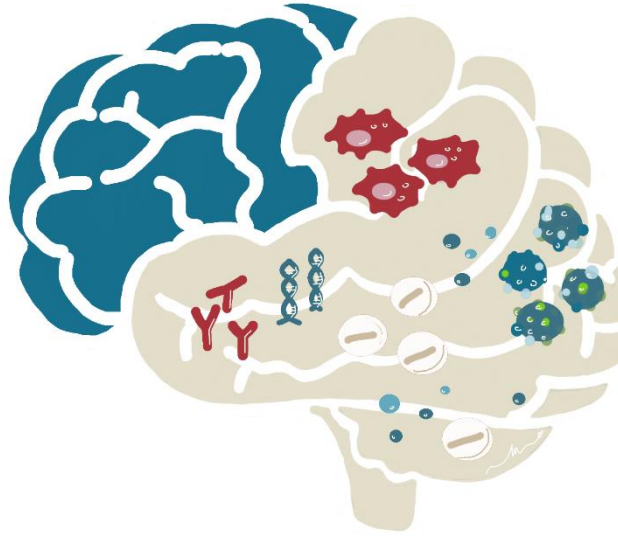
Institute of Neuroimmunology
Slovak Academy of Sciences



SUMMER SCHOOLS 2023

I. DRUG DISCOVERY: FOCUS ON BIOLOGICS

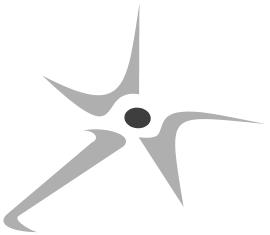
II. DIAGNOSTIC TOOLS AND BIOMARKERS

Smolenice, June 2023

DRUG DISCOVERY: FOCUS ON BIOLOGICS DIAGNOSTIC TOOLS AND BIOMARKERS

InterTAU Summer School I. & ADDIT-CE Summer School I.

Smolenice Castle, Slovakia
June 18-24, 2023
June 20-24, 2023



Organized by
Institute of Neuroimmunology,
Slovak Academy of Sciences
Co-organized with InterTAU and ADDIT-CE consortia

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INTERTAU SUMMER SCHOOL

18.6. Sunday

17.00 **Introductory lecture InterTAU Summer School**
– Jozef Hritz, Rostislav Skrabana

19.00 *Get together dinner – InterTAU school and participants of Advances in Experimental Neuroimmunology 2023*

19.6. Monday
(jointly with "Advances in Experimental Neuroimmunology")

9.00-12.00 **Morning Session I.**

9.00 **Understanding Brain Disorders** (45') – *Tomas Hromadka*

9.45 **Application of *in situ* cryo-ET: Axon branching story** (45') – *Hana Nedozralova*

10.30 *Coffee break*

11.00 **Blood-brain barrier transport of kynurenines: immunomodulatory and neuroprotective role in the transgenic model for Tauopathy** (30') – *Petra Majerova*

11.30 **Directed evolution of antibodies for detection of low abundant biomarkers in body fluids** (30') – *Jozef Hanes*

12.00 **Shifting the detection paradigm for low abundant neurobiomarkers using an ultrasensitive digital immunoassay** (30') – *Stanislav Kukla*

12.30 *Lunch*

14.00-17.00 **Afternoon Session II.**

14.00 **Universal functions of the milk protein lactoferrin – why not in the brain?** (30') – *Vladimir Leksa*

14.30 **Structural analysis of protein assemblies by solid-state NMR**
(60') – *Kristaps Jaudzems*

15.30 *Coffee break*

16.00 **Effect of Nonconcussive Repetitive Head Impacts on Tau181/Total Tau in Plasma of Young Elite Soccer Players**
(30') – *Martin Cente*

16.30 **Heatstroke-induced late-onset neurological deficits in mice caused by Purkinje cell degeneration, demyelination, and synaptic impairment at cerebellum**
(30') – *Kazuyuki Miyamoto*

17.00 **Stem cell-based therapy for dogs and cats with spinal cord injury**
(30') – *Jana Farbakova*

17.30 **InterTAU project meeting I**
(hybrid form, PIs and management only)

19.00 *Dinner*

20.6. Tuesday

8.20-12.05 Morning Session III.

8.20 **Practical aspects of drug development I.**
(45') – *Branislav Kovacech*

9.05 *Coffee break*

9.20 **Practical aspects of drug development II.**
(45') – *Branislav Kovacech*

10.05 *Coffee break*

10:15 – 12:20 **Short talks**
(10'; jointly with "Advances in Experimental Neuroimmunology")

10:15 **Identification of tau interacting proteins and associated molecular pathways in vivo**
– Jakub Sinsky

10:25 **miRNAs as primary molecular players in repetitive head impacts – association with signaling pathways leading to neurodegeneration** – *Sara Porubska*

10:35 **Debunking the Myth: Chronic Inflammation Might Not Be Fueling Tau Pathology** – *Neha Basheer*

10:45 **Mechanism of tau protein transport through the Blood-CSF barrier** – *Krutika Khiratkar*

10:55 **The association between cellular senescence and progression of neurodegenerative diseases: From traditional marker screenings to the generation of novel senescence-reporting neuronal cell lines** – *Kristina Macova*

11:05 **Effect of AD-Tau aggregation on mitochondria in Tau FRET biosensor cell model** – *Muhammad Khalid Muhammadi*

11.15 *Coffee break*

11:30 **The interconnection of senescence and alpha-synuclein related pathologies in neurodegeneration** – *Miraj Ud Din Momand*

11:40 **Construction of CDR3-phage library for selection of neutralizing peptides against tick-borne encephalitis virus** – *Tomas Malarik*

11:50 **Animal models of TBI, first experience at ISNI** – *Marian Horvath*

12:00 **Investigation of metastable conformations of the neuronal protein Tau to design novel anti-AD drug candidates** – *Stefana Njemoga*

12:10 **Mesenchymal stem cell therapy for the treatment of traumatic brain injury** – *Andrej Durgala*

12.30 *Lunch*

14.00-17.00 **Afternoon Session IV.**

14.00 **Rational Structure-Based Design: from Small Molecules to Biologics I. Principles** (45') – *Radovan Dvorsky*

14.45 *Coffee break*

15.05 **Rational Structure-Based Design: from Small Molecules to Biologics II. Applications** (45') – *Radovan Dvorsky*

15.50 *Coffee break*

16.05 **Round table discussion with panellists**

INTERTAU/ADDIT-CE SUMMER SCHOOL

17.00 **Introductory lecture ADDIT-CE Summer School with participation of InterTAU students** – *Jozef Hritz, Rostislav Skrabana*

19.00 *Get together dinner – InterTAU and ADDIT-CE Summer Schools*

21.6. Wednesday

8.30-12.00 Morning Session V./I.

8.30 **Transgenic animal models of tauopathy and their significance** (15' + 40' + 20') – *Peter Neradil, Tomas Smolek, Petra Majerova*

9.45 *Coffee break*

10.00 **Models of alpha-synuclein propagation in neurons and its impact on mitochondrial function, cellular ion homeostasis and the neuronal transcriptome and proteome** (45') – *Jochen Prehn*

10.45 *Coffee break*

11.15 **Identifying a new aggregation hotspot in Alzheimer's disease: Opportunities for drug discovery** (45') – *Michal Nemergut*

12.00 *Lunch*

13.30-17.00 **Afternoon Session VI./II.**

13.30 **A Path to Discovering Causal Insights in Multi-Omics Data Analyses** (45' + 45') – *Chiara Pastrello*

15.00 *Coffee break*

15.20 **Directed evolution of therapeutic proteins** (45') – *Maria Tomkova*

16.05 *Coffee break*

16.20 **High-sensitive ELISA methods and their applications in clinics** (40') – *Jozef Hanes*

17.00 **InterTAU project meeting II**
(hybrid form, PIs and management only)

18.30 *Dinner*

22.6. Thursday

9.30-12.30 **Morning Session VII./III.**

9.30 **Extracellular transmission in health and disease** (45') – *Eva Syková*

10.15 *Coffee break*

10.30 **Role of perineuronal nets and diffuse extracellular matrix in brain plasticity** (45') – *Eva Syková*

11.15 *Coffee break*

11.30 **Auditory System in Health and Disease** (45') – *Josef Syka*

12.15 *Lunch*

14.00 – 19.00 **no lectures, individual programme or programme in groups of special interests (sporting and cultural activities)**

19.00 *Dinner*

23.6. Friday

9.00-12.00 **Morning Session VIII./IV.**

9.00 **Molecular methods used in clinical laboratory – from the past to the future** (45') – *Miroslav Tomka*

9.45 *Coffee break*

10.00 **Comparative genomic hybridization in diagnostics of neurodevelopmental disorders** (45') – *Miroslav Tomka*

10.45 *Coffee break*

11.15 **Student flash talks I.**

12.00 *Lunch*

14.00-17.00 **Afternoon Session IX./V.**

14.00 **Clinical trials in reality: why do we need to wait so long for a wonder drug?** (45') – *Beata Cecetkova, Jana Gregorova*

14.45 *Coffee break*

15.00 **Periphery-brain crosstalk in exercise-induced brain plasticity: non-pharmacological approach** (45') – *Barbara Ukropcova*

15.45 *Coffee break*

16.15 **Students flash talks II.**

17.00 **ADDIT-CE consortia workshop – new grant application(s) – possibilities, ideas, inspirations**

18.30 *Dinner*

24.6. Saturday

9.00-11.30 **Morning Session X./VI.**

9.00 **Structural aspects of antibody engineering for therapy and diagnostics** (45') – *Ondrej Cehlar*

9.45 *Coffee break*

10.00 **Round table discussion with panellists**

11.00 CONCLUDING REMARKS

12.00 *Lunch*

ABSTRACTS

UNDERSTANDING BRAIN DISORDERS

T. Hromadka

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APPLICATION OF *IN SITU* CRYO-ET: AXON BRANCHING STORY

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In situ cryo-electron tomography is an emerging imaging technique able to visualize cellular features in a near-native environment at nanometre resolution. In contrast to electron microscopy methods used for biology, vitrified cryo-ET samples do not suffer from the artefacts introduced by the traditional sample fixation and the advance in focus ion beam milling technology now allows the preparation of thin sample sections (or lamella) without the deformations of mechanical microsectioning.
This presentation aims to introduce the methodology of cryo-ET for cellular biology purposes with a focus on neurobiology application which will be demonstrated in the study of axon branching [1].
Axon branching is an important process in neuron development. However, our understanding of branch formation is sparse due to the lack of direct in-depth observations. Using *in situ* cellular cryo-ET on primary mouse neurons, we directly visualized the remodeling of organelles and cytoskeleton structures at axon branches.

1. H. Nedozralova, N. Basnet, I. Ibiricu, S. Bodakuntla, C. Biertümpfel, N. Mizuno, *J Cell Biol*., 2022 Apr 4;221(4):e202106086. doi: 10.1083/jcb.202106086.

This work has received funding from Czech Science Foundation (22-15175I).

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 17.

BLOOD-BRAIN BARRIER TRANSPORT OF KYNURENINES: IMMUNOMODULATORY AND NEUROPROTECTIVE ROLE IN THE TRANSGENIC MODEL FOR TAUOPATHY

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The blood-brain barrier (BBB) represents the bottleneck in brain drug development and is the single most important factor limiting the future growth of neurotherapeutics. Essentially 100% of large-molecule pharmaceutics, including peptides, recombinant proteins, monoclonal antibodies, RNA interference (RNAi)-based drugs and gene therapies and >98% of small-molecule drugs do not cross the BBB. Tauopathies are a heterogeneous group of neurodegenerative disorders, pathologically characterized by neuronal and/or glial intracellular inclusions of the microtubule-binding protein, tau. The pathogenesis of tauopathies has been associated with systemic inflammation and disruption of the serotonergic signaling system. A common link between neuroinflammation and disruption of the serotonergic signaling system is the catabolism of the essential amino acid- L- tryptophan (L-TRP). Levels of kynurenines can be altered in aging and neuropathological conditions leading to decreased biosynthesis of nicotinamide. Several studies indicate the significant involvement of the kynurenine pathway (KP) in the pathogenesis of Alzheimer´s disease (AD). Kynurenines exert immunomodulatory and neuroactive properties and can influence the central nervous system. However, neuroprotective kynurenic acid has limited ability to cross the BBB, therefore different analogs are necessary to exploit its therapeutic potential. In the present study, we used a modified synthesized neuroprotective derivate of kynurenic acid N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamid (KYNA-1) to modulate neurofibrillary pathology in the brain. The analog was intraperitoneally administrated into a transgenic rat model for tauopathies. KYNA-1 treatment induced a decrease in glial fibrillary acid protein (GFAP) levels and a reduction in sarkosyl-insoluble tau. We found a significant dose-dependent reduction in GFAP levels after KYNA-1 treatment.

Acknowledgement: VEGA 2/0129/21, APVV-21-0321, APVV-18-0302

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 18.

DIRECTED EVOLUTION OF ANTIBODIES FOR DETECTION OF LOW ABUNDANT BIOMARKERS IN BODY FLUIDS

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Alzheimer's disease (AD) is a neurodegenerative brain disorder in which progressive dementia occurs. The causes are still not clear. To date, there is no cure for AD and no FDA-approved biomarker. We developed a novel pT217 tau ELISA assay, which measures phosphorylated tau proteins on Thr217 in cerebrospinal fluid [1]. We showed that this novel assay can distinguish AD from healthy individuals and AD from other neurodegenerative diseases with very high sensitivity and specificity and is more sensitive and specific compared to other currently used biomarkers. Because this assay was not sensitive enough to measure pT217 tau species in blood we improved the affinity of pT217-tau binding antibody by ribosome display. In collaboration with our partner, we developed a pT217 assay for blood and showed that it was possible to measure pT217 tau in all AD and control plasma samples.

1. Hanes, J., Kovac, A., Kvartsberg, H., Kontsekova, E., Fialova, L., Katina, S., Kovacech, B., Stevens, E., Hort, J., Vyhnalek, M., Boonkamp, L., Novak, M., Zetterberg, H., Hansson, O., Scheltens, P., Blennow, K., Teunissen, C.E. and Zilka, N. (2020) Evaluation of a novel immunoassay to detect p-Tau Thr217 in the CSF to distinguish Alzheimer disease from other dementias. Neurology 95:e3026-e3035

Acknowledgement: APVV-21-0254, VEGA 2/0123/21

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 19.

SHIFTING THE DETECTION PARADIGM FOR LOW ABUNDANT NEUROBIOMARKERS USING AN ULTRASENSITIVE DIGITAL IMMUNOASSAY

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Merck Life Science, spol. s r.o. Na Hřebenech II 1718/10, 140 00 Praha, Nusle

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UNIVERSAL FUNCTIONS OF THE MILK PROTEIN LACTOFERRIN – WHY NOT IN THE BRAIN?

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Lactoferrin, a member of the lactotransferrin family of iron-binding glycoproteins, is present in most human exocrine fluids, particularly mother milk. Both human and bovine lactoferrin exhibit a plethora of biological activities, including an iron sequestration, a blockade of proteases, or a direct modulation of immune cells. Via these activities lactoferrin plays manifold roles in antimicrobial host defense. Furthermore, antitumor functions have also been attributed to lactoferrin. The ingested lactoferrin is cleaved upon digestion in the gastrointestinal tract, yielding bioactive peptides called lactoferricins and lactoferrampins, which preserve and even augment some activities of the intact protein. Altogether, these properties make lactoferrin a cheap and widely available candidate for supplementary therapy in management of infectious diseases, including COVID-19. Here, I will focus on the role of lactoferrin in regulation of pericellular proteolysis and discuss its possible implications in neurobiology.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 20

STRUCTURAL ANALYSIS OF PROTEIN ASSEMBLIES BY SOLID-STATE NMR

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The atomic-level characterization of non-diffracting protein assemblies such as fibrillar aggregates and viral capsids is one of the greatest challenges of modern structural biology, as well as a fundamental step for the design of effective treatments. Over the last decades, solid-state NMR (ssNMR) has developed into a powerful structural tool for studying structure and dynamics of solid biological samples at atomic resolution. However, the inherently low sensitivity and poor resolution of the technique has limited its applicability to small proteins that can be tightly packed at a high molar concentration, while large proteins or multi-domain assemblies were mostly inaccessible to site-specific ssNMR studies. This has been recently overcome by the introduction of faster spinning probes, which facilitate the use of proton-detected ssNMR experiments [1], as well as by dynamic nuclear polarization (DNP), which allows transfer of polarization from the unpaired electrons of a paramagnetic center to the surrounding nuclei, and can enhance the sensitivity of ssNMR experiments by several orders of magnitude [2-3]. In my presentation, I will provide an overview of state-of-the-art ssNMR techniques for structural biology applications as well as show examples of ssNMR and DNP structural studies of different non-diffracting samples [4-6].

1. T. Le Marchand, T. Schubeis, M. Bonaccorsi, P. Paluch, D. Lalli, A.J. Pell, L.B. Andreas, K. Jaudzems, J. Stanek, G. Pintacuda, *Chem. Rev.*, **122,** (2022), 9943.
2. K. Jaudzems, A. Bertarello, S.R. Chaudhari, A. Pica, D. Cala-De Paepe, E. Barbet-Massin, A.J. Pell, I. Akopjana, S. Kotelovica, D. Gajan, O. Ouari, K. Tars, G. Pintacuda, A. Lesage, *Angew. Chem. Int. Ed.*, **57,** (2018), 7458.
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5. K. Jaudzems, A. Kirsteina, T. Schubeis, G. Casano, O. Ouari, J. Bogans, A. Kazaks, K. Tars, A. Lesage, G. Pintacuda, *Angew. Chem. Int. Ed.*, **60,** (2021), 12847.
6. J. Fridmanis, Z. Toleikis, T. Sneideris, M. Ziaunys, R. Bobrovs, V. Smirnovas, K. Jaudzems, *Int. J. Mol. Sci.*, **22,** (2021), 9635.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 21

EFFECT OF NONCONCUSSIVE REPETITIVE HEAD IMPACTS ON TAU_{181}/TOTAL TAU IN PLASMA OF YOUNG ELITE SOCCER PLAYERS

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Head impacts resulting in traumatic brain injury (TBI) are a significant risk factor for the development of sporadic tauopathies such as chronic traumatic encephalopathy, Alzheimer's disease, and other neurocognitive disorders. However, the data on the consequences of low-intensity repetitive head impacts that are not followed by loss of consciousness (nonconcussive head impacts) are inconsistent. Therefore, it is not clear whether or not such mild head impacts are associated with neuropathology.
In this study, we investigated the effect of heading in soccer on the plasma level of total and phosphorylated tau protein (p-tau181). The experimental cohort included young elite soccer players who performed intense physical activity with and without heading the ball.
The primary study outcomes were the levels of total tau protein and p-tau181 in plasma samples and the cognitive status of the study participants (n=37).
We found significantly elevated levels of total tau and p-tau181 in the plasma of soccer players 1 hour after physical exercise (tau, 1.4-fold; $P < .001$; p-tau181, 1.4-fold; $P < .001$) and repetitive head impacts (tau, 1.3-fold; $P < .001$; p-tau181, 1.5-fold; $P < .001$). The ratio of p-tau181 to tau was significantly higher 1 hour after exercise and heading training, and remained elevated specifically in the heading group even after 24 hours (1.2-fold; $P = .002$). Performance in cognitive tests revealed a significant decline in focused attention and cognitive flexibility after physical exercise and heading training, while the physical exercise of higher intensity without heading training was associated with a greater negative cognitive performance than heading only.

The increase of p-tau181 levels relative to tau after 24 hours indicates an acute enrichment of phosphorylated tau fraction in the periphery of head impacted individuals. These findings are potentially relevant for the assessment of the consequences of nonconcussive repetitive head impacts, such as heading in soccer, in association with an increased risk of developing neurodegenerative disorders later in life.

This research was cofunded by the Slovak Research and Development Agency (grant Nos. APVV-17-0668, APVV-19-0568, APVV-20-0615) and the Ministry of Education, Science, Research and Sport of the Slovak Republic (grants VEGA 2/0118/19, 2/0153/22 and ERA-NET Neuron JTC2019 Neu-Vasc).

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 22-23

HEATSTROKE-INDUCED LATE-ONSET NEUROLOGICAL DEFICITS IN MICE CAUSED BY PURKINJE CELL DEGENERATION, DEMYELINATION, AND SYNAPTIC IMPAIRMENT AT THE CEREBELLUM

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Global warming increases the incidence and severity of heatstroke near future [1]. After a heatstroke, patients who exhibit certain neurological symptoms, such as disorientation, wobbling, and vertigo—are bedridden, thereby preventing patients from returning to normal life, and suggesting cerebellar damage [2]. Moreover, several interesting reports showed that neurological deficits appeared several weeks after heatstroke rather than immediately afterward [3], and some cases improved with time [4], while others are permanent. However, it is not well understood how these late-onset neurological deficits post-heatstroke appear and, in some cases, improve over time. Therefore, we focused on the influence of heatstroke on the cerebellum using the mice heatstroke model. In our study, motor coordination disorder significantly appeared 3 weeks post heatstroke and gradually improved to some extent. Demyelination was detected at 1 and 3 weeks after heatstroke in the cerebellum and improved in 9 weeks. It was not found in the corpus callosum. The Purkinje cell numbers significantly decreased at 1-, 3-, and 9-weeks post heatstroke. The intensity of synaptophysin and postsynaptic density-95 temporarily appeared to attenuate at 3 weeks and recovered at 9 weeks post heatstroke. Our results suggested that permanent Purkinje cells loss, transient demyelination and synaptic impairment at cerebellum might correlated with delayed cerebellar ataxia of heatstroke. In further, we will investigate the mechanism of the neurodegeneration after heatstroke to investigate the new treatment.

1. Meehl, G. A. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 2004, 305, 994-997.
2. Lawton, E. Review article: environmental heatstroke and long-term clinical neurological outcomes: A literature review of case reports and case series 2000-2016. Emerg. Med. Australia 2019, 31, 163-173.
3. Jung, I. Delayed vestibulopathy after heat exposure. J. Neurol 2017, 264, 49-53.
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This work was supported by research grants JSPS KAKENHI Grant Numbers 19K09442.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 24

STEM CELLS BASED THERAPY FOR DOGS AND CATS WITH SPINAL CORD INJURY

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Neurological diseases resulting from damage to the spine and spinal cord are among the most common in the clinical practice of small animals. They are often caused by numerous external and internal factors. For animals and their owners, spinal cord injuries (SCI) are often frustrating, and they are also associated with an unfavourable prognosis in terms of recovery of the function in the affected limb or body part. In dogs, SCI is usually presented with sensory and motor deficits accompanied by ataxia, pain, paresis or paralysis, and urinary disturbances. Despite the large number of treatment strategies used in experimental and clinical settings, prognosis and outcome after SCI are still largely unpredictable in animals and people, as well. Prognosis is primarily related to early and correctly selected diagnostics and subsequent adequate therapy. In addition, the final prognosis is influenced by factors such as the cause and strength of the traumatic spinal cord injury, the time interval from the trauma to the correctly selected therapy, and the overall management of the neurological patient in the preoperative and postoperative periods.

In our clinical research, we are dealing with the possibility of improving the prognosis in such affected dogs and cats by applying therapy based on stem cells. The ideal therapy is based on the local application of a conditioned medium derived from stem or progenitor cells of mesenchymal and neural origin. In the case of spinal cord involvement, our route is a combination of intravenous, intrathecal and epidural administration to maximize the potential effect of this stem cell product. During the study, we focus on the evaluation of the clinical neurological status through the neurological score, the measurement of muscle group circumferences, the monitoring of urinary function, and blood parameters.

This therapy appears to be very promising, in safety studies, we have not reported the occurrence of serious adverse side effects, in clinical studies, we have achieved positive (yet unpublished) results after spinal cord trauma in dogs and cats.

Supported by: VEGA 1/0376/20, APVV 19-0193

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 25

PRACTICAL ASPECTS OF DRUG DEVELOPMENT

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Hundreds of substances are investigated every year by pharmaceutical and biotechnology companies, as well as at academic research institutions, for their potential to treat diseases. Only a small number will be tested in patients, and only a fraction of these will ever have clinical results good enough to reach the market. To obtain marketing authorisation, medicine developers need to submit data characterising their newly-developed medicine, including the manufacturing processes and specifications, efficacy in laboratory studies, benefits and side effects observed in patients, and how risks will be managed, as well as the proposed information to be provided to patients and doctors. A designated regulatory body then carries out a thorough assessment of these data to decide whether or not the medicine is safe, effective and of good quality and is therefore suitable for use in patients. The body of European Union legislation in the pharmaceutical sector, and supporting guidelines, are compiled in the publication "The rules governing medicinal products in the European Union", also known as EudraLex. In the USA, a similar extensive legislation, "The Federal Food, Drug, and Cosmetic Act", codified in Title 21 Chapter 9 of the United States Code.

IDENTIFICATION OF TAU INTERACTING PROTEINS AND ASSOCIATED MOLECULAR PATHWAYS IN VIVO

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Tauopathies, the most representative among which is Alzheimer's disease (AD), are currently nearly incurable neurodegenerative diseases characterized by tau protein pathology [1]. Tau protein, which is mainly present in neurons, plays several physiological functions under normal conditions, such as stabilization of microtubules, participation in the regulation of synaptic plasticity, or protective interactions with DNA and RNA [2,3]. In AD and other tauopathies, tau protein undergoes pathological modifications that lead to the several pathological processes in neurons and the brain, such as its self-aggregation, disruption of intracellular transport and protein homeostasis, neuronal death, and chronic inflammation [4,5]. Worldwide, an increasing number of patients are suffering from AD, and although several therapeutic approaches have been developed to target tau pathology, there is still no reliable treatment [6]. Since formation of protein complexes and protein-protein interactions (PPIs) are the fundamental pillars for molecular pathways and all biological processes, disruption of the physiological interactome of the tau protein is likely the cause of the development of pathological forms of tau [7]. The relevance of this topic is evident from the number of known tau interacting proteins (TIPs), which have been more than doubled in the last 5 years [8]. The yet known TIPs offers a lot of opportunities for the investigation of molecular pathways affecting tau pathology. However, the number of studies that confirmed TIPs *in vivo* is limited. The majority of studies reporting TIPs used conventional approaches of molecular biology, which, despite the great robustness, are not capable to capture weak and transient PPIs. Because also weak and transient PPIs can have a substantial effect on cellular signalling and processes, there is a growing need to identify those TIPs using biochemical approaches competent to capturing also weak and transient interactions, such as chemical crosslinking [9], proximity-labelling [10], FRET [11] and other biophysical methods. Researchers at the Institute of Neuroimmunology developed unique transgenic animal models with tau pathology similar to Alzheimer's disease [12–15]. We used one of these models, namely the SHR72 rat, for the identification of novel TIPs. We applied *in vivo* crosslinking strategy, where both strong and weak interactions could be identified in the brains of SHR72 rats. After identification of purified TIPs, we validated them using coimmunoprecipitation

and colocalization experiments in animal and cellular models. Furthermore, bioinformatic analysis revealed several associated molecular pathways which may play a role in tau pathology.

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This work was supported by the Slovak Research and Development Agency (grant nos. APVV-16-0531, APVV-18-0302 and APVV-21-0254), the Scientific Grant Agency of the Ministry of Education of the Slovak Republic (grant nos. VEGA 2/0088/18, VEGA 2/0148/18 and VEGA 2/0123/21), and the Ministry of Health of the Slovak Republic under the project registration number 2018/24-SAV-2. Computational analyses were supported in part by the Natural Sciences Research Council (NSERC no. 203475), the Canada Foundation for Innovation (CFI nos. 225404 and 30865), the Ontario Research Fund (RDI no. 34876), the Ontario Research Fund (GL2-01-030), IBM, and the Ian Lawson van Toch Fund to I.J.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 29-30

miRNAs AS PRIMARY MOLECULAR PLAYERS IN REPETITIVE HEAD IMPACTS – ASSOCIATION WITH SIGNALING PATHWAYS LEADING TO NEURODEGENERATION

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Traumatic brain injuries (TBI) belong to major health problem in contemporary society. From the total number of TBI cases, up to 90% represent mild TBI, while the most at-risk groups include children, people of productive age, and athletes actively practicing in contact sports, who are repeatedly exposed to head impacts of various intensities without any external head protection during training and matches. The association between this kind of head injury and the later development of neurodegeneration, especially chronic traumatic encephalopathy (CTE) has already been described. However, the potential connection between sub-concussive head impacts and the later development of neurodegeneration has not been studied yet. Although, there are many examples of contact sport players who suffered neuropsychiatric disorders eventually neurodegeneration after retirement. Here we have decided to study the effect of non-concussive repetitive head impact in soccer players via analyzing their peripheral blood.

In our study, we focused on the identification of molecular mediators and peripheral biomarkers induced by mild non-concussive head impact. We have collected blood samples from 46 soccer players, students of the Faculty of Physical Education and Sports, Comenius University, in Bratislava. Participants underwent two types of training, heading and identical physical exercise without heading as a control experiment. Timepoints for blood withdrawal were set before the training session, 1 hour, and 24 hours after the training. Using qRT-PCR arrays, we identified a set of 117 unique deregulated microRNAs as primary biomarker candidates after the training sessions. Out of these primary hits, 37 molecules were validated using qRT-PCR assays in the large cohort of subjects. The panel of 33 significantly deregulated miRNAs we used as entry data for a comprehensive and integrative computational biology platform analysis with a focus on the identification of neuro-associated and recovery pathways in the acute phase after RHIs.

The validated miRNAs were also used in the correlation analysis with results of the

previous work, obtained in collaboration with our research team[1], to which miRNA analyses followed. We identified eight miRNAs which profile correlated with Tau protein levels, p-Tau_{181} ratio, percentage of maximum heart rate, results of TMT-B cognitive testing, and a number of headers during the training.
Understanding the deregulation of molecular pathways caused by RHIs may help elucidate not only pathogenic mechanisms after head trauma but also the mechanisms that can lead to spontaneous recovery after mild head injuries.

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This research was funded by the Slovak Research and Development Agency (grant Nos. APVV-17-0668, APVV-19-0568, APVV-20-0615) and the Ministry of Education, Science, Research and Sport of the Slovak Republic (grants VEGA 2/0118/19, 2/0153/22).

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 31-32

DEBUNKING THE MYTH: CHRONIC INFLAMMATION MIGHT NOT BE FUELLING TAU PATHOLOGY

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Alzheimer's disease (AD) is a complex disorder neuropathologically defined by amyloid plaques, cerebral amyloid angiopathy, and neurofibrillary pathology [1, 2]. Further, it's associated with astrogliosis and microglial activation, hinting at a neural immune response [3]. Chronic inflammation, a defining feature of AD, plays a pivotal role in its pathogenesis and progression, with neuroinflammation displaying potential for both beneficial and detrimental effects. Furthermore, individual differences in neuroinflammatory status can uniquely impact tau pathology. Our study explored the effects of chronic systemic inflammation on tau pathology in the R3m/4 mouse model of AD, a model expressing truncated tau (151–391/3R) found in early-stage AD and presenting with brainstem tangle-like pathology. Chronic immune system activation was achieved using bacterial endotoxin (lipopolysaccharide, LPS). Our findings demonstrated a notable decrease in hyperphosphorylated tau at AD-specific epitopes in the hippocampus, and tangle pathology in the brainstem. These results lend credence to the hypothesis that chronic neuroinflammation can differentially influence AD pathology. The role of neuroinflammation in AD progression should therefore be considered contextually and not as a uniform phenomenon.

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This work is supported by APVV-20-0331, APVV-19-0585, APVV-20-0585, and VEGA 2/0127/22 grant.

Reprinted from Advances in Experimental Neuroimmunology 2023, AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 33

MECHANISM OF TAU PROTEIN TRANSPORT THROUGH THE BLOOD-CSF BARRIER

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Tauopathies are referred as the pathological aggregation of tau proteins in the brain in various neurodegenerative diseases. The blood-cerebrospinal fluid (BCSF) barrier is one of the most important barriers in the central nervous system. The Tau proteins have been found in the extracellular space before the neuronal death which indicates its secretion by neuronal cells. This project aims to study the molecular and cellular mechanism of efflux of the tau proteins to and from the brain using the BCSF barrier which was developed by using the primary choroid plexus epithelial cells. The in vitro results showed that the proline-rich region containing tau proteins has highest transportation rate. The findings were confirmed by the in vivo study in SHR24 transgenic rats that showed the interaction of N-terminal and proline rich region tau protein with the choroid epithelial cells. The data confirms the role of BCSFB and tau proteins and its active transportation across the barrier.

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This work was supported by research grants VEGA 2/0123/21 and APVV-21-0254.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 34

THE ASSOCIATION BETWEEN CELLULAR SENESCENCE AND PROGRESSION OF NEURODEGENERATIVE DISEASES: FROM TRADITIONAL MARKER SCREENINGS TO THE GENERATION OF NOVEL SENESCENCE-REPORTING NEURONAL CELL LINES

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In the last few decades, the average age of populations, especially in developed countries, has notably increased. This seemingly positive result of an improved healthcare system and better living conditions has, however, led to a rising prevalence of age-related pathologies, including metabolic disorders, cardiovascular diseases, cancer, and neurodegeneration. Despite being very heterogeneous in the sense of initial cause and manifestations, the majority of these chronic conditions is accompanied by a common biological process called senescence [1, 2].

Although primarily recognized as an essential tumor-suppressor mechanism that prevents the proliferation of damaged and potentially malignant cells, persisting senescence has been linked to the onset and/or progression of multiple neurodegenerative diseases (NDs), such as the second most common ND, Parkinson's disease (PD) [1, 2]. Moreover, despite the initial association only with proliferating cells, groundbreaking discoveries revealed that even post-mitotic fully differentiated neurons can develop a senescent-like phenotype (e.g. increased senescence-associated β galactosidase (SA-β gal.) activity; elevated levels of cyclin-dependent kinase inhibitors $p16^{INK4a}$ and $p21^{Waf1/Cip1}$, pro-inflammatory molecules, and anti-apoptotic proteins) [2, 3]. However, it is not clear yet (i) whether the load of senescent cells in an organism correlates with the stage of NDs and (ii) if elevated levels of these features, specifically in neurons, are involved in the pathogenesis of chronic conditions.

Therefore, the aim of our research is to establish a new approach of analysis and address these crucial yet unresolved questions. Firstly, we analyze the correlation between senescence induction and progression of PD pathology in human patients. We are using different screening methods (e.g. qPCR, WB, ICC) to monitor and quantify expression levels of the main senescence hallmarks (e.g. SA-β gal. activity, $p16^{INK4a}$, $p21^{Waf1/Cip1}$) initially in peripheral tissues and later on in post-mortem brains. In parallel, to analyze the neuronal senescence-like response, we successfully developed previously missing molecular tools that can be stably integrated into the genome of various cell types, including human neurons, to report senescence induction. These DNA constructs, created by molecular cloning, consist of a reporter gene (encoding either a red fluorescence protein Tomato or a luciferase NanoLuc) placed in between two 500-650 bp long

sequences amplified from either $p16^{INK4a}$ or $p21^{Waf1/Cip1}$ alleles. By employing the CRISPR/Cas9 targeting technique, we knocked-in our donor constructs into the genome of human neural progenitor ReNVM cells to generate novel senescence-reporting cell lines. These genome-modified cells are being used to induce neuronal senescence and analyze the features and consequences of the resulting phenotype. Subsequently, we sought to identify the main regulators of this process by modifying the expression of various senescence-associated genes.

Thus far, our preliminary results revealed elevated levels of several senescence markers (e.g. SA-β gal. activity, $p16^{INK4a,}$ and $p21^{Waf1/Cip1}$) in fibroblasts from PD patients compared to controls, and initial trials of senescence induction in generated senescence-reporting neural cell lines resulted in several cells positive for Tomato fluorescence. We believe that our progressing research can help to clarify the yet unknown manifestations, and consequences of neuronal senescence-like response and evaluate the role of this process in PD.

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This project was supported by: Grant UK for young scientists UK/137/2021, DoktoGrant APP0311, Doktorand APP0407, APVV-19-0585, VEGA2/0158/21, SASPRO 2_1085/01/02, APVV-20-0331.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 35-36

EFFECT OF AD-TAU AGGREGATION ON MITOCHONDRIA IN TAU FRET BIOSENSOR CELL MODEL

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Alzheimer's disease (AD) is the most commonly occurring neurodegenerative disorder. It is characterized by the accumulation of amyloid β (Aβ) plaques and neurofibrillary tau tangles that are associated with progressive cognitive impairment and memory loss [1,2]. A large sum of data reports mitochondria malfunction, energy metabolism, and oxidative damage in AD [3,5,6,7,8]. However, the direct effect of tau pathology on mitochondria is not clearly understood and little is known about its mechanism.

Here we aim to illustrate the effect of AD-tau, on mitochondria using tau FRET biosensor cell model. FRET biosensor cell model is a quantitative, ultrasensitive and specific assay for detecting tau aggregation in-vitro. This model is derived from transducing HEK 293T cell with two separate lentivirus constructs encoding recombinant P301S-tau tagged with either cyan fluorescence protein (CFP) or yellow fluorescence protein (YFP). Introduction of potent tau seeds into these cells leads to nucleation of endogenous tau reporter proteins that produces FRET signal which can be detected through florescence microscopy and quantified using flow cytometry [9].

We treated FRET biosensor cell models with specific concentration of human AD brain derived pathological tau. After 48 hours of incubation, tau aggregation was validated on florescence/ confocal microscopy. Red mito-tracker dye, which is a molecular probe that specifically stains mitochondria was used to facilitate their visualization. We used flow cytometry to measure the combined FRET densities of both AD-tau and mitochondria, as per standard protocol.

Aggregation of AD-tau in FRET biosensor cells were successfully validated after spontaneous uptake. We found that tau aggregation reduced the mitochondrial membrane potential in these cells, analysed by flow cytometry. Co-localization of FRET signal and red mito-tracker demonstrated a probable effect on the distribution of mitochondria inside the cell.

Based on these results, we can ascertain that tau aggregation in FRET biosensor cells may affect mitochondrial membrane potential however additional investigation is needed to confirm this effect.

This work is supported by APVV-20-0331, APVV-19-0585, APVV-20-0585, and VEGA 2/0127/22grant.

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Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 37-38

THE INTERCONNECTION OF SENESCENCE AND ALPHA-SYNUCLEIN RELATED PATHOLOGIES IN NEURODEGENERATION

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Parkinson's disease (PD) is a neurodegenerative disease, affecting primarily the population over the age of 65 [1]. Prominent hallmarks of PD are accumulation of intracytoplasmic protein inclusions called Lewy bodies (LB) and progressive loss of dopaminergic neurons [2]. LB mainly consists of a protein called alpha synuclein (a-Syn), which is a natively disordered protein predominantly expressed in the brain. The duplication or triplication of SNCA (gene encoding a-Syn) locus was observed in some families with PD and its higher expression level has been suggested to play a critical role in the pathogenesis of PD [3]. Accumulation of LB leads to various detrimental effects including dysfunction of mitochondria, oxidative stress and synaptic dysfunction [4]. However, the precise molecular and cellular mechanism of a-Syn involvement in neuronal death resulting in neurodegeneration remains unclear. Some of these toxic effects are related to the process of senescence, which is a cellular mechanism resulting in the cell cycle arrest protecting the organism against the growth of severely damaged cells. Despite being an important tumour-suppression mechanism, senescence is also believed to be a major contributor to ageing and age related diseases.
Understanding the pathological changes, diagnosis, and treatment of PD is one of the pressing issues of present-day neuroscience, necessitating the development of robust disease models. In our study we created novel neuronal cell lines stably overexpressing a-Syn (wild type or its PD-related mutated variant A53T) tagged with GFP in uniform genetic background, enabling more consistent data acquisition. These newly generated cell models were then separated into three types each, based on the expression levels of a-Syn using cell sorting. We then analysed cell viability, expression levels using Western blot and qPCR and compared biological effects triggered in these cell models as the result of alterations of a-Syn levels, comparing the effects of overexpression of a-Syn among themselves and with cells not overexpressing a-Syn. We further plan to correlate the readouts with senescence markers (SA-beta-galactosidase staining and analysis of p16INK4a, p21Waf/Cip1, IL-6, TNFα expression).

Our study aims to enhance understanding of a-Syn related pathologies in PD and contribute to the establishment of innovative approaches for its treatment.

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This study was supported by: APP0398, APVV-19-0585, APVV-20-0331, VEGA2/0158/21, SASPRO 2_1085/01/02, ICGEB grant CRP/SVK22-04_EC.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 39-40

CONSTRUCTION OF CDR3-PHAGE LIBRARY FOR SELECTION OF NEUTRALIZING PEPTIDES AGAINST TICK-BORNE ENCEPHALITIS VIRUS

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Tick-borne encephalitis virus (TBEV) causes severe infections of central nervous system and more then 12 000 cases are being reported each year. Several technologies are being developed for treatment, diagnosis and prevention of the infection. However, we still lack the effective therapeutics that could prevent severe and life-threating TBEV infection. We are aiming to develop of structurally constrained CDR3 peptides (Cys-CDR3-Cys) recognizing specifically protein E of the TBEV. Here, the CDR3 is derived from heavy chain only antibodies of llama (*Lama glama*). We immunized llama with recombinant DIII subunit of E protein. After six booster doses, mRNA from the B-lymphocytes of the llama was isolated and reverse transcribed. CDR3 region was amplified using degenerated primers. Primers were designed as 63-mer (reverse) and 35-mer (forward) with overhangs that contained restriction sites for NcoI (C/CATGG) and NotI (GC/GGCCG) restriction enzymes. The amplified product of CDR3 region was 150 bp in size. The PCR product was cloned into pSEX81 phagemid and transformed into the *E. coli* XL1-blue in order to generate CDR3- *E. coli* library. For obtaining CDR3-*E. coli* library, 15 electroporations were performed to electrocompetent *E. coli* XL-1 blue. For the growth of clones, only one plate (17.8 cm in diameter) supported with 1% glucose and Carbenicillin was used. Clones were selected based on antibiotic resistance for Carbenicillin at the concentration of 1 µg/100 ml. The estimated library size was calculated as $1.1x10^{10}$ of clones. Clones grown on the 2xTY plates were scrapped in 50% glycerol and stored at -80 until use. A loopful of library was streaked on 2xTY-carbenicillin plate to separate the clones. DNA from the clones was isolated and subjected to the sequencing using phagemid specific primers. PCR products from 16 clones were sequenced, sequences were translated in-silico into amino acids and aligned. Based on the alignment we deduced high diversity of constructed CDR3-*E. coli* library 87.5%. The library was subjected to the phage packaging wherein CDR3-*E. coli* library was grown in 2xTY- carbenicillin till OD_{600} 0.5, superinfected with hyperphage (Progen, Germany) and escaped phages were purified with NaCl-PEG precipitation. Total $1.61x10^{13}$ phages were obtained and stored in -80C until their use in bio-

panning against TBEV and WNV. We hypothesized that structurally constrained CDR3 selected specifically against DIII domain of protein E, which is the only binding site of flaviviruses to the host cell, could block the binding of virus to the host cell receptor and thus its cell entry.

We acknowledge funding for this research by APVV through the project APVV-18-0259, VEGA (projects - 1/0381/23; VEGA - 1/0348/22). KK and EM are funded from DSV-ITMS2014+ project code NFP313010V455. We thank KEGA (007UVLF-4/2021) for purchase of graphpad. Development of dendrimers is funded by ERA-NET project EU-RONANOMED2021-105.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 41-42

ANIMAL MODELS OF TBI, FIRST EXPERIENCE AT NIU SAS

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Traumatic brain injury (TBI) is sustained by tens of millions of patients each year. TBI of mild severity, characterized by a transient disruption of brain function, is the most prevalent form. Understanding the underlying mechanisms and developing effective therapeutic interventions for mTBI necessitate the use of appropriate animal models that mimic the human condition. In this study, we aimed to develop a mouse model of mTBI to provide a reliable and reproducible experimental platform for investigating the pathophysiology and potential therapies.

The mouse model was established by utilizing an electromagnetic controlled cortical impact device that delivered a controlled force to the closed skull of anesthetized mice. The impact parameters, including the impactor tip size, velocity, dwell time, and depth, were carefully optimized to induce a mild injury with minimal mortality and consistent behavioural deficits. Various outcome measures, such as neurobehavioral assessments, histological analysis, and biochemical assays, are being employed to evaluate the severity and progression of the injury.

The mouse model of mTBI in development holds great promise for advancing our understanding of the underlying mechanisms and exploring potential therapeutic interventions for this prevalent form of TBI. The model's reproducibility and similarity to the human condition make it an invaluable tool for preclinical studies aiming to unravel the complex pathophysiology of mTBI and evaluate the efficacy of novel treatment strategies. Ultimately, this model may contribute to the development of effective interventions and improve outcomes for individuals suffering from mTBI.

This research supported by the Slovak Research and Development Agency (grant Nos. APVV-17-0668, APVV-19-0568, APVV-20-0615) and the Ministry of Education, Science, Research and Sport of the Slovak Republic (grants VEGA 2/0118/19, 2/0153/22).

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 43

INVESTIGATION OF METASTABLE CONFORMATIONS OF THE NEURONAL PROTEIN TAU TO DESIGN NOVEL ANTI-AD DRUG CANDIDATES

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Unlike globular proteins with stable 3D structures, intrinsically disordered proteins exist as a highly dynamic system composed of interchanging conformations, which complicates their structural studies. These disordered proteins are often subjected to a self-aggregation process yielding insoluble protein aggregates that damage neurons, as in the case of various neurodegenerative diseases. Intracellular inclusions of protein tau are present in the brains of Alzheimer's disease patients [1]. The aim of the research is to identify metastable tau conformations against which inhibitors of aggregation can be designed. To achieve our objectives, we combine X-ray crystallography with molecular dynamics simulations and molecular docking experiments [2-3]. Future plans include screening the virtual library of small molecules to select potential molecules that affect the aggregation of tau.

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This work was supported by grant no. APVV 21-0479, VEGA 2/0125/23 and MSCA-RISE no. 873127.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, p 44

MESENCHYMAL STEM CELL THERAPY FOR THE TREATMENT OF TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is a major cause of injury-related mortality and morbidity. TBI can occur due to head impacts such as sports-related injuries, accidental fall, domestic violence, automobile accidents, or blast-related injuries. Those who survive TBI may experience a range of symptoms, including cognitive and memory impairments, vision and hearing loss, movement disorders, and various psychological challenges. Based on the severity of primary impact or damage as well as the extent of secondary damage, TBIs can be categorized into mild, moderate, and severe [1-2]. The treatment of TBI induced diseases are still symptomatic and very challenging. Clinical trials for drugs aimed at restoring neuronal loss have not been successful so far. However regenerative approaches for TBI treatment using mesenchymal stem cells (MSCs) seem to be promising [3]. The success of stem cell therapy depends on optimal transplantation time point and route of administration. Also, differentiation of transplanted MSCs is considered as an important step of successful therapy. MSCs have potential to differentiate into a variety of adult cells, like chondrogenic, osteogenic, and neural progenitors [4]. Preclinical research has demonstrated that transplanting MSCs has the potential to decrease secondary neurodegeneration and neuroinflammation, stimulate neurogenesis and angiogenesis, and enhance overall functional outcomes in experimental animals.

In the project, we are focussing on the efficient preparation of mouse MSCs cultures, expansion and subsequent differentiation into nerve cells. For differentiation, we used different combinations of inducers such as β-mercaptoethanol, forskolin or IBMX. So far, we analysed the stem cells by immunofluorescence via confocal microscopy and detected the expression of tau protein, β-tubulin III and nestin. Our results indicate that the differentiation of stem cells into nerve cells is challenging and requires further experimentation.

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Acknowledgment: This research was cofunded by the Slovak Research and Development Agency (grant Nos. APVV-17-0668, APVV-19-0568, APVV-20-0615). The authors thank Dr. Marina Uhart, Prof. Diego Bustos and Mr. Lautaro Rivera for their help and introduction to the mesenchymal stem cells preparation, culture and characterization.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 45-46

RATIONAL STRUCTURE-BASED DESIGN: FROM SMALL MOLECULES TO BIOLOGICS

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One of the main principle for rational design is based on structure. The structure in this regards means conventional position of atoms in 3D space. The design can include any chemical entities, from small organic molecules, through oligomers to polymers such as proteins, nucleic acids, and polysaccharides. In the case of polymers, the sequence of monomeric units is a crucial aspect referred to as the 'primary' structure. However, what interests us is the 3D structure. Why? Because it enables us to calculate the interactions between designed entities and surrounding biological components, such as proteins, nucleic acids, membranes, and polysaccharides. From that calculations one can assess the properties of new or modified, chemical or biological, entities.

That word 'calculation' actually represent the word 'rational' in the name of the method as its scope and intention is really to be able to make prediction just on the base of input information and computing. Unfortunately, reality is much complex and the process of rational design must encompass a wide range of techniques and methods, to reach its goal.

My lecture will introduce basic principles of computational methods used in rational design [1], how and where they can be used, their abilities, scopes, and limitations. Computation in this regard does not mean only an application of physical laws and mathematics. It also includes other scientific disciplines, informatics and of course artificial intelligence.

Although the rational structure-based design described above can be used in many fields, focus will be put on the design of drugs and medicaments [2]. For them, not only the actual interaction with target spot in bio-system but also properties like side effects or the impact on environment are very crucial, and the involvement of their assessment in the course of design will be exercised as well.

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TRANSGENIC ANIMAL MODELS OF TAUOPATHY AND THEIR SIGNIFICANCE

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Alzheimer's disease (AD) is the most prevalent type of tauopathy and the leader in whole dementia spectrum of diseases. From clinical point of view, AD is characterized by memory deficits, spatial disorientation, deterioration in sleep-wake cycle and in the later stage by impairment of motoric functions. From histopathological perspective, typical features of AD are pathological deposits of amyloid beta and tau aggregates, which is accompanied by neuroinflammation. Despite intensive and extended research there is still widespread debate about AD cause(s) and no effective treatments exist so far [1].

Amazing benefits for *in vivo* study of gene function in AD come from genetically modified animal models. However, none of the existing models fully reproduces the complete spectrum of this insidious human disease. Interestingly, from tau perspective, vast majority of existing studies has been done on rodents which expressed a mutated form of tau, ignoring the fact that 90% of AD are sporadic and these mutations are absent.

In this overview, after an introduction into the problematic of animal models in general, we will talk about main features of rodent animal models (mice and rats), which express human truncated tau and therefore are closer to sporadic form of tauopathy. These models were developed and characterized at Axon Neuroscience and Institute of Neuroimmunology in the last 20 years. Finally, we will present a study on transgenic rats investigating changes in metabolic pathways associated with tau-induced neurodegeneration. We identified several metabolic changes connected with tauopathy process, which can aid a development of a panel of biomarkers for diagnostics and monitoring of clinical trials in humans.

Our special thanks belong to all students and technicians contributing to this study.

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MODELS OF ALPHA-SYNUCLEIN PROPAGATION IN NEURONS AND ITS IMPACT ON MITOCHONDRIAL FUNCTION, CELLULAR ION HOMEOSTASIS AND THE NEURONAL TRANSCRIPTOME AND PROTEOME

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IDENTIFYING A NEW AGGREGATION HOTSPOT IN ALZHEIMER'S DISEASE: OPPORTUNITIES FOR DRUG DISCOVERY

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Apolipoprotein E (ApoE) ε4 genotype is the most prevalent risk factor for late-onset Alzheimer's Disease (AD) [1]. Although ApoE4 differs from its non-pathological ApoE3 isoform only by the C112R mutation, the molecular mechanism of its proteinopathy is unknown. Here, we reveal the molecular mechanism of ApoE4 aggregation using a combination of experimental and computational techniques, including X-ray crystallography, site-directed mutagenesis, hydrogen-deuterium mass spectrometry (HDX-MS), static light scattering and molecular dynamics simulations. Treatment of ApoE ε3/ ε3 and ε4/ ε4 cerebral organoids with tramiprosate was used to compare the effect of tramiprosate on ApoE4 aggregation at the cellular level.

We found that C112R substitution in ApoE4 induces long-distance (>15 Å) conformational changes leading to the formation of a V-shaped dimeric unit that is geometrically different and more aggregation-prone than the ApoE3 structure. AD drug candidate tramiprosate and its metabolite 3-sulfopropanoic acid induce ApoE3-like conformational behavior in ApoE4 and reduce its aggregation propensity. Analysis of ApoE ε4/ε4 cerebral organoids treated with tramiprosate revealed its effect on cholesteryl esters, the storage products of excess cholesterol.

Our results connect the ApoE4 structure with its aggregation propensity, providing a new druggable target for neurodegeneration and ageing.

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A PATH TO DISCOVERING CAUSAL INSIGHTS IN MULTI-OMICS DATA ANALYSES

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Mass spectrometry, sequencing, and other high-throughput assays generate data to investigate the role, expression, and features of multiple molecules at the same time. When combined with integrative computational biology analysis and data mining, these analyses can lead to the identification of key players in the disease or mechanism being studied. To further comprehend the molecular landscape of the condition being investigated, it is important, if possible, to integrate results from multiple high-throughput studies across different molecules.

A drawback of this approach is the amount of data resulting from the analyses, even after filtering out outliers, contaminants, and less-significant results. To help with this, multiple databases have been developed to provide annotations and characterize links connecting molecules to identify prioritized targets of validation or key components to explain specific mechanisms.

The path from results to insights varies depending on the research question and the molecules at hand. Physical protein interactions can provide connections among proteins and highlight central proteins, provide context annotations, identify complexes, and reveal functional and causal relationships. MicroRNA:target predictions are used to link microRNAs and the genes they regulate, and can be used to prioritize microRNAs, gene targets, or the contexts and relevant pathways to investigate. Transcription factor:target predictions are important to identify master regulators of the disrupted genes or proteins being studied. Pathways and Gene Ontology annotations provide a context in which the molecules perform their functions.

The integration of these analyses and annotations creates a path that leads the researcher to discover important molecules and key mechanisms to select for validation or further investigation. Knowledge of how to combine the workflows, and of the best features and drawbacks of each step is necessary to follow the path correctly and to be aware of the possible limitations of the identified insights and causal relationships. These topics will be discussed and supported by examples using tools such as IID (http://ophid.utoronto.ca/iid), mirDIP (http://ophid.utoronto.ca/mirDIP), pathDIP (http://ophid.utoronto.ca/pathDIP), Catrin (http://ophid.utoronto.ca/Catrin) and Enrichr (https://maayanlab.cloud/Enrichr).

DIRECTED EVOLUTION OF THERAPEUTIC PROTEINS

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Directed evolution represents a powerful strategy for the development of therapeutic proteins with improved properties [1]. By harnessing the principles of evolution, researchers can rapidly generate protein variants that exhibit enhanced affinity, stability, or specificity, enabling the creation of more effective and tailored therapeutics. The process of directed evolution involves iterative cycles of mutagenesis, selection, and amplification. There are several techniques of directed evolution that can be generally divided into cellular approaches, including phage and yeast display, and cell-free approaches represented by ribosome or mRNA display.

We employed ribosome display to improve a thrombolytic agent, staphylokinase (SAK). The usage of currently available thrombolytics is limited due to their high immunogenicity and haemorrhagic complications. SAK is a single-chain extracellular protein secreted by *Staphylococcus aureus*. It initiates the fibrinolytic cascade to help invading bacteria move deeper into the tissues. Owing to its thrombolytic properties and high fibrin specificity, it is considered a promising new thrombolytic agent. However, not all SAK features are optimized for its practical applications, leaving room for improvement. Engineering the affinity and selectivity of SAK could increase its residence time on plasmin, thus reducing the severity of side effects.

Directed evolution has been used to optimize the activity, stability and solubility of therapeutic proteins. Continued advancements in directed evolution techniques and technologies hold great promise for the future of protein engineering and the development of novel therapeutic interventions.

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This work was supported by Slovak Research and Development Agency through the project APVV-20-0340 and by the grant agency of the Ministry of Education, Science, Research, and Sport of the Slovak Republic, grant no. VEGA 1/0074/22.

HIGH-SENSITIVE ELISA METHODS AND THEIR APPLICATIONS IN CLINICS

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The number of people with dementia worldwide is estimated at 55.2 million and is expected to triple by 2050. Currently, there are therapies for only some neurodegenerative diseases, such as multiple sclerosis. However, there are several drugs in development that could be successful soon for the treatment of, for example, Alzheimer's disease, the most common form of dementia, as well as other dementias. In addition to drug development, scientific teams around the world are working to identify biomarkers that could reliably monitor the therapy of these diseases. Various biomarkers are used for this purpose, such as a) clinical symptoms, b) neurophysiology, c) biomarkers in cerebrospinal fluid (CSF), blood, or certain tissues, and d) neuroimaging methods. The aim of this presentation is to introduce the audience to highly sensitive immunochemical methods and their use for the determination of biomarkers in CSF and blood which can be used for diagnosis and monitoring of therapy of neurodegenerative diseases.

EXTRACELLULAR TRANSMISSION IN HEALTH AND DISEASE. ROLE OF PERINEURONAL NETS AND DIFFUSE EXTRACELLULAR MATRIX IN BRAIN PLASTICITY

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Extracellular matrix (ECM) is a network of macromolecules secreted by neurons and glia. ECM has a crucial role in modulating synapse formation, neuroplasticity, neural cell migration, differentiation, axonal growth, sprouting and pathfinding [1]. Moreover, ECM remodeling also significantly influences extrasynaptic (volume) transmission, which is mediated by diffusion of neuroactive substances in the extracellular space [2, 3]. ECM has two forms, a diffuse ECM (dECM) and perineuronal nets (PNNs). Both forms of ECM influence brain development, aging, brain and spinal cord injury, progression of brain disorders e.g. ALS, Alzheimer´s disease, schizophrenia etc. Using RTI (real time iontophoretic method) we can study absolute changes in ECS volume and geometry. With DW-MRI we can study changes of mean diffusibility and fractional anisotropy. Disruption of PNNs and dECM accompanied by changes in ECS diffusion parameters has been demonstrated during aging and pathological states such as Alzheimer´s disease, neuroblastoma, brain and spinal cord injury and others [2]. It will be presented, how remodeling of dECM and PNNs can influence a disease progression and neuroplasticity. Both, dECM and PNNs, can be modified, e.g. by stem cells application [4], by manipulation of the chondroitin sulphate proteoglycans (CSPG) and hyaluronan synthesis (HA). ECS is wider and plasticity higher during development than in adult and aged brain. It can be therefore beneficial to modulate dECM, PNNs and ECS volume for treatment of neurological disorders in adulthood or aging. The observed beneficial effects of drugs which are removing ECM for treatment of CNS diseases and for a support of neuroregeneration may be related to the enhanced extrasynaptic communication due to changes in the brain ECS volume, diffusibility and transport of solutes in ECS.

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Supported by APVV-200331.

AUDITORY SYSTEM IN HEALTH AND DISEASE

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Human auditory system is under normal conditions capable to perceive sound frequencies from approximately 100 Hz to 20 kHz. The upper frequency range decreases slowly since adolescence resulting in elderly in deterioration of the speech perception particularly in a noisy environment. The frequency span is different in experimental animals used in the auditory research (rats. mice, guinea pigs) mostly limiting the low frequency hearing and reaching 60-80 kHz in the upper hearing range.

Sensory perception in the inner ear is based on the function of one row of inner hair cells and three rows of outer hair cells. Outer hair cells contain contractile protein prestin, the contraction of which results in a production of so called oto-acoustic emissions. The measurement of oto-acoustic emissions is used in neonates for checking the hearing function. Two in one thousand neonates are born usually deaf, there are approximately 150 known genes that may cause deafness. In the central Europe approximately 50% cases of deafness are caused by mutation of the GJB2 gene. This is a gene coding protein of the gap junction in the organ of Corti, called connexin 26. Inner ear dysfunction, resulting ultimately in a loss of hearing, may be the result of an excessive noise exposure or may be produced by exposure to ototoxic drugs, such as aminoglycoside antibiotics or cisplatin.

Hearing deteriorates with aging in experimental animals such as rats in a similar manner to that of other mammals: hearing thresholds increase, while DPOAE amplitudes, the number of inner and outer hair cells, and the number of ribbon synapses all decrease. Fischer 344 rats represent a specific type of age-related hearing loss that is based on pathological changes in the stria vascularis as well as in the spiral ligament and is present particularly in males. Age-related changes in the inner ear are accompanied by changes in the central auditory system with slight non-significant decreases in the total number of neurons. However, there are specific significant decreases in some types of neuronal populations (inhibitory interneurons, neurons immunoreactive for calcium binding proteins, SMI-32). In principle, major age-related functional decline corresponds with a decline in the inhibition. Aging is accompanied by a deterioration in the processing of temporal parameters of acoustical signals, expressed as decreased neuronal synchronization, lower processing speed, or lower sensitivity to very short gaps.

Age-related hearing loss in humans starts with increasing thresholds in high frequencies of sound and slowly spreads to low frequencies. Presbycusis is accompanied by decreases of the amplitudes of oto-acoustic emissions, decreases in the speech com-

prehension especially in the presence of backgound noise and decreases of the temporal processing of sound, expressed as increases of the gap detection thresholds. In addition, deteriorates space hearing based on interaural time delay.

The treatment of presbycusis is mostly based on amplification of the incoming sound provided by hearing aids. However, in serious cases of the age-related hearing loss are used as a treatment these days cochlear implants. Cochlear implants were developed in seventies of the last century for a treatment of deafness and serve mostly for recovery of hearing in children that were born deaf or lost their hearing for different reasons.

MOLECULAR METHODS USED IN CLINICAL LABORATORY – FROM THE PAST TO THE FUTURE

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It is estimated that humans have between 20,000 and 25,000 genes. Many of them might cause the problems for person`s health even life if affected. Accurate and timely diagnostics is the key for identifying a disease, prognosis and, if available, for the treatment. Many molecular methods have been developed in scientific laboratories which are nowadays used as routine techniques in clinical laboratories. This presentation describes some of them.

COMPARATIVE GENOMIC HYBRIDIZATION IN DIAGNOSTICS OF NEURODEVELOPMENTAL DISORDERS

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Neurodevelopmental disorders are group of disorders affecting the development of the central nervous system, which in turn, affect the higher brain functions such as learning, memory, emotion control and social interactions. One of the best-known disorders of this group is autism spectrum disorders, followed by attention deficit hyperactivity disorder, Rett syndrome and Asperger's syndrome. Molecular methods, like comparative genomic hybridization, play the key role in diagnostics of neurodevelopmental disorders.

CLINICAL TRIALS IN REALITY: WHY DO WE NEED TO WAIT SO LONG FOR A WONDER DRUG?

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Synopsis:
- Introduction into practical aspects of clinical trials in a medical facility
- Basic rules and procedures (good clinical practice, legal framework)
- A handbook of clinical trial: preparation, realization, evaluation
- Participants in the process of clinical trial and their roles
- Communication above everything else
- Academic research and its peculiarities

PERIPHERY-BRAIN CROSSTALK IN EXERCISE-INDUCED BRAIN PLASTICITY: NON-PHARMACOLOGICAL APPROACH

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Multidomain lifestyle interventions have a potential to slow down or even reverse aging-associated cognitive decline and reduce the risk of neurodegenerative disease development. As their integral component, intensive physical activity stimulates multiple mechanisms that act in parallel to meet increased energy demands and work load. However, the systemic adaptive response to acute intensive exercise goes beyond the immediate metabolic needs, resulting in structural and functional changes at many different organs, including the brain. This process occurs in a highly coordinated manner, synchronized at a systemic level by bioactive molecules, exerkines. Exerkines are released into systemic circulation from skeletal muscle, adipose tissue, liver and other organs during or shortly after physical exertion, and include a wide spectrum of molecules – metabolites, proteins, lipids and microRNAs. Some exerkines have neuroprotective potential, and it was demonstrated using the animal models that they can cross the blood brain barrier and enhance brain plasticity, cognitive functions and exert an antidepressogenic effects. We and others have shown that some of these molecules are distinctly regulated in blood and cerebrospinal fluid in response to an acute exercise bout in humans. Better understanding of the molecular mechanisms involved in the exercise-induced periphery-brain crosstalk may help identify new targets of treatment for neurodegenerative diseases.

Grant support: ADDIT CE Widera -Horizon Europe

STRUCTURAL ASPECTS OF ANTIBODY ENGINEERING FOR THERAPY AND DIAGNOSTICS

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Synopsis:

- Nature of the recognition of a target molecule and a candidate therapeutic monoclonal antibody
- The atomic level structural description of the complexes formed between candidate antibodies and their respective targets
- Focus on disordered peptide and protein targets in the case of neurodegenerative diseases
- Deciphering mode of action of a candidate therapeutic antibody
- Improvement of affinity and efficacy
- Specific targeting of the most toxic forms: monomers, oligomers or fibrils?

FLASH TALK ABSTRACTS

IDENTIFICATION OF BIOMARKERS IN THE EARLY PHASE OF CTE

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Chronic traumatic encephalopathy (CTE) is defined as a progressive neurodegenerative disease, which is caused by episodic or repetitive head impact and rotational force on the brain [1-2]. The mild traumatic brain injury (mTBI), is relatively often associated with head injuries of athletes participating in contact sports. Current literature suggests that TBI makes up 10% to 15% of all sports-related injuries. CTE presents clinically after a prolonged latent period as a composite syndrome and can be diagnosed by image analyses just in late stages of the disease or by postmortem immunohistochemistry. Linking the behavioral symptoms specifically to CTE during the life is challenging since they are very common also in the general population without the history of TBI. Therefore, CTE has been the subject of extensive research in recent years. Although some progress was made in understanding its risk factors and disease mechanisms, important research questions remain, including the development of biomarkers for early detection, differential diagnosis and treatment, as it is also in the case of other neurodegenerative diseases.

Mouse models of TBI are therefore of great value for mimicking CTE. They allow for detailed characterization of the behavior and associated molecular and histopathological changes in the brain, and as such, represent the unique opportunity to explore underlying molecular mechanisms induced by TBI. Importantly, the recent studies suggest that suitable biomarkers for the early diagnosis of concussion could be miRNA [3].

The aim of our study is identification of molecular markers for early detection of specific pathological pathways induced by TBI. We are focusing on deregulated microRNAs in brain tissue, which can be detected also in peripheral body fluids. Our effort may lead to discovery of new biomarkers and identification of novel therapeutic targets for treatment of TBI, eventually CTE.

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NEUROBEHAVIOURAL TESTING OF MICE SUBJECTED TO MILD TRAUMATIC BRAIN INJURY

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Mild traumatic brain injury (mTBI) is a prevalent neurological condition characterized by a transient disruption of brain function due to mechanical impact or acceleration-deceleration forces. Animal models, particularly mouse models, are widely used to investigate the pathophysiological mechanisms and evaluate potential therapeutic interventions for mTBI. Behavioural testing in mouse models provides valuable insights into the cognitive, motor, and emotional deficits associated with mTBI.

Here we summarize the key findings and methodologies employed in the behavioural testing of a mouse model of mTBI. The model employs a controlled impact device, which delivers a precise and reproducible impact to the head of the mouse. Behavioural assessments were conducted at various time points post-injury, including pre-injury baseline measurements, acute phase (hours to days after injury), and chronic phase (weeks to months after injury).

Cognitive functions were evaluated using a battery of tests, such as the novel object recognition, and Y maze. Given the phenotypical profile of the transgenic mouse model expressing truncated tau protein, which features motoric impairment it is important to assess the motor functions. This will be performed using the rotarod test and beam walking.

In order to facilitate our understanding of mTBI pathology in humans, we are on the way of developing an experimental model of mTBI that recapitulates all the key aspects of human mTBI as closely as possible.

This work has been supported by competitive academic grants VEGA 2/0153/22, APVV-19-0568 and APVV-20-0615.

PRODUCTION OF ANTI-DENGUE APTAMERS USING DENGUE VIRUS LIKE PARTICLES

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Dengue is considered as a Neglected tropical disease (NTD) by WHO causing periodic endemics. In recent years, there has been a rise in cases of dengue haemorrhagic fever (DHF) implying that virus is developing neurotropic affinity. There is no approved therapeutic agent for human use, but for prevention, CYD-TDV Dengavaxia (Sanofi Pasteur Inc.), a live, tetravalent, FDA-approved vaccine for people with a history of infection, is available. Other potential candidates are Takeda (TAK-003) and TV003/TV005 developed by National Institute of Allergy and Infectious Diseases. The Dengue virus belongs to flaviviridae family, and has a positive, single stranded and enveloped RNA. The molecular structure is composed of three structural and seven nonstructural proteins [1]. The Envelope protein consists of three ectodomains viz., domain I, II and III. The Domain III of E protein facilitates the binding of the DENV to different receptors of cells [2]. Hence there arises the need to produce a blocking agent that would bind to the of E protein and thereby inhibit the binding of the virus to the cells. Aptamers can be considered to be a suitable candidate for therapeutic purposes due to its ability to accurately bind to the target, smaller size, zero cross reactions, low toxicity and low cost production [3]. The first aptamer produced against dengue was able to bind to DENV-2 envelop protein domain III (D3) and also neutralize the infections caused by all four serotypes of DENVs [4]. Further, first RNA based aptamer was produced against the 5´-UTR (untranslated regions) coding for polyprotein, which lead to structural changes of the proteins [5]. In the current study we have successively produced single stranded aptamer library that can specifically bind to Dengue virus serotype 2 VLP (Native antigen) using *Systematic Evolution of Ligands by Exponential Enrichment* (SELEX). A total of eight rounds of SELEX were performed and the binding affinity of the aptamers was analysed using digoxigenin (DIG) ELISA. The pool of specific aptamers were cloned, and after colony qPCR, the clones were categorised according to the data obtained after melt curve analysis. Ten groups of potential candidates were made with similar melting temperatures. These aptamers can be used as a therapeutic agent in a *nano drug delivery system* (nDDS) by conjugating it with nanocarriers like dendrimers which would help it cross the blood-brain barrier and bind specifically to domain III and thereby inhibit binding of the virus to the cells.

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We acknowledge funding for this research by APVV through the project APVV-18-0259, VEGA (projects - 1/0381/23; VEGA - 1/0348/22) and ERA-NET project EURONANOMED2021-105. We thank KEGA (007UVLF-4/2021) and NFP313010V455 for purchase of bioinformatic software. KK is funded by NFP313010V455.

Reprinted from Advances in Experimental Neuroimmunology 2023,
AHO5 Dunajska Luzna 2023, ISBN 978-80-69025-00-4, pp 50-51

STUDY OF CONFORMATIONAL CHANGES OF TAU(210-240) UPON MULTIPLE PHOSPHORYLATIONS USING MOLECULAR DYNAMICS SIMULATIONS

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It is challenging to elucidate the conformational dynamics of intrinsically disordered proteins (IDPs) regulated by post-translational modifications (PTMs) such as phosphorylation. Tau is a well-known IDP, found hyper-phosphorylated in Alzheimer's disease (AD) in humans [1]. The proline-rich domain of tau directly interacts with its partner proteins such as BIN1, 14-3-3 etc. All atoms molecular dynamic (MD) simulation studies have been performed in microsecond time scale for wildtype and four phosphorylated (pT212, pT217, pT231, pS235) tau(210-240) peptide using three different temperature variants (278K, 298K and 310K) and two different force field parameters (AMBER99SB-ILDN and CHARMM36m) with TIP4PD water model as these force fields parameters combine with the water model worked the better for IDPs found from our group previous studies [2, 3]. These four-phosphorylations cause increase in compactness of the peptide resulting bent conformation. From the experimental studies we found the binding affinity reduced by 12-folds between SH3 domain of BIN1 protein and tau(210-240). The binding of associated proteins like BIN1 with tau may alter by the strong salt bridges, forming nearby lysine and arginine due to the phosphorylation [4]. Phosphorylation induces a strong structural transition, with tau(210-240) favouring a bent conformation. The MD simulation results were verified using NMR experimental parameters like chemical shift and ^{3}J-coupling [4].

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Computational resources were supplied by the metacentrum and IT4 Innovations National Supercomputing Center (OPEN-17-7), project (e-INFRA CZ 90140 and LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructures. KB and JH were funded by the Ministry of Education, Youth, and Sport of the Czech Republic (MEYS CR), grant number LTAUSA18168 (Inter-Excellence Inter-Action), Czech Science Foundation [GF20-05789L] and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 873127 – InterTAU. KB is also supported by Brno Ph.D. Talent Scholarship – funded by the Brno City Municipality, Brno, Czech Republic.

THE TRYPTOPHAN-KYNURENINE PATHWAY –- A THERAPEUTIC STRATEGY FOR NEUROPROTECTION IN TAUOPATHIES

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Alzheimer's is a chronic devastating neurodegenerative disease characterized by its neuropathological hallmarks that include the deposition of intracellular neurofibrillary tangles and extracellular amyloid proteins accompanied with neuronal loss [1, 2]. The current study deals with the tryptophan-kynurenine pathway (TKP) associated with this disease. Physiologically, this pathway regulates the metabolism of tryptophan and forms the neuroprotective metabolite, kynurenic acid. In Neuroinflammatory conditions, the inflammatory marker released and increased oxidative stress shifts the pathway and increases the production of neurotoxic metabolite known as the Quinolinic acid that causes neuronal damage by overstimulation of excitotoxic N-methyl D-aspartate (NMDA) receptor [3,4]. This project aims to influence the metabolism of tryptophan into the production of neuroprotective kynurenic acid and thus regulate the neuroinflammation in the animal model of tauopathy.

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This work was supported by research grants VEGA 2/0129/21, VEGA 2/0078/22, and APVV-21-0321.

COULD SENESCENT-LIKE NEURONS REPRESENT NEW DIAGNOSTIC AND THERAPEUTIC TARGETS IN NEURODEGENERATIVE DISEASES? THE GENERATION OF NOVEL CELL LINES FOR ANALYSIS OF NEURONAL SENESCENCE-LIKE RESPONSE

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Neurodegenerative diseases (NDs) are very heterogeneous and progressive age-related pathologies that are becoming increasingly prevalent worldwide. Despite the great interest and extensive research, the underlying pathogenic mechanism that initiates the onset of these conditions remains unknown. However, looking closely at a cellular process called senescence could bring valuable insights.

Even though primarily recognized as a protective tumor-suppressor mechanism, persisting senescence has been linked to the onset and/or progression of multiple health-deteriorating conditions, including neurodegeneration [1]. Moreover, its initial association only with proliferating cells has been challenged after discovering that even post-mitotic fully differentiated neurons can develop a senescent-like phenotype (e.g. increased senescence-associated β galactosidase activity; elevated levels of cyclin-dependent kinase inhibitors $p16^{INK4a}$ and $p21^{Waf1/Cip1}$, pro-inflammatory molecules, and anti-apoptotic proteins) [1, 2]. However, it is not clear yet whether elevated levels of these features specifically in neurons are involved in the pathogenesis of NDs and, thus, if senescent-like neurons could represent new diagnostic and therapeutic targets in these diseases.

Therefore, the aim of our research is to establish a new approach of analysis and address these crucial yet unresolved questions. Firstly, we successfully developed previously missing molecular tools that can be stably integrated into the genome of various cell types, including human neurons, to report senescence induction. These DNA constructs consist of a reporter gene (encoding either a red fluorescence protein Tomato or a luciferase NanoLuc) placed in between two 500-650 bp long homology arms amplified from either $p16^{INK4a}$ or $p21^{Waf1/Cip1}$ -encoding genes. By employing the CRISPR/Cas9 targeting technique, we knocked-in our donor constructs into the genome of human neural progenitor ReNVM cells to generate novel senescence-reporting cell lines. These genome-modified cells are being used to induce neuronal senescence and analyze features of the resulting phenotype. Subsequently, we aim to identify the main regulators of this process by modifying the expression of various senescence-related genes. We believe that results obtained in our laboratory can con-

tribute to clarifying the yet unknown mechanisms, manifestations, and consequences of senescence induced in neurons and also help to evaluate the role of this process in neurodegeneration.

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This project was supported by: Grant UK for young scientists UK/137/2021, DoktoGrant APP0407, APVV-19-0585, VEGA2/0158/21, SASPRO 2_1085/01/02, APVV-20-0331.

THE GENERATION OF RENVM CELLULAR MODELS FOR THE ANALYSIS OF ALPHA-SYNUCLEIN RELATED PATHOLOGIES IN PARKINSON'S DISEASE

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Understanding the pathological changes, diagnosis and treatment of Parkinson's disease (PD) represents a significant challenge in the field of neuroscience underscoring the importance of creating robust disease models. While cellular models are effective tools, the in vitro culturing of post mitotic neuronal cells presents challenges, necessitating the development of new resources for the in-depth neurobiology studies at cellular level. RenVM cell line, which is an immortalised human neural progenitor cell line with the capability to differentiate into dopaminergic neurons represent one such resource. This cell line was derived from the ventral mesencephalon region of the human fetal brain, immortalised by retroviral transduction and remains stable up to 45 passages. Thus, it represents a suitable choice to recapitulate the physiological process in vitro and serves as a valuable tool for investigating neurodegenerative diseases including PD.

Presence of pathological protein inclusions called Lewy bodies (LB) is one of the prominent hallmarks of PD. The main component of LB is a protein called alpha synuclein (a-Syn). Which is a natively unstructured protein primarily expressed in the brain. The precise mechanism of accumulation of a-Syn in LB and subsequent neuronal death still remains elusive. To describe this phenomenon in greater detail we generated novel neuronal cell lines in RenVM cells, with stable overexpression of a-Syn both wild type (WT) or its PD related mutant A53T tagged with GFP employing lentivirus transduction. These cell lines were then separated into three subtypes each, based on the expression levels of GFP. Subsequently, these cells were analysed for a-Syn levels through Western blot and qPCR. Next we analysed the cytotoxic effects of these overexpressions comparing them among themselves as well as with cells not overexpressing a-Syn.

We further plan to differentiate these cell lines into dopaminergic neurons and analyse them for the effects of overexpression of both types of a-Syn on intracellular oxidative stress levels, mitochondrial health, and senescence response. As an added advantage, RenVM can also be used for a variety of research applications such as

studies of neurotoxicity, electrophysiology, neurotransmitter and receptor functions. Our study aims to contribute to a better understanding of cellular processes resulting in the aggregation of a-Syn, subsequent neuronal death and neurodegeneration.

This study was supported by: APP0398, APVV-19-0585, APVV-20-0331, VEGA2/0158/21, SASPRO 2_1085/01/02, ICGEB grant CRP/SVK22-04_EC.

MITOCHONDRIAL TRANSPLANTATION AS A NEW THERAPEUTIC STRATEGY TO TREAT ALZHEIMER'S DISEASE (AD)

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Nowadays, millions of people are suffering with dementia and this number is increasing rapidly. Until 2050, the number of patients is expected to exceed 130 million [1]. The most prevalent dementia is Alzheimer's disease (AD). Typical clinical symptoms are cognitive decline, memory impairment and loss of orientation. Pathological hallmarks include presence of two insoluble protein aggregates, tau neurofibrillary tangles and beta amyloid plaques [2].

Mitochondrial dysfunction is known to be one of the causes of progression of AD. However, the exact mechanism is not very well understood [3]. Being the powerhouse, it provides Adenosine Tri-Phosphate (ATP) to the neurons, regulate calcium homeostasis and apoptotic process [4]. Several clinical studies on post-mortem AD brains have reported the alteration of complex I and IV in mitochondria, leading to their inability to generate ATP [5,8]. Moreover, few studies have also demonstrated that tau pathology induces functional and structural changes in mitochondria [6,7].

Mitochondrial transplantation is a novel strategy for the treatment of mitochondrial dysfunction overcoming the limitations of conventional pharmacological agents [9,10]. Behind the procedure is the transfer of functional exogenous mitochondria into defective, energy-depleted cells aiming to prevent the onset or progression of mitochondrial diseases [11,12]. Mitochondrial transplantation might be a helpful and suitable strategy for the treatment of Alzheimer´s disease (AD). Exogenous mitochondria will protect cells from oxidative stress and restore the functional properties of the cells. In future, the combination of the mitochondrial transplantation with the disease-modifying therapy (active or passive tau immunotherapy) may halt the disease progress or might be used for preventive strategies.

We aim to validate the mitochondrial dysfunction in both in-vitro and in-vivo models of tauopathy. For which we will be using FRET biosensor cells and transgenic rat models expressing human truncated tau protein. We will perform the functional analysis of mitochondria via respirometry, morphological analysis by Transmission electron microscopy (TEM) and confocal microscopy. Additionally, we will explore mitochondrial transfer efficiency through various administration methods such as intracerebral administration, intravenous injections or alternatively administration by nebulization with an aim to develop novel treatment approaches for AD and related tauopathies.

This work is supported by APVV-20-0331, APVV-19-0585, APVV-20-0585, and VEGA 2/0127/22grant.

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STRUCTURAL STUDY OF AGGREGATION-PRONE TAU321-391 FRAGMENT ASSOCIATED WITH ALZHEIMER`S DISEASE

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Protein tau occupies wide conformational space, while certain tau conformations can mediate protein aggregation associated with Alzheimer´s disease and other tauopathies. The epitope of monoclonal antibody DC11, tau321-391, is believed to represent the intermediate structure between physiological and pathological aggregation-prone tau conformation [1]. Also, the ability of tau321-391 to self-aggregate was proven before. Therefore, investigation of mentioned structure can provide insights into the pathological conversion of tau and thus provide basics for anti-aggregation drug development. The structure of the DC11 Fab fragment was obtained using X-ray crystallography followed by structure determination using molecular replacement. During the next steps, antibody structure was used in molecular docking experiments to further examine the structural characteristics of the tau321-391 fragment. Future plans will involve performing molecular dynamics simulations of DC11 Fab/tau321-391 result complexes.

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This work was supported by grant no. APVV 21-0479, VEGA 2/0125/23 and MSCA-RISE no. 873127.

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The meetings were supported by projects
H2020-MSCA-RISE "InterTAU" (GA 873127) and
HORIZON-WIDERA-2022-ACCESS project "ADDIT-CE" (GA 101087124).

All contributions were selected by scientific programme committee and abstracts were peer-reviewed.

Vydalo vydavateľstvo AHO5, Dunajská Lužná,
v júni 2023 ako svoju 35. publikáciu.

Spoluvydavateľ:
Neuroimunologický ústav SAV, v.v.i.

Editor: Peter Filipčík, Rostislav Skrabana
Sadzba: Peter Blaho

ISBN 978-80-973697-9-8

This event has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 873127.

This event has received funding from the European Union's Horizon Europe Widening participation and strengthening the European Research Area programme under the Excellence Hubs grant agreement No 101087124.

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

Funded by the European Union

ISBN 978-80-973697-9-8

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