tissue integrity, and using both fixators in studies will be beneficial in demonstrating morphological structures.

Keywords: Bouin, fixation, formaldehyde, histopathology, testis.

Oral Presentation

NOVEL INHAT REPRESSOR (NIR) MAY REGULATE VARICOCELE-INDUCED APOPTOSIS THROUGH P53 IN RAT TESTICULAR TISSUE

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Introduction: Varicocele is a common cause of male infertility, and lead to oxidative stress. Various studies of testicular tissues of males with varicocele have shown that apoptosis is increased in developing germ cells. The P53 plays a role in DNA repair mechanism and induces apoptosis. Nir (novel INHAT repressor) which inhibits P53-mediated gene transactivation by blocking histon acetylation controls cell proliferation and apoptosis.

Purpose: The apoptotic processes of varicocele still are not known exactly. Currently, there is no evidence of Nir protein expression in varicocele-induced testicular tissues in any studies. The aim of this study was to investigate associated of Nir and P53 on testicular damage caused by varicocele.

Material-Method: Thirty male wistar rats were randomly divided into three groups as control, sham and varicocele (VC). The VC model was established by partial ligation of the left renal vein, and the left testes were taken from all groups after 13 weeks of varicocele formation. First of all, the obtained sections were stained with Hematoxylin-Eosin and the degrees of seminiferous tubule degeneration were evaluated under the light microscope. The TUNEL assay was used to detect apoptotic cells. Nir, p53 and HIF-1α expressions were determined using Immunohistochemistry and Western blot techniques. One-way ANOVA was used to determine the statistical significance between the results obtained from the groups.

Results: The expressions of Nir, P53, and HIF-1 were significantly increased ($P < 0.05$) in the varicocele group according to the results of Immunohistochemistry and Western blot analysis. In addition, an increase in both apoptotic germ cell number and seminiferous tubule degeneration was observed in the varicocele group.

Conclusion: In conclusion, increased expression of P53 (as Nir interacting partner) in varicocele group, appears as a result of varicocele-induced apoptosis. HIF-1α protein which increased with hypoxia in rats with varicocele, regulates apoptosis-related downstream gene, P53. Increased Nir expression can block P53 activity and therefore the Nir signaling can play a protective role in this process.

Oral Presentation

ARID3A AND ARID3B DIRECTLY REGULATES LNCRNAS MALAT1 AND NORAD IN NSCLC

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Introduction/Aim: Lung cancer is the leading cause of cancer-related deaths with poor prognosis. Early diagnosis and effective treatment strategies are keys for survival efforts. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers. Long non-coding RNAs (lncRNAs) play vital roles in many biological processes. Their dysregulation causes carcinogenesis and may influence the pathways that might be crucial therapeutic targets in various types of cancers. Dysregulation of lncRNAs metastasis associated lung adenocarcinoma transcript 1 (MALAT1) and non-coding RNA activated by DNA damage (NORAD) leads to the neoplastic initiation, progression, metastasis, tumor angiogenesis, chemoresistance and genomic instability in NSCLC. Both MALAT1 and NORAD are important for cell cycle progression as they directly regulate the expression of the transcription factor E2F1. The expression of p53 target genes is also affected by MALAT1 by repressing p53 promoter activity followed by cell cycle progression, and NORAD is indirectly regulated by p53. The AT-rich interaction domain (ARID) family of DNA-binding proteins, especially ARID3A and ARID3B are involved in various biological processes, including cell proliferation, differentiation, and development. ARID3A and ARID3B play important roles in E2F-dependent transcription and are transcriptional targets of p53. The pivotal function in promoting either cellular proliferation by pRb-E2F or growth arrest by p53 pathways implies a tight and finely tuned regulation. Both ARID3A, ARID3B and lncRNAs MALAT1, NORAD are involved in pRb-E2F and p53 pathways. In this study, we tried to find probable interactive and functional connectivity among ARID3A, ARID3B, lncRNAs MALAT1 and NORAD in NSCLC.

Materials and Methods: Overexpression of ARID3A and ARID3B was achieved using recombinant expression plasmids. As a NSCLC cell line model A549 cells were used. After successful overexpression of ARID3A and ARID3B, their effect on lncRNAs MALAT1 and NORAD were investigatedusing Real-time quantitative reverse transcription-PCR.Fold change analysis of MALAT1 and NORAD were done using $2^{(-\Delta\Delta Ct)}$ method. Resulting findings were statistically evaluated using GraphPad Prism 8 and paired t test was applied.

Results: As a result, it was found that the expression of MALAT1 and NORAD was increased 8.3-fold and 9.7-fold, respectively, in lung cancer cells overexpressing ARID3A. In addition, the expression of MALAT1 and NORAD was found to be increased 3.4-fold and 3-fold, respectively, in lung cancer cells overexpressing ARID3B compared with the control group. These results were found to be statistically significant.

Conclusion: Our results indicate that ARID3Aand ARID3B regulates MALAT1 and NORAD in NSCLC. We show here that activities of MALAT1 and NORADwere significantly increased after overexpressing ARID3A and ARID3B. To conclude, we can say that ARID3A and ARID3B might have significant rolesin the oncogenic

activity of MALAT1 and NORAD in NSCLC.Thus, ARID3A and ARID3B can be used as a therapeutic target in NSCLCas they directlycontrol the expression of MALAT1 and NORAD.

Keywords: ARID3A, ARID3B, MALAT1, NORAD, Non-small-cell lung carcinoma

Oral Presentation

THE USE OF CRISPR/DCAS9 ENGINEERED MSCS TO PREVENT CISPLATIN-INDUCED CELLULAR DAMAGE IN HUMAN GRANULOSA CELLS

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Introduction & Objective of the study: Primary ovarian insufficiency (POI), also known as premature ovarian failure, is described as losing ovarian function before 40 years of age. Infertility, sings of estrogen insufficiency, and amenorrhea constitute the clinical appearance of POI. POI can be induced by chemotherapy and the number of young POI patients are increasing due to application of chemotherapy treatments worldwide. Cisplatin is a common chemotherapeutic used to treat a variety of cancer types, usually in adjunction with other chemotherapeutics and radiotherapy. Cisplatin has a broad effect on all ovarian cortical and stromal cells and as the effect on granulosa cells directly interfere with folliculogenesis and oogenesis, ovarian function is seriously diminished leading to POI. Stem cells have been offered for supportive treatment in POI, such as mesenchymal stem cells (MSCs) that can be obtained from various adult and fetal tissues. CRISPR/Cas systems are currently addressed to be the most widely used gene editing systems and dCas9 (dead Cas9) is a CRISPR tool aimed at targeted activation without use of endonucleases. One of the various activators of dCas9 system is SAM (synergestic activation mediator) that uses specifically designed sgRNAs, VP64 fusion protein and MS2 proteins, which then recruit additional activation domains as HS1 and p65. Exosomes are extracellular vesicles that range between 30–150 nm. These vesicles have been shown to be found in different biological fluids and as they can carry RNAs, proteins, and lipids, they can be used to transfer their content to target cells to modify genes or proteins in target cells. This study has been designed to assess the effect of dCas9 engineered cisplatin resistant MSCs and their exosomes for preventing cisplatin-induced damage on cisplatin-applied on human granulosa cells (hGRC1).

Materials & Methods: hGRC1 and MSC cell lines were used throughout the experimental set-up. A cisplatin-resistant mesenchymal stem cell line has been established via use of CRISPR/dCas9 activation system with specific sgRNAs. Exosomes from both normal and cisplatin resistant MSCs have been obtained. For exosome isolation the MSC/resistant MSC culture medium without serum was collected after 48 h, centrifugated for 15 min at 10,000 g centrifugation and 4 °C. The supernatant was filtered through a 0.22 µm filter (Millipore) and eventually ultracentrifuged at 100,000 g for 5h at 4 °C. The exosome pellets were resuspended in PBS, characterization was achieved by Western blotting.As previous studies showed that 3-dimensional cultures of MSCs were more effective in treatments compared to 2-dimensional sytems Spheroids of both normal and resistant MSCs were formed via the Hanging Drop Technique.The cultures of MSCs, and their products with human granulosa cells were maintained on Transwell® systems by seeding of MSCs on the bottom and seeding of hGRC1 cells on the membrane insert. In all groups hGRC1 cells were co-cultured with: i. normal MSC, ii.resistant MSCs, iii. exosome of normal MSC, iv. exosome of resistant MSC, v. normal MSC+its exosome, vii.

resistant MSC+its exosome, vii.normal MSC spheroid, viii. resistant MSC spheroid. For the same in vitro co-cultures cisplatin treatment ann control groups were established. In the cisplatin group, cisplatin (6.25ug/ml) was given to the common medium (DMEM-HG, 10% FBS 1%P/S). The dose of cisplatin was detected by a CTG assay. In the control group no cisplatin has been used in the culture medium. At 24, 48, 72, and 96 h after administration of cisplatin the medium was collected in both groups. Apoptosis, inflammation and functional markers as BCL-2, Bax etc, IL-1Ra, IL-4, IL-6, IL-10, cleaved caspase 3, FSH-R, LH-R were demonstrated by the qPCR, IF and Western Blotting. The activated gene was also checked by qPCR to see the increased expression due to use of dCas9 activation system.

Results: Cisplatin resistancy was successfully maintained by dCas9 activation system depicted by the increase gene expression. The exosomes of both normal and resistant MSCs were obtained and characterize successfully with positive CD63, CD 9 and negative Calnexin. As normal and resistant MSC (R-MSC) groups, Caspase, Bax and Bcl-2 were all decreased in R-MSC groups ($p<0.05$). In The exosome+R-MSC, only exosome and spheroid groups ILR-a and IL-4 were decreased, the decline was most evident in the exosome+R-MSC group however this finging was significant ($p<0.5$). FSH and LH receptors were elevated compared to normal MSC groups, but this finding was not significant.

Conclusion: These findings suggest that MSCs can be engineered via dCas9 for drug resistance. Resistant MSCs and their exosomes may be an alternative cellular and gene treatment. In vivo trials of the same system can reveal the ability of the engineered cells on protection of ovarian tissue during chemotherapy.

Keywords: Mesenchymal Stem Cells, Cisplatin, CRISPR/dCas9, exosomes

Oral Presentation

THERAPEUTIC EFFECTS OF SILYMARIN ON PACLITAXEL-INDUCED LUNG TOXICITY IN RATS

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Objectives: Paclitaxel (PAX) is a chemotherapeutic agent used to treat cancer, but it has toxic effects on various organs. Silymarin (SIL) is a natural flavonoid derived from the plant Silybum marinum, called milk thistle. Many studies have shown that this compound has antimitotic, antioxidative, and anti-inflammatory effects. In this study, paclitaxel-induced lung injury and the therapeutic effect of silymarin were investigated.

Methods: Rats were administered paclitaxel 2 mg/kg intraperitoneally for five consecutive days and then 200 mg/kg oral silymarin for ten consecutive days. The lung tissues were stained with Crossman modified Mallory's triple staining for histopathologic examination. And the lung tissues were immunohistochemically stained with anti-Bax, anti-Bcl-2, anti-iNOS, anti-Nf-kB-p65, anti-IL-6, and anti-MUC1 antibodies. Also, IL-6, Caspase-3, Bcl-2, P2x7, and NF-κB p65 protein expression levels were determined by western blot analysis. Biochemically, SOD activity and CAT, GPx, GSH, and MDA levels were evaluated in lung tissues.

Results: Histopathologic changes (increase in alveolar septa, thickness, inflammatory cells, and endothelial degenerations) were intensely observed in the lung tissue of the PAX group. Also, Bax, iNOS, Nf-kb-p65, and IL-6 immune reactivities were lower in the SIL-PAX group compared to PAX groups. However, the immunopositivity in Bcl-2 and MUC1 immune reactivities were higher in the SIL-PAX group than in the PAX group. IL-6, Caspase-3, P2x7, and NF-κB p65 protein expression levels were higher in the PAX group than SIL-PAX group. In biochemical analysis, antioxidant enzyme (SOD, CAT, GSH, and GPx) levels increased in the SIL-PAX group compared to the PAX group. A significant decrease was observed in MDA levels in the PAX group compared to other groups. While IL-6, Caspase-3, P2x7, and NF-kB p65 immunoreactivity was decreased in the SIL-PAX group, Bcl-2 immunoreactivity was increased.

Conclusion: As a result of the study, it was determined that silymarin improved the existing lung damage via anti-inflammatory and antioxidant effects. Also, more detailed molecular studies are still required for the understanding of underlined mechanism.

Keywords: Paclitaxel, Silymarin, Lung damage

Oral Presentation

A NEW COATING MATERIAL FOR THE FAST PRODUCTION OF 3D STEM CELL SPHEROIDS

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Introduction: Two-dimensional (2D) cell culture methods, which are easy to implement at a low cost are inadequate for imitating the 3D environment of the body, therefore reduces the value. Various 3D culture methods such as spheroid/organoid systems, microfluidics and 3D bioprinting were developed to overcome this problem. Mesenchymal Stem Cells (MSCs), which are responsible for maintaining homeostasis in the body and have high differentiation potential. When MSCs were cultured as spheroids, biological and physiological responses were found to be similar to *in vivo*. However, current methods used to create spheroids are costly, takes longer time or lead MSCs to differentiate.

Aim: In this study, we developed a novel biocompatible hydrogel material, analyzed spheroid formation properties of MSCs, cultured on the material, and compared with existing spheroid making methods.

Material-Method: MSCs were cultured on Noble agar (NA) and our material, named as SpheroMake (SM) in addition to standard hanging drop (HD) method. MSCs on NA and SM were cultured for 5 days while HD only cultured for 3 days. Spheroids were photographed and diameters measured each day. At the end of the experiments, spheroids collected for Flow cytometry, RNA isolation and IF stainings. RNAs were converted to cDNA and analyzed with real time PCR for stem cell genes. Spheroids were disintegrated via trypsinization for flow cytometer and MSC markers were measured. For IF stainings, spheroids were transferred to standard culture plates and allowed to attach for 12 h. IF staining were performed within culture plates following the routine protocol.

Results: Spheroid size varied greatly in NA group but found to be uniform in SM group. FC analyzes showed around %20 decrease in HD group while similar in NA and SM groups with 2D controls for CD73. CD90 and CD105 were 15% lower for experiment groups. IF stainings showed higher levels of CD44, integrin, vimentin and fibronectin and FGF-2, and lower levels of Caspase-3 in SM group than other experiment groups. Gene expression levels of Sox-2 and CD44 were also higher in SM group. Nestin expression levels of SM group were lower than 2D controls but higher than other 3D groups.

Conclusion: We determined that the material we developed has advantages over other 3D methods in terms of providing the appropriate spheroid diameter, keeping the spheroids stable, maintaining viability and stem cell gene expressions. We believe that we can create a 3D culture material that will better mimic the *in vivo* environment.

Keywords: Mesenchymal Stem Cell (MSC), Three-Dimensional (3D) Cell Culture, Spheroid, Hydrogel, Static Suspension Culture.

Oral Presentation

VALIDATION OF RNAS EFFECTIVE IN RELAPSE DEVELOPMENT AFTER LIVER TRANSPLANTATION IN HBV-INFECTED HEPG2 CELLS

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Bacground: Hepatocellular carcinoma (HCC) caused by hepatitis B (HBV) and C (HCV) is an important health problem as one of the most common neoplasms in the world. Today, orthotopic liver transplantation (OLT) is applied in the treatment of HCC patients, especially those with cirrhosis and no local or distant metastases. However, post-transplant recurrence is one of the most important factors reducing success and is associated with poor prognosis in patients receiving this treatment.

Purpose: In our previous studies, microRNAs (miRNA), non-coding long RNAs (Long-non coding RNA: LncRNA), which are effective in the development of recurrence after OLT in HCC tumors, were examined and miR-21, LncRNA PVT1, LncRNA MALAT1 and LncRNA SNGH16 were found to be highly expressed. In our study, it was aimed to infect HEPG2 cells with HBV and to analyze the expression profiles of miR-21, PVT1, MALAT1 and SNGH16 RNAs in these cells.

Material-Method: At 2 to 6 days after plating, HepG2 cells were infected with 1 ml of HBV particlesfrompatientserum. The medium was then changed every 2days, harvested,and tested for HBV antigens. Detection of HBsAg and pre-Santigens in culture medium by Mo-RIAs. We investigated the levels of miR-21, LncRNA PVT1, LncRNA MALAT1 and LncRNA SNGH16 in viral-HEPG2 cells by using RT-PCR. HEPG2 and HUVEC were used control cells.

Results: The expression levels of LncRNA PVT1, LncRNA MALAT1 and LncRNA SNGH16 were higher in viral-HEPG2 compared with HUVEC ($p = 0.016$, $p = 0.033$, $p = 0.004$, and $p = 0.001$, respectively). High levels of miR-21 and LncRNA MALAT1 were associated with viral cell formation in HCC ($p = 0.011$ and $p = 0.024$, respectively) (This study was supported by the Science Foundation of Bursa Uludag University (THIZ-2021-573).

Conclusion: In our study, ncRNAs that were found to be associated with relapse after OLT in our patient-based studies were examined and it was determined that miR-21 and LncRNA MALAT1 contributed to the formation of viral HCC.

Oral Presentation

THE DNA DOUBLE-STRAND BREAK REPAIR PROTEINS RAD51, BRCA1 AND RP70 EXHIBIT SIGNIFICANT CHANGES IN THE POSTNATAL MOUSE OVARIES FROM EARLY TO AGED TERMS

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Introduction: DNA double-strand breaks (DSBs) gradually increase in the mouse ovaries during biological aging, which leads to decreasing oocyte quality. Underlying factors causing increased DSBs are not fully determined yet. As is known, γH2AX is a commonly used biomarker for marking DSBs. DBSs are mainly repaired by the homologous recombination (HR) pathway in an error free manner. The RAD51, BRCA1, and RP70 proteins are the main components of HR pathway. Deficiencies of these proteins lead to various types of defects in the oocytes and granulosa cells.

Purpose: The aim of the present study is to determine the spatiotemporal distributions and relative levels of the RAD51, BRCA1, and RPA70 proteins in the postnatal mouse ovaries from prepuberty to late aged periods.

Material-method: We created five groups from Balb/C female mice as follows: prepuberty (3-week-old; n=6), puberty (7-week-old; n=7), postpuberty (18-week-old; n=7), early aged (52-week-old; n=7), and late aged (60-week-old; n=7). Immunostaining of the RAD51, BRCA1, RPA70, and γH2AX proteins was evaluated using immunohistochemistry. The data were evaluated using one-way ANOVA and Tukey post hoc test. $P<0.05$ was considered statistically significant.

Results: In all groups, γH2AX was localized in the nuclei of the oocytes and granulosa cells, and there were weak cytoplasmic immunoreactions. We did not observe any statistical differences for the γH2AX levels among groups ($P>0.05$, Fig. 1A). The HR-repair related proteins BRCA1, RAD51, and RPA70 exhibited both nuclear and cytoplasmic immunostaining in the ovarian cells including oocytes and granulosa cells of the follicles from primordial to antral stages. Also, there were weak expressions of these proteins in the stromal cells. The relative BRCA1 levels gradually increased from prepuberty to early aged groups ($P<0.01$, Fig. 1B), and sharply decreased in the late aged group ($P<0.001$). On the other hand, there was a gradual decrease in the RAD51 levels from prepuberty to late aged groups ($P<0.01$, Fig. 2A). We also found that RPA70 levels gradually decreased from prepuberty to early/late aged groups ($P<0.01$). Notably, the early and late aged groups possessed lower RPA70 levels when compared to the remained group ($P<0.01$, Fig. 2B).

Conclusions: The changed HR repair protein levels in the aged groups may be associated with the altered DSB repair activity in the aged ovarian cells including oocytes and granulosa cells. Further studies are required to determine the molecular mechanisms resulting in decrease or increase of these protein levels during ovarian aging.

Keywords: DNA double-strand break, HR repair, oocytes, granulosa cells, ovarian aging.

Oral Presentation

IS MELATONIN POSSIBLY THE NEW HOPE IN POLYCYSTIC OVARY SYNDROME RELATED INFERTILITY? AN EXPERIMENTAL

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Introduction: Polycystic ovary syndrome is an endocrine disorder that is seen in women of reproductive age and has been gradually increasing over the years. The mechanism of syndrome is still not understood.

Purpose: The purpose of this study is to determine the possible effects of melatonin on MT1 (melatonin receptor 1) receptor, GDF9 (Growth differentiation factor-9) and BMP15 (bone morphogenetic protein 15) growth factors in polycystic ovary syndrome

Material-Method: Thirty-two 6-8 week old *Sprague-Dawley* rats were randomly divided into 4 groups (n=8) as sham, melatonin, PCOS, PCOS+melatonin group. At the end of the 21st day, Hematoxylin-Eosin staining, MT1, GDF9, BMP15 immunohistochemical labeling, western blot and quantitative real-time polymerase chain reaction (qPCR) analyses were performed. Serum LH/FSH levels and colpo-cytological examinations were also studied...

Results: Melatonin supplementation has increased the expression levels of MT1 receptor, GDF9 and BMP15 in PCOS at protein and mRNA levels.

Conclusion: This results showed that melatonin supplementation reduced the symptoms of PCOS. Melatonin was found to be effective via MT1 receptor in the pathogenesis of PCOS and suppress the transport pathways of GDF9 to granulosa cells in antral follicles.

Keywords: Polycystic Ovary Syndrome, Melatonin, MT1 Receptor, GDF9, BMP15

Oral Presentation

EFFECTS OF GALLIC ACID ON CYCLOPHOSPHAMIDE-INDUCED BLADDER INJURY IN RATS

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Introduction: Cyclophosphamide (CP), an alkylating agent, has cytotoxic, antineoplastic, immunosuppressive, and anti-inflammatory effects. In addition to the treatment of many malignant diseases, it is widely used in the treatment of autoimmune diseases such as vasculitis, scleroderma, systemic lupus erythematosus, Wegener's granulomatosis, and microscopic polyangiitis. CP is a prodrug and is metabolized by cytochrome P450 in the liver and turns into metabolites. Metabolites bind to DNA, leading to disruption of DNA synthesis and cell death. Gallic acid (GA) is an important polyphenolic substance found in green tea, grapes, red wine, mango, walnuts, etc. GA is a powerful, natural antioxidant and has many biological and pharmacological activities such as scavenging free radicals, anti-inflammatory, antimutagenic, anticarcinogenic, antiapoptotic.

Objective: In this study, it was aimed to investigate the effects of GA against bladder damage that may occur with the use of CP.

Material-Method: 32 female Spraque Dawley rats weighing 200-250 g were divided into 4 groups. Control group (n:8); No application was made. CP group(n:8); On the seventh day of the experiment, a single dose of CP (150mg/kg) was administered intraperitoneally. GA group (n:8); From the first day of the experiment, gallic acid was administered by oral gavage at a daily dose of 40mg/kg for 8 days. CP+GA group (n:8): From the first day of the experiment, 40mg/kg gallic acid was administered by oral gavage daily for 8 days, and on the 7th day, 150mg/kg intraperitoneal CP was administered. At the end of the study, bladder tissue was removed from the rats under anesthesia. The animals were sacrificed. Routine tissue follow-up was performed, tissues were blocked. Sections taken were stained with hematoxylin and eosin, Masson's trichrome. In the histopathological evaluation, denudation of the surface epithelium, edema in the lamina propria, and inflammatory cell infiltration were evaluated by scoring (0:none-mild, 1:moderate, 2:intense). TUNEL stain was used to evaluate apoptosis. The apoptotic index (AI) was calculated.

Results: In the histopathological evaluation, it was observed that the epithelium was in a smooth structure and the lamina propria structure was normal in the control group. The GA group was similar to the control group. In the CP group, epithelial denudation, severe edema in the lamina propria and inflammatory cell infiltration were observed. In the CP(+)GA group, pathological the findings were considerably reduced. There was no significant difference between the groups for the apoptotic index.

Conclusion: It was concluded that GA may be effective in preventing the damage caused by CP in the bladder.

Keywords: Gallic acid, cyclophosphamide, bladder

Oral Presentation

EFFECTS OF ROSMARINIC ACID AND QUERCETIN ON LIVER, SMALL INTESTINE DAMAGE EXPERIMENTALLY CREATED WITH METHOTREXATE IN RAT

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Introduction: Methotrexate (MTX) is a drug widely used in the treatment of various cancers and inflammatory diseases. However, it has side effects in organs belonging to many systems such as the gastrointestinal system and hematological system. Quercetin (QCT) is a powerful antioxidant that prevents oxidative damage and cell death by scavenging oxygen radicals. It has anti-inflammatory effects. Rosmarinic acid (RA) is natural phenolic compound. It has antioxidant and anti-inflammatory effects.

Objective: In this study, it was aimed to compare the effects of QCT and RA against liver and small intestine damage that may occur due to MTX use.

Material-Method: 40 male Spraque Dawley rats weighting were divided into 5 groups. Control group; No application was made. MTX group; On the fifth day of the experiment, a single dose of MTX (30mg/kg) was administered intraperitoneally. MTX+QCT group; From the first day of the experiment, 30mg/kg QCT was administered daily by oral gavage for 7 days, and 30mg/kg MTX was administered intraperitoneally on the 5th day. MTX+RA group: From the 1st day of the experiment, 30mg/kg RA was administered daily for 7 days by oral gavage, and on the 5th day 30mg/kg MTX was administered intraperitoneally. MTX+QCT+RA group: From the 1st day of the experiment, 15mg/kg QCT and 15mg/kg RA administered daily for 7 days by oral gavage and on the 5th day 30mg/kg MTX was administered intraperitoneally. At the end of the study, liver and small intestine tissue were removed. Sections taken were stained with hematoxylin and eosin, Masson's trichrome. Histopathological evaluations were made using scoring (0:none-mild, 1:moderate, 2:intense).

Results: It was observed that the liver in the control group had normal structure. In the MTX group, intense edema, bleeding, severe necrotic cell and inflammatory cell infiltration, were observed especially in the portal area. In the treatment groups, pathological findings were considerably reduced. In the small intestine tissue, it was observed that the epithelium and the connective tissue were normal appearance in the control group. In the MTX group, degeneration of the surface epithelium, villous fusion, inflammatory cell infiltration and areas of bleeding in the mucosa were observed. In the MTX+QCT group, pathological findings were considerably reduced. In the MTX+RA, MTX+QCT+RA groups, pathological findings slightly decreased.

Conclusion: It was concluded that QCT and RA may be effective in preventing liver damage caused by MTX. It was concluded that QCT may be more effective than RA in preventing small intestine damage caused by MTX.

Keywords: Methotrexate, quercetin, rosmarinic acid, liver, small intestine tissue

Oral Presentation

IMPLANTATION POTENTIAL IN ORGANOID FROM MOUSE ENDOMETRIUM

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Introductıon: The endometrium, which has a hormonally regulated menstrual cycle every month, is one of the most dynamic tissues in the human body. Due to this dynamic structure, its molecular structure, which is still not fully elucidated, complicates diagnosis and treatment approaches in implantation problems. Organoid cultures are known to have a high potential to transform drug development and disease research, as drug testing and disease studies have traditionally mostly relied on 2-dimensional (2D) in vitro cell culture analyzes or animal models. Animal models are important for basic and applied research, but are time-consuming, expensive, and often limited to species-specific anatomy and physiology, limiting their use. Therefore, physiologically in vitro tissue models are necessary to study human biology and medicine.

Aim: In this study, we were aimed to create an endometrial organoid from mice, to examine the implantation potential of blastocysts in the formed endometrial organoid, and to examine the role of human-derived mesenchymal stem cells in both organoid formation and implantation success.

Materıal Metods: For this purpose, 6-8 weeks old Balb-c female mice in estrus period were used. Simultaneously, on embryonic 3,5. blastocysts collected daily were added according to study groups and cultured. Study groups; endometrial organoid culture in proliferation stage (group 1), 48 hours indirect co-culture with proliferation stage endometrial organoid hMSC (Human mesenchimal stem cell; group 2), endometrial organoid culture in secretion stage (group 3a), endometrial organoid blastocyte culture in secretion stage (group 3b), endometrial organoid hMSC was created as 48 hours indirect co-culture (group 4a) and secretory endometrial organoid hMSC 48 hours indirect co-culture blastocyst culture (group 4b). After the study, the distribution of LIFR (Lokemia inhibitor factor receptor), pan-cytokeratin, mucin 1, MMP9 (matrix metalloproteinase 9) and CD146 for immunohistochemical analysis with hematoxylin-eosin staining for model histochemical analysis was evaluated using image j analysis method.

Results: According to the study groups, gland and epithelial structures were clearly seen in Hematoxylin-Eosin staining. In indirect immunohistochemistry staining, protein immunoreactivities examined according to study groups were found to be different, reflecting normal endometrial tissue.

Conclusion: It was concluded that the in vitro endometrial organoid model could be created due to the significant differences between the protocol and the factors used in the study and the proliferation-secretory period, the groups in which hMSC and blastocyst were added.

Keywords: endometrium, organoid, implantation, infertility

Oral Presentation

INVESTIGATION OF THE EFFECTS OF MONENSIN ON SH-SY5Y NEUROBLASTOMA CELL PROLIFERATION THAT MEDIATED BY PI3K/AKT SIGNALING PATHWAY

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Introduction: Neuroblastoma is the most common extracranial childhood tumor and accounts for approximately 15% of pediatric cancer-related deaths. Further studies are needed to identify potential therapeutic targets for neuroblastoma. Monensin is an ionophore antibiotic obtained from *streptomyces cinnamonensis* with known antibacterial and antiparasitic effects. To our knowledge, no study has been reported the effects of monensin on SH-SY5Y neuroblastoma cells through targeting the PI3K/AKT signaling pathway.

Objective: It was aimed to investigate the effects of monensin and its combined treatment with rapamycin on SH-SY5Y neuroblastoma cell proliferation mediated by the PI3K/AKT signaling pathway.

Material-Method: The effective dosage of the molecules on the cell viability of the SH-SY5Y neuroblastoma cells was determined by preliminary studies using mTOR inhibitor rapamycin concentrations of 50, 100, 200, 400, 800 nM and monensin concentrations of 4, 8 µM as well as with the combinations of these concentrations. Cell viability was evaluated by 2,3 -Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide method (XTT). These preliminary studies showed that 72 hours of treatment of monensin and rapamycin at the lowest concentrations (50 nM rapamycin and 4 µM monensin) acts synergistically as a combination treatment in SH-SY5Y cells when analyzed with Chou-Talalay combination index (CI<1). Using the determined dosages, four experimental groups were generated including control, monensin (4 µM), rapamycin (50 nM) and rapamycin+monensin (50 nM rapamycin + 4 µM monensin) groups. The expression differences of PI3K/P-PI3K and AKT/P-AKT proteins were analyzed by immunohistochemistry and the expression profiles of the genes involved in the mTOR pathway were assessed by Real-time PCR. Data were statistically analyzed with SPSS 23, comparison between groups was made with one-way ANOVA followed by Tukey or Tamhane test.

Results: Immunohistochemical analyses revealed that PI3K, P-PI3K, AKT, P-AKT expressing cell numbers were significantly decreased in the monensin group when compared to control group ($p<0.05$). In the rapamycin group the mean number of PI3K-, P-PI3K-, AKT-, P-AKT- immunopositive cell numbers were also reduced compared to control group ($p<0.05$). Similarly, the average number of cells expressing all the above-mentioned proteins was significantly decreased in the rapamycin+monensin group ($p<0.05$). Gene expression studies showed that the combination of monensin and rapamycin treatment caused a significant decrease in the mRNA levels of the proteins involved in the PI3K/AKT signaling pathway including AKT, DEPTOR, MAPK1, mTOR and p21ras ($p<0.05$).

Conclusion: Our results showed that the combination of monensin and rapamycin treatment has a significant inhibitory effect on SH-SY5Y cell proliferation by targeting the PI3K/AKT signaling pathway. It is suggested

that monensin and its combination with rapamycin may be an effective therapeutic candidate for the treatment of neuroblastoma.

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Oral Presentation

PHOENIXIN-14 IMMUNOREACTIVITY IN RAT KIDNEY TISSUE

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Introduction: The diverse biological effects of neuropeptides are of wide interest to researchers. Different identification strategies allow the discovery of novel peptides, including identification from biological activities, receptor or genomic approaches. Phoenixin (Pnx), a novel endogenous neuropeptide, mainly exists as two active isoforms, phoenixin-14 (Pnx-14) and phoenixin-20 (Pnx-20). Pnx is a highly conserved peptide across different species including human, rat, mouse, porcine, and canine. Although it has not yet been ten years since then, many studies have shown that Pnx exerts a variety of biological effects. Interestingly, Pnx immunoreactivity was detected in various peripheral tissues, including heart, thymus, stomach, and spleen, with the highest expression in hypothalamus in rats. This study was performed to study the investigation of immunoreactive distribution of Pnx-14 in the kidney tissues of male rats.

Materials and Methods: Six adult Sprague-Dawley male rats, 3-4 months old, were used in the study. After the rats were decapitated, laparotomy procedures were performed quickly, and kidney tissues were taken and fixed in 10% buffered neutral formalin. Immunohistochemical evaluations were performed on tissue sections following routine histological steps. Immunohistochemical staining procedure was applied according to the avidin-biotin-peroxidase complex method. Staining was completed using 3-amino-9-ethylcarbazole (AEC) chromogen. Sections were counterstained with Mayer's hematoxylin. Immunoreactivity in kidney sections was graded by semi-quantitative scoring system (-; no staining, +; weak, ++; moderate, +++; strong).

Results: Our results demonstrated that strong Pnx-14 immunoreactivity (+++) in tubules of kidney sections (Figure 1)

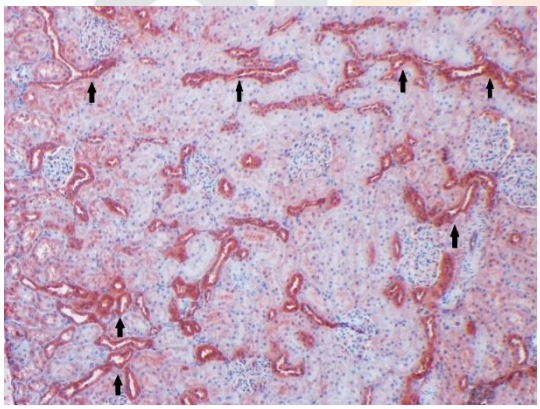

Figure 1. Strong Pnx-14 immunoreactivity (arrows) in rat kidney tissue (IHC, Mayer's haematoxylin counterstain, Magnification x100)

Conclusion: It was concluded that neuropeptide Pnx-14 participates in the regulation of various homeostatic functions by its potent immunoreactivity in the kidney and could potentially be an important regulator of renal physiology.

Keywords: Phoenixin-14, immunohistochemistry, kidney, rat.

Oral Presentation

ARE TESTICULAR STROMAL CELLS A VALUABLE RESOURCE THAT HAS BEEN OVERLOOKED UNTIL NOW?

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İntroduction: The testicles are a pair of organs located in the scrotum. Each testicle is surrounded by a thick, tight case of connective tissue called tunica albuginea. The inner part of the capsule is vascular-rich loose connective tissue called tunica vasculosa. The testicle is divided into lobules by septums in the connective tissue structure that extends from the capsule. The testicle consists of the seminiferous tubules and the interstitium in the connective tissue structure. Seminiferous tubules have germinal cells and sertoli cells. Interitisum includes peritubular myoid cells, collagen fibers, fibroblasts, leydig cells, macrophages, lymphatic ducts and vascular structures. Leydig cells regulate the functions of spermatogonial stem cells (SSC) by secreting the insulin growth factor (IGF) and colony-stimulating factor (CSF1). Macrophages provide proliferation and differentiation of SSC by secreting CSF1 and retinoic acid (RA). Vascular structures with secretions of CSF1 and endothelial growth factor (VEGF); Peritubular myoid cells are effective in the self-renewal and proliferation of SSC with their secretion of neurotrophic factor (GDNF) and CSF1 originating from the glial cell line. Seminiferous tubule cells in the niche of the SSC contribute as intrinsic factors, while interstitium cells outside the niche play important roles in regulating the functions of the SSC as extrinsic factors.

Purpose: To isolate testicular stromal cells, to grow them in a culture medium and to characterize them.

Materials and Methods: Testicular tissue from two 20-day-old male Wistar Albino rats was removed in sterile conditions and placed in falcon tubes with a medium in them. Then the laminary was broken into small pieces in the cabinet, creating explant cell cultures. Flow cytometry analyses were performed for the characterization of testicular stromal cells that migrate from tissues and multiply in culture containers.

Results: In the examination performed with a phase-contrast microscope, it was observed that the cells adhered to the culture dishes and their morphological appearance were similar to fibroblasts. In flow cytometry analysis, cells expressed CD54 and CD90, which are mesenchymal stem cell markers, but did not express CD29; It was determined that it expressed CD45, which is a hematopoietic stem cell marker.

Conclusion: In patients who will undergo orchiectomy for a testicular tumor or any other reason, either freezing of the cell suspension (cryopreservation) or freezing of tissue fragments with spermatozoa resort in to preserve fertilization. Then, with the dissolving of the material, cryoprotectants are removed and sperm can be used. In Tese (Testicular sperm extraction) operations, sperm are searched in the seminiferous tubules, ICSI (intracytoplasmic sperm injection) is performed when found, and unused mature germ cells are frozen. However,

valuable sources such as spermatogonial stem cells and testicular stromal cells, which are known to be contained in Tese material, are not hidden. The fact that these cells are also frozen and used later may bring different perspectives to research on the treatment of azoospermia patients, which is an important cause of male infertility.

Oral Presentation

CELL DEATH IN HEREDITARY RETINAL DEGENERATIONS: PARTHANATOS AND PARP INHIBITION

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Purpose: PARP (poly-ADP-ribose polymerase) which has a very important role in maintaining cell survival in case of mild DNA damage, and leads to cell death if DNA damage is severe. This PARP-dependent death mechanism was named “PARthanatos”. Increased Poly-ADP ribose polymerase (PARP) activity (Parthanatos) is a common factor among several RP mouse and rat models carrying different mutations including PRPH2. PRPH2/rd2 mutation causes slow retinal degeneration in rd2 mouse model. PARP inhibitors *(PIs)* that are currently used or are being tested in the clinical studies can be used as therapeutic agents in retinal degenerations. Here, we compared the neuroprotective effect of two different PARP inhibitors on rd2 slow retinal degeneration.

Methods: Retinal explants were prepared with retinal pigment epithelium (RPE) at postnatal day 9 (PN9). Retinal explants were treated with two different PARP inhibitors at different concentrations at PN11. One retina was used as a control, while the other was included in the treatment group. The treatment was terminated at PN18, the day when photoreceptor degeneration peaked. DAB, TUNEL, DAPI and Rho stainings were performed to observe PAR accumulation, determine photoreceptor cell death, measure the thickness of the Outer Nuclear Layer (ONL) containing photoreceptors, and compare the amounts of Rhodopsin involved in ONL formation.

Results: PAR accumulation was significantly reduced for the treated groups. The most effective concentrations were 100nM for *PI1* and 3nM for *PI2* (untreated: 0.34±0.05 SEM n=6, 100nM *PI1*: 0.15±0.02 SEM n=5 p=0.0007, 3nM *PI2*: 0.14±0.01 SEM n=5 p=0.0003). According to TUNEL, both PARP inhibitors significantly reduced photoreceptor cell death (untreated: 1.35±0.07 SEM n=7, 100nM *PI1*: 0.74±0.11 SEM n=6 p=0.0001, 3nM *PI2:* 0.4±0.04 SEM n=5 p<0.0001). And the measurement of retinal thickness showed significant increase for both treated groups (untreated: 36294±1616 SEM n=7, 100nM *PI1*:51399±1775 SEM n=6, 3nM *PI2:*56871±885.8 SEM n=5). It was determined that the expression of Rhodopsin in ONL increased in both treated groups (untreated: 1.66±0.19 SEM n=3, 100nM *PI1*: 2.88±0.43 SEM n=3 p=0.0660, 3nM *PI2*: 3.27±0.30 SEM n=3 p=0.0231).

Conclusion: PARP may have important functions as it regulates the cellular components during photoreceptor degeneration. Excessive PARP activation is occurred during photoreceptor cell death. PARP inhibition could open new treatment strategies for a variety of hereditary photoreceptor dystrophies.

Oral Presentation

EFFECT OF SEROTONIN, SELENIUM AND ZINC ON MOTILITY, APOPTOSIS AND REACTIVE OXYGEN SPICES OF HUMAN SPERM

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Background/aim: Infertility affects nearly 15% of couples all over the world. Half of infertility cases are exclusively caused by a male factor according to the recent studies. Besides concentration and morphology, decrease in motility is directly associated with infertility. Current assisted reproduction treatment approaches are based on selecting spermatozoa with the highest motility. We aimed to evaluate the possible beneficial effect of serotonin (5- HT), selenium (Se), Zinc (Zn) supplementation on sperm motility and viability in this study.

Materials and methods: Semen samples were obtained from 150 patients between the ages of 28 and 40 years old with 3–5 days of sexual abstinence. Following liquefaction, sperm analysis was performed according to the WHO manual criteria. Different concentrations of 5-HT, Se, Zn were dissolved in Human Tubal Fluid (HTF) media. All samples were washed with density gradient or swim-up techniques. After semen preparation techniques, sperm number and motility were evaluated. Also, the level of sperm apoptosis and intracellular reactive oxygen species (ROS) were measured with flow cytometry.

Results: Progressive motility is an important condition for achieving ovum fertilization in the female reproductive tract during the capacitation. The percentage of fast progressive (+4), slow progressive (+3), non-progressive (+2) and immotile (+1) sperm cells were calculated for each concentration. After washing, there were significant increases of fast progressive motility compared with control groups. While the mean +4 sperm motility was % 14 in control groups, it was increased to %21 in the combination of 5-HT-Se-Zn. Also, sperm apoptosis decreased around %11 and ROS levels decreased %12,60 up to control groups.

Conclusion: Our in vitro study has shown that incubation with 5-HT, Se, Zn has beneficial effects on sperm motility, sperm viability and ROS levels. These molecules have key roles in vitro during the preparation of semen assisted reproductive techniques to improve motility and viability.

Keywords: infertility, sperm motility, serotonin

Oral Presentation

VERIFICATION OF BEAS-2B CELLS VIA CONFOCAL MICROSCOPY AS A MODEL FOR *DIABETES MELLITUS* RELATED PATHOLOGIES OF THE HUMAN AIRWAYS

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Introduction: DM *(Diabetes mellitus)* is a systemic disorder characterized with elevated levels of blood sugar and excessive ROS (Reactive oxygen species) accumulation. Induction of ROS production through high glucose exposure is often used as a model for DM related pathologies *in vitro.* However, investigation of these pathologies in human airways are limited, due to high antioxidant capacity of both primary and immortalized bronchial epithelial cells.

Purpose: The aim of this study was to introduce an immortalized human bronchial epithelial cell line (BEAS-2B, ATCC) as an alternative model for *in vitro* studies in the field of diabetic disorders, through evaluation of ROS production via confocal microscopy.

Material – Method: BEAS-2B cells were incubated in low (2 mM) and high (10, 25 and 50 mM) glucose conditions in RPMI media containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml-µg/ml penicillin-streptomycin (PS) and 2.5 mg/ml amphotericin B (AMP-B) in a humidified atmosphere of 5% CO_2 at 37°C. ROS levels were determined for each experimental group by Leica TCS SP5 confocal microscope equipped with 488-nm argon and 543-nm helium neon lasers. BEAS-2B cells were loaded with 10 µM of cell-permeant 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA, Invitrogen) for 60 min at RT and a total of maximum fluorescent intensity within the range of 30-70 cells from 10 random areas was measured. Cell proliferation and viability were evaluated with an automated cell counter (Vi-Cell, Beckman Coulter). Data were analyzed with one-way ANOVA and Dunnett's test was applied for multiple comparisons. The level of significance was set to $p < 0.05$.

Results: ROS levels were increased with increasing doses of glucose when compared to low glucose treated cells (Figure 1A). The level of significance was p=0,0032 for 25 mM and p=0,0004 for 50 mM group. Cell proliferation and viability were also decreased in a dose-dependent manner (Figure 2B and C). Destructive effects of elevated ROS levels were significant in 25 (p=0.007 and p=0.0005) and 50 mM groups (p=0.0005 and p<0.0001) for proliferation and viability, respectively (** for p <0.005, *** for p<0.0005 and **** for p<0.0001).

Conclusion: Increased intracellular ROS levels in BEAS-2B cells was measured via confocal microscopy. The method was replicable and was used to evaluate ROS production following high glucose exposure *in vitro.* In conclusion, high glucose exposure decreases proliferation and viability of BEAS-2B cells, making them a suitable candidate for an *in vitro* model for DM.

Oral Presentation

THREE-DIMENSIONAL MODELLING OF HASSALL'S CORPUSCLES

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Introduction: Thymus is the primary lymphoid organ and thymic epithelial cells carry out critical functions for T-cell development. Hassall's corpuscles composed of type VI thymic epithelial cells are the unique structures of thymus. In the past, they were thought to be a "thymus dump/cemetery" because of their degenerating components. However in recent studies it is shown that Hassall's corpuscles represent functionally active components of thymic microenvironment regulating the differentiation and selection of T lymphocytes secreting cytokines, growth factors, chemokines. Although the development and functional importance of Hassall's corpuscles were still under investigation, lack of sufficient information about their structural organization especially whether or not they connected to each other.

Purpose: Aim of this project is to determine whether the Hassall's corpuscles are connected to each other structurally using serial sections from rat thymus, and to reconstruct their three-dimensional (3D) image using a digital imaging program.

Material-Method: This study was approved by Baskent University Ethical Committee for Experimental Resarch on Animals (Project no: DA 19/05) and supported by Baskent University Research Fund. Thymuses of two healthy adult, female, Sprague-Dawley rats were used. Plastic blocks were prepared and semi-thin serial sections of 200 nanometers thick were taken. Toluidine blue stained sections were examined by LEICA DM3000 light microscope and digital images were obtained by using LEICA DFC500 camera (which is attached to LEICA DM3000) with LAS *(Leica Application Suite)* software. The obtained digital images were transformed into 3D images by using TT3D-BMMP *(TanTuna 3D-Başkent Measuring and Modelling Programme)* developed by Prof. Erhan Kızıltan (Head of Biophysics Department).

Results: It was observed that many Hassall's corpuscles were interconnected to each other in serial sections which were used to create 3D reconstructed samples. Because of the difficulties to obtain thousands of serial section to reconstitute entire medulla of thymuses we examined different 3D reconstructed compartments of medulla and we observed interconnected Hassall's corpuscles in all samples.

Conclusion: As Hassall's corpuscles do not represent inert structures, they possibly play a critical role in the elimination of T lymphocytes following their differentiation, selection and maturation processes. The structural confluency of these unique structures may have a significant role in their function which remains to be further elucated.

Keywords: Hassall's Corpuscles, Three-dimensional modeling, Thymus, TT3D-BMMP

Oral Presentation

A RAT MODEL OF POLYCYSTIC OVARY SYNDROME INDUCED BY ULIPRISTAL ACETATE, DIETHYLSTILBESTROL AND PROGESTERONE

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Introduction: Polycystic ovary syndrome (PCOS) is an endocrine disorder that is characterized by polycystic ovaries, oligo-/anovulation and hyperandrogenism. The detailed pathophysiology of PCOS is not fully understood. Appropriate animal models can be used to investigate PCOS pathogenesis. The animal models for PCOS involve the administration of estrogens, anti-progesterone or androgens.

Purpose: In this study, our intention has been to contribute to research and development of PCOS with the proposed model which is created differently from the existing methodologies (e.g., mifepristone, estradiol valerate).

Material-Method: 13 female Wistar-albino rats were randomly divided into two groups: control group and model group. We used Diethylstilbestrol (DES) administration rather than commonly used estrogen forms in PCOS models and Ulipristal acetate (UPA) administration as antiprogestin instead of mifepristone which is preferred in mainstream methodologies. Progesterone was administered to reveal the antagonist effect of UPA. In PCOS model group, oral DES (1,35 mg/kg/day) and intramuscular progesterone (1mg/kg) were administered 3 times a week for 5 weeks. Experimental PCOS was induced with 5 weeks of oral UPA (3 mg/kg/day) administration on previously DES and progesterone administered rats. To determine the effects of UPA, DES and progesterone, groups were evaluated in terms of histopathological features. The diameter of the follicle and the thickness of the granulosa cell layer were measured in the largest follicle of the section.

Results: After histochemical analysis, it was determined that our model group exhibits cystic follicles and thin granulosa cell layers compatible with PCOS. After high progesterone, several detected corpus luteums appeared to shrink in size due to degeneration which occurs before maturation by negative inhibition of progesterone. It was noticed that some corpus luteums were enlarged because UPA prevented progesterone negative feedback. The diameter of the largest follicle increased and the thickness of the granulosa cell layer decreased in the model group according to the statistical analysis ($p<0,05$).

Conclusion: UPA and DES with progesterone administration on rats cause ovarian changes such as cystic follicles and thin granulosa cell layers to emerge similar to those encountered in PCOS patients. Our study will contribute to the relevant literature on UPA and DES. In future studies, endocrine disruptions in this model should be clarified. Animal models facilitate a better understanding of the pathogenesis of PCOS besides aiding the development of potential therapeutics for PCOS. We established an alternative PCOS rat model that shows the ovarian disturbances of the human PCOS phenotype.

Oral Presentation

HISTOLOGICAL COMPARATIVE EVALUATION OF MESNA AND TAURINE IN BONE MORE DAMAGE ASSOCIATED WITH IFOSFAMIDE CHEMOTHERAPY: EXPERIMENTAL RAT STUDY

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Aim: The use of intravenous MESNA seems to be the only option for the prevention of hemorrhagic cystitis due to ifosfamide chemotherapy, and there is no accepted agent for the treatment after the development of hemorrhagic cystitis. In this study, it was aimed to evaluate the effects of prophylactic taurine administration versus prophylactic mesna administration and taurine therapy on bone marrow histology in rats treated with ifosfamide chemotherapy.

Method: Randomized 7 groups were formed from 42 Wistar Albino male rats. Groups; 1. Group: 4. and 5th groups control, 2nd group: 6th group control, 3rd group: 7th group control, 4th group: experimental model group (400mg/kg/IP single dose ifosfomid), 5th group: MESNA prophylaxis group (provided with three intraperitoneal injections 5 minutes before, 2 and 6 hours after ifosfamide injections), group 6: taurine prophylaxis (0.5 g/kg/day taurine was administered orally for seven consecutive days before ifosfamide injection), group 7: Taurine treatment (a single dose of oral taurine 0.5 g/kg/day was administered 24 hours after ifosfamide injections). The control groups were also given saline injections and oral administrations at the same time as the conjugate groups. After 24 hours of follow-up, the subjects were sacrificed and their femurs were kept in 10% formaldehyde for 48-72 hours. After the first fixation, the samples were taken to 4% formic acid and decalcified. Decalcified samples were followed by light microscopy. Hematoxylin&eosin and Wright-Giemsa stainings were performed by taking 5-6 micrometer thick sections from the blocks obtained. Sections were immunohistochemically labeled with anti-CD68 (Thermo Scientific-Ref: MS-397-R7 Lot: 2005A) antibody to assess macrophage density and anti-VEGFR-2 (Bioss-bs10412R) antibody to assess vascularity. The findings were evaluated considering the histomorphological change. Histomorphological findings were scored from 0 (best) to 3 (worst). Differences between the groups were evaluated with the one-way ANOVA test. When a difference was found, the groups were compared in pairs with the post-hoc TUKEY HSD test, and the findings with a p value less than 0.05 were considered significant.

Results: In the groups in which MESNA and taurine prophylaxis were applied, it was observed that histomorphological damage in the bone marrow was partially prevented compared to the ifosfomite group. It was noted that macrophage activity was high in the ifosfamide group, while this effect was partially reduced in the prophylactic MESNA and taurine treatment group. A varying level of angiogenesis stimulation was detected in all groups.

Conclusıon: As a result, it was concluded that taurine may also be effective in minimizing the negative effects of ifosfamide, a chemotherapeutic agent, on the bone marrow, apart from prophylactic MESNA.

Keywords: ifosfamide, taurine, MESNA, bone marrow

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Oral Presentation

THE ROLE OF C-ABL TYROSINE KINASE IN CELL FATE SPECIFICATION AND MORPHOGENESIS IN PREIMPLANTATION MOUSE EMBRYO

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Introduction: Preimplantation mouse embryonic development process includes important stages such as compaction, polarization and cell fate determination following mitotic divisions. c-Abl tyrosine kinase, an important oncogene localized in the nucleus and cytoplasm and capable of nuclear-cytoplasmic shuttle, is activated when DNA damage occurs and plays a role in the DNA repair mechanism. In this study we hypothesized that c-Abl may be involved in cell fate specification during preimplantation embryonic development in mouse.

Aim: We aimed to show the effect of c-Abl activation by PDGF-AA in cell fate specification and blastocyst formation.

Material-Method: 6w C57BL6 female mice were superovulated with 5IU of PMSG and then 48 hours later 5IU of hCG. 20 hours after hCG injection, embryos were collected at the zygote stage and cultured for 96 hour. We designed 3 groups as control (KSOM+AA), 20 and 50 ng/mL PDGF-AA in KSOM+AA. All embryos from experimental groups were evaluated for morphokinetic parameters. We used immunofluorescent staining to determine localization of c-Abl, YAP, F-actin and PARD6 by using confocal microscope. We detected *c-Abl*, *Yap*, trophoectodermal cell markers *Tead and Cdx2 and* inner cell markers *Oct4 and Nanog* expression at the mRNA levels using the qRT-PCR.

Results and Conclusion: All embryos from experimental groups were developed from the zygote to the blastocyst stage properly, there is no significant difference between groups for developmental potential. We observed membranous F-actin expression in all groups of embryos. PARD6 expression was observed on the apical region in the control and 20 ng/mL PDGF-AA groups, while it was detected weaker in the 50 ng/mL PDGF-AA group. We detected strong nuclear YAP expression in trophoectodermal cells in control group but 20 and 50 ng/mL PDGF-AA groups embryos showed nuclear and cytoplasmic YAP localization in trophoectoderm. We detected increased c-*Abl* expression in 20 and 50 ng/mL PDGF-AA groups of embryos, and the level of *Tead4* was decreased. Additionally, we determined decreased level of *Cdx2* in PDGF-AA groups. There were no significant difference the expression levels of *Nanog* and *Oct4*. As a conclusion, we showed that c-Abl may play an important role in cell specification and cell fate determination in preimplantation mouse embryonic development. (#YAP-AP-SAB-21019)

Keywords: c-Abl, PDGF-AA, cell fate, mouse, preimplantation embryo.

Oral Presentation

DOES GINKGO BILOBA HAVE THE PROTECTIVE EFFECT ON KIDNEY PRENATALLY EXPOSED TO CYCLOPHOSPHAMIDE IN WISTAR ALBINO RAT: A STEREOLOGICAL STUDY

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Introduction: With the growing incidence of cancer, the use of chemotherapy drugs has raised in recent years. Cyclophosphamide (CP) is one of the chemotherapy drugs that are commonly used to treat different types of cancer, but its side effects have increased public health concern.

Aim: The present study was aimed to investigate the adverse effects of prenatal CP exposure on kidney of 32-day-old rats. Another objective was to survey where ginkgo biloba (GK) would ameliorate the renal alteration caused by CP.

Material and method: In this study, 20 pregnant female rats (aged 12-week-old; weighing 230-280 gr) were divided into five groups: control, vehicle, CP (20 mg/kg, intraperitoneal, single dose), GK (80 mg/kg, oral), and CP+GK. After parturition, 2 female pups were randomly selected from each female rat, so that each group was composed of 8 pups. At the end of 32 day, animals underwent cardiac perfusion, then kidneys were removed immediately. All sections were also stained with haematoxylin and eosin for stereological examination.

Result: Our stereological findings revealed that the total number of glomeruli was significantly lower in the CP group than the control group ($p<0.05$). To the contrary, the mean volume of kidney, medulla, and cortex was significantly increased in the CP group compared to the control group ($p<0.05$). In the CP+GK group, there was a significant increase in the total number of glomeruli and a decrease in the mean volume of kidney, medulla, and cortex compared to the CP group ($p<0.05$).

Conclusion: We speculated that prenatal exposure to CP caused a deleterious effect on the kidney, and that administration of GK ameliorated the renal alteration induced by CP.

Oral Presentation

STRESS-ACTIVATED JNK/P38 MAPK SIGNALING AND CIRCADIAN RHYTHM CHANGES IN THE MATERNAL OVARY DURING PREGNANCY AND LACTATION PERIODS

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Introduction: Circadian rhythm changes induce multiple pathophysiological alternations in the female reproductive system. Circadian clocks in the ovary may play a role particularly in the timing of ovulation and folliculogenesis. The effects of oxidative stress and dysregulated circadian rhythms have been the subject of intense investigations, and research into the molecular mechanisms linking the two is crucial to elucidate the basis of their connection. Among the many signaling pathways that respond to stress, mitogen-activated protein kinase (MAPK) family members are important for the maintenance of cells. Both Jun N-terminal kinases (JNKs) and p38-MAPK are simultaneously activated in response to a variety of cellular and environmental stresses. However, the potential relationship between circadian rhythm changes and JNK/p38 signaling pathway in the maternal ovary has not been previously investigated during pregnancy and lactation periods.

Purpose: This study aims to investigate the potential relationship between circadian rhythm alterations and JNK/p38 MAPK signaling pathway in the maternal ovary during pregnancy and lactation periods.

Material-Method: Pregnant rats were kept at control (12 hours light/12 hours dark), short day (8 hours light/16 hours dark) and long day (16 hours light/8 hours dark) conditions during pregnancy and lactation periods. The mothers were sacrificed at the end of the lactation period. The ovarian tissues were removed, and further processed for immunohistochemical analysis. Expression and localization of p-JNK and p-p38 proteins in the ovarian follicles (primordial, primary, secondary, preantral, antral) were evaluated by immunohistochemistry. The staining intensity (0: no staining, 1: weak, 2: moderate, 3: strong) and stained area percentage (1 for <50%; 2 for ≥50%) were multiplied to establish a weighted score.

Results: Cellular localization of p-JNK and p-p38 MAPKs was observed in oocytes and granulosa cells in rat ovaries. The expression of p-JNK protein showed a significant increase in granulosa cells from antral follicles ($p<0.05$). However, p-p38 expression was significantly increased in the primary follicles in the long day groups ($p<0.05$). There were no significant differences between control versus short day groups for both p-JNK and p-p38 proteins in the ovarian follicles.

Conclusion: The current study indicates that stress-responsive JNK/p38 MAPK signaling pathway is activated in the ovarian follicles due to long day circadian conditions during pregnancy and lactation periods. Thus, it will be of interest to determine if circadian rhythmicity is in response to rhythmic maternal secretions of hormones, or by a local ovarian clock mechanism within the ovary or both.

Poster Presentation

THE EFFECT OF TYROSINE KINASE INHIBITOR NINTEDANIB ON POST-TRAUMATIC PROLIFERATIVE VITREORETINOPATHY

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Introduction: Proliferative vitreoretinopathy (PVR) is most common cause of rhegmatogenous retinal detachment surgery failure. It is a serious complication of ocular trauma and characterized by contraction of membranes on both sides in vitreous cavity close to retinal surface. Cytokines and growth factors are considered important molecules in pathogenesis of PVR. Nintedanib (BIBF1120) is the first tyrosine kinase inhibitor approved by FDA for treatment of Idiopathic Pulmonary Fibrosis. It is small molecule tyrosine kinase inhibitor that targets PDGF receptor-α and -β, FGF receptor-1–3, VEGF receptor-1–3. Nintedanib competitively binds to ATP-binding site of these receptors, thereby blocking intracellular signaling. It has been shown that intravitreal/oral Nintedanib reduces neovascularization, does not increase vaso-obliteration, and increases normal retinal vascularization.

Purpose: In our study, it was aimed to demonstrate that intravitreal nintedanib prevents PVR formation on PVR model induced rabbit eyes by clinical and histological examination.

Material-Method: 12 rabbits were divided into two groups; control and nintedanib. Eye of each rabbit was injured with 23G needle, causing puncture injury, and dispase solution was injected just above it. After induction of PVR model, PBS was injected into midvitreus of control group and 0.5% liposomal nintedanib was injected into midvitreus of nintenib group. They were examined weekly for four weeks, and, eyes were enucleated. Enucleated eyes were examined histologically with azan trichrome dye and marked with anti-collagen-1-antibody by immunofluorescence method then intensity measurement was performed.

Results: Posterior part of eyeball was noted that choriocapillary layer was much thinner in experimental group than control group. The difference between groups most probably due to edema caused by the dilatation of veins in control group whereas the potential edema-reducing effect of nintedanib administeration in experimental group. Consistent with epiretinal membrane expected to be observed in disease model, fibrotic membrane formation extending towards the lens were also observed in front of retina and ciliary body of control group. However fibrotic membranes decreased in front of retina or were absent in the front of iris in nintedanib application group. (Figure 1). Terms of corrected-total-cell-fluorescence (CTCF) levels of subjects in control and those nintedanib treated groups are given in Table 1. According to mann-whitney-u test results applied, CTCF

level of subjects administered nintedanib was found to be statistically significantly lower than control group. (p:0,004)

Conclusion: Nintedanib has been shown to reduce the occurrence of PVR without any significant side effects. So nintedanib may have potential property in treatment and prophylaxis of PVR.

Poster Presentation

INVESTIGATION OF THE EFFECT OF HALOFUGINONE ON EPIDURAL FIBROSIS IN A RAT LAMINECTOMY MODEL

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Introduction: Lumbar disc herniation, which is the most common cause of low back pain, requires medical and/or surgical treatment. Epidural fibrosis is the most common cause of unsuccessful back surgery syndrome, in which complaints such as low back and leg pain continues after spinal surgery. It causes scar tissue around the neural tissue and compresses it and causes radicular pain by disrupting the nutrition of the nerve roots. Halofuginone has been shown to inhibit type 1 collagen synthesis and have an antifibrotic effect when administered orally and/or systemically in studies.

Purpose: It was aimed to investigate the antifibrotic effect of locally applied Halofuginone in the lumbar laminectomy model created in rats.

Material and method: A total of 21 rats were used in 3 separate groups and 7 Wistar Albino male rats (n=7) in each group. 7 of them were divided into the control group that underwent laminectomy, 7 of them were divided into the group in which hydrogel was placed in the laminectomy area, and 7 of them were divided into the group in which Halofuginone-loaded hydrogel was placed in the laminectomy area. Tissues including spinal column, vertebral column, paravertebral muscles and fascia were taken from rats sacrificed on the post-operative 21st day, 1 cm above and below the laminectomy site. They were washed with saline, kept in formaldehyde solution for a week, and after decalcification, they were dehydrated and taken into paraffin blocks. After the 5-6 µm serial sections were obtained, the area of epidural fibrosis and the degree of adhesion of the fibrosis were analyzed by staining with hematoxylin-eosin (HE) and Masson's trichrome stain.

Results: There are intense fibrosis and active fibroblasts in the control group (Figure 1), a membranous structure that can prevent fibrosis formation by forming a mechanical barrier in the hydrogel group (Figure 2), and the low distribution of fibrous tissue in the hydrogel and Halofuginone groups (Figure 3), as well as the enlargement of the vascular structures in some regions. Active osteoblasts were present. In addition, there was no infiltrative inflammatory cell, no foreign body reaction, and no new bone formation that could fill the laminectomy area was observed in all three groups.

Conclusion: Epidural fibrosis as a scar tissue around the neuronal tissue were not totally prevented by Halofuginone-loaded hydrogel. Epidural fibrosis area in square millimeters was evaluated statistically in all three groups, it was determined that there was no significant difference.

Poster Presentation

INVESTIGATION OF THE PRESENCE OF FATTY ACID AMIDE HYDROLASE ENZYME IN THE HISTOPATHOLOGIES OF PSORIASIS, CUTANEOUS LUPUS ERYTHEMATOSUS AND LICHEN SCLEROSIS

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Introduction: The endocannabinoid system (ECS) is responsible for the differentiation, apoptosis and division of keratinocytes and the management of inflammatory processes in the skin. ECS is a potential target for the autoinflammatory diseases with hyperkeratinization because of its inflammatory effects regulating cell division and differentiation. It performs these tasks with CB1/2 receptors found in the blood vessels, immune system cells, nerve endings and keratinocytes in the skin. The most known ligands of the CB1/2 receptor are N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG). Fatty acid amide hydrolase (FAAH) is the degradation enzyme of CB1/2 receptor ligand AEA.

Purpose: To investigate the role of FAAH in the pathophysiology of psoriasis, cutaneous lupus erythematosus (CLE) and lichen sclerosis (LS) with hyperkeratinization.

Material-Method: In the skin biopsy samples of 12 patients diagnosed with psoriasis, cutaneous lupus erythematosus and lichen sclerosis immunohistochemical expression of FAAH is investigated by streptavidin-biotin technique. The percentages of immunolabeled areas were calculated with the ImageJ 1.53k program. Statistical analysis was performed by using SPSS Statistics v26.0 program.

Results: In all of the groups FAAH expression was observed in the epidermis. Immunoexpression was decreased in the apical parts of epidermis in psoriasis and lichen sclerosis groups but in the CLE group expression was observed in certain parts of the epidermis. In the CLE group, FAAH expression was not observed in the keratin plugs but cytoplasmic expression in the keratinocytes was present in all the layers of epidermis. Nuclear FAAH expression was present in 5 samples of psoriasis and 4 samples of LS samples besides cytoplasmic expression. In parakeratinized regions of the epidermis no expression was present in all of the groups. In the expression of FAAH, there was no statistically significant difference between the groups according to the positive areas but in the CLE and psoriasis groups more strong expression was present in the epidermis and sweat glands when compared with the LS group.

Conclusion: FAAH although shows different expression patterns in CLE, LS and psoriasis, may have an important role in the hyperkeratinization process in these diseases. Investigation of the roles of enzymes in the synthesis of AEA and the other receptors of endocannaboid system can give important data in the histopathogenesis of these diseases.

Poster Presentation

ANALYSIS OF SERUM MATRILIN-3 CONCENTRATION AS A PUTATIVE BIOMARKER IN THE CLINICAL DIAGNOSIS AND PROGRESSION PROCESS OF OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) is one of the most common musculoskeletal diseases worldwide. The primary pathology seen in OA can be defined as degradation in the joint cartilage, damage and consequently joint failure. It is known that the tissue, in which oligomeric extracellular adapter proteins belonging to the Matrilin family are most intensely expressed, is cartilage tissue. Matrilin-3 (MATN3), a member of this family, is an important and regulatory component of the extracellular matrix of articular cartilage. It has been shown that the enzymes that cause degradation in the joint cartilage during the OA process cause the proteolytic release of MATN3 into the synovial fluid, thus increasing the concentration of MATN3 in the synovial fluid. In the literature, there are studies showing that MATN3, whose concentration increases in synovial fluid, can also be detected in serum taken from the OA patients.

Purpose: In our project carried out in the light of this information, the hypothesis that MATN3 may be an important biomarker in the clinical diagnosis of OA and follow-up of its progression has been tested.

Material-Method: In line with the purpose of the project, 108 patients aged 40 and over, who applied to the Republic of Turkey, Ministry of Health, Ankara Atatürk Training and Research Hospital and Ankara City Hospital, Orthopedics and Traumatology Clinics with knee joint complaints and diagnosed with knee joint OA according to the American College of Rheumatology (ACR) criteria, and a control group of 33 young patients under the age of 40 without knee joint complaints were included in the project after obtaining their volunteer consent. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC Index) scoring was applied to all participants. OA cases included in the study were graded based on the Kellgren-Lawrence classification after radiological examination of the knee joint. Peripheral blood samples of 1-2 ml were taken from the OA cases and control group participants included in the project, serum was separated from the blood samples and the samples were analyzed in terms of MATN3 concentration by ELISA method.

Results: Our results showed that serum MATN3 concentration did not differ significantly between the OA and control groups, and serum MATN3 concentration was not a reliable biomarker in the diagnosis and progression of OA.

Conclusion: It can be concluded that radiographic examination methods still maintain their primary value in the clinical diagnosis of OA and during its progression.

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Poster Presentation

POSSIBLE ROLES OF ANGIOGENIC FACTORS IN IMPLANTATION, DECIDUALIZATION AND PLACENTATION PROCESSES IN THE RAT ENDOMETRIUM

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Introduction: Implantation in mammals is one of the complex biological events that occur as a result of the dialogue between the blastocyst stage embryo and the receptive endometrium. Ang-1 (Angiopoietin-1), ET-1 (Endothelin-1), and Cdh-5 (VE-Cadherin) proteins play a critical role in mediating the interaction between the endothelium and surrounding matrix and mesenchyme in vascular proliferation during embryo invasion.

Objective: We aimed to investigate the possible roles above-mentioned proteins in angiogenesis by determining their immunolocalizations in the estrus cycle and gestational days in rat endometrium.

Material-Methods: Immunofluorescence staining was applied to sections taken from uterine tissues. In addition, the mRNA expressions of the above-mentioned proteins were determined by the Real-Time PCR method from the uterine endometrium.

Results: Cdh5 was found to have very strong immunolocalization especially in proestrus, estrus and metaestrus stages; ET-1, on the other hand, showed very strong immunostaining in the endothelium of endometrial blood vessels in diestrus stage. It was determined that all 3 proteins had relatively weak immunostaining in the luminal and glandular epithelia during all cycle stages. Ang-1 was detected to have very strong reaction in the cytoplasm of stromal cells, especially in the proestrus and diestrus stages. At days 7.5 and 8.5 of gestation, ET-1 and Cdh5 showed strong/very strong staining in the endothelium of blood vessels in the mesometrial area. Immunolocalization of Ang-1 was relatively strong in the cytoplasm of undifferentiated stromal cells.

Real-Time PCR analyzes demonstrated that ET-1 gene expression was upregulated (8.87-fold, 55.45%) in the transition from proestrus to estrus, while Cdh-5 and Ang-1 gene expressions were downregulated by 0.36-fold (15.8%) and 0.52-fold (31.2%) respectively. When transitioning from the diestrus to proestrus, the inverse relationship between the ET-1 and Cdh-5 genes was disrupted and the expressions of both genes were downregulated. When the gestational days were examined, the inverse relationship between Cdh-5 and Ang-1 was remarkable. Although ET-1 gene expression decreased relatively from the days 8.5 to the 9.5 of pregnancy, it was upregulated during all days of pregnancy. Accordingly, it was determined that there was no correlation between genes, and each gene exhibited different patterns in terms of gestational days and cycle stages.

Conclusion: It supports the idea that the studied angiogenetic factor proteins may be important modulators in the changes in the oestrus cycle and angiogenic events occurring in embryo implantation, and may play important roles in critical functions such as implantation, decidualization and placentation in the human endometrium.

Poster Presentation

LONG-TERM EFFECTS OF LPS-INDUCED NEUROINFLAMMATION ON DIFFERENT BRAIN AREAS

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Introduction: The growing body of evidence revealed the crucial role of neuroinflammation and microglial activation in the initiation and progression of neurodegenerative disorders. Microglia are activated in response to a neuronal injury or other insult and release either proinflammatory factors that exacerbate neurotoxicity or anti-inflammatory neuroprotective molecules that aid in wound healing and tissue repair, depending on the type and amount of stimuli. Prolonged microglial activation harms surrounding healthy brain tissue, and chemicals produced by dead or dying neurons aggravate chronic microglial activation, resulting in progressive neuron loss.

Purpose: The aim of the study was to investigate the possible morpho-functional consequences of chronic neuroinflammation on the hippocampus and prefrontal cortex.

Materials and methods. The study was conducted on 8 weeks old male mice (n=30), that have been divided into 2 groups, control and experimental. The model of chronic neuroinflammation was created by intraperitoneal injections of lipopolysaccharide (LPS) for 7 days (750 µg/kg, Escherichia coli serotype: 055:B5; Sigma–Aldrich, St. Louis, MO, USA) into experimental animal group. Control group have recieved the same amount of saline. Five weeks after the last LPS injection mice were sacrificed, hippocampus homogenates were examined for inflammatory cytokines. Additionally, the serial brain tissue sections were examined for ionized calcium-binding adaptor molecule 1 (IBA-1) immunostaining.

Results: The levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the brain homogenates were increased 5 weeks after the LPS treatment. We performed stereological counting of IBA-1+ cells per unit volume in the different regions of the hippocampus and in prefrontal cortex (PFC). The results demonstrated significant increase in the absolute counts of microglial cells in different areas of hippocampus as well as in the entire hippocampus, reflecting ongoing chronic neuroinflammatory process ($p < 0.05$). Similar results were obtained from microglia counting in PFC area ($p < 0.05$).

Conclusion: These findings show that peripheral inflammation in adult mice can stimulate brain microglia to create chronically increased proinflammatory factors along with persistent rise in the number of microglial cells compared to control animals.

Poster Presentation

IN VITRO INVESTIGATION OF THE EFFECT OF ASPROSIN HORMONE ON WOUND HEALING

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Introduction: Cutaneous wounds are an important health problem over the world. Various substances have been studied for the treatment of cutaneous wounds for centuries, new ones are being added every day, and the information obtained is being updated.

Objective: In this study, it was aimed to investigate the possible effects of the asprosin hormone, an adipokine that is newly discovered and affects many systems, on wound healing in vitro.

Material and Methods: The fibroblast cells obtained from the HDF (Human Dermal Fibroblast) cell line were seeded in the plates, and their confluence was provided. Then, the plates were divided into control, 24-and 48-hour groups. A wound model was ensured with the "Wound Scratch Assay". The asprosin hormone was administered at the determined doses (50 ng/ml, 100 ng/ml, 150 ng/ml, and 200 ng/ml) in the plates. Cell viabilities were analyzed by the MTT method and the immunoreactivities of IL-1, IL-10, fibronectin, and collagen by the immunocytochemical method in all groups. Comparisons between groups were analyzed using the One-way-ANOVA test.

Results: After the wound scratch assay, wound areas were closed in all groups given the asprosin hormone before the control group, but the earliest closure was observed at a 200 ng/ml dose. Cell viability was observed at higher levels in all groups compared to the control, and it was the highest at the dose of 200 ng/ml. In the immunohistochemical examination, it was seen that immunopositive staining of IL-1 was not different between control and asprosin given groups. A significant increase was detected in the IRS score of IL-10 at 150 ng/ml and 200 ng/ml doses of asprosin hormone in the 48-hour groups. Although IRS of fibronectin was more pronounced in 48-hour groups administered asprosin at a dose of 200 ng/ml, it was determined that fibronectin exhibited a higher IRS score at 100 ng/ml, 150 ng/ml, and 200 ng/ml doses at 24th and 48th hours. The assessment of immunocytochemical staining of collagen was detected the higher IRS scores in the 48-hour groups at 100 ng/ml, 150 ng/ml, and 200 ng/ml doses compared to the control group.

Conclusion: Our study has shown that the asprosin hormone increases cell proliferation and improves wound healing by increasing collagen and fibronectin formation and exhibiting anti-inflammatory effects. As a result, it has been thought that the asprosine hormone could be an agent that can be used for the treatment of wounds in the future.

Poster Presentation

THE INVESTIGATION OF APELIN AND APELIN RECEPTOR (APJ) EXPRESSIONS IN MOUSE ENDOMETRIUM DURING PERI-IMPLANTATION PERIOD

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Introduction: Fertilization, pre-implantation embryo development, implantation, and decidualization are critical for a healthy pregnancy. Successful implantation requires a competent blastocyst and receptive uterus. Apelin was first purified from bovine stomach extracts. Apelin is synthesized as pre-proapelin and undergoes enzymatic degradation. The apelin receptor (APJ) is a member of the G protein-related receptors. The apelin/APJ system plays important and diverse roles such as blood pressure regulation, angiogenesis, cell proliferation, apoptosis, and immune response.

Aim: Apelin is known to be expressed in the human placenta. The presence of apelin in the human placenta points out the importance of this peptide for pregnancy. However, the role of the apelin/APJ system in embryo implantation and decidualization is not known. We hypothesized that the apelin/APJ system has a role in uteri during the peri-implantation period in mice. In this study, we aimed to determine the localization and expressions of apelin and APJ in peri-implantation period mouse uteri and implantation sites.

Material&Methods: 6-8 weeks old Balb/C female and 8 weeks old Balb/C male mice were used. Non-pregnant female mice in the estrous phase were included in the estrous group. Two female mice were left in the same cage with male mice overnight for mating and the vaginal plug was checked the next morning. Female mice with a vaginal plug were admitted on the 1st day of pregnancy, thereby forming the experimental groups; D1: 1st day, D4: 4th day, D5: 5th day, D6: 6th day and D8: 8th day of pregnancy. The localization and expressions of apelin and APJ in peri-implantation period mouse uteri and implantation sites were determined via immunohistochemistry and Western blot techniques, respectively.

Results: Apelin and APJ were expressed in luminal and gland epithelium, and stroma in all experimental groups. Apelin had two isoforms, which were approximately 8 and 16 kDa. Apelin expressions gradually upregulated from the estrous phase to the 8th day of pregnancy. APJ expressions gradually upregulated from the estrous phase to the 4t^{h} day of pregnancy and reached the highest level on this day of pregnancy. APJ expressions gradually downregulated from day 4 to day 8 of pregnancy

Conclusion: Our results suggest that the apelin/APJ system may be involved in angiogenesis and proliferation during embryo development, implantation, decidualization, and placentation processes. We believe that our results will guide future studies and help elucidate the underlying causes of various pathologies such as implantation failure and pregnancy losses.

Keywords: apelin, APJ, mouse, implantation, decidualization

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Poster Presentation

INVESTIGATION OF WOUND HEALING POTENTIALS OF PINE BARK EXTRACTS AND DEVELOPMENT OF GEL FORMULATION

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Objectives: Wound is deterioration of tissues that make up skin or mucosa and disruption of normal skin structure as a result of physical or chemical damage. Wound healing is a process consists of biochemical and cellular events leading to repair of damaged tissues. This study aims to determine wound healing activity of pine bark extracts and developing a gel formulation.

Methods: For this purpose, extracts of *Pinus brutia*, *Pinus sylvestris* and *Pinus pinea* barks which are pine species grown in Turkey, were used. Cytotoxic and wound healing doses of pine bark extracts were determined by *in vitro* cell culture assays. Human Foreskin Fibroblasts were used for cell culture assays. Wound healing results were evaluated statistically. *Pinus brutia* bark extract containing Carbopol® 940 gel formulations were prepared. Physical appearance, homogeneity and color properties of the formulations were evaluated at room temperature. Viscosity and pH measurements and rheology analysis were conducted for characterization of the gel formulations. *In vitro* dissolution study of the formulations were performed by using diyalysis bag method and drug release kinetics were calculated.

Results: Based on the data obtained from the cytotoxicity test, non-cytotoxic dose ranges were determined for the extracts, in the wound healing test for each pine extract It was decided to apply 6 different concentrations, including mL. After the *in vitro* wound model application of each pinus type, distances were analyzed using inverted misrocopy. When the values of the control group and the 0th and 24th hours of each dose are evaluated within themselves, $p = 0.007$ for the control group, $p = 0.003$ for the dose of 1 µg/ mL, $p = 0.008$ for the dose of 10 µg / mL, and 40 µg/ mL and 80 µg/ mL. It was found statistically significant for doses ($p < 0.001$). It was decided to prepare the gel formulations and to use *Pinus brutia* extract at a concentration of 20 µg/ mL in the next steps. Rheological properties gel formulations were fitted to non- Newtonian plastic flow curves. The viscosity of gel formulations were increased when the Carbopol® 940 concentration was increased. It was found that *in vitro* dissolution rate was decreased when the Carbopol® 940 concentration was increased.

Conclusion: Wound healing activity of *Pinus Brutia* extract was found to be significantly higher than the control group. *Pinus Brutia* extract containing gel formulation was developed for wound healing.

Keywords: wound healing; Carbopol® 940; gel; pine bark extract

Poster Presentation

A HISTOLOGICAL APPROACH TO A PATIENT WITH PRIMARY CILIARY DYSKINESIA: CILIUM PREASSEMBLY PROTEINS IN THE PRESENCE OF THE OUTER DYNEIN ARM DEFECT

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Introduction: Primary ciliary dyskinesia (PCD) is a rare and autosomal recessive disease, characterized by sinopulmonary infections and caused by a defect in the motile cilium/flagellum. Defects of the structural proteins contained in the cilium, which has a microtubule arrangement in the axoneme structure (9+2), are observed in individuals with PCD due to their genetic heterogeneity. After being synthesized in the ribosomes, the outer dynein motor proteins (ODA) are folded with chaperones in the endoplasmic reticulum (ER) and their production is completed in the cytoplasm with preassembly proteins. Zinc finger MYND domain-containing protein 10 (ZMYND10); is the protein that is responsible for the pre-production of ODA and ciliary structure elements.

Purpose: To demonstrate that disease in a PCD patient with ODA defect may be due to the defect in cilium preassembly factors.

Material and method: A patient, aged 19 female, who were genetically pre-diagnosed with PCD (ODA defect) in Turkey, was included in this study. The physical examination, high speed video microscoby (HSVM) findings and nasal swab samples were obtained from Hacettepe University, Department of Pediatric Pulmonology. The nasal swab samples were double-labeled with DNAH5-Acetylated beta tubulin and RSPH9-ZMYND10 antibodies and evaluated under fluorescent microscope. Fluorescence measurements were evaluated from at least 3 micrographs of the patient and control individual. Measurements were calculated according to the corrected total cell fluorescence (CTCF) formula. Statistical significance was checked according to the averages in the Graphpad Prism program.

Results: Acetylated beta tubulin and DNAH5 immunoflourescence labelings were performed. The DNAH5 and acetylated beta tubulin were positive along the cilium of the control. The acetylated beta tubulin labeling was positive and DNAH5 labeling was negative for the patient's sample (Figure 1).

Conclusion: The DNAH5 mutation of the patient might be associated with ZMYND10 defect. As a result, using histological approaches to identify the cilium preassembly protein may aid in the study of the mechanisms behind the ODA protein deficiency.

Poster Presentation

MOLECULAR CLOCK PROTEINS ARE EXPRESSED IN MOUSE PENILE CAVERNOUS TISSUE: FIRST EVIDENCE FOR THE PRESENCE OF CIRCADIAN RHYTHM IN PENIS

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Introduction: The circadian rhythm is a biological process involving physiological changes that occur rhythmically in the day and night cycle in the body in a 24-hour period. Circadian rhythm is controlled by the molecular clock genes/proteins Clock, Bmal1, Per (Per1, Per2, Per3), Cry (Cry1 and Cry2) and Npas2. Obesity, type 2 diabetes mellitus, various cancers, cardiovascular and neurodegenerative diseases are associated with sleep disorders. Sleep disturbances in men have been associated with decreased erectile function, although the underlying molecular mechanism is not yet known. Moreover, whether molecular clock proteins are expressed in the penis is unknown.

Purpose: The aim of this study is to assess the expression of molecular clock proteins in proximal part of the mouse penis.

Material-method: Male mice (BALB/C breed adult 6 weeks old) with normal circadian rhythm (maintained in 12 hours light-12 hours dark light cycle) were used and their penile tissue (n=3) were obtained. Protein expression and localization of Bmal1, Clock, Npas2, Per1, Per2, Per3, Cry1, and Cry2 in proximal part of penis that includes dorsal vein, dorsal artery, corpus cavernosum, corpus spongiosum, nerve bundle was evaluated on paraffin sections by immunohistochemistry. ImageJ analysis was performed to quantify the protein expression.

Results: Bmal1 protein expression was intense and cytoplasmic in penile corpus cavernosum and corpus spongiosum. Protein expression of Clock and Cry2 was intense and cytoplasmic in all compartments of proximal penile tissue. Weak nuclear Npas2 expression was present in the cavernous tissue. Intense expression of Per1 was present in the cavernous tissue while its expression was low in corpus spongiosum. Per3 expression was low in the cavernous tissue. Per2 and Cry1 expression was not present in any compartment of the proximal penile tissue.

Conclusion: To our knowledge, this is the first evidence in the literature that molecular clock proteins are expressed in mouse proximal penile tissue and suggests basic evidence for the possible presence of circadian rhythm-controlled regulation of erection mechanism. Our results highlighted the rationale to investigate the possible effects of these molecular clock genes on the mechanism of erection and erectile dysfunction that may be related to sleep disorders in males with sleep disorders such as shift workers.

Poster Presentation

INVESTIGATION OF THE EFFECT OF HAFNIUM CHLORIDE ON SPERM VIABILITY AND MOTILITY EXAMINATION OF NORMOSPERMIC CASES

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Hafnium alloys are used in medical applications due to their biocompatibility and high corrosion resistance. Osteogenic and antimicrobial activities have been demonstrated in surgical implants. It has been used in the treatment of sarcoma. A sensor based on hafnium nanoparticles has been reported for the detection of COVID-19. Till now no study was found on the sperm effects of hafnium, which enters our daily lives, both for humans and animal species. In our study, semen samples were collected and analyzed according to WHO 2010 criteria. Twenty normospermic specimens were included in the study. After washing and centrifugation, 3 groups were formed as control, hafnium chloride 2 mg/ml and 4 mg/ml. Mobility and viability were checked in all groups at the 20th and 40th minutes. In this study, it was determined that hafnium chloride had negative effects on sperm motility and viability. It has been demonstrated that there is a potential danger in newly used tools. It is important for public health that country regulations show sensitivity in this regard.

Poster Presentation

STRESS-INDUCED JNK/P38 MAPK SIGNALING IN RAT TESTIS AFTER ACUTE AND CHRONIC EXPOSURE TO 2100 MHZ RADIO FREQUENCY RADIATION

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Introduction: Exposure to radio frequency radiation (RFR) has increased dramatically in years due to the widespread use of electronic devices, particularly mobile phones. An important consideration is the possible effects of these devices on human health. However, the pathophysiological mechanism underlying the health effects associated with RFR is not well known. One of the most important effects of RFR is that it causes oxidative stress by increasing the level of free radicals. Activation of mitogen-activated protein kinases (MAPKs) is critical in the oxidative stress-mediated apoptosis-associated signaling pathway. Both JNK and p38 MAPKs are considered to be stress-activated protein kinases (SAPKs) that are preferentially activated by cell stress-inducing signals, including oxidative stress or environmental stress.

Purpose: This study aimed to investigate whether JNK/p38 MAPK signaling pathway involves in the rat testis after exposure to 2100 MHz RFR during acute and chronic periods.

Material-Method: Rats were divided into sham groups, and groups exposed to 2100 MHz RFR for 2 h/day for acute (1 week) or chronic (10 weeks) periods (n=6/group). Localization and expression level of p-JNK and p-p38 proteins were evaluated by immunohistochemistry and western blot methods, and further quantified with ImageJ analysis.

Results: p-JNK immunostaining showed a nuclear staining pattern in spermatogonia, spermatocytes, the heads of round and elongated spermatids. Some of the Leydig cells in the interstitial areas also showed a strong immunostaining. p-p38 was detected predominantly in spermatocytes with a nuclear localization. However, round spermatids and elongated spermatids were also positively stained in some of the seminiferous tubules beside to Leydig cells in the interstitial areas. Both p-JNK and p-p38 protein intensities were significantly higher in groups exposed to 2100 MHz RFR in acute ($p < .001$) and chronic ($p < .001$) periods as compared to related sham groups. Western blot analysis also supported the data that there were statistically significant increases for both p-JNK and p-p38 proteins in testicular lysates from RFR groups when compared to sham groups in acute ($p < .001$) and chronic ($p < .001$) periods.

Conclusion: Our data demonstrate that acute and chronic exposure of 2100 MHz RFR causes sequential activation of JNK/p38 MAPK signaling pathway. Increased expressions of p-JNK and p-p38 proteins supports the idea that SAPKs are activated in rat testis after exposure to 2100 MHz RFR. Therefore, the duration and

exposure periods seem to be crucial for heavy mobile phone users and a special attention needs to be given to reducing potential negative health effects.

Poster Presentation

PROSPECTIVE AND RETROSPECTIVE INVESTIGATION OF THE EFFECTS OF NEW GENERATION INCUBATORS AND CONVENTIONAL INCUBATORS ON EMBRYO DEVELOPMENT

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Infertility, that is, the inability to have children despite being desired, is an important problem in many societies. By definition, failure to achieve pregnancy for at least 1 year without using any contraceptive method is called infertility. All of the processes that enable infertile couples to have children are called Assisted Reproductive Techniques. These techniques are; IUI (Intrauterine insemination), IVF (In vitro fertilization), ICSI (Intra cytoplasmic sperm injection). The most important devices of assisted reproductive techniques laboratory are incubators. The entire process, from the preparation of the medium to the embryo transfer, takes place almost entirely in the incubators. CO_2, O_2, humidity and temperature settings in the air in the incubators are very important for embryos. Even minimal changes in these (0.01 unit gas change and 0.01°C temperature change) can affect the development of embryos or cause them to lose strength. Therefore, incubators should be closely monitored and kept under control. As technologies have improved, incubators have changed. While the first conventional incubators had only a CO_2 sensor, that is, they could only adjust the amount of CO_2 from the air they took in; Today, newly released incubators have mix gas control, namely CO_2, $O_2$and N sensors, and we can adjust all of them from outside. The latest benchtop incubators also offer smaller volume culture areas and faster temperature regulation technology to improve the environmental balance of gametes and developing embryos. In recent studies, embryo development, clinical pregnancy and morphology follow-up in a conventional incubator with a benchtop incubator were investigated. In this study, it was determined that the clinical pregnancy rate was 20% higher and the 5th Day transfer rate was higher than the embryos followed with the benchtop incubator from randomly selected patients. In the light of these studies, we investigated embryo development, morphology, clinical pregnancy, and media pH between our conventional incubator and benchtop incubator as a retrospective and prospective study in our Assisted Reproductive Techniques clinic. Preliminary results of our study, we regulated the pH of the medium in different CO_2 with a conventional incubator and we did not find a significant difference in embryo development rates.

Poster Presentation

EFFECTS OF *CORIANDRUM SATIVUM* ON DISTANT ORGAN APOPTOSIS AND INFLAMMATION DUE TO LIVER ISCHEMIA/REPERFUSION INJURY

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Introduction: Ischemia and reperfusion injury (IR) in organs is one of the most important reason of morbidity and mortality. As a result of liver IR, the increase of inflammatory mediators leads to tissue damage. Liver IR damage often affects distant organs such as, small intestine, kidney and lung. In addition inflammatory cells increase in the organs, and also causes apoptotic cell death with increased oxidative stress. In the small intestine, mucosal degeneration, loss of villi, disruption of the intestinal barrier, changes in the microbiota profile and bacterial translocation occur. There is still no clinical solution for primary or secondary IR damage in various organs. The *Coriandrum sativum* is a culinary herb has been widely used in traditional medicine and it has antioxidant and antiinflammatory effects on liver damage.

Purpose: The aim of this study was to investigate the antiinflammatory and antiapoptotic effects of *Coriandrum sativum* (CS) extract on distant organ intestine, lung and kidney after liver ischemia reperfusion injury.

Material and Methods: Sham, IR, IR+CS and CS groups were formed. Sixty minutes of ischemia and 60 minutes of reperfusion were performed in appropriate groups. Hepatic IR was performed by Pringle Maneuver. In the treatment group, 300mg/kg/day *Coriandrum sativum* was administered by gavage. Intestine, lung and kidney tissues were fixed in 4% paraformaldehyde. Tissues were evaluated and scored in terms of cell degeneration, inflammation and congestion as well as Cas-3 and CD31 immunostaining were carried out. Renal enzymes, creatine and urea levels were measured in serum. Obtained data analysed and compared between groups using statistical methods.

Results and Conclusion: In the sham and CS group, the small intestinal villi structures were normal. After ischemia-reperfusion, a decrease in villi size, diffuse degeneration, epithelial cell shedding and extensive congestion in the capillaries were observed. Meanwhile, the number of degenerated villi and congestion were decreased in the IR+CS group. Due to IR damage, increased congestion and distal tubule enlargement were detected in the interalveolar septum of the lungs and in the capillaries between the kidney tubules. Structural influence by IR damage was decreased in the presence of CS. It was observed that the cells stained with Cas-3 and CD31(+) were increased in the lung, kidney and small intestine tissues of the IR group, and decreased in the IR+CS group. Histological scoring results indicated that, in the group using CS against IR damage, significant improvement was observed in the kidneys and intestines. However, there was a reduction in the lungs, it was not significant statistically. Kidney enzymes urea and creatinine levels were significantly increased in the IR group and decreased in the IR+CS group. Although the CS given in IR injury reduced the urea level, it was not found to be statistically significant. It was observed that liver IR damage caused changes in distant organs, especially in

the small intestine, lung and kidneys. Moreover, damaging effects of IR as well as apoptosis and inflammation were found to be decreased in the groups treated with CS.

Keywords: Liver ischemia, *Coriandrum sativum*, kidney, lung, intestine

Poster Presentation

THE ROLE OF CGAS/STING PATHWAY IN THE EMBRYO DEVELOPMENT

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Introduction: The cyclic GMP AMP synthase (cGAS) / stimulator of interferon genes (STING) pathway recognizes cytosolic deoxyribonucleic acid (DNA) and activates inflammatory processes. This pathway is expected to be suppressed in fertilization. If this pathway is not suppressed, inflammatory processes can lead to failure of fertilization by activating or impaired early embryonic development.

Aim: The major aim of this study was the identifying the role of cGAS / STING signaling in embryo development.

Methods: The experiment was divided into two groups: (1) Fertilization group (embryonic day 1; n: 15) and (2) Implantation group (embryonic day 4.5; n: 15). In fertilization group, embryos were collected and immunofluorescent staining was performed with cGAS, STING, NLR Family Pyrin Domain Containing 14 (NLRP14), Interferon regulatory factor 3 (IRF3) and Interferon (IFN) primary antibodies. In implantation group, hematoxylin-eosin staining and indirect immunohistochemistry with cGAS, STING, NLRP14, IRF3 and IFN primary antibodies were applied.

Results: High levels of cGAS and STING immunoreactivities, moderate IRF3 and IFN immunoreactivities and negative NLRP14 immunoreactivity were detected after fertilization on embryonic day 1 and 4,5 (Figure 1 and 2).

Conclusion: In all groups immunoreactivity levels of the cGAS / STING pathway molecules and NLRP14, the negative regulatory of this pathway were determined. In particular, NLRP14 is important in suppressing the nucleic acid- sensing pathway in fertilization and early embryo development and should be considered in unexplained infertility cases.

Keywords: cGAS, fertilization, implantation, NLRP14, STING

Poster Presentation

TOPICAL INTRANASAL INSULIN ENHANCES THE HEALING OF NASAL MUCOSA

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Introduction: Sinonasal surgeries are one of the most performed group of ear, nose, throat surgical procedures. The post-operative wound care mainly consists of nasal irrigations, lubrication of nasal mucosa. It is important to improve the post-operative wound healing both in surgical and public health perspective[1]. The rationale of this study is that the topical insulin promotes healing of acute tympanic membrane, increases the rate of closure, fibroblastic activity, collagen production and epithelial thickness migration according to various *in vivo* and *in vitro* human and animal studies. The hypothesis is that the topical intranasal insulin application may enhance the healing of nasal mucosa.

Purpose: The aim of this study is to evaluate the inductive effect of topical intranasal insulin application on healing process of nasal mucosa.

Material-Method: The animal experiments were approved by Kobay Laboratories Animal Experimentation Local Ethics Committee (323/2018). Left nasal mucosa of 48 Wistar rats of 10-12 weeks old, weighing between 250-300 grams has removed via 1.9 mm curette. Then, 24 of rats were applied 1 cc of 5 IU/ml regular insulin diluted with 0.9 % physiological serum, while the other 24 were treated with 1 cc of 0.9 % saline 3 times a day as control group. On days 5, 10, and 15, the animals were sacrificed and histomorphometric evaluation was performed by scoring the ciliated cell loss, inflammation, edema, goblet cell loss, defect length and defect area was quantitated on Hematoxylin-Eosin and Masson Trichrome stained sections (Leica, LASv3).

Results: Macroscopically, there were no incidence of infection and mucosal synechia. On day 5, the reduction of defect size was 56% in insulin group vs 21% in saline group (p=0.006). On day 10, reduction was 79% in insulin group vs 62% in saline group (p=0.034). On day 15, groups treated with insulin had complete closure vs 37% full closure in saline group (100% vs 92% epithelial defect reduction, p=0.036). Both inflammation and edema were decreased and less apparent at insulin group on day 15 (p=0.023; p=0.006, respectively).

Conclusion: The study output revealed that topical intranasal insulin application increases the healing and closure rate of nasal mucosal wound and reduces the inflammation and edema during the healing process. Those preliminary data present a foundation for further studies that focus on mechanism of action of insulin on nasal wound healing process for possible clinical application.

Keywords: Wound Healing, Nasal Mucosa, Insulin

Poster Presentation

INVESTIGATION OF CHRONIC ALCOHOL CONSUMPTION AND PROTECTIVE ROLE OF BORIC ACID IN HEPATITIS B VIRUS (HBV)-TRANSGENIC MICE

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Introduction: Chronic alcoholism and hepatitis B virus (HBV) infection are among the most important public health problems in the World. Both of these are problems that the target the liver, which can cause liver cancer, cirrhosis and hepatitis. HBV markers increase in the serum of chronic alcholics. Boric acid has antioxidant properties and reduces apoptosis.

Aim: To investigate the effects of alcohol use on HBV gene expression, biochemical and histological parameters in HBV transgenic mice and the possible protective role of boric acid.

Material and methods:HBV transgenic mice were divided into 4 groups: control(C) (izocaloric dextrose, by gavage,for a month), alcohol(A) (first week 1.5g/kg/day etanol, second week 3g/kg/day etanol,third and fourth weeks 6g/kg/day etanol, by gavage) , alcohol+boric acid(A+B) (first week 1.5g/kg/day etanol+50mg/kg/day boric acid, second week 3g/kg/day etanol+50mg/kg/day boric acid, third and fourth weeks 6g/kg/day etanol+50mg/kg/day boric acid, by gavage); and boric acid(B) (50mg/kd/day boric acid,by gavage, for a month). At the end of the experiment, blood samples were obtained from the heart and livers were removed for the biochemical and histopathological evaluations. After the routine histological protocols, sections were stained with H&E and TUNNEL apoptosis kit. Total antioxidant capacity (TAS), total oxidant capacity (TOS) were examined in the blood samples. The expression levels of HBV DNA, caspase-3 and cytocrome c were determined by qPCR, one of the molecular biological markers.

Results: By the histomorphological examinations, hepatocytes with a ground-glass appearance specific to HBV infection were observed in group C. Unlike other groups, group A had edematous areas, more mononuclear cell infiltrations in the parenchyma and the glycogen density in all hepatocytes was markedly decreased. It was observed that mononuclear cell infiltrations were less and hepatocyte glycogen densities were equal in all parenchyma cells in the A+B group. Compared to the C and A groups, the connective tissue increase was observed less in the A+B group around the portal areas, central vein and sinusoids.

A+B group depicted significant decrease in TUNEL positivity in comparison to A group($p<0.05$). The OSI was found to be significantly high in the A group in comparison to the C and B group ($p<0.05$, $p<0.001$ respectively). Cytochrome c and APAF-1 expression decreased significantly in A+B group in comparison to A group ($p<0.05$, $p<0.01$ respectively). Caspase 3 expression was significantly high in A group($p<0.001$).

Discussion: These results showed that alcohol increases liver damage caused by HBV, and boric acid reduces damage by antioxidant mechanism.

Poster Presentation

IN VITRO CYTOTOXICITY ASSESSMENT OF 3D PRINTED TEMPORARY DENTAL RESTORATIONS

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Introduction: Computer-aided design/computer-aided manufacturing (CAD/CAM) approaches replaced the traditional mould-based manufacturing (MBM) for polymethyl methacrylate (PMMA)-based temporary dental restorations due to shorter manufacturing periods and aesthetics[1]. However; wear of the scraper tips, insufficient precision and loss of raw materials are major disadvantages. Additive manufacturing by 3D printing may overcome drawbacks of CAD/CAM and MBM if 3D printed PMMA could present a biocompatible restorative device.

Purpose: We aimed to compare biocompatibility of polymethyl methacrylate (PMMA) temporary dental restorations manufactured by 3D printing (Unwashed/washed), CAD/CAM subtractive manufacturing and traditional MBM on L929 mouse fibroblasts.

Material-Method: Temporary dental replacements were produced with MBM, CAD/CAM subtraction and 3D printing (UV polymerization and optional ethanol wash for the surface cleaning) with PMMA as disks (5 mm diameter and 2 mm height) and sterilized by autoclaving. Cytotoxicity assay was implemented with L929 mouse fibroblasts as described by "ISO 10993-5:2009 Biological Evaluation of Medical Devices" standard. Samples were incubated in culture media (DMEM-LG with 10% FBS, 1% Pen-Strep and 0.1% L-Glutamine) for 24 hours to extract cytotoxic materials. Subsequently, L929 mouse fibroblasts were incubated with extraction media and XTT assay determined cell viability for 24, 48 and 72 hours. 10% of Triton X-100 and culture media constituted positive and negative control groups of the study, respectively.

Results: The number of live cells on MBM, CAD/CAM, unwashed/washed 3D printed devices were similar compared to negative control at 24 h ($p>0.05$ for each). The 3D printed unwashed samples presented a significant decrease ($p=0.0003$) in viability, while 3D printed washed group was similar to the negative control at 48 hours. Both unwashed and washed 3D printed groups exhibited a significant decline compared to negative control in 72 hours ($p=0.0262$ and $p=0.0001$, respectively). Positive control showed lowest absorbance values at all time points validating the assay.

Conclusion: Our study provides a comparison of temporary dental restoration manufacturing approaches and describes the cytotoxic profile of 3D printing in PMMA-based restorations. Cytotoxicity assay revealed increased cytotoxicity of 3D printed restorations compared to the traditional and CAD/CAM methods independently from the ethanol wash. Optimization of printing and polymerization procedures are required to achieve biocompatible materials before broad clinical usage of 3D printing in the fabrication of temporary dental restorations.

Keywords: 3D Printing, CAD/CAM, Temporary Dental Restorations, Biocompatibility, Cytotoxicity

Hacettepe University Scientific Research Projects Coordination Unit funded the study (THD-2021-19240).

Poster Presentation

3D BIOPRINTING, CELL CULTURE AND HISTOLOGICAL EVALUATION OF 3D PRINTED SKIN TISSUE ENGINEERING PRODUCTS FOR REGENERATIVE MEDICINE APPLICATION

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Objective: In this study, production of a printable bio-ink (cell-hydrogel mixture), and bioprinting of keratinocytes were aimed. Cell proliferation, viability, distribution and morphology were evaluated within the epidermis-like hydrogels.

Methods: Keratinocytes from epidermis layer, that is the upperlayer of skin tissue, were used. Human keratinocyte cell line (HS2) that had been characterized before were suspended with in different hydrogels and their mixtures to obtain the most suitable hydrogel for bioprinting. Bioprinted skin tissues were tested by MTT assay for viability analysis; and hematoxylin- eosin staining was performed for determination of cell distribution and morphology 1, 4 and 7 days after the beginning of the culture.

Results: According to the MTT results, cell viability was above 50% of that of control 2-dimensional cultures. MTT results are indicative of cell attachment and viability within the hydrogel structure. The resulting cell-laden polymeric hydrogel constructs were determined to be suitable for histological cross-sectioning, and hematoxylin/eosin histological staining indicated that cells were distributed homogenously within the hydrogels; and they retained their viability.

Conclusion: It was shown that epidermis-like tissues were successfully produced using 3D bioprinting, and keratinocytes were able to attach the hydrogels and retain their viability. The se hydrogel bioinks have the potential for skin tissue engineering applications, and particularly in fast and personalized treatment of burns in dermislayer.

Poster Presentation

BIOINK DESIGN, BIOPRINTING OF 3D BONE MODEL AND IN VITRO ANALYSIS

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Objective: Main purpose of the study is designing bioink for bone tissue engineering, and the bioprinting of bone model with hydrogels. *in vitro* analysis of bioprinted hydrogel are examined.

Methods: SAOS-2, cell line were used. Cells were suspended with in hydrogel for bioprinting. Bioprinted bone tissues were tested by MTT assay for viability analysis; and von Kossa and Alizarin red-S stainings was performed for determination of cell distribution and morphology tissue emerged 14, 21 and 28 days after the beginning of the culture.

Results: According to the MTT results, cell viability at tissues was 80% before the bioprinting. MTT results are indicative of cell attachment and viability within the hydrogel structure. The resulting cell-laden polymeric hydrogel constructs were determined to be suitable for histological cross-sectioning, and stainings indicated that cells were distributed homogenously within the hydrogels; and they retained their viability.

Conclusion: It was shown that bone like tissues were successfully produced using 3D bioprinting, and osteocytes were able to attach the hydrogels and retain their viability. This hydrogel bioink have the potential for bone tissue engineering applications, and particularly in fast and personalized treatment of bone regeneration

Poster Presentation

IMMUNOHISTOCHEMICAL DETERMINATION OF EXCITATORY INPUTS ON NESFATIN-1 NEURONS

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Introduction: Peptidergic neurons are controlled under the influence of both peripheral and central signals. Central control is provided by neurons containing different neurotransmitters. Nesfatin-1 is an anorexigenic peptide that suppresses food intake and is synthesized and secreted by neurons located in the hypothalamus.

Purpose: In this study, it was aimed to show the presence of excitatory innervation on nesfatin-1 neurons. In this direction, in order to investigate whether the axon terminations in contact with nesfatin-1 neurons are glutamatergic, immunohistochemical approach was employed against vesicular glutamate transporter proteins (VGluT2 and VGluT3), which have been used as markers to identify neurons that use glutamate as a neurotransmitter.

Material and Method: Male (n=5) and female (n=5) rats were used in the study. Dual peroxidase immunohistochemistry on floating vibratome sections was performed using nesfatin-1 primary antibody with either of the primary antibodies of VGluT proteins. Nesfatin-1 neurons located in the hypothalamic supraoptic nucleus were microscopically analyzed in terms of contacts with the axonal terminations containing the relevant VGluT protein. In accordance with the literature, nesfatin-1 neurons that possess at least one contact was considered as "innervated" and the ratio of such neurons to the number of all nesfatin-1-labeled neurons was obtained. These numbers were then compared between transporter proteins, and possible gender-related differences between male and female subjects were examined.

Results: While nesfatin-1 reaction was determined in brown reaction with diaminobenzidine in dual immunohistochemistry applied on the sections, VGluT proteins were labeled in black with nickel-intensified diaminobenzidine. The number of VGluT2-contacting nesfatin-1 neurons was compared between the male (88,71±4,05%) and female (69,05±6.06%) rats and determined to have a statistically significant difference (p=0.027). Although the number of VGluT3-contacting nesfatin-1 neurons was found to be higher in male rats (66.58±4,96%) when compared to female rats (53,02±3,84%), this difference was not statistically significant (p=0.063). The ratio of VGluT2-contacting nesfatin-1 neurons was higher than VGluT3-contacting neurons in both genders, however a statistically significant difference was calculated only in male rats (p=0.009).

Conclusion: Demonstrating the existence of VGluT-positive contacts on nesfatin-1 neurons suggested that glutamate may be effective in the regulation of the functions of nesfatin-1 neurons. Since there is little information in terms of central control of the nesfatin-1 neurons in the literature, the results of this study would fill an important gap and allow further studies to be planned.

Keywords: Nesfatin-1, glutamate, VGluT, hypothalamus, supraoptic nucleus, immunohistochemistry.

This study was supported by a grant from the Scientific Research Projects Foundation of the Bursa Uludag University, Bursa, Turkey [Project No: TTU-2021-376].

Poster Presentation

IMMUNOHISTOCHEMICAL LOCALIZATION OF R-SPONDIN EXPRESSING NEURONS IN THE RAT HYPOTHALAMUS

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Introduction: The hypothalamus plays a key role in the regulation of feeding behavior. Several hypothalamic nuclei, including the arcuate nucleus (ARC), paraventricular nucleus (PVN), and ventromedial nucleus (VMH) of the hypothalamus, are involved in energy homeostasis and feeding. R-spondin (Rspo) family proteins were identified as ligands of the leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4) subfamily. In mammals, the Rspo protein family consists of four members, namely Rspo1 through Rspo4. Rspos bind to LGR4 and enhance Wnt/β-catenin signaling. Although the anorexigenic effect of R-spondin proteins was reported in the literature, there is no study showing the localization of Rspo-positive neurons in the hypothalamus.

Objectives: In this study, we investigated the presence and distribution of neurons expressing Rspo 1 or Rspo 3 proteins in different hypothalamic nuclei using immunohistochemical method.

Material-Method: Male rats (n=5) were sacrificed by transcardiac perfusion fixation method under deep ether anesthesia. Fixation was carried out with a mixture of 4% paraformaldehyde (PFA) and 7.5% picric acid buffered with phosphate. Immunohistochemical staining with Rspo1 and Rspo3 antibodies was performed on free-floating sections obtained from subjects who were not exposed to any treatment. Rspo 1 or 3 immunoreactivity was revealed using DAB.

Results: As a result of the immunohistochemical studies, it was determined that neurons expressing the Rspo 1 protein were found in large numbers in the supraoptic nucleus (SON), suprachiasmatic nucleus (SCh), anterior paraventricular nucleus (PVA), periventricular hypothalamic nucleus (PeV), anterior hypothalamic area, magnocellular preoptic nucleus (MCPO) and the lateral hypothalamic area (LH) from the hypothalamic nuclei, while they were localized in fewer numbers in the arcuate nucleus. Rspo 3 protein expression was found in neurons localized in the hypothalamic nuclei SON, PVN, PeV, ARC, VMH, LH, anterior parvicellular nucleus (PaAP) and zona inserta (ZI). In addition, neuron groups synthesizing both peptides were found in the cortex and hippocampus regions.

Conclusion: Our data indicate that Rspo 1 and 3 proteins are expressed in hypothalamic energy homeostatic areas, thus these proteins may be involved in the regulation of food intake. The results of this study may lead to the planning of further histological, physiological and pharmacological studies. This study was supported by a grant from the Scientific Research Projects Foundation of the Bursa Uludag University, Bursa, Turkey [Project No: THIZ-2021-645].

Poster Presentation

EFFECTS OF ROYAL JELLY AND ITS INGREDIENT 10-HDA ON NEUROGENESIS IN RAT MODEL OF EXPERIMENTAL STROKE

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Introduction: Stroke is a significant cause of mortality and morbidity and divided into two categories: ischemic and hemorrhagic stroke. Ischemic stroke is one of the leading causes of death and disability worldwide. Generation of new neuronal cells in the injured brain is considered to be important for healing after stroke.

Purpose: We examined the effect of royal jelly and its ingredient 10-HDA, the compounds that are known for inhibiting several pathological processes involved in stroke pathophysiology, on the proliferation of neural stem cells in the subventricular zone (SVZ) and hippocampus of rats after middle cerebral artery occlusion (MCAO).

Material-Method: The rats (n=5/group) were divided into four groups including sham, control, royal jelly (100 mg/kg/day) and 10-HDA (3.1 mg/kg/day) treatment groups. Rats were treated for either 7 or 28 days and immunohistochemical staining for *bromodeoxyuridine (*BrdU) or doublecortin (DCX) was performed to identify progenitor cells and proliferating cells. BrdU-positive cells counted in the ipsilateral hippocampus using ImageJ software. These numbers were expressed as the numbers of cells per square millimeter. DCX immunoreactivity was calculated in the ipsilateral SVZ. DCX-immunoreactivity was estimated by using the ImageJ software to measure optical density (OD). Data were statistically analyzed between groups.

Results: Compared with the sham, control or royal jelly groups, the rats in the 10-HDA group showed significantly increased number of BrdU-positive cells in the ipsilateral hippocampus in 7-day treatment group ($p=0.006$, $p=0.004$, $p=0.008$ respectively). The number of ipsilateral BrdU-positive cells in 10-HDA was significantly greater than that in the sham, control and royal jelly groups ($p<0.001$) after 28-day treatment. In the ipsilateral hemisphere, DCX immunopositivity significantly increased 2-folds in the royal jelly group ($p=0.03$) and 1.5-fold in the 10-HDA group ($p=0.044$) compared to the sham group. DCX expression in the ipsilateral area was significantly higher in the royal jelly administered group than in the control group of 7-day treatment ($p=0.010$). In 28-day treatment, DCX immunoreactivity in the ipsilateral area was found to be significantly higher in royal jelly group when compared to the sham group ($p<0.001$).

Conclusion: This study showed that royal jelly or 10-HDA treatment can be effective in increasing the number of proliferating cells as well as the newly generated neurons after experimental ischemia-reperfusion. It is suggested that royal jelly and/or 10-HDA possess potential to be used in the treatment of ischemic stroke.

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Poster Presentation

THE EFFECT OF ANOMALIES IN OOCYTES COLLECTED IN IVF PROCEDURES ON TREATMENT OUTCOMES

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Today, there is an increased focus on noninvasive embryo quality assessment techniques in IVF treatments. One of the most important of these is to examine the embryo with polarization microscopy. In our study, we examined the cytoplasmic anomalies that we think affect embryo quality by polarization microscopy and examined the relationship of these anomalies with factors such as age, fertilization rate and beta hCG positivity. According to our results, fertilization rate decreases with increasing age and the rate of nonfertilized/dead oocytes increases (Table-1). The percentage of nonfertilized/dead oocytes increased statistically significantly at the age of 35 years and above compared to those under 35 years (p=0.027). In conclusion, despite the limited number of oocytes studied, oocyte anomalies continue to be important as an embryo selection criterion. The presence of oocyte abnormalities and the advanced age (≥35 years) of the infertile woman may be determinative in predicting fertilization failure.

Keywords: Oocyte anomaly, fertilization, IVF

Poster Presentation

NEUROPROTECTIVE EFFECTS OF ROYAL JELLY AND ITS INGREDIENT 10-HDA AGAINST CEREBRAL ISCHEMIA-REPERFUSION INJURY IN RAT

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Introduction: Ischemic stroke is one of the most common causes of death worldwide and remains a serious threat to human life. It can be a result of multiple events such as embolism with a cardiac origin, occlusion of vessels in the brain, and atherosclerosis affecting the cerebral circulation. There is an unmet need for drugs that can effectively treat ischemic stroke.

Purpose: The present study explored the neuroprotective effects of royal jelly and its ingredient 10-HDA in a rat model of middle cerebral artery occlusion (MCAO), and evaluated the pathophysiology underlying its effects.

Material-Method: In this study rats (n=5/group) were used and there were four groups, sham, control, royal jelly (100 mg/kg/day) and 10-HDA (3.1 mg/kg/day). Two treatment groups were formed, short- and long-term (7 and 28 days, respectively). Following the completion of the experiment, immunohistochemical analyses were performed on the brain sections. Immunohistochemistry staining for glial fibrillary acidic protein (GFAP) and neuronal nuclei protein (NeuN) was performed in order to determine astrocytic reaction and neuronal cell loss, respectively. Changes in the GFAP-immunoreactivity was estimated using the ImageJ software by measuring optical density (OD). NeuN-positive cell counts were obtained using ImageJ software in both the ipsilateral and contralateral hemispheres at the same coordinates and the change in the neuronal cell number between the intact and lesioned hemisphere was determined. Data were statistically analyzed between groups.

Results: It was determined that GFAP-positivity significantly ($p<0.001$) increased in the ipsilateral hemisphere in the control, royal jelly and 10-HDA groups of both short- and long-trem treatments. In addition, it was found that the amount of GFAP-positivity in the ipsilateral hemisphere of the royal jelly and 10-HDA ($p<0.05$) groups was significantly higher when compared to the control and sham groups. A statistically significant increase in the prevention of neuronal loss was found between 10-HAD group and control or royal jelly groups of the 7-day treatment. In the 28-day treatment group, when 10-HDA (26.97%) application was compared with the control (54.03%) group, it was found that 10-HDA effectively protected neuronal loss ($p=0.038$).

Conclusion: This study showed that royal jelly and 10-HDA treatments can trigger astrogliosis and prevent neuronal loss following the experimental model of ischemic stroke. It is suggested that royal jelly and 10-HDA might have a potential for a novel treatment approach for ischemic stroke disease.

This study was supported by TUBİTAK 118S391.

Poster Presentation

IVF LABORATUVARINDA OOSİT MAYOZ MEKİĞİ DEĞERLENDİRMESİNİN İNFERTİLİTE TEDAVİ BAŞARISINDAKİ YERİ

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Özet: Açıklanamayan infertilite olgularının arttığı IVF vakalarında noninvazif yöntemlerle oosit kalitesi değerlendirilmesi önemlidir. Çalışmamızda retrospektif olarak 2021 yılında infertilite nedeniyle Gülhane EAH ÜYTE Merkezi'ne başvuran hastaların verileri incelenmiştir. ICSI (Intra Cytoplasmic Sperm Injection) sırasında polarizasyon mikroskopi sistemi (PolScope) kullanılarak mayoz mekiği (MM) varlığı ve oositin 1. Polar Body (PB)'sine göre konumu değerlendirilmiştir. PolScope eşliğinde ICSI yapılan 70 hastanın 341 ICSI uygulama sayısı için MM varlığı/yokluğu ve açılı konumları için yaşlar arası görülme yüzdesi, intrasitoplazmik (İS) ve ekstrasitoplazmik (ES) oosit anomalisi yüzdesi, fertilizasyon başarısı, embriyo kalitesi (EK) ve β-hCG(+)'liği değerlendirilmiştir. Yaşa göre MM varlığı/yokluğu/açılı konum yüzdesi değerlendirildiğinde her iki yaş grubu için aralarında istatistiksel anlamlı farklılık yoktur. MM varlığı/yokluğu/açılı konumda olması ile İS, ES, AY oosit gruplarında ve EK arasında istatistiksel anlamlı farklılık ve korelasyon saptanmamıştır. Benzer şekilde fertilizasyon ile MM değerlendirildiğinde MM olan ve açılı konumdaki oositlerle istatistiksel anlamlı farklılık yokken; MM olmayanların non fertilize/ölen yüzdesi arasında pozitif zayıf korelasyon vardır (r=0,289; p=0,015). MM ile yaş grupları arasında anlamlı farklılık olmasa da MM yokluğu fertilizasyon başarısını olumsuz etkilemektedir. MM varlığı/yokluğu/açılı konumda ile β-hCG(+)'liği arasında da istatistiksel olarak anlamlı farlılılık olmamasına rağmen medyan (%25-75) değerleri sırasıyla 47,22(25 -66,66); 13,39(0 -50) ve 25(0 -55,55) olarak hesaplanmıştır. MM varlığında MM yokluğuna göre daha yüksek medyan değerleri oluşu örneklem sayısı artırıldığında β-hCG(+)'liği ile arasında istatistiksel anlamlılık oluşabileceğini öngörebilir. Sonuç olarak mayoz mekiği varlığınındeğerlendirilmesi oosit seçimiyle fertilizasyon başarısının ve β-hCG(+)'liğinin öngörülmesinde belirleyici olabilir.

Anahtar Kelimeler: Oosit mayoz mekiği, Polscope.

Oral Presentation

INVESTIGATION OF CERAMIDASE INHIBITION-MEDIATED CYTOTOXICITY AND CELL DEATH PATHWAYS

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Introduction: Cancer occurs in tissues and organs as a result of uncontrolled division of normal cells due to different reasons. Cancer cells have the ability to invade and metastasize to other tissues. Breast cancer is the most common type of cancer in women in the world and in our country. Current strategies as chemotherapy, radiotherapy, surgical interventions and hormonal applications are not sufficient treatments and there is a need to develop new therapeutic targets, applications and treatment agents. In recent years, the relationship between sphingolipid metabolism and cancer has started to draw attention in cancer studies. Especially due to their role in controlling signaling in the cells, sphingolipids are molecules with strong potential to develop therapeutic strategies in cancer and many other diseases and are seen as therapeutic targets. Ceramide and sphingosine-1-phosphate, which are intracellular sphingolipid molecules, are converted into each other enzymatically. A high amount of intracellular ceramide leads the cell to apoptosis, while a high level of sphingosine-1-phosphate promotes cell survival. Based on these, targeting the sphingolipid metabolism, increasing the intracellular amount of ceramide, which has proapoptotic properties, and decreasing the amount of the sphingosine-1-phosphate molecule, which triggers proliferation and suppresses apoptosis, may be an alternative method in preventing cancer.

Objective: The aim of this study is to investigate the cytotoxic effect and cell death pattern in MCF-7 and MDA-MB231 breast cancer cells treated with Ceranib-2 molecule, a ceramidase inhibitor.

Materials and Methods: The cytotoxic effect on MCF-7 and MDA-MB231 cells was investigated by MTT test, anexin-V test was performed to determine the triggered death pattern, and morphological changes were detected by confocal microscopic method.
Results: Ceranib-2 showed cytotoxicity at low doses in MCF-7 and MDA-MB231 cells. This cytotoxic value caused morphological changes in cells, which are apoptotic indicators. As a result of Anexin-V application, Ceranib-2 was found to trigger apoptosis in MCF-7 and MDA-MB231 cells.
Conclusion: Ceranib-2, MCF-7 and MDA-MB231 ceramidase inhibitors showed cytotoxic effects in breast cancer cells in a dose- and time-dependent manner at low doses. These results showed that intracellular ceramides constitute an alternative target for cancer therapy. The induced apoptosis and the detected morphological changes highlighted the potential of Ceranib-2 molecule for designing new drugs and constituted a research proposal for advanced pharmacokinetic, therapeutic and theranostic effects.
Keywords: Breast cancer, Cytotoxicity, Apoptosis, Ceramide, Ceranib-2.*This study was supported by Scientific Research Projects Unit of Eskişehir Technical University.*

Poster Presentation

EFFECT OF SYSTEMIC OXYTOCIN ADMINISTRATION ON NEW BONE FORMATION IN RABBIT MANDIBLE

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The main disadvantage of distraction osteogenesis is the prolonged treatment protocol. Recently, oxytocin (OT) has been found to have anabolic effects on bone metabolism. In this experimental study, the effects of OT on the mandibular distraction gap in rabbits at 2 different distraction rates were evaluated. This experimental study was conducted on 28 male New Zealand white rabbits. The animals were divided into 3 experimental groups and 1 control group. Group A (control group, n = 7) consisted of animals with distraction at a rate of 1 mm/day, and group B (n = 7) consisted of animals with a distraction rate of 2 mm/day; groups A and B received postoperative saline solution injection. Group C (n = 7) consisted of animals with distraction at a rate of 1 mm/day, and group D (n = 7) consisted of animals with a distraction rate of 2 mm/day; postoperative OT injection was performed in groups C and D. Tissue specimens were retrieved, fixed, decalcified, and dehydrated before embedding in paraffin. Hematoxylin eosin and Masson's trichrome stained 3 to-4 micrometer-thick sections were quantitatively evaluated for overall morphology and, the new bone formation within the distraction area. The new trabecular bone area (TA) was measured in the whole thickness of the distraction zone (DZ) of each specimen and the percentage (TA/DZ) was calculated. In total 1023, cross sectional images were reconstructed from whole volume in a high-resolution, desktop Micro-CT system. TV, BV, BV/TV, Tb. Th, Tb. Sp, Tb.Pf, and Conn.Dn. parameters were calculated three dimensionally (3D) based on the volume of the ROI. Both histomorphologic and micro–computed tomography evaluations showed increased bone healing in the OT-treated groups (Figure 1 and 2, respectively). Based on the evaluation of both the histomorphometric and micro–computed tomographic data, systemic OT administration was found to increase new bone formation and bone healing with distraction osteogenesis.

Poster Presentation

EFFECT OF SHORT ABSTINENCE PERIOD ON SEMEN PARAMETERS OF NORMOZOOSPERMIC HEAVY SMOKER MEN: A PROSPECTIVE COMPARATIVE STUDY

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Introduction: In the process from spermatogenesis and spermiogenesis to ejaculation, testis, epididymis, vas deferens and accessory reproductive glands function. Toxic agents to which spermatozoa will be exposed at these stages can affect the quality of spermatozoa and adversely affect parameters such as fertilization, embryo quality, pregnancy and the health status of the born offspring. This interaction occurs most strikingly during the storage process of spermatozoa in the epididymis. Cigarette consumption, which is in the first place in the etiology of mortality and morbidity, causes excessive production of various toxic agents, especially reactive oxygen radicals (ROR) in the body. At the same time, the effect of cigarette consumption on the success rates of assisted reproductive techniques (ART) has been extensively studied. In this study, the possible positive effects of short absinence time on semen parameters, sperm chromatin integrity and sperm DNA fragmentation by reducing the ROR exposure of spermatozoa in the epididymis in men with heavy smoking were investigated by comparing them with control groups.

Materials and methods: After 7 and 1 days of sexual abstinence, semen samples were collected by masturbation from heavy smokers (≥20) and non-smoker normozoospermic volunteers. Following liquefaction, spermatozoon concentration (with Neubauer hemocytometry), motility (with computer-assisted sperm analysis), morphology (with Diff-Quick stain), persistent histone (with aniline blue stain) and viability (with eosin nigrosine stain) rates were evaluated. DNA fragmentation (TUNEL labeling) rates and intracellular ROR levels (DCFDA staining) were determined by the flow cytometry method. In addition, malondialdehyde (MDA) levels in seminal plasma obtained after centrifugation were measured spectrophotometrically.

Results: In all groups, semen volume and total sperm count increased significantly in direct proportion to the duration of sexual abstinence. No significant difference was observed between concentration, motility, morphology, persisted histone, viability, DNA fragmentation, and intracellular ROR levels in smokers and non-smokers. It was determined that the duration of sexual abstinence did not cause a significant change in these parameters. After long periods of sexual abstinence, seminal plasma MDA levels were found to be significantly higher in the non-smoker group.

Conclusion: The World Health Organization recommends sexual abstinence for 2 to 7 days for men who apply for semen analysis. In the light of the findings obtained in our study, it was concluded that these recommended times were optimal and that despite heavy cigarette consumption, semen parameters, DNA fragmentation rates and chromatin packaging levels were not affected. Although our findings are in line with similar studies in the literature, studies that evaluate clinical data and include patients with abnormal semen parameters are needed. As a result, it was evaluated that there is no need to recommend a short period of sexual abstinence in normozoospermic cases of heavy smokers who applied to ART clinics.

Invited Speaker

MORPHOMETRY OF MICROSCOPICAL IMAGES: WHAT DOES IT MEAN?

Alistair WARREN

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Morphometry simply means measuring shape. However, the consequences of having to cut thin sections in order to visualise tissues and cells in Histology results in loss of important structural information in the resulting images. To recover this information we use the techniques of Stereology. Many definitions of stereology exist, depending on the viewpoint of the individual, but a useful starting point is: "Stereology provides meaningful quantitative descriptions of the geometry of 3D structures from measurements that are made on 2D images"- Basic Stereology for Biologists and Neuroscientists, 2012, West, M.J., Cold Spring Harbor Laboratory Press, N.Y. By using a range of mathematical and statistical formulae applied to carefully obtained random and/ or systematically samples, valuable biological information can be obtained from histological sections.

In this presentation I will describe some of the stereological approaches we have taken to estimate features such as number, volume, thickness and surface area of tissue components from human and non-human tissues including reproductive and central nervous systems at the light and electron microscopical levels. These analyses were used to obtain information that cannot be obtained in other ways and which can help us to understand function, development and pathology of cells and tissues in efficient and effective ways. The events studied include changes in the human endometrium at carefully timed stages around the late proliferative and early luteal phases in order to explore the time around implantation, comparisons of endometrial cells grown as *in vitro* tissue models with those examined from *in vivo* biopsies, and the impact of hormones and other factors on tissue culture models that are shown to contain cells that closely resemble those seen *in vivo*.

Invited Speaker

HİSTOLOJİ, EMBRİYOLOJİ VE İLGİLİ ALANLARDA TÜRKİYE'DE 140 YILDA YAYIMLANAN KİTAPLARIN ANALİZİ

Prof. Dr. Alp Can

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Günümüze dek tıp, veteriner, diş ve fen fakültelerinin histoloji ve embriyoloji eğitimlerinde kaynak kitap gereksinimini karşılamak üzere çok sayıda eser yayımlanmış durumda. Bu da bizi üniversite eğitiminin özellikle ilk yıllarındaki temel alan derslerinde fazlasıyla söz sahibi kılıyor. Biz, bu alanda eserler vermiş olan üç öğretim üyesi olarak meslek alanımızın Türkçe literatüre ne oranda, ne zamandan bu yana katkı verdiğini anlamaya dönük bir arşiv analizi yaptık. Bunu yaparken salt yayımlanan eserlerin dökümünü yapmak değil aynı zamanda yazılı belge üzerinden alanımızın tarihini elimizdeki tüm araştırma imkanlarını kullanarak gözler önüne sermek istedik. Tüm eserleri bir araya getirmek için kendi kütüphanelerimizin yanı sıra alandaki öğretim üyelerinin kütüphanelerinden, kitap veya kısa not şeklinde yayımladığı bazı derlemelerden çok yararlandık. En büyük eksikliğin, yayınevlerinin kendi bastıkları eserleri arşivlerinde bulundurmuyor olmasıydı. Fiziksel olarak bir kopyasını bile bulamadığımız eserler oldu. Hatta, yazarında bile bulamadığımız eserlerle karşılaştık; birkaç eserin sadece kapak fotoğrafına ulaşılabildik. Ancak gelinen noktada bulanamayan hiçbir eser olmadı; yani bu arşiv çalışması tam bir listeyle gerçekleştirildi. Arşiv çalışmasına başlarken bir sınır çizmeye karar verdik. Alanımızda yazılmış eserleri toplarken 140 yıl içinde farklı yayın biçimleri, konu içerikleri ve yazım şekilleriyle karşılaştık. Bu listenin kapsamını histoloji ve embriyoloji alanları temel olmak üzere tıp, veteriner, diş hekimliği ve fen fakültelerinde bu alanda ders veren ve eğitim-öğretim etkinliklerinde fiilen çalışan bilim insanlarının yazmış olduğu kitap, atlas ve benzeri basılıp yayınlanmış eserlerle sınırlı tuttuk. Buna karşın, laboratuvar kılavuzu ve notlarını, TUS, DUS ve benzeri sınavlar için hazırlanmış açıklamalı soru kitaplarını, dar bir öğrenci grubu için yazılmış föy ve notları, bizim dışımızdaki alanlarda çalışan kişilerin yazdığı eserleri kapsam dışında bıraktık. Alanımızın basılı tarihine topluca baktığımızda 203 adet özgün (telif) esere karşın sadece 39 çeviri eserin yayımlandığını görüyoruz. Bu durum alanımız yazarlarının kendi konusunda ne denli yetkin olduklarının bir kanıtı sayılabilir. Çeviri eser yayımlamak her ne kadar önemli olsa da özgün eserler her zaman daha değerli kabul edilir. Öte yandan hücre, kök hücre ve sitoloji alanlarında verilen eser sayısı 18 olarak karşımıza çıktı. İn vitro fertilizasyon ve klinik embriyoloji konuları çoğunlukla bizim alanımızın profesyonelleri tarafından kaleme alınmış durumda. Piyasada bu konuda yazılmış kitapların neredeyse tümünde yer almaktayız. Elinizdeki bu arşiv çalışmasının sonuçlarına göre 8 adet telif eser ve 1 adet çeviri eser bizler tarafından Türkçe literatüre kazandırılmış durumda. Son olarak mikroskopi alanında verilen eserlere göz attığımızda, özellikle yaşam bilimleri alanında kullanılan ışık ve elektron mikroskoplar ve buna ilişkin preparasyon hazırlama tekniklerinde toplam 9 eser karşımıza çıktı. Bunların hemen hepsi özgün eserler olarak literatüre katkı sağlamış durumda. Ortaya çıkan devasa liste kanımızca bundan sonra kitap veya atlas yayınlayacak olan kişiler için de göz atılması gereken bir belge olma özelliğini taşımakta. Listede en dikkati çeken noktanın eserlerin çok büyük bir bölümünün öğrenci düzeyinde yazılmış olması. Bir başka deyişle; temel okuyucu olarak sürekli yenilenen öğrenciler kabul edilip bunlara yönelik olarak temel bilgilerin sunulmuş olması. Kuşkusuz bu tür kaynak

kitapların varlığı bu alanların temel eğitimlerinde büyük bir boşluk doldurmakta. Bunun yayınevleri açısından da ekonomik bir kazanç konusu olduğu çok açık. Yayınevleri sürekli yenilenen okuyucuyu (fakültelerin birinci ve ikinci sınıfına başlayan öğrenciler) tercih ediyor ve baskı sayılarını öğrenci sayısına göre belirliyor. Yazar da buna göre telif ücreti alıyor. Ancak geldiğimiz noktada histoloji veya embriyoloji gibi çok geniş kapsamı olan konuların yanı sıra daha dar alanda yazılmış eserlere de talep olabilir, olmalı da. Son yıllarda çıkan bazı eserlerin buna yönelik olduğunu görmek çok sevindirici. Umarız bu eserlerin yazarları emeklerinin maddi ve manevi karşılığını alıyorlardır. Kitabın veri toplama ve değerlendirme süreçlerinde çok değerli emeklerini büyük bir heyecan ve özveriyle harcayan diğer iki yazarımız Prof. Dr. Meltem Kuruş ve Prof. Dr. Yiğit Uyanıkgil'e teşekkür ederim.

Invited Speaker

SIGNAL TRANSDUCTION CASCADES IN CANCER AND LINCHPIN ROLE IN SHAPING THE TUMOR MICROENVIRONMENT

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Cancer is a deadly disease and existing clinical therapies gradually become ineffective against drug-resistant primary tumors and metastasis. Natural products have gained attention because of their remarkable pharmacological properties. It is evident that natural products have the ability to inhibit the growth of primary and secondary tumors. We have focused on oxidative stress-inducing activities of natural products. Moreover, natural products have also been shown to regulate non-coding RNAs and apoptosis. These aspects are intriguing and many natural products have been shown to inhibit secondary growth in distant organs. We have attempted to provide an overview of our research work in tumor-bearing mice. Alnustone significantly inhibited tumor growth of HepG2 xenografts. Mere15, an anticancer polypeptide extracted from the marine species *Meretrix meretrix*. Mere15 inhibited tumor growth significantly in xenograft nude mice bearing NCI-H460 cancer cells.

Invited Speaker

HYPOTHALAMIC CIRCUITS CONTROLLING ENERGY METABOLISM: USE OF ELECTROPHYSIOLOGY, FLUORESCENT MICROSCOPY AND OPTO/CHEMOGENETIC METHODS WITH TRANSGENIC MICE MODELS

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Obesity has become a major public health problem worldwide. The fundamental cause of obesity is the energy imbalance between calorie intake and expenditure. However, genetics, individual, social and environmental factors also play role in the etiology of this complex metabolic disorder. The hypothalamic arcuate nucleus (ARC) Agouti-Related Peptide (AgRP) and Proopiomelanocortin (POMC) neurons play important roles in hunger and satiety feelings, respectively. Our recent findings have shown the importance of Tyrosine hydroxylase (TH) neurons in the hypothalamic feeding pathways (Aklan et al, 2020). These three circuits process and maintain energy balance. Signaling between the brain and peripheral factors is usually impaired in obesity and metabolic syndrome. Recent discovery of opto/chemogenetic techniques have allowed neuroscientists to better elucidate the hypothalamic circuits controlling energy metabolism by selective excitation or inhibition of specific neuron groups. Use of viral vectors and transgenic mice technology are very useful tools in examining AgRP, POMC and TH projections and circuitry in the brain. In this presentation, utilization of electrophysiology (including patch-clamp technique), opto/chemogenetic and fluorescent microscopy methods in neuroendocrine research will be reviewed.

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Invited Speaker

OOCYTE CRYOPRESERVATION: HOW FAR WE HAVE COME

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The Oocyte cryopreservation became one of most important fertility treatment options in the last decade especially after the scientific and technological improvements in embryo and oocyte vitrification. In the past, slow freezing of human oocytes was yielding very poor survival and life birth rates therefore oocyte cryopreservation was excepted as an experimental procedure back then.

The improvements in the last decade include the optimization of the vitrification solutions, improvements in the carrier systems and new automation approaches in vitrification technologies. In the first part of the talk, I will summarize the history of oocyte freezing and the current success rates in USA and Europe. the efforts for improving the success. In the second part, important factors that determine the success and carrier systems used during oocyte vitrification and warming procedure. In the last part of my talk, I will give some examples about the automation and standardization efforts in oocyte vitrification which are the two important factors for the future success of the oocyte freezing.

Invited Speaker

THE EFFECTS OF MATERNAL CIRCADIAN RHYTHM DISRUPTION ON MATERNAL, FETAL, PLACENTAL, AND OFFSPRING HEALTH: SHEDDING LIGHT ON THE MECHANISM OF FETAL GROWTH RESTRICTION

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The world economy is based on a 24/7 alert society and shift work or jet travel across time zones disrupts circadian rhythm in pregnant women. Gestational circadian rhythm disruption causes fetal growth restriction (FGR) that affects 3-7% of all births, and emerging evidence indicates that FGR babies are prone to non-communicable chronic diseases (NCDs) such as metabolic syndrome and cardiovascular (CV) disorders in their later life. However, epidemiological studies cannot establish a direct causal relationship. Effects of maternal chrono-disruption (CD) on the health of the mother, fetus, and offspring have never been evaluated in rodents, and humans with an integrated approach and underlying mechanisms are unknown. Biomarker studies are needed to assess maternal exposure to CD and prevent NCDs in adulthood. The main objective we explored was the idea that gestational CD causes direct effects on maternal as well as on fetal, neonatal, and adult health through placental insufficiency. To restore or prevent FGR caused by CD utilizing chrono- correction and melatonin administration was tested as the further objective. We found that due to gestational CD; in the placenta, rhythmic expression of circadian clock genes is lost, trophoblast invasion/vascular defects are present, and FGR is the outcome. When we followed up on the pups born from CD mothers, we found that their growth was retarded. Chrono-correction and melatonin administration was effective in diminishing some effects of CD on placental function and pup weights. Moreover, when they reached adulthood, they showed learning difficulties and endothelial dysfunction. Our research defines how in utero CD affects health in two generations and provides important knowledge on preventing adverse outcomes of maternal CD from a trans-generational perspective. Research utilizing human trophoblast cells and placenta organoids is already planned and will be the next ambitious agenda to study and translate such effects to humans.

Invited Speaker

INFECTIONS AT THE MATERNAL-FETAL INTERFACE: IMPACT OF DECIDUAL CELLS IN VIRAL INFECTIONS OF TROPHOBLASTS

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Throughout pregnancy, the placenta is a specialized tissue that acts as a physical and immunological barrier against invading pathogens to protect the developing fetus from infectious agents. Unlike most viruses, which cannot cross the placental barrier, Zika virus (ZIKV), varicella zoster, rubella, and cytomegalovirus are transmitted from the mother to the fetus by infecting various placental cell types. Serving as major antecedents of infection-related global morbidity and mortality during pregnancy, these pathogens cause congenital anomalies defects *e.g.*, microcephaly, retinopathy and placental dysfunction resulting in adverse pregnancy outcomes such as preeclampsia, preterm birth, fetal growth restriction and/or miscarriage. Maternal and fetal immunity are critical for infection clearance, while pathogens have evolved to evade both innate and adaptive immune responses that restrict infection spread. Therefore, pregnant women represent a vulnerable population for viral infections since pregnancy confers a unique immune status that facilitates maternal tolerance of the semi-allogenic fetus and enables viral infections. Better understanding of the role and mechanism(s) responsible for viral infections during pregnancy has become increasingly relevant because of the risk of current pandemic.

Fetal dissemination of any infectious agent requires transmission through the placenta, which is attached to the immunologically active uterine decidua. The decidua which is comprised of decidualized stromal cells, epithelial cells, blood vessels and maternal immune cells. undergoes complex changes to promote immunological tolerance to the semi-allogenic fetus as well as host defense against pathogens. However, downregulated maternal immunity, hormonal changes, and mechanical adaptation (*e.g.*, restricted lung expansion) make the pregnant woman more susceptible to pathogens. Depending on the infectious agent or timing of the infection during gestation, fetal pathology can range from mild to severe, and even fatal. For examples, vertical transmission of ZIKV causes placental dysfunction and elicit severe fetal defects. At the maternal-fetal interface, syncytiotrophoblasts are less-permissive to ZIKV, thereby preventing ZIKV transmission to the underlying cytotrophoblasts and/or other cells in the villi. However, anchoring villi are tightly attached to the decidua and their cytotrophoblastic cell columns are ZIKV-permissive, suggesting this location as the most likely site of ZIKV vertical transmission. Thus, at the maternal-fetal interface, maternal decidual cells are highly permissive to ZIKV infection and likely act as both a reservoir and source of ZIKV transmission to adjacent anchoring villi.

This topic focuses on summarizing the putative mechanism(s) responsible for recent pandemic ZIKV and SARS-CoV 2 infections at the maternal-fetal interface, thus highlighting the role of maternal and fetal cells in infection.

Invited Speaker

FROM ANIMAL MODELS TO THE TREATMENT OF COMPLEX NEURODEGENERATIVE DISORDERS OF HUMANS

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Neurodegenerative disorders are characterized by progressive loss of neurons in certain regions of the nervous system and despite differences in phenotypic characteristics, common mechanisms play roles in their etiopathogenesis. Treating or delaying onset of neurodegenerative diseases is critical not only for patients, but also for caregivers and healthcare systems. At this point, animal models are extremely useful in understanding the pathophysiology of neurodegenerative diseases since they might reproduce different aspects of the disease as defining symptoms and/or histopathological lesions. In recent years, many studies have been carried out on the roles of certain proteins that are prone to misfolding, especially in motor neuron damage and aging-related diseases. Patients who suffer from amyotrophic lateral sclerosis (ALS) or a related disorder frontotemporal dementia (FTD) have characteristic protein aggregates in their brains. These aggregates are toxic to neurons and contain DNA/RNA-binding proteins. How exactly cytoplasmic aggregates kill motor neurons still remains to be unraveled. However, novel animal models have provided certain hints indicating that main cellular pathways, including gene processing, protein metabolism, oxidative stress and axonal transport are involved in this degeneration process. We have recently produced an animal model in which TDP-43 proteinopathy was developed specifically in cortical motor neurons of rats by using the virus-promoter dual targeting method, to investigate the phenotypic and histomorphological alterations. Depending on the differences in the experimental model, virus titers, age and gender various levels of motor deficits and ultrastructural changes occurred in the central nervous system of these animals. Understanding the motor neuron biology and pathologic mechanisms involved different predispositions could provide new insight into how motor neuron diseases progress. Different models to be created with this technique will enable the enlightenment of the molecular mechanisms of other pathological processes related to the nervous system and trial of new therapeutic approaches in neurodegenerative diseases.

Invited Speaker

THE FATE OF CELL THAT CANNOT DIE: CANCER

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Hanahan and Weinberg have kept saying for years that one of the hallmarks of cancer cell was the resistance to cell death, primarily apoptosis, which is also arguably known as programmed cell death. In addition to this, cancer cell resists to anticancer drugs by escaping from cell death. There are numerous ways of escaping such as mutations on cell death receptors, overexpression of antiapoptotic mollecules or down regulation of proapoptotic molecules, excessive level of telomerase, lack of immune surveilance.

Cell death has actually ben ignored for a long years. However, since the middle of 1990s, its importance has gained big attention and a firm link has been established between cancer and cell death. Therefore, success in cancer treatment also relies on cell death mechanisms / apoptotic machinary. Its role in especially in the prediction of prognosis could be invaluable for the oncologists. Our group was the first to show the relationship between baseline apoptotic rate and survival of cancer patients although its clinical application is still debateable for some reasons.

In this talk, there will be a critical debate against what Hanahan and Weinberg says. They say resistance to cell death is the hallmark of cancer and i will say that this is wrong! It is actually not the resistance but the facilitance.

Invited Speaker

PHYSICAL ACTIVITY AS A NEW FRONTIER TO PREVENT OSTEOARTHRITIS: THE SYNOVIUM THEORY

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The purpose of this study was to investigate the influence of moderate physical activity (MPA) on the expression of osteoarthritis (OA)-related (IL-1β, IL-6, TNF-α, MMP-13) and anti-inflammatory and chondroprotective (IL-4, IL-10, lubricin) biomarkers in the synovium of an OA-induced rat model. A total of 32 rats were divided into four groups: Control rats (Group 1); rats performing MPA (Group 2); anterior cruciate ligament transection (ACLT)-rats with OA (Group 3); and, ACLT-rats performing MPA (Group 4). Analyses were performed using Hematoxylin & Eosin (H & E) staining, histomorphometry and immunohistochemistry. In Group 3, OA biomarkers were significantly increased, whereas, IL-4, IL-10, and lubricin were significantly lower than in the other experimental groups. We hypothesize that MPA might partake in rescuing type B synoviocyte dysfunction at the early stages of OA, delaying the progression of the disease.

Invited Speaker

KANSER KÖK HÜCRESİ VE EMBRİYONİK MİKROÇEVRE

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Kanser hastalarında prognozdan sorumlu olduğu düşünülen ve bilim insanları tarafından yapılan pek çok çalışmaya konu olan Kanser Kök Hücreleri (KKH) ilerleyen araştırmalarla farklı yönleri ile de tartışılmaktadır. Tümör dokusunun heterojen yapısı düşünüldüğünde kanser kök hücrelerinin sürekli olarak ekstrinsik ve intrinsik faktörlerden etkilenen dinamik popülasyonlar oluşturduğu ortaya çıkmıştır. Bu etkiler tümörde artmış immun evazyon, azalmış köklülük yolakları, artmış plastisite ve yüksek epitelial mezenkimal geçiş özelliğinin oluşmasına ve böylelikle tümörün yayılmasına neden olmaktadır. Kanser çevresini oluşturan stromal hücreler, immun hücreler, kanser geliştiren faktörler, hipoksik bölgeler ve ekstraselüler matriks yapısı, kanser fenotipi modülasyonunda büyük rol oynamaktadır. MSC'lerinin kanser hücresi tarafından salgılanan çözünür faktörlere maruz kalması, onları kemoterapiye dirençli kanser kök hücresi benzeri hücrelere dönüştürmektedir. Bu hücrelerin TGF-β ile daha fazla uyarılması, mezenkimal fenotiplerini ve invazyon potansiyellerini arttırmaktadır. Tümör stromasının önemli bir bileşeni olan kanserle ilişkili fibroblastların, Hepatocyte büyüme faktörü (HGF) ve onun reseptörü mesenchymal epithelial transition factor (c-Met) salınımı yoluyla karaciğer kanserini başlatan hücreleri düzenlediği bulunmuştur. IF düzenleyici faktör-7, gliomada anjiyogenezi ve heterojeniteyi indüklemek için inflamatuar bir mikroçevre üretmekte ve glioma hücrelerini, interlökin-6'yı serbest bırakarak kök hücre elde etmek için stimüle etmektedir. Sonuçta, CSC'lerinin temel özellikleri ve heterojenliği, kanser mikro ortamında ortaya çıkan farklı dış faktörlerden büyük ölçüde etkilenmektedir.

Kanser kök hücrelerini etkileyen moleküller yolaklar, embriyonik dönemde salgılanan pek çok molekülle benzerlik göstermektedir. Bu benzerlik kansere neden olan moleküler mekanizmaların çözülmesinde embriyonik mikroçevreden faydalanılabileceği fikrini ortaya çıkarmaktadır. Erken gelişimsel sinyaller doğal olarak proto-onkogenlerin ekspresyonunu baskıladığından, embriyonik mikro ortamın tümör gelişimine izin vermediği bilinmektedir. Benzer bir şekilde, embriyonik kök hücre kültürü sırasında erken embriyonik ortamı taklit etmenin kanser hücrelerinin onkojenik fenotiplerini baskıladığı gösterilmiştir. Hücre dışı matriks; embriyonik gelişim sırasında, normal gelişimde ve kök hücre biyolojisinde hücrelerin farklı soylara farklılaşmasında, hücre göçünde ve hücre çoğalmasında kritik bir rol oynar. Kök hücreler ve mikroçevreleri arasındaki bu karmaşık ilişki, hücre kaderinin belirlenmesinde de oldukça önemlidir. Bununla beraber embriyonik mikroçevrenin yanı sıra, embriyonik kök hücre mikroçevresinin de invazyon ve tümörojenite özellikleri ile çeşitli kanser hücrelerinin davranışlarını değiştirdiği gösterilmiştir. Embriyonik mikroçevre, kök hücre popülasyonunun büyümesini sürdürme ve düzenleme işlevi gören anahtar düzenleyici ipuçlarına ve sinyal moleküllerine sahip olduğundan, embriyonik kök hücre mikroçevresinin, plastik fenotipleri normalleştirerek hücre biyolojisini etkileyebileceği varsayılmaktadır. Ayrıca insan embriyonik kök hücrelerinin mikroçevresinin, agresif kanser hücrelerini daha az agresif bir duruma değiştirebildiği ve yeniden programlayabildiği ortaya çıkarılmıştır.

Invited Speaker

A TALE OF TWO SIDES: LEFT-RIGHT ORGAN ASYMMETRY IN EMBRYONIC DEVELOPMENT

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The external body plan of most metazoans exhibits bilateral symmetry. However, various internal organs display asymmetric positioning in the left-right (L/R) axis that is fundamental to organ packaging and function. During organogenesis, the primordia of the unpaired organs of the chest and abdomen first appear in the midline and then lateralize. All these asymmetries are established by complex genetic and epigenetic cascades. L/R defects in humans are phenotypically variable and genetically heterogeneous. The normal asymmetric arrangement of the organs is called *situs solitus* and occurs in 99% of humans. However, clinically significant laterality defects arise in around 1/10,000 births. Thus, we need to better understand the establishment of organ laterality during embryogenesis and therefore, it is important to identify genes and mechanisms that are involved in organ positioning. In vertebrates, left identity is mediated by the left-specific Nodal-Pitx2 axis that is repressed on the right-hand side by the epithelial-mesenchymal transition (EMT) inducer Snail1. Despite some existing evidence, it remains unclear whether an equivalent instructive pathway provides right-hand-specific information to the embryo. We have identified, in zebrafish, BMP mediates the L/R asymmetric activation of another EMT inducer, Prrx1a, in the lateral plate mesoderm with higher levels on the right. Prrx1a drives L/R differential cell movements towards the midline, leading to a leftward displacement of the cardiac posterior pole through an actomyosin-dependent mechanism. Downregulation of Prrx1a prevents heart looping and leads to mesocardia. Thus, two parallel and mutually repressed pathways exist in the left and right LPM. Activation of Nodal (on the left) and BMP (on the right) converge on the asymmetric activation of two paired-like homeodomain transcription factors, Pitx2 and Prrx1, respectively. This mechanism is conserved in the chicken embryo, and in the mouse SNAIL1 acts in a similar manner to Prrx1a in zebrafish and PRRX1 in the chick. Thus, a differential L/R EMT produces asymmetric cell movements and forces, more prominent from the right, that drive heart laterality in vertebrates.

Invited Speaker

KANSER VE HÜCRE ÖLÜMÜNDE SERAMİDAZLARIN İNHİBİSYONU

Hatice Mehtap KUTLU

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Seramid, uzunluğu C14-26 arasında değişen yağ açil zincirine bağlanmış bir amid olan sfingozinden oluşmaktadır. Seramid, sfingomiyelin, seramid-1-fosfat ve glukozil seramid gibi hidrofilik baş grubundan oluşan kompleks sfingolipidlerin metabolik ve yapısal öncülüdür. Sfingolipidler, membran çift tabakalı lipid yapısının elemanı olup, membranın akışkanlığını düzenlemektedirler. Sfingolipidler, hücrelerin farklı açılardan çoğalmasını, büyümesini ve antikanser terapötikleri etkileyen biyoefektör moleküllerdir. Sfingolipid metabolizmasındaki merkezi molekül olan seramid genel olarak hücre büyümesini baskılama, apaptozu tetikleme ya da senesensi düzenleme gibi antiproliferatif yanıtlara aracılık etmektedir. Diğer taraftan sfingozin-1 fosfat ise, zıt bir role sahip olup, kanser hücrelerinin büyümesi ve anjiyogenezi tetikleme gibi rolleri üstlenmiştir. Seramid ve sfingozin-1 -fosfatın oluşumunda bir seri düzenleyici metabolik enzim rol almaktadır. Bu enzimler pekçok hücre içi ve hücre dışı sinyaller ile birlikte sfingolipid aracılı yanıtların ileticisidirler. Kanser hücrelerinin çoğalmasının ve terapisinin içindeki anahtar rollerinin yanı sıra seramid üretiminin azaltılması ya da artırılmış sfingozin-1 fosfat seviyeleri ilaç ile indüklenen apoptoza karşı direnç geliştirme ve böylece hücrenin ölümden kaçmasına sebep olmaktadır. Hücre içi seramid seviyeleri yükseldiğinde apoptoz tetiklenmektedir. Yapılan çalışmalar seramid ve seramid-1-fosfatın intrinsik etki mekanizmalarının anlaşılmasının kanserle savaşmamızda yeni terapilere kapı açabileceğini göstermektedir. Endojen (iç) seramid seviyeleri, bütünleşmiş ve kompleks metabolik yolaklarla kontrol edilmektedir. Bu yolaklardan her biri birçok özel enzim ile katalizlenmektedir. Endojen seramid, sfingomiyelini hidrolize edip seramid oluşturan sfingomiyelinazların aktivasyonuna ilaveten *de novo* yolak ile de oluşabilmektedir. De novo yolakta, serin ve palmitoil KoA serin palmitoil transferaz ile 3-ketosfinganin'e dönüştürülmektedir. Seramid, büyümenin durması, veya apoptozun düzenlenmesine aracılık ederken Bu fonksiyonlardan bazıları yeni sfingolipid-protein etkileşimleriyle kontrol edilebilmektedir. Kanser sinyalleşme yolaklarında önemli düzenleme rolü olan seramid yapılı protein fosfataz ve kinazlar doğrudan hedeflerdir. Seramid ile aktifleşen protein fosfatazların seramid aracılı aktivasyonu Bcl-2 familyası proteinleri, siklin bağımlı kinazlar, Rb ve c-Myc onkoproteini gibi farklı hedefler ile düzenlenmektedir. Katepsin D, seramid etkileşimi sonucunda seramid bağlayan ve apoptozu tetikleyen diğer bir proteindir. Asit seramidazlar insanda görülen birçok kanser türünde çok eksprese olmaktadır ve bu durum apoptoza direnç geliştirme ile sonuçlanmaktadır. Biz de çalışmalarımızda seramidaz enzimini hedefleyerek inhibisyonunu sağlayacak molekülleri ve yeni sentezlenecek olan nanopartikül formülasyonlarının hücrelerde meydana getirebileceği ince yapısal ve morfolojik değişiklikleri, sitotoksik, antiproliferatif ve proapoptotik antikanser etkilerinin ve hücre ölüm mekanizmalarının in-vitro ve in-vivo olarak araştırılmasını hedefledik. Kanser hücrelerinde apoptozu tetiklemek için hem ekzojen seramid analoglarını hem de hücre içi seviyelerini değiştirerek tedavide yeni terapötiklerin kullanımını araştırmaktayız.

Invited Speaker

HÜCRE DİNAMİĞİ VE HÜCRE DAVRANIŞI ÜZERİNE BÜTÜNSEL BAKIŞ

Prof Dr. Hüseyin AKTUĞ

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Hücre; çevreyle madde alışverişi yapabilme yetisi ve gelişme yetisi gösteren, kendi populasyonunu büyütme ve kendi kendine üreme yeteneğine sahip, hareket edebilen ve uyarılabilen bir durumda olan yapıdır. Her hücre, hücre dışı sinyal moleküllerinin özgül kombinasyonuna_yanıt verecek şekilde hücre içi dinamiklerle cevaba programlıdır. Hücre polaritesi; hücre içindeki şekil, yapı ve fonksiyondaki mekansal farklılıkları ifade eder. Hemen hemen tüm hücre tipleri, bir çeşit polarite sergiler. Hücre polaritesi hücre içindeki asimetrileri ifade eder. Hücre yapısı, şekli ve içeriği de bundan oldukça etkilenir. Hücre bölünme paternleri incelendiğinde asimetrik ve simetrik bölünme biçimleri karşımıza çıkar. Asimetrik bölünme; belirleyici anahtar faktörler ve organeller açısından eşit olmayan bölünme (Numb, Brat, PAR, aPKC) olarak tanımlanır. Gelişim ve Farklılaşma; Asimetrik bölünme ve progenitör hücrelerinin farklılaşması ile anahtar polarite proteinlerinin eşit olmayan dağılımı gelişim ve farklılaşma konularında önem taşımaktadır. Organizmada fonksiyonel ve yapı olarak farklılaşmada planar polaritenin gerekliliği ile karşılaşılmaktadır. (Göz, Kulak, Kanat vb…) Hücre migrasyonu yine önemli bir kavram olup; Cdc42, Rac and PAR gibi proteinlerin polarite aracılığı ile up-regulasyonu ve redistribusyonu mevcuttur. Hücre içi iskeletten aktin organizasyonu ve mikroçevrenin buna etkisi sinyale doğru migrasyon başlamasında öne çıkmaktadır. Özelleşmiş bazı hücre türlerinin, spermin daima polarize olması gibi, silya-flagella yapılarının migrasyona yardımcı olması örnek olarak sunulabilir. Diğer hücrelerde lamellipod ve filopodlar aracılığı ile stimuluslara cevap, Rho ailesi proteinlerinin aktivasyonu ve bunun da aktin zincirleri üzerine büyüme ve yapışma üstünden etkisi de önem taşımaktadır. Senkronize hücre hareketlerinde hücre-hücre temasının devamı da ek olarak gerekli bir durumdur. Kendini Yenileme (Self-renewal); İki ana mekanizma üzerinden sağlanır. Obligatory asimetrik replikasyon; Kök hücre bölünür bir tane kök hücre diğeri diferansiye yavru hücre oluşur. Stokastik diferansiasyon; Bir kök hücreden iki diferansiye yavru hücre, diğer kök hücreden iki (orijinal-eş) kök hücre oluşur. Hücresel Metabolizma; Metabolizma, hücrenin madde ve enerji kaynaklarının idame süreci olarak tanımlanabilir. Enerji ve çeşitleri ile bunların yönetildiği biyokimyasal süreçlerdir. Termodinamik; maddelerdeki enerji dönüşümlerinin incelenmesi süreci olarak değerlendirilen bir süreçtir. İncelenen maddeye sistem, bunun dışında kalan objelere de çevre adı verilir. Entropi ve biyolojik düzen arasındaki ilişki oldukça önem gösterilen bir alan olarak öne çıkmaktadır. Organizmalar çevreleri ile enerji ve madde alışverişi yaparlar. Daha az organize maddeleri kullanarak daha düzenli yapılar oluştururlar (örn; protein sentezi). Meydana gelen her enerji dönüşümü entropide artış oluşturur. Metabolizma fonksiyonunun en iyi bilinen örneklerinden biri Warburg etkisidir. Bu özel metabolik durum yüksek oranda proliferasyon gösteren *kanser hücreleri* ve *embriyo gelişiminde* yaygın olarak görülmektedir. Sonuç olarak; hücre polaritesi, hücre pozisyonu, lokal mikro çevre etkileri, sinyal aktivitesi, hücre metabolizması, epigenetik ve transkripsiyonel faktörler ile bunların karşılıklı etkileşimlerinin hepsi bir bütün olarak "HÜCRE KADERİNİ- CELL FATE" belirlemektedir. Hücre kaderi üzerine mekanik faktörlerin, hücre dışı komponentlerin ve biyofiziksel değişkenlerin araştırılması da günümüzde hücre biyolojisinin anlaşılmasına katkı sağlayarak büyük bir ivmeyle

gelişme göstermektedir. Multidisipliner bakış açısı ile hücre içinde ve dışında fizik, kimya ve biyolojik süreçlerin takibi ve entegrasyonu mutlak gerekli olarak karşımızda durmaktadır.

Invited Speaker

KÖK HÜCRE LABORATUVARINDA 'KİLOMETRE TAŞLARI'

Prof. Dr. Meltem ÖZGÜNER

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'Başlangıcından beri organizmaların nasıl geliştiğini görenler onlar hakkında en iyi gözleme sahip olacaktır' Yüzyıllar öncesinde ünlü filozof Aristotle'ın belirttiği gibi embriyologlar insan organizmasının gelişimi hakkında en iyi gözlem ve bilgiye sahip kişilerdir. Bir başka açıdan değerlendirdiğimizde ise *Histoloji ve Embriyoloji Bilim Dalı* insan kök hücresi ve işlevleri hakkında detaylı bilgi sahibi olmayı gerektirmektedir. Türkiye'de kök hücre laboratuvarları; 1) Kök Hücre Araştırma Laboratuvarları, 2) Kök Hücre ve Hücresel Ürünler Üretim Laboratuvarları, 3) Klinik İlişkili Kök Hücre Laboratuvarları- Hematopoetik Kök Hücre Nakil Üniteleri ve In Vitro Fertilizasyon (IVF) Üniteleri olarak sınıflandırılabilir.

Hematopoetik Kök Hücre Nakli Laboratuvarları, hücre işleme (kültür) laboratuvarı ve hücre dondurma /saklama (kryoprezervasyon) ünitesinden oluşmaktadır. Bu laboratuvarlarda kök hücre elde edilme basamağından hastaya nakline kadar tüm aşamalarda Histologların önemli rolleri vardır. Hücre kültürü kök hücre laboratuvarlarının en önemli basamağıdır. CFU-assay ile HKH potansiyeli ölçüldüğü gibi, MKH kültür ile araştırma çalışmaları yapılmaktadır. Farklı kök hücre tipleri için farklı kültür ortamları hazırlamak gerekmektedir.

Hücre İzolasyonu (Ayrıştırma) /Seleksiyonu (Seçimi) / Deplesyonu (Uzaklaştırma): Kök Hücreler kaynağından (Sıvılardan veya Dokulardan) elde edildikten sonra hücre izolasyon, seleksiyon veya uzaklaştırma amaçlı basit santrifüj yöntemlerinden, ileri teknolojik cihazlara kadar farklı tekniklerden faydalanılmaktadır. Günümüzde, bu amaçla kullanılan Aferez cihazlarının, MACS ve Flow-Sitometri/ Akım Sitometri –FACS cihazlarının kullanımı ve veri analizi sertifikasyon gerektirmektedir. Bu teknikler hakkında uzman düzeyinde bilgi sahibi olmak özellikle kök hücre laboratuvarlarında görev alacak Histologlar için şarttır.

Hücre karakterizasyonu (Tanımlama): Bu amaçla kök hücre kültür ortamında immunhistokimyasal teknikler kullanılarak tanımlanabileceği gibiileri teknoloji lazer ışınları aracılığıyla floresanla işaretli hücreleri analiz edebilen Flow-Sitometri/ Akım Sitometri –FACS cihazları kullanılarak da tanımlanabilmektedir. Bu metotla, dakikalar içinde pekçok hücresel parametre ölçümü yapılabilmektedir. Flow-Sitometri cihazları kullanılarak hücrelerin; immunfenotipleri, canlılık analizleri/ apopitoz, enzim içerikleri, membran potansiyelleri, DNA içerikleri analiz edilebilmektedir. Günümüzde tüm hücre bilimcilerin (histolog, patolog, immunolog) bu yöntemlerle elde edilen verilerin analizini yapabilmesi gerekmektedir.

Kryoprezervasyon: Kök hücrelerin; tüm fiziksel ve biyokimyasal reaksiyonlarını durdurarak, dondurup saklamak ve ihtiyaç olduğunda çözüp kullanabilmek amacıyla uygulanan kryoprezervasyon yöntemleri ayrı bir bilim dalına konu bile olabilir. Kryoprezervasyonun standardizasyonu zor olduğu için yeni stratejilere ve metotlara açık bir işlemdir. Yine bu alanda bilgili ve tecrübeli Histolog ve Embriyologlara ihtiyaç vardır.

Kök Hücre laboratuvarlarını kurmak ve sorumluluğunu almak esasen Histolog-Embriyologların görevidir; bu nedenle tüm asistanlarımızın kök hücre kaynaklarından hücre elde etme teknikleri, hücre ayrıştırma yöntemleri,

hücrenin fiziksel, kimyasal, genomik yapısını analiz eden testler, kryoprezervasyon yöntemleri hakkında gerekli bilgileri yüksek lisans, doktora, uzmanlık eğitimleri boyunca almaları çok önemlidir.

Invited Speaker

STEM CELL EXOSOME-NANOCOMPOSITE BASED THERAPIES FOR TARGETED BONE REGENERATION: ARE WE READY FOR TRANSLATION?

Prof. Petek KORKUSUZ

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Absence of high-level verification and operational restrictions impede our understanding of stem cell therapies and nanoscale biomaterial applications for bone regeneration. Widespread off-label administration and direct-to-consumer marketing necessitate high-quality preclinical and clinical data before prescription of personalized cell therapeutics. Inconsistent terminology, classification and regulations for heterogeneous cell populations as advanced medicinal products may present effects that are not primarily attributed to medicinal capacity of the therapeutic agent. The literature reviews have tended to take a positive view of stem cells and nanoscale molecules as promising potential treatment alternatives for osteoarthritis, osteoporosis, vertebral repair. However, effective translation of targeted nanoscale cell therapies might be possible by coupling the *in vitro* study data with preclinical and clinical trials. This talk summarizes the output of three coupled experimental studies concerning mesenchymal stem cell exosome and growth factor-based therapies implemented by nanoscale bone matrix mimicking composites. The boron-containing nanohydroxyapatite composites increase mineralization acting on Wnt, TGF-β signalling pathways, and enhance response to stress on human osteoblasts. Intramedullary application of boron-nanohydroxyapatite increases new bone formation and mineralization in osteoporotic ovariectomized rabbit model. Low dose rhBMP2 containing nanohydroxyapatite-PEG/PLA stimulates proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells (BMMSCs) in a tight therapeutic window. The novel nanohydroxyapatite/PLA-PEG/BMP-2 composite material is validated in a vertebral posterolateral spinal fusion animal model by enabling efficient bone formation by BMP-2. Calcium deficient hydroxyapatite-annexin-V bound allogeneic BMMSC exosomes induce proliferation and matrix mineralization of human osteoblasts. The new complex could have a regenerative potential in bone diseases as an autogenic and/or allogeneic tool after *in vivo* and clinical validation. This suggests that the application of MSC allogenic exosomes for bone disorders is still at the initial stage, and more evidence regarding the therapeutic effect, targeted signalling pathways, and other mechanisms are needed. In conclusion those randomized controlled preclinical trials that have been published from our lab to date nano-molecule implemented stem cell therapy increasing bone mineralization, bone volume, and the regeneration. bring new therapeutic ideas and directions to the clinical treatment of musculoskeletal conditions such as osteoporosis, osteoarthritis, osteonecrosis and fracture repair. High-quality translational studies are needed to complement the existing clinical prescriptions.

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(**) Patents pending (#TR201608109B and #TR021/002221).

Invited Speaker

ELECTRON MICROSCOPY OF MICROORGANISMS

Prof. Serap ARBAK

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Electron microscopy is an important technique that could be used to investigate the ultrastructural features of the microorganisms. Negative staining in transmission electron microscope (TEM) has been used for many years to investigate the microorganisms. As a limited factor, it needed an adequate concentration of bacterial cells or virus particles. Recent development of high-quality polycarbonate filters allowed scanning (SEM) and transmission (TEM) electron microscopical identification of viruses with as little as 5000 total particles per sample. The optimum pore size can be selected to collect any virus or bacterial species. This presentation includes bacteria and virus SEM and TEM processing methods of Acıbadem Mehmet Ali Aydınlar University, School of Medicine, Department of Histology and Embryology. For SEM -bacterial sample preparation, we prefer in our lab the membrane filters which were resistant to alcohols and amyl acetate. For TEM, following fixation by glutaraldehyde, centrifugation and postfixation in OsO_4, agar-embedded bacterial samples are subjected to the next steps of TEM processing. These SEM and TEM methods were recently used in our lab in a multidiscipilinary scientific project in which we investigated the ultrastructural changes in the bacteria trigerred by antimicrobial peptides (AMPs). A novel antimicrobial peptide, D-TN6 peptide, has been developed synthetically in Acıbadem Mehmet Ali Aydınlar University. Electron microscopy techniques (SEM and TEM) were used to reveal its antimicrobial effect on bacteria. Ultrastructural results suggested that this novel antimicrobial peptide was quite effective against resistant strains, E. Coli and S. Aureus. Isolated Sars-CoV-2 virus particles were also ultrastructurally investigated in our lab in the very early days of pandemics. We had successfully processed and investigated them by the above-mentioned TEM techniques including both Epon-embedding and negative staining.

Invited Speaker

APPLICATIONS OF ADVANCED MICROSCOPIC IMAGING TECHNIQUES IN LIFE SCIENCES

Asst. Prof. Dr. Sercin KARAHUSEYINOGLU [1,2]

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Microscopic imaging is vital for visualization of diverse structures that range between micro scales to even pico scales. Some of the microscopes we use today are indispensable to accomplish interventions on tissues and cells. The conventional brightfield microscope is still an extensively used instrument in almost all cell and tissue biology related fields, microbiology, parasitology, etc. due to its simple design, multiple options for contrast imaging and reasonable price. On the other hand, in research practice more advanced systems are needed to reach higher resolving power. While atomic force microscopes offer such resolution, in life sciences electron microscopes are still among the most commonly used microscopes for sub-micron scale imaging. However, when spatiotemporal resolution is considered, microscopy systems that rely on fluorescence are of great value due to the fact that even a simple and inexpensive epi-fluorescence microscope enables the researcher to witness the life-cycle of cells and to interfere cellular functions. The advanced fluorescence-based imaging systems that are equipped with confocal, multiphoton, super-resolution and light sheet microscopes are well-established and emerging tools for visualization of biological/biomedical samples, temporally at x, y, z directions in a discriminative manner due to use of fluorescence label-based probing methods. The fluorescence probes that enclose a huge variety of fluorophores, very skilled detectors, superior computer systems and softwares along with the cutting-edge technology have enabled the researchers to reveal what has not been seen in and outside the cell more than ever. As for most of the occasions laser scanning confocal microscopes are more than enough for a spatial resolving power of 200-500 nm in thin sections, super-resolving systems capable of sub-diffraction imaging that increase the resolving power up to 20-50 nm, are required to distinguish very close structures inside and outside the cells. Super-resolution systems that either decrease the point spread function or enable stochastic switching of single molecules, are not convenient to be used for thick samples, as brain cortex, zebra fish larvae, etc. The thick samples can be visualized efficiently by both lightsheet and multiphoton microscopes. However, due to the refractive environment of biological samples, light sheet imaging requires a transparent sample, while multiphoton imaging enables use of live animals (e.g., mouse) as non-transparent samples. Our studies reveal the differences between, sample preparation, imaging strategy for different types of advanced systems besides advantages and limitations of each system.

Invited Speaker

TGFB-INDUCED CELL CYCLE ARREST IN PROSTATE EPITHELIAL CELLS: A NOVEL ROLE OF JUND

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Paracrine signaling between epithelial and mesenchymal cells, involving TGFβ family members and androgen receptor (AR), instructs prostate morphogenesis during the prostate development process. Adults have a prostate epithelial component that is comprised of a bilayer of two major cell types, luminal and basal cells. Luminal cells exhibit columnar morphology and represent the exocrine component of the epithelium, express AR, and secrete prostate-specific antigen (PSA). Once terminally differentiated, luminal epithelial cells do not undergo cell proliferation. TGFβ, secreted by stromal cells, plays a significant role in the inhibition of cell proliferation and maintenance of differentiated function and morphology in luminal epithelial cells. The inhibitory effects of TGFβ on cell proliferation are exerted via Smad signaling leading to the up-regulation of cyclin-dependent kinase inhibitors and down-regulation of Id1 and cMYC in target cells. The loss of TGFβ effects on the inhibition of cell proliferation results in increased cell division and de-differentiation of columnar epithelial cells resulting in several lesions including carcinogenesis. Our recent studies have identified an additional novel mechanism through which TGFβ inhibits cell proliferation in human prostate epithelial cells. This novel mechanism depends on the TGFβ-induced down-regulation of JunD, a member of the AP-1 family of transcription factors. We have shown that JunD is essential for proliferation in human normal prostate epithelial and prostate cancer cell lines and plays a significant role in the inhibitory effects of TGFβ on cell proliferation. TGFβ treatment leads to the specific proteasomal degradation of JunD in prostate epithelial cells which is required for its inhibitory effects on cell proliferation. Down-regulation of JunD in prostate epithelial cells leads to the reduced expression of several genes, including Id1 and cMYC which are required for cell proliferation. Our studies also identified constitutive photomorphogenic 1 protein (COP1) as a candidate E3-ligase which is required for TGFβ-dependent proteasomal degradation of JunD in prostate epithelial cells. The absence or down-regulation of COP-1 leads to the lack of TGFβ-induced degradation of JunD and a resistance to its effects on cell proliferation. We conclude that the inhibitory effects of TGFβ in prostate epithelial cells are, in part, dependent on the proteasomal degradation of JunD which plays an essential role in cell proliferation. We also suggest that COP1 activity is required for degradation of the JunD protein in response to TGFβ. The absence of this mechanism will lead to uncontrolled cell proliferation and carcinogenesis in prostate epithelial cells.

Invited Speaker

OVARIAN CRYOPRESERVATION AND RE-TRANSPLANTATION: UPDATES AND NATIONAL OUTCOMES

Dr. Sinan OZKAVUKCU

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Fertility preservation (FP) has become an indispensable and special field for assisted reproduction technologies (ART) with increasing demand. This demand fuels the creation of novel medical indications, in line with the recent technological developments. It is inevitable that FP needs the effective utilization of cryopreservation, a routine technique in ART in terms of vitrification applications. In addition to vitrification, slow freezing still seems to be on the agenda for the cryopreservation of gonads, when gamete freezing is not possible in numerous clinical scenarios. Especially in female patients, where controlled ovarian hyperstimulation is contraindicated, ovarian freezing is an indispensable gold standard method. It was considered an experimental procedure until 2019, but due to many reports indicating live births and the potential of superiority with respect to oocyte freezing, it is no longer considered experimental. Indications for ovarian tissue cryopreservation, standard operational procedures, and necessary training should be set by each ART center willing to adapt and offer this technique to their patients. As a result of basic/clinical research, standardization efforts, and growing experience within the last two decades, we are proud to be able to publish the first live birth case of Turkey after the re-transplantation of frozen-thawed ovarian tissues at Ankara University ART center. This case has many unique novelties as it is the second case in the world reporting a successful outcome from a leukemia survivor and displaying histologic evidence of functionality and safety of the transplanted tissue strips. Despite many controversial opinions, ovarian cryopreservation may still be a unique option in patients with blood-born malignities, and with a strict follow-up, and crucial planning, the risk of reintroducing the malignity during re-transplantation can be minimalized with vital decision-making strategies undertaken by an experienced team. This presentation will also be the first announcement of the second live birth after ovarian thawing and re-transplantation in Turkey. It should be kept in mind that maintenance of standardization and quality control must be sustained for many years for these outcomes to emerge. Patients who apply for fertility preservation may need a long period of time, sometimes 15-20 years, for the request to use their tissues. For this reason, it should be considered that the storage time of the samples after cryopreservation may take decades, and it is of great importance that the centers make long-time infrastructure planning. An updated literature review in this field will also be discussed in the lecture.

Invited Speaker

3D BIOPRINTING OF CANCER MODELS FOR IMMUNOTHERAPHY

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We bioprinted a novel dynamic-flow-based vascularized breast tumor model, consisting of heterotypic breast tumor spheroids and a perfusable vasculature. The tumor spheroids demonstrated different tumor angiogenesis and cancer invasion behaviors depending on the distance from the vasculature. The model has been treated with a chemotherapy drug, doxorubicin, and exhibited a dose-dependent drug response. CAR T cells perfused through the vasculature resulted in extensive CAR T cell recruitment and activation and were effective in killing tumor spheroids and primary tumor organoids. The presented physiologically relevant 3D tumor model has shown the potential for future translation of anti-cancer therapies to personalized medicine.

Invited Speaker

STEM CELL AND REGENERATIVE MEDICINE

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Because of diseases people can loss their tissues and organs. Humans have less regenerative capacity.Therefore, tissue or organ transplantaion takes central role in the treatments. Because of the difficulty of financial situation or finding sufficient amount and competible between donor and host.Scientist have to find innovative and effective treatment solutions. Regenerative medicine is the field that can give the changes to combine cellular therapy and engineering principles.So,reliable, non-allergic, high efficiency treatment options can be created. 3D printing, extracellular vesicles,hydrogels,nanoparticles, MSCs these innovative technologies have role. Stem cells are unspecified cells.They are exist in embryonic, fetal or adult stages of life.Due to the potency, these cells can differentiate different type of cells and create tissues and organs. In the adult stages stem cells are more specific to tissue. Therefore mainly their aim is repair injured tissue.Yet,few properties are common for stem cells, 1- self-renewal,2-clonality,3-differentiate different type of tissues. Basicly, stem cells can be classify into as totipotent, pluripotent, multipotent and unipotent. Totipotent stem cells that present in blastocyst stages so these cells have the highest differentiation and renawal capacity.Pluripotent stem cells presents in the early stages of embryonic stem cells differentiage into 3 germ layer which are mesoderm, endoderm and exoderm. When the cells matured,stem cells gained multipotent differentiate capacity. MSCs are undifferentiated multipotent cells that have self-renewal and have ability to differentiate into diverse cell types such as adipose, chondrocyte and osteocyte cells. MSCs can be isolated from several sources like adipose tissue, placental tissue, umbilical cord, Wharton's jelly and dental tissues.According to the Interntational Society of Cellular Therapy (ISCT) to characterize the MSC, positive and negative expressed surface markes for instance as positive markes CD73, CD90 and CD105; as negative markers CD14, CD11b, CD19, HLA-DR must be shown.Also,by inducing proper agent differentiation into the adipocyte, osteocyte, chondrocyte must be shown. MSCs can be used in regenerative medicine as a therapeutic agent in various disease because of its easy isolation, differentiation, regenerate healthy tissues,immunomodulatory properties.Such as inflammatory diseases, Schizophrenia, Atopic Dermatitis, Asthma, Crohn,Fibrosis, Sepsis etc.Besides, MSCs are anti-antinflammatory because of absence of HLA-DR expression and CD80, CD86,CD40 as HLA-DR's co-stimulatory molecules.

MSCs give opportunity to use allogenic and xenogenic in regenerative medicine.When MSC and regenerative medicine used together efficiency and reliability of the treatment might be increased.

Invited Speaker

PURE COLLAGEN BIOTEXTILE SLING FOR TREATMENT OF STRESS URINARY INCONTINENCE

Prof. Dr. Ozan AKKUS

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Stress urinary incontinence (SUI) is an involuntary loss of urine on effort, physical exertion, sneezing or coughing (SUI) and impacts ~1/3 of women over 50 years of age. SUI occurs predominantly due to parity related pelvic trauma to the connective tissue. For surgical management, synthetic midurethral slings (MUS) are implanted in ~400,000 patients/year in the United States alone. Failure rates for MUS were reported to be 16-19% within two years with no strong evidence for which intervention is best to utilize after failure. PP slings are relatively contraindicated in patients undergoing revision of failed slings and during concurrent urethral surgery. As a result, the search for alternative biomaterials for sling repair is ongoing. Our study aimed to evaluate in vivo response to collagen sling implanted in an ovine model. Collagen threads were fabricated by electrocompaction, threads were filament wound as a sling, and crosslinked in genipin. Collagen slings were implanted suburethrally mimicking the transvaginal tape technique. Main study groups were: Collagen sling (n = 3, 6 months) and PP sling (n = 3, 6 months). Collagen sling was also tested at 3-weeks (n = 1) to observe early-stage tissue response and one-year (n = 2) to assess biomaterial longevity on a preliminary capacity. Collagen slings healed to a fibrous ligament texture as of six months and maintained such texture at one year time point. Histological scoring indicated a biocompatible response to genipin crosslinked electrocompacted collagen sling with no adverse events. All study groups exhibited complete tissue ingrowth along with interstitial *de novo* collagen deposition at all time points. Collagen threads induced orderly *de novo* collagen deposition that was aligned along long axes of threads. Tissue infiltrated collagen slings that were explanted at 6 and 12 months presented similar structural strength with native tissues such as vagina and the fascia, and PP (Lynx) slings ($p<0.05$). With the limitation of low number of animals per time point in hindsight, this preliminary study justifies evaluation of collagen slings in a larger sample size of animals, particularly to assess persistence of ligamentous tissue response over longer durations than one- year. Biocompatibility, structural robustness, and physical conformation to the complex, dynamic suburethral environment in a human-sized animal model highlights collagen slings as a potentially useful biomaterial for pelvic floor and reconstructive surgeries.

Invited Speaker

IQGAP-RELATED PROTEIN IQGC FINELY TUNES CELLULAR FEEDING

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Cellular feeding describes different ways of nutrient uptake. Metazoan cells surrounded by extracellular fluids internalize nutrients via membrane transporters or by receptor-mediated endocytosis. Mammalian cells are also capable of a bulk, nonspecific uptake of extracellular fluid, named macropinocytosis, but to what extent is this process used for feeding is still not clear. On the other hand, macropinocytosis and phagocytosis (particle uptake), collectively termed large-scale endocytosis (LSE), is the only way of nutrient uptake for a unicellular, soil-living amoeba that predominantly feeds on bacteria. LSE requires extensive remodelling of the actin cytoskeleton to produce macropinocytic or phagocytic cups. GTPases of the Ras, Rho and Rab families regulate the organization of these protrusions. Ras proteins promote macropinocytosis in both mammalian cells and amoeba *Dictyostelium*. Moreover, tumour cells with overly activated Ras use this ancient process for feeding on proteins in the microenvironment scarce in amino acids. This finding underlines the importance of both timely activation and inactivation of Ras GTPases for normal cell functioning. Inactivation of Ras GTPases is catalysed by RasGAPs (*GTPase activating proteins*) that promote intrinsic GTP-hydrolytic activity of a GTPase. We investigated the biological function of an IQGAP-related protein IqgC in *Dictyostelium discoideum*. Unlike other IQGAPs that have lost GAP activity, IqgC turned out to be a genuine RasGAP that exerts GAP activity toward human H-Ras and its endogenous partner, RasG. Using confocal microscopy and functional assays with both IqgC *knock-out* and overexpressing cells, we demonstrated that IqgC localizes to macropinocytic and phagocytic cups, and negatively regulates both types of LSE. More specifically, we showed that IqgC restricts the size of macropinocytic cups. Further, IqgC colocalizes with the active Ras on macropinosome, and using *rasG* null cells or mutated IqgC, we proved RasG indispensable for IqgC recruitment to the forming macropinosome. However, IqgC stayed on the internalized macropinosome even after Ras has dissociated from the vesicle. This suggested novel, RasG-independent functions of IqgC, probably in early endosome maturation. Indeed, using GST-Rab5A-pull-down assay with purified IqgC, we found an early endosome marker, Rab5A, to be a direct interactor of IqgC. Moreover, IqgC and Rab5A colocalize on the primary macropinocytic vesicle. RasG is essential for IqgC loading to macropinocytic cups where IqgC suppresses RasG activity to finely tune nutrient uptake. However, after Ras has dissociated, IqgC interacts with another small GTPase, Rab5A, but the functional consequences of this interaction are currently unknown.

Invited Speaker

PREİMPLANTASYON GENETİK TANI; BİYOLOJİ, TEKNOLOJİ VE KLİNİK SONUÇLAR

Berrin AVCI

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Doğal fertilizasyon veya üremeye yardımcı tedavi uygulamalarıyla (ART) gelişen erken insan embriyolarında, embriyoların canlılığını ve gelişimsel potansiyelini tehlikeye atan yüksek oranda kromozom anomalisi vardır. Bu sitogenetik kusurlar implantasyon başarısızlıklarına, gebelik kayıplarına neden olur ve doğuştan gelen bozuklukların büyük bir kısmından sorumludur. Bu nedenle, infertilite tedavisi gören bir hastaların embriyolarının intrauterin transferden önce kromozomal anormallikler açısından değerlendirerek transferi klinik sonuçları iyileştirmek açısından önemlidir. Anöploid embriyoların tanımlanması için yapılan preimplantasyon genetik testi (PGT-A), in vitro fertilizasyon (IVF) sırasında normal kromozom yapısına (euploid) sahip olan preimplantasyon embriyoları tanımlamayı amaçlar. Çağdaş yöntemler, tüm kromozom anormallikleri (örneğin, monozomi/trizomi) saptamaktadır. Teknik iyileştirmeler ve artan çözünürlük ve hassasiyet ile kromozomal mozaikliğin tanımlanması ve ayrıca segmental delesyonlar ve duplikasyonlar gibi alt kromozomal anormalliklerin saptanması mümkündür. PGT-A'nın önceki sürümleri, embriyo biyopsi zamanına bağlı olarak travma, biyopsi sonrası embriyo gelişimsek potansiyelinde azalma, ayrıca teknolojinin tüm kromozomların analizini mümkün kılmaması nedeniyle etkin olarak kabul görülmemiştir. Yakın zamanlarda, tüm kromozomların analiz edilmesini sağlayan teknolojilerin ortaya çıkması ve daha az travmatik blastosist evresi trofoektoderm biyopsisine geçiş, PGT-A'nın etkinliğini arttırmıştır. Bununla birlikte in vitro embriyo kültür süresinin uzaması ve buna bağlı embriyo kaybı, mozaik embriyoların transfer kararının hala tartışmalı konular arasında yer alması, embriyo biyopsisi ve kriyoprezervasyon protokollerinin optimum şartlarda gerçekleştirilmesi gerekliliği nedeniyle PGT-A uygulamalarında birçok faktörün dikkate alınmasını gerektirir. Embriyoya zarar vermeden, non-invaziv yaklaşımlarla embriyonun genetik yapısını tanımlamaya yönelik uygulamalar güncelliğini korumaktadır. Ayrıca bu PGT-A uygulamasının hasta yaşı, infertilite etyolojisi, prognozu, siklus maliyeti açısından etkinliğine yönelik tartışmalar devam etmektedir.

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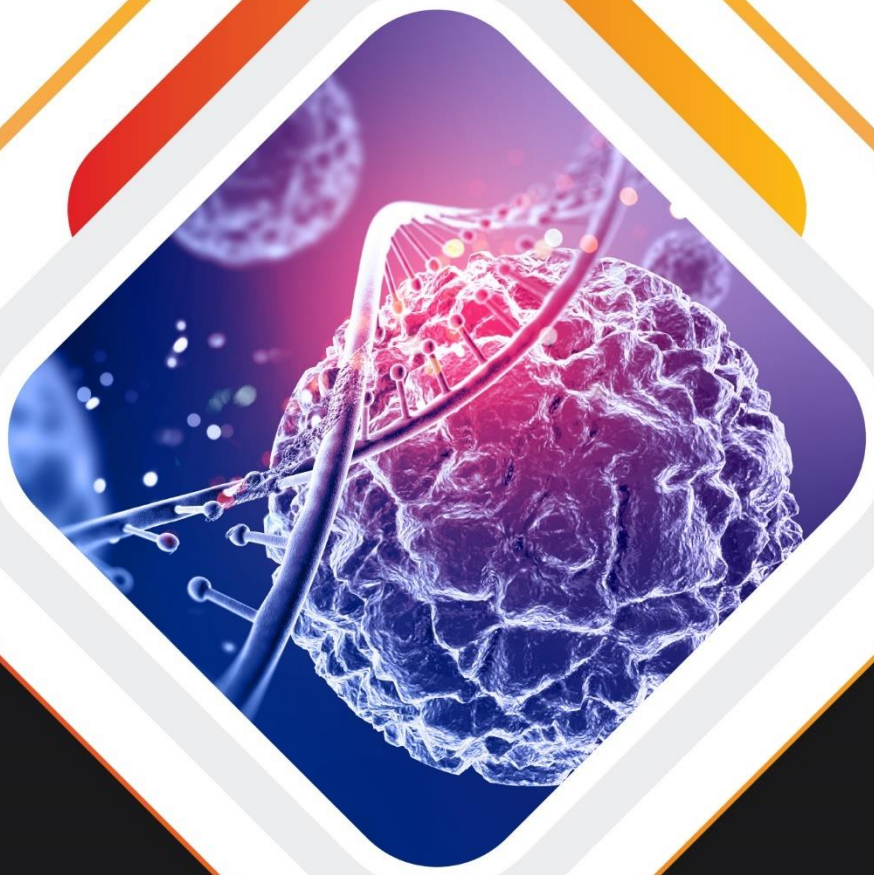

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2022

15. ULUSAL – 1. ULUSLARARASI HİSTOLOJİ VE EMBRİYOLOJİ KONGRESİ
26-28 MAYIS 2022 TÜRKİYE

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NICHE 2022'YE HOŞGELDİNİZ

Türk Histoloji ve Embriyoloji Ailesinin Değerli Üyeleri,

15. Ulusal – 1. Uluslararası Histoloji ve Embriyoloji Kongresi (NICHE2022), Türk Histoloji ve Embriyoloji Derneği tarafından 26 – 28 Mayıs 2022 tarihleri arasında çevrimiçi olarak düzenlenecektir. Sizleri kongremize davet etmekten büyük mutluluk ve gurur duymaktayız.

Türk Histoloji ve Embriyoloji Derneği 16. Dönem Yönetim Kurulu olarak bu kongrenin yüz yüze yapılabilmesi için yoğun çabalarımıza rağmen, içinde bulunduğumuz pandemi koşullarının artan bir ivmeyle seyretmesi ve yine ülkemizdeki ekonomik hareketlilik nedeniyle bu yıl 15. 'sini düzenleyeceğimiz kongremiz, çevrimiçi ve Uluslararası niteliğe sahip olarak gerçekleştirilecektir.

Alanlarında söz sahibi değerli araştırmacıların konferanslarının yanı sıra, güncel araştırmaların sonuçlarının paylaşılacağı oturumlara yer verileceği ve yurt dışından konularında uzman bilim insanlarının da davet edileceği kongremiz çok değerli katılımlarınız ile gerçekleşecektir. Bu anlamda kongremizde sizlerle ekran başında bile olsa buluşacak olmanın sevinç ve heyecanını yaşamaktayız.

15. Ulusal – 1. Uluslararası Histoloji ve Embriyoloji Kongresinde gelişim biyolojisi, moleküler biyoloji ve hücre biyolojisi, kök hücre, hücresel tedaviler, rejeneratif ve rekonstrüktif tıp, yardımlı üreme teknikleri, tümör biyolojisi, biyomedikal mühendisliği, biyoteknoloji, biyoinformatik ve sinirbilim gibi bizleri çok yakından ilgilendiren güncel konularda multidisipliner katkılar ile bilimsel bir ortam oluşturulması amaçlanmaktadır.

Kongremiz uluslararası kongre şartlarına uygun olarak düzenlenecek, özet ve tam metin bildirilerin kongre kitapçığında yer alması ve yayınlanmış tam metin olarak sunulması şartı yerine getirilecektir.

Katılımlarınız ve paylaşacağınız bilgi ve deneyimlerinizle bilimsel anlamda zenginleşeceğinden emin olduğumuz 15. Ulusal – 1. Uluslararası Histoloji ve Embriyoloji Kongresine sizleri davet eder, en derin sevgi ve saygılarımı sunarım.

Prof. Dr. Gamze TANRIÖVER

Kongre Başkanı

Sözel Bildiri

FERTİL VE İNFERTİL ERKEKLERDE SPERM KASPAZ-3 AKTİVİTESİNİN DEĞERLENDİRİLMESİ

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Amaç: Konsantrasyon, motilite ve morfoloji gibi semen parametreleri erkeklerin fertilite potansiyelinin değerlendirilmesine yönelik ilk ve ana adımdır. Ancak sperm parametre anormalliklerinden hangisinin sperm DNA hasarı ile en çok ilişkili olduğu hala belirsizdir. Amacımız Normozoospermili, Astenozoospermili ve Teratozoospermili erkek bireylerde Kaspaz-3 aktivitesinin değerlendirilmesidir

Materyal-Metod: Rutin semen analizi yapılan normozoospermili (n=15), astenozoospermili (n=15) ve teratozoospermili (n=15) bireylerden alınan semen örnekleri 1500 rpm'de 10 dk santrifüj edildikten sonra süpernatant hızlıca uzaklaştırılıp, pellet elde edilmiştir. Pellete; 1/1 oranında kültür medyumu ilave edilip homojenize edilmiştir. Normozoospermili, astenozoospermili ve teratozoospermili semen örneklerinde immünohistokimyasal boyama ile Kaspaz-3 aktivitesi değerlendirilmiştir.

Bulgular: Apoptotik sperm sayısı; teratozoospermili ve astenozoospermili erkeklerde normozoospermili erkeklere göre anlamlı derecede yüksek çıkmıştır. Teratozoospermili ve astenozoospermili bireyler kendi aralarında karşılaştırıldığında ise Teratozoospermili erkeklerde Kaspaz-3 pozitif sperm sayısı Astenozoospermili erkeklere göre yüksek çıkmasına rağmen anlamlılık gözlenmemiştir.

Sonuç: Sperm anormalliklerine sahip erkeklerle olan bu çalışmada, sperm Kaspaz-3 aktivitesi; sperm motilitesi ve normal sperm morfolojisi ile negatif korelasyon göstermiştir. En yüksek Kaspaz-3 aktivitesi teratozoospermili erkeklerde gözlenmiştir. Astenozoospermili erkeklerde gözlenen yüksek Kaspaz-3 seviyesi ise apoptotik hasarın spermin mitokondrial DNA'sı ile ilgili olduğunu düşündürmektedir.

Anahtar Kelimeler: Astenozoospermi, Kaspaz-3, Erkek İnfertilitesi, Teratozoospermi

Sözel Bildiri

SIÇANLARDA FLUOKSETİN HİDROKLORÜRÜN NEDEN OLDUĞU TESTİS HASARINDA KAFEİK ASİT FENETİL ESTERİN DEĞERLENDİRİLMESİ

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Giriş: Günümüzde artan psikolojik bozukluklara bağlı olarak antidepresanların kullanımları giderek artmaktadır. Serotonin geri alım inhibitörleri sınıfından bir antidepresan olan fluoksetin hidroklorürürün (FLX) oksidatif stresi arttırdığı ve erkek genital sisteminde hasara neden olduğu raporlanmıştır. Kafeik asit fenetil ester (CAPE) flavonoid grup bileşiğidir ve antioksidatif, antimikrobiyal, antienflamatuvar etkileri yapılan çalışmalarda gösterilmiştir. Literatürde FLX'in testis dokusunda hasar oluşturduğunu gösteren çalışmalar olmasına rağmen, bu hasar üzerine CAPE'nin etkisini gösteren bir çalışma bulunmamaktadır. Yapılan bu çalışmada, FLX uygulanan sıçanların testis dokusunda oluşan değişiklikler üzerine CAPE'nin olası koruyucu etkilerinin histolojik olarak gösterilmesi hedeflenmiştir.

Amaç: FLX kullanımının testis dokusu dahil birçok dokuda hasar oluşturduğu biliniyor olsa da, bu hasarın nasıl önlenebileceği konusunda yeterli çalışma bulunmamaktadır. Çalışmamızda FLX kaynaklı testis hasarına karşı CAPE'nin olası koruyucu etkilerini histolojik olarak göstermek ve literatüre yeni bir bakış açısı kazandırmayı amaçladık.

Materyal – Metot: Çalışmada 25 adet erkek Wistar Albino sıçan kullanıldı. Denekler kontrol 1, kontrol 2, FLX, FLX+CAPE ve CAPE olmak üzere 5 gruba ayrıldı. Kontrol 1 grubuna (n=5) %0.9'luk serum fizyolojik, Kontrol 2 grubuna (n=5) dimetil sülfoksit (DMSO), FLX grubuna (n=5) 10 mg/kg/gün FLX, CAPE grubuna (n=5) 10 µmol/kg/gün CAPE ve CAPE+FLX grubuna (n=5) ise 10 mg/kg/gün FLX ve beraberinde 10 µmol/kg/gün CAPE 4 hafta boyunca intraperitoneal olarak enjekte edildi. 4 hafta sonunda deneklerin vücut ağırlıkları ölçülüp sakrifiye edildi ve çıkarılan sağ testis dokularının ağırlığı ölçülüp fiksatife alındı. Biyokimyasal analizler için kan örnekleri alındı. Genel morfolojik yapıyı değerlendirmek için histokimyasal boyamalar yapıldı. İmmunohistokimyasal incelemeler için Kaspaz-3, Bcl-2 ve PCNA işaretlemeleri yapıldı.

Bulgular: Yapılan ölçümler ve histolojik değerlendirmeler sonucunda; vücut ağırlığı, sağ testis ağırlığı, seminifer tübül çapı ve germinal epitel kalınlığının FLX grubunda kontrol gruplarına ve CAPE grubuna kıyasla anlamlı bir şekilde azaldığı belirlenirken, FLX+CAPE grubunda anlamlı bir şekilde arttığı saptandı. Johnsen skorlamasında FLX grubu değerlerinin kontrol gruplarına göre anlamlı bir şekilde azaldığı belirlenirken, FLX+CAPE grubu değerlerinin FLX grubuna göre anlamlı bir şekilde arttığı saptanmıştır. Leydig hücrelerinde kaspaz-3 ekspresyonu ve seminifer tübüllerde Bcl-2 ekspresyonunun FLX grubunda kontrol ve CAPE gruplarına göre anlamlı bir şekilde arttığı, FLX+CAPE grubunda ise azaldığı saptandı. PCNA indeksinin FLX grubunda

kontrol ve CAPE gruplarına göre anlamlı bir şekilde azaldığı, FLX+CAPE grubunda FLX grubuna göre anlamlı şekilde arttığı saptandı. Biyokimyasal analizlerde MDA ve SOD düzeyinin FLX grubunda arttığı, CAT düzeyinde anlamlı bir değişiklik olmadığı gözlendi .

Sonuç: Bulgularımız FLX'in oksidatif stres ile testiste yapısal bozukluklar oluşturduğunu, apoptozu indüklediğini; CAPE'nin antioksidan etkisiyle, oluşan yapısal hasarı, apoptozu azalttığını ve proliferasyon indeksini arttırdığını göstermektedir.

Anahtar Kelimeler: Fluoksetin; CAPE; Testis; İmmunohistokimya; Apoptoz

Sözel Bildiri

İNSAN İMMUNOGLOBULİN A NEFROPATİSİNDE GLOMERÜLER MEZANGİYAL HÜCRELERDEKİ VE PODOSİTLERDEKİ DEĞİŞİKLİKLERİN İMMÜNELEKTRON MİKROSKOPİ YÖNTEMİYLE İNCELENMESİ

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Giriş: İmmunglobulin A nefropatisi (İgAN) tüm dünyada yaygın olarak görülen bir hastalıktır. Böbrek glomerüllerinde bulunan mezangiyal hücrelerin hücreler arası matriksinde (mezangiyum) İgA birikimiyle karakterize olan bu hastalıkta, proteinüri tablosu gelişmektedir. Bunun sonucunda glomerüler filtrasyonda aksaklıklar oluşmaktadır. Glomerüler filtrasyonda görev alan podositlerin ayaksı uzantıları arasında bulunan slit diafram proteinlerinden biri olan nefrinin İgAN'de, immünelektron mikroskobik yöntemle yapılan incelemede azaldığına yönelik az sayıda çalışma bulunmaktadır. Patern tanıma reseptör ailesindeki toll benzeri reseptörlerden biri olan Toll-like reseptör 4 (TLR-4) ve fibroziste görev alan TGF-β1 (transforming growth factor β1) proteinlerinin İgAN patogenezinde önemli roller oynadığı düşünülmüştür. Fakat TLR-4 ve TGF-β1 proteinlerinin podositlerde, glomerüler endotelyal hücrelerde ve mezangiyal hücrelerdeki değişiklikleri immunelektron mikroskobik yöntemle incelenmemiştir.

Amaç: Bu çalışmada nefrinin, TLR-4 ve TGF-β1 proteinlerinin İgAN'de glomerüler hücrelerdeki olası değişikliklerinin incelenmesi amaçlandı.

Materyal – Metot: Çalışmamızda MEÜ Tıp Fakültesi Histoloji ve Embriyoloji AD'daki Elektron Mikroskopi Laboratuvarına gönderilen, patolojik ve klinik olarak İgAN tanısı almış 16 kişi hasta grubu olarak; tubuloinsterstisyel nefrit tanılı ve glomerülleri normal olan 16 kişi de kontrol grubu olarak çalışmaya dahil edildi. Kontrol ve İgAN grubu glomerülleri ultrastrüktürel düzeyde incelenip, anti-nefrin, anti-TLR-4 ve anti-TGF-β1 antikorları ile immünelektron mikroskobik olarak işaretlendi ve altın partikülleri sayıldı. Ayrıca filtrasyon yarıkları sayıldı ve slit diafram yüzdeleri belirlendi.

Bulgular: Yapılan işaretlemelerin incelenmesi ve istatistiksel olarak değerlendirilmesi sonucu podositlerdeki nefrinin, filtrasyon yarıklarının ve slit diafram yüzdesinin İgAN grubunda istatistiksel olarak anlamlı bir şekilde azaldığı bulunurken; TLR-4 ve TGF-β1 proteinlerinin podositlerde, glomerüler endotelyal hücrelerde ve mezangiyal hücrelerde istatistiksel olarak anlamlı bir şekilde arttığı belirlendi.

Sonuç:Çalışmamızda ulaştığımız bulgular ışığında İgAN'de mezangiyal hücre proliferasyonu ve mezangiyal matriks artışı görülmesinin yanı sıra, hücreler arası etkileşim nedeniyle podositlerin ve glomerüler endotelyal hücrelerin de etkilendiği, TLR-4 ekspresyonunun bu hastalıkta artmasıyla çeşitli yolakların aktive olabileceği ve TGF-β1 ekspresyonuna dolaylı olarak etki ederek, İgAN'de görülen fibrozise sebep olabileceği düşünüldü.

Anahtar Kelimeler: TLR-4; TGF-β1; nefrin; İgAN; mezangiyal hücre

Sözel Bildiri

DENTAL PULPA KAYNAKLI KÖK HÜCRELERİN KÖKLÜLÜK VE HÜCRESEL KARAKTERİSTİKLERİ ÜZERİNE DÜŞÜK VE YÜKSEK DOZ İBUPROFENİN ETKİLERİ

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Giriş: Dental pulpa kaynaklı mezenkimal kök hücreler (DPKH'ler), elde edilme kolaylığı, rejeneratif tıp ve doku mühendisliğinde kullanılmaları nedeniyle önemli yere sahiptir. DPKH'lerin biyolojisini anlamak adına gerçekleştirilecek çalışmalar rejeneratif tıp alanında bu kaynağın etkin bir şekilde kullanılmasını sağlayacaktır. Steroid olmayan anti-enflamatuvar ilaçlar (NSAID'ler) erişebilirliği nedeniyle dikkat çekmektedir. Bir NSAID olan ibuprofen ağrı, ateş ve iltihabı tedavi etmek için kullanılır. Ibuprofenin DPKH'ler üzerindeki etkisi henüz araştırılmamıştır.

Amaç: Düşük ve yüksek dozlarda ibuprofenin DPKH'lerde köklülük ve hücresel karakteristikleri nasıl etkilediğinin araştırılmasıdır.

Materyal-Metot: DPKH'ler insan dişlerinden izole edilerek alfa-MEM ve %20 fetal dana serumu (FBS) ile kültürlendi. Kültürlenen DPKH'ler akış sitometri ve farklılaşma testleri ile karakterize edildi. Karakterizasyonu tamamlanan DPKH'lere düşük dozda (0.1 mmol/L) ve yüksek dozda (3 mmol/L) ibuprofen 24, 48 ve 72 saat olmak üzere üç ayrı zaman diliminde uygulandı. İlaç uygulanmayan hücreler kontrol grubu olarak kullanıldı. Gruplar arasında CD44, CD73, CD90 ve CD45 yüzey belirteç ifadeleri immün floresan boyama ile analiz edildi. Biyolojik aktivite test kitleri kullanılarak gruplar arasında mitokondriyal membran depolarizasyonu, H2A.X aktivasyonuna bağlı DNA hasarı, canlılık ve hücre döngüsü analizleri ürün prosedürüne göre Muse Cell Analyzer (Millipore) cihazında gerçekleştirildi. Gruplar arasında hücresel proliferasyon MTT testi ile ölçüldü. İstatistiksel analizler Graphpad Prism yazılımı kullanılarak gerçekleştirildi. $P<0.05$ olan veriler anlamlı kabul edildi.

Bulgular: Yüksek doz ibuprofenin CD44 ve CD73 ifadesini DPKH'lerde anlamlı bir şekilde artırdığı tespit edildi ($p<0.05$). CD90 ifadesinin de düşük doz ibuprofen ile şiddetli bir şekilde arttığı görüldü ($p<0.05$). Yüksek doz ibuprofen DPKH'lerde mitokondri membran depolarizasyonunu kayda değer bir şekilde azalttı ($p<0.05$). Düşük doz ibuprofen uygulaması 24. saatte anlamlı bir şekilde toplam depolarizasyonu azaltsa da ($p=0.009$) 48. saatte şiddetli bir şekilde artırdığı tespit edildi ($p<0.05$). DPKH'lerde DNA hasarının yüksek doz ibuprofen uygulaması ile anlamlı bir şekilde düştüğü tespit edildi ($p<0.001$). Düşük ve yüksek doz ibuprofenin DPKH'lerde DNA hasarını azaltıcı etkisine bağlı olarak hücre canlılık yüzdesi ibuprofen uygulanan gruplarda anlamlı bir şekilde artmıştır ($p<0.05$). Yüksek doz ibuprofenin DPKH'lerde özellikle 24 ve 48. saatlerde mitotik aktiviteyi artırdığı tespit edildi ($p<0.05$). DPKH'lerde proliferasyon, yüksek doz ibuprofen uygulaması sonucu mitoz evresindeki artışa paralel olarak kontrol ve düşük doz ibuprofen gruplarına göre artmıştır ($p<0.05$). Proliferasyon bulgularımızın hücre döngüsü analizlerini desteklediği görülmüştür.

Sonuç: Düşük doz ibuprofen uygulamasına göre yüksek doz ibuprofenin DPKH immünofenotiplerini daha iyi hale getirdiği, mitokodri membran depolarizasyonunu azaltmada daha kritik olduğu, DNA hasarını azaltarak hücre canlılığını artırdığı ve mitotik aktiviteyi yükselterek proliferasyonu indükleyici etki yaptığı bulunmuştur. Rejeneratif tıp ve hücresel tedavilerde DPKH kullanımında ibuprofen kombinasyonu kök hücre tedavisini daha efektif hale getirebilir.

Sözel Bildiri

KUERSETİN VE 5-FLOROURASİL'İN HGS-27 MİDE KANSERİ HÜCRELERİ ÜZERİNE TRPV1 KANALLARI ARACILI ETKİLERİNİN İNCELENMESİ

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Giriş-Amaç: Mide kanseri dünya genelinde görülen kanserlerin %10'nunu oluşturmaktadır ve kötü bir prognoza sahiptir. 5-Florourasil (5-FU) pankreas, yemek borusu, göğüs ve mide kanseri de dahil olmak üzere bir çok kanser türünde kullanılan kemoterapötik bir ilaçtır. Kuersetin ise polifenolik bir bileşiğe sahip olup diyetlerimizde tükettiğimiz besinlerde yer almakta ve kanser hastalıklarının moleküler mekanizmaları üzerine etkileri araştırılmaktadır. Bizde çalışmamızda kuersetinin tek başına ve 5-FU ile birlikte kombinasyonuyla HGS-27 mide kanser hücre hattı üzerine etkilerini TRPV1 kanal aktivasyonu yoluyla araştırmayı planladık.

Materyal-Metod: Hücreler kontrol grubu, 5-FU, Kuersetin ve 5-FU+Kuersetin olmak üzere dört gruba ayrılmıştır. Tüm gruplar, analizden önce veya analiz sırasında bir TRPV1 kanal agonisti olan kapsaisin ile uyarıldı. Reaktif oksijen türleri (ROS), kaspaz-3, kaspaz-9, mitokondriyal depolarizasyon ve apoptoz seviyeleri ölçüldü. Veriler tek yönlü ANOVA testleri kullanılarak değerlendirildi.

Bulgular: ROS üretimi, apoptoz, kaspaz-3, kaspaz-9 ve mitokondriyal depolarizasyon değerleri 5-FU verilen grubun kontrol grubuna göre anlamlı derecede yüksek çıktı ($p<0.001$). Kuersetin verilen grubunda kontrol grubuna göre değerleri anlamlı derecede yüksek bulundu ($p<0.001$). 5-FU+Kuersetin verilen grupta ise kontrol, 5-FU ve quercetin verilen gruplara göre değerlerin daha yüksek olduğu saptandı ($p<0.001$).

Sonuç: Bu sonuçlar doğrultusunda 5-FU ve kuersetinin birlikte verildiği kombinasyonun TRPV1 kanal aktivasyonunun aracılık ettiği apoptotik etki sebebiyle mide kanserlerine karşı güçlü bir ilaç olarak kullanılabileceği sonucu elde edilmiştir.

Anahtar Kelimeler: Kuersetin, HGS-27, 5-Florourasil, TRPV1, Apoptoz.

Sözel Bildiri

DMU-212 PROSTAT KANSERİ HÜCRELERİNDE LNCRNA EKSPRESYON PROFİLLERİNİ DÜZENLER

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Giriş: Prostat kanseri (PCa), erkeklerde görülen en sık ikinci kanser türüdür. PCa klinik olarak, yavaş başlayarak hızla ilerleyebilen ve ölümcül olabilen heterojen bir seyir izlemektedir. Resveratrol, kırmızı üzümde bulunan, potansiyel bir kemopreventif ve kemoterapötik ajandır. Resveratrol çeşitli kanserlerde anti-tümörijenik etkiye sahiptir. Sentezlenen resveratrol analogları arasında yer alan DMU-212, çeşitli kanser türlerine karşı yüksek seçicilik ve aktivite göstermektedir ve resveratrole göre daha güçlü bir antitümör aktiviteye sahiptir. Proteine kodlanmayan 200 nükleotidden uzun RNA molekülleri olan lncRNA'lar onkogenik ve tümör supresör özelliklere sahiptirler.

Amaç: Önceki çalışmalarımız ile DMU-212'nin prostat kanseri hücrelerindeki üzerinde anti-kanser etkisi olduğu belirlenmiştir. Çalışmamızda DMU-212'nin PCa hücreleri üzerindeki anti-kanser etkinliğinde rol oynayan lncRNA'ların tanımlanması amaçlanmıştır.

Materyal-Metod: Çalışmada PCa hücre modeli olarak PC-3 (androjen bağımsız) ve LNCaP (androjen bağımlı) kullanıldı. DMU-212'nin PCa hücreleri üzerinde belirlenen sitotoksik dozu (PC-3 için IC_{50}: 1,83 µM, LNCaP için IC_{50}: 1,02 µM) hücrelerinde uygulandı ve tRNA izolasyonu gerçekleştirildi. Doksan lncRNA'nın ekspresyon seviyeleri qRT-PCR ile kantite edildi. LncRNA'ların rölatif ekspresyon seviyeleri $2^{-\Delta\Delta CT}$ metodu ile hesaplandı. LncRNA ekspresyonundaki ±2 katlık değişiklikler ve p değeri <0.05 anlamlı kabul edildi.

Bulgular: Çalışmada PC-3 hücrelerinde DMU-212 uygulaması sonrasında özellikle prostat kanserinde tümör büyümesini inhibe eden GAS5 ifadesinde 12,76 kat artış ve prostat kanseri hücrelerinin proliferasyonunu ve metastazını arttıran KCNQ1OT1 ifadesinde 2 kat azalma gözlenmiştir. LNCaP hücrelerinde ise DMU-212 uygulaması anti-NOS2A ifadesini 6,44 kat arttırırken, KRAS onkogenini aktive eden KRASP ifadesini 5,06 kat azaltmıştır. DMU-212'nin PCa hücreleri üzerindeki anti-kanser etkiliğine çok lncRNA'nın dahil olduğu belirlenmiştir.

Sonuç: Kanserlerde önemli görevleri olduğu bilinen lncRNA'ların düzenlenmesi, PCa hücrelerinin hedeflenmesinde DMU-212'nin umut verici bir tedavi stratejisi olabileceğini düşündürmektedir.

Sözel Bildiri

TRİPLE NEGATİF MEME KANSERİNDE ATR İNHİBİTÖRÜNÜN ANTİ-KANSER ETKİSİNİN BELİRLENMESİ

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Giriş: Triple negative meme kanseri (TNMK) östrojen reseptörü, progesteron reseptörü ve insan epidermal büyüme faktörü reseptörü 2 genlerinin eksprese olmaması ile karakterize, yüksek metastaz ve rekürrens potansiyeline sahip meme kanseri alt tipidir. Heterojen yapısından dolayı mevcut tedavi yöntemlerine dirençli olan TNMK hastalarına yenilikçi tedavi yaklaşımların geliştirilmesi gerekmektedir. Ataksi telenjiektazi ve Rad3 ile ilişkili (ATR), DNA'da meydana gelen hasar yanıtında ve replikasyon çatalının stabilitesinde önemli role sahip bir protein kinazdır. TNMK hücreleri genellikle *ATM* veya *TP53* mutasyonlarından dolayı hayatta kalabilmek için spesifik olarak ATR-Chk1'e bağlı DNA hasar yanıtına ihtiyaç duymaktadır. Bu kapsamda, ATR inhibitörleri DNA onarımının doğru gerçekleşmediği kanser tiplerinde yeni terapötik bir tedavi seçeneği olarak dikkat çekmektedir. Ancak, TNMK etkisine dair henüz literatürde sınırlı sayıda çalışma bulunmaktadır.

Amaç: Mevcut çalışmada ilk kez MDA-MB-231 TNMK hücre hattında bir ATR inhibitörü olarak Elimusertib'in anti-kanser etkisinin belirlenmesi amaçlanmaktadır.

Materyal-Metot: Elimusertib'in MDA-MB-231 hücrelerindeki etkin doz ve saati WST-1 analizi ile belirlendikten sonra, Annexin V, hücre siklusu ve mitokondri boyaması ile apoptotik etkisi belirlenmiştir.

Bulgular: WST-1 sonçlarına göre, 24, 48, 72 ve 96 saat boyunca 1-8 µM Elimusertib uygulanan MDA-MB-231 hücrelerinde zamana ve doza bağlı olarak canlılığın anlamlı bir şekilde azaldığı belirlenmiştir ($p<0.05$). 72 ve 96 saat boyunca 8 µM elimusertib uygulanan hücrelerde canlılık oranı sırasıyla %59.8 ve %46.3 olarak analiz edilmiştir. ($p<0.01$). Ayrıca, Elimusertib'in kontrol grubuna TNMK hücrelerinde sırasıyla %41.5 ve %58.9 toplam apoptotik hücre ölümüne ve G0/G1 fazında artışa neden olduğuda tespit edilmiştir ($p<0.01$). Bunun yanısıra, Elimusertib uygulanan hücrelerde mitokondri membran potansiyelinin bozulduğuda görüntülenmiştir.

Sonuç: Elimusertib'in MDA-MB-231 TNMK hücrelerinde potansiyel anti-kanser etkisi ilk kez ortaya konmuştur. Ancak ATR inhibisyonunun DNA hasar yanıtında moleküler düzeyde etkisinin aydınlatılmasına yönelik ileri çalışmaların yapılması gerekmektedir.

Anahtar Kelimeler: Triple negative meme kanseri, ATR inhibitörü, Elimusertib

"Bu çalışma Sakarya Üniversitesi 2022-7-24-28 numaralı Bilimsel Araştırma Projesi ile desteklenmiştir."

Sözel Bildiri

SIÇAN ENDOMETRİUMUNDA CAS-9, TCF VE LEF-1 İFADELERİ İLE HÜCRE TRANSKRİPSİYONU VE APOPTOZ REAKSİYONUNUN DÜZENLENMESİ

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Giriş: Uterin desidualizasyon, endometrial stromal hücrelerin desidual hücrelere dönüşme süreci olarak ifade edilir. Bu süreç, hücre çoğalması, doku yeniden şekillenmesi, farklılaşması ve hücre transkripsiyonu ile karakterize edilir.

Amaç: Bu çalışmada başarılı devam eden gebelik süreçleri için önemli olan desidualizasyon ve trofoblast farklılaşması ile Cas-9 (Caspase-9), Lef-1 (Lenfoid Enhancer Factor) ve TCF (T-Cell Factor) proteinlerinin lokalizasyonu ve mRNA ekspresyonunun değişip değişmediği araştırılmıştır.

Materyal-Metot: Dişi sıçanlar, östrus siklusu ve erken gebelik günleri olarak 2 gruba ayrıldı. Elde edilen uterin dokularda Cas-9, Lef-1 ve Tcf proteinlerinin aktivitelerinin çeşitli sinyallerle nasıl düzenlendiği immünfloresan mikroskop ve Real-Time PCR yöntemleri kullanılarak belirlendi.

Bulgular: Proöstrus'ta Cas-9, endometrial bazal stroma alanlarında Lef-1 ve Tcf'ye göre en güçlü immünlokalizasyona sahipti. Lef-1 ve Tcf'in lokalizasyonu hemen hemen benzerdi. Östrus evresinde luminal epiteldeki en güçlü immün boyanma sırasıyla Cas-9 ve Lef-1'e aitti. Lef-1 ve Tcf subluminal stromal alanlarda zayıf olarak ifade edildi. Tcf sadece endometrial bazal stromal alanlarda ve miyometrial-perimetrial doku alanlarında güçlüydü. Metaöstrus evresinde Cas-9 luminal epitelde en güçlü lokalizasyonu gösterdi. Tcf ve Lef-1 molekülleri, glandular epitel alanlarında Cas-9'dan daha güçlü immünlokalizasyona sahipti. Gebeliğin 7,5. gününde PDZ (primer desidual zon)'de Tcf ve Cas-9 proteinleri sırasıyla çok güçlü ve güçlü ekspresyon seviyeleri gösterirken, SDZ (sekonder desidual zon)'de daha az güçlü olduğu izlendi. Tcf ve Lef-1 özellikle desidual hücrelerde lokalize olmuştur. Gebeliğin 8,5. gününde Lef-1 immünlokalizasyonu anti-mezometrial ve mezometrial bölgedeki desidual hücre çekirdeklerinde çok güçlüydü. Ayrıca GTC (dev trofoblast hücreleri), trilaminar germ diski hücrelerinin çekirdekleri ve hücre sınırlarında da çok güçlü ifadeye sahipti. Tcf proteini de desidual hücre çekirdeklerinde, PDZ ve SDZ'de çok güçlü immün boyanma özelliği gösterdi. 9,5. gebelik gününde ise Cas-9 lokalizasyonu, EPC (ektoplasental koni hücreleri)'nin çevresinde ve GTC'de güçlü ifade edildiği gözlendi. Lef-1 molekülü, özellikle GTC ve desidual hücre çekirdeklerinde kritik bir lokalizasyona sahipti. Real Time PCR sonuçları her üç genin de ilerleyen gebelik günlerinde farklı gen ifadesine sahip olduğunu gösterdi. Cas-9 ve Tcf geni özellikle gebeliğin 8,5. gününde çok az gen ifadesi gösterirken, 9,5. günde ise gen ifadesi artmıştır. 7,5. günde Lef-1 genine ait ekspresyon görülmezken, 8,5 ve 9,5. günlerde Lef mRNA gen ifadesi de orta seviyede izlenmiştir.

Sonuçlar: Her üç molekülün de implantasyon aşamasında programlı hücre ölümü ve hücre transkripsiyonunun önemli bir aracısı olarak rol oynayabileceği söylenebilir. Tcf ve Lef-1'in olgun desidua da güçlü ve geniş ekspresyonu nedeniyle, desidual reaksiyonun yeni bir belirteci olarak kullanılabileceği önerilebilir. Cas-9 ise siklus ve ilerleyen gebelik günleri için programlı hücre ölümünün kritik düzenleyicisi olabilir. Caspase-9, Tcf ve Lef-1 moleküllerinin ekspresyonunu ve aktivitesini düzenlemede etkili olan sinyal moleküllerini belirlemek için daha fazla çalışmaya ihtiyaç vardır.

Sözel Bildiri

SARS-COV-2 İLE ENFEKTE HASTALARIN SEMEN PARAMETRELERİNİN ANALİZİ

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Giriş: Pandeminin resmi başlangıç tarihi olan Aralık 2019'da Çin'de başlayan koronavirüs hastalığı 2019 (COVİD-19)'un etkeni olan şiddetli akut solunum sendromu koronavirüs 2 (SARS− COV-2), dünya çapında hızla yayıldı. Dünya Sağlık Örgütü (DSÖ), 11 Mart 2020'de salgını küresel bir salgın olarak ilan etti. 25 Mart 2022 itibariyle dünya çapında 476 milyondan fazla doğrulanmış vaka ve yaklaşık 6.1 milyon ölüm bildirilmiştir. Ciddi solunum semptomları ve yüksek ölüm riski olan akut solunum sıkıntısı sendromuna yol açan, öncelikle akciğerleri hedef alan hastalık; kalp, böbrek, karaciğer ve testis gibi diğer organlarda da hasarlar oluşturabilmektedir. Yapılan çalışmalarda erkeklerin kadınlardan daha çok hastalığa yakalandığı ve hastalığa bağlı ölüm oranlarının erkeklerde daha çok olduğunu gösterilmiştir.

Amaç: COVİD-19'un sperm parametreleri üzerine etkilerini göstermeyi amaçladık.

Materyal-Metot: Çalışmamıza 1 Ocak 2021-1 Temmuz 2021 tarihleri arasında Malatya Eğitim ve Araştırma Hastanesi'ne başvuran ve sperm analizi yapılan yaşları 17-68 arasında değişen 510 hasta dahil edildi. Retrospektif olarak gerçekleştirdiğimiz çalışmada, hastane verilerinde PCR ile doğrulanmış COVİD-19 pozitifliği olup olmamasına göre kontrol ve hasta gruplarının sperm analiz sonuçları karşılaştırılmıştır. Kronik hastalığı olanlar ile azospermisi olanlar ve sperm parametreleri cihaz tarafından ölçülemeyen hastalar çalışmaya dahil edilmedi. 54 vakanın SARS-CoV-2 pozitif olduğu PCR ile doğrulanmıştır. İyileşen COVİD-19 hastalarının semen analizi yapılmıştır. COVİD-19 (+) olan hastalar, hastanede yatarak tedavi altına alınmamış, hastalığı hafif geçiren hastalardır. İyileşen COVİD-19 hastalarının PCR ile doğrulanmasının ardından ortalama 157 (24-355) gün geçtikten sonra semen analizleri yapılmıştır. Hastalığın kısa ve uzun vadeli etkilerinin değerlendirilebilmesi açısından hastalar iyileşmeden sonra 90 günden az ve 90 günden fazla geçenler olarak da gruplandırılmıştır.

Bulgular: Çalışmamızda COVİD-19 ile enfekte olan ve olmayan kişilerinin semen analiz sonuçları ile hastalar ve kontrol grubu iyileşme zaman dilimine göre semen sonuçları açısından karşılaştırıldığında gruplar arasında istatistiksel olarak önemli farklılık olmadığı bulunmuştur.

Sonuç: SARS-CoV-2 enfeksiyonunun erkek fertilitesi üzerine etkilerini değerlendirmek için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: COVİD-19, SARS-CoV-2, Erkek Üreme Sistemi, Sperm, Sperm Analizi.

Sözel Bildiri

TESTİSTEKİ SAVAŞIN KAZANANI KİM? SİKLOFOSFAMİD Mİ, PTEROSTİLBEN Mİ?

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Giriş: Siklofosfamid (CP) klinikte kullanımı kaçınılmaz olan bir antineoplastik ajandır. Birçok organda olduğu gibi testis ve epididim dokularında oksidatif stresi indükleyebilir. Ejakulattaki sperm parametrelerini bozabilir ve bu etkinin sonucunda da erkek infertilitesine neden olabilir. Pterostilben (Ptr); üzüm, yaban mersini, kırmızı sandalağacı gibi bitkilerde bulunan antimikrobiyal bir maddedir. Antioksidan ve antiapoptotik etkileri olan Ptr; kardiyovasküler hastalıklar, hiperglisemi, bazı kanser türleri, inflamatuvar hastalıklar gibi oksidatif stresle ilişkili patolojik durumlara karşı tedavi edici etkiye sahiptir.

Amaç: Bu çalışmada oksidatif etkileri olan CP'nin neden olduğu erişkin sıçan testis hasarının engellenmesi ve/veya iyileştirilmesine kuvvetli bir antioksidan olan Ptr'in etkisi araştırıldı.

Materyal-Metot: Araştırmada kullanılmak üzere 21 adet Sprague Dawley ırkı erişkin erkek sıçan rastgele 3 eşit gruba (kontrol (K), CP ve CP+Ptr) ayrıldı. Siklofosfamid grubuna 9. gün tek doz 200 mg/kg ip. CP verildi. CP+Ptr grubuna ise CP 9. gün tek doz 200 mg/kg ip., Ptr ise 14 gün boyunca oral 40 mg/kg dozunda uygulandı. Deneyin 15. günü denekler tartılıp sakrifiye edilerek sol testis ile epididim dokuları çıkarıldı. Kauda epididimden elde edilen sperm örneklerinde sperm sayısı, motilitesi, canlılığı ve morfolojisi analiz edildi. Kaput epididimiste Hematoksilen & eozin (H&E) ve testis dokusunun bir yarısında H&E, Masson Trichrome ve Periyodik Asit Schiff (PAS) boyama yöntemleriyle histopatolojik incelemeler yapıldı. Testis dokusunun diğer yarısında ise biyokimyasal olarak malondialdehit (MDA), süperoksit dismutaz (SOD), katalaz (CAT), total antioksidan durum (TAS), total oksidan durum (TOS) ve oksidatif stres indeksi (OSI), kaspaz-3 ve testis doku testosteron düzeylerine bakıldı.

Bulgular: Vücut ağırlığı ölçümlerinde CP+Ptr grubunda K grubuna göre anlamlı azalma vardı. Histolojik olarak CP grubuna ait testislerde; seminifer tübül atrofisi, bazal membranda düzensizlik ve ayrılma, interstisyumda ödem ve germinal epitelde ayrılma görüldü. Bu bulgular, CP+Ptr grubunda oldukça azalmıştı. Hem CP hem de CP+Ptr grubunda belirgin immatür germinal hücre dökülmesi (GHD) vardı ve Modifiye Johsen Skoru (MJS) kontrole göre düşüktü. Sperm analizinde sperm sayı, motilite, canlılık ve normal morfolojinin CP grubunda

bozulduğu; CP+Ptr grubunda ise morfolojinin düzeldiği görüldü. Biyokimyasal olarak CP grubunda CAT ve doku testosteronunda azalma, TOS'ta artış mevcuttu. CP+Ptr grubunda ise CP'ye göre TOS azalırken; CAT arttı. Grup CP'ye ait epididim dokularında K ve CP+Ptr gruplarından farklı olarak berrak hücrelerde azalma, halo hücrelerinde artış ve bazı hücrelerde sitoplazmik vakuoller izlendi.

Sonuç: Elde edilen bulgulara göre Ptr uygulamasının; CP'nin indüklediği sıçan testis ile epididim hasarlarını ve sperm morfolojisindeki bozukluğu azaltabileceği söylenebilir.

Anahtar Kelimeler: sıçan, testis, sperm analizi, pterostilben, siklofosfamid.

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Sözel Bildiri

DOKSORUBİSİN İLE İNDÜKLENEN SIÇAN OVARYUM HASARINDA *TARAXACUM OFFİCİNALE'NİN* KORUYUCU ETKİSİ

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Giriş: Doksorubisin kanser tedavisinde kullanılan antrasiklin türevi bir antibiyotiktir ve DNA içine interkalasyon yapar. Hematolojik kanserler ve birçok karsinoma tipinde kullanılmaktadır. Doksorubisin, ovaryumda oosit ve folliküller üzerinde toksisiteye neden olmaktadır. Taraxacum officinale (Karahindiba), papatyagiller familyasına ait bir bitkidir ve hücre hasarında koruyucu etkiye sahip antioksidan özellikteki beta-karoteni içerir. Serbest radikaller üzerindeki etkisine ek olarak Doksorubisin ile indüklenmiş ovaryum hasarında vazodilatasyona ve kan basıncında düşmeye neden olur. Böylece hücre içine kalsiyum girişini engelleyerek koruyucu etki gösterir. Bu çalışmada sıçanlarda oluşturulan Doksorubisin'e bağlı ovaryum doku hasarını önlemede Taraxacum officinale'nin tedavi etkinliğinin değerlendirilmesi hedeflendi.

Gereç-Yöntem: Wistar albino cinsi 40 dişi sıçan 4 gruba ayrıldı. Grup I (n=10); kontrol grubu. Herhangi bir ilaç verilmedi. Grup II (n=10) 100 mg/kg Taraxacum officinale gavaj yolu ile 10 gün verildi. Grup III (n=10) 40 mg/kg doksorubisin tek doz intraperitoneal olarak çalışmanın sekizinci günü verildi. Grup IV (n=10) 100 mg/kg Taraxacum officinale gavaj yolu ile 10 gün verildi ardından sekizinci gün tek doz 40 mg/kg doksorubisin i.p. enjeksiyon yapıldı. Onuncu günün sonunda ovaryumlar cerrahi olarak çıkarılarak % 10'luk Formaldehit solüsyonunda fikse edildi ve doku takibi yapılarak parafin bloklara gömüldü. Ovaryum dokularından alınan 5 µm kalınlığındaki kesitler, morfolojik değerlendirme için H&E ve masson trikrom ile boyandı. TUNEL yöntemi ile ovaryan folliküller apoptotik aktivasyonu açısından değerlendirildi. Kan malondialdehit (MDA) seviyeleri ve katalaz (CAT) ve süperoksit dismutaz (SOD) aktiviteleri ölçüldü.

Bulgular: Follikül sayımı yapılan gruplar değerlendirildiğinde primer folliküller düzeyinde istatistiksel anlamlılık görülmezken, doksorubisin grubunda(Grup III) primordiyal follikül sayılarında anlamlı düzeyde azalma belirlendi ($p<0.05$). Doksorubisin grubu diğer gruplarla karşılaştırıldığında kontrol, Taraxacum officinale (Grup II) ve doksorubisin+Taraxacum officinale gruplarına göre sekonder follikül sayılarında azalmalar görülürken, atretik follikül sayılarında ise artış olduğu görüldü ($p<0.05$) .Ovaryan folliküller apoptotik aktivasyonu açısından TUNEL yöntemi ile değerlendirildiğinde ise hematoksilen eosinle yapılan değerlendirmeyle uyumlu olduğu görüldü. Doksorubisin uygulanan gruptaki apoptotik hücre sayısının doksorubisin+ Taraxacum officinale grubuna göre anlamlı düzeyde fazla olduğu görüldü. Doksorubisin+Taraxacum officinale grubunda sekonder ve graaf follikül sayılarının kontrol ve Taraxacum officinale grubu ile benzer olduğu görüldü ve atretik follikül sayılarının ise doksorubisin grubuna göre anlamlı düzeyde azaldığı saptandı ($p<0.05$). Doksorubisin grubunda sekonder ve graaf folliküllerin granüloza hücrelerinde piknotik görünümlü nukleuslar, sekonder folliküllerde daha belirgin olmak üzere, tüm folliküllerde ve bağ dokusu alanlarda dejenerasyon bulguları görüldü.

Sonuç: Doksorubisin uygulanan ratların ovaryumunda korteks damarlarında fibrozis oluştuğu, follikül kaybı olduğu ve ovaryum dokusu skarlaşmasının primordiyal folliküller üzerinde toksisiteye neden olduğu gözlendi. Bu çalışmada Doksorubisin ile indüklenen sıçan ovaryum hasarında Taraxacum officinale'nin koruyucu etkisinin olduğu saptandı.

Anahtar Kelimeler: Doksorubisin, Taraxacum officinale, Apoptoz, Ovaryum hasarı

Sözel Bildiri

OVARYUM YÜZEY EPİTELİNİN İZOLASYONU VE ÇOĞALTILMASI

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Giriş: Ovaryumun yüzeyi tek katlı kübik ve bazı bölgelerde yassı epitel ile döşelidir ve altında bazal membran vardır. Germinal epitel olarak bilinen bu sellüler tabaka mezovaryumu kaplayan mezotelyum ile devamlılık göstermektedir. Yüzey epiteli ile korteksin arasında sıkı bağ dokusu tabakası olan tunika albuginea yer almaktadır. Silya ve apikal mikrovilluslara sahip olan ovaryum yüzey epiteli tip 7,8,18 ve 19 keratinleri içerir. Ovaryum kanserlerinin yaklaşık olarak %85'ne neden olan yüzey epitelinde Oct 4 ve SSEA-4, Sox-2 ve Nanog gibi kök hücre belirteçleri eksprese edilir. Overyan siklusta yüzey epiteli siklik değişikliklere bağlı olarak morfolojik değişikliklere uğramakta ve prolifere olmaktadır. Yüzey epiteli ovulasyonda hasar gören yüzeyi eski haline getirmek için çoğalır ve farklılaşır. Hem epitelyal hem de mezenkimal belirteçleri (vimentin) ifade eden ovaryum yüzey epitelinde FSH, androjen, progesteron, östrojen ve TSH reseptörlerinin bulunduğu gösterilmiştir. Ovaryumun yapısal ve fonksiyonel birimleri olan foliküller tunika albuginea ve korteks tabakasında bulunmaktadır. Ovaryumda en çok bulunan folikül çeşidi primordiyal foliküldür. Puberteden sonra primordiyal foliküller seçilerek büyümeye başlarlar. Oositin çevresindeki folikül hücrelerinin dış yüzeyi bazal lamina ile sınırlandırılmıştır. Daha sonra oluşan primer folikülün granüloza hücreleri prolifere olunca tekal hücreler stromal hücrelerden farklılanırlar. Bu foliküller gelişimlerini sekonder ve graff folikül olarak sürdürürler. Ovulasyon, graaf folikülünden sekonder oositin atılması surecidir. Bir dogma olarak şimdiye kadar yetişkin ovaryumunda yeni foliküllerin ortaya çıkmadığı ve tüm foliküllerin fetal dönemde meydana geldiği görüşü kabul edilmiştir. Bazı araştırmacılar ise postnatal ooogenez (neo-oogenez) veya neofolikülogenez kavramlarını ortaya atmışlar ve yaptıkları çalışmalarla bu hipotezlerini ispat etmeye çalışmışlardır.

Amaç: Bizim bu çalışmadaki amacımız kültür ortamında ovaryum yüzey epitelini izole etmek, çoğaltmak ve özelliklerini incelemektir.

Gereç-Yöntem: İki tane prepubertal dönemdeki (4 haftalık) dişi sıçanların ovaryumları steril şartlarda alındı. İlk önce stereo mikroskop altında çevresindeki adipoz dokusu eksize edildi. Daha sonra tunika albuginea tabakası ve altındaki bölümler uzaklaştırıldı. Kalan materyal iki petri kabında küçük parçalara ayrılarak eksplant hücre kültürü oluşturulurdu.

Bulgular: Hücreler doku parçalarından yaklaşık olarak 3 gün sonra sonra migrasyona uğradı. 7. günde hücrelerin petri kabını doldurduğu ve morfolojik görünümlerinin Arnavut kaldırımı (cobblestone) şeklinde olduğu görüldü. Faz kontrast mikroskobisi altında Thoma lamıyla yapılan sayımda kültür kaplarında sırasıyla 1x 10^6 ve 2x 10^6 hücrenin ürediği gözlemlendi. Bazı alanlarda ise primordiyal folikül benzeri yapıların oluştuğu görüldü.

Sonuç: Ovaryum yüzey epitelinin yanı sıra overyan stromal hücrelerin de izole edilmesi, üretilmesi ve ko-kültürlerin yapılarak birbirleriyle etkileşimlerinin gözlemlenmesi bizlere oositlerin nişleri hakkında değerli bilgiler verebilir.

Anahtar Kelimeler: Ovaryum yüzey epiteli, hücre kültür tekniği, ovaryum, overyan stromal hücre

Sözel Bildiri

ASPALATHUS LİNERAİS (ROOİBOS) EKSTRAKTININ SIÇAN TESTİS DOKUSUNDA AKUT DÖNEMDE MELAMİNİN OLUŞTURDUĞU HASAR ÜZERİNE KORUYUCU ETKİSİNİN İNCELENMESİ

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Amaç: Kanserojen etkili endüstriyel kimyasal olan melaminin testis üzerinde oksidatif stres, apoptozu indükleme, seminifer tubullerde morfolojik değişikliklere neden olduğu bilinmektedir. Bu çalışmada melaminin testis dokusunda yaptığı harabiyeti üzerine rooibos ekstraktının akut dönemdeki iyileştirici etkilerini histopatolojik olarak incelemeyi amaçladık.

Materyal ve Metot: 8 haftalık, 18 adet Wistar-albino sıçanlar kontrol (K) , melamin (M), melamin+ rooibos (M+R) gruplarına ayrıldı. Melamin 3 gün oral gavaj-Rooibos 28 gün IP olarak uygulandı. Ketamin anestezisi altında çıkarılan testis dokusu formaldehit ve bouinde fikse edilerek rutin histolojik doku takip edildi. Kesitler 5µm kalınlığında alınarak H&E, Masson, PAS, IHC, TUNEL boyamalar yapıldı.

Bulgular: Kontrol grubu ile kıyaslandığında melamin grubunda seminifer tübül çapında ve alanında azalma, rooibos grubunda ise artış istatistiksel olarak ($p<0.05$) anlamlı bulundu. Kontrol grubunun H&E boyamasında; seminifer tübüller incelendiğinde spermatogenik seri hücrelerin ve Sertoli hücrelerinin normal görünümde olduğu görüldü. Tübüllerin bazal membranının intakt olduğu, peritubular miyoid hücrelerin, interstisyel bağ doku alanındaki yapıların ve Leydig hücrelerinin normal histolojik görüntüde olduğu gözlendi. Melamin grubunda regresif seminifer tübüllere rastlandı. Seminifer tübüllerin lümeninde spermatogenik hücre döküntüleri görüldü. Spermatogenik seri hücrelerinde yer yer vakuolizasyon ve ayrışmalar görüldü. Rooibos grubundaki testislerin histolojik görüntüleri kontrol grubuna benzerdi. Spermatogenik hücreler normale yakın, lümende spermatozoalarında varlığı görüntülendi. Kontrol grubunun PAS boyalı kesitleri incelendiğinde; spermatogenik seri hücreler içerisinde sperm akrozomlarının ve seminifer tubullerin düzgün, kesintisiz PAS (+) bir bazal membranla çevrelendiği görüldü. Melamin grubunda, bazal membranlarında PAS pozitifliğin zayıf olduğu ve yer yer kesintiye uğradığı görüldü. İnterstisyel alanda Leydig hücrelerinde dejenerasyon ve ödem izlendi. Rooibos grubunda kontrol grubuna benzer şekilde seminifer tubullerin normale yakın morfolojik bulgusu ile vasküler konjesyonda ve bazal membrandaki ayrışmada iyileşme olduğu gözlendi.

Kontrol grubu Masson Trikrom boyalı kesitler incelendiğinde, bağ doku kapsülü olan tunika albuginea ve yoğun dizilimli kollajen lifler gözlendi. Melamin grubunda ise, kapsülde ayrılmalar, dejeneratif ve nekrotik seminifer tubuller izlendi. Rooibos gurubunda, melamin grubuna nazaran intersitisyel doku alanındaki hasar ve ödemde azalma görüldü. Tübüler atrofide azalma olup, kontrol grubuyla benzer özellikler bulundu. Kontrol grubu ve rooibos grubuna ait testis dokularında bulunan seminifer tübüllerdeki hücrelerde çok az sayıda TUNEL pozitif işaretlenmiş hücrelere rastlandı. Melamin grubunda ise seminifer tubullerdeki TUNEL pozitif hücrelerin sayısında artış olduğu gözlendi. Hücreler arası sıkı bağlantı proteinleri olan okludin, klaudinin

immunohistokimyasal incelemelerinde kontrol ve M+R grubunda sertoli hücrelerinde ekspresyonun güçlü olduğu gözlendi, ancak melamin grubunda kan testis bariyerinin bozulmasına bağlı olarak okludin, klaudinin ekspresyonun azaldığı tespit edildi.

Sonuç: Çalışmamızda, Melaminin seminifer tubul bütünlüğünü ve kan testis bariyerini bozduğu, Rooibosun bu dejenerasyonları iyileştirici etki gösterilerek toksisiteye bağlı infertiliteyi iyileştirmede katkı sağladığı düşünüldü.

Sözel Bildiri

EPİDİDİMAL BEYAZ YAĞ DOKUSUNUN *İN VİTRO* SPERMATOGENEZ ÜZERİNE ETKİSİNİN İKİ FARKLI KÜLTÜR PLATFORMUNDA KARŞILAŞTIRILMASI

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Giriş: Çocukluk çağı kanser hastalarında uygulanan tedaviler testis germ hücreleri ve mikroçevresine hasar vererek hastaların %46'sının infertil olmasına neden olmaktadır. Epididimal beyaz yağ dokusunun (EBYD) spermatogenez için gerekli olan faktörleri ürettiği ve uzaklaştırıldığında spermatogenezin inhibe olduğu bilinmektedir[1]. Yenidoğan fare testisleri, üç boyutlu hava sıvı interfaz (HSİ) ve U plak (UP) teknikleri ile kültüre edildiğinde EBYD'nin *in vitro* spermatogenezi destekleyeceği varsayılmıştır.

Amaç: Bu çalışmada EBYD ile desteklenen testislerin *in vitro* koşularda UP ve HSİ kültür teknikleri ile kültürü sonucunda spermatogonyal kök hücre (SKH) havuzunun korunması ve sperm elde edilmesi amaçlanmıştır.

Materyal-Metot: Üç boyutlu HSİ ve UP teknikleri ile sinjeneik C57BL/6 fare yetişkin EBYD uygulanan yenidoğan testis kültür ve kokültürlerinde EBYD'nin, 1, 3, 4 ve 6. haftalarda *in vitro* spermatogeneze etkisi histomorfometrik ve immünohistokimyasal olarak değerlendirilmiştir.

Bulgular: Seminifer tübül epitel alanı, tüm HSİ kültür platformlarında 3. haftaya kadar artmış ($p=0,0001$) sonra sabit olarak gözlenirken, tüm UP kültür platformlarında 1. haftadan 6. haftaya kadar değişiklik gözlenmemiştir. Bir ($p<0,0001$), üç ($p=0,0009$), dört ($p=0,0001$) ve altıncı ($p<0,0001$) haftalarda HSİ kokültür platformunda tübül lümen alanı UP kokültür platformuna göre anlamlı olarak daha yüksek bulunmuştur. HSİ tekniğinin UP tekniğiyle karşılaştırıldığında SKH sayısı üzerinde 3. ve 6. haftalarda arttırıcı etkisi olduğu görülmüştür ($p<0,0001$). Epididimal beyaz yağ dokusunun HSİ kokültür platformunda 3. haftadan sonra SKH havuzunu koruyucu etkisi gözlenmiştir. Farklılaşan spermatogonyumların sayısı HSİ tekniğiyle, UP tekniğine göre 1, 3, 4 ve 6. haftalarda artmış ve EBYD'nin olumlu etkisi gözlenmiştir. Epididimal beyaz yağ dokusu, HSİ kokültür platformlarında 1, 3, 4 ve 6. haftalarda spermatosit sayısını kontrol grubuna göre değiştirmezken, UP kokültür platformlarında 3. haftada spermatosit sayısını kontrol grubuna göre anlamlı olarak azaltmıştır ($p=0,0348$). Epididimal beyaz yağ dokusu, HSİ kokültür platformlarında spermatid sayısını kontrol grubuna göre 3. ($p=0,0006$) ve 4. ($p=0,0203$) haftalarda anlamlı olarak arttırırken UP kokültür platformlarında spermatid sayısını arttıcı etkisi gözlenmemiştir.

Sonuç: Bu çalışma kapsamında tüm kültür platformlarında *in vitro* spermatogenez yuvarlak spermatid aşamasına kadar tamamlanmıştır. Germ hücre farklılaşması ve SKH havuzunun korunması bakımından karşılaştırıldığında EBYD ile desteklenen HSİ tekniğinin UP tekniğine göre daha başarılı olduğu görülmüştür. Epididimal beyaz yağ

dokusunun SKH havuzu ve *in vitro* spermatogenez üzerine HSİ tekniği ile olumlu etkisi ortaya konduğundan, çocukluk çağı kanser hastalarında sperm eldesini sağlayacak üç boyutlu testis organ kültürlerine iyi bir aday olabileceği saptanmıştır.

Anahtar Kelimeler: Erkek İnfertilitesi, Organ Kültürü, *In Vitro* Spermatogenez

Bu çalışma, Hacettepe Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından TSA-2021-18974 numaralı Kapsamlı Araştırma Projesi ile desteklenmiştir.

Sözel Bildiri

KEMİK TÜMÖRLERİNDE KANNABİNOİDLERİN RESEPTÖR ARACILI ANTİPROLİFERATİF VE APOPTOTİK ETKİLERİNİN ARAŞTIRILMASI

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Giriş: Osteosarkom, çocuklarda ve gençlerde yaygın rastlanan, hızlı metastaz yaptığından mortalitesi yüksek kemik tümörüdür. Klinikte osteosarkom tedavisinde uygulanan kemoterapötiklerin sınırlı küratif etkisi ve yüksek yan etki potansiyelleri yeni ilaç adaylarının araştırılmasını önemli kılmaktadır. Sistemik dağılımları ve hücre ölümü ile ilgili düzenleyici etkileri nedeniyle epitelial kanserlerde ilaç adayı olarak araştırılan kannabinoidlerin osteoblast, osteoklast ve osteoprogenitör hücrelerde klasik kannabinoid 1 reseptörü (CB1) ve klasik kannabinoid 2 reseptörleri (CB2) aracılığıyla proliferasyonu düzenleyebildiği bildirilmiştir. Bu çalışmada sentetik spesifik CB2 reseptör agonisti CB65'in Saos-2 ve MG63 osteosarkom hücre hatlarındaki etkili dozu tespit edilebilir, proliferasyonu azaltıcı ve apoptozu indükleyici etkisi ortaya konabilirse, osteosarkom için potansiyel ilaç adayı olabileceği varsayılmıştır.

Amaç: Bu çalışma kapsamında yeni sentetik, spesifik CB2 kannabinoid reseptör agonisti CB65'in *in vitro* koşullarda MG63 ve Saos-2 osteosarkom hücre hatlarında, CB2 reseptörü aracılı doz ve zaman bağımlı apoptotik ve antiproliferatif etkinliğinin değerlendirilmesi amaçlanmıştır.

Materyal-Metot: MG63 ve Saos-2 hücre hatlarındaki CB1/CB2 reseptör profili akım sitometrisi yöntemi ile saptanmış, 10^{-12} – 10^{-6} M doz aralığında CB65'in hücrelerdeki antiproliferatif ve apoptotik etkinliği WST-1, gerçek zamanlı proliferasyon analizi ve akım sitometrisi yöntemleri ile değerlendirilmiştir.

Bulgular: MG63 hücrelerinde hücre içi CB1 ve CB2 reseptör ekspresyon seviyeleri sırasıyla %61.56 ve %27.34 zardaki CB1 ve CB2 reseptör seviyeleri %80.35 ve %79.37 olarak ölçülmüştür. Saos-2 hücrelerinde hücre içi CB1 ve CB2 reseptör ekspresyon seviyeleri %59.44 ve %61.52 saptanmıştır; membranındaki CB1 ve CB2 reseptörlerinde %23.87 ve %31.72 olarak ölçülmüştür. Sentetik spesifik CB2 agonisti CB65 10^{-12} – 10^{-8} M doz aralığında, MG63 ve Saos-2 hücre hatlarında sırasıyla 1. Günde ve 2. günde proliferasyonu azaltıcı etki göstermiştir ($p<0.05$). Gerçek zamanlı proliferasyon analizinde CB65 için ED50 MG63 hücrelerinde $3.88x10^{-10}$ M ve Saos-2 hücrelerinde $4.95x10^{-11}$ M olarak hesaplanmıştır. Antiproliferatif etki CB2 antagonisti AM630 uygulaması ile bloke olmuştur. CB65 gerçek zamanlı terapötik penceresinde uygulandığında Saos-2 hücrelerinde ilk 24 saatte %19.06, MG63 hücrelerinde ilk 48 saatte %7.31 oranında geç apoptozu indüklemiştir (Kontrole göre $p<0.05$).

Sonuç: Bu çalışma ile ilk kez sentetik spesifik CB2 agonisti CB65'in osteosarkom hücre hatlarındaki gerçek zamanlı antiproliferatif ve apoptotik etkisi ortaya konarak *in vitro* koşullardaki terapötik penceresi belirlenmiştir. Yağ yapılı kannabinoid agonisti CB65 etkili dozda uzun süreli salımının sağlanması ve *in vivo* koşullarda validasyonu sonrası kliniğe aktarılabilecek bir tedavi seçeneği olabilir.

Anahtar Kelimeler: osteosarkom, kannabinoid, CB2 reseptörü, CB65, ilaç

Çalışma TYL-2021-19573 numaralı Hacettepe Üniversitesi Bilimsel Araştırma Koordinasyon Birimi kapsamında desteklenmiştir.

Sözel Bildiri

GLİOBLASTOMA MULTİFORME TEDAVİSİNDE UMUT VERİCİ BİR AJAN OLAN ERİANİNİN APOPTOTİK YOLAKLAR ARACILIĞIYLA U373 VE A172 GLİOBLASTOMA HÜCRELERİNİN PROLİFERASYON VE MİGRASYONU ÜZERİNE ETKİLERİNİN ARAŞTIRILMASI

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Giriş: Glioblastoma (GBM), yetişkinlerde en agresif, yaygın ve ölümcül primer intrakraniyal beyin tümörüdür. Dendrobium özündeki yeni bir dibenzil bileşiği olan erianinin anti-tümör aktivitesi, daha önce GBM kanserinde gösterilmemiştir.

Amaç: Bu çalışmada, insan U373 ve A172 GBM kanser hücrelerinde erianinin antikanser aktivitesi ve altında yatan mekanizmaları araştırılmıştır.

Materyal-Metot: Monensinin hücre canlılığı, invazyon ve migrasyon üzerine etkileri sırasıyla, XTT (2,3-bis (2-methoxy-4 nitro-5- sulfophenyl)-2H-tetrazolium-5-carboxanilide), matrijel invazyon ve wound healing, apoptoz üzerine etkileri ise RT-PZR (gerçek-zamanlı polimeraz zincir reaksiyonu) ve Annexin V yöntemleriyle belirlenmiştir.

Bulgular: Erianinin U373 ve A172 hücrelerinde IC50 (yarı en yüksek inhibitör konsantrasyon) değeri 48. saatte sırasıyla, 16 ve 64 mikromolar olarak belirlenmiştir. Erianin ayrıca *Bcl-2* (B-hücreli lenfoma 2), *caspase-8, caspase-9* ve *TRADD* (tümör nekroz faktör reseptörü tip 1-ilişkili ölüm domain proteini) gen ifadelerini azaltarak ve *caspase-3*, *BID* (BH3 etkileşimli domain ölüm agonisti) gen ifadelerini artırarak GBM hücrelerinin apoptozunu tetiklemiştir ($P<0.05$). Ek olarak, erianin, U373 ve A172 hücrelerinde apoptotik hücre sayısını anlamlı olarak arttırmıştır. U373 ve A172 GBM hücrelerinde apoptotik hücre oranı sırasıyla %32 ($P=0.000$) ve %7 ($P=0.01$) iken bu değerler kontrol gruplarında %3 ve %1'dir. A172 Erianin, U373 ve A172 hücrelerinde invazyon ve migrasyonu anlamlı olarak azaltmıştır. U373 hücrelerinde erianin uygulanan grupta invaze olan hücre sayısı ortalama 1478 ($P=0.000$) iken A172 hücrelerinde 329 ($P=0.000$)'dır. Bu değerler U373 ve A172 kontrol gruplarında sırayla 2964.33 ve 835'dır. U373 ve A172 hücrelerinde yara mesafesi kontrol gruplarında sırasıyla 71 ± 4.58 ve 77 ± 9.64 iken erianin uygulanan gruplarda 185 ± 26.57 ($P=0.002$) ve 136 ± 7.54 ($P=0.001$)'dır.

Sonuç: Birlikte ele alındığında, sonuçlarımız erianinin GBM kanserinin tedavisinde güçlü apoptotik etkilere sahip yeni terapötik anti-kanser ilaç bileşeni olabileceğini düşündürmektedir.

Anahtar Kelimeler: Erianin, apoptoz, glioblastoma.

Sözel Bildiri

SOSYAL IZOLASYON VE ÇEVRE ZENGINLEŞTIRMENIN YAVRU SIÇANLARDA HIPOKAMPUSTA NÖRON SAYISI VE KISA SÜRELI BELLEK ÜZERINE ETKILERI

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Giriş: Standart yaşam koşullarında tek birey olarak yaşamayı ifade eden "Sosyal İzolasyon (Sİ)", öğrenme ve bellek gibi hipokampus ile ilgili bazı işlevleri etkileyen, duygudurum bozukluklarına yol açabilen bir stres şeklidir. "Çevresel zenginleştirme (ÇZ)", laboratuvar hayvanlarının standart barınma koşullarından daha fazla fiziksel ve/veya sosyal uyarıma maruz bırakmak için kullanılan bir terimdir ve iki şekilde sınıflandırılır: [1] Fiziksel çevre zenginleştirme; artırılmış zemin alanı ve egzersiz, oyuncaklar gibi değişiklikleri ifade eder. [2] Sosyal zenginleştirme; hayvanları gruplar halinde barındırma ve hayvan ile bakıcısı arasındaki dinamik etkileşimleri ifade eder. İdeal olan hem sosyal hem de fiziksel zenginleştirme unsurlarının bir kombinasyonudur.

Amaç: Bu çalışmada yavru sıçanlarda sütten kesme döneminde sosyal izolasyonun veya çevresel zenginleştirmenin hipokampusta nöron sayısı üzerine etkilerinin araştırılması ve elde edilen histolojik verilerin özgün davranış testleri ile karşılaştırılması amaçlanmıştır.

Materyal-Metot:

21 günlük 50-60 g ağırlıkta Wistar albino dişi ve erkek sıçanlar kullanıldı.

1. Grup: Kontrol grubu (n=7)

2. Grup: Sİ grubu (n=7)

3. Grup: ÇZ grubu (n=7)

Sosyal izolasyon modeli oluşturmak için; sıçanlar tek kişilik kafeslerde barındırıldı. Çevre zenginleştirme modelinde ise yavrular; 3 veya 4'lü gruplar halinde, içinde çeşitli kemirgen egzersiz topları, kemirmek için oyuncaklar ve tüneller bulunan kalın altlıklı geniş kafeslerde barındırıldı. Kontrol grubu sıçanlar; standart laboratuvar kafeslerinde, kafes başına 3 veya 4 sıçan olacak şekilde barındırıldı. 28 gün bitiminde sıçanlara anksiyete değerlendirmesi için bilye gömme ve açık alan testleri, kısa süreli bellek değerlendirmesi için yeni obje tanıma testi olmak üzere üç farklı davranış testi uygulandı. Sonrasında perfüzyon fiksasyonu ile sıçanlardan uzaklaştırılan beyinler parafin blok haline getirildi. Bloklardan elde edilen 5µm kalınlıktaki koronal parafin kesitler krezil viyole metodu ile boyandı. Seviyeleri belirlenen kesitlerde hücre sayımları yapıldı. Histolojik sonuçlar ve davranış test sonuçları istatistiksel olarak değerlendirildi.

Bulgular: Kontrol ve ÇZ grubuna göre Sİ grubunda nöron sayılarında belirlenen azalış ve kontrol grubuna göre ÇZ grubunda belirlenen nöronal artış istatistiksel olarak anlamlı bulundu. Uygulanan üç ayrı davranış testi sonuçlarının, histolojik bulguları desteklediği saptandı.

Sonuç: Sosyal izolasyonun sütten kesme döneminde hipokampal nörogenezi olumsuz etkilediği, buna karşılık fiziksel ve sosyal zenginleştirme birlikteliği ile sağlanan çevresel zenginleştirmenin nörogenezi arttırmak yolu ile hem öğrenme ve kısa süreli bellek gibi entelektüel alanlarda hem de depresyon ve anksiyete gibi psikolojik durumların önlenmesi üzerinde olumlu etki gösterdiği sonucuna varıldı. Sonuçların günümüzdeki pandemi koşullarında çocuk yetiştirilmesine katkı sağlayacağı düşünüldü.

Sözel Bildiri

SOSYAL İZOLASYONUN YAVRU SIÇANLARDA MERKEZİ SİNİR SİSTEMİNDE MİYELİNİZASYON ÜZERİNE ETKİLERİNİN BİLİŞSEL VE BELLEK DAVRANIŞLARI İLE KOMPARATİF ANALİZİ

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Giriş: Sosyal bağlar yavrulara güven verir, yavruların hayatta kalmasını destekler ve enerji harcama ihtiyacını azaltır. "Sosyal izolasyon (Sİ)" gibi durumlarda sosyal etkileşimin kısıtlanması, fiziksel ve bilişsel sağlığa zarar verir. Sİ, merkezi sinir sisteminde çok sayıda öğrenme ve hafıza gibi davranışsal, morfolojik ve işlevsel anormalliklere neden olur. Son zamanlarda yapılan çalışmalar, sütten kesme sonrası izolasyonun uzamsal hafızayı ve sosyal hafızayı bozabileceğini göstermektedir.

Amaç: Bu çalışmada yavru sıçanlarda sütten kesme döneminde sosyal izolasyonun merkezi sinir sisteminde miyelinizasyon üzerine etkilerinin araştırılması ve elde edilen histolojik verilerin davranış testleri ile karşılaştırılması amaçlanmıştır.

Materyal-Metot: 21 günlük 50-60 g ağırlıkta Wistar albino dişi ve erkek sıçanlar iki gruba ayrıldı: 1. Grup: Kontrol grubu (n=7), 2. Grup: Sİ grubu (n=7). Sosyal izolasyon modeli oluşturmak için; sıçanlar tek kişilik kafeslerde barındırıldı. Kontrol grubu sıçanlar; standart laboratuvar kafeslerinde, kafes başına 3 veya 4 sıçan olacak şekilde barındırıldı. 28 gün bitiminde sıçanlara anksiyete değerlendirmesi için bilye gömme ve açık alan testleri, kısa süreli bellek değerlendirmesi için yeni obje tanıma testi olmak üzere üç farklı davranış testi uygulandı. Sonrasında perfüzyon fiksasyonu ile sıçanlardan uzaklaştırılan beyinler parafin blok haline getirildi. Bloklardan elde edilen 5µm kalınlıktaki koronal parafin kesitler miyelin değerlendirmesi için luksol fast blue metodu ile boyandı. Seviyeleri belirlenen kesitlerde korpus kallozum kalınlıkları ölçüldü ve boyanma yoğunlukları değerlendirildi. Histolojik bulgular ve davranış test sonuçları istatistiksel olarak değerlendirilerek karşılaştırıldı.

Bulgular: Kontrol grubuna göre Sİ grubunda korpus kallozum kalınlıklarında saptanan azalışlar, istatistiksel olarak anlamlı bulundu. Uygulanan üç ayrı davranış testi sonuçları ile histolojik bulguların birbiri ile korele olduğu belirlendi.

Sonuç: Sütten kesme çağındaki sosyal izolasyonun merkezi sinir sisteminde miyelinizasyonu olumsuz etkilediği, buna bağlı olarak depresyon ve anksiyete davranışlarının geliştiği, öğrenme ve kısa süreli bellek gelişiminin yetersiz olduğu sonucuna varıldı.

Sözel Bildiri

İNSAN ENDOMETRİYAL ADENOKARSİNOM HÜCRE HATTINDA GALEKTİN-1 VE GALEKTİN-3 EKSPRESYONLARININ HORMONAL DÜZENLENMESİ

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Giriş: Galektinler yaygın şekilde eksprese edilen lektin sınıfı karbonhidrat bağlayıcı bir protein grubunu temsil ederler. Galektinlerin inhibe edildiği çalışmalar aracılığıyla galektinlerin immün ve enflamatuar yanıtlar, tümör gelişimi, nöral dejenerasyon, ateroskleroz, diyabet ve yara onarımı gibi çeşitli fizyolojik ve patolojik süreçlerde önemli roller oynadığı gösterilmiştir. İnsan endometriyumunda Galektinlerin farklı tipleri bildirilmiştir. Bunlardan özellikle Galektin-1 (Gal-1) ve Galektin-3 (Gal-3) ekspresyonları yüksek oranlarda bulunmuştur. İmmunohistokimyasal çalışmalar, Gal-1'in daha çok endometriyal stromada, Gal-3'ün ise endometriyal glandüler epitelde bulunduğuna ve bu galektinlerin menstrual döngü boyunca farklı ekspresyon paternleri gösterdiklerine işaret etmektedir. Menstrual döngü süresince galektinlerin ekspresyonlarının değişiyor olmasından yola çıkarak, çalışmamızda Gal-1 ve Gal-3'ün ekspresyonunun ovaryan hormonlar ile düzenleniyor olabileceği hipotezi kurulmuştur.

Amaç: Çalışmamızda bir endometriyal adenokarsinom (Ishikawa) hücre hattına östradiol (E2) ve progesteronun (P4) ayrı ayrı veya birlikte verilmesi ile Gal-1 ve Gal-3 mRNA ve protein düzeyinde meydana gelen değişikliklerinin belirlenmesi amaçlanmıştır.

Materyal ve Metod: Ishikawa hücre kültürlerinde E2 ve P4 hormon dozlarına ve zamana bağlı deney grupları oluşturulmuş, hücrelerden RNA izole edilerek gerçek zamanlı PCR yöntemi ile Gal-1 ve Gal-3 mRNA düzeyleri, ve hücre kültür süpernatantları toplanarak ELISA ile salgısal Gal-1 ve Gal-3 protein miktarları değerlendirilmiştir.

Bulgular: Gal-1 mRNA düzeyleri E2'nin farklı dozlarında anlamlı bir değişiklik göstermezken, P4'ün 10^{-9} mol/L konsantrasyonunda kontrole göre anlamlı olarak yüksek bulunmuştur. E2'nin 10^{-7} mol/L konsantrasyonu Gal-3 mRNA düzeylerini kontrole kıyasla anlamlı bir şekilde artırmıştır. E2 ve P4'ün ayrı uygulanması 24, 48 ve 72 saat sonunda Gal-1 ekspresyonunda anlamlı bir değişikliğe neden olmazken, birlikte verilmesinin kontrole kıyasla neredeyse inhibisyona yol açacak derecede Gal-1 ekspresyonunu düşürdüğü görülmüştür. E2 uygulanması 24, 48 ve 72 saat süren deneylerde Gal-3 ekspresyonunu anlamlı olarak değiştirmezken P4 uygulanması 72. saatte E2 uygulamasına göre Gal-3 ekspresyonunu anlamlı olarak artırmıştır. P4 ile E2'nin birlikte hücrelere verilmesi Gal-3 mRNA düzeylerini düşürmemiştir. Gal-1 salgısal protein miktarı E2 ve P4'ün konsantrasyonlarına göre bir anlamlı değişiklik göstermezken Gal-3 miktarı P4'ün tüm konsantrasyonlarında kontrole göre anlamlı olarak artmıştır. Gal-1 protein miktarı E2 ve P4'ün birlikte uygulandığı durumda 48 saatte

anlamlı olarak düşmüştür. E2 ve P4 ile 24 saatlik inkübasyon sadece P4 ile 24 saatlik inkübasyon sonucu elde edilen Gal-3 protein miktarını anlamlı şekilde azaltmıştır.

Sonuç: Ishikawa hücrelerinde Gal-1 ve Gal-3 mRNA düzeyleri ve salgısal protein miktarı ovaryan hormonlarla zamana ve doza bağlı olarak değişmektedir. Hem Gal-1 hem de Gal-3 ekspresyonu östradiole kıyasla progesterona daha duyarlıdır. Patogenezinde Gal-1 ve Gal-3'ün rol aldığı bilinen ve prognozunu hormonların etkilediği hastalıklarda galektinlerin hormonlarla düzenlendiğinin göz önünde bulundurulması, tanı/tedavi yöntemlerinin belirlenmesinde önem arz etmektedir.

Sözel Bildiri

MONENSİNİN APOPTOZ ARACILI SH-SY5Y NÖROBLASTOM HÜCRE PROLİFERASYON VE MİGRASYONU ÜZERİNE ETKİLERİNİN ARAŞTIRILMASI

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Giriş: Nöroblastom, nöral krest kökenli ve çocuklarda en sık görülen ekstrakranial solid tümördür. Nöroblastom tedavisinde kullanılan mevcut tedaviler klinik olarak agresif seyreden bu hastalığın uzun süreli tedavisinde etkili değildirler. Bu yüzden hastalığın tedavisinde kullanılmak üzere yeni antikanser ilaçların keşfedilmesi gerekmektedir. Monensin *Streptomyces Cinnamonensis*'den izole edilen ve kanser hücrelerin gelişimini inhibe eden iyonofor bir antibiyotiktir; ancak insan nöroblastom kanser hücreleri üzerindeki antikanser etkileri ve etki mekanizması henüz bilinmemektedir.

Amaç: Bu çalışmanın amacı, monensinin SH-SY5Y nöroblastom hücre proliferasyon, migrasyon, invazyon, koloni formasyonu ve apoptoz mekanizmaları üzerindeki etkilerini araştırmaktır.

Materyal-Metot: Monensinin hücre canlılığı üzerine etkileri XTT ile, hücre invazyon, migrasyon ve koloni oluşumu üzerine etkileri sırasıyla, matrijel-invazyon, yara iyileşmesi ve koloni formasyon testi ile monensinin apoptoz üzerine etkileri ise RT-PCR, TUNEL, Western Blot ve Annexin V yöntemleri kullanılarak belirlenmiştir.

Bulgular: Yaptığımız çalışmada monensinin nöroblastom hücre proliferasyon, invazyon, migrasyon ve koloni oluşumunu baskıladığını gösterilmiştir. Monensinin SH-SY5Y nöroblastom hücrelerinde IC_{50} dozu 48 saatte 16 µM olarak bulunmuştur. Monensin uygulaması SH-SY5Y hücrelerinin migrasyonunu anlamlı olarak azaltmıştır ($p<0,001$). 8, 16 ve 32 µM monensin uygulanan gruplarda invaze olan hücre sayısı sırasıyla 308 ($p<0,001$), 178 ($p<0,001$), ve 123 ($p<0,001$) iken bu sayı kontrol grubunda 633 olarak belirlenmiştir. Kontrol grubunda koloni sayısı 101 iken 8, 16 ve 32 µM monensin uygulanan gruplarda sırasıyla 7 ($p<0,001$), 3 ($p<0,001$) ve 0 ($p<0,001$)'dır. Dahası bu çalışmada monensinin nöroblastom hücrelerinin apoptozunu tetikleyerek hücre proliferasyon ve migrasyonunu inhibe ettiği de bildirilmiştir. Monensin nöroblastom hücrelerinde kaspaz-3, kaspaz-7, DR5, MCL1, NOXA, BIRC3, BCLAF, BMF, BAD ekspresyonlarını arttırarak ve kaspaz-9 ekspresyonunu azaltarak apoptoza neden olmuştur. Annexin V sonuçlarında apoptotik hücre oranı 8, 16 ve 32 µM monensin uygulanan grupta sırasıyla, %9.66±0.01 ($p<0.001$), 29.28±0.88 ($p<0.01$) ve 62.55±2.36 ($p<0.01$) olarak bulunmuştur. TUNEL sonuçlarında ise bu değerler sırasıyla; %35±2 ($p<0.001$), 34±0,57 ($p<0.001$) ve 75±2.51 ($p<0.001$) olarak belirlenmiştir.

Sonuç: Yaptığımız deneylerden elde ettiğimiz sonuçlarımız monensinin pediatrik nöroblastom tedavisinde güvenli ve etkin bir terapötik ilaç adayı olabileceğini önermektedir.

Anahtar Kelimeler: Nöroblastom, apoptoz, monensin.

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Sözel Bildiri

YUKARI KIZILIRMAK HAVZASINDA (SİVAS) YAŞAYAN *ALBURNUS CHALCOİDES* (GÜLDENSTADT, 1772) POPÜLASYONUNDA KLORÜR HÜCRELERİNİN HİSTOLOJİK ANALİZİ

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Balıklarda solungaçların gaz değişimi yanında su ve iyon değişimi yaparak boşaltım ve osmoregülasyonda görev aldığı bilinmektedir. Solungaçlarda yer alan klorür hücrelerinin iyonik düzenleme, asit-baz dengesi ve gaz transferindeki rolü üzerine birçok araştırma mevcuttur. Sunulan çalışmada Yukarı Kızılırmak Havzası (Sivas)'nda yaşayan *Alburnus chalcoides* (tatlı su kolyozu)'in iki farklı populayonundan (İmranlı-Zara ve Zara-Sivas arası bölgeler) elde edilen balık örneklerinde klorür hücrelerinin varlığı histolojik olarak incelenmiştir. Örnekleme bölgelerinde suyun elektriksel iletkenlik değerinin 0.2-1.12 mS (İmranlı-Zara) ile 4.7-5.7 mS (Zara-Sivas) arasında değiştiği belirlenmiştir. Her iki bölgeden yakalanan balıkların solungaçları çıkarıldıktan sonra bouin çözeltisinde tespit edilmiş, rutin protokolü takiben, parafin içinde bloklanan örneklerden 7 µm'lik kesitler alınmıştır. Hazırlanan preparatlar histolojik inceleme için hematoksilen-eosin ve masson-trikrom ile boyandıktan sonra değerlendirilmiştir. Klorür hücreleri sekonder lamellerin kaidesinde ve inter-lamellar bölgede yer almaktadır. Armut biçimli klorür hücrelerinin kaide kısmında yuvarlak bir çekirdek bulunur. Mitokondri ve endoplazmik retikulumca zengindir. Birinci bölgeye oranla yaklaşık 6 ile 20 kat daha tuzlu olan ikinci bölgede yaşayan tatlı su kolyozu örneklerinin solungaçlarında, birim alanda tespit edilen klorür hücre sayısı oldukça daha fazladır. Klorür hücrelerinin çevresinde destek hücreleri ve mukus hücreleri gözlenmiştir.

Anahtar Kelimeler: *Alburnus chalcoides*, klorür hücresi, Yukarı Kızılırmak Havzası, histoloji.

Sözel Bildiri

KOLİSTİNE BAĞLI KARACİĞER HASARINA KARŞI MEZENKİMAL KÖK HÜCRELERİN ETKİSİ

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Kolistin, çoklu ilaca dirençli gram-negatif bakteriyel enfeksiyonlara karşı tedavi için kullanılan bir glikopeptid antibiyotiktir. Mezenkimal kök hücreler (MSC) ise, birçok hastalıkta terapötik aracılar olarak kapsamlı bir şekilde çalışılmaktadır. Bu hücreler, nispeten kolay genişletilebildikleri gibi, güçlü anti-inflamatuar, immünomodülatör ve pro-anjiyojenik etkilere sahip oldukları ve bağışıklık sistemini uyarma riski düşük olduğundan dolayı tedavi için özellikle dikkat çekicidir. Bu çalışmada Kolistin'in karaciğerde oluşturacağı toksisiteye karşı mezenkimal kök hücrelerin olası koruyucu etkisinin belirlenmesi amaçlanmıştır. Çalışmada 40 adet wistar albino dişi sıçan her grupta 10 adet olacak şekilde; kontrol, kolistin (36 mg/kg i.p), MSC ($1x10^6$ 100µl/rat i.v), MSC ($1x10^6$ 100µl/rat i.v) + kolistin (36 mg/kg i.p) gruplarına ayrıldı. Deney sonunda, karaciğer dokuları eksize edildi ve rutin histolojik doku takibi basamaklarından geçirilerek parafine gömüldü. Hazırlanan parafin bloklardan alınan 5µm kalınlığındaki kesitler histopatolojik değerlendirme için Hematoksilen&Eozin ve Masson trikrom ile boyanarak ışık mikroskobunda incelendi. TNF-α ve PCNA ekspresyonları immünohistokimya metodu ile incelenmiştir. Işık mikroskobik bulgulara göre, kontrol grubuna ait karaciğer dokularının normal histolojik bir görünüme sahip olduğu gözlendi. İyi tanımlanmış çekirdeklere sahip ana karaciğer hücreleri olan hepatositler, lobül içinde ışınsal olarak düzenlenmişti. Kolistin grubuna ait karaciğer bölümlerinde, lökosit infiltrasyonu, hepatosit hücrelerinde hasar ve hemoraji gibi dejeneratif değişiklikler görülmüştür. Bu görünümler, kolistine maruz kalmanın sıçan karaciğerinde ciddi hasara neden olduğunu göstermektedir. Kolistin grubu karaciğer dokusunda TNF-α ve PCNA ekspresyon yoğunlukları, diğer gruplar ile karşılaştırıldığında anlamlı farklılık gösterdi. MSC+kolistin grubunda ise, kolistinin neden olduğu karaciğer hasarında MSC'nin koruyucu rolünü göstermiştir. Bu bulgular, MSC'nin kolistinin neden olduğu karaciğer hasarını önlemek ve kolistin kaynaklı karaciğer fonksiyonunu iyileştirmek için uygun bir farmasötik müdahale olabileceğini düşündürmektedir.

Anahtar Kelimeler: Karaciğer, Kolistin, MSC, PCNA

Sözel Bildiri

METOTREKSAT UYARILI RAT TESTIS HASARINDA TIMOKINON'UN ETKISI

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Giriş: Kemoterapi kanser tedavisinde kullanılmasıyla birlikte kemoterapi ilaçlarının yan etkileri de mevcuttur. Kemoterapide kullanılan ilaçlardan biri olan metotreksat (MTX) özellikle meme kanseri ve romatoid artritte kullanılmaktadır. Metotreksat'ın faydalarının yanı sıra testis üzerinde önemli yan etkileri mevcuttur. Metotreksat sperm sayısının azalması, seminifer tübül hücrelerinin dejenerasyonu ve testosteron seviyesinin azalması gibi önemli yan etkilere sahip olup testiste oksidatif hasara yol açmaktadır. MTX uyarılı testis hasarında oksidan/antioksidan denge bozulmaktadır. Nükleer faktör eritroid 2 ile ilişkili faktör 2 (NRF2), oksidatif stresin önlenmesinde önemli rol oynar. Heme oksijenaz-1 (HO-1), hücresel homeostazın korunması için önemlidir. Nrf2 ile HO-1 koordineli olarak hareket etmektedirler ve oksidan/antioksidan dengenin sağlanmasında önemli rol oynarlar. Nigella sativa tohumu yağının aktif bir bileşeni olan Timokinon, bir antioksidan, anti-inflamatuar ve antitümör ajan olarak kullanılır.

Amaç: Bu çalışmada Metotreksat'ın sebep olduğu testis hasarına karşı Timokinon'un koruyucu etkisini araştırmayı amaçladık.

Materyal-Metot:

Gruplar aşağıdaki şekilde belirtildi;

1. Kontrol grubuna (n:8) 10 gün boyunca intraperitoneal serum fizyolojik uygulandı.
2. Zeytinyağı grubuna (n:8) 10 gün boyunca intraperitoneal zeytinyağı uygulandı.
3. Metotreksat grubuna (n:8) deneyin 1. günü 20 mg/kg tek doz MTX intraperitoneal yolla uygulandı.
4. Timokinon Grubuna (n:8) 10 mg/kg THQ intraperitoneal olarak 10 gün boyunca uygulandı.
5. MTX+THQ grubuna (n:8) (MTX: (20 mg/kg 1. gün tek doz intraperitoneal yolla) ve THQ: 10mg/kg intraperitoneal olarak 10 gün boyunca uygulandı.

Deneyin 10. gününde son enjeksiyondan 2 saat sonra yüksek doz ketamin+ksilazin anestezisi altında ratların hayatlarına son verildi. Sakrifikasyon sonrası epididimisden alınan örneklerden sperm sayısı belirlendi. Ratlardan elde edilen testis ve epididymis ağırlıkları ölçüldü. Deney sonunda ratların testis dokuları alınıp fiksasyon ve doku takibi işlemlerinden sonra 5µ kalınlığında kesitler alındı. Hematoksilen&eozin boyama ile seminifer tübül hasarı belirlendi. NRF2 ve HO-1 antikorları immünohistokimya ile değerledirildi.

İstatistiksel analiz: Veriler ortalama ± SD olarak ifade edildi. One-way ANOVA testi ve Tukey's post-hoc testi ile analiz yapıldı. Analizlerde $p < 0.05$ anlamlı olarak kabul edildi.

Bulgular: Deney sonunda elde edilen testis ağırlığı, epididimis ağırlığı, sperm sayısı ve ileri hareketli sperm sayısı MTX grubunda kontrol grubuna göre istatistiksel olarak anlamlı seviyede azaldı. Hematoksilen&eozin

boyama sonucunda MTX grubunda seminifer tübül epitel hücre serisinde azalma ve tübül boyutunda azalma görüldü. NRF2 ve HO-1 antikor ifadesi MTX grubunda kontrol grubuna göre istatistiksel olarak anlamlı miktarda azaldı. MTX+THQ grubunda bu hasarlar istatistiksel olarak önemli seviyede düzeldi.

Sonuç: Bizim sonuçlarımız, MTX uyarılı testis hasarını azaltmada THQ'nun NRF2/ HO-1 aktivitesini artırarak antioksidan dengeyi sağlayıp testiküler fonksiyonu düzeltmede rol oynadığını göstermektedir.

Sözel Bildiri

GBM HÜCRELERİNİN CANLILIĞI VE MİGRASYONUNUN DURDURULMASINDA; İNDOKSİMOD İLE BİRLİKTE MELATONİN UYGULAMASI ETKİN MİDİR?

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Giriş: Glioblastoma Multiforme (GBM), santral sinir sisteminin en sık görülen malign tümörüdür. Yüksek invazivliği ve heterojenliği nedeniyle, cerrahi rezeksiyon, radyoterapi ve eş zamanlı kemoterapiyi içeren klinik tedaviler, GBM hastalarının sağkalımında çok etken rol oynayamamaktadır. Bu yüzden günümüzde hastaların sağkalımını artıracak kombine tedaviler araştırılmaktadır. IDO; triptofanın (Trp) kinurenine (Kyn) dönüşmesini sağlayarak T hücrelerinin işlevini kaybetmesine neden olan bir enzimdir. İndoksimod ise, düşük Trp ve yüksek Kyn seviyesinin yarattığı immün baskılayıcı etkileri tersine çeviren, özellikle tümör mikroçevresinde T hücre aktivasyonunu destekleyen bir IDO inhibitörüdür. Melatonin, immün sistemi güçlendirici etkisi olduğu ve kansere karşı savunmada doğal öldürücü (NK) hücrelerin artışına yol açtığı bilinen bir ajandır.

Amaç: İndoksimod ile Melatonin birlikte uygulandığında hücre migrasyonunu yavaşlatır hipotezinden yola çıkarak; U87 hücre hattında, İndoksimod'un tek başına ve Melatonin ile birlikte uygulanmasının hücre migrasyonu ve canlılığı üzerindeki etkisinin değerlendirilmesi amaçlanmıştır.

Materyal-Metot: U87 hücre hattında, İndoksimod ve Melatonin için IC_{50} değerleri belirlendi. Takiben hücrelere İndoksimod ve Melatonin bireysel ve kombine şekilde uygulandı. Hücreler Tripan Mavisi ile boyanarak; 24, 48 ve 72. saatlerde canlılıkları analiz edildi. Ayrıca 0, 6, 12, 24, 36 ve 48. saatlerde yara iyileşme testi yapıldı ve hücrelerin migrasyon hızları değerlendirildi.

Bulgular: İlk 24 saatte İndoksimod ve Melatonin'in bireysel uygulamalarında; İndoksimod'un hücre sağkalımını kontrol hücrelerine kıyasla etkilemediği ancak; Melatonin'in canlı hücre sayısını anlamlı şekilde azalttığı görüldü. Hücre migrasyonunda ise İndoksimod ve Melatonin'in bireysel uygulamalarında kontrole kıyasla bir değişim gözlenmedi. Kombinasyon dozlarında; ilk 24 saatte hücre canlılığı açısından ciddi bir etki görülmezken hücre migrasyonun yavaşladığı görüldü. 48. saate gelindiğinde; İndoksimod ve Melatonin'in bireysel ve kombine uygulamalarında; hem hücre sağkalımında hem de migrasyonunda kontrole kıyasla azalma dikkati çekti. 72. saate gelindiğinde tüm deney gruplarında hücre sağkalımında anlamlı bir şekilde azalma gözlendi.

Sonuç: Çalışmamızla İndoksimod ve Melatonin'in GBM hücrelerine birlikte uygulanmasının hücre canlılığını ve migrasyonunu zamana bağlı olarak azalttığı gösterilmiştir. Çalışmamız henüz tanımlayıcı bir çalışma niteliğinde

olup radyoterapi ve kemoterapinin eklenmesiyle oluşturulacak yeni gruplar doğrultusunda elde edilecek verilerle kapsamlı olarak değerlendirilecektir.

Anahtar Kelimeler: Glioblastoma Multiforme, U87MG, İndoksimod, Melatonin, Hücre Migrasyonu, Hücre Canlılığı