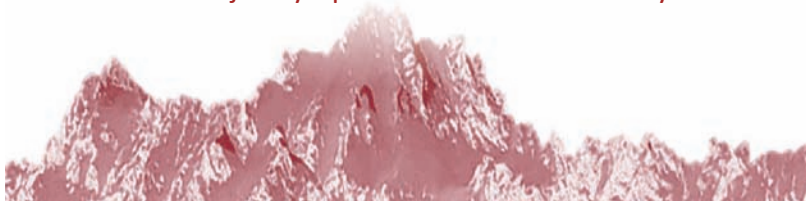




MORPHOLOGY 2019

45th International Congress of Slovak Anatomical Society
56th Lojda Symposium on Histochemistry



Conference Programme and Abstracts



5–7 September 2019, Štrbské Pleso, High Tatras, Slovak Republic

Slovak Medical Association
Slovak Anatomical Society
Department of Anatomy, Faculty of Medicine,
Pavol Jozef Šafárik University in Košice



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56th Lojda Symposium on Histochemistry

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5 - 7 September, 2019
Hotel Panorama Resort **** Štrbské Pleso, High Tatras, Slovak Republic

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Editors: doc. MVDr. Květuše Lovásová, PhD., doc. MUDr. Adriana Boleková, PhD.,
prof. MVDr. Silvia Rybárová, PhD., doc. MUDr. Ingrid Hodorová, PhD.

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Dear Colleagues and Friends of morphology,

it is my great pleasure to invite you to the Morphology 2019 – 45th International Congress of Slovak Anatomical Society and 56th Lojda Symposium on Histochemistry which will take place in High Tatras, at Štrbské Pleso, which are one of the most beautiful sights in Slovakia. I am already excited about the programme of the event which will include insightful presentations and round discussions about the recent scientific findings in morphology complemented with a rich social programme.

Speaking of experience from my previous years, I have always left the congress inspired, and I am certain that this year's programme will be as inspiring and stimulating as the previous ones were. Surrounded by the nature of High Tatras, we will have plenty of possibilities to visit the famous gem of Slovakia and walk across the picturesque Tatra countryside.

On behalf of the Organising Committee, I am already enthusiastic about meeting you all at Panorama Resort **** hotel where the Morphology 2019 International Congress will be held. Thank you for your effort. We look forward to seeing you at Štrbské Pleso.

Ingrid Hodorová
President of the Morphology 2019

CONFERENCE INFORMATION

Organizers

Slovak Anatomical Society
Department of Anatomy, Faculty of Medicine,
Pavol Jozef Šafárik University in Košice

HONORARY COMMITTEE

prof. RNDr. Pavol Sovák, CSc. - Rector of the Pavol Jozef Šafárik University in Košice
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Pavol Jozef Šafárik University in Košice
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- doc. MVDr. Květuše Lovásová, PhD. - Organizing and Scientific Secretary

Members:

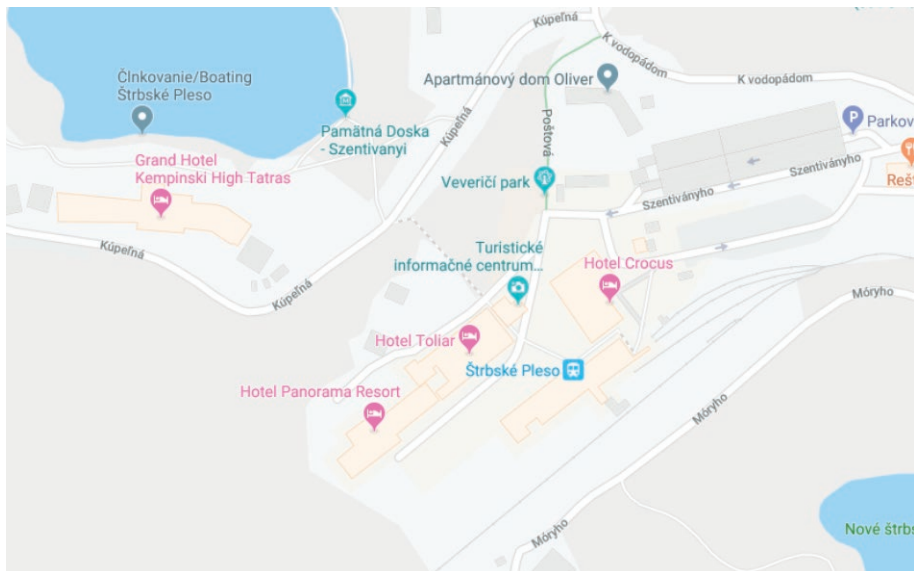
- prof. MVDr. Silvia Rybárová, PhD.
- doc. MUDr. Adriana Boleková, PhD.
- doc. MVDr. Jozef Mihalik, CSc.
- MUDr. Dalibor Kolesár, PhD.
- MUDr. Janka Vecanová, PhD.
- Mária Orgonášová

ORGANIZING SUPPORT

- SOLEN s.r.o.

CONFERENCE VENUE

Hotel Panorama Resort **** Štrbské Pleso, High Tatras, Slovak Republic



For detailed information see the websites:
<https://hotelpanorama.sk/en/contact/>
<https://www.strbskepleso.sk/sluzby/infocentrum>

IMPORTANT INFORMATION

LANGUAGE

- Oral presentations will be presented in Czech, Slovak or English
- Posters may be in Czech, Slovak or English
- Abstracts must be in English

ORAL PRESENTATION

- Please prepare your slides in ppt format
- All speeches, except invited lectures, are scheduled for 12 minutes (10 minutes presentation + 2 minutes discussion)

POSTER PRESENTATION

- Recommended poster size is 70 cm (width) × 90 cm (height)
- Authors are expected to be present during the poster sessions

Welcome Party

Thursday, September 5, 2019, Hotel's Restaurant, Hotel Panorama Resort ****
Štrbské Pleso

The programme starts at 19.00. The ticket to Welcome Party is included in the conference fee, get it at the registration desk.

Social Evening

Friday, September 6, 2019, Hotel Restaurant, Hotel Panorama Resort ****
Štrbské Pleso

The programme starts at 19.00. Please note that the Social Evening is not included in the conference fee. Please buy a ticket at the registration desk.

MORPHOLOGY 2019

45th International Congress of Slovak Anatomical Society
56th Lojda Symposium on Histochemistry

PROGRAMME AT A GLANCE

Thursday September 5, 2019		Hotel Panorama Resort **** ŠTRBSKÉ PLESO, HIGH TATRAS SLOVAK REPUBLIC	
14.00-19.00	Registration (Hotel's Entrance Corridor)		
16.30-17.00	Coffee Break (Hotel's Restaurant)		
17.00-17.30	Opening Ceremony with Welcome Speeches (Congress Hall Bystrá)		
17.30-18.15	Invited Lectures (Congress Hall Bystrá)		
18.15-18.30	Sponsor Lecture (Congress Hall Bystrá)		
18.30-18.45	Cultural Programme (Congress Hall Bystrá)		
19.00-22.00	Welcome Party (Hotel's Restaurant)		
Friday September 6, 2019	Congress Hall Bystrá	Lounge Trigan	
8.30-17.30	Registration (Hotel's Entrance Corridor)		
9.00-10.00	Clinical and Imaging Anatomy + Varia Chairs: Lovásová K., Báča V.	Histology and Embryology 1 Chairs: Adamkov M., Mihalik J.	
10.00-10.30	Coffee Break (Hotel's Restaurant)		
10.30-11.45 Bystrá	Teaching Anatomy Chairs: Mechírová E., Sedmera D.	Histology and Embryology 2 + Anatomy – Head and Neck, Trunk, Locomotor System, CNS and PNS Chairs: Kubíková E., Naňka O.	
10.30-11.00 Trigan			
11.45-12.00	Collective Photography		
12.00-13.00	Lunch (Hotel's Restaurant)		
13.00-13.30	Poster Session 1		
13.30-14.45	Developmental Biology, Molecular and Cell Biology 1 Chairs: Hodorová I., Smetana K.		
14.45-15.15	Coffee Break (Hotel's Restaurant)		
15.15-16.30	Histochemistry and Cytochemistry 1 + Sponsor Lecture Chairs: Rybárová S., Tóth Š.		
16.30-17.00	CAS Plenary Meeting, CSHC Plenary Meeting	SAS Plenary Meeting	
19.00-24.00	Social Evening (Hotel's Restaurant)		
Saturday September 7, 2019	Congress Hall Bystrá	Lounge Trigan	
8.30-9.00	Registration (Hotel's Entrance Corridor)		
9.00-10.15	Developmental Biology, Molecular and Cell Biology 2 Chairs: Boleková A., Mokřý J.	Histochemistry and Cytochemistry 2 Chairs: Domoráková I., Výbohová D.	
10.15-10.45	Coffee Break (Hotel's Restaurant)		
10.45-11.15	Poster Session 2		
11.30-12.00	Closing Ceremony (Congress Hall Bystrá)		
12.00-13.00	Lunch (Hotel's Restaurant)		



THURSDAY SEPTEMBER 5

14.00-19.00	Registration (Hotel's Entrance Corridor)
16.30-17.00	Coffee Break (Hotel's Restaurant)
	Congress Hall Bystrá
17.00-17.30	Opening Ceremony with Welcome Speeches
17.30-18.15	Invited Lectures
17.30-17.45	Interleukin-6 contributes to initiation of neuronal regeneration program in the remote dorsal root ganglia neurons after sciatic nerve injury <i>Dubový P, Hradilová-Sviženská I, Klusáková I, Brázda V, Joukal M.</i>
17.45-18.00	Association of induced hyperhomocysteinemia with Alzheimer's disease-like neurodegeneration in rat cortical neurons after global ischemia-reperfusion injury <i>Kovalská M, Tóthová B, Kovalská L, Tatarková Z, Kalenská D, Tomaščová A, Adamkov M, Lehotský J.</i>
18.00-18.15	Košice anatomy – insight into history <i>Hodorová I.</i>
18.15-18.30	Sponsor Lecture Elsevier: XXI century medical education <i>Warmínska-Marczak M.</i>
18.30-18.45	Cultural Programme
19.00-22.00	Welcome Party (Hotel's Restaurant)

FRIDAY SEPTEMBER 6

8.30-17.30	Registration (Hotel's Entrance Corridor)	
	Congress Hall Bystrá	Lounge Trigan
9.00-10.00	Clinical and Imaging Anatomy + Varia Chairs: Lovásová K., Báča V.	Histology and Embryology 1 Chairs: Adamkov M., Mihalik J.
9.00-9.15	Experience with practical training of bronchoscopy in the educational center for anatomy, endoscopy and miniinvasive techniques <i>Báča V, Bartáková H, Votruba J, Otahal M.</i>	Ionizing radiation-induced metabolic and histopathologic changes in the rat spinal cord <i>Bálenťová S, Hnilicová P, Kalenská D, Murín P, Hajtmanová E, Lehotský J, Adamkov M.</i>
9.15-9.30	Pelvic bone stiffness as a descriptor of bone quality: pilot study and feasibility <i>Kuchař M., Henyš P.</i>	Role of hyperhomocysteinemia in neurodegeneration in rat entorhinal cortex-hippocampal system after global brain ischemia: A progression of Alzheimer's disease-like pathological features? <i>Kovalská M., Hnilicová P, Kalenská D, Tóthová B, Adamkov M, Lehotský J.</i>



9.30-9.45	Morphological and functional aspects of neurogenic pulmonary edema <i>Šedý J.</i>	GPx4 in preimplantation embryos and female genital organs of rat <i>Kreheľová A., Mihalík J., Kovaříková V., Domoráková I., Solár P., Pavliuk-Karachentseva A., Rybárová S., Hodorová I.</i>
9.45-10.00	Revised anatomical and histological terminology of the ear <i>Kachlík D., Broman J., Tubbs R.S., Baud R., ten Donkelaar H.J.</i>	Wnt signalling pathway in cervical cancer <i>Mešťanová V., Klinerová B., Škuciová V., Drahošová S., Adamkov M.</i>
10.00-10.30	Coffee Break (Hotel's Restaurant)	
	Congress Hall Bystrá	Lounge Trigan
10.30-11.45 Bystrá 10.30-11.00 Trigan	Teaching Anatomy Chairs: Mechírová E., Sedmera D.	Histology and Embryology 2 + Anatomy – Head and Neck, Trunk, Locomotor System, CNS and PNS Chairs: Naňka O., Kubíková E.
10.30-10.45	Introduction to brain-based educational methods <i>Boleková A., Výbohová D., Hešková G., Hodorová I.</i>	Methods for visualisation of phagocytic apoptotic cells in mouse blastocysts <i>Pisko J., Kovaříková V., Fabian D.</i>
10.45-11.00	The challenge for teachers of anatomy: the birth year 2000 comes <i>Hájek P.</i>	Lymphatic drainage system in brain dura <i>Maloveská M., Danko J., Humeník F., Mojžišová Z., Krešáková L., Vdoviaková K., Teley J., Čížek M., Murgoci A-N., Čížková D.</i>
11.00-11.15	Case reports – motivational component of anatomy education <i>Lisková K., Hájek P.</i>	
11.15-11.30	Evaluation of the effectiveness of the presentation of virtual histology slides by students during classes <i>Tauber Z., Čížková K., Lichnovská R., Lacey H., Erdosová B., Žižka R., Kamarád V.</i>	
11.30-11.45	Producing cadaveric videos for studying anatomical structures of head and neck <i>Vecanová J., Kluchová D., Matěffy S., Hvizdošová N., Hodorová I., Bona M., Majerník J.</i>	
11.45-12.00	Collective Photography	
12.00-13.00	Lunch (Hotel's Restaurant)	



Congress Hall Bystrá

13.00-13.30	Poster Session 1 1-28	
13.30-14.45	Developmental Biology, Molecular and Cell Biology 1 Chairs: Hodorová I., Smetana K.	
13.30-13.45	Western blotting and colorectal lesions <i>Adamkov M., Drahošová S., Tatarková Z.</i>	
13.45-14.00	A microscopic view on the glycocalyx of the endothelial cells and erythrocytes <i>Čížková D., Bezrouk A., Mokrý J., Zadák Z.</i>	
14.00-14.15	Derivation of primitive neural stem cells from murine embryonic stem cells <i>Mokrý J., Písal R.V., Chvátalová J., Voborníková M.</i>	
14.15-14.30	Development of atrial form and function <i>Sedmera D.</i>	
14.30-14.45	Cancer ecosystem and its role in tumor progression <i>Smetana K. Jr., Dvořánková B., Kodet O., Szabo P., Strnadová K., Kolář M., Kupcová-Skalníková H., Lacina L.</i>	
14.45-15.15	Coffee Break (Hotel's Restaurant)	
	Congress Hall Bystrá	
15.15-16.15	Histochemistry and Cytochemistry 1 Chairs: Rybářová S., Tóth Š.	
15.15-15.30	Isolation of enriched microglia from rat neocortex <i>Čížková D., Murgoci A-N., Cubínková V., Danko J., Humeník F., Mojžišová Z., Maloveská M., Krešáková L., Vdoviaková K., Teleky J.</i>	
15.30-15.45	Raman spectroscopy and blood leukocytes research <i>Křížková V., Buřka J., Vaňková L., Hrubá K., Jíchová H., Šigutová P., Štambachová A., Nevoral J., Maršálová L., Bouř P.</i>	
15.45-16.00	Clearance of lead oxide nanoparticles following subchronic inhalation <i>Smutná T., Dumková J., Vrlíková L., Kotasová H., Dočekal B., Čapka L., Tvrdohová M., Mikuška P., Večeřa Z., Křůmal K., Coufalík P., Vaculovič T., Hamp l., Buchtová M.</i>	
16.00-16.15	Subarachnoid hemorrhage induces cellular and enzymatic changes in the choroid plexus <i>Solár P., Dubový P., Jančálek R., Joukal M.</i>	
16.15-16.30	Sponsor Lecture IPPM: Fekálna mikrobiálna transplantácia – jej možnosti využitia <i>Kuchta M.</i>	
16.30-17.00	CAS Plenary Meeting, CSHC Plenary Meeting (Congress Hall Bystrá)	SAS Plenary Meeting (Lounge Trigan)
19.00-24.00	Social Evening (Hotel's Restaurant)	



SATURDAY SEPTEMBER 7		
8.30-9.00	Registration (Hotel's Entrance Corridor)	
	Congress Hall Bystrá	Lounge Trigan
9.00-10.15	Developmental Biology, Molecular and Cell Biology 2 Chairs: Boleková A., Mokrý J.	Histochemistry and Cytochemistry 2 Chairs: Domoráková I., Výbohová D.
9.00-9.15	Majority of cerebrospinal fluid-contacting neurons in the spinal cord of C57Bl/6N mice is present in ectopic position unlike in other studied experimental mice strains and mammalian species <i>Ševc J., Tonelli Gombalová Z., Košuth J., Alexovič Matiašová A., Daxnerová Z.</i>	Expression of Toll-like and Formyl peptide receptors in choroid plexus after subarachnoid haemorrhage <i>Bental I., Solár P., Levin S., Zamani A., Dubový P., Joukal M.</i>
9.15-9.30	Impact of canine mesenchymal stem cells products on angiogenesis in chicken chorioallantoic membrane <i>Humeník F., Mojžišová Z., Danko J., Mudroňová D., Farbáková J., Maloveská M., Petrovová E., Čížek M., Čížková D.</i>	Relationship between PD-L1 expression and TILs in malignant melanomas <i>Škuciová V., Drahošová S., Výbohová D., Adamkov M.</i>
9.30-9.45	The role of the fractalkine/CX3CR1 in the development of the trigeminal neuropathic pain <i>Kubičková L., Klusáková I., Dubový P.</i>	The effect of betanin parenteral pretreatment on liver parenchyma after jejunal ischemia reepfusion injury <i>Tóth Š., Urda M., Kalpakidis T., Pribula M., Kušnier M., Mechírová E., Maretta M.</i>
9.45-10.00	Characteristics of canine umbilical cord mesenchymal stem cells <i>Mojžišová Z., Humeník F., Mudroňová D., Madari A., Farbáková J., Horňáková L., Danko J., Maloveská M., Čížková D.</i>	Paclitaxel treatment activates receptors for mitochondrial damage associated molecular patterns in the choroid plexus <i>Zamani A., Kubičková L., Dubový P., Joukal M.</i>
10.00-10.15	Rudimentary structures and their fates in the mouse dentition <i>Hovořáková M., Steklíková K., Dalecká L., Pasovská M., Zahradníček O., Tucker A.S.</i>	Expression of sodium channel nav 1.8 in dorsal root ganglia after paclitaxel treatment <i>Levin S., Dubový P., Joukal M.</i>
10.15-10.45	Coffee Break (Hotel's Restaurant)	
10.45-11.15	Poster Session 2 29-52 (Congress Hall Bystrá)	
11.30-12.00	Closing Ceremony (Congress Hall Bystrá)	
12.00-13.00	Lunch (Hotel's Restaurant)	

ANATOMY – HEAD AND NECK, TRUNK, LOCOMOTOR SYSTEM, CNS AND PNS

- 1. Domoráková Iveta** Ischemia-reperfusion and bradykinin postconditioning: morphometric evaluation of the edematous grey matter lesions in rabbit spinal cord
Domoráková I., Fagová Z., Danková M., Mechírová E., Stebnický M.
- 2. Korim Filip** Preparation method of ligamentous apparatus specimen of the stifle joint in horses
Korim F., Žert Z., Karamanová M., Horňáková P.
- 3. Krešáková Lenka** Anatomic investigation of the cranial cardiac vein in rats
Krešáková L., Vdoviaková K., Petrovová E.
- 4. Šimaiová Veronika** Articular cartilage defects regeneration using a chitosan based scaffold in the sheep
Šimaiová V., Tomčo M., Holovská K., Krešáková L., Vdoviaková K., Petrovová E.
- 5. Vdoviaková Katarína** The importance of the rat jejunal arteries in the experimental surgery
Vdoviaková K., Krešáková L., Petrovová E., Šimaiová V.

HISTOLOGY AND EMBRYOLOGY

- 6. Almášiová Viera** Influence of electromagnetic radiation on kidney structure of prenatally exposed rats
Almášiová V., Holovská K., Andrašková S., Cigánková V.
- 7. Hamouzová Paula** Canine and feline mast cells: Using different fixation fluids
Hamouzová P., Čížek P., Bartošková A.
- 8. Holovská Katarína** The effects of prenatal exposure to electromagnetic radiation on rat liver
Holovská K., Almášiová V., Andrašková S., Beňová K., Cigánková V.
- 9. Kadlecová Simona** Confirmation of ultrastructural findings by immunofluorescent examination in primary ciliary dyskinesia
Kadlecová S., Martinů V., Pohunek P., Uhlík J.
- 10. Kučera Tomáš** The increased number of mast cells in epicardial adipose tissue is associated with coronary artery disease
Rozsíválová K., Pierzynová A., Kloučková J., Lindner J., Lipš M., Haluzík M., Kučera T.
- 11. Kvasilová Alena** The development of the cardiac conduction system in avians: chick versus other species
Kvasilová A., Olejníčková V., Gregorovičová M., Sedmera D.
- 12. Pierzynová Aneta** Myocardial and adipose tissue CD68+ cells are associated with cardiovascular and metabolic disease in heart surgery human patients
Pierzynová A., Šrámek J., Cinkajzlová A., Kratochvílová H., Lindner J., Haluzík M., Kučera T.
- 13. Polakovičová Simona** The morphological structure of the human pineal gland at light microscopic and electron microscopic levels
Polakovičová S., Liška J., Rísová V., Kopáni M., Krivošíková L., Polák Š.

- 14. Šrámek Jaromír** Mathematical morphology as a tool for quantitative description of an adipose tissue on the histological slide
Šrámek J., Kučera T.
- 15. Teshayev Shuxrat** Morphological changes of small intestine and testes in norm, in irradiation and under the influence of the biostimulant – ASD-2
Teshayev Sh., Haribova E., Radjabov A., Shukurov I., Khojiev D., Khasanova D., Baymuradov R.
- 16. Uhlík Jiří** Upregulated vessel formation in the contracted side of the clubfoot
Novotný T., Uhlík J., Eckhardt A., Ošťádal M.
- 17. Vohníková Markéta** Proportions of the growing chick embryo body wall on embryonic day 6
Vohníková M., Škapová K., Hubičková Heringová L., Maňáková E., Zemanová Z.
- 18. Vrabcová Michaela** Hypothalamic changes associated with chronic liquid nutrition feeding
Vrabcová M., Polák Š.

HISTOCHEMISTRY AND CYTOCHEMISTRY

- 19. Danková Marianna** The effects of remote conditioning on spinal cord ischemia/reperfusion injury in rabbit
Danková M., Domoráková I., Fagová Z., Stebnický M., Mechírová E.
- 20. Fagová Zuzana** Bradykinin postconditioning influences neuronal survival and HSP-70 immunoreaction in the rabbit spinal cord after ischemia
Fagová Z., Domoráková I., Danková M., Mechírová E., Stebnický M.
- 21. Hladová Alena** Expression of survivin and paclitaxel resistance in rat mammary tumor cells
Hladová A., Rybárová S., Hodorová I.
- 22. Hvizdošová Natália** Fluoro-jade positive neurons in the contralateral hemisphere after focal cerebral ischemia of rat
Hvizdošová N., Horváthová F., Kluchová D., Kolesár D., Matéffy S., Vecanová J., Bona M.
- 23. Kolesár Dalibor** Immunohistochemical analysis of the dorsal root entry zone and the dorsal funiculus of the spinal cord in rat
Kolesár D., Maršala J.†, Filipčík P.
- 24. Kováčová Zuzana** Immunohistochemistry of colorectal carcinoma: expression of survivin
Kováčová Z., Hodorová I.
- 25. Mechírová Eva** 5G4 immunohistochemistry in the enteric nervous system
Mechírová E., Škorvánek M., Tóth Š., Gdovinová Z.
- 26. Rybárová Silvia** p53 expression in relation to paclitaxel resistance in rat mammary tumor cells
Rybárová S., Hladová A., Hodorová I.
- 27. Pavliuk-Karachevtseva Andriana** GPx8 expression in cancer cells of colorectal carcinoma
Pavliuk-Karachevtseva A., Krehelová A., Mihalik J., Hodorová I.
- 28. Ševčíková Zuzana** The effect of FGF2 in wound healing
Ševčíková Z., Danešová M., Buchtová M.

CLINICAL AND IMAGING ANATOMY

- 29. Al-Redouan Azzat** Scapula – new anatomical terms of not yet described structures: a modified Delphi approach
Al-Redouan A., Kachlík D.
- 30. Bartoš Martin** Micro-CT evaluation of heart architecture in experimental animals
Bartoš M., Olejníčková V., Kvasilová A., Gregorovičová M., Sedmera D.
- 31. Bevízová Katarína** Morphometric study of variable origin of the recurrent artery of Heubner
Bevízová K., El Falougy H., Zohdi V., Dovalová D., Mifkovič A., Kubíková E.
- 32. Dovalová Daniela** Comparative anatomy of human and animal eyeballs
Dovalová D., Zohdi V., El Falougy H., Mifkovič A., Bevízová K., Kubíková E.
- 33. El Falougy Hisham** Determination of anatomical extent of the urinary bladder cancer
El Falougy H., Bevízová K., Zohdi V., Dovalová D., Mifkovič A., Kubíková E.
- 34. Herma Tomáš** Variation of a duplicated superficial branch of radial nerve and a two-bellied brachoradialis muscle: epidemiology and clinical significance
Herma T., Kachlík D.
- 35. Lovásová Květuše** Geometric and morphometric contribution to the maxillary sinus visualization
Lovásová K., Hudák R., Tóth T., Rajtuková V., Živčák J., Kachlík D., Boleková A., Hodorová I.
- 36. Mifkovič Andrej** Macroscopic relations of internal capsule and its connections
Mifkovič A., Zohdi V., El Falougy H., Dovalová D., Bevízová K., Kubíková E.
- 37. Musil Vladimír** Anatomical study of arteria iliaca externa – case study of patient with hip joint pain
Musil V., Šeremeta M., Krbcová-Moudrá V., Riedlová J., Mrzílková J., Zach P., Lami J.
- 38. Naňka Ondřej** Anatomical study of fibular notch
Naňka O., Fojtík P., Bartoníček J.
- 39. Stingl Josef** Vascular supply of the subepicardial nerves and Purkinje fibers of the porcine heart
Stingl J., Musil V., Šach J., Vránová J., Suchomel Z., Kudrna V., Riedlová J., Kachlík D., Zach P.
- 40. Teleky Jana** X-ray computed tomography as a tool for determining enamel thickness
Teleky J., Maloveská M., Krešáková L., Tóth T.

TEACHING ANATOMY

- 41. Výbohová Desanka** What is more effective for academic performance in anatomy – intensive anatomy course or regular weekly practicals?
Výbohová D., Boleková A., Hešková G., Mellová Y.

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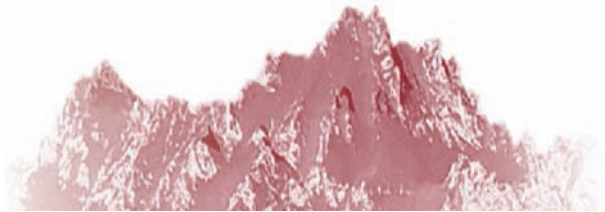
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ABSTRACTS



INTERLEUKIN-6 CONTRIBUTES TO INITIATION OF NEURONAL REGENERATION PROGRAM IN THE REMOTE DORSAL ROOT GANGLIA NEURONS AFTER SCIATIC NERVE INJURY

Dubový P., Hradilová-Sviženská I., Klusáková I., Brázda V., Joukal M.

Department of Anatomy, Cellular and Molecular Research Group, Faculty of Medicine, Masaryk University, Kamenice 3, Brno, CZ-62500, Czech Republic

To assess the potential role of IL-6 in sciatic nerve injury-induced activation of a pro-regenerative state in remote dorsal root ganglia (DRG) neurons, we compared protein levels of SCG-10 and activated STAT3, as well as axon regeneration in IL-6 knockout (IL-6ko) mice and their wild-type (WT) counterparts.

Unilateral sciatic nerve compression and transection upregulated SCG-10 protein levels and activated STAT3 in DRG neurons not only in lumbar but also in cervical segments of WT mice. A pro-regenerative state induced by prior sciatic nerve lesion in cervical DRG neurons of WT mice was also shown by testing for axon regeneration in crushed ulnar nerve.

DRG neurons from IL-6ko mice also displayed bilaterally increased levels of SCG-10 and STAT3 in both lumbar and cervical segments after sciatic nerve lesions. However, levels of SCG-10 protein in lumbar and cervical DRG of IL-6ko mice were significantly lower than those of their WT counterparts. Sciatic nerve injury induced a lower level of SCG-10 in cervical DRG of IL-6ko than WT mice, and this correlates with significantly shorter regeneration of axons distal to the crushed ulnar nerve. These results suggest that IL-6 contributes, at the very least, to initiation of the neuronal regeneration program in remote DRG neurons after unilateral sciatic nerve injury.

KOŠICE ANATOMY – INSIGHT INTO HISTORY

Hodorová I.

Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: ingrid.hodorova@upjs.sk

The Department of Anatomy was founded by MUDr. Vladimír Munka on 1st August 1949. Prof. MUDr. Vladimír Munka, DrSc. was the first Head of Anatomy Department at the Faculty of Medicine in Košice and he was also one of the founders of the morphology practice in Košice. After graduating from Comenius University in 1948, he became a research assistant at the Department of Normal Anatomy at the Faculty of Medicine in Bratislava. As a young and inexperienced assistant, he was sent to Kosice. Prof. MUDr. Vladimír Munka, DrSc. established the anatomy practice in Košice from the grounds, having only a couple of empty rooms to work in. Eventually, he managed to obtain the necessary equipment for the laboratories and autopsy rooms. In his career as an academic, he specialised in research of the lymphatic system. Prof. MUDr. Vladimír Munka, DrSc identified the trend and started gathering materials and equipment for studying the lymphatic system in humans and experimental animals. His research made a valuable contribution to the global anatomic study lungs as did his lecture on The Pulmonary and Cardiac Regional Lymph Nodes presented at the 11th International Anatomic Congress in Mexico City. He initiated the creation of the first textbook on Systematic Anatomy and was a continuous member of the Czechoslovak Anatomic Society. He was also an editorial board member of the academic magazine Folia Morphologica and he was in the committees responsible for awarding academic titles CSc. and DrSc. Pavol Jozef Šafárik University in Košice awarded him with the Silver Medal, and he also received the Honorary Medal from the Medical Faculty. On the 7th of August 1986, the whole community of medical practitioners gathered to say the “last goodbye” to the first professor of anatomy and a member of the academic senate at the Medical Faculty of Pavol Jozef Šafárik University in Košice.

The following head of the Anatomy Department was Prof. MUDr. Alojz Gomboš, DrSc. He was in his fourth year of medical studies when he began teaching a course on Human Anatomy at the Faculty of Medicine. His expertise in the field was also recognised abroad. A two-year-long tenure at the Faculty of Medicine in Bagdad and Malta allowed him to bring different and modern approaches back to Kosice where he established the Laboratory for Experimental Morphology at the Department of Anatomy. Since 1986 until 1989, he was also a director at the Department of Histology and Embryology at the Faculty. His research and academic work were indeed very productive. In collaboration with the team of surgeons, he created an operational guideline for kidney transplantations. The research on cardiovascular and cerebrovascular medicine focused on neurotomical intervention of lymphatic organs and

heart also belonged to his areas of specialisation. From 1994 to 1996, he led the research team responsible for the research of Functional Morphology of Mutual Relationships of Nervous, endocrine and lymphatic system. Many of his research outcomes were presented at national and international scientific conferences. Prof. MUDr. Alojz Gomboš, DrSc. passed away in 2000.

For over 70 years, the Department has been gradually improving the quality of teaching and the departmental premises and facilities, including dissecting rooms, lecture theatres and many more seminar rooms.

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ASSOCIATION OF INDUCED HYPERHOMOCYSTEINEMIA WITH ALZHEIMER'S DISEASE-LIKE NEURODEGENERATION IN RAT CORTICAL NEURONS AFTER GLOBAL ISCHEMIA-REPERFUSION INJURY

Kovalská M.¹, Tóthová B.², Kovalská L.³, Tatarková Z.⁴, Kalenská D.⁵, Tomašcová A.², Adamkov M.¹, Lehotský J.^{2,4}

- 1 *Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, Slovak Republic*
- 2 *Biomedical Center Martin, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, Slovak Republic*
- 3 *Institute of Maxillofacial Surgery, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, , Martin, Slovak Republic*
- 4 *Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, , Martin, Slovak Republic*
- 5 *Department of Anatomy, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, Slovak Republic*

e-mail: kovalska2@uniba.sk

Introduction: Alzheimer's disease (AD) belongs to the most severe neurodegenerative diseases typical for massive neuronal loss leading to dementia and death. Its pathology manifests mainly in the hippocampus and certain layers in the cerebral cortex, despite of the fact of the different vulnerability of cortical neurons to the neurodegeneration. The precise etiology of AD is unclear. Several risk factors have been suggested, including elevated levels of homocysteine in plasma (hHcy) and brain stroke. hHcy is assumed to be an independent, strong risk factor for both dementia and stroke. Though, the precise molecular background of these mechanisms is not completely understood.

Material and Methods: Adult male Wistar rats were treated by subcutaneous injection of 0.45 $\mu\text{mol/g}$ of Hcy in duration of 14 days. At the day 15, rats underwent global ischemia-reperfusion injury (IRI) developed by 4-vessels occlusion lasting for 15 minutes. The reperfusion period was set up to 72 hours. After sacrifice, the brains were harvested, frozen, cut and then processed for biochemical, histo-morphological as well as immunofluorescence analyses.

Results: The results showed that induction of hHcy with subsequent IRI aggravate the neuronal cell death in brain cortex. We demonstrated degeneration of cortical neurons, alterations in number and morphology of tissue astrocytes and dysregulation of oxidative balance with increased membrane protein oxidation. Moreover, an immunohistochemical analysis of tau protein and β -amyloid peptide showed that combination of hHcy with the IRI might lead to the progression of AD-like pathological features.

Conclusion: Our results suggest that the combination of the two risk factors hHcy and IRI, aggravates the neurodegenerative process and leads to the development of AD-like pathology in cerebral cortex.

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WESTERN BLOTTING AND COLORECTAL LESIONS

Adamkov M.¹, *Drahošová S.*², *Tatarková Z.*³

¹ *Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovak Republic*

² *Hermes LabSystems, s.r.o., Bratislava, Slovak Republic*

³ *Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovak Republic*

e-mail: marian.adamkov@jfmmed.unibsk

Introduction: Multifunctional protein survivin plays an essential role in negative regulation of apoptosis. It is widely expressed in the most human cancers and also in premalignant entities. Due to large quantitative differences in survivin expression degree in normal adult tissues and in corresponding premalignant and malignant lesions, survivin appears to represent a promising tumor biomarker and a prognostic factor.

Material and Methods: Using western blotting, we examined survivin expression in 16 colorectal adenomas and 16 colorectal carcinomas to determine its association with degree of dysplasia and localization.

Results: Overall, protein in question shows significant differences between proximal and distal localization of colorectal lesions. Furthermore, there are significant differences between adenomas and carcinomas in a given localization.

Conclusions: Beside immunohistochemistry, western blot analysis reveals that survivin may represent the most promising diagnostic, differential-diagnostic and prognostic biomarker which should be introduced in histological evaluation of colorectal lesions.

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EXPERIENCE WITH PRACTICAL TRAINING OF BRONCHOSCOPY IN THE EDUCATIONAL CENTER FOR ANATOMY, ENDOSCOPY AND MININVASIVE TECHNIQUES

Báča V.^{1,2}, **Bartáková H.**³, **Votruba J.**³, **Kachlík D.**¹, **Otáhal M.**⁴

¹ *Educational Center for Anatomy, Endoscopy and Miniinvasive Techniques, Third Faculty of Medicine, Charles University, Prague, Czech Republic*

² *Department of Health Care Studies, College of Polytechnics Jihlava, Czech Republic*

³ *Department of Pulmonology, First Faculty of Medicine, Charles University and VFN, Prague, Czech Republic*

⁴ *Department of Anesthesiology and Critical Care, First Faculty of Medicine, Charles University and VFN, Prague, Czech Republic*

e-mail: vaclav.baca@vspj.cz

Introduction: Practical teaching of clinical medical skills, for example bronchoscopy, at the departments of anatomy is one of the most sought-after ways of improving qualification not in the pulmonology only but in the intensive care too. Knowledge of anatomy during examination or therapeutic intervention requires sufficient anatomy knowledge. We have been teaching bronchoscopy in our Educational Center since 2012. Lectures on cadavers led by pulmonologists and clinical anatomists is very reasonable approach of teaching.

Methods: From 2012-2015 practical courses were organized and evaluated, donor bodies embalmed according to Thiel's method were used, and then the questionnaires were e-mailed to former participants focused on impact of the workshop on their clinical practice and a need of their follow-up education.

Results: 12 practical courses for 141 participants were organized and evaluated, overall, 98 questionnaires were returned and 77% of participants declared application of practical skills obtained in the workshop in their clinical practice, 74% declared usage of the theoretical know-how, more than 90% expressed their wish to take a part in advanced training.

Conclusions: For clinical experts in different branches hands-on courses and demonstrations of flexible bronchoscopy on cadavers is one of the possibility how to increase knowledge of anatomy for clinical needs and finally increase the quality of the medical care including the expansion of specialized postgraduate education in miniinvasive techniques.

IONIZING RADIATION-INDUCED METABOLIC AND HISTOPATHOLOGIC CHANGES IN THE RAT SPINAL CORD

Báľentová S.¹, Hnilicová P.², Kalenská D.³, Muriň P.⁴, Hajtmanová E.⁴, Lehotský J.^{2,3}, Adamkov M.¹

¹ Institute of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

² Division of Neurosciences, Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

³ Institute of Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

⁴ Department of Radiotherapy and Oncology, Martin University Hospital, Martin, Slovak Republic

e-mail: balentova@uniba.sk

Introduction: Head and neck cancer represent approximately 4% of all malignant tumors in Europe and United States. Nowadays, thanks to more advanced irradiation techniques, the adverse effects of radiotherapy caused by spinal radiation are minimized. Although sufficient clinical knowledge regarding the side effects of radiotherapy, there is still a lack of experimental studies to broaden the knowledge in this field.

Material and methods: Adult male Wistar rats were divided into a control (n = 10) group and a second group (n = 10), which was irradiated with a total dose of ionizing gamma (cD = 16Gy, divided into 2x8Gy, once weekly for 2 weeks). The irradiated volume included spinal segments C1-T2. 11-12 weeks after irradiation, we examined C2-C4 spinal cord segments by in vivo proton magnetic resonance spectroscopy (1H MRS). In the selected spinal cord segments, metabolite concentrations and possible incidence of inflammatory and necrotic changes were evaluated. After sacrificing the animals for 12-13 weeks after irradiation, we identified the markers of individual cell types in spinal cord tissue sections by immunofluorescence staining and analyzed the quantitative changes by image analysis.

Results: Based on ¹H MRS examination, we found a significant decrease in the ratio of total N-acetylaspartate to total creatine (tNAA / tCr) and total choline to tCr (tCho / tCr). Although quantitative analysis of tissue preparations is still under assessment, preliminary data indicate an increase in the number of astrocytes in the gray matter and changes in the density of myelin sheets in the white matter of the spinal cord.

Conclusions: Fractionated irradiation of the spinal cord has led to the development of subacute metabolic and histopathological changes that may result in the development of late symptoms. Applying these findings in human medicine, late symptoms after spinal cord irradiation could be very serious health complications for cancer patients.

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EXPRESSION OF TOLL-LIKE AND FORMYL PEPTIDE RECEPTORS IN CHOROID PLEXUS AFTER SUBARACHNOID HEMORRHAGE

Bental I., Solár P., Levin S., Zamani A., Dubový P., Joukal M.

Department of Anatomy, Faculty of Medicine, Masaryk University in Brno, Czech Republic

e-mail: mjoukal@med.muni.cz

Introduction: A subarachnoid hemorrhage is a life threatening state which can be caused by aneurysm rupture, arteriovenous malformation or trauma. In our previous experiments, we found cellular immune reaction in choroid plexus after SAH. Therefore, the main aim of presented study was to investigate receptor mediated reaction to SAH. We focused on the expression of toll-like receptors (TLR) 4, 9 and formyl-peptide receptor (FPR) 2 after SAH.

Material and Methods: The experiments were performed on 16 Wistar rats (males, 250g). SAH was induced by injection of blood into the cisterna magna, while control group received artificial CSF. The animals were left to survive for 1, 3, and 7 days from the application. After time of survival, the SAH and control together with naïve rats were perfused transcardially with Zamboni's fixative. Coronal cryostat sections through the brains were cut and immunostained for TLR4, TLR9, and FPR2.

Results: The presence of TLR4, TLR9, and FPR2 immunofluorescence were found in CP cuboidal cells in all animal groups. SAH animals have shown increased expression of TLR 4 in the plasma membrane after 1 day, and TLR 9 in the cytoplasm after 7 days, in comparison to control and naïve groups. CP of control group have shown increased expression TLR4 and TLR9 in comparison to naïve group. The expression of FPR2 among the groups did not shown any significant change.

Conclusions: In conclusion, we found upregulation of TLR4 and TLR9 in CP cuboidal cells of SAH animals and control, probably due to blood degeneration products and increased intracranial pressure. The increased expression of TLR4 during day 1 and TLR9 during day 7, correlates with active and regenerative phase of inflammation after SAH, respectively.

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INTRODUCTION TO BRAIN-BASED EDUCATIONAL METHODS

Boleková A.¹, **Výbohová D.**², **Hešková G.**², **Hodorová I.**¹

¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

e-mail: adriana.bolekova@upjs.sk

Introduction: Brain-based education is highly engaging and practical teaching tool with countless strategies of teaching with the brain in mind. This method is the purposeful engagement of strategies based not on myths but on principles derived from solid brain research in education.

Material and Methods: Brain-based educational results will be used during the teaching and learning which discover the brain's learning methods by research-based and practical strategies.

Results: In this work, the impact of brain based learning, with the core principles and with critical links to student achievement are introduced. This shows how much more influence teachers on students have than they thought. Teachers need to know how way of teaching can grow new brain cells, how the brain responds to emotions, what is the key to our memory etc. This type of education regulates cognition, mood, and memory of students. Brains can change, if you know how to do it.

Conclusions: The brain-compatible education is the new science of learning. The background of knowledge about and practical skills are important for academic success of teachers and intervene in positive changes. Change the way and accomplish it with our own learning will have positively impact to work with students.

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A MICROSCOPIC VIEW ON THE GLYCOCALYX OF THE ENDOTHELIAL CELLS AND ERYTHROCYTES

Čížková D.¹, Bezrouk A.², Mokřý J.¹, Zadák Z.³

¹ Department of Histology and Embryology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

² Department of Medical Biophysics, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

³ Department of Research and Development, University Hospital, Hradec Králové, Czech Republic

e-mail: cizkovad@lfhk.cuni.cz

Introduction: The glycocalyx is a gel-like structure on the luminal surface of the endothelial cells and erythrocytes formed by 3D network of membrane-bound proteoglycans and glycoproteins and integrated soluble molecules with many functions in vascular physiology and pathology. Because it is a highly dynamic and fragile structure and traditional tissue processing usually results in a partial or complete loss of its components, its visualization is greatly challenging.

Material and Methods: In our work we visualized the glycocalyx of the endothelial cells and erythrocytes by TEM and lectin histochemistry for subsequent evaluation of newly developed infusion solutions. In conventional TEM, polysaccharides interacts poorly with common postfixation solutions and thus the glycocalyx is indistinguishable from its environment. The cationic dyes binding to the negative surface of the glycocalyx and forming electron-dense contrast enable its visualization. We applied the cationized ferritin to visualize the glycocalyx of the HUVEC cells and of the human erythrocytes. We also used fluorochrome conjugated lectin WGA that selectively binds to N-acetylglucosamine and sialic acid residues as constituents of the glycocalyx to detect this structure in the human kidney and erythrocytes.

Conclusions: Together with other advanced methods such as cryo-TEM, cryo-SEM, FIB-SEM and AFM, microscopic techniques are currently crucial and promising for studying the glycocalyx of the endothelial cells and erythrocytes.

This work was supported by **PROGRES Q40/06** and **Q40/09** from MŠMT.

ISOLATION OF ENRICHED MICROGLIA FROM RAT NEOCORTEX

Čížková D.^{1,2}, Murgoci A-N.¹, Cubínková V.¹, Danko J.², Humeník F.², Mojžisová Z.², Maloveská M.², Krešáková K.², Vdoviaková K.², Teleky J.²

¹ Institute of Neuroimmunology, Slovak Academy of Science, Bratislava, Slovak Republic

² Institute of Anatomy, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

e-mail: cizkova.dasa@gmail.com

Introduction: Microglia possess a brain resident macrophages that are important sentinels constituting the first line of response to injury, infection or inflammatory processes. They strongly interact with other brain cells regulating neural circuits and synaptic transmission. It has been established that microglia derived from different anatomical CNS regions have an anatomical territory-specific phenotype. The aim of present study was to examine the efficacy of magnetic separation technique on purity of microglia population isolated from neocortex of rats.

Material and Methods: Neural progenitors isolated from brain cortex of rat neonatal pups P1-P6 were assessed for efficacy of CD11b/c MicroBeads separation, by performing immunocytochemistry using antibodies against CD11b receptor and Iba1 protein in primary microglia culture after 10 days cultivation.

Results: Based on magnetic separation - CD11b antibody attached to microbeads we have confirmed enhanced purity of microglia derived from neonatal rats between P1-P6. The analyses of confocal images showed that >94 % of cells are positive for CD11b antibody and for Iba1 antibody, thus confirming microglia phenotype. Microglia showed heterogeneous cell population including amoeboid shape as well as ramified morphology. This pattern is in line with *in vitro* data indicating typical morphology of primary microglia isolated by different protocols.

Conclusions: In conclusion, our data clearly confirmed that neonatal cortex derived from new born rats up to 6 days contain microglia expressing CD11b and less myelin which is beneficial for specific separation procedure. In conclusion, magnetic separation is an efficient tool to obtain enriched population of microglia which serves as valuable source of primary culture for different *in vitro* experiments.

The study was supported by the Grants APVV 15-0613, ERANET-AxonRepair, VEGA 1/0571/17, VEGA 2/0146/19, IGA UVLF 02/2019, IGA UVLF 06/2018.

THE CHALLENGE FOR TEACHERS OF ANATOMY: THE BIRTH YEAR 2000 COMES

Hájek P.

Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

e-mail: hajekp@lfhk.cuni.cz

Medical faculties in the Czech Republic are forced to admit an extra 15% of students in the General Medicine branch. Nevertheless, the profession suffers from a bad reputation which discourages elite students from their studies. Moreover, generations change: our experience together with sociological analyses show a decline in both mental and motivational aspects in recent years.

At our department about one or two decades ago, we have been producing simplified educational materials for easier learning of our students. As the time goes, it becomes obvious that now it is time to prepare materials beyond pre-selected phrases and statements, for stimulation of thinking. Provided that 4th year students forget to “guesstimate” 80% of anatomy, we are justified to reduce ballast description. We may decrease the number of terms but we should not reduce associations in anatomy.

We fight with students’ passivity in laboratory classes, which is why we are considering the use of worksheets for some topics. For each topic we already prepared case reports, in which we present real problems in medicine and their solutions based on anatomical knowledge (presented separately). Peer-to-peer education was our dream, but was not possible due to a disagreement with the previous management of our faculty. Currently it is limited, owing to the crossing of schedules.

Implementation of modern educational methods causes a big discussion. The author accepts that devices like Virtual Dissection Tables or project Human Anatomy Virtual Reality, could motivate the students to enroll into the faculty and also make the studies more entertaining. The question is if such expensive tools will help the students pass the 1st year of Medical school, or if it is more a pride of the Dean’s office and a benefit for commercial companies. The author would like to discuss alternative views and opposing arguments.

RUDIMENTARY STRUCTURES AND THEIR FATES IN THE MOUSE DENTITION

Hovořáková M.¹, **Steklíková K.**^{1,2}, **Dalecká L.**^{1,3}, **Pasovská M.**^{1,3}, **Zahradníček O.**¹,
Tucker A.S.^{1,4}

¹ *Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic*

² *Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic*

³ *Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, Czech Republic*

⁴ *Craniofacial Development & Stem Cell Biology Division, King's College London, London, United Kingdom*

e-mail: maria.hovorakova@iem.cas.cz

Introduction: During embryogenesis, the developing tooth germ forms when the oral epithelium buds into the underlying neural crest derived mesenchyme. This budding is a common mechanism for the development of many different skin appendages (hairs, scales, feathers, teeth, glands). During the development of all these epithelial organs, epithelial cells play a key role in controlling the morphogenesis of the organ and express specific sets of signalling molecules. In the early tooth germ a specific group of these epithelial cells have been characterized as signalling centres.

Material and Methods: To help to elucidate the unsolved questions on early dental development in the mouse we use cell lineage tracing experiments, in situ hybridization and immunostaining, combined with in-vitro culturing experiments and modern imaging technologies.

Results and Conclusions: Here we show that the formation of functional molars and incisors in mice is anticipated by rudimentary tooth buds that appear anteriorly and earlier than the tooth germs of the functional teeth in both molar and incisor areas. These rudimentary structures reach distinct stages of development and are suppressed or incorporated into the functional teeth. They form their own signalling centres expressing signalling molecules, such as Sonic hedgehog, that control the cells around them to form distinct morphologies. We focus on how the early signaling centers in the odontogenic areas are determined and how they relate to later forming signalling centres that control tooth shape. We also document that the early events in the oral cavity are common for teeth and other adjacent organs, eg. oral vestibule.

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IMPACT OF CANINE MESENCHYMAL STEM CELLS PRODUCTS ON ANGIOGENESIS IN CHICKEN CHORIOALLANTOIC MEMBRANE

Humenik F.¹, **Mojžišová Z.**¹, **Danko J.**¹, **Mudroňová D.**¹, **Farbáková J.**¹, **Maloveská M.**¹, **Petrovová E.**¹, **Čížek M.**¹, **Čížková D.**^{1,2}

¹ *Institute of Anatomy, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*

² *Institute of Neuroimmunology, Slovak Academy of Science, Bratislava, Slovak Republic*

e-mail: filip.humenik@gmail.com

Introduction: Many pathological conditions are associated with disorders of angiogenesis, such as diabetic neuropathy, myocardial infarction or CNS trauma. Bone marrow derived-mesenchymal stem cells (BMMSC) release via their secretomes bioactive molecules that may be analyzed for functional angiogenesis in the chicken chorioallantoic membrane (CAM) assay.

Material and Methods: Thus, in present study, under well-defined standardized isolation protocols the multipotent differentiation and specific surface markers of canine bone marrow MSCs were documented. Furthermore, BMMSC secretomes (containing bioactive molecules) or culture medium (DMEM) were injected on CAM at ED6 and after 72hours angiogenesis was evaluated.

Results: Data from CAM assay showed that secretome contain functional pro-angiogenic molecules. CAM treated with BMMSC secretome revealed enhanced density of vessels and their bifurcations (47.250 ± 9.7) in comparison to control (medium only, 27.625 ± 7.981) after 72h. In addition, the intervascular distance was lower and the large vessels in some cases further enhance their diameter.

Conclusions: In conclusion, we confirmed our hypothesis that canine BMMSC secretome can stimulate angiogenesis in chicken CAM model. Moreover, here we suggest that MSC release a variety of bioactive molecules (VEGF) with strong paracrine component which may pronounce therapeutic potential in revascularization. However, extensive experimental or pre-clinical trials testing canine sources needs to be performed in order to better understand their angiogenic action in regenerative veterinary medicine.

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REVISED ANATOMICAL AND HISTOLOGICAL TERMINOLOGY OF THE EAR

Kachlík D. ^{1,2}, Broman J. ³, Tubbs R. S. ⁴, Baud R. ⁵, ten Donkelaar H. J. ⁶

¹ Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, Czech Republic

² Department of Health Care Studies, College of Polytechnics Jihlava, Czech Republic

³ Department of Clinical and Experimental Medicine, University of Linköping, Sweden

⁴ Seattle Science Foundation, Seattle, WA, USA

⁵ Service of Medical Informatics, University Hospitals of Geneva, Switzerland

⁶ Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands

e-mail: david.kachlik@lfmotol.cuni.cz

The Working Group Neuroanatomy (WGN) of the Federative International Programme on Anatomical Terminology (FIPAT), a commission of the International Federation of Associations of Anatomists (IFAA) processed and prepared a proposal revised morphological nomenclature of the ear, concerning its bony and soft tissues as well as vessels and nerves.

It combines the anatomy and histology of the external, middle and internal ear.

The document is part of Terminologia Neuroanatomica (TNA), accepted by the Executive Committee of IFAA in September 2016 and published online in February 2017, freely accessible at <http://www.FIPAT.library.dal.ca>. The TNA document is composed of five columns (Latin term; Latin synonyms; British English term; American English term; English synonyms and related terms including eponyms). This contribution sums up and explains the fundamental changes.

MUTUAL RELATION BETWEEN STAT3 AND CCL2 IN NEURONS OF SPINAL GANGLIA

Kokošová V., Klusáková I., Hradilová-Sviženská I., Joukal M., Dubový P.

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

e-mail: viktorija.kokosova@mail.muni.cz

Introduction: Transcription factor STAT3 and chemokine CCL2 play a key role in the successful regeneration of injured axons. Overexpression of CCL2 in undamaged spinal ganglia (SG) neurons leads to activation of STAT3, but STAT3 is activated in SG neurons of CCL2 $-/-$ mice after sciatic nerve transection. Goal of our study was to contribute to elucidation of mutual relation between STAT3 and CCL2 in SG neurons.

Material and Methods: Wistar rats were operated on complete sciatic nerve transection (CSNT) and subsequent intrathecal application of either AG490 (CSNT+AG490) or artificial cerebrospinal fluid (CSNT+ACSF). Naive rats were used as another control. Immunofluorescence intensity (IFI) of activated STAT3 and CCL2 was measured bilaterally in SG neurons of L4 segments by image analysis.

Results: SG neurons of naive rats displayed very low IFI of both STAT3 and CCL2, while CSNT with AG490 or ACSF treatment induced their significant increase. Significantly lower IFI of STAT3 and CCL2 was detected in medium- and small-sized neurons of CSNT+AG490 compared to CSNT+ACSF treated rats.

Conclusions: Blocking of Jak2 kinase (AG490) not only led to decrease of activated STAT3 but also CCL2 in medium- and small-sized SG neurons after sciatic nerve transection. These results suggested that activation of STAT3 is associated with increased CCL2 in axotomized SG neurons of medium and small size. Since IFI of CCL2 was still higher in SG neurons of CSNT+AG490 and CSNT+ACSF treated rats than those of naive control animals, other factors are considered in regulation of CCL2 besides activation of STAT3.

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ROLE OF HYPERHOMOCYSTEINEMIA IN NEURODEGENERATION IN RAT ENTORHINAL CORTEX-HIPPOCAMPAL SYSTEM AFTER GLOBAL BRAIN ISCHEMIA: A PROGRESSION OF ALZHEIMER'S DISEASE-LIKE PATHOLOGICAL FEATURES?

Kovalská M.¹, Hnilicová P.², Kalenská D.², Tóthová B.³, Adamkov M.¹, Lehotský J.^{2,3}

¹ Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, Slovak Republic

² Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, Slovak Republic

³ Biomedical Center Martin, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, Slovak Republic

e-mail: kovalska2@uniba.sk

Introduction: The hippocampus has a crucial role in spatial navigation and memory processes. The major interface of cortico-hippocampal circuits involves the entorhinal cortex (EC), and constantly being studied for Alzheimer's disease (AD) pathology. AD is an irreversible neurodegenerative disorder with pathological processes invariably involving the EC-hippocampal (ECH) system leading to dementia and death. The precise etiology of AD is uncertain. Several risk factors have been suggested, including elevated levels of homocysteine (hHcy) in plasma, and stroke. HHcy is regarded as an independent, strong risk factor for both dementia and stroke. Although, the underlying molecular background of these mechanisms is not completely comprehended.

Material and Methods: After 4 weeks of high methionine diet at a dose 2 g/kg of animal weight/day, adult male Wistar rats underwent global brain ischemia induced by 4-vessel occlusion lasting for 15 min followed by reperfusion period of 72 hours. We explored the changes in ECH of rat brain using the proton magnetic resonance spectroscopy (¹H MRS), involving 7T MR scanner. After sacrifice, the brains were harvested, frozen, cut and then processed for histo-morphological as well as immunofluorescence analyses.

Results: The results showed that induction of hHcy with subsequent ischemia-reperfusion injury (IRI) might aggravate the neuronal cell death in the ECH system. We observed degeneration of selective vulnerable neurons in combined (hHcy + IRI) group. Decrease of volume in grey matter, alterations in number and morphology of astrocytes, neurons and their processes was demonstrated in the mentioned group as well as presence of proteins associated with AD development.

Conclusion: Our results suggest that the combination of the two risk factors hHcy and IRI, aggravates the neurodegenerative process and possibly indorse the development of AD-like pathology.

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GPX4 IN PREIMPLANTATION EMBRYOS AND FEMALE GENITAL ORGANS OF RAT

Krehelová A.¹, Mihalik J.¹, Kovaříková V.², Domoráková I.³, Solár P.⁴, Pavliuk-Karachentseva A.¹, Rybárová S.¹, Hodorová I.¹

¹ Department of Anatomy, Faculty of Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice, Slovak Republic

³ Department of Histology and Embryology, Faculty of Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

⁴ Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: andrea.krehelova@student.upjs.sk

Introduction: The imbalance between amount of reactive oxygen species (ROS) and antioxidants can result in pathologies. Mammals defense against high ROS levels by using many antioxidant systems, one of them are glutathione peroxidases (GPx). While GPx4 plays important role in male infertility, we decided to find out if this enzyme plays similar role during preimplantation period of pregnancy.

Material and Methods: 15 rat females were overdosed by anesthetic on the 1., 3. and 5. day of pregnancy. Uterine horns, oviducts and ovaries were removed and embryos were flushed out with PBS+BSA solution. GPx4 was visualized using specific FITC-conjugated antibody and embryos were observed under confocal microscope. Removed organs were stored in paraformaldehyde and subsequently were used for immunohistochemical detection of GPx4, which was visualized by DAB.

Results: Oocytes were characterized by uniformly diffuse GPx4 distribution in cytoplasm. In zygotes, GPx4 began to concentrate into clusters mainly around the nuclei. Same phenomenon was observed from 2-cell embryos to blastocysts. Degenerated embryos were characterized by inhomogeneous arrangement of GPx4 with diffusion distribution and clustering. GPx4 was found in all observed organs under light microscope. Enzyme occurs in all cell types in uterus, such as epithelium, uterine glands and myometrium. In oviduct GPx4 was found in kinocilia, epithelium and muscle layer. In ovary protein was detected in corpus luteum and oocytes.

Conclusions: GPx4 was detected in all developmental stages of rat preimplantation embryos and all female genital organs. On the base of our results can be assumed that GPx4 plays important role in preimplantation period of pregnancy and in correct embryo development.

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RAMAN SPECTROSCOPY AND BLOOD LEUKOCYTES RESEARCH

Křížková V.¹, **Bufka J.**^{1,2,3}, **Vaňková L.**^{1,2}, **Hrubá K.**^{1,2}, **Jíchová H.**¹, **Šígutová P.**⁴,
Štambachová A.⁴, **Nevoral J.**^{1,2}, **Maršálová L.**¹, **Bouř P.**³

¹ *Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University, Czech Republic*

² *Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Czech Republic*

³ *Institute of Clinical Biochemistry and Hematology, Teaching Hospital in Pilsen, Czech Republic*

⁴ *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Science, Prague, Czech Republic*

e-mail: vera.krizkova@lfp.cuni.cz

Introduction: Raman spectroscopy (RS) is based on the interaction of electromagnetic radiation with sample molecules. During the interaction, vibrational and rotational states of molecules may be changed. These changes are specifically identified. Atoms have different masses and in molecules they are connected by different bonds. Associated vibrations of various bonds, atomic groups and studied molecules are different, which results in characteristic Raman spectra - "fingerprint" spectral patterns of a molecule. The spectroscopy can be used to analyze all kind of substances (specific for the cell type).

Material and Methods: Vein blood of healthy donors (EDTA-anticoagulated specimen) was applied to a standard clean glass slide: it was fixed in May-Grünwald solution and then it was Giemsa-Romanowski stained in cooperation with experts of Institute of Clinical Biochemistry and Hematology, Teaching Hospital in Pilsen (accredited Institute with blood smear certification). Leukocytes were identified in mentioned specimens and analyzed by means of RS at chosen time intervals.

Results: First important step was optimization of Raman spectroscopy basic parameters. After that it was possible to obtain Raman peaks specific for each type of blood leukocyte with possible inter-individual variability. Gained Raman peaks corresponding to particular molecular vibration and rotation of the leukocyte type will be demonstrated in presentation.

Conclusions: The Raman spectroscopy has been established for biomedical purposes and clinical medicine research (blood leukocytes changes in some diseases). We will present general and inter-individual features in Raman spectra.

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THE ROLE OF THE FRACTALKINE/CX3CR1 IN THE DEVELOPMENT OF THE TRIGEMINAL NEUROPATHIC PAIN

Kubičková L., Klusáková I., Dubový P.

Department of Anatomy, Cellular and Molecular Research Group, Faculty of Medicine, Masaryk University, Brno, Czech Republic

e-mail: Lucie.kubickova@med.muni.cz

Introduction: Cytokines and chemokines, released during peripheral nerve injury, contribute to induction and maintenance of neuropathic pain. Expression and cellular detection of CX3CL1 and CX3CR1 was investigated in the trigeminal subnucleus caudalis (TSC) following unilateral ligature of the infraorbital nerve (IONL).

Material and Methods: The IONL- and sham-operated rats survived for 1, 3, 7, and 14 days (n=6 each group). Von Frey monofilaments was used for measurement of mechanical allodynia. The rats were perfused with Zamboni solution and the brainstem was dissected. Transverse cryostat sections (12 µm) were immunostained to explore cellular distribution of CX3CL1 and CX3CR1.

Results: Tactile hypersensitivity was significantly increased bilaterally in vibrissal pads of sham- and IONL-operated animals from day 1 to 7 with tendency for normalization in sham controls during next days. CX3CL1 immunostaining was found bilaterally in TSC neurons 1 day after IONL, but in later periods of survival the immunoreaction was detected ipsilaterally in astrocytes with a weak intensity in neurons. On contralateral side of IONL- and sham-operated rats was CX3CL1 found only in neurons. In contrast, CX3CR1 was present only in astrocytes during all periods of survival with significant increase from 3 to 7 days in TSC ipsilateral to IONL compared to naïve and sham-operated controls. In contralateral side and both sides of sham-operated rats, CX3CR1 immunostaining was observed in neurons of TSC 1 and 14 days after operation while the immunoreaction was found in both neurons and astrocytes at 3 and 7 days from IONL.

Conclusions: We found different cellular distribution of CX3CL1 and CX3CR1 in TSC during various periods of survival. This result revealed CX3CL1/CX3CR1 signaling system in TSC via astroglial-neuronal interactions participating in orofacial neuropathic pain.

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PRINCIPAL PELVIC BONE STIFFNESS AS A DESCRIPTOR OF BONE QUALITY: PILOT STUDY AND FEASIBILITY

Kučař M.¹, **Henyš P.**²

¹ *Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic*

² *Department of Technologies and Structures, Textile Faculty, Technical University in Liberec, Czech Republic*

e-mail: kucharm@lfhl.cuni.cz

Introduction: Bone stiffness is one of the fundamental biomechanical property defining the overall bone quality as it contributes to ability of the bone to resist deformation. To derive bone structural stiffness the standard compression/bending or tensile tests are traditionally deployed. There is, however, a number of limitations using these methods as their strongly depend on chosen bone attachments and direction of load applied. In our work we attempt to develop a new method of bone stiffness evaluation independent on mechanical testing and capable to quantify differences of overall stiffness with changing shape, bone density and structure. We focused on pelvic bone as an integral part of the hip joint and of complex geometry, nevertheless not so frequently tested as the long bones.

Material and Methods: The proposed method is based on the hypothesis that there exists a deformation mode of a bone, for which the bone has the smallest stiffness. The smallest stiffness and corresponding deformation mode of the bone were estimated from the decomposition of stiffness matrix computed by finite element method. Left and right pelvic bones were segmented from routine CT scans acquired from the internal database of the Faculty hospital in Hradec Králové. In order to split up the bone density and shape effects, the deformable registration method was employed. The digital bone twins were segmented with semi-automatic interactive graphCut algorithm.

Results: For all tested patients a characteristic deformable mode and associated smallest stiffness were found. Those smallest stiffness were sensitive for both arbitrary density and shape variations. Further, it was found that variations in bone mass and smallest stiffness are predominantly given by variations in shape rather than density.

Conclusions: The principal (smallest) stiffness measure provides a unique characteristic of bone and it is potentially independent on bone load as it actually computes the worst load scenario.

EXPRESSION OF SODIUM CHANNEL NAV 1.8 IN DORSAL ROOT GANGLIA AFTER PACLITAXEL TREATMENT

Levin S., Dubový P., Joukal M.

Department of Anatomy, Faculty of Medicine, Masaryk University Brno, Czech Republic

e-mail: mjoukal@med.muni.cz

Introduction: The chemotherapeutic agent Paclitaxel causes the adverse reaction of peripheral neuropathy. The Nav sodium channel family has an important role in pain sensation. The subtype Nav 1.8 is the more abundantly expressed Tetrodotoxin resistant (TTX-R) sodium channel expressed in nociceptive Dorsal Root Ganglia (DRG) neurons. The aim of our study was to assess possible changes in Nav 1.8 channel expression in the DRG after intraperitoneal Paclitaxel application.

Material and methods: Wistar rats (n=27, males) were used in our experiments. Intraperitoneal injections of Paclitaxel in 4 doses with a cumulative dose of 8 mg/kg were performed on experimental rats, while control animals received only the excipient (alcohol and cremophor EL; 1:1). The animals were left to survive for 1, 7, 14 and 21 days from the last application. Experimental and control rats were sacrificed together with naïve rats and perfused transcardially by Zamboni's fixative. Longitudinal cryostat sections through the lumbar DRGs were cut and immunostained for Nav 1.8. The intensity of Nav 1.8 immunofluorescence in the DRGs was measured and statistically analyzed.

Results: The presence of Nav 1.8 immunofluorescence was found in lumbar DRG neurons in naïve, control and Paclitaxel treated animals. Statistically significant increase in Nav 1.8 immunofluorescence was found in the DRGs of Paclitaxel treated animals in comparison to both control and naïve animals. Interestingly, positivity was also detected in vessels' walls within the DRG.

Conclusions: Our results indicate that DRG neurons react to Paclitaxel application by increasing Nav 1.8 channel expression. These changes might be associated with the symptoms of chemotherapy induced peripheral neuropathy.

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CASE REPORTS – MOTIVATIONAL COMPONENT OF ANATOMY EDUCATION

Lisková K., Hájek P.

Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

e-mail: hajekp@lfhk.cuni.cz

Introduction: The main impulse to create this project was the curriculum of the General Medicine study program. As of now, students get their first contact with patients after two years of theoretical training. This demotivation partakes in outflow of students during early years. Besides this long-term issue, we wanted to address the universal passivity and lack of motivation in the current generation of students.

Material and Methods: We collected 57 case reports – 15 concerning muscular system, 7 on cardiovascular system, 15 on splanchnology and 20 on nervous system. Therefore, we covered the topics of all practical classes in both terms. We aimed for diversity of cases in various age groups, while preferring common diagnoses that are regularly encountered in medical practice, yet rarely seen in scientific articles. Main sources were my personal experience in our university hospital, as well as recounting of clinical teachers and various web sources.

Results: Case reports were chosen to be applicable to certain anatomical topics and show the importance of anatomy in medical practice. Bearing in mind that we present the cases to grammar school graduates unexperienced in medicine, we added a brief explanation of pathophysiology of some conditions. Professional medical terms (e.g. names of procedures) were also explained when needed. To promote active thinking, each case report contains an anatomical question presented to the student in a critical point. The difficulty is set so they can answer it with knowledge needed to be prepared for corresponding practical class. The literary aspect to the case reports was also important to us. We strived to bring the whole story of the patient including their social background, prognosis and alternative solutions of their condition, to create more engaging experience for our students.

Conclusions: Our work is published in the form of a Moodle course as a part of preparation for practical classes.

LYMPHATIC DRAINAGE SYSTEM IN BRAIN DURA

Maloveská M.¹, Danko J.¹, Humeník F.¹, Mojžišová Z.¹, Krešáková L.¹, Vdoviaková K.¹, Teleky J.¹, Čížek M.¹, Murgoci A-N.², Čížková D.^{1,2}

¹ Department of Anatomy, Histology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovak Republic

² Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovak Republic

e-mail: marcela.maloveska@uvlf.sk

Introduction: Recently discovered meningeal lymphatic system draining the central nervous system has redefined our understanding of brain cleaning. Most studies on meningeal lymphatics have been conducted in mice. In present study we have characterized the morphology, density and directing of meningeal lymphatic vessels in rat and pig. Dura mater of rat and swine is relatively thick and solid and can be removed from the inner site of the skull by the standard neurosurgical procedures. But due to its different arrangement (compare to dura of spinal cord) is firmly attached to the adjacent skull and therefore can be easily torn during isolation.

Material and Methods: Isolated dura from three different anatomical areas was sectioned (in cryocut), and processed for immunohistochemistry with specific antibodies (Prox-1, Lyve-1). Using confocal scanning and standard fluorescence microscopy, we serially imaged rat and swine dura mater and evaluated the pattern of lymphatic vasculature in studied areas.

Results: Here we confirm by immunohistochemical analyses of lymphatic vessel that their distribution point to at least three distinct lymphatic drainage pathways from the brain: along central venous sinuses (sinus sagittalis dorsalis) in falx cerebri, through lamina cribrosa ossis ethmoidalis (olfactory route) and the third pathway was confirmed around the openings in the basal part of the skull at the exit of cranial nerves and blood vessels. Higher magnification views showed that brain dura reveals a more complex lymphatic vasculature with a different density depending on their topography. The highest density of lymphatic vasculature was demonstrated directly in area of skull base compared to falx cerebri.

Conclusions: Studies characterizing the CNS lymphatic system from morphological point of view may subsequently provide insight in to the physiological functionality and possible role in neurodegenerative disorders development.

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Wnt SIGNALLING PATHWAY IN CERVICAL CANCER

Mešťanová V.¹, Klinerová B.¹, Škuciová V.², Drahošová S.³, Adamkov M.¹

¹ Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovak Republic

² Patológia s.r.o., Martin, Slovak Republic

³ Hermes LabSystems, s.r.o., Bratislava, Slovak Republic

e-mail: veronika.mestanova@uniba.sk

Introduction: Cervical cancer is one of the primary lethal malignancies for women worldwide, simultaneously one of the most preventable types of cancer. Drug resistance development and occurrence of tumor dissemination remain a challenge to struggle within therapeutic approaches. Potential bond to cervical atypia and cancer is Wnt signaling pathway with related downstream effectors participating on cell differentiation, proliferation and migration. Activation or inhibition of intracellular signal transduction factors in T cell factor (TCF)/ β -catenin family can manage cancer cell growth and viability. Deregulated expression of survivin by TCF/ β -catenin disrupts the equilibrium between cell proliferation and apoptosis that plays an essential role in tumorigenesis.

Material and Methods: Histomorphological and immunohistochemical study was performed on archived formalin-fixed, paraffin-embedded tissue blocks from diagnostically closed cases of precancerous and cancerous cervical lesions. Expression of surviving – member of IAP family and β -catenin – transcriptional coactivator of canonical Wnt pathway, are categorized in terms of percentage of positive cells, subcellular localization and immunoreaction intensity.

Results: Based on preliminary results and review of literature, authors assume that combination of histomorphological criteria, Wnt signaling components and survivin expression might be more effective for diagnosis, stratification of patients and prognosis of cervical cancer.

Conclusions: Correlation between survivin and Wnt signaling pathway components might imply their clinical role in cervical cancer effective prevention, diagnosis and novel therapeutical strategies. Assessment of aberrant abovementioned proteins/factors expression might indicate their crucial predictive and prognostic significance in cervical cancer management.

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CHARACTERISTICS OF CANINE UMBILICAL CORD MESENCHYMAL STEM CELLS

Mojžišová Z.¹, Humeník F.¹, Mudroňová D.², Mađari A.³, Farbáková J.³, Horňáková L.³, Danko J.¹, Maloveská M.¹, Čížková D.¹

¹ Institute of Anatomy, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

² Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovak Republic

³ University Veterinary Hospital, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

e-mail: mozes.zuz@gmail.com

Introduction: Mesenchymal stem cells (MSC) are multipotent cells derived from various sources such as bone marrow, adipose tissue or umbilical cord. MSC under standard conditions are able to differentiate into osteocytes, chondrocytes and adipocytes. General data indicate that MCS derived from UC represent more primitive population, which can have the greatest impact on the patients suffering from various diseases.

Material and Methods: For the isolation we used UC of dogs from caesarean sections and induced abortion realized under strictly sterile conditions. UC were washed and dissected and digested in 0.1% collagenase, using standard protocol. Afterwards, isolated cells were suspended and seeded in 25 cm² flasks, maintained at 37°C under standard cultivation conditions. After 24 h incubation, floating cells were removed and culture medium was replaced every 3-4 days. Adherent cells were cultured until reaching 90% confluence and after 3 passages processed for CD expression and three lineage differentiation.

Results: With this protocol we gained from 3-4 g UC tissue an average of 3.34 x10⁶ cells, with an average of 96. 1% cellular viability and excellent proliferative capacity till passage 4. During *in vitro* cultivation cells adhere to the plastic and showed fibroblastic-like morphology. Primary cultures reached 80-85% confluence within 3-5 days. We characterized cells at passage 3, using flow cytometry with CD markers, revealing CD29+, CD90+ (>90%) and CD45+ (<4%) as well as multilineage properties confirmed by Alcian Blue, Alizarin Red and Oil Red.

Conclusions: In conclusion, our data indicate that we were able to isolate highly viable MSC from dog UC. These cells meet the criteria for MSCs and are good candidate for clinical application in veterinary medicine.

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DERIVATION OF PRIMITIVE NEURAL STEM CELLS FROM MURINE EMBRYONIC STEM CELLS

Mokry J., Pisal R. V., Chvátalová J., Voborníková M.

Department of Histology and Embryology, Charles University, Medical Faculty in Hradec Králové, Hradec Králové, Czech Republic

e-mail: mokry@lfhk.cuni.cz

Introduction: Neural stem cells (NSCs) are principal cells responsible for development of the nervous tissue. Studies of signaling pathways involved in neurogenesis have to use primitive NSCs that act as the early precursor of definitive NSCs. Modification of crucial signaling involved in early neurogenesis permitted us to establish a new approach for derivation of primitive NSCs from pluripotent stem cells.

Material and Methods: Mouse ES D3 cells were cultured in a complete 2i medium; signaling pathways were suppressed with specific inhibitors. After 6 days gene expression was examined with qRT-PCR; expression at protein level was confirmed with two-step immunofluorescence.

Results: By inhibiting Wnt, TGF-beta and BMP signaling in the presence or absence of LIF and examining expression of pluripotent (pou5f1, Sox2, Nanog) and early neurogenic markers (ccdc141, Sox1, Pax6 and nestin) we were able to identify conditions for transition of pluripotent ES cells into primitive NSCs. Our results give evidence that Wnt signaling is not crucial for derivation of primitive NSCs instead cytoplasmic beta-catenin stabilization is crucial. Primitive NSCs are not regionally specific, coexpress pluripotent and neurogenic markers, which indicates they are only partially committed to neural cell lineage.

Conclusions: Primitive NSCs allow in vitro studies of influence of signaling pathways on onset of neurogenesis. Derivation of primitive NSCs has potential to extend the spectrum of in vitro studies on neurogenesis.

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METHODS FOR VISUALISATION OF PHAGOCYTOTIC APOPTOTIC CELLS IN MOUSE BLASTOCYSTS

Pisko J., Kovaříková V., Fabian D.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Science, Bratislava, Slovak Republic

e-mail: pisko@saske.sk

Introduction: Aim of our study was to standardize methodology for evaluation of *physiological capacity* of embryonic cells to eliminate apoptotic cells by phagocytosis in *in vivo* obtained blastocysts by means of fluorescence morphological staining followed by confocal laser scanning microscopy.

Material and Methods: Expanded blastocysts were obtained from CD-1 mice stimulated by hormonal treatment. *In vivo* developed blastocysts were freshly isolated from fertilized females on Day 4 of pregnancy. For visualization of apoptotic / dead cells in blastocysts TUNEL assay and Annexin V-FITC / propidium iodide assay combined with Hoechst 33342 DNA staining have been used. The incidence of phagocytosis was evaluated by the visualization of acid organelles in cytoplasm using LysoTracker test and by F-actin in plasma membrane using Phalloidin staining.

Results: Average number of apoptotic cells showing presence of specific DNA fragmentation in nucleoplasm (TUNNEL assay) and/or fragmented (condensed) morphology of nuclei in 112 evaluated mouse blastocysts was 5.56. However, incidence of apoptotic cells showing phosphatidylserine translocation on cytoplasmatic membrane (Annexin V assay) was only occasional (4.44 % of identified dead cells). The majority of identified apoptotic cells showed presence of acid organelles in cytoplasm. Based on these findings, the LysoTracker test appears to be inappropriate method for the evaluation of phagocytosis. On the other hand, 26.21% of identified apoptotic cells showed internalization in cytoplasm of neighboring cells, i.e. they were phagocytized. However, we were not able to classify 30.34% of apoptotic cells as phagocytized or not, because of massive degradation of F-actin in apoptotic cells.

Conclusions: Our findings suggest lowered expression of signal for phagocytosis (phosphatidylserine flip) in embryonic apoptotic cells. However, this fact does not lower the ability of relatively high number of neighboring blastomeres to act as non-professional phagocytes and to perform phagocytosis of dying cells.

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DEVELOPMENT OF ATRIAL FORM AND FUNCTION

Sedmera D.

Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic

e-mail: david.sedmera@lf1.cuni.cz

The atria serve as a contractile blood reservoir of the cardiac pump. Morphologically, the pectinate muscles, or their relative extent, are used to distinguish the morphologically left and right atrial appendages. They are also considered to be the substrate of preferential conduction pathways within the atria. This notion is corroborated by developmental studies, which identified the main bundle in the roof of the chick embryonic atria as a substrate of interatrial conduction pathway.

During embryogenesis, the atria and ventricles are soon distinguished from the persisting tubular segments (atrioventricular canal, outflow tract) by disappearance of the cardiac jelly and formation of pectinate muscles and trabeculae, respectively. While the process of ventricular trabeculation received considerable attention in the past as a mechanism to increase cardiac mass prior to development of coronary vasculature and forming preferential pathways for rapid impulse conduction, the formation of the pectinate muscles in the atria is considerably less intensely studied. In this presentation, I will review current knowledge of development of atrial myoarchitecture and electrophysiology in popular vertebrate model species, and also provide comparative data from non-model organisms. I will also discuss recent experimental studies to provide support for functional concepts of roles of pectinate muscles and developmental basis of atrial arrhythmias.

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CANCER ECOSYSTEM AND ITS ROLE IN TUMOR PROGRESSION

Smetana K. Jr., Dvořánková B., Kodet O., Szabo P., Strnadová K., Kolář M., Kupcová-Skalníková H., Lacina L.

Charles University, 1st Faculty of Medicine, Institute of Anatomy & Department of Dermatovenereology & BIOCEV

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic

Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague, Vestec, Liběchov

e-mail: karel.smetana@lf1.cuni.cz

Cancer incidence is growing due to the increase of life expectancy worldwide. Despite the progress in cancer diagnostics and therapy, new therapeutic approaches are necessary to treat the old and therapeutically fragile polymorbid patients. One of new strategies is possibility to manipulate the cancer ecosystem. Malignant tumors are composed not only from cancer cells but also from many types of noncancerous cells that clearly influence biological properties of tumors concerning the local aggressiveness and lymphatic/distant metastazation. Cancer-associated fibroblasts, and leukocytes (Treg, TCD8+, neutrophils, macrophages...) orchestrate in support of cancer growth and metastazation. The mutual exchange of many growth factors, cytokines and chemokines between cancer cells and noncancerous cells of tumor ecosystem play a crucial role in malignant disease progression. Moreover, they also influence metabolism of whole patient body and they are behind cancer cachexia and wasting. The decoding of this crosstalk may be a new challenge for cancer treatment.

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CLEARANCE OF LEAD OXIDE NANOPARTICLES FOLLOWING SUBCHRONIC INHALATION

Smutná T.^{1,2}, **Dumková J.**^{1,3}, **Vrlíková L.**², **Kotasová H.**¹, **Dočekal B.**⁴, **Čapka L.**⁴, **Tvrdoňová M.**⁵, **Mikuška P.**⁴, **Večeřa Z.**⁴, **Křůmal K.**⁴, **Coufalík P.**⁴, **Vaculovič T.**⁵, **Hampl A.**¹, **Buchtová M.**^{2,3}

¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

² Institute of Animal Physiology and Genetics, v.v.i., Czech Academy of Sciences, Brno, Czech Republic

³ Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

⁴ Institute of Analytical Chemistry, v.v.i., Czech Academy of Sciences, Brno, Czech Republic

⁵ Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic

e-mail: buchtova@iach.cz

Introduction: Lead oxide nanoparticles (PbO-NPs) are able to pass lung barrier, and induce the pathological changes in primary (lung) and secondary target organs (liver, kidney). However, the ability of these target organs to eliminate PbO-NPs is still not clear. This study is focused on the clearance of inhaled PbO-NPs from different organs.

Material and Methods: Adult female mice (CD-1 (ICR) BR strain) were placed into whole body inhalation chambers and exposed to PbO-NPs with a mass concentration 78.0 µg PbO/m³ for 6 weeks (PbO/6w) or 11 weeks (PbO/11w). Clearance group (PbO/cl) inhaled air with PbO-NPs for 6 weeks, and then inhaled clean air for 5 weeks. The control groups were inhaling clean air. At the end of the exposure periods, the target organs (lung, liver, and kidney) were weighted and collected for chemical, histopathological, histochemical, immunohistochemical, and immunofluorescent analysis, LA-ICP-MS and for the study of gene expression.

Results: The histopathological analysis of PbO-exposed lung revealed a chronic inflammation. Clearance of PbO-NPs in the lung was sufficient after 5 weeks of clean air inhalation. Chemical analysis and LA-ICP-MS confirmed that the lead was fairly eliminated. In liver, the inhalation of PbO-NPs caused steatohepatitis and the remodeling of liver parenchyma. The content of lead was decreased practically to the values of controls after clearance. The qPCR method confirmed that *Abcc2* and *Abcc3* are involved in clearance of PbO-NPs from liver, and *Abcg2*

from lungs. LA-ICP-MS analysis confirmed occurrence of lead in kidney cortex even after clearance period.

Conclusions: The study determined the effective clearance of inhaled PbO-NPs from lung and liver. The genes encoding ABC transporters were identified as genes significantly deregulated during PbO-NPs inhalation, and after it.

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SUBARACHNOID HEMORRHAGE INDUCES CELLULAR AND ENZYMATIC CHANGES IN THE CHOROID PLEXUS

Solár P.^{1,2}, **Dubový P.**¹, **Jančálek R.**², **Joukal M.**¹

¹ Department of Anatomy, Division of Neuroanatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

² Department of Neurosurgery, Faculty of Medicine, Masaryk University and St. Anne's University Hospital, Brno, Czech Republic

e-mail: mjoukal@med.muni.cz

Introduction: The subarachnoid hemorrhage (SAH) is a specific form of hemorrhagic stroke. Our previous experiments revealed that SAH induces inflammatory reaction in the choroid plexus (CP). The aim of presented work is to provide an overview of CP cellular and enzymatic reaction to SAH.

Material and methods: SAH was induced by application non-heparinized autologous blood (SAH group) or ACSF (ACSF group) into the cisterna magna and animals were left to survive for 1, 3 and 7 days. The brain sections of naive, SAH and ACSF groups of animals were immunostained under identical conditions with anti-CD68 (ED1), anti-CD163 (ED2), anti-CCR7, anti-CD206, anti-CD3, anti MHC II, anti-Ki67, anti- heme-oxygenase-1 (HO-1), and anti-biliverdin reductase (BVR) antibodies. Immunohistochemical staining of HO-1 and BVR was confirmed by Western blot analysis.

Results: The number of MHC II+ cells as well as ED1+ macrophages gradually increased with duration after SAH or ACSF application while the number of ED2+ macrophages increased in all periods after SAH. Immunostaining of CCR7+ cells showed gradually decrease in both SAH and ACSF groups of animals. The number of CD206+ cells increased with duration after SAH and decreased after ACSF injection. CD3 immunostaining did not reveal T cells in the CP of any group of the animals. Ki-67 immunostaining showed gradually increased proliferation following SAH and decreased with duration after ACSF application. Increased expression of HO-1 and BVR was found in all periods following SAH.

Conclusions: Our results demonstrate that CP responds with immune cellular and enzymatic changes at different time periods following the application of blood or ACSF. These findings indicate that not only blood degradation products but also increased intracranial pressure after SAH contributes to cellular and enzymatic changes in the CP following SAH.

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MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF NEUROGENIC PULMONARY EDEMA

Šedý J.

Department of Anatomy, Medical Faculty, Palacky University, Olomouc, Czech Republic

e-mail: jirisedy@jirisedy.cz

Neurogenic pulmonary edema (NPE) is a relatively infrequent, but important clinical entity. Its development is related to severe injury of the central nervous system. The center of NPE is most likely a group of rostral ventrolateral nuclei of the medulla oblongata, which trigger its development on the basis of the hyperactivation of afferent neural pathways and a sudden increase of intracranial pressure. In the pathogenesis of NPE, the sympathetic nervous system plays a major role, starting a cascade of processes that lead to interstitial and intraalveolar edema, together with significant hemorrhage. In the diagnosis of NPE, physical examination and X-ray imaging play a crucial role. The differential diagnosis of NPE is not easy, but the chances of reaching the proper diagnosis are increased when central nervous system injury and pulmonary disturbances are found together. To date, no targeted curative treatment exists, thus therapy is mainly supportive and symptomatic. The most important treatment modalities are the continuous monitoring of the patient's status, the position of the patient and oxygenation and ventilation support. Currently, several NPE models have been developed, which can be used to study the etiopathogenesis and treatment of NPE. The main aim of experiments is to develop a preventive and therapeutic approach, able to prevent and treat NPE. From this point of view, the most suitable is the use of atropine.

MAJORITY OF CEREBROSPINAL FLUID-CONTACTING NEURONS IN THE SPINAL CORD OF C57BL/6N MICE IS PRESENT IN ECTOPIC POSITION UNLIKE IN OTHER STUDIED EXPERIMENTAL MICE STRAINS AND MAMMALIAN SPECIES

Ševc J., Tonelli Gombalová Z., Košuth J., Alexovič Matiašová A., Daxnerová Z.

Department of Cell Biology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: juraj.sevc@upjs.sk

Introduction: Cerebrospinal fluid contacting neurons (CSF-cNs) represent a specific class of neurons located in close vicinity of spinal cord central canal and brain ventricles.

Material and Methods: All analyzes were performed using immunofluorescence molecular biology methods on spinal cord tissue of C57Bl/6N, C57Bl/6J and Balb/C mice strains, Wistar rats and New Zealand rabbits.

Results: In contrast with the knowledge gained from other vertebrate species, we found that vast majority of CSF-cNs in the spinal cord of C57Bl/6N mice is located in ectopic distal position; beyond the border of ependymal lining, in ventral part of gray commissure or even in anterior white commissure. However, we found that small number of ectopic CSF-cNs is present also in the spinal cords of other investigated mammalian species. Similarly, as the proximal populations, ectopic CSF-cNs retain PKD2L1-immunoreactivity. On the other side, they show rather multipolar morphology lacking the thick dendrite contacting central canal lumen. Ectopic CSF-cNs in the spinal cord of C57Bl/6N mice emerge during the whole period devoted to production of CSF-cNs and reach their ventral destinations during first postnatal weeks. Employing *in silico* analyses, we ranked the polymorphisms in C57Bl/6N substrain and selected *Crb1* or *Cyfp2* genes as the most probable candidates, whose product dysfunction might be responsible for the ectopic distribution of CSF-cNs. Employment of spinal cord transection model mimicking the disruption of CSF flow on Balb/C mice with normal CSF-cN distribution, did not result in changes neither in CSF-cN abundance, nor in their arrangement below the site of injury.

Conclusions: Our results suggest that contrary to results obtained on zebrafish, mammalian CSF-cNs may not be necessarily dependent on the contact with CSF under certain circumstances. Moreover, the role of ectopic subpopulation remains undisclosed.

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RELATIONSHIP BETWEEN PD-L1 EXPRESSION AND TILs IN MALIGNANT MELANOMAS

Škuciová V.^{1,4}, *Drahošová S.*², *Výbohová D.*³, *Adamkov M.*⁴

¹ *Alphamedical s.r.o., Martin, Slovak Republic*

² *Hermes LabSystems, Bratislava, Slovak Republic*

³ *Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic*

⁴ *Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic*

e-mail: veronikaskuciova@gmail.com

Introduction: Programmed death ligand 1 (PD-L1) expression can provide significant value to predict response following immunotherapy in malignant melanoma (MM). In primary melanomas, most studies point to a favorable prognostic effect of TILs. Our aim was to examine the association of tumor-infiltrating lymphocyte (TIL) count, PD-L1 level and clinicopathological and prognostic factors such as number of mitotic figures, Clark's and Breslow's levels of invasion in our set of malignant melanomas.

Material and Methods: We performed immunohistochemistry for PD-L1 and CD8 on 56 formalin-fixed paraffin-embedded specimens from patients with cutaneous or metastatic malignant melanomas. PD-L1 expression level was determined by immunohistochemistry (clone 28-8) and tumor proportion score (TPS) was evaluated. CD8+ TIL expression was classified as grade 0, 1+, 2+, and 3+ based on density and distribution of the infiltrating lymphocytes.

Results: PD-L1 expression was detected in 20 cases (35,71%) out of 56 MM. Expression of PD-L1 on tumor cells was significantly correlated with higher TILs infiltrate in the tumor microenvironment ($p = 0,038$). Conversely, lower TIL score correlates with poor prognostic clinicopathological factors such as higher number of mitotic figures ($p = 0,005$) Clark's level ($p = 0,007$) and Breslow's depth ($p = 0,010$).

Conclusions: Assessment of PD-L1 expression in combination with CD8+ TIL density may be a useful predictive marker. These two entities are highly correlated each other. The presence of tumour-infiltrating lymphocytes (TILs) seems to be favourable prognostic factor.

EVALUATION OF THE EFFECTIVENESS OF THE PRESENTATION OF VIRTUAL HISTOLOGY SLIDES BY STUDENTS DURING CLASSES

Tauber Z.², **Čížková K.**¹, **Lichnovská R.**¹, **Lacey H.**¹, **Erdošová B.**¹, **Žižka R.**², **Kamarád V.**¹

¹ Department of Histology and Embryology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic

² Czech Educational and Dental Research Innovative Group, Brno, Czech Republic

e-mail: zdenek.tauber@gmail.com

Introduction: Virtual microscopy, used as a method to teach histology, has many undeniable advantages. This paper describes the results of a recent pilot project which combined the modern teaching methods of active learning; where students themselves present histological slides, and use of the virtual microscopy system.

Methods: A structured questionnaire was used to evaluate student responses.

Results: We found that a combination of both electronic materials and textbooks was commonly used by students to prepare for practical teaching sessions; with electronic resources being used regularly by the majority of students. Cooperation between student groups during the preparation for individual presentations was seen to be beneficial by a majority of dentistry students; they reported that the introduction of student led to presentations improved their quality of preparation for practical lessons, as well as increasing their participation and activity level in the lessons themselves. That in turn facilitated an improvement of their presentation skills and expressive ability. Conversely, only a minority of general medicine students expressed similar opinion. This difference was statistically significant.

Conclusions: Our findings confirm that there are differences in motivation and approaches between both groups of students; which should be taken into account and which could lead to differentiation of future curricula for both study courses.

THE EFFECT OF BETANIN PARENTERAL PRETREATMENT ON LIVER PARENCHYMA AFTER JEJUNAL ISCHEMIA-REPEFUSION INJURY

Toth Š.¹, *Urda M.*¹, *Kalpavidis T.*¹, *Pribula M.*¹, *Kušnier M.*¹, *Mechírová E.*¹, *Maretta M.*²

¹ *Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic*

² *Department of Neurology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic*

e-mail: stefan.toth1@upjs.sk

Introduction: Ischemia-reperfusion injury of jejunum leads to pathological changes in distant organs, such as liver. Betanin is known for its antioxidant properties, and it is found mostly in vegetables. The aim of present study was to determine effect of betanin on liver parenchyma after jejunal ischemic-reperfusion injury.

Material and Methods: Pathogen-free Charles River Wistar adult male outbred rats (n=42) were randomly divided into 2 groups: experimental group B with betanin pretreatment and control group A (n=21) without pretreatment (n=21). After 1 hour of ischemia by superior mesenteric artery occlusion 1, 4 and 24 hours of reperfusion followed.

Results: Results of histological analyses showed lower index of histopathological injury and central vein diameter reduction in liver parenchyma. Mild inflammatory response (expressed by MPO and COX-2 positive cells) and modulation of antioxidant tissue capacity (expressed by catalase positive cells) in betanin pretreated experimental group B was detected. Regeneration/proliferation index of injured tissue expressed by MCM2 positive cells was also lower in group B with betanin pretreatment.

Conclusions: We conclude that betanin parenteral pretreatment attenuated liver parenchyma injuries sustained due to jejunal ischemia-reperfusion injury.

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PRODUCING CADAVERIC VIDEOS FOR STUDYING ANATOMICAL STRUCTURES OF HEAD AND NECK

Vecanová J.¹, Kluchová D.¹, Matéffy S.², Hvizdošová N.¹, Hodorová I.¹, Bona M.³, Majerník J.⁴

¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Alpha medical s.r.o., Diagnostic Center of Pathology, Member of the Unilabs Group, Prešov, Slovak Republic

³ Department of Medical Physiology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

⁴ Department of Medical Informatics, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: janka.vecanova@upjs.sk

Introduction: The regions of the head and neck are complex and clinically important, but it takes hours of study and dissections in order to understand it anatomically. Currently, many textbooks and anatomical atlases utilize hand-drawn or computer-generated images and the knowledge gained from these images is difficult to transfer to a real body. Therefore, our team is working to compile a database of dissecting based videos that are primarily directed to beginning medical students to promote deep learning and meaningful understanding of the subject.

Material and Methods: Two human donor formaldehyde-fixed cadavers were used for dissection and recording individual video-clips in order to describe particular human body parts. The dissections were done by skilled anatomists, using the finest surgical techniques. The images were recorded by using a broadcast-quality, digital video camera Canon G10 mounted on a rotating arm. The names of structures, when seen for the first time, appear on screen in labels as a learning reinforcement. Video processing, adding descriptions, and uploading commentary has been done in computer programme Sony Vegas Pro.

Results: Our team has created 20 short videos of the head and neck regions highlighting complex spatial relationship of various structures in the human body, which is the very essence of gross anatomy.

Conclusions: Our collection of videos has been incredibly well-received by students and new educators in Anatomy. Students feel more motivated by the use of audiovisual technology

and appreciate viewing videos at home as many times as they wish that results to better learning. Our future goal is to expand the array of topics, such as brain sections and to increase productivity of videos via the addition of team members and to create multimedia atlas of human body.

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PACLITAXEL TREATMENT ACTIVATES RECEPTORS FOR MITOCHONDRIAL DAMAGE ASSOCIATED MOLECULAR PATTERNS IN THE CHOROID PLEXUS

Zamani A., Kubičková L., Dubový P., Joukal M.

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

e-mail: mjoukal@med.muni.cz

Introduction: A major side effect of chemotherapeutic agent paclitaxel is peripheral neuropathy caused by mitochondrial degeneration in axons of peripheral nerves. We hypothesize that released damage-associated molecular patterns (DAMPs) from degenerated axons may affect the blood-cerebrospinal fluid (B-CSF) barrier in the choroid plexus (CP) via toll-like receptor 9 (TLR9) and formyl peptide receptor 2 (FPR2). Therefore, we aimed to investigate the expression of TLR9 and FPR2 in the CP after paclitaxel treatment.

Material and Methods: In our experiments, we intraperitoneally injected 4 doses (1, 3, 5, and 7 days; cumulative 8 mg/kg) of Paclitaxel or vehiculum (control) in Wistar rats. The animals were sacrificed 1, 7, 14 and 21 days after the last application together with naive rats. We analysed the expression level of TLR9 and FPR2 using immunohistochemical detection and quantification in choroid plexus.

Results: We found TLR9 and FPR2 immunopositivity in the cytoplasm and the plasma membrane of CP epithelial cells. TLR9 and FPR2 expression level were significantly higher in the CP when examined one day after the last IP injection of paclitaxel, in comparison with controls and naive. Interestingly, the expression level further increased with the time, being highest at 21 days after the last paclitaxel injection.

Conclusions: Our data show that paclitaxel application causes indirect molecular changes in the CP, based on the release of DAMPs. Up-regulation of TLR9 and FPR2 could potentially result in the release of pro-inflammatory cytokines and alter the structure of tight-junction proteins, which are essential for the functionality of the B-CSF barrier. Our data provides the possible molecular mechanism of the neuroinflammation in the CNS caused by paclitaxel.

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INFLUENCE OF ELECTROMAGNETIC RADIATION ON KIDNEY STRUCTURE OF PRENATALLY EXPOSED RATS

Almášiová V., Holovská K., Andrašková S., Cigánková V.

Department of Anatomy, Histology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

e-mail: viera.almasiova@uvlf.sk

Introduction: Because of rapid proliferation and differentiation of embryonic tissues, they are considered extremely sensitive to many environmental factors. The recent massive expansion of wireless technologies has raised concerns about the potential health risks related to long-term exposure of fetuses and newborns. The aim of this study was to investigate the influence of non-ionizing electromagnetic radiation on kidney of prenatally exposed rats.

Material and Methods: The experimental animals were exposed to a whole-body pulsed microwave irradiation at a frequency of 2.45 GHz and mean power density of 2.8 mW/cm² for 2 hours a day throughout their entire intrauterine development. Uniformity of the electromagnetic radiation in the purpose-designed chamber was assessed via a spectral analyser. Five weeks old rats were sacrificed and their kidneys were processed for histopathological evaluation by a common histological technique.

Results: The kidney parenchyma of the experimental animals revealed serious diffuse degenerative changes. The most pronounced structural abnormalities were observed within the renal corpuscles. They invariably had an irregular shape and their urinary spaces appeared significantly enlarged. Several renal corpuscles showed obvious signs of an advanced atrophy. The uriniferous tubules as well as collecting ducts had uneven shape and their epithelial lining was atypical. The epithelial cells had irregularly shaped nuclei and clearly vacuolized cytoplasm. The brush border of proximal tubules was affected. The interstitial spaces were typical in appearance. No necrotizing cells or inflammatory infiltrates were observed in the kidney parenchyma.

Conclusions: The observed histopathological changes in the kidney cortex and medulla resulted from the whole-body microwave radiation, which acted solely in the course of prenatal development. The presented data support the theory that prenatal exposure to a non-ionizing electromagnetic radiation may adversely affect the development of definitive organs, including the kidneys.

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SCAPULA – NEW ANATOMICAL TERMS OF NOT YET DESCRIBED STRUCTURES: A MODIFIED DELPHI APPROACH

Al-Redouan A., Kachlik D.

Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, Czech Republic

e-mail: azzat.al-redouan@lfmotol.cuni.cz

Introduction: The scapula structures that have not yet been assigned by anatomical terms generate a challenge in scientific observations as well in clinical applications. The morphology and topography at such small spaces should be described in a more straightforward wording with less descriptive sentences to avoid ambiguity in communication. We redefined the scapula as a lamina with projecting processes marked by landmarks defining not yet described additional topographical spaces.

Material and Methods: Descriptive study of structures was observed on routinely encountered dry scapulae and radiographs of the shoulder. A set of proposed terms was generated to be newly incorporated into Terminologia Anatomica, etymology of anatomical terminology was considered. In addition, contributed terms commonly encountered through the literature concerning the scapula were revised. A modified Delphi method was implemented to assess the degree of consensus among 20 nominated experts within the field of scapula research and clinical anatomy terminology.

Results: Proposal of new terms define grossly visible structures on the scapula that have not been described yet, including a call to unify contributed terms that have not been codified and are often used interchangeably within different scientific fields.

Conclusions: Incorporating these terms into the anatomical nomenclature would facilitate communication accuracy and eliminate ambiguity.

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MICRO-CT EVALUATION OF HEART ARCHITECTURE IN EXPERIMENTAL ANIMALS

Bartoš M.¹, **Olejníčková V.**^{1,2}, **Kvasilová A.**¹, **Gregorovičová M.**^{1,2}, **Sedmera D.**^{1,2}

¹ *Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic*

² *Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic*

e-mail: martin.bartos@lf1.cuni.cz

Introduction: Knowledge of heart morphology in experimental animals under different conditions is important because of its relevance to study of human cardiovascular diseases. Precise 3D visualization needed for its evaluation may be complicated in specimens of small size (micro- or millimeters). Micro-CT is a high-resolution imaging method offering visualizations and data analysis (e.g. structure quantification). Thanks to its non-destructive nature, specimen may be subjected to further examination or stored.

Material and Methods: Different specimens of excised hearts (adult/embryo; mouse, rat, chicken) were scanned using ex-vivo micro-CT SkyScan 1272 (Bruker micro-CT, Belgium). Scanning parameters were set based on specimen size and structure. Pixel size values ranged from 1 μm to 8 μm . Specimens were contrasted using PTA (phosphotungstic acid), iodine solution or osmium tetroxide. Vascular system was contrasted using Microfil (FlowTech, USA) injection in selected specimens.

Results: 2D (virtual sections) and 3D (volume and surface rendering) visualizations allowing studying of specimen structure are presented. Heart tissue was sufficiently X-ray contrasted in all specimens. Tissue shrinkage was observed. PTA resulted in better contrast in adults, iodine in embryos. Embryonic tissue generally presents lower contrast compared to adult tissue. Contrast vessels can be easily segmented and evaluated. In adult specimens certain parts of cardiac conduction system are noticeable.

Conclusions: Micro-CT is largely applicable in cardiac morphology evaluation in both adult and embryonic specimens. Micro-CT presents many benefits (e.g. non-destructivity, time efficacy) and can be combined with conventional approaches (e.g. histology).

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MORPHOMETRIC STUDY OF VARIABLE ORIGIN OF THE RECURRENT ARTERY OF HEUBNER

Bevızová K., El Falougy H., Zohdi V., Dovalová D., Mifkovič A., Kubíková E.

Institute of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

e-mail: katarina.bevizova@uniba.sk

Introduction: The recurrent artery of Heubner (RAH) is the largest vessel of the medial lenticulostriate arteries. It supplies many deep structures, mainly the corpus striatum, the globus pallidus, and the anterior crus of the internal capsule. The aim of the present paper was studying the morphological variations of the RAH and its diameter in relation to different areas of origin.

Material and Methods: The series contained the records from 183 formalin-fixed adult human brains. The calibrated digital images of the studied brains were evaluated and measured by Image J, which can calculate the number of pixels and convert them to metric measures.

Results: The RAH arose most often from the postcommunicating part of the anterior cerebral artery (47.81%). It originated from the precommunicating part of the anterior cerebral artery in 3.55% and at the level of the anterior communicating artery in 43.4% of cases. The RAH was missing in 5.19% and doubled in 6.28% of cases. The mean outer diameter of the RAH was 0.6 mm. The maximal measured diameter was 1.34 mm, and the minimal diameter was 0.19 mm.

Conclusions: The awareness of the various anatomical and morphometric variations of the RAH is essential in planning the neurosurgical procedures to avoid unexpected neurological complications.

THE EFFECTS OF REMOTE CONDITIONING ON SPINAL CORD ISCHEMIA/REPERFUSION INJURY IN RABBIT

Danková M.¹, **Domoráková I.**¹, **Fagová Z.**¹, **Stebnický M.**², **Mechírová E.**¹

¹ *Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic*

² *2nd Department of Surgery and L. Pasteur University Hospital Košice, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic*

e-mail: marianna.dankova@upjs.sk

Introduction: We aimed to determinate the effect of remote conditioning on the grey matter of rabbit spinal cord by functional assessment of hind limbs and immunohistochemical method.

Material and Methods: Ischemia (I) of the rabbit spinal cord was induced by occlusion of the aorta below the left renal artery for 20 min and reperfusion period (R) was 24 or 72 h. Remote conditioning was realized as preconditioning (PerC) – during last 12 min of ischemia or postconditioning (PostC) – in 1st or 3rd h of reperfusion. Both types of remote conditioning were induced by compression with a tourniquet of left fore limb in three cycles of 2 min of ischemia followed by 2 min of reperfusion. New Zealand rabbits were divided into 9 groups: control group (1), ischemic groups: 20I/24R (2) and 20I/72R (3) and experimental groups with remote conditioning: 20I/PerC/24R (4), 20I/PerC/72R (5) and 20I/24R/PostC 1hR (6), 20I/72R/PostC 1hR (7), 20I/24R/PostC 3hR (8) 20I/72R/PostC 3hR (9).

Results: The highest and therefore the best value of Tarlov score was obtained in animals in the experimental group 20I/24R/PostC 1hR (6). This obtained value was statistically significant (*p<0.5) compared to the ischemic group 20I/72R (3). Statistically significant decrease (**p<0.01) of Tarlov score was recorded 72 h after 20 min of ischemia in the ischemic group (3) vs. control group (1).

Conclusions: The study demonstrates that remote conditioning has beneficial effect on hind limbs function and reduces spinal cord I/R injury in rabbits.

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ISCHEMIA-REPERFUSION AND BRADYKININ POSTCONDITIONING: MORPHOMETRIC EVALUATION OF THE EDEMATOUS GREY MATTER LESIONS IN RABBIT SPINAL CORD

Domoráková I.¹, *Fagová Z.*¹, *Danková M.*¹, *Mechírová E.*¹, *Stebnický M.*²

¹ *Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic*

² *2nd Department of Surgery, Faculty of Medicine, Pavol Jozef Šafárik University in Košice and L. Pasteur University Hospital, Košice, Slovak Republic*

e-mail: iveta.domorakova@upjs.sk

Introduction: Paraplegia of hind limbs and neurodegeneration is a common sign after lumbar spinal cord ischemia-reperfusion in rabbits. We investigated potential effect of bradykinin postconditioning on neuronal survival and incidence of grey matter lesions.

Material and Methods: New Zealand male rabbits (n=48) were divided into sham control group (n=6), ischemia/reperfusion group (I/R; n=6) and four bradykinin postconditioned groups (postBr1, postBr6, postBr12, postBr24; each of n=9). Single dose of bradykinin was applied (i.p.) at 1, 6, 12 or 24 h after 20 min of ischemia, induced by abdominal aorta occlusion below the left renal artery. After 3 days of reperfusion animals were transcardially perfused with paraformaldehyde and samples from lumbar spinal cord were embedded to paraffin. Sections 8µm thick were stained by Nissl method. In the grey matter of L₅ segments edematous foci were measured with light microscope OLYMPUS BX50 with a digital camera OLYMPUS SP350 and QuickPHOTO Industrial 2.3 image analyser (Promicra).

Results: In each group, three sections from left and right sites of spinal cord grey matter were evaluated. I/R group showed neurodegeneration, large edematous foci, both in anterior and posterior horns. In all postBr groups, motor neurons with typical round euchromatic nuclei and prominent Nissl substance in perikaryon were found around smaller irregular edematous areas in the grey matter. In those animals function of hind limbs evaluated by Tarlov score was improved vs. I/R group. The percentage of edematous area from total area of spinal cord grey matter was as follows: I/R (87.5% injured), postBr1 (16%), postBr6 (41%), postBr12 (47%) and postBr24 (28%).

Conclusions: Bradykinin postconditioning protects spinal cord neurons against 20 min of ischemia and 3 days of reperfusion in the rabbits. However, bradykinin postconditioning applied 6 and 12 hours after ischemia showed lesser extent of protection.

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COMPARATIVE ANATOMY OF HUMAN AND ANIMAL EYEBALLS

Dovalová D., Zohdi V., El Falougy H., Miřkovič A., Bevízová K., Kubíková E.

Institute of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

e-mail: daniela.dovalova@fmed.uniba.sk

Introduction: In higher organisms the eye collects light from the environment, regulates its intensity, focuses, forms an image, converts this image into a set of electrical impulses transmitting them into the visual cortex through the optic pathway. Morphologically the porcine eye is structurally similar to human eye. The deer visual system is designed to function in extremely low-light conditions. The aim of this work was to study, observe and compare the morphology of the human, porcine and deer eyes.

Material and Methods: Two human eyes were obtained from 70 years old male cadaver, ten porcine eyes from a local butcher shop and four deer eyes from a local hunting association. The eyes were fixed in 10% formalin, processed by the paraffin embedding technique and stained with hematoxylin-eosin. The photo-micrographs were taken by light microscope equipped with digital camera.

Results: The porcine corneal thickness was twice that of the human cornea lacking Bowman's layer. The radius of the human lens had following parameters: anterior thickness was 10 mm, posterior 6 mm and central lens 4 mm. These values are smaller than those of the porcine eye. The porcine retina has displayed great similarity to the human retina. Deer ciliary muscles surrounding the lens were small with thick lens. These muscles are too weak to change the shape of a thick lens. The tapetum lucidum is a shiny, blue-green colored membrane attached to the deer retina enhancing their vision in low light. It does not exist in human nor porcine eye. Human pupil is round, whereas deer have a horizontal slit pupil that extends almost the entire width of the eye. The deer retina has a horizontal visual streak with cones highly concentrated along a wide band.

Conclusion: This work described the anatomy of the deer, porcine and human eyeballs collected from our histological study.

MITOCHONDRIAL CALCIUM RETENTION CAPACITY AND MITOCHONDRIAL MEMBRANE PERMEABILITY TRANSITION PORE

Endlicher R.^{1,2}, *Drahota Z.*³, *Červinková Z.*²

¹ Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

² Department of Physiology, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

³ Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

e-mail: endlicherr@lfhk.cuni.cz

Introduction: The mitochondrial permeability transition pore (MPTP) is a non-specific channel on the inner mitochondrial membrane that plays an important role in cell metabolism. It protects cells against oxidative stress and triggers processes resulting in cell death. Currently, method for determining mitochondrial retention capacity for calcium (CRC) is used to evaluate the MPTP function. The goal of our study was to compare the CRC values under different experimental conditions.

Material and Methods: All experiments were performed on liver mitochondria isolated from adult male Wistar rats. CRC was evaluated using the membrane-impermeable fluorescent probe Calcium Green-5N. This probe enables to monitor Ca²⁺ movements across the mitochondrial membrane. CRC indicates the amount of Ca²⁺ that must accumulate in mitochondria to induce pore opening.

Results: We observed that CRC is just an arbitrary value that can be modified by: A) the medium composition, B) the calcium concentration used to trigger MPTP opening, C) the time interval between calcium additions and D) the calcium/mitochondrial protein ratio in the medium. We found that all measured parameters caused significant changes in CRC values

Conclusions: Value of the CRC of rat liver mitochondria is highly dependent on the experimental conditions used. Our data show that this method of determining CRC value must be standardized for obtaining reproducible and generally comparable CRC data from different laboratories.

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BRADYKININ POSTCONDITIONING INFLUENCES NEURONAL SURVIVAL AND HSP-70 IMMUNOREACTION IN THE RABBIT SPINAL CORD AFTER ISCHEMIA

Fağová Z.¹, **Domoráková I.**¹, **Danková M.**¹, **Mechírová E.**¹, **Stebnický M.**²

¹ Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² 2nd Department of Surgery, Faculty of Medicine, Pavol Jozef Šafárik University in Košice and L. Pasteur University Hospital, Košice, Slovak Republic

e-mail: zfagova@gmail.com

Introduction: Elimination of damaged proteins is possible mechanism of ischemic tolerance acquisition. Heat-shock protein HSP-70 is produced especially in stress conditions such as ischemia and reperfusion. HSP-70 is involved in replacement of denatured proteins with newly synthesized proteins which prevents protein aggregation.

Material and Methods: New Zealand white rabbits (n=48) were divided into control group (n=6), ischemia/reperfusion group (I/R; n=6) and 4 bradykinin postconditioned groups (postBr1, postBr6, postBr12, postBr24; each group n=9) with bradykinin applied 1, 6, 12 or 24 h after 20 min of ischemia. Ischemia was induced by occlusion of the aorta below *a. renalis sinistra*. Reperfusion lasted 3 days. Paraffin sections from lumbar spinal cord segments were processed for NeuN (detection of surviving neurons) and HSP-70 immunohistochemistry. Number of surviving neurons was counted and HSP-70 immunoreaction was evaluated in anterior horns neurons of the rabbit spinal cord.

Results: Significant decrease in number of NeuN positive neurons was observed in I/R and also in all bradykinin postconditioned groups vs. control group. Furthermore, non-significant increase of NeuN positive neurons was registered in all bradykinin postconditioned groups vs. I/R group. Control group showed neurons with pale nuclei and HSP-70 positive cytoplasm. In I/R group, shrunken neurons with pyknotic nuclei and HSP-70 positive cytoplasm were found. In bradykinin postconditioned groups, surviving neurons cytoplasm was the same as in control group. Moreover, in postBr1 and postBr6 HSP-70 positive nuclei of surviving neurons were observed. Nuclear HSP-70 positivity was rarely present in postBr12 group. On the other hand, postBr24 nuclei did not exhibit HSP-70 positivity.

Conclusions: Bradykinin postconditioning confirmed beneficial effect on neuronal survival in the rabbit spinal cord after ischemia. Immunohistochemical analysis showed influence of bradykinin postconditioning on HSP-70 immunoreaction in neurons in anterior horns of the spinal cord.

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DETERMINATION OF ANATOMICAL EXTENT OF THE URINARY BLADDER CANCER

El Falougy H., Bevízová K., Zohdi V., Dovalová D., Mifkovič A., Kubíková E.

Institute of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

e-mail: hisham.elfalougy@fmed.uniba.sk

Introduction: The urinary bladder is a hollow muscular organ which lies entirely in the lesser pelvis when it is empty. The function of the bladder is to collect and empty urine. Malignant cancers of the urinary bladder are the second most common malignancy of the urinary system, and the fourth most common malignancy of all cancer, especially in men. The aim of this study was a retrospective analysis focused on the anatomical extent of bladder tumors (staging).

Material and Methods: We have analyzed the data of 244 patients (202 males, 42 females) with diagnosed bladder cancer of different type. The age of our patients ranged from 36 to 98 years.

Results: In 124 cases, it was *Ta*, noninvasive papillary tumor. In 32 cases it was tumor *T1*, this tumor has spread to the lamina propria mucosae, but it did not involve the bladder wall muscles. In 83 cases the tumor has spread to the muscle of the bladder wall, *T2*. In 41 cases the tumor has spread to the inner half of the muscle of the bladder wall *T2a*. In 42 cases the tumor has spread to the outer half of the muscle *T2b*. In 3 cases the tumor has grown into the perivesical tissue, as seen through a microscope *T3a*. In 2 cases the tumor has spread to the uterus or vagina *T4a*.

Conclusions: The correct determination of the anatomical extent of the tumor is the basis for establishing a proper diagnosis and successful treatment.

CANINE AND FELINE MAST CELLS: USING DIFFERENT FIXATION FLUIDS

Hamouzová P.¹, Čížek P.¹, Bartošková A.²

¹ Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

² Institute of Lifelong Learning, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

e-mail: hamouzovap@vfu.cz

Introduction: Effect of the used fixation fluid on the total number of mast cells (MCs) detected by metachromatic staining can differ among organs and species. The effect of various fixation fluids on MCs' detection was not known in canine or feline ovaries and uteri. The aim of this study was to evaluate the usability of different fixative fluids in above mentioned organs of domestic carnivores.

Material and Methods: All animals were kept as pets and were spayed for therapeutic reasons. No experimental procedure was performed on the animals. Samples were fixed in formalin, Carnoy's fluid (C), Mota's basic lead acetate (BLA), and isotonic formaldehyde-acetic acid (IFAA), processed by standard methodology and examined under the light microscope. Toluidine blue staining was used and MCs were counted in various parts of the ovaries and uteri.

Results: In the ovaries of both species, the numbers of MCs were significantly higher in C than in formalin. No significant differences were found between C and BLA (tested only in cats). In some parts of the uterus, numbers of MCs were significantly higher in C, BLA, and IFAA compared to formalin, in other parts only in C and BLA compared to formalin and in other parts also in BLA compared to IFAA.

Conclusions: The majority of MCs were formalin-sensitive in the parts of examined organs. Thus, using of formalin is not recommended. Conclusion of this study contributes to improve the methodology for MCs' detection in the canine and feline female reproductive organs.

VARIATION OF A DUPLICATED SUPERFICIAL BRANCH OF RADIAL NERVE AND A TWO-BELLIED BRACHORADIALIS MUSCLE: EPIDEMIOLOGY AND CLINICAL SIGNIFICANCE

Herma T., Kachlík D.

Department of Anatomy, 2nd Faculty of Medicine, Charles University, Czech Republic

e-mail: tomas.herma@seznam.cz

Introduction: We have found an interesting variation of two-bellied brachioradialis muscle (BM) and duplicated superficial branch of radial nerve (SBRN) during a routine student dissection course at the 2nd Faculty of Medicine, Charles University. The BM had one origin and insertion and under each of the bellies ran one SBRN. This variation was described only indirectly by Murphy and Blair and in was a cause of the Wartenberg's syndrome in a patient. The aim of our study was to determine the prevalence of this variation in the population.

Material and Methods: We have dissected 132 upper limbs (57 left, 75 right). Each variation of SBRN or BM was photographed and measured.

Results: We have found two cases of duplicated SBRN and two-bellied BM. In both cases, the SBRN was impinged by a muscle bundles connecting the two muscle bellies together. The prevalence of this variation in our sample was 1,51%. We have also found one case (0,76%) of duplicated SBRN without the duplication of the BM belly. The total prevalence of duplicated SBRN is 2,3%.

Conclusions: The variation of duplicated SBRN and BM is relatively common in Central Europe and rarely may be a cause of Wartenberg's syndrome.

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EXPRESSION OF SURVIVIN AND PACLITAXEL RESISTANCE IN RAT MAMMARY TUMOR CELLS

Hladová A., Rybárová S., Hodorová I.

Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: alena.hladova@student.upjs.sk

Introduction: Survivin is the smallest member of the Apoptosis Protein Inhibitor family, overexpressed in most human tumors, including mammary gland tumors. Overexpression of survivin is generally associated with tumor progression, drug resistance and poor prognosis. The goal of this study was to observe the impact of paclitaxel therapy on the expression of survivin in chemically-induced mammary tumors.

Material and Methods: Thirty-one rat females were used in this study. Mammary tumors were induced by 7,12 dimethylbenz(a)anthracene (DMBA). Rats were divided into two groups, a group treated by paclitaxel and a non-treated (control) group that was administered the physiological solution. According to the tumor stage, they were subdivided into carcinoma in situ (CIS) and invasive carcinoma (IC). To detect the expression of survivin, the immunohistochemical staining (IHC) was used. For statistical analysis, we have used the Chi-square test.

Results: A total of 74 samples of mammary gland tumors were analyzed. Survivin was expressed in 49 (77,8%) of 63 samples in the treated group and 7 (63,6%) of 11 samples in the non-treated group ($p < 0,05$). The difference in survivin expression between IC treated vs. IC non-treated tumors was statistically significant ($p < 0,05$), but in CIS treated group compared with non-treated group was no statistical significance.

Conclusions: Paclitaxel-induced survivin overexpression in mammary tumors seems to correlate with resistance to drug therapy. Therefore, survivin could be a useful marker for improving treatment outcomes. Learning more about survivin's role in drug resistance will be a necessary task for future studies.

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THE EFFECTS OF PRENATAL EXPOSURE TO ELECTROMAGNETIC RADIATION ON RAT LIVER

Holovská K.¹, **Almášiová V.**¹, **Andrašková S.**¹, **Beňová K.**², **Cigánková V.**¹

¹ *Department of anatomy, histology and physiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*

² *Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*

e-mail: katarina.holovska@uvlf.sk

Introduction: The aim of this study was to observe morphological changes in rat liver after prenatal exposure to electromagnetic radiation (EMR).

Material a Methods: The experimental animals were daily exposed to a pulsed microwave radiation at a frequency of 2.45 GHz and mean power density of 2.8 mW/cm² for 2 hours, throughout pregnancy. After delivery, the liver of 5-week old offspring was subjected to histopathological evaluation.

Results: The samples for light and electron microscopy (TEM) were processed by a common histological technique. In experimental group, the structure of the liver was not markedly altered. The hepatocytes had normal shape and size. Small inflammatory foci were observed in some lobuli. Examination by TEM revealed ultrastructural changes of varying extent. Significant changes were detected in the wall of liver sinusoids. Fenestrations in the sinusoidal endothelial cells (SEC) were enlarged. In these areas the space of Disse was dilated and irregular. In many regions, SEC were separated from the surface of hepatocytes which were subsequently directly exposed to blood. The basal surfaces of the cell membranes either contained few and irregular microvilli or remained smooth. A significant increase in the number of vesicles was observed. The vesicles of different shapes and sizes were usually located in the basal part of cells.

Conclusions: Our examinations demonstrated an adverse effect of EMR on ultrastructure of rat liver.

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PERINEURAL INVASION OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK – THE BEGINNING OF THE PATH

Hurník P. ^{1,2,3}, Židlík V. ¹, Žiak D. ¹, Sporková M. ^{1,2}, Štembírek J. ⁴, Čermáková Z. ^{6,7}, Putnová B. ⁵, Buchtová M. ⁵

¹ Department of Pathology, University Hospital Ostrava, Ostrava, Czech Republic

² Department of Pathology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

³ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

⁴ Department of Oral and Maxillofacial Surgery, University Hospital Ostrava, Ostrava, Czech Republic

⁵ Institute of Animal Physiology and Genetics, The Czech Academy of Sciences, Brno, Czech Republic

⁶ Department of Oncology, University Hospital Ostrava, Ostrava, Czech Republic

⁷ Department of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

e-mail: pavel.hurnik@gmail.com

Introduction: Perineural invasion has been defined as the ability of cancer cells to invade through, in or around nerves and is clinically associated with elevated recurrence and diminished survival. It was defined more than 100 years ago and it is morphologically described in solid head and neck tumors, prostate, pancreatic or colorectal cancer. Only a few years ago, the attention was devoted to the factors leading to it.

Material and Methods: We have retrospectively analysed cases of 532 patients with squamous cell carcinoma of head and neck in age from 33 to 86 years. In all cases, cervical block dissection was also performed with the resection of the main tumor. Patients without cervical block dissection were excluded. All cases were paraffin embedded and 3 µm sections were stained by means of the hematoxylin and eosin. The tumor size, depth of invasion, the presence and morphology of perineural invasion and the presence of lymphangiogenesis and hemangiogenesis were evaluated.

Results: From the total number of 532 cases, 70 (13,15%) cases with perineural invasion, 22 (4,13%) with hemangiogenesis and 60 (11,27%) with lymphangiogenesis were encountered. Generalisation to ipsilateral lymph nodes was found in 249 (46,80%) cases, contralateral generalisation was present in 39 (7,33%) cases. These factors were statistically compared with final statistical significance.

Conclusions: We focused mostly on the perineural invasion (PNI) which is probably based on the chemotropism of tumor cells that can be stimulated by nerve tissue to further growth. The interactions between tumor and neural cells is not only limited to cell migration and tumor growth from the primary location but such interaction can also stimulate axonogenesis or extend the nerves themselves together with increasing number of axons. The subject of our further study and this poster will be the analysis of changes in epithelial-mezenchymal transformation and the presence and distribution of chemoattractans of nerve tissue.

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FLUORO-JADE POSITIVE NEURONS IN THE CONTRALATERAL HEMISPHERE AFTER FOCAL CEREBRAL ISCHEMIA OF RAT

Hvizdošová N.¹, Horváthová F.⁴, Kluchová D.¹, Kolesár D.¹, Matěffy S.², Vecanová J.¹, Bona M.³

¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Alpha medical s.r.o., Diagnostic Center of Pathology, Member of the Unilabs Group, Prešov, Slovak Republic

³ Department of Medical Physiology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

⁴ T-Systems Slovakia s.r.o., Telekom IT, DTO-CC, LCC, Košice, Slovak Republic

e-mail: natalia.hvizdosova@upjs.sk

Introduction: Stroke induced ischemia has influence on the directly affected as well as on the contralateral hemisphere. Cells in the contralateral hemisphere start to degenerate in a pattern related to their sensitivity to ischemia. The aim of this paper is to analyse the number of degenerating neurons in regions of the contralateral hemisphere, the brain hemisphere not directly affected by ischemia.

Material and Methods: 24 male Wistar rats were used to evaluate the presence of Fluoro-Jade C positive cells. Animals were randomly divided in 4 subgroups (6 animals in each) according to duration of post-ischemic reperfusion: 90 minutes of ischemia followed by one (IR 1d), three (IR 3d) and seven days (IR 7d) recirculation, including control subgroup (C). For visualization of degenerating neurons, the staining with Fluoro-Jade C was used.

Results: In the cortex, number of dying cells started to increase at the 3dt post-ischemic day only in contra-penumbra region. The peak was reached at the 7th day in the contra-penumbra and in the contra-core. In striatum degenerating neurons seemed to be significantly increased in both regions from the 1st day of post-ischemic recovery. The highest count of Fluoro-Jade C positive neurons was observed in contra-core on the 3dt and stayed significantly elevated up to 7th post-surgery day. In contra-penumbra, Fluoro-Jade C positive cells slightly decreased with ongoing postischemic reperfusion.

Conclusions: Our results clearly show that neurodegeneration affects neurons in regions close to ictus and in the contralateral hemisphere as well. Fluoro-Jade C positive cells emerged earlier in ischemia more sensitive cells of striatum, later in more resistant cortical neurons.

Our results demonstrate that brain ischemia affects the whole brain response, which is very important in understanding the pathophysiology of the ischemic condition.

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CONFIRMATION OF ULTRASTRUCTURAL FINDINGS BY IMMUNOFLOUORESCENT EXAMINATION IN PRIMARY CILIARY DYSKINESIA

Kadlecová S.¹, **Martinů V.**², **Pohunek P.**², **Uhlík J.**¹

¹ *Department of Histology and Embryology, Second Faculty of Medicine, Charles University, Prague, Czech Republic*

² *Department of Paediatrics, Second Faculty of Medicine and University Hospital Motol, Charles University, Prague, Czech Republic*

e-mail: simona.kadlecova@lfmotol.cuni.cz

Introduction: Primary ciliary dyskinesia (PCD) is a rare autosomal recessive disease affecting cilia and manifesting mainly in respiratory system. Due to the highly complex structure of cilia, the disease is very heterogenous. Currently, more than 40 causal genes are known and the number is still increasing. That is why the genetic examination of PCD is complicated and unavailable in the Czech Republic. Various PCD types can manifest by different severity of clinical symptoms. Thus, the knowledge of the specific PCD type is important. We present two PCD patients, in which the diagnostic combination of transmission electron microscopic (TEM) and immunofluorescent (IF) examinations was used.

Material and Methods: The material for the TEM and IF examinations was collected by endobronchial or nasal brush biopsy. The TEM was performed in resin contrasted sections. The IF of four proteins used as markers of various axonemal structures followed.

Results: The patient 1 (P1) showed a high rate of central pair defects often associated with peripheral doublet dislocations. The patient 2 (P2) displayed a quantitatively significant microtubular disorganization of ciliary axonemes together with missing inner dynein arms. The IF of P1 revealed a negative marker of radial spokes RSPH9. In P2, two axonemal proteins GAS8 and DNALI1 were missing from cilia. This finding is described only in two types of PCD – CCDC39 and CCDC40.

Conclusions: The combination of TEM and IF examinations confirmed the diagnosis of PCD and determined its very probable type in both patients.

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IMMUNOHISTOCHEMICAL ANALYSIS OF THE DORSAL ROOT ENTRY ZONE AND DORSAL FUNICULUS OF THE SPINAL CORD IN RAT

Kolesár D.¹, **Maršala J.**^{2†}, **Filipčík P.**³

¹ Department of Anatomy, Pavol Jozef Šafarik University in Košice, Košice, Slovak Republic

² Institute of Neurobiology, Slovak Academy of Sciences in Košice, Košice, Slovak Republic

³ Institute of Neuroimmunology, Slovak Academy of Sciences in Bratislava, Bratislava, Slovak Republic

e-mail: dalibor.kolesar@upjs.sk

Introduction: Dorsal root entry zone (DREZ) is region on the posterolateral sulcus of the spinal cord where the dorsal rootlets of the corresponding spinal nerves enter into the spinal cord. It represents site where structures of the peripheral nervous system are connected to structures of the central nervous system.

Material and Methods: We used immunocytochemical methods to detect neuronal nitric oxide synthase-immunoreactive (nNOS-IR), parvalbumin-immunoreactive (PV-IR) and calcitonin gene-related peptide-immunoreactive (CGRP-IR) axonal profiles in DREZ and adjacent dorsal column regions of the spinal cord.

Results: We found thick nNOS-IR and PV-IR axons entering the spinal cord in massive dorsomedially situated medial bundle and thin nNOS-IR and CGRP-IR axons in thinner ventrolaterally oriented lateral bundle. In addition, we observed presence of thick heavily myelinated nNOS-IR and PV-IR axons in deep portions of the dorsal column mainly in region situated dorsomedially to the dorsal horn of the spinal cord.

Conclusions: We demonstrated for the first time that there is segregation of thick heavily myelinated nNOS-IR and PV-IR A α / β fibers and thin poorly myelinated A δ and unmyelinated C nNOS-IR and CGRP-IR fibers in DREZ, the former making massive medial bundle and the latter less prominent lateral bundle of the dorsal rootlets of the spinal nerve.

PREPARATION METHOD OF LIGAMENTOUS APPARATUS SPECIMEN OF THE STIFLE JOINT IN HORSES

Korim F., Žert Z., Karamanová M., Horňáková P.

Equine Clinic, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

e-mail: filkorim@gmail.com

Introduction: Progress of the new surgical and imaging approaches in equine medicine requires excellent anatomy knowledge. The anatomy of the stifle joint in horses is complicated and depends upon new realistic models for study and training. Surgeries performed in stifle joint are mostly because of osteochondral defects, meniscal trauma, cruciate ligaments trauma, ruptures, and other. Ultrasound examination of the stifle joint is complicated because many other structures cover the ligaments and menisci. The goal of this work was to prepare new real models of stifle joint's ligaments.

Material and Methods: In our work we used 8 equine hind limbs (Slovakian warmblood breed). The hind limbs were ex-articulated in the *articulatio coxae* and perfused through *arteria femoralis* by 0,9% saline. The knee joints were dissected and samples (n=2) were macerated, (n=4) were fixed in „our solution“ and (n=2) fixed in thymol solution. All stifle joints were dried at laboratory temperature and observed in detail. We evaluated plasticity of tissue, colour, odour, leakage of blood, and fat.

Results: Samples processed by maceration technique on day 10 were completely destroyed by maceration medium. Samples prepared in „our solution“ were fixed 14 days. After fixation the colour was white in all structures. During process of drying, menisci were yellowed, ligaments stayed white and cartilages were removed. Plasticity of tissue was bad, because all structures have dried out too much. However, thymol's fixation preserved articular cartilage and plasticity of cartilage. The colour of all structures was brown. We observed small movements of joints. Blood leakage and fat leaking were present in two samples fixed in „our solution“.

Conclusion: The key to preparation of stifle joint specimen is excellent dissection of structures. Our fixation solution have very good characteristics. On the other hand maceration is not a suitable process of preparation, however in the preparation of tarsal joint specimen it showed excellent features. Our samples are fixed, odourless and allow long years use during teaching process (under gradual and post gradual) and scientific research.

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IMMUNOHISTOCHEMISTRY OF COLORECTAL CARCINOMA: EXPRESSION OF SURVIVIN

Kováčová Z., Hodorová I.

Department of Anatomy, Faculty of Medicine, P. J. Šafárik University in Košice, Košice, Slovak Republic

e-mail: zuzana.kovacova@upjs.sk

Introduction: Colorectal cancer is the third most common type of cancer and the fourth most common cause of cancer-related mortality globally. Therefore, scientists have been searching for specific biomarkers or potential prognostic factors that could be applied in the diagnosis and treatment monitoring of patients with colon cancer or could affect the character of the tumour cell. Survivin is a member of the inhibitor of apoptosis (IAP) protein family. It prevents apoptosis through the inhibition of caspase 3 and caspase 7. The protein takes part in the process of mitosis, it is involved in a spindle formation, and can also cause chemoresistance or angiogenesis. In contrast to the other IAPs, the expression of survivin is minimal in normal tissues, therefore, it has become a lead target for a tumour diagnostic and prognostic as well as for anti-cancer therapies. For these reasons, we have decided to detect survivin in colon cancer cells.

Material and Methods: We have used a three-step indirect immunohistochemical method for the detection of survivin (using of monoclonal mouse anti-human survivin antibody, clone 12C4, Dako North America, Inc.) in 74 specimens of human colorectal adenocarcinomas. Eventually, we have evaluated specimens using qualitative and quantitative analysis (a semi-quantitative method).

Results: A positive immunoreaction was observed in 15 cases (12 positive and 3 high positive samples) that presents 20,27%. Survivin was detected only in cytoplasm. A negative immunoreaction was observed in 59 cases (42 negative and 17 low positive samples) that presents 79,73%. There was no significant association among the expression of survivin and grading of the tumour (Chi-squared test; $p > 0,05$).

Conclusion: In this pilot study we have detected survivin in 20,27% of cases. Our results show no significant association among survivin expression and grading of the tumour. It is necessary to find an association between survivin expression and the other clinical-pathological parameters as patient's age, patient's sex, a staging of tumour and presence or absence of metastasis or compare the expression of survivin in colon cancer cells with other potential prognostic factors.

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THE EFFECT OF ASPARTAME AND SODIUM NITRITE ON MOUSE PREIMPLANTATION EMBRYOS *IN VITRO*

Kovaříková V., Šefčíková Z., Špírková A., Kšiňanová M., Koppel J., Číkoš Š.

Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovak Republic

e-mail: kovarikova@saske.sk

Introduction: Food additives are substances added to food to preserve flavour or enhance its taste, appearance, or other qualities. In the European Union (EU) all food additives are identified by an E number. For the evaluation of potential embryo-toxicity, we have selected two additives: aspartame and sodium nitrite. Aspartame is an artificial sweetener used as a sugar substitute in some foods and beverages. In the European Union, it is codified as E951. Sodium nitrite is a food additive that gives cured meats their characteristic red colour and flavour, it inhibits the growth of bacterial spores that cause botulism. It is coded as E250 by the European Union.

Material and Methods: Design of our experiment was as follows. We obtained 2-cell embryos from oviducts of hormonally stimulated and mated mouse females. Embryos were divided into 4 groups. Control group was cultivated in KSOM medium, experimental groups were cultivated in KSOM medium with addition of aspartame or sodium nitrite. 3 different concentrations (1, 10, and 100 μM) for each additive was used. After 72- hours of cultivation we used vital propidium iodide staining and TUNEL assay for the evaluation of cell death *in vitro* developed blastocysts. Embryos were then analyzed under fluorescence microscope.

Results: For aspartame at 10 and 100 μM we found significant decrease of mean cell number per blastocyst and significant increase in dead cell numbers in blastocyst. For sodium nitrite at 10 and 100 μM there was significant increase in dead cell numbers in blastocyst. Developmental analysis were performed also. The majority of embryos cultivated with aspartame reached blastocyst stage. At 1 μM concentration it was 92.5 %, at 10 μM 85% and at 100 μM 87.5% of embryos. Similar result for sodium nitrite was observed. At 1 μM concentration it was 100%, at 10 μM 100%, and at 100 μM 93.33% of embryos.

Conclusions: Our results indicate that the presence of aspartame and sodium nitrite at concentrations 10 μM and higher in the microenvironment of developing embryo can influence quality of obtained blastocysts.

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ANATOMIC INVESTIGATION OF THE CRANIAL CARDIAC VEIN IN RATS

Krešáková L., Vdoviaková K., Petrovová E.

Department of Anatomy, Histology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

e-mail: lenka.kresakova@uvlf.sk

Introduction: Rat models that mimic human cardiac diseases are standard, and useful models to study cardiovascular disease. The cardiac venous system is a target for the delivery of drugs, angiogenetic growth factors, gene vectors, cardioprotective reagents, and stem cells. The aim of this study was to describe the anatomic continuity and clinical significance of the cranial cardiac vein.

Material and methods: The analysis of cranial cardiac vein was performed using a corrosion cast method and perfusion of colored latex in 36 adult, healthy Wistar rats of both sexes.

Results: The cranial cardiac vein was located between the right conal and right cardiac vein, drained the blood from the cranial part of the proximal third of the right ventricle, advanced dorsocaudally, and terminated in the right atrium. The cranial cardiac vein was inconsistent and was present in only 58,3% of cases. This vein drained the right ventricle as a single vein (7 cases, 19,4%) or as a tributary of the right cardiac vein (10 cases, 27,8%) or merged with other veins. In 3 cases (8,3%), the cranial cardiac vein constituted a common trunk with the major caudal and right cardiac veins, and in 1 case (2,8%), the cranial cardiac vein was united with major caudal, right cardiac, and minor caudal veins.

Conclusions: Knowledge of the arrangement of the coronary veins is necessary to optimal clinical cardiac procedures. Some interventional techniques can use the access to the myocardium provided by this vein.

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DEVELOPMENT OF THE CARDIAC CONDUCTION SYSTEM IN AVIANS: CHICK VERSUS OTHER SPECIES

Kvasilová A.¹, **Olejníčková V.**^{1,2}, **Gregorovičová M.**^{1,2}, **Sedmera D.**^{1,2}

¹ *Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic*

² *Institute of Physiology, The Czech Academy of Sciences, Charles University, Prague, Czech Republic*

e-mail: alena.kvasilova@lf1.cuni.cz

Introduction: Easy accessibility of the chick embryo makes it a particularly suitable model for study of the developing cardiac conduction system (CCS) in the avian embryo. Could it be really regarded as a representative and universal model for all avians?

Material and Methods: The hearts of avian species available to us (e.g. chick, duck, goose, pheasant, quail) were collected at various stages of embryonic development. We performed staining on alternating serial paraffin sections using histology (Hematoxylin&Eosin&Alcian Blue) and immunohistochemistry staining with Human Natural Killer-1 (HNK-1) antibody to visualize the positivity in specialized myocyte populations of CCS, and co-staining with different myocardial markers.

Results: We focused on the myocardium of the sinus venosus, the atrioventricular conduction axis, and the extracellular matrix of the cardiac cushions/valves. Meanwhile, HNK-1 visualization has not shown significant differences in the positivity of the specialized structures belonging to the central CCS (SAN, AV myocardium, His bundle and proximal bundle branches) in our studied group of the avian hearts. In all our examined species, the HNK-1 was expressed by the cardiac nerve fibers and ganglia, epicardium, valve mesenchyme, and subendocardium.

Conclusions: According to our observations, we conclude that the chick embryo could be regarded as an optimal avian representative and it is appropriate and sufficient for the study of the development CCS.

The study was supported by the Grant Agency of the Charles University No. 1456217, Ministry of Education, Youth and Sports of the Czech Republic PROGRES-Q38/LF1, and Czech health research council of the Ministry of Health of the Czech Republic No. 17-28265A.

MORPHOLOGICAL VARIATION AND DISTRIBUTION OF NON-METRIC TRAITS IN HUMAN AURICLE

Lakatosová K.

Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

e-mail: kladia.lakatosova@lfhk.cuni.cz

Introduction: Auricle is characterized by unique signs, which are individually variable and some of them do not change their morphology even with age. Due to these unique traits, auricle can be used in forensic practice as an individual parameter. The expansion of 3D visual technologies caused, that 3D models are gradually replacing 2D recordings. Following research concerns reevaluation of definitions, which are concerning morphology of auricles and which were published so far. Furthermore, it compares reliability of original definitions with adapted and newly designed definitions. Resulting definitions were used for processing of image atlas with insights of 3D models.

Material and Methods: As material were used 3D models from database of 3D human faces FIDENTIS, created by members of Laboratory of Morphology and Forensic Anthropology, Department of Anthropology, Masaryk University, using scanners Vectra XT and Vectra M1 (Canfield Inc.) 3D models were visualized in editing program MeschLab. On the models, the signs were graded at different time horizons. The choice of individual signs was made based on previous research. The concordance rate between the individual evaluations was calculated using the Fleiss Kappa coefficient. The statistical significance of variations in individual evaluations was quantified using the chi-square test.

Results: The results of this research were determined relevance of selected signs to identification by usage of 3D model of auricle. The next part was determination of definitions, which are the most reliable for evaluation of 3D models. Besides that, the charts of frequency representation of individual sign demonstrations were made.

Conclusions: The auricle has been shown to be a suitable parameter for individual identification. It was found which morphological features are applicable for its assessment on 3D records. Previous definitions for ear evaluation have been reviewed and new assessment approaches have been developed.

GEOMETRIC AND MORPHOMETRIC CONTRIBUTION TO THE MAXILLARY SINUS VISUALIZATION

Lovásová K.¹, Hudák R.², Tóth T.², Rajtuková V.², Živčák J.², Kachlík D.³, Boleková A.¹,
Hodorová I.¹

¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Department of Biomedical Engineering and Measurement, Faculty of Mechanical Engineering, Technical University of Kosice, Slovak Republic

³ Department of Anatomy, 2nd Faculty of Medicine Charles University, Prague, Czech Republic

e-mail: kvetuse.lovasova@upjs.sk

Introduction: 3D reconstruction and the scan of the maxillary sinus (MS) can be used in forensic anthropology, for criminal investigations, determination of unknown subjects and also in cases of orthodontic patients who should be treated by placement of mini-implants to present the illusion of depth. This study is focused on identifying the MS geometry and morphology in different ageing processes in specific samples of the population. Main aim of this study was to use the high-end software for the analysis and visualization of computed tomography data to increase it into the future oriented development of maxillary sinus imaging.

Material and Methods: Parameters – length, width and height of solid impressions were measured and compared due to validation by using of two different 3D (coordinate) measurement software Netfabb® for 3D printing and VG Studio Max 2.2 with a specified 3D reference system of coordinates x, y, z. The impression diameters were measured in 3D space according three coordinates x, y and z. Reproducibility and accuracy of measurements were assessed by comparing of the two different software measurements by two different independent operators. Isometric type of projection was used in both software to visualise 3D objects. All measured values were recorded and graphically evaluated.

Results: This study analysed morphological comparative and descriptive data for the MS evaluation. All observations and measurements were made to define the MS morphology. The different anatomical dimensions of the MS were obtained from 3D projection images with CAD/CAM software.

Conclusions: With the unique anatomical documentation and 3D projection we would like to present new and modern view on the MS geometry and morphology in a sample of the Slovak population. The studies of different populations may provide relevant data for

comparative analysis and better understanding of regional and word differences with respect to environmental and social influence.

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5G4 IMMUNOHISTOCHEMISTRY IN THE ENTERIC NERVOUS SYSTEM

Mechirová E.¹, Škorvánek M.², Tóth Š.¹, Gdovinová Z.²

¹ Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Department of Neurology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice and L. Pasteur University Hospital, Košice, Slovak Republic

e-mail: eva.mechirova@upjs.sk

Introduction: Aggregated α -synuclein, the main component of Lewy bodies and neurites associated with Parkinson's disease is found also in the enteric nervous system (ENS). To visualize this disease associated α -synuclein in colonic mucosa and submucosa of living patients we used a monoclonal antibody 5G4 (Analytic Jena Roboscreen) and the results were compared to anti-phosphorylated α -synuclein monoclonal antibody (pSyn#64, Wako Chemicals) immunostaining.

Material and Methods: Our cohort contains 182 patients (168 controls, 7 PD patients, 7 prodromal patients). After routine colonoscopy we evaluated the bioptic material from ascendent and sigmoid colon as well as upper limb using a monoclonal 5G4 antibody and anti-phosphorylated α -synuclein monoclonal antibody.

Results: 5G4 strongly binds to the high molecular weight fraction of β -sheet rich oligomers. No binding to primarily disordered oligomers or monomers was observed.

We documented strong 5G4 positive nerve fibers in colonic submucosa and mucosa and ganglionic cells in the submucous nerve plexus. In the sections from upper limb skin, 5G4 and phosphorylated α -synuclein positivity was detected among smooth muscle cells of m. arrector pili and in perivascular localization.

Conclusions: The novel 5G4 and anti-phosphorylated α -synuclein monoclonal antibodies can serve as biomarkers for detection of Parkinson's disease also in prodromal premotor period.

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MACROSCOPIC RELATIONS OF INTERNAL CAPSULE AND ITS CONNECTIONS

Mifkovič A., Zohdi V., El Falougy H., Dovalová D., Bevízová K., Kubíková E.

Institute of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

e-mail: andrej.mifkovic@fmed.uniba.sk

Introduction: Even though the brain gross anatomy has been studied for years, the intrinsic micro-anatomical study of the white matter is still neglected. Part of the white matter structures of the brain are association fibers and internal capsule. The internal capsule lies between the lenticular and caudate nuclei.

Materials and Methods: Dissection was performed on five formalin-perfused human brains. It was used medial and lateral approach of dissection including a transverse section.

Results: Structure and topography of the internal capsule was described by transverse section of the brain. Medial approach of dissection revealed the association fibers of cingulate gyrus, superior longitudinal fasciculus, corona radiata and internal capsule. Using a lateral approach of dissection it was described insula, lentiform complex, part of the internal capsule, superior longitudinal fasciculus and short U-shaped association fibers. After superficial dissection of pons and cerebral crus it was found continuation of pyramidal tract from posterior limb of internal capsule to cerebral crus. Dissection from lateral aspect of hemisphere exposed the interconnections of neighbouring sulci with short U-shaped association fibers.

Conclusion: The dissection of white matter of the brain and thus the tracts, is very important not only from educational point of view but most importantly, from clinical point of view. It is crucial for medical students to realize and to understand the 3-dimensional topography of white matter in the brain. For physicians is very important to evaluate various revolutionary imaging methods as well as to be able to diagnose lesions and compressions of white matter.

GPX8 IN PREIMPLANTATION EMBRYOS AND FEMALE GENITAL ORGANS OF RAT

Mihalik J.¹, Kreheľová A.¹, Kovaříková V.², Domoráková I.³, Solár P.⁴, Pavliuk-Karachentseva A.¹, Rybárová S.¹, Hodorová I.¹

¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Košice, Slovak Republic

³ Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

⁴ Department of Medical Biology, Faculty of Medicine, Pavol Jozef Šafárik University, in Košice, Košice, Slovak Republic

e-mail: jozef.mihalik@upjs.sk

Introduction: Oxidative stress, which may contribute to the development of various diseases, such as cancer, diabetes, inflammation, and infertility, is a result of imbalance between reactive oxygen species and antioxidant defense system. Glutathione peroxidases (GPx) are one part of such defense mechanism. The total lack of information as to GPx8 distribution in female genital organs and preimplantation embryos prompted us to start investigation into this area.

Material and Methods: 15 sexually matured rat females were killed by a lethal dose of anesthetics. Uterine horns, oviducts, and ovaries were removed on 1., 3., and 5. day of pregnancy and embryos were flushed out with PBS+BSA solution. GPx8 in embryos was visualized under confocal microscope using specific FITC-conjugated antibody and enzyme in genital organs was detected under light microscope by immunohistochemical method employing DAB as chromogen.

Results: GPx8 in oocytes was uniformly diffused in cytoplasm, while in zygotes was concentrated into clusters around the nuclei. The same concentrated distribution was observed till blastocysts developmental stage. In female genital organs was GPx8 found predominantly in blood vessels. Moreover, enzyme occurs also in uterine glands, in cells of connective tissue of oviduct and in granulosa lutein cells of corpus luteum.

Conclusions: We found out that GPx8, the last discovered antioxidant enzyme from GPx family, is present in rat oocytes, preimplantation embryos and female genital organs. Further experiments are needed to describe functional role of this protein during pregnancy.

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DETERMINATION OF NEURAL STEM CELL POTENTIAL IN VITRO CONDITIONS UNDER NORMAL AND PATHOLOGIC CONDITIONS AT VARIOUS STAGES OF POSTNATAL ONTOGENESIS OF RAT SPINAL CORD

Mochnacký F.¹, **Ševc J.**¹, **Slovinská L.**², **Alexovič Matiašová A.**¹, **Daxnerová Z.**¹

¹ Department of Cell Biology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

² Institute of Neurobiology of Biomedical Research Center, Slovak Academy of Sciences, Košice, Slovak Republic

e-mail: juraj.sevc@upjs.sk

Introduction: Neural stem cells (NSCs) are capable of self-renewal and multilineage differentiation. Normally, the NSCs are quiescent, but under pathologic condition, as spinal cord injury, they could become active. The aim of our study was to specify the neurogenic potential of NSCs derived from central canal lining *in vitro* conditions at various stages of postnatal development of rat, before and after stimulation by spinal cord injury (SCI).

Material and Methods: Wistar rats at following age: postnatal day 7 (P7, early postnatal development), P29 (late postnatal development) and P120+ (adulthood) were subjected to minimal SCI. Intact and sham operated animals at the same age were used as controls. 4 days postop, animals were sacrificed, lesioned spinal cords were isolated and cultivated *in vitro* for 12 days in proliferation media and another 14 days in medium without growth factors for induction of differentiation.

Results: During early postnatal development, the NSCs formed neurospheres with largest diameter (almost 3x larger compared to adulthood) and in higher numbers (2x compared to adulthood). The size and count of neurospheres decreased during ontogenesis. After SCI, we did not observe significant changes in count and size of neurospheres in any of tested groups. Neurogenic potential of NSCs was highest during early postnatal development (3,5x compared to late postnatal development and adulthood). After minimal SCI, neurogenic potential increased only at late postnatal development (7,7x compared to sham controls). Potential of NSCs to form oligodendrocytes did not changed during postnatal development but in adulthood, we observed 5,5x increase after SCI.

Conclusions: Our results indicate that during early postnatal development possess NSCs higher mitotic and neurogenic potential and therefore the best regenerative capacity. SCI effectively stimulates NSCs in late postnatal development which should be promising steppingstone for future studies.

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ANATOMICAL STUDY OF ARTERIA ILIACA EXTERNA – CASE STUDY OF PATIENT WITH HIP JOINT PAIN

Musil V.¹, Šeremeta M.^{2,3}, Krbcová-Moudrá V.², Riedlová J.², Mržílková J.², Zach P.², Lami J.³

¹ Centre of Scientific Information, Third Faculty of Medicine, Charles University, Prague, Czech Republic

² Department of Anatomy, Third Faculty of Medicine, Charles University, Prague, Czech Republic

³ Centre of Nuclear Medicine Inc., Prague, Czech Republic

e-mail: vladimir.musil@lf3.cuni.cz

Introduction: Isolated aneurysm of arteria iliaca externa is serious but not very common disease. Due to anatomical localization it is hard to make correct diagnosis. The disease usually manifests by not much pronounced clinical symptoms so that it's catchment is mostly random.

Material and Methods: In the submitted contribution is documented case study of 64-years old female with marked hip joint pain. Following examination techniques were deployed: X-ray of the hip joint, three-fold skeletal scintigraphy, sonography of arteria iliaca externa and CT scan of arteria iliaca externa.

Results: Aneurysm of arteria iliaca externa was diagnosed by combination of above mentioned examinations at the Nuclear Medicine Department.

Conclusions: Hip joint pain was attributed to aneurysm with possible nerve plexus in the abdominal/pelvic region irritation.

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ANATOMICAL STUDY OF FIBULAR NOTCH

Naňka O., Fojtík P., Bartoniček J.

Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic

e-mail: Ondrej.Nanka@lf1.cuni.cz

Introduction: One of the problems of ankle traumatology today is the reduction of the distal fibula to fibular notch of tibia and the evaluation of the quality of the reduction on CT. Another problem is the optimal height for inserting the syndesmal screw. Therefore, we decided to carry out an anatomical study in order to obtain relevant data.

Material and Methods: 261 adult tibia from the Pachner Collection were studied. We measured the length of the tibia, the height of the fibular notch, the width of the notch at the widest point and finally the depth measured to the incisural line joint, again 3 and 10 mm above the top of the facies articularis inferior tibiae and at the deepest point, and the distance of this deepest point from the top facies articularis inferior tibiae.

Results: Average width at facies articularis inferior tibiae was 2.36 ± 0.36 cm. 3 mm above this area was 2.2 ± 0.22 cm wide. Ten millimetres above this area was 1.89 ± 0.23 cm wide. The average height was 4.25 ± 0.56 cm. Depth 10 mm above facies articularis inferior tibiae was 0.41 ± 0.12 cm, 3 mm above this area 0.38 ± 0.12 cm. The largest depth of notch was 0.45 ± 0.12 cm. This place was between the previous measurement levels, typically 5 mm above facies articularis inferior tibiae, approximately at the apex of the incisural crests.

Conclusions: The depth of the fibular notch is greatest at the level of the incisural crests which is usually 5-6 mm above the facies articularis inferior tibiae. Therefore, the position of the distal fibula in the notch should be evaluated on CT sections at this level. The optimal height for inserting the syndesmal screw is 3 ± 0.5 cm above the top of the facies articularis inferior tibiae.

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GPx8 EXPRESSION IN CANCER CELLS OF COLORECTAL CARCINOMA

Pavliuk-Karachevtseva A., Krehelova A., Mihalik J., Hodorova I.

Department of Anatomy, Faculty of Medicine, P.J. Šafárik University in Kosice, Kosice, Slovak Republic

e-mail: andriana.pavliuk-karachevtseva@upjs.sk

Introduction: GPx8 is a mammalian heteromeric cysteine-rich glutathione peroxidase (GPx), contained in the endoplasmic reticulum membrane. It has a similar structure and functions with other members of the family of GPxs. This enzyme is transcriptionally regulated by HIF α and modulates the growth factor signalling in HeLa cells. Numerous studies show an increase in the level of different types of GPxs in tumour tissues and affected lymph nodes, expression of the GPx genes in cancer cells. But at the same time little is known about the role of the oxidation of the oxidative stress in the metastatic lymph nodes. It has been described in the colorectal cancer cells that the increase of intracellular reactive species is first associated with cell growth and invasion. Nowadays colorectal carcinoma is one of the most common types of cancerous tumours. It reaches 10% of all cancer incidents, ranked third in the prevalence and fourth in morbidity among all cases of cancer. It is necessary to improve our knowledge about colorectal carcinoma for the understanding of reasons and mechanisms and for improving the diagnostic, treatment and prognosis to patients.

Material and methods: In our studies, we used the three-step indirect immunohistochemical method of detection of GPx8 (using the rabbit polyclonal antibody to GPx8 Biorbyt) in 58 specimens of the human colorectal adenocarcinoma. Then, we evaluated specimens, using qualitative and quantitative analysis (semi-quantitative method).

Results: The immunohistochemical staining showed high cytoplasmic positivity in 13 cases (12 positives and 1 high positive samples) that presents 22,41%. Negative immunological reaction was observed in 45 cases (39 negative and 6 low positive samples) that presents 77,59%.

Conclusions: Our research shows the positive immunological reaction to the anti-GPx8 antibody in 22,41% concerning the cases of human colorectal adenocarcinoma specimens. In our future studies, we will compare the expression of GPx8 with the expression of GPx4 and GPx1 in colorectal carcinoma. This can help us to find the relationship between colorectal carcinoma and oxidative stress. Further studying of the pathophysiological mechanisms of the onset and course of the disease can enhance the diagnostic of the tumour, the providing of new treatments, improve the prognosis to patients with colorectal carcinoma.

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MYOCARDIAL AND ADIPOSE TISSUE CD68+ CELLS ARE ASSOCIATED WITH CARDIOVASCULAR AND METABOLIC DISEASE IN HEART SURGERY HUMAN PATIENTS

Pierzynová A.¹, Šrámek J.¹, Cinkajzlová A.², Kratochvílová H.², Lindner J.³, Haluzík M.², Kučera T.¹

¹ *Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, Czech Republic*

² *Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*

³ *2nd Department of Surgery – Department of Cardiovascular Surgery, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

e-mail: Aneta.Pierzynova@lf1.cuni.cz

Introduction: Macrophages alone and in the form of crown-like structures (CLS) are a potent source of pro-inflammatory cytokines in adipose tissue and myocardium. Macrophages can contribute to the pathogenesis of obesity-associated disorders including type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD). We compared M1 and M2 populations and occurrence of CLS in the right atrium of myocardium (RA), epicardial (EAT) and subcutaneous (SAT) adipose tissue.

Material and Methods: Samples of human tissue were obtained during elective heart surgery (non-obese, n=34; obese, n=24). The macrophages were visualized by immunohistochemical method. A fraction of EAT and SAT samples was analysed using flow cytometry.

Results: Pro-inflammatory M1 phenotype of macrophages in adipose tissue was strongly associated with presence of the CLS. Presence of CLS in SAT is associated with all analysed pathologies, presence of CLS in EAT is associated with obesity and CAD. SAT macrophages were more frequent in CAD compared to non-CAD patients (p-Value=0.012). RA macrophages were more abundant in obese compared to non-obese patients (p-Value=0.045).

Conclusions: This result demonstrates that the phenotype of macrophages is important for development of pathological processes in the adipose tissue associated with obesity, CAD and T2DM. The hypothesis that therapeutic modulation of SAT and EAT inflammation may represent a target for treatment of obesity.

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THE MORPHOLOGICAL STRUCTURE OF THE HUMAN PINEAL GLAND AT LIGHT AND ELECTRON MICROSCOPIC LEVELS

Polakovičová S.¹, Liška J.¹, Rísová V.¹, Kopáni M.², Krivošíková L.³, Polák Š.¹

¹ *Institute of Histology and Embryology, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovak Republic*

² *Institute of Biophysics, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovak Republic*

³ *Institute of Pathological Anatomy, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovak Republic*

e-mail: simona.polakovicova@fmed.uniba.sk

Introduction: This small neuroendocrine gland secreting the hormone melatonin is located on the roof of the third ventricle. The parenchyma contains, in addition to the pinealocytes and supporting interstitial neuroglial cells also brain sand (acervulus). These calcified structures were described firstly in 18th century. In order to provide more detailed information about morphology light and electron microscope were used.

Material and methods: Samples of the human pineal gland were obtained at the Institute of Pathological Anatomy Faculty of Medicine, Comenius University in Bratislava, Slovakia. The study protocol was approved by the ethical committee. Tissue samples were processed by routine histological technique. For electron microscopy (TEM and SEM) the human pineal glands were fixed in glutaraldehyde and prepared by routine technique.

Results: At the light and electron microscopic observation normal architecture of the pineal gland was revealed. The interstitial cells were smaller than the pinealocytes, have darker triangular nucleus and cytoplasm. In the parenchyma of the gland mostly homogenous intrapineal acervulus in various size was recognized, extrapineal acervulus was observed only rarely. For more detailed classification we used SEM by freeze-etching technique. This technique enabled to recognize mulberry-like acervulus. Immunohistochemical analysis revealed nerve fibers and some types of neurons.

Conclusions: The great amount of research over the last decades of the past and this century led to better understanding the morphology, development and function of the pineal gland. Research in humans is so complicated, because of the small sizes of the gland that make it difficult to obtain the material. Human material is usually obtained from the necropsy, but that is exposed to autolysis. Nevertheless our research on the human tissue can clarify to

some extent questions about the structure and function of this miraculous and interesting gland.

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MICRO-CT VISUALIZATION OF METAPHYSEAL GROWTH PLATE AS A TOOL TO EVALUATE THE MODE OF GROWTH IN IGUANID LIZARDS

Riedlová J. ¹, Mrzilková J. ¹, Frýdlová P. ², Dudák J. ³, Žemlička J. ³, Šeremeta M. ^{1,4}, Křemen J. ¹, Zach P. ¹

¹ Department of Anatomy, Third Faculty of Medicine, Charles University, Prague, Czech Republic

² Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

³ Institute of Experimental and Applied Physics, Czech Technical University in Prague, Czech Republic

⁴ Centre of Nuclear Medicine Ltd., Prague, Czech Republic

e-mail: jana.mrzilkova@lf3.cuni.cz

Introduction: Growth plate cartilage (GPC) is responsible for the growth of long bones due to endochondral ossification, which is the main mechanism of longitudinal skeletal growth in tetrapods. Degradation of GPC is a sign of determinate growth as it arrests the growth irreversibly. By contrast, indeterminate growth requires the persistence of GPC throughout the entire life. The goals of our study were (1) to verify that μ CT has sufficient resolution and contrast for unambiguous detection of the presence/absence of growth plates in long bones, and (2) to reinvestigate mode of growth in lizards, which is usually considered as indeterminate.

Material and Methods: To visualize GPC on the proximal part of the femur, we employed micro-radiography and micro-computed tomography. Micro-radiography was used for the initial visualization. In smaller samples and/or in bones with the progress of growth plate degradation, we employed μ CT with high magnification and spatial resolution capability suitable for the following 3D image analyses. The imaging systems were equipped with semiconductor hybrid pixel detectors based on the Timepix technology. The high-resolution set-up features a micro-focus X-ray tube and a large field of view. Thanks to the cone-beam imaging geometry, it is suitable for imaging with high magnification factors and spatial resolution better than 5 μ m.

Results: We analysed 95 long bones of iguanid lizards and found that the employment of micro-radiography and μ CT enables detailed visualization of GPC.

Conclusions: The universality of indeterminate growth of squamate reptiles has been challenged again. We suggest that the dual type of growth represents an extreme case of heterochrony.

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THE INCREASED NUMBER OF MAST CELLS IN EPICARDIAL ADIPOSE TISSUE IS ASSOCIATED WITH CORONARY ARTERY DISEASE

Rozsivalová K.¹, **Pierzynová A.**¹, **Kloučková J.**^{2,3}, **Lindner J.**⁴, **Lipš M.**⁵, **Haluzík M.**^{2,3}, **Kučera T.**¹

¹ *Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, Czech Republic*

² *Institute of Medical Biochemistry & Laboratory Diagnostics, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

³ *Centre for Experimental Medicine and Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*

⁴ *Second Department of Surgery – Department of Cardiovascular Surgery, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

⁵ *Department of Anesthesiology, Resuscitation and Intensive Medicine, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

e-mail: tkucer@lf1.cuni.cz

Introduction: Inflammation of adipose tissue can affect its metabolic activity associated with the pathogenesis of coronary artery disease (CAD). Mast cells represent an important component of the innate defense system. In our work, we quantified mast cell counts in epicardial adipose tissue (EAT), subcutaneous adipose tissue (SAT), and atrial myocardium (AM) in patients undergoing open heart surgery with various metabolic diseases.

Material and Methods: Biopsic samples of EAT (n=44), SAT (n = 42) and AM (n=17) were fixed by 4% paraformaldehyde and embedded into paraffin. All participants signed a written informed consent prior to the enrollment into the study and the study was approved by Human Ethics Review Board. An anti-mast cell tryptase antibody was used for immunohistochemical detection and quantification of mast cells. The expression of CD117 and chymase was also demonstrated immunohistochemically.

Results: In ET of patients with CAD, a higher number of mast cells was found compared to patients without CAD (3.7 ± 2.6 vs. 2.1 ± 1.2 cells / mm²). Higher number of mast cells in ET of patients with obesity and type 2 diabetes mellitus (DM2T) was not statistically significant. In ST and AM there was no difference in the number of mast cells in patients with obesity and without obesity, neither in patients with CAD or without CAD nor in patients with DM2T and without DM2T. Mast cells in ST, ET and AM expressed CD117 and chymase.

Conclusions: An increased number of mast cells in ET patients with CAD may indicate the specific role of these inflammatory cells in relation to epicardial adipose tissue and coronary arteries affected by atherosclerosis.

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p53 EXPRESSION IN RELATION TO PACLITAXEL RESISTANCE IN RAT MAMMARY TUMOR CELLS

Rybárová S., Hladová A., Hodorová I.

Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: silvia.rybarova@upjs.sk

Introduction: p53 regulates cell growth and plays an important role in inducing apoptosis of cells. Mutations of p53 gene produce dysfunctional p53. Mutant p53 lives much longer than functional wild-type, which leads to high IHC detectable p53 protein levels in transformed cells. p53 is in stabilized form, which is inactive form. Abnormalities in p53 expression are common in breast cancer, lung cancer and many other malignancies. The goal of this study is to find out, if the paclitaxel therapy of breast cancer influences the expression of p53.

Material and Methods: Thirty-one rat females were used in this study. Mammary tumors (N=88) were induced by 7,12 dimethylbenz(a)anthracene (DMBA). Rats were divided into two groups, a group treated by paclitaxel and a non-treated (control) group that was administered the physiological solution instead of DMBA. According to the tumor stage, they were subdivided into carcinoma in situ (CIS) and invasive carcinoma (IC). To detect the expression of p53, the immunohistochemical staining (IHC) was used.

Results: A total of 88 tissue samples of mammary gland tumors were analyzed. p53 was expressed in all tissue samples (88 samples=100%). Moreover, there was no difference in p53 expression between treated and non-treated group. The difference in p53 expression between IC treated vs IC non-treated tumors, also between CIS treated vs CIS non-treated tumors were not statistically significant.

Conclusions: Overexpression of p53 were seen in all samples of both groups, treated and control (nontreated) groups, IC and CIS tumors. Therefore, according to our results, the expression of p53 was not influenced by paclitaxel treatment. p53 is in general good marker for drug resistance in many cancers. Its expression in rat mammary malignancy is very high, but according to results of our study, with no influence of paclitaxel therapy and type of tumor.

The study was supported by the Grants VEGA 1/0204/16, 1/0173/19, and KEGA 019 UPJŠ-4/2017.

THE EFFECT OF FGF2 IN WOUND HEALING

Ševčíková Z.¹, Danešová M.², Buchtová M.¹

¹ Institute of Animal Physiology and Genetics, The Czech Academy of Sciences, Brno, Czech Republic

² Enantis, Brno, Czech Republic

e-mail: buchtova@iach.cz, sevcikova618@gmail.com

Introduction: Chronic wounds represent important problem for individual human patients, who can suffer physically or mentally, as well as for the human society. One of the possible cures could be Fibroblast growth factor 2 (FGF2). Unstable form of this protein has been proven to demonstrate significant positive effects in wound healing. We propose that stable form of FGF2 (FGF2-STAB) will enable us to improve efficiency of wound healing. Our research was focused on histopathological analyses of skin wounds treated by recombinant human FGF2 (FGF2-WT) or its stable form (FGF2-STAB) manufactured by Enantis.

Material and Methods: Two wounds were generated in the dorsal area of diabetic male rats. Each wound was treated by scaffolds soaked with FGF2-WT or FGF2-STAB. One of the wounds was collected after 14 days of healing, the second wound after 28 days.

Basic histological staining Haematoxylin-Eosin to analyze general effect of this protein in wounded tissue was used. Collagen fibers maturation was evaluated by special staining using Sirius Red. Immunohistochemical labeling of Ki67 and PCNA proteins were used as well as alpha SMA analysis and pan-cytokeratin staining. TUNEL assay uncovered the distribution of apoptotic cells in treated tissues. MPO labeling and mastocytes visualization was used for the comparison of inflammatory tissues response.

Results: Histopathological analyses uncovered reduced panniculitis in rats treated by FGF2-STAB. Immunohistochemical analyses revealed decreased amount of myofibroblasts in FGF2-STAB treated samples. Moreover, we found increased cell proliferation in tissues treated by both forms of FGF2 proteins. The number of inflammatory cells was reduced in the animals treated by FGF2-STAB compared to untreated animals.

Conclusions: Our study revealed that the speed and even the quality of skin wound healing was enhanced by FGF2-STAB treatment in comparison to controls. Based on our results, we predict FGF2-STAB as possible future treatment for wound healing. However, more analyses confirming its harmlessness in experimental animals will be necessary.

Acknowledgement: The study was supported by the European Union's Horizon 2020 Programme SME Instrument **GA No. 756471**.

MATHEMATICAL MORPHOLOGY AS A TOOL FOR QUANTITATIVE DESCRIPTION OF AN ADIPOSE TISSUE ON THE HISTOLOGICAL SLIDE

Šrámek J., Kučera T.

Institute of Histology & Embryology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

e-mail: jsram@lf1.cuni.cz

Introduction: Obesity is associated with both morphological and functional disturbances in the white adipose tissue. Direct analysis of morphological changes of adipocytes is difficult, therefore analysis of 2D sections is the widely used approach. We have used approach based on the mathematical morphology for quantitative analysis of histological slides of adipose tissue.

Material and Methods: We have used a collection of hematoxylin-eosin samples of human adipose tissue. From each sample, at least five representative images have been taken. Analysis of images has been performed using MATLAB® (MathWorks®, ver. R2018b) and Image Processing Toolbox. Images have been binarized. Successive opening with the structuring element „disk“ with increasing diameter has been used to produce the granulometric spectrum. Finally, quantiles of the granulometric spectrum of have been used for comparison of spectra from different sites. For purposes of stability analysis, the same analysis has been performed for different parameters of binarization.

Results: Obtained spectra are stable with respect to small disturbances of parameters. Shape of spectra from different sanoles differs.

Conclusions: Proposed approach to analysis of morphology of adipose tissue seems to be a useful tool for comparison of the morphology of histological samples of adipose tissue.

*The study was supported by **PROGRES Q25**.*

POTENTIAL OF R2 RUDIMENT TO FORM A TOOTH IN ISOLATION REFLECTS THE ANCESTRAL PATTERN OF ODONTOLOGY

Steklíková K.^{1,2}, Tucker A. S.^{1,3}, Hovořáková M.¹

¹ *Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic*

² *Department of Cell Biology, Faculty of Science, Charles University, Czech Republic*

³ *King's College London, Craniofacial Development & Stem Cell Biology Division, London, United Kingdom*

e-mail: klara.steklikova@iem.cas.cz

Introduction: During early sequential development of mouse molars we observe rudimentary tooth bud structure known as R2 which is incorporated into the nascent first molar during its development. R2 initiates in the antemolar diastema, where premolars in murine ancestors' used to be located. Here we have focused on the tooth forming potential of R2.

Material and Methods: Embryos manifesting green fluorescence in *Shh* expressing cells were used for tissue cultures. Left and right emerging tooth germs at 13.5 and 14.3 dpc were dissected. One pair of the germs was cut into an anterior rudiment, corresponding to R2, and posterior parts, corresponding to the future M1, and both parts were cultured separately. Contralateral intact tooth germs dissected from the same specimen were used as control. The germs were cultured for 6 days.

Results: The anterior part of the tooth germ, developed into a discrete tooth germ when experimentally separated from the posterior part. In cultures of 14.3 dpc tooth germs, signalling centre was recovered in the anterior part after 24 hours in culture. After two days, a signalling centre appeared also in the posterior part and this part formed a similarly sized tooth as the anterior part. In the culture dissected at 13.5 dpc we observed development of two teeth from the posterior part. In total three tooth germs were formed from the dissected dental tissue. In the intact placodes, two molars appeared with the second molar (M2) forming after the third day of culture. As expected M2 was smaller than M1 in these cultures, reflecting differences in timing of initiation and final tooth size.

Conclusions: Rudimentary structures seem to preserve odontogenic potential, which can be revealed by interruption of their natural dynamics. This supports the theory that developmental process likely reflects the evolutionary history of species.

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VASCULAR SUPPLY OF THE SUBEPICARDIAL NERVES AND PURKINJE FIBERS OF THE PORCINE HEART

*Stingl J.*¹, *Musil V.*², *Šach J.*³, *Vránová J.*⁴, *Suchomel Z.*⁵, *Kudrna V.*⁶, *Riedlová J.*¹,
*Kachlik D.*⁷, *Zach P.*¹

¹ *Department of Anatomy, Third Faculty of Medicine, Charles University, Prague, Czech Republic*

² *Centre of Scientific Information, Third Faculty of Medicine, Charles University, Prague, Czech Republic*

³ *Department of Pathology, Third Faculty of Medicine, Charles University and Teaching Hospital Kralovske Vinohrady, Prague, Czech Republic*

⁴ *Department of Medical Biophysics and Informatics, Third Faculty of Medicine, Charles University, Prague, Czech Republic*

⁵ *Department of Pediatrics, Hospital Teplice, Teplice, Czech Republic*

⁶ *Institute of Animal Science, Prague, Czech Republic*

⁷ *Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, Czech Republic*

e-mail: vladimir.musil@lf3.cuni.cz

Introduction: The purpose of the study was the morphological analysis of the vasa nervorum, supplying the subepicardial nerves and Purkinje fibers on the porcine heart.

Material and Methods: The study was performed on 20 hearts of healthy pigs. The tissue blocks for the histological analysis were cut out from the upper, middle and lower segment of the anterior interventricular region, fixed in formalin and stained with HE, van Gieson, and CD 34. Transversal sections were analysed.

Results: The microscopical analysis have brought following results: 317 subepicardial nerves were identified, with a range of diameter of 15-347 μm . Their vasa nervorum were found in 75% of cases. In this group of vascularised nerves 43.75% were the perivascular nerves, while 56.25% of them supplied freely coursing subepicardial nerves. The presence of the vasa nervorum was independent on the diameter of the nerves, and on their topography. In the complexes of Purkinje fibers, their striking supply by blood vessels was found only in two exceptional cases.

Conclusions: The majority of the subepicardial nerves of the porcine heart possess well developed vasa nervorum. This fact may play a significant role in the modulation of the cardiac nerves, for instance by the retrograde application of the neuro-stimulating medications, currently used in the invasive cardiology and in the cardiac surgery.

*The study was supported by the **Project Q41**.*

ARTICULAR CARTILAGE DEFECTS REGENERATION USING A CHITOSAN BASED SCAFFOLD IN THE SHEEP

Šimaiová V.¹, Tomčo M.¹, Holovská K.², Krešáková L.¹, Vdoviaková K.¹, Petrovová E.¹

¹ Department of Anatomy, Histology and Physiology, Institute of Anatomy, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

² Department of Anatomy, Histology and Physiology, Institute of Histology and Embryology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

e-mail: veronika.simaiova@uvlf.sk

Introduction: Since cartilage is avascular, aneural, allymphatic and contains only a small population of single cell type (chondrocytes), its ability to regenerate after injury is limited.

Material and Methods: By the experimental 2 years old Merino and Valaška female sheeps (n=6) were performed full thickness defect on the knee joint in the trochlea of the femur using the osteochondral autograft transfer system. After that, the defect sites were filled with chitosan-based composite biopolymer material. In control animals (n=2) the cartilage defect was not filled with biomaterial. At the end of the 6-month monitoring period, samples were taken from the implantation site for histological analysis, stained with hematoxylin-eosin and safranin-O.

Results: Histological examination in the experimental animals showed defect completely filled with hyaline-like cartilage tissue. The most frequently noticed changes were: reduction in matrix staining intensity due to reduced GAG content, chondrocytes were not arranged in zones, cell clumps were formed at the periphery of the newly formed tissue, and the presence of slots and a thinner subchondral plate were also observed.

Conclusion: Based on our partial results we can assume a positive effect of applied biomaterial on the process of osteochondral defect regeneration in articular cartilage.

The study was supported by the Grants VEGA No. 1/0050/19 and APVV-17-0110.

X-RAY COMPUTED TOMOGRAPHY AS A TOOL FOR DETERMINING ENAMEL THICKNESS

Teleký J.¹, Maloveská M.¹, Krešáková L.¹, Tóth T.²

¹ Department of Anatomy, Histology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, Košice, Slovak Republic

² Department of biomedical engineering and measurement, Faculty of mechanical Engineering of Technical university of Košice, Košice, Slovak Republic

e-mail: jana.teleký@uvlf.sk

Introduction: X-ray computed tomography is a fast-growing method in scientific research applications that allows for non-destructive imaging of morphological structures. The aim of this paper is to provide the enamel thickness of incisors in two species: beaver and hare.

Material and Methods: Attention has focused on incisors of European beaver (*Castor fiber*) and European hare (*Lepus europaeus*). The both species originated from Slovakia. The teeth inside the skull and mandible were scanned by two types of Computer Tomography using Metrotom 1500 from Carl Zeiss (beaver) and nano CT using Phoenix from GE (hare). The material comprised 50 teeth, 30 of hare (15 of the mandible, 15 of the maxilla) and 20 of beaver (10 of the mandible, 10 of the maxilla). Since the density of the tooth layers differs, the thickness of the incisor's enamel is measurable. The enamel was measured in a labio-lingual direction, at a right angle to the transversal plane.

Results: The results of measurements were evaluated using software Volume Graphics VG Studio MAX 2.2. Although the both animals species had different body weight and way of life, the presented CT imaging revealed that the enamel of mandibular incisors was thicker than the enamel incisors in maxilla in both of them. Interestingly, the different values were in different part of tooth in beaver and hare, too.

Conclusions: Use of CT has a big advantage, it makes use of computer-processed combinations of many X-ray measurements taken from different angles to produce cross-sectional images of specific areas of a scanned object, allowing the user to see inside the object without cutting.

The study was supported by the **Projects VEGA 1/0571/17 and KEGA 013UVLF-4/2017.**

MORPHOLOGICAL CHANGES OF SMALL INTESTINE AND TESTES IN NORM, IN IRRADIATION AND UNDER THE INFLUENCE OF THE BIOSTIMULANT – ASD-2

Teshayev Sh.¹, Haribova E.², Radjabov A.¹, Shukurov I.³, Khojiev D.¹, Khasanova D.¹,
Baymuradov R.¹

¹ Department of Anatomy, Clinical Anatomy (OSTA), Bukhara State Medical Institute, Bukhara, Uzbekistan

² Department of Human Morphology, Moscow State University of Medicine and Dentistry, Moscow, Russia

³ Department of Medical chemistry, Biochemistry, Bukhara State Medical Institute, Bukhara, Uzbekistan

e-mail: teshayev@mail.ru

Introduction: Biostimulants, including ASD-2, has immunomodulating properties that can be studied on the digestive and reproductive system.

Material and methods: An experimental study was conducted on the material of the small intestine and testes of 60 white male rats, weighing from 210 to 280 g. The material was distributed into 3 groups: I – intact control group (n=20); II – rats with irradiation (n=20); III – rats (n=20), which were administered for 20 days solution of a biostimulant at a dose of 0.01 ml / kg in relation to distilled water 1:5 intragastrically during irradiation.

Results: The form of grouped lymphoid nodules in rats of the experimental group, in contrast to the control to move from the initial part to the final part of the small intestine, varies from oval to rounded and irregular. The number of single nodules increases, which was not observed in a healthy group. The control group of rats had mainly rounded (46.2%) or oval (51.2%), rarely irregular or quadrangular (2.6%) forms, and irradiated mainly irregular (34.2%) and rounded (52.5%), rarely oval (13.3%). In the 2-group rats at mature age (90 days old), the diameter of the convoluted tubules compared to the control is 23% less, their cross-sectional area lags by 41%, (in the group receiving the biostimulant, 11% and 26%, respectively). When exposed to a biostimulant (against a background of radiation), the process of spermatogenesis is 10-12 days late, and when exposed to only radiation lateness is 22-28 days.

Conclusions: With the correction of ASD by fraction 2, the restoration of plaques from the irregular to the round or oval form is carried out, and the number of single nodules increases, which was not observed in the irradiated and healthy group. Moreover, the biostimulant reduces the pathological effect of radiation sickness on the structural elements of the testes.

UPREGULATED VESSEL FORMATION IN THE CONTRACTED SIDE OF THE CLUBFOOT

Novotný T.^{1,2,3}, **Uhlík J.**², Eckhardt A.³, Ošťádal M.⁴

¹ Department of Orthopaedics, Masaryk Hospital, Ústí nad Labem, Czech Republic

² Department of Histology and Embryology, Second Faculty of Medicine, Charles University, Prague, Czech Republic

³ Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

⁴ Department of Orthopaedics, University Hospital Bulovka, Charles University, Prague, Czech Republic

e-mail: tomas.novotny@kzcr.eu

Introduction: The clubfoot deformity belongs to the group of fibroproliferative disorders. Even though it is one of the most common birth defects, its origin remains still unknown. Our study aimed on the morphological comparison of contracted and non-contracted clubfoot tissue in order to find changes helping to elucidate the clubfoot etiology.

Materials and Methods: In our study, we used the immunohistochemistry, light microscopy and image analysis. We compared the expression of vascular endothelial growth factor (VEGF) and the level of vascularization between contracted clubfoot tissue, i.e. the medial side of the foot (M-side), and non-contracted clubfoot tissue, i.e. the lateral side of the foot (L-side). Samples from ten patients were analyzed (N=10 for M-side, N=10 for L-side). The differences in quantification between the L- and M-sides were checked for normality (Q-Q plot) and compared by two-sample t-test. The significance level to reject the null hypothesis was set to 0.05.

Results: We observed a significantly higher expression of VEGF as well as a significantly higher level of vascularization in the contracted clubfoot tissue compared to the non-contracted clubfoot tissue.

Conclusions: Our findings are in consensus with our previously published results that showed an active remodeling process in the fibrous tissue of contracted (medial) part of the foot in the clubfoot deformity. We found, that the soft tissue remodeling and fibroproliferation are accompanied by the upregulated vessel formation in the medial side of the clubfoot. These findings may contribute to the recognition of the clubfoot etiology and to the further development of our therapeutic strategies.

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THE IMPORTANCE OF THE RAT JEJUNAL ARTERIES IN THE EXPERIMENTAL SURGERY

Vdoviaková K., Krešáková L., Petrovová E., Šimaiová V.

Department of Anatomy, Histology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovak Republic

e-mail: katarina.vdoviakova@uvlf.sk

Introduction: The present time brings the rapid development and new advances in the surgical research of the experimental medicine and in its further education. The objective of this study was investigated the morphometric parameters, the anatomical arrangement and number of jejunal arteries and their relationship to the intestinal length in rats.

Material and Methods: The experiment was carried out on 50 one-year-old laboratory rats, breed Wistar of both sexes and weighing approximately 350 – 520 g. We measured the length of the jejunum after midlaparotomy and then we prepared corrosion casts of the arteries of the jejunum.

Results: We observed that the jejunal arteries originated from the cranial mesenteric artery, which is the thickest branch of the abdominal aorta. In 26 % we observed 14 jejunal trunks, and 4 jejunal arteries which originated separately from the cranial mesenteric artery. In 26 % we detected 16 jejunal trunks, and 3 jejunal arteries which originated separately from the cranial mesenteric artery. In 24 % we found 18, and in another 24 % cases, 20 jejunal trunks.

Conclusions: The results of our study showed that the number of jejunal trunks and jejunal arteries increased continually with the elongation of the rat intestine and body weight. Our results point to the importance of research of the circulatory system of the rat intestine for future experimental transplantation of digestive organs.

*The study was supported by the **Project No. 1/0050/19.***

GASTROINTESTINAL MICROBIOTA AS A BIOMARKER OF PARKINSON'S DISEASE

Ventosa J. R., Škorvánek M.

Department of Neurology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Louis Pasteur University Hospital, Košice, Slovak Republic

e-mail: joaquim_ventosa@yahoo.com

Introduction: Parkinson's disease (PD) is a chronic neurodegenerative condition, characterized by gradual loss of dopaminergic neurons. Usually when diagnosed, it is already in its late motor phase. Thus, biomarkers such as the gastrointestinal microbiota are being researched, to allow for an earlier diagnosis, and to further understand the pathophysiology of this disease. Considering this, our aim was to investigate if there are specific compositions of gastrointestinal microbiota in PD patients, different from those observed in healthy people, and in prodromal patients, with a high risk of developing PD.

Material and Methods: 15 stool samples were collected – 5 from healthy controls, 5 from prodromal patients, and 5 from PD patients. These subjects followed inclusion and exclusion criteria outlined by the Movement Disorder Society (MDS). After, DNA was extracted and displayed in denaturing gel gradient electrophoresis (DGGE) profiles, as DNA fragments of different sizes, to compare the separate composition of *Eubacteria*, *Bacteroidetes*, *Firmicutes*, *Blautia coccooides* and *Lactobacillus*, between each one of the samples.

Results: A specific size of DNA fragment was identified in the DGGE profile for *Blautia coccooides*, which was present in 5 out all 5 healthy controls, in 4 out of 5 prodromal patients, and only in 2 out of 5 PD patients.

Conclusions: This could demonstrate a decrease in the concentration of *Blautia coccooides* with the progression of PD, already evidenced in prodromal patients, and reinforce the hypothesis of the gastrointestinal microbiota as a PD biomarker.

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PROPORTIONS OF GROWING CHICK EMBRYO BODY WALL ON EMBRYONIC DAY 6

Vohníková M., Škapová K., Hubičková Heringová L., Maňáková E., Zemanová Z.

Department of Histology and Embryology, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic

e-mail: zemanovazma@gmail.com

Introduction: Development of the ventral body wall (BW) of chick embryos in embryonic day 6 (ED6) was studied, and changes of proportions of the primary and secondary body wall (BW-I) and (BW-II) were noted. Asymmetry in the length and thickness of the BW-II was detected, so we aimed to evaluate these parameters at three different sites down the embryo's long axis.

Materials and methods: 5µm thick transverse sections of the embryos at three levels: (A) "liver and heart, (B) "liver" and near umbilicus (C) "liver and stomach" were prepared. Using the CellSens software, we measured: body circumference, BW-I length and area and BW-II length (distance between the dorsal midline and the transition between BW-I and BW-II).

Results: The BW circumference is greatest at level A; BW-II is longest there with similar values at either sides. At levels B and C BW-II is longer on the left and the difference increases craniocaudally. BW-I is longest at level A and shortest at level B. The thickness of BW-I increases towards the umbilicus.

Conclusions: On ED6 the length of BW-I and BW-II varies between A, B, and C. Cranially, the BW is longest close to the heart (approx. level A). The BW initially develops faster on the left with the right side catching up later. Close to the umbilicus BW-I is the thickest and second longest, likely due to fast growth of BW-I there. Growth of the BW conspicuously coincides with the growth of internal organs.

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HYPOTHALAMIC CHANGES ASSOCIATED WITH CHRONIC LIQUID NUTRITION FEEDING

Vrabcová M., Polák Š.

*Institute of Histology and Embryology, Faculty of Medicine, Comenius University in Bratislava,
Bratislava, Slovak Republic*

e-mail: michaela.vrabcova@fmed.uniba.sk

Hypothalamic neurons play a key role in the regulation of energy metabolism. The chronic intake of liquid nutrition is accompanied by the development of obesity in rats. The increased amount of fats and especially carbohydrates contained in the specific type of liquid nutrition disturb energy balance associated with local inflammation and leptin/insulin resistance in the hypothalamus. These „adverse“ hypothalamic changes involves the activation of glial cells, a process called gliosis characterized by proliferation and morphological rearrangement of the microglia and astrocytes. Proinflammatory signals and gliosis lead to disruption of functions and loss of hypothalamic neurons; consequences associated with development of obesity and associated metabolic disorders.

WHAT IS MORE EFFECTIVE FOR ACADEMIC PERFORMANCE IN ANATOMY – INTENSIVE ANATOMY COURSE OR REGULAR WEEKLY PRACTICALS?

Výbohová D.¹, **Boleková A.**², **Hešková G.**¹, **Mellová Y.**¹

¹ Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

² Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: vybohova@jfmed.uniba.sk

Introduction: The objective of this study was to determine which design of the anatomy course is more effective for the academic success.

Material and Methods: The academic performance (scores of mid semester tests, final exam written test, final practical and oral exam) of 458 students was analysed considering the anatomy course design. First group students (n=382) attended regular weekly practicals and lectures during semesters - 6,5 academic hours (AH) per week during 26 weeks. Second group students (n=76) attended intensive anatomy course during one month studying the only anatomy - 42 AH per week during 4 weeks.

Results: Statistical analysis revealed that students who attended regular weekly practicals and lectures achieved significantly better score in mid semester viscerology tests, in final practical and oral exam than the students who attended intensive month-long anatomy course. However, there was no significant difference in scores of mid semester osteo-myo-arthrology tests, and written final exam test. Interestingly, when we compared the test scores between the groups separately for female and male students, results showed that score differences were significant only in female students not in male ones.

Conclusions: We can assume that the course with regular weekly practicals and lectures is more effective for the academic success of female students, however, the academic performance in male students was comparable in both types of courses.

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DETECTION OF CELL APOPTOSIS AND NECROSIS USING IMAGE ANALYSIS

Weismann P.¹, *El Falougy H.*¹, *Bohumel T.*², *Ždímalová M.*², *Plachá-Gregorovská K.*³, *Kubíková E.*¹

¹ *Institute of Anatomy, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovak Republic*

² *Slovak University of Technology in Bratislava, Bratislava, Slovak Republic*

³ *Institute of Experimental Pharmacology and Toxicology Slovak Academy of Science, Bratislava, Slovak Republic*

e-mail: peter.weismann@fmed.uniba.sk

Introduction: Assessment of cell apoptosis and necrosis is often used in the study of neurodegenerative diseases, as well as in the development of various drugs. Several different procedures have been progressively developed to study different mechanisms of cell death. Since the beginning of the study of apoptosis, it is known that cells in more advanced stages exhibit morphological changes characterized by nuclear and cytoplasmic condensation leading to fragmentation. This can be observed by light microscopy and evaluated by image analysis.

Material and Methods: We have developed a computer program for image segmentation and subsequent detection of apoptosis/necrosis and transient stage of the tested cells in histological preparations. For the analysis, we used histological preparations of rat brains after hypoxia from the CA1 region of the hippocampus.

Results: Our computer program with built-in graph-cutting algorithms can separate monitored cells/neurons from the surroundings and can detect the occurrence of apoptosis, transition stage, and necrosis based on form factor evaluation. Once set up correctly, the computer program also works automatically.

Conclusions: The initial efficiency of the program is around 90%. However, it can be increased by adjusting the conditions depending on the quality of staining and image pre-processing. The program allows additional manual classification of cells into evaluated groups.

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RELATIONSHIP BETWEEN THE CENTRAL CANAL LINING CELLS AND BLOOD VESSEL SYSTEM IN THE DEVELOPING SPINAL CORD OF POSTNATAL RAT

Zrubáková J., Alexovič Matiašová A., Ševc J., Daxnerová Z.

Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

e-mail: anna.alexovic.matiasova@upjs.sk

Introduction: The central canal (CC) lining of spinal cord (SC) represents a barrier separating cerebrospinal fluid from nervous tissue. In the adult rodents, CC lining is formed by the heterogeneous population of cells, mostly ependymocytes. Since the developmental processes occurring in this area after birth are not completely understood, we focused on relationship between cells of the CC lining and the blood vessel system of SC of Wistar rats in this study.

Material and Methods: Distribution of blood vessels (BVs), fractones and Reissner's fibre in CC area of rat spinal cord was analysed at following age: postnatal day 8 (P8), P29 and P90. All analyses were performed using immunofluorescence methods and confocal microscopy.

Results: During the postnatal development, gradual reorganization of both; the CC lining and the vascular system in its vicinity was observed. At P8, we noticed presence of Laminin⁺ BVs which are longitudinally oriented on coronal sections of SC ($7,07 \pm 2,21$ BVs/section) and contacted by Vimentin⁺ basal processes of cells present in the lateral walls of the CC lining. Vimentin⁺ cells of the dorsal and ventral poles contacted *pia mater*. In the period between P8 ($0,32 \pm 0,52$ BVs/section) and P90 ($7,48 \pm 3,62$ BVs/section) the number of transversally oriented BVs increased, indicating the growth of BVs along the longitudinal axis of SC. Basal processes of cells located in lateral walls of CC lining undergo gradual undulation. We observed Laminin⁺ fractones between ependymocytes in the adult animals. In addition, the analyses of BVs using anti CD31-antibody revealed the presence of Reissner's fibre in the lumen of the CC in each time-points.

Conclusions: Despite the unknown relationship between the ependymal cells and the BVs and unknown function of the Reissner's fibre or fractones in the SC, our results indicate that these compartments could be functionally closely related.

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AUTHORS INDEX

A

Adamkov
Al-Redouan
Alexovič
Almášiová
Andrašková

B

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Bevízová
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Dalecká
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Danková

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