Acknowledgment. „This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-4906, within PNCDI III.

Ageing

P-01.5-001
DNA polymerase Lambda: a new putative mitochondrial DNA polymerase?

A.M. Teixeira*, S.J. Allison, T.K. Kinnunen, D.J. Clarke, C.D.O. Cooper*
Department of Biological and Geographical Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, United Kingdom

DNA polymerases are key proteins in genome replication and repair. There are 16 human DNA polymerases, and they are classified in 4 families, according to their amino acid sequence and structure, and PrimPol that belongs to the Archaea-Eukaryotic Primase family. Their main roles are in the nucleus, with exception of DNA polymerase gamma (Pol γ) that is responsible for mitochondrial DNA (mtDNA) maintenance, and PrimPol with functions within both compartments. However recently, additional DNA polymerases (specifically Pol ζ or REV3L, Pol β and Pol θ) have been found in the mitochondria, interacting with other mitochondrial proteins. The aim of this project is to identify if further additional DNA polymerases localise to the mitochondria and, if so, to understand if they influence positively or negatively the integrity of mtDNA, particularly in the acquisition of genome mutations and their impact in aging and life- and healthspan. To study the potential of further DNA polymerases localising to mitochondria, subcellular fractionation was performed after inducing different DNA lesions, followed by protein expression detection of native or epitope-tagged DNA polymerases. DNA polymerase lambda (Pol λ) was the main DNA polymerase targeted and in addition to its nuclear expression, Pol λ was also detected in the mitochondrial fraction. More studies are needed to assess and confirm Pol λ mitochondrial localisation, particularly confocal microscopy to determine the spatial location of Pol λ without possible contamination between organelles from fractionation approaches. Moreover, gene knockdown and mitochondrial metabolism assays will be performed to understand if Pol λ is involved in mtDNA physiological processes, and aging studies will be done using *Caenorhabditis elegans* to assess the influence of DNA polymerases in the mitochondrial genome. *The authors marked with an asterisk equally contributed to the work.

P-01.5-002
GABAergic system of subfornical organ in adult and aged rats

V. Razenkova, D. Korzhevskii
FSBSI Institute of Experimental Medicine, Saint Petersburg, Russia

The subfornical organ (SFO) is one of the circumventricular organs (CVOs) of the brain located in the roof of the third ventricle. Due to its high vascularization and lacking blood–brain barrier, SFO is affected by humoral signals and thus mediates blood–brain communication. Despite notable interest in the SFO, the effects of ageing which occur in one of the major neurotransmitter system of CNS here remain to be clearly established. In this study we examined GABAergic system of SFO using immunohistochemical methods. The brain of adult (4-6 months) and old (23 months) male Wistar rats was explored (n = 3 for each age). Serial frontal sections were prepared approximately at the 1.4 mm behind Bregma. Antibodies against glutamate decarboxylase 67 (GAD67) were used in order to visualize GABAergic structures. It was established that GABAergic structures are ubiquitous within SFO. GABAergic neurons tend to locate in the peripheral regions near ventricle and, moreover, one can notice sparse subependymal GABAergic neurons. GABAergic terminals are common in SFO and tend to form nets around not only individual cells, but also cell clusters and perivascular space of fenestrated capillaries and septal veins. There is also a considerable amount of axon terminals in subependymal area. In old rat's brain tissue, the intensity of immunohistochemical reaction is significantly lower. In the lateral part of SFO near septal veins we found GABAergic cells, which seem to be absent in SFO of adult animals. The results obtained suggest the estimated broadening of GABAergic cell population and general degradation of GABAergic innervation in SFO during aging. The reported study was funded by RSF according to the research project № 22-25-00105, https://rscf.ru/project/22-25-00105/.

P-01.5-003
Analyzing *Origanum vulgare* ssp. hirtum (Lamiaceae) essential oil for neuroprotective potential against scopolamine-induced zebrafish (*Danio rerio*) model

L. Hritcu[1], L. Capatina[1], E.M. Napoli[2], G. Ruberto [2]
[1]*Alexandru Ioan Cuza University of Iasi, Iasi, Romania,* [2]*Institute of Biomolecular Chemistry, National Research Council ICB-CNR, Catania, Italy*

Origanum vulgare ssp. hirtum has been used as medicinal herb promoting antioxidant, anti-inflammatory, antimicrobial and neuroprotective activities. We investigated the protective effects and the mechanism of *O. vulgare* ssp. hirtum essential oil (OEO) on cognitive impairment and brain oxidative stress in a scopolamine (Sco)-induced a zebrafish (*Danio rerio*) model of cognitive impairment. Our results showed that exposure to Sco (100 μM) leads to anxiety, spatial memory, and response to novelty dysfunctions, whereas the administration of OEO (25, 150, and 300 μL/L, once daily for 13 days) reduced anxiety-like behavior and improved cognitive ability, which was confirmed by behavioral tests such as the novel tank-diving test (NTT), Y-maze test and novel object recognition test (NOR) in zebrafish. Additionally, Sco-induced brain oxidative stress and increasing of acetylcholinesterase (AChE) activity were attenuated by the administration of OEO. The gas chromatography-mass spectrometry (GC-MS) analyses were used to elucidate the OEO composition, being thymol (38.82%), p-cymene (20.28%), and γ-terpinene (19.58%) as the main identified components. These findings suggested the ability of OEO to revert the Sco-induced cognitive deficits by restoring the cholinergic system activity and brain antioxidant status. Thus, OEO could be used as perspective sources of bioactive compounds, displaying valuable biological activities, with potential pharmaceutical applications. Acknowledgment: Authors are thankful to Romanian Ministry of Research, Innovation and Digitization, within Program 1 –

Development of the national RD system, Subprogram 1.2 – Institutional Performance – RDI excellence funding projects, Contract no.11PFE/30.12.2021, for financial support

P-01.5-004
Effect of hesperidin, hesperetin, rutinose, and rhamnose on skin aging

R. Sklenarova[1], D. Skarupova[1], J. Hanyk[1], J. Ulrichova[1], V. Kren[2], P. Bojarova[2], K. Brodsky[2], J. Vostalova[1], J. Frankova[1]

[1]*Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic,* [2]*Laboratory of Biotransformation, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

Skin aging is a complex physiological process caused by internal (chronological) and external factors. Chronological aging is associated with spontaneous accumulation of modified biomolecules by non-enzymatic glycation and formation of advanced glycation end-products (AGE). AGE cause oxidative stress, tissue damage, inflammation, and the development of complications of diabetes. Therefore, it is useful to reduce their formation, and the use of natural substances is one of the possible prevention strategies. In our study, we focused on the molecular mechanism of the antiglycation effect and the comparison of hesperidin, hesperetin, rutinose, and rhamnose. Hesperidin and its aglycone hesperetin are classified as natural flavonoids that are compounds with anti-inflammatory and antioxidant potential. Hesperidin has also been shown to be a potent anti-photoaging factor by regulating metalloproteinases and inflammatory interleukins. Hesperidin is glycosylated by rutinose, which is composed of rhamnose and glucose. The cytotoxicity of our selected substances was evaluated using the MTT assay on primary cultures of normal human dermal fibroblast (NHDF). Subtoxic concentrations of hesperidin, hesperetin (1-25 μM), rhamnose, and rutinose (1-25 mM) were tested on the skin aging model. The antisenescent activity of the test compounds was evaluated by measuring the level of pro-inflammatory cytokines (IL-6, IL-8) and matrix metalloproteinases. The results of each method (ELISA and MTT) will be discussed in the poster presentation. Acknowledgements: This work was supported by the student grant: Skin anti-aging study of hesperidin, hesperetin, rutinose, and rhamnose: Comparative study (Registration number: DSGC-2021-0065). This student grant is funding from the OP VVV Project No. CZ.02.2.69/0.0/0.0/19_073/0016713, Improving Schematics of Doctoral Student Grant Competition and their Pilot Implementation. Project MEYS LTC20069 is acknowledged as well.

P-01.5-005
Molecular alterations in ageing sperm and their relevance for male fertility decline

J. Santiago[1], J. V. Silva[1,2,3], M. A. S. Santos[1], M. Fardilha[1]

[1]*iBiMED – Institute of Biomedicine, University of Aveiro, AVEIRO, Portugal,* [2]*QOPNA & LAQV, Department of Chemistry, University of Aveiro, AVEIRO, Portugal,* [3]*Unit for Multidisciplinary Research in Biomedicine, Abel Salazar Institute of Biomedical Sciences (UMIB-ICBAS), University of Porto, Porto, Portugal*

Male fertility is strongly affected by environment and lifestyle. Advanced paternal age has been linked with changes in testicular structure and function, impaired semen parameters and DNA integrity, lower pregnancy rates and decline in offspring fitness. The sperm quality decline with ageing has also been associated with an increase in oxidative stress. However, only a few studies reported the deregulation of sperm proteins or RNAs associated with this risk factor for male infertility. This work aimed to study the ageing-related alterations in human sperm protein and small RNA content possibly responsible for the age-associated decline in male fertility. To do so, 120 Portuguese men from the Aveiro region (Portugal), aged between 19 and 56 years old were included in this study. Basic semen analyses were performed on all sperm samples according to WHO's guidelines. To avoid contamination by somatic cells, density gradient sperm selection was performed. The proteome of 19 normozoospermic human sperm samples divided into four groups according to men's age was evaluated by quantitative proteomic analysis. The small RNA content of 16 human sperm samples was investigated using small RNA sequencing. Our data showed no correlation between paternal age (mean age 35.2 ± 6.32 years) and the seminal parameters examined. Proteomic analysis revealed 46 differentially expressed proteins (DEPs) between groups. Gene ontology analysis of all deregulated sperm proteins shows that response to unfolded protein, positive regulation of mitochondrion organization and apoptotic process, negative regulation of phosphoprotein phosphatase activity, and spermatogenesis are common biological processes affected. Transcriptomic analysis identified 5 differentially expressed miRNAs (DEMs) between groups. The DEPs and DEMs here identified could help to elucidate and/or became potential diagnostic markers for the age-associated decline in human sperm quality.

P-01.5-006
Collagenase, elastase, hyaluronidase inhibition by hesperidin and its structural part hesperetin, rutinose, and rhamnose

J. Hanyk[1], D. Škařupová[1], R. Sklenářová[1], J. Ulrichová[1], V. Křen[2], P. Bojarová[2], K. Brodsky[2], J. Franková[1], J. Vostálová[1]

[1]*Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University in Olomouc, Olomouc, Czech Republic,* [2]*Laboratory of Biotransformation, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

Skin aging is a multisystemic degenerative process characterized by phenotypic and functional changes of cutaneous cells. These changes are accompanied by structural disturbances in extracellular matrix components such as collagen, elastin, and hyaluronic acid caused by the increasing activity of collagenase, elastase, and hyaluronidase. One of the most important ways to prevent skin aging is the topical application of dermatological preparations containing active compounds that inhibit collagenase, elastase, and hyaluronidase. For this purpose, naturally occurring compounds derived from plants have been investigated in dermatology. The flavonoid hesperidin and its aglycone form hesperetin, both abundant in citrus fruits, possess antioxidant, anti-inflammatory, and free radical scavenging activity. Hesperidin has also been shown to be an anti-aging and anti-photoaging agent. Rutinose, which is the structural component of hesperidin, consists of rhamnose and glucose. The anti-aging effect of rhamnose has been partially documented, but rutinose has not yet been studied. The aim of this study was to determine the ability of hesperidin, hesperetin, rutinose, and rhamnose to inhibit enzymes associated with skin aging (collagenase, elastase,

FEBS Open Bio **12** (Suppl. S1) (2022) 67–336 DOI: 10.1002/2211-5463.13440