Abstracts

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CORRELATION OF MISMATCH REPAIR PROTEINS MLH1, MSH2, MSH6 AND PMS2 WITH ANTIAPOPTOTIC PROTEIN SURVIVIN IN COLON LESIONS

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Introduction: Defective function of mismatch repair proteins (MMRP) contributes to development of variety of cancers. Antiapoptotic protein survivin is involved in pathogenesis of human malignancies and it is widely expressed in majority of malignant tissues. Both MMRP and survivin are considered to be powerful prognostic parameters. The purpose of this study was to determine the association of MMRP with survivin in benign and malignant colon lesions. Methods: The MMRP status and survivin were retrospectively assessed by immunohistochemistry on paraffin-embedded formalin-fixed samples from 41 benign colon polyps and 73 grade 1 and 2 colon carcinomas. Results: In polyps, 34/41 samples were positive for MLH1 staining (82,9%), 38/41 for MSH2 and MSH6 stainings (92,7%), and 35/41 for PMS2 staining (85,4%). Survivin expression was found in 1/41 sample only (2,4%). In panel of carcinomas, immunoreaction was detected in 64/73 samples for MLH1 (87,7%), 72/73 samples for MSH2 (98,6%), 71/73 for MSH6 samples (97,3%), and 67/73 for PMS2 samples (91,8%). Survivin was shown in 24/73 samples (32,9%). In carcinomas, the statistical analysis confirmed a significant correlation between the expression of MMRP and survivin ($p<0,001$), a significant difference in the intensity of MMRP and survivin immunoreactivity ($p<0,001$), and a significant difference in percentage of MMRP and survivin labeled cells ($p<0,001$). Furthermore, the correlation was found in panel of polyps between expression of MMRP and survivin ($p<0,001$). Conclusions: Our results suggest that MMRP may suppress the survivin expression and its antiapoptotic activity in grade 1 and 2 colon carcinoma. This work was supported by project VEGA 1/0050/11.

CLINICAL ANATOMY TEACHING ON CADAVERS IN PREGRADUATE AND POSTGRADUATE MEDICAL EDUCATION

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A rapid increase of the importance of mini-invasive techniques in many medical disciplines, ranging from internal medicine (bronchoscopy, gastroscopy) to surgical disciplines (laparoscopy, thoracoscopy) occurs in last ten years. Then the growing need for mastering these techniques, both in terms of theory and practical skills, in particular increases too. In response we built a unique multimedia educational centre for topographic and clinical anatomy, endoscopy and microsurgery at our Anatomical Institute. The Educational Centre offers cadavers fixed by a special method for a repeated use, in order to imitate different medical procedures. We fully equipped a small operating theatre, rooms are interconnected by an audio-video network with a wi-fi Internet. We equipped also a graphic studio for creation of educational materials. Demonstration for medical students includes simulations of arthroscopies, laparoscopies, bronchoscopies and gastroscopies. For young physicians training courses are organized, to get acquainted with instruments for a wide range of endoscopic and microsurgical procedures in a similar topographical situation. Postgradual students can use e-learning materials designed for the selected practical course in advance to their enrollment. Distant educational components together with the practical

cadaver courses are a good approach in clinical anatomy education and surgical skills improvement. The centre provides an advanced approach to the practical training in the field of mini-invasive and endoscopic methods concentrated in one place and, in fact, represents the maximum progress achievable in this respect, before the students enter the real world of medical practice. Supported by RP MSMT and EU Funds OPPC CZ216/3100/24018 (INO/02/01/0017/ 2010).

NEW REMARKS TO VISUALISATION AND IDENTIFICATION OF IMMUNOCOMPETENT CELLS IN HUMAN CORNEA

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The corneal transplantation is currently common procedure - in the Czech Republic it is about 600 operations per year. There is a lot of conditions which have influence to grafts survival, e.g. number of endothelial cells present in the donor cornea, immunological mechanisms. A principle of white elements series naturally occurring in the cornea is unclear. The aim was to innovate and improve procedure for display cells of white line in the various layers of the cornea. The physiological corneas were dissected and examined under a surgical microscope, and only those without any signs of inflammation or other abnormalities were used in the studies. The corneas were cleared from attached lens, conjunctiva, and excess limbal tissue. The corneal stroma and epithelium were then separated after a 20-minute incubation at 37°C in PBS containing 20 mM EDTA. After separation, the corneal stromas were fixed for 30 minutes at 4°C in 1% paraformaldehyde-PBS followed by extensive washing with PBS. After fixation, the corneal tissue was permeabilized with 0,2-0,5% TWEEN20 aqueous solution for 20 minutes at 37°C. Following corneal tissue was incubated overnight at 4°C with 100 µL antibody antihuman CD45 FITC/CD14 –PE DUAL TAG TM diluted in 0,2% TWEEN/PBS. Tissues stained with primary antibody were fixed again with 1% paraformaldehyde-PBS, rinsed with PBS, placed on slides, mounted with Prolong Gold Antifade Reagent (Life Technologies), and coverslipped. All slides were examined by fluorescence microscopy on a 1 × 40 microscope (Nikon, Eclipse). The procedure showed individual immuno-competent cells. Cells were evaluated from the quantity and type point of view. This is a preliminary work and it is taken as a first step in research of cross-linking methodology to reduce rejection episodes in corneal transplantation. Supported by GAUK 282011/2011

RADIATION-INDUCED LONG-TERM ALTERATIONS IN THE FOREBRAIN UNDER EXPERIMENTAL CONDITIONS

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Introduction: Ionizing radiation can induce significant injury to normal brain structures. The present study was dealed with effect of fractionated doses of gamma rays on specific cell types housed the rat brain's neurogenic region. Methods: Adult male Wistar rats received whole-body exposure with fractionated doses of gamma rays (a total dose of 5Gy) and were investigated thirty, and sixty days later. Immunohistochemistry and confocal microscopy were used to determine density of young neurons - neuroblasts derived from anterior subventricular zone (SVZa) and brain resident and activated microglia distributed along and/or adjacent to subventricular zone–olfactory bulb axis (SVZ-OB axis). Cell counting was performed in four anatomical parts along the well defined pathway, known as the rostral migratory stream (RMS) represented by the SVZa, vertical arm, elbow and horizontal arm of the RMS. Results: Gradual decline of neuroblasts was seen in course of sixty days after radiation treatment mostly visible in rostral parts of the migratory pathway. Population of resident and activated microglia showed different dynamic of cell distribution, however data about their cell density are still under evaluation. Conclusion: Preliminary data suggested that radiation response of young neurons arisen from the SVZa may play contributory role in development of more adverse radiation-induced late effects and therefore may have implications for clinical radiotherapy. Supported by a VEGA grant 1/0050/11 and projects "center of excellency for research in personalized therapy (CEVYPET)", code: 26220120053 and “Identification of novel markers in the diagnostic panel of neurological diseases" co-financed from EU sources and European Regional Development Fund.

CLINICAL AND CLINICALLY ORIENTED ANATOMY FOR MEDICAL STUDENTS

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Medicine curriculum in the University of Cordoba was changed in 1973. Until that time Anatomy was developed into four different courses: descriptive anatomy, topographic anatomy, surgical technique and surgical anatomy in the first 4 years of the career. Since 1973 the course of Normal Anatomy (gross anatomy) curriculum included radiological aspects.

Later (1980) endoscopic contents were added. However, those topics were only developed by some professors during theoretical lessons as illustrative examples of clinical application of anatomical knowledge. After 1990 new contents related to diagnostic images were introduced. On 1999, the governmental entity that regulates university careers determined that clinical aspects had to be included in medical basic courses and Anatomy had to specifically involve the identification of anatomical structures in diagnostic images and surface anatomy. Since then, our theoretical lessons include clinical concepts for all subjects, practical activities are developed not only with cadavers but also with diagnostic images and clinical aspects are also evaluated in all instances. Depending on institutional possibilities, students visit some hospitals to observe the practical application of their anatomical knowledge; and depending on their particular interest, they can choose an optional course related to a specific anatomical region or medical specialty. Our students have received with enthusiasm these clinical contents as they feel they motivate their study and provide the basement for that knowledge. Our curriculum does not include a course of Clinical Anatomy. We are developing an optional course of Clinical Anatomy in English, but it only develops some few topics. An introduction of clinical anatomy contents is taught at the beginning of each clinical course, but usually they are not developed by the professors of Anatomy. However, the tradition of our university determines that most of the clinical professors (mainly in surgical courses) have spent some time in Anatomy at any moment of his/her training.

NADPH-d HISTOCHEMICAL STUDY OF THE RAT HIPPOCAMPUS

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Introduction: The hippocampus is a complex region of the brain that has an important clinical significance. Unlike humans, the hippocampus in the rat is located on the dorsal side of the thalamus. The aim of this study was to investigate the presence of nitrergic neurons in the rat hippocampus. Methods: Study was carried out on 12 Wistar rats of the both sexes at the age of 3 months under physiological conditions. The coronal sections of each brain sample were examined by nicotinamide dinucleotide phosphate-diaphorase (NADPH-d) histochemical method. Results: The distribution of the NADPH-d positive neurons in hippocampus and dentate gyrus of adult rat was investigated. Two thirds of the NADPH-d neurons were located in hippocampal regions CA1, CA2 and CA3, one third was found in the dentate gyrus. The highest number of labelled cells was located in the radiate layer. In the CA1 and CA2 areas of hippocampus the NADPH-d positive interneurons were found mostly in pyramidal and radiate layers, whilst in the CA3 region they were distributed throughout the thickness of whole pyramidal layer. The NADPH-d positive cells in the dentate gyrus were located near the granular layer. Most of the stained neurons were bipolar and oriented radially in the pyramidal layer of CA1 and CA2. In CA3 layer they were uniform in the size and in multipolar or fusiform shape. Conclusions: According to our investigation we can conclude, that the distribution of the NADPH-d positive cell was higher in the ventral part of the rat hippocampus. There were observed no differences between the NADPH-d positive neurons of both hemispheres. Supported by Grant VEGA 1/0154/11.

FORMATION OF TOOTH-BONE INTERFACE IN REPTILES

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Vertebrates exhibit a great variability in type of tooth attachment, which is also depending on the existence or absence of the teeth replacement. Acrodont, pleurodont and thecodont attachments with several subtypes can be distinguished. In reptiles, we can find state of complete ankylosis of tooth into the jaw as well as teeth localized in the alveolar bone accompanied by periodontal apparatus similar to mammals. Acrodonta class (comprises chameleons and agamas) is characteristic by the direct connection of dental tissues to the jaw surface by mineralized connective tissue. This fusion starts in chameleons during the course of their prehatching stages. Developmental processes and molecular bone/tooth communication, which participate to tooth attachment into surrounding bones, have not been fully elucidated yet. We analyzed the origin of chameleons embryonic connective tissues produced between the tooth and the bone. Transversal histological sections of mandibles were used from selected embryonic stages. We applied Hematoxyline-Eosin staining and immunohistochemical analysis on alternative sections. Furthermore, detailed views of tooth/bone attachment were obtained by microCT analysis of chameleon heads. The tooth base in the chameleon formed after mineralization of the tooth crown. The enamel layer did not develop in the area of the tooth base and thus the tooth base was formed only by the dentine. Odontoblasts produced a layer of predentine that connected the dentin to the supporting bone, with both tooth and bone protruding out of the oral cavity and acting as a functional unit. CT scan showed that tooth and bones were separated by bony lamellae whose resorption connected the tooth pulp and the bone marrow at post-hatching stages. In contrast to chameleon, the ankylosis is pathological phenomena in mammals following the tooth implantation or tooth eruption failures. Teeth mal-functions arise with disturbances in the occlusion. Furthermore, the alveolar bone shows a progressive demineralization, which leads to tooth loss. As cellular and molecular mechanisms of ankylosis development are not known, chameleons may provide new and useful information to study the molecular interaction at

