Session 7: Oxidative stress in health and disease

Lectures

L.07.1

Redox Eustress: Homeostasis is Homeodynamics

Helmut Sies

Institute for Biochemistry and Molecular Biology I, Heinrich-Heine-University Düsseldorf, Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
Helmut Sies <sies@hhu.de>

Maintaining redox homeostasis in cells and organs is a continuously ongoing challenge. The redox balance between oxidants and reductants is regulated by powerful enzyme systems in response to endogenous and exogenous signaling processes. The positioning of the redox state is characterized by ongoing oscillations around a steady state redox set-point. The overall redox set-point, however, is an umbrella term, also called redox tone, which is under spatiotemporal control. Considerable redox gradients exist between subcellular organelles, and there are circadian fluctuations. Given these considerations, it becomes evident that the so-called 'resting state' is highly dynamic, suggesting that 'homeodynamics' describes the basal cellular redox state more adequately than 'homeostasis'. This is reflected by the term 'eustress' to denote the physiological range of deviations from the redox set-point, in contrast to 'distress' at supraphysiological redox challenge, which causes biomolecular damage.

L.07.2

Aging and failing cellular proteodynamics: a lesson from centenarians

Jacek M. Witkowski

Department of Pathophysiology, Medical University of Gdańsk, Poland
Jacek Maciej Witkowski <jawit@gumed.edu.pl>

Proteodynamics includes protein production, maturation, maintenance and degradation, and is affected by aging and related oxidative stress. Proteomes of aging individuals become functionally impaired as *geroproteomes*, participating in cellular senescence. Aging of the immune system results in increased susceptibility, severity and mortality due to infectious diseases and malignancies. Aging-associated conversion of immune cell proteomes to geroproteomes, in which more and more proteins are underproduced, defective and not properly eliminated, precipitates inaccurate behavior of the system. Mechanistically, the immune system aging includes immunosenescence (similar, although not identical with generalized cellular senescence) and inflammaging (aging-related increase of proinflammatory readiness). An important mechanism of posttranslational protein modification likely involved in immunosenescence and inflammaging is their limited modulatory proteolysis by specialized proteases, including the calpain-calpastatin system (CCS). We have demonstrated that CCS activity is necessary for effective T cell response to stimulation and that its activity includes modification of some crucial signalling molecules. Also, we have demonstrated that with aging amounts and activities of CCS decrease, directly participating in immunosenescence. Centenarians seem to retain most (if not all) features of their proteomes (including robust CCS) into old age which is likely deciding of their longevity.

L.07.3

AKT and JNK kinases in iron-mediated oxidative stress

Jędrzej Antosiewicz, Andżeliką Borkowska, Małgorzata Halon-Golabek

Department of Bioenergetics and Physiology of Exercise, Medical University of Gdańsk, Poland
Jedrzej Antosiewicz <jant@gumed.edu.pl>

Iron is indispensable for most cellular processes, including DNA synthesis, cellular respiration, oxygen transport, steroid synthesis, etc. Conversely, iron can induce iron-dependent formation of reactive oxygen species and oxidative stress. One of the factors which determine iron toxicity is its excessive accumulation in several tissues, including skeletal muscle, heart, liver, pancreas, etc. Several reports demonstrate that iron accumulation in tissue increases the risk of diseases like diabetes, cancer, and heart attack.
Here we will ponder two critical questions concerning iron toxicity: What is the mechanism of tissue iron accumulation, and why is iron toxic even if it is stored in ferritin and handled by other specialised proteins which protect it from free radical-generating reactions? We hypothesised that an impaired Akt-FOXO3a signalling pathway, which is characteristic of insulin-resistant tissue, triggers changes in iron metabolism. Our data clearly show that impairment of the Akt signalling pathway leads to activation of FOXO3a, a transcriptional factor upregulating ferritin. An increase in cellular ferritin leads to a decrease in the labile iron pool, which elevates iron transport into a cell. Moreover, we provide experimental evidence that incell culture, iron-dependent ROS formation is mediated by the JNK1-ITCH-p66shc signalling pathway.

Posters

P.07.1

Oxidative stress contributes to carboplatin and paclitaxel-dependent premature senescence of normal peritoneal cells

Szymon Rutecki, Agnieszka Leśniewska-Bocianowska, Julia Matuszewska, Daniel Rychlewski, Aleksandra Leśniewska, Justyna Mikuła-Pietrasik, Krzysztof Książek

Department of Pathophysiology of Ageing and Civilization Diseases, Poznan University of Medical Sciences, Poznan, Poland
Szymon Rutecki <srutecki@student.ump.edu.pl>

Oxidative stress participates in cellular senescence. Here, we investigated whether it plays a role in senescence of peritoneal mesothelial cells (PMCs) and fibroblasts (PFBs) subjected to carboplatin (CPT) and paclitaxel (PCT). These drugs are used to treat ovarian cancer (OC), and PMCs and PFBs are critical for OC metastases. We show that PMCs and PFBs senesce prematurely upon CPT and PCT exposure. In PMCs, this state is telomere-independent, mediated by p16, AKT, and STAT3, whereas in PFBs, it proceeds in a telomere-dependent manner, engaging p21, p53, and ERK1/2. Both cell types develop SASP via p38 MAPK, NF-kB, STAT3, Notch1, and JAK3 signaling. Drug-treated cells release increased amounts of superoxides and peroxides and accumulate damaged DNA and proteins. Activities of cytochrome c oxidase and NADH dehydrogenase are elevated. They also have reduced inner mitochondrial membrane potential, which in PMCs is compensated by PGC-1a-dependent induction of mitochondria biogenesis. Senescent PFBs display increased activity of antioxidants, SOD and CAT. Upon drug-treated cell protection against oxidative stress with spin-trap ROS scavenger, the expression of senescence indices declined. Collectively, our study shows that CPT+PCT can promote the senescence of normal peritoneal cells, and this paradoxical effect opposing therapeutic needs, proceeds via increased oxidative stress.

Acknowledgements

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P.07.2

Reactive nitrogen species (RNS)-dependent formation of macrophage extracellular traps (METs) by bone marrow-derived macrophages (BMDMs)

Dominika Drab[1,2], Michal Santocki[1], Elzbieta Kolaczkowska[1]

[1]Laboratory of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland, [2]Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, Krakow, Poland

Dominika Drab <dominika.drab@doctoral.uj.edu.pl>

Extracellular trap (ET) formation has been extensively studied in neutrophils, but other innate immune cells such as macrophages are also capable of releasing extracellular DNA decorated with nuclear and granular proteins. Macrophage extracellular traps (METs) represent a novel tool in the repertoire of macrophage defense against pathogens. To date, knowledge of the mechanisms involved in their formation is still fragmentary. The aim of this study was to validate MET formation by bone marrow-derived macrophages (BMDMs) and to verify a role of reactive nitrogen species (RNS) in MET formation. Our studies were performed on BMDMs obtained from bone marrow (BM) cells of C57Bl/6J male mice. BMs were differentiated into naïve BMDMs ($F4/80^+$, $CD11a^+$; flow cytometry) using conditioned medium from mouse fibroblast cell line L929. BMDMs were stimulated with a nitric oxide synthase (NOS) inhibitor (L-NAME) or a nitric oxide donor (SNAP) or LPS. Sytox Green DNA stain and antibodies against MET components, histones (H2A.X) and MMP-9, were used for MET visualization by confocal microscopy. Fully differentiated and functional macrophages (adherent, metabolically active, $iNOS^+$, NO producing) were able to form METs upon stimulation. Most importantly, we showed for the first time that MET release depends on RNS, as L-NAME inhibited MET formation by BMDMs upon stimulation with LPS, and SNAP induced METs.

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P.07.3

Assessment of lipid peroxidation products in schizophrenia patients

Aleksandra Polikowska[1], Małgorzata Goszka[1], Patrycja Stodolak[1], Natalia Serwin[1], Anna Michalczyk[2], Jerzy Samochowiec[2], Barbara Dołęgowska[1], Elżbieta Cecerska-Heryć[1]

[1]Department of Laboratory Medicine, Pomeranian Medical University, Szczecin, Poland; [2]Clinic of Psychiatry, Pomeranian Medical University, Szczecin, Poland

Aleksandra Polikowska <polikowska.aleksandra@gmail.com>

Introduction: Schizophrenia (SCZ) is a mental disorder with complex etiology and chronicity. Oxidative stress (OS) may play a role in the pathophysiology of SCZ. OS is a condition caused by excess reactive oxygen species and impaired antioxidant defenses consisting of antioxidant enzymes, which can lead to lipid peroxidation.
Aim of the study: Determination of the relationship between the levels of selected lipid peroxidation products depending on the type of SCZ.
Material and methods: The study included 150 people. Blood was collected in clot tubes. The study group consisted of 116 patients of the Psychiatric Clinic in Szczecin diagnosed with schizophrenia (7 women, 21 men with SCZ deficit); (24 women, 20 men with SCZ without deficit); (17 women, 13 men with first psychosis); (7 women and 7 men ultra-high risk of psychosis). The control group comprised 34 healthy people (19 women, 15 men). Lipid peroxidation product levels were tested by ELISA. Statistical analysis performed by RStudio.
Results: Statistical analysis showed a significant relationship between MDA, 4-HNE, 8-iso-PGF(2alpha), and the type of schizophrenia ($p<0.05$).
Conclusions: Patients with schizophrenia experience chronic oxidative stress, as evidenced by elevated levels of lipid peroxidation products: iso-PGF(2alpha), MDA, and altered levels of 4-HNE, suggesting that OS develops in the early stages of the disease and persists throughout the illness, suggesting that OS may be one of the causes of schizophrenia.

P.07.4

Exploring the antioxidant potential of purple potato extract: A comprehensive study on lipid membrane protection, anti-radical and antidiabetic activity

Paulina Strugała-Danak, Aleksandra Włoch, Natalia Mrowińska, Hanna Pruchnik, Janina Gabrielska

Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
Paulina Strugała-Danak <paulina.strugala@upwr.edu.pl>

The aim of the project was to investigate antioxidant potential of the extract which was obtained from the *Vitelotte* variety (VE) of purple potatoes. The study involved determining ant-oxidative activity in relation to model lipid membranes, where peroxidation was induced by UVB radiation and a chemical compound called AAPH. Additionally, both antiradical activity against free radical DPPH$^{\bullet}$ and antidiabetic activity obtained in a way of inhibiting α-glucosidase were specified. The toxicity of the extract which was exerted on erythrocyte cells was also examined in a way of measuring hemolytic activity.
The investigation of antioxidant activity conducted on phosphatidylcholine liposomes revealed that the extract provided more effective protection to the model lipid membrane against AAPH-induced oxidation compared to UVB-induced oxidation. Moreover, VE exhibited antiradical properties, confirming its ability to undergo oxidation-reduction in neutralizing stable, free radical DPPH$^{\bullet}$. It was demonstrated that VE inhibited the activity of α-glucosidase enzyme under *in vitro* conditions. Finally, the study showed that VE did not cause hemolysis of red blood cells and, therefore, did not have any toxic effects.
The research results suggest that VE could have applications in the pharmaceutical industry as an ingredient in dietary supplements. As a proven antioxidant, the extract is capable of delaying aging processes *in vivo* and may also be used in order to support diabetes treatment.

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P.07.5

Assessment of oxidative potential of compounds in the development of gut microbiota dysbiosis

Anna Lichota, Monika Sienkiewicz

Department of Pharmaceutical Microbiology and Microbiological Diagnostics, Medical University of Lodz, Lodz, Poland
Anna Lichota <anna.lichota@umed.lodz.pl>

Gut microbial dysbiosis is a condition characterized by a decrease in microbial diversity, quantity, and function. Changes in gut microbial diversity result in the dysfunction of the intestinal barrier, thus leading to multiple inflammatory diseases. This imbalanced microbiota is unable to protect from pathogens, that can trigger inflammation and lead to the development of chronic diseases. Gut microbial dysbiosis is the most frequently caused by the use of antimicrobial drugs including antibiotics. The aim of the study was to investigate antioxidant properties of carvacrol, geraniol (natural compounds), rifaximin and sulfasalazine on selected bacterial strains of gut microbiota. Carvacrol and geraniol are secondary metabolites of plants with good antimicrobial activity. They are characterized by antimicrobial, anti-inflammatory properties, is used in cosmetic and food industries. Rifaximin is antibiotic commonly used for the treatment of traveller's diarrhoea. Whereas sulfasalazine is used to treat and prevent chronic inflammatory bowel diseases. The study determined the concentration of hydroperoxides, as well as carbonyl groups and free thiol groups by *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212). The research allowed to determine oxidative potential of compounds against bacteria living in gut microbiota. Obtained results indicate that sulfasalazine and geraniol to a greater extent cause oxidative damage in bacterial cells.

P.07.6

Polyphenol-rich composition AP119 shows antioxidant and anti-inflammatory properties in human Hek293 cells and PBMCs.

Karolina Niska[1], Patrycja Bloch[1], Alina Mieczkowska[1], Nikola Sobczak[1], Krzysztof Lemke[1], Barbara Khaidakov[1]

[1]AronPharma Ltd., Gdańsk, Poland; [2]Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-Universität München, Germany
Karolina Niska <karolina.niska@aronpharma.pl>

Polyphenols have been extensively studied for their potential health benefits. They exhibit potent antioxidant activity, primarily attributed to their ability to scavenge free radicals and reduce oxidative stress and inflammation. This study aimed to evaluate the properties of AP119 composition in Hek293 and peripheral blood mononuclear cells (PBMCs). Hek293 were treated with AP119 and H2O2 as a positive control, and the expression level of selected markers associated with oxidative stress and inflammation were analyzed using qPCR. GSH level was evaluated using Glutathione Assay Kit. NO and ROS level at PBMCs were measured with Griess and DCFDA assay respectively and IL-2 and IFN- γ level were measured using ELISA. AP119 treatment significantly reduced the level of *Nrf-2, SOD-2, HO-1* expression and GSH in Hek293 cells when compared to H2O2-treated positive control. Furthermore, AP119 decreased the level of IL-2 and IFN-γ protein in PHA-L stimulated PBMCs. PBMCs pre-incubation with AP119 also resulted in an increase of NO level and a reduction of H2O2-induced ROS production. Overall, these findings highlight the potential of AP119 composition as a natural antioxidant for cellular protection against oxidative stress and inflammation. Further studies are warranted to deeper elucidate the underlying molecular mechanisms and evaluate the potential of AP119 in various oxidative stress-related conditions.

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P.07.7

A Design of Experiments Strategy to select a unique composition of extracts rich in polyphenols

Laura Banaszkiewicz, Julian Guzowski, Patrycja Bloch, Karolina Niska, Alina Mieczkowska, Michał Dobkowski, Krzysztof Lemke, Barbara Khaidakov

AronPharma Ltd., Gdańsk, Poland
Laura Banaszkiewicz <barbara.khaidakov@aronpharma.pl>

Many extracts rich in polyphenols have been extensively described in literature. Much less data can be found on the properties of extracts mixtures, which, due to their unique composition, can show multidirectional effects. The aim of the present study was to establish the best experimental conditions that lead to the extracts richest in polyphenolic compounds with potentiated biological activity, high bioavailability and stability of active substances. In this regard, a D-Optimal design of experiments (DoE) method was applied. The input variables were the following data: content of polyphenols/anthocyanins, antioxidant and anti-inflammatory properties for single extracts. The results based on the prediction of Design-Expert® showed several mixtures with the optimized composition of single extracts to obtain blends with desired properties. The selected mixtures were then further studied. Their bioavailability was assessed using Caco-2 in vitro model. Antioxidant potential was measured with DPPH and ORAC assays, anti-inflammatory properties were measured using ELISA and stability of polyphenols and anthocyanins was evaluated in accelerated aging tests in the stability chamber (40°C, 75% RH). Obtained results highlight the potential of mixtures of extracts to offer enhanced benefits and indicate that the proportion of components in the mixture is the main factor involved in determining its properties.

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P.07.8

Nitroxide-containing amphiphilic polymers mitigate oxidative stress in human neuroblastoma SH-SY5Y cells induced by overexpression of tau protein

Natalia Pieńkowska[1], Margaret Fahnestock[2], Crystal Mahadeo[2], Izabela Zaborniak[3], Paweł Chmielarz[3], Grzegorz Bartosz[1], Izabela Sadowska-Bartosz[1]*

[1]Laboratory of Analytical Biochemistry, Institute of Food Technology, College of Natural Sciences, University of Rzeszów, Rzeszów, Poland; [2]Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada; [3]Department of Physical Chemistry, Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland

Izabela Irmina Sadowska-Bartosz <isadowska@poczta.fm>

Tau protein is a microtubule-associated protein, mainly expressed in the neurons, closely associated with the proper functioning of the cytoskeletal network. The aim of this study was to examine whether overexpression of tau protein leads to changes in the redox status of human neuroblastoma SH-SY5Y cells. Non-differentiated SH-SY5Y cells were stably transfected with a plasmid carrying the sequence of hTau40 (the longest human tau isoform). The level of reactive oxygen species (ROS) was elevated in tau-overexpressing cells (TAU cells) as compared with cells transfected with the empty vector (EP cells). The level of reduced glutathione was increased in TAU cells, due to the overproduction of this antioxidant as an adaptation to oxidative stress (OS). The TAU cells had elevated mitochondrial mass. They were more sensitive to hydrogen peroxide and delphinidin compared to EP controls. These results indicate that overexpression of the tau protein imposes OS on the cells. The nitroxide 4-amino-TEMPO and nitroxide-containing nanoparticles (NPs) mitigated OS in TAU cells, decreasing the level of ROS. NPs lowered the level of lipid peroxidation and mitochondrial mass in both TAU and EP cells. These results suggest that NPs may be promising candidates to mitigate neuronal OS and delay the progress of tauopathies.

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P.07.9

Antioxidant properties and antiproliferative effects of various forms of garlic

Paulina Furdak[1,4], Natalia Pieńkowska[1], Ireneusz Kapusta[2], Grzegorz Bartosz[3], Izabela Sadowska-Bartosz[1]

[1]Laboratory of Analytical Biochemistry, [2]Department of Food Technology and Human Nutrition and [3]Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, College of Natural Sciences, University of Rzeszów, Rzeszów, Poland; [4]Doctoral School of the University of Rzeszów, University of Rzeszów, Rzeszów, Poland

Grzegorz Bartosz <isadowska@poczta.fm>

Examination of extracts of various common fruits and vegetables showed an efficient inhibition of proliferation of human ovarian cancer cells by garlic extracts. Antioxidant capacity, polyphenol content, flavonoid content and organosulfur compounds content of acetone, ethanol and phosphate-buffered saline (PBS) extracts of fresh Polish and Spanish garlic, black and granulated garlic, as well as fresh and dried bear's garlic were compared. Considerable differences were found between various garlic extracts. Extracts of black and granulated garlic showed the lowest antioxidant capacity. A significant correlation was found between the content of phenolic compounds and the antioxidant capacity assayed by the $ABTS^{\bullet}$ decolorization and FRAP methods, and between the results of the FRAP and $DPPH^{\bullet}$ decolorization assays. Garlic extracts inhibited the proliferation of PEO1 and SKOV3 ovary cancer cells and, usually to a smaller extent, MRC-5 fibroblasts. PBS extract of fresh Spanish garlic were most efficient in the inhibition of proliferation of PEO1 cells (IC_{50} of 0.71 μg/100 μL medium) while acetone extract of Polish garlic caused the highest inhibition of proliferation of SKOV3 cells (IC_{50} of 6.0 μg/100 μL medium). The results demonstrate that phenolic compounds are the main determinants of the antioxidant capacity but, in contrast to organosulfur compounds, they do not contribute significantly to the antiproliferative effect of garlic extracts against human ovarian cancer cells.

P.07.10

Formation of a purple product in the reaction of ABTS• with proteins

Kacper Kut[1],Grzegorz Bartosz [2],
Ireneusz Stefaniuk[3], Izabela Sadowska-Bartosz[1]

[1]Laboratory of Analytical Biochemistry and 2Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, and [3]Institute of Materials Engineering, College of Natural Sciences, University of Rzeszów, Rzeszów, Poland
Kacpar Kut <isadowska@poczta.fm>

Decolorization of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) free radical (ABTS•) is a popular method of assay of the antioxidant capacity of food, beverages, and biological fluids. We observed that the reaction of ABTS• with various proteins leads not only to the ABTS• reduction of also to the appearance of a purple color (absorption maximum at 550-560 nm) visible after total ABTS• reduction. The aim of this study was to characterize the formation and explain the nature of the product responsible for the appearance of this color. The purple color co-precipitated with protein, and was diminished by reducing agents. A similar color was generated by tyrosine upon reaction with ABTS•. The most feasible explanation of the color formation is the addiction of ABTS• to proteins tyrosyl radicals formed by protein reactions with ABTS•. The product formation was decreased by the nitration of BSA tyrosine residues. The formation of the purple product of tyrosine was optimal at pH 6.5. A pH decrease induced a bathochromic shift of the spectra of the product. The product was not a free radical as demonstrated by EPR spectroscopy. Another byproduct of the reaction of ABTS• with tyrosine and proteins was dityrosine. These byproducts can contribute to the non-stoichiometry of the antioxidant assays with ABTS•. Formation of the purple ABTS adduct may be a useful index of radical addition reactions of protein tyrosine residues.

P.07.11

Effect of 6-hydroxydopamine on the glutathione level in SH-SY5Y human neuroblastoma cells

Natalia Pieńkowska[1], Paulina Furdak[1],
Grzegorz Bartosz[2], Izabela Sadowska-Bartosz[1]

[1]Laboratory of Analytical Biochemistry, and [2]Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, College of Natural Sciences, University of Rzeszów, Rzeszów, Poland
Paulina Furdak <paulinaf2@o2.pl>

Treatment of human neuroblastoma SH-SY5Y cells with a catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA) is an in vitro experimental model of Parkinson disease (PD). A decrease in the glutathione (GSH) content occurs in PD. Higher concentrations of 6-OHDA lower the GSH level in SH-SY5Y cells, nonetheless we and other authors found a considerable increase in these cells' GSH content after 24 h treatment with 60 μM 6-OHDA. A synthetic antioxidant, 4-aminotetramethylpiperidine-1-oxyl (4-AT) exerted a similar effect. The aim of the present study was to explain this surprising effect by monitoring the time course of changes in the levels of reduced and oxidized glutathione (GSSG), the level of reactive oxygen species and activities of enzymes of glutathione metabolism after treatment of the cells with 60 μM 6-OHDA and/or 4-AT for 30 min – 24 h. A transient decrease in the level of GSH and decrease in the activities of glutathione peroxidase, glutathione reductase, glutathione S-transferase and γ-glutamyl-cysteine ligase activities were found followed by normalization or overshoot of the GSH level and enzyme activities. Increased activity of γ-glutamyl-cysteine ligase activity starting after 4 h was responsible for the elevation of the level of GSH in cells treated with 6-OHDA, 4-AT, and both compounds.

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P.07.12

Elevated level of DNA damage and impaired DNA repair of oxidative DNA lesions in patients with multiple sclerosis

Beata Filipek, Tomasz Popławski

Department of Pharmaceutical Microbiology and Biochemistry, Medical University of Lodz, Poland
Tomasz Poplawski <tomasz.poplawski@umed.lodz.pl>

Multiple sclerosis (MS) is a common neurodegenerative disease in which axon demyelination and nerve cell death follow the inflammatory process. We assumed that MS pathogenesis include damage to cell DNA caused by oxidative stress. The further fate of the cells depends on the effectiveness of DNA repair. The aim of this work was to analyze the sensitivity of peripheral blood mononuclear cells (PBMC) isolated from MS patients to DNA damaging agents inducing oxidative DNA lesions - tert-Butyl hydroperoxide (TBH) and calculate the repair efficiency. We included 30 MA patients and 30 healthy controls and used alkaline version of comet assay with modifications to measure sensitivity to DNA damaging agents and DNA repair efficiency. We found an increased number of oxidative DNA lesions in the MS patients as compared to the controls. Exposure to TBH evoked similar increased damage in both groups, but we observed statistically higher PMBC sensitivity to TBH (MS-25.3 *vs* 10.6 in control). Examination of the repair kinetics of both groups revealed that the DNA lesions induced by TBH were more efficiently repaired in the controls than in the patients (ROC area curve 0.69; $p<0.05$ for TBH). These data suggests impaired repair of oxidative DNA lesions in MS patients.

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P.07.13

The association of base-excision repair genes expression and the occurrence of non-alcoholic fatty liver disease

Sylwia Ziółkowska[1], Piotr Czarny[1], Magdalena Ejsmont[1], Kinga Jarmusz[1], Maciej Kosmalski[2], Tadeusz Pietras[2], Maciej Jabłkowski[3], Janusz Szemraj[1]

[1]Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland; ; [2]Department of Clinical Pharmacology, Medical University of Lodz, Lodz, Poland; ; [3]Department of Infectious and Liver Diseases, Medical University of Lodz, Lodz, Poland.
Sylwia Ziółkowska <sylwia.ziolkowska@umed.lodz.pl>

Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is one of the most common disorders affecting the liver, since NAFLD can be observed in about 25% of the population. Recent literature shows the link between NAFLD and increased production of reactive oxygen species (ROS), which can be produced in mitochondria. Due to a high number of mitochondria present in hepatocytes, it makes the liver is particularly vulnerable to oxidative stress. It can lead to an oxidative damage in the mitochondrial DNA (mtDNA), and a base excision repair (BER) is main DNA repair pathway responsible for dealing with these lesions.
Method: The studied groups have 84 NAFLD patients and 40 controls. RNA was isolated from blood samples and transcribed into cDNA. Gene expression was determined by TaqMan probes and qPCR. *APEX1, NEIL1, POLG, LIG1, LIG3, XRCC1, PARP1, FEN1* were chosen as genes of interest, while *GAPDH* as a reference gene. Statistical analysis was performed using GraphPad Prism 8 and results were calculated using the $2^{-\Delta Ct}$ method.
Results: The findings present that all the eight studied genes were up-regulated in the comparison to the controls. The differences between groups expressed as p-value were below 0.001 for each of the tested genes implying strong association.
Conclusion: The results imply that genes involved in BER repair pathway may influence the development of NAFLD. The data allows to suspect, that the requirement of DNA repair system is increased in the steatosis.