METABOLOMICS CIRCLE
WROCŁAW 2023

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Metabolomics Circle Wrocław 2023

January 27–28, 2023

9th Conference of the Polish Metabolomic Society

BOOK OF ABSTRACTS

POLSKIE TOWARZYSTWO METABOLOMICZNE

Wrocław University of Science and Technology

Oficyna Wydawnicza Politechniki Wrocławskiej

Metabolomics Circle

9th Conference of the Polish Metabolomic Society
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Speakers
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– Age-related memory formation defects are accompanied by the metabolic profile

Mariusz Bromke
– Metabolomic analysis of a Pseudomonas-resistant versus a susceptible Arabidopsis accession

Michał Markuszewski
– Metabolomics in clinical perspective – analysis of gastrointestinal stromal tumour samples

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DEMOCRATIZATION OF METABOLOMICS IN LIFE AND HEALTH SCIENCES

MAIN LECTURE

Nicola Zamboni

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The metabolomics community has made stunning collective progress towards providing a deep molecular characterization on all kinds of specimens at scale, fast, and with efficient use of resources. Upon solving most bottlenecks in data acquisition, untapped opportunities and new challenges emerge. Moving forward, what is needed to fully seize the unique potential of metabolomics and promote its capillary adoption in biomedical research? In this lecture, I will present two current developments of our lab.

SERUM METABOLOMICS AND FECAL MICROBIOTA ANALYSIS IN NEWLY DIAGNOSED CHILDREN WITH INFLAMMATORY BOWEL DISEASE

Michal Ciborowski[1], Katarzyna Zdanowicz[2], Karolina Pietrowska[1], Rafal Kucharski[3], Aleksandra Czajkowska[2], Joanna Godzien[1], Jaroslaw Daniluk[3], Dariusz Marek Lebensztejn[2], Adam Kretowski[1, 4], Urszula Daniluk[2]

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Inflammatory bowel disease (IBD) is a condition characterized by chronic inflammation of the gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are the most common types of IBD. Whilst the pathogenesis of IBD remains unclear, its incidence in both children and adults is increasing. Dysregulation of microbiota have recently been hypothesized as a possible cause of IBD development. To increase the knowledge of the intestinal microbiota and metabolites in IBD we performed serum metabolomics and analyzed the intestinal microbiome of treatment-naive pediatric patients with IBD and healthy controls. The study group consisted of children with IBD (n = 18) and healthy controls (n = 10). IBD children were diagnosed with CD (n = 9) or UC (n = 9). Bacterial abundance in fecal samples was evaluated using a 16S rRNA DNA-based test. Serum untargeted metabolomics was estimated using liquid chromatography coupled with mass spectrometry (LC-MS). The majority of significant metabolites belonged to phospholipids and were downregulated in the CD and UC groups in comparison to the control group. One metabolite was found significant in CD vs UC comparison, which in combination with certain inflammatory markers composes a potential diagnostic panel capable of discriminating between CD and UC children with high specificity and sensitivity. Analysis of fecal microbiome showed significant differences in the 25 families, 52 genera and 79 species of bacteria between the patients with IBD and the control group. Six species of bacteria were detected only in children with IBD (*Bacteroides clarus*, *Haemophilus quentini*, *Sporolactobacillus putidus*, *Pasteurella pneumotropica*, *Prevotella oris*, *Sporolactobacillus putidus*). The analysis of the correlation between the bacterial species predominant in IBD patients, metabolites and lipids showed over 200 significant correlations. Based on the microbiome's composition, a PCA model was built showing a spontaneous separation of IBD patients into two groups, independently of IBD type. Then, serum metabolic profiles of IBD patients were analyzed using the grouping of the patients observed on above-mentioned PCA model. OPLS-DA modelling showed that these the two groups of patients have also distinct serum metabolic profiles. In total, 12 metabolites were found significantly different between these groups. IBD patients and healthy children have different metabolic and intestinal microbiota profiles. Our results suggest that host metabolites are related to the composition of the gut microbiota and likely influence each other and may determine the outcome of IBD.

The study was supported by the Medical University of Bialystok (SUB/1/DN/22/001/4406).

PHARMACOLOGIC MODULATION OF PLATELET ENERGY METABOLISM AS A WAY TO DEVELOP INNOVATIVE ANTIPLATELET THERAPIES

Patrycja Kaczara[1], Kamil Przyborowski[1], Marta Lech[1], Anna Kurpinska[1], Barbara Sitek[1], Stefan Chłopicki[1, 2]

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Thrombosis, which is associated with various cardiovascular diseases, is one of the leading clinical concerns associated with high morbidity and mortality. Despite antithrombotic treatment, the risk of cardiovascular events related to thrombosis is not eliminated, moreover, unwanted side effects, such as bleeding, can occur. Platelets represent important players in vascular thrombosis, and antiplatelet drugs are widely used to reduce thrombosis. However, some patients with metabolic diseases show platelet hyperreactivity despite the use of antiplatelet drugs, suggesting that platelet reactivity is intrinsically related to altered energy metabolism, an effect that might not be inhibited by current antiplatelet strategies. Therefore, new strategies are needed to limit platelet hyperreactivity, without interference with their basic function. The aim of this work was to investigate the effects of modulation of energy metabolism pathways on platelet aggregation and to define whether the antiplatelet effects of known antiplatelet mediators, such as nitric oxide (NO) and prostacyclin (PGI_2), but also carbon monoxide (CO), are related to modulation of energy metabolism in platelets.

We exposed platelets isolated from healthy volunteers to: 1) metabolic inhibitors: 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), oligomycin, 2-deoxy-D-glucose, 2) CO (released by CORM-A1) and 3) antiplatelet mediators: carbaprostacyclin ($cPGI_2$; acting *via* the cAMP-dependent mechanism) and NO (delivered by PAPA NONOate; acting *via* the cGMP-dependent mechanism). The effects of all tested compounds on platelet aggregation, oxidative phosphorylation, and glycolysis were analyzed, respectively, by light transmission aggregometry and the Seahorse XFe technique. Furthermore, the effects of CORM-A1 on platelet energy metabolism were analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS)-based metabolomics (described previously by Kaczara et al., ATVB, 2020; 40:2376-2390).

The CORM-A1-induced inhibition of platelet aggregation was mediated by the inhibition of both ATP-generation processes, mitochondrial respiration at the level of cytochrome c oxidase, and glycolysis, at the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), attributed to cytosolic NAD^+ depletion. 3PO inhibited platelet aggregation and this effect was associated with reduced oxidative phosphorylation and glycolysis. In turn, neither $cPGI_2$ nor PAPA NONOate at concentrations that substantially inhibited platelet aggregation, did not reduce oxidative phosphorylation or glycolysis. The combination of 3PO, $cPGI_2$ and PAPA NONO at concentrations, at which each of them individually only slightly affected platelet aggregation, allowed platelet aggregation to be substantially reduced, suggesting their synergistic activity.

The results of our study reveal the platelet-specific action of CO on energy metabolism pathways that is not recapitulated by NO or PGI_2. Furthermore, we demonstrated that pharmacological modulation of platelet energy metabolism might increase the antiplatelet efficacy of some other antiplatelet strategies.

Acknowledgments: The National Science Centre Poland [OPUS 2021/41/B/NZ7/01426].

VASCULAR INFLAMMATION INDUCED BY IL-1B MODULATES VASCULAR METABOLISM AND FUNCTION IN THE ISOLATED MURINE AORTA

Agnieszka Karaś[1], Patrycja Kaczara[1], Anna Kurpińska[1], Mariola Olkowicz[1], Anna Bar[1], Stefan Chłopicki[1, 2]

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Vascular inflammation is one of the major drivers of atherosclerosis and cardiovascular diseases and is associated with endothelial dysfunction and arterial stiffness, phenotypes that predict morbidity and mortality. Yet, the metabolic response of the vascular wall cells to proinflammatory activation is still vague, and it remains unclear how it affects vessel function. Most studies in this field tend to focus on the metabolism of the isolated endothelial or vascular smooth muscle cells cultured alone. Such approaches do not take into account metabolic crosstalk between cells in a tissue, and understanding of the influence of metabolic changes on vascular physiology is limited. In this study, we aimed to investigate effects of acute vascular inflammation on energy metabolism of vascular wall in relation to endothelial function.

Studies were performed in young and aged healthy C57BL/6 mice to test whether vascular ageing affect acute vascular metabolic and function response. IL1β was chosen to induce vascular inflammation by *ex vivo* stimulation of the isolated aorta. The influence of acute inflammation on vascular metabolism was studied on functional level using unique approach of analysis of vascular bioenergetics in isolated aortic rings *ex vivo* by Seahorse XFe96 Extracellular Flux Analyzer. To explore in detail the metabolic response to acute inflammation targeted metabolomic analysis in the aortic tissue was performed using LC/MS/MS method. Finally, endothelial function was assessed based on the magnitude of Ach-induced vasodilation using wire myography. The results revealed that IL1β increased basal respiration linked to ATP production and glycolysis and the effect was observed 2 and 24 hours after IL1β. Surprisingly, metabolic activation preceded severe endothelial dysfunction caused by IL1β in young mice that was detected 24 hours but not 2 hours after IL1β. In contrast to young mice, in aged mice IL1β did not induce activation of energy metabolism and endothelial dysfunction induced by IL1β seemed to be blunted. Targeted metabolomic analysis in the aortic tissue revealed robust alterations in concentration of various metabolites suggesting distinct metabolic response to acute inflammation induced by IL1β in young as compared with aged mice including altered concentration of some metabolites of TCA cycle (citrate, malate), lactate and pyruvate, as well as changes in arginine and cysteine metabolism.

In summary, acute vascular inflammation induces activation of vascular energy metabolism and endothelial dysfunction, and these responses were considerably altered in aged mice. Further identification of major metabolic pathway effecting vascular inflammation phenotype might bring novel insight into metabolic mechanisms of inflammaging.

This research was funded in part by The National Science Centre grant PRELUDIUM 2021/41/N/NZ5/03396.

ALTERATIONS OF NAD+ METABOLOMIC FOOTPRINT IN HUNTINGTON'S DISEASE

Marta Tomczyk[1], Małgorzata Presler[1], Agata Jedrzejewska[1], Magdalena Podlacha[2], Daniel Zielonka[3], Oliwia Krol[1], Michal Mielcarek[4], Karolina Pierzynowska[2], Mark Isalan[4], Grzegorz Wegrzyn[2], Ryszard T. Smolenski[1], Ewa M. Slominska[1]

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The role of nicotinamide adenine dinucleotide (NAD+) metabolism is extensively investigated for a role in the development of neurodegenerative disorders such as Huntington's disease (HD). Besides neurodegeneration, HD is accompanied by skeletal muscle atrophy, and cardiomyopathy and currently it is considered a multi-system disorder. Experimental studies related to NAD+ in HD were focused so far on brain metabolism. This work aimed to investigate the transcripts and levels of proteins involved in NAD+ synthesis and consuming pathways as well as NAD+ metabolites degradation in the striatum, heart, and skeletal muscle of the HD experimental mice model. Moreover, to assess the possible usage of disrupted NAD+ metabolism in HD diagnosis, the levels of NAD+ metabolites were measured in the serum of symptomatic HD mice model and plasma and blood of HD patients relative to healthy controls.

Striatum, heart, skeletal muscle, and serum were collected from 28 weeks of age R6/1 mice as well as wild-type littermates (n = 8). Plasma samples from HD patients and control patients (n = 5) were obtained from the Polish center of the European Huntington's Disease Network in Poznan. Transcripts *of Nmrk1*, *Nmnat1*, *Nampt*, *Nt5e*, *Pnp*, *Nmnt*, *Aox1*, *Parp1*, *Sirt1*, *Sirt2*, *Sirt3*, *Nadk2* were measured with RT-PCR. Cardiac and skeletal muscle PARP1, SIRT1, SIRT2, and SIRT3 levels were analyzed with ELISA tests. The liquid chromatography linked to mass spectrometry or HPLC was used to determine mice/patients' serum/plasma/blood NAD+ metabolites concentration.

We noted reduced transcripts of genes involved NAD+ salvage pathway accompanied by reduced *Sirt1*, *Parp1,* and *Nadk* levels in the skeletal muscle of the HD mouse model. In HD-affected hearts, increased nicotinamide degradation (*Nampt*) and heightened *Sirt 1* and *Nadk* transcripts were found. Altered cardiac and skeletal muscle mRNA levels of sirtuins and PARP1 were confirmed with the protein levels via ELISA tests. Analysis of striatal transcripts of genes involved in NAD+ metabolism in HD mouse brain revealed reduced *Nmrk1*, *Nadk* and *Parp1* as well as *Pnp* mRNA levels. However, an increased striatal transcript of *Aox1* was established. Moreover, analysis of HD mouse model serum highlighted raised concentrations of nicotinamide and 4-pyridone-3-carboxamide-1-β-D-ribonucleoside (4PYR). Interestingly, increased 4PYR levels were noted also in HD patients relative to healthy controls, which correlated with HD disease burden and CAG repeat size, and was accompanied by massively elevated erythrocyte concentration of phosphorylated 4PYR metabolite – 4PYTP.

To summarize, disrupted patterns of NAD+ metabolism in HD were reflected by increased serum/plasma concentration of 4PYR in both pre-clinical and clinical settings. Furthermore, 4PYR concentration correlated with HD severity. Thus, monitoring the concentration of this compound in HD might be valuable for the assessment of the disease progression and to validate the effectiveness of HD therapies.

EFFECTS OF COQ10 SUPPLEMENTATION IN STATIN-TREATED PATIENTS

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Statins are commonly used in the therapy of patients with hyperlipidemia. They are competitive inhibitors of 3-hydroxy-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which block the mevalonate pathway, leading to reduced cholesterol levels in the blood. Blocking this pathway at the early stage also decreases other products like ubiquinone (coenzyme Q10). A low level of CoQ10 is postulated as one of the reasons for myalgia, which is a side effect observed in the group of statin-treated patients. The results of the effectiveness of CoQ10 supplementation in statin users as a prevention of myalgia are still contradictory. The main aim of the presented research was an evaluation of the molecular effects of CoQ10 supplementation in statin-treated patients who suffer from myalgia and patients without side effects.

The study group consisted of 26 statin users, 13 of them suffered from myalgia, and the next 13 used statin without noting side effects related to muscles. Both groups were supplemented with CoQ10 for 8 weeks. Metabolic fingerprinting based on LC-QTOF-MS was performed on the plasma samples obtained from patients before CoQ10 supplementation and collected at the end of the intervention.

Univariate nor multivariate analysis did not show significant differences caused by the supplementation of CoQ10 in patients with myalgia. For statin-treated patients without side effects, subtle differences were detected. Only 6 metabolites significantly differentiate plasma metabolic profiles of patients without myalgia before and after CoQ10 supplementation.

Supplementation of CoQ10 does not significantly change the metabolic profile of patients treated with statins. This observation is convergent with many scientific reports and supports the theory that supplementation of CoQ10 does not resolve problems with statin-induced myalgia.

This study is funded by a grant from the National Science Centre, Poland (2020/37/N/NZ4/03903).

MARKERS FOR COVID-19 DISEASE PROGRESSION: COMPREHENSIVE LC-MS CHARACTERIZATION OF THE METABOLOME

Shaufa Sharif[1], Eleanor Matthews[1], Adam King[2], Lisa Reid[2], Mark Towers[2], **Bartosz Rasiński**[3], Steve Fowler[1], Lee Gethings[2], Robert Plumb[2], Angela Simpson[1], Tim Felton[1], Clare Mills[1]

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Over two years since the WHO declared the start of the COVID-19 pandemic, COVID-19 remains a major public health crisis across the globe. Where outbreaks stretch local healthcare services, rapid clinical decision making is vitally important to identify patients most at risk of severe disease. Using an LC-MS metabolomics approach to profile serum from COVID-19 inpatients over the course of their hospital stay, we identify and characterise prognostic biomarkers to support the development of mass spectrometry rapid clinical screening tools. Previous works within the group have identified changes to serum lipid, protein, and metabolite profiles associated with severe allergic reaction, helping to elucidate the underlying immunochemical mechanics. We expect to achieve similar results with regards to COVID-19 progression, and are hoping to characterise immune disfunction as a consequence of SARS-CoV-2 infection with findings potentially applicable to the clinical management of other immune pathologies that are not exclusive to COVID-19.

IDENTIFICATION OF KNOWN AND UNKNOWN COMPOUNDS BY HR LC-MS/MS

Rafał Szewczyk

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Compound identification is one the key aspects of qualitative analysis in targeted and untargeted analytics. High resolution mass spectrometry (HR-MS) has become the major tool for this kind of analysis because of its day-to-day reproducibility, very good mass accuracy, broad linearity and high full scanning speeds achieved in the newest hardware on the market, especially in QTOF instruments. HR-MS data analysis is also strongly supported by software tools. Targeted analysis includes MRMhr or TOF MS scanning while for untargeted analysis data dependent analysis (DDA) or data independent analysis (DIA) are used. With faster scanning and stronger software support DIA slowly becomes a major way for targeted or retrospective quantitation and typical untargeted qualitative analysis. During lecture selected examples of workflows applied to compounds presence confirmation in targeted quantitative analysis and unknown compounds identification will be presented.

Examples of targeted analysis include quantitative forensic analysis of anabolic steroids and psychoactive compounds in urine and blood samples with MRMhr multilevel confirmation of compound presence that includes scoring for mass defect of pseudomolecular ion, isotope distribution, target fragment mass defect, mass spectrum database match and retention time. The benefits of novel electron activated dissociation (EAD) gaining steroids selectivity in targeted analysis will be presented.

For untargeted analysis and unknowns identification DDA and DIA results and workflows will be presented and discussed with the focus on manual and software supported chemical structure proposal, mass spectrum align and result verification. The selected examples include diclofenac metabolites identification, hydroxyzine contaminants identification and loperamide metabolism during 120 h of cell culture.

With unparallel selectivity, sensitivity and scan speed novel HR-MS/MS methods gives accurate results in much shorter analytical methods while software tools strongly supports qualitative data analysis. The benefits of EAD – alternative to standard collision induced dissociation (CID) fragmentations opened completely new level of MS/MS data. Still, the knowledge of fragmentation rules and experience is important in unknown compound identification.

AGE-RELATED MEMORY FORMATION DEFECTS ARE ACCOMPANIED BY THE METABOLIC PROFILE

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Inhibition of glycogen degradation in the brain of young animals has been shown to block memory formation and disrupt the Long Term Potentiation (LTP). Unexpectedly, inhibition of glycogen phosphorylase (Pyg) activity, an enzyme indispensable for glycogen breakdown, significantly improved the LTP induction in hippocampal brain slices of old animals [PMID:26101857]. Based on this we hypothesized that inhibition of Pyg activity using BAY U6751 (Pyg inhibitor) may be used for the improvement of age-associated deficits of memory. To verify this hypothesis, young (1 mth) and old (18–21 mth) mice were treated for two weeks with BAY. To evaluate the long-term general memory formation, we performed a Novel Object Recognition test with a 6h inter-trail interval (ITI). Our results revealed significantly improved memory formation in old mice treated with BAY (OLD+BAY, $p = 0.004$). Analysing the dendrite spines, we found that the BAY influenced their morphology in the hippocampus only. Thus, to measure hippocampus-dependent spatial memory formation we performed the Object Location Test (30' ITI). BAY treated old mice spent significantly more time exploring the familiar displaced object than the familiar non-displaced one ($p = 0.0001$), compared to the control group ($p = 0.42$). Moreover, we also observed a shortening of time to the first exploration in the OLD+BAY group ($p = 0.0001$).

Pyg is an enzyme of basal energy metabolism thus it might have been expected that its inhibition would disturb physiological parameters. However, two-week treatment of animals with BAY had no effect on body mass index and blood glucose level, as well as we didn't observe differences in mice behaviour in the Rotarod and Open-field tests.

The assumption that the astrocyte-neuron metabolic coupling plays a key role in the phenomenon of plasticity of neural networks and the formation of memory traces is quite attractive [PMID: 21376239]. We used the NMR method to investigate the metabolomic fingerprint and find potential biomarkers that estimated the condition and function of nervous tissue at different ages. The frozen tissues were extracted under Folch's procedure. Metabolomic analysis identified 27 metabolites involved in various biochemical pathways (energy metabolism, neurotransmitter metabolism, cell membrane metabolism, and astrocyte-neuron metabolism). The found metabolites were common for all three analyzed regions of the brain (hippocampus, cortex, and cerebellum). The comparison of the experiment groups allowed for the identification metabolites with statistically significant changes in their relative concentrations ($p < 0.05$). Interestingly, we observed decrease signals for γ-aminobutyric acid, glutamate, and aspartate in the old mice group. Those difference are diminished in BAY treated old animals. Clear differences between neurotransmitters, in compared animal groups, may indicate the implications of changes in cognitive abilities and the formation of new memory traces relevant to age.

HIGH MASS AND SPATIAL RESOLUTION IMAGING OF BRAIN LIPIDS USING AP-MALDI-ORBITRAP MS

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Many diseases of the brain including multiple sclerosis share common characteristics of neurodegeneration – sustained changes in organ structure and accumulation of toxic metabolites. Currently, the level/degree of demyelinization is assessed based on total brain myelin or myelin protein staining. The former lacks spatial information while the latter is characterized by high spatial resolution but does not give insight into structural lipid changes or the surrounding healthy tissue. A method allowing for their spatial and semi-quantitative analysis would allow better understanding of brain pathology.

The loss of spatial information during extraction from tissue is a severe limiting factor of traditional liquid chromatography-based lipidomics. Mass spectrometry imaging (MSI) conserves this information and has been previously performed on brain tissue at spatial resolutions of less than 10 µm. Preserving sub-cellular resolution while maintaining lipid annotation count of lower resolution workflows remains a significant and partially unsolved challenge, most often requiring custom-made, one-of equipment with many operational drawbacks.

Herein we present a novel atmospheric-pressure matrix-assisted laser desorption ionization mass spectrometry (AP-MALDI-MS) approach for determining the lipid composition of brain tissue in an untargeted manner using commercially available equipment (MassTech AP-MALDI (ng) UHR and Thermo Scientific Orbitrap Exploris 480). α-Cyano-4-hydroxycinnamic acid matrix was applied to tissue samples with a SunChrom SunCollect sprayer, followed by a 10% isopropyl alcohol recrystallization step. A wild-type mouse coronal *corpus callosum* section of 30 µm thickness was imaged at 20 µm spatial and 240,000 mass resolution. The MetaSpace2020 service was used to annotate lipid species to acquired spectra, resulting in 329 annotations at false discovery rate (FDR) of 20%. The locations of annotated lipid species were compared with standard myelin stainings and matched to appropriate anatomical structures of mouse *corpus callosum* using the Allen Institute Brain Atlas. Acquired myelin lipid spectra were successfully colocalized with this data, revealing a rich and diverse molecular composition. Additionally, a white matter section of a consecutive brain slice was successfully imaged at 5 µm spatial and 240,000 mass resolution, maintaining a very high annotation count of 226 at 20% FDR despite a 16-fold decrease in ablated matrix area per pixel.

The developed workflow for both screening (20 µm resolution) and precise (5 µm resolution) imaging could become a valuable tool for the elucidation of brain disease mechanics. The technique described greatly lowers the barrier for entry into MSI as well as increases the practicality of the method and its speed compared to traditional data acquisition using vacuum-based sources and cyclotron-based mass spectrometry, all while maintaining record-high lipid annotation counts.

DEVELOPMENT AND VALIDATION OF A FAST, SELECTIVE, AND SENSITIVE LC-MS METHOD FOR QUANTIFICATION OF GLYCOGENOLYSIS PHOSPHORYLASE INHIBITOR (BAY U6751) IN MOUSE TISSUES

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Introduction: BAY U6751 hydrate is a noncompetitive inhibitor of glycogen phosphorylase (PYG) – a critical enzyme that catalysis the first step of glycogen breakdown (glycogenolysis). Currently known, that enzymatic defects along this pathway are associated with numerous pathophysiological conditions. Thus, growing interest in the development of potent and specific glycogen phosphorylase inhibitors seems to be justified. The perfect example seems to be the use of glycogen phosphorylase hepatic inhibitors for the discovery of novel antihyperglycemic drugs in type 2 diabetes (Treadway et al., 2001). Beyond the canonical role of glycogenolysis, particularly intriguing is the meaning of glycogen metabolism in the memory paradigm, which was presented in two flagship publications (Suzuki et al., 2011), (Gibbs et al., 2006). The main goal of the current study was to develop a fast, sensitive, and selective LC-MS method, which allows determining the level of BAY in mouse tissues such as the liver, heart, and muscle as well as three regions of the brain: hippocampus, cortex, and cerebellum.

Methods: All experiments were conducted with one-month male mice (C57BL6), which were divided into two groups: mice treated with BAY, and the control group. After scarifying the mice, the tissues were immediately frozen under liquid nitrogen and stored until LC-MS analysis. Sample preparation included homogenized with 1 ml of cold methanol and an internal standard. Next, the supernatant was collected and evaporated. The dried samples were then dissolved in methanol and immediately analyzed by LC-MS.

Results: The linearity in the range of 10 ng/mL–550 ng/mL, had a correlation coefficient 0.9996. The limit of detection (LOD) was 7.76 ng/mL and the limit of quantification (LOQ) was 23.29 ng/mL in tissue matrix samples, respectively. The CV value within run precision and between run precision did not exceed ±15% for QC samples. The extraction recovery was in the range of 89.44%–98.70% for lower, medium, and high concentrations.

Conclusions: In summary, we present the newly developed LC-MS method allowing the analyses of BAY in tissues. The developed method was validated and met all requirements regarding selectivity, carry over, the limit of detection, the limit of quantification, precision, accuracy, stability, extraction recovery, and matrix effect.

COATED BLADE SPRAY MASS SPECTROMETRY AS A USEFUL TOOL FOR RAPID ANALYSIS IN BIOMEDICAL APPLICATIONS

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Mass-spectrometry-based techniques offer high specificity and sensitivity, which has made them a popular choice for analyzing the chemical composition of biological samples. Consequently, by omitting chromatographic separation and time-consuming sample separation step, analysis time using mass spectrometry could be shortened. Due to this solution, results are obtained in a few seconds but at the same time matrix effect may occur resulting in irreproducible data. Mentioned problems can be solved by introducing of sample preparation step that does not compromise the speed of analysis but improves the quality of the results. One such solution is coated blade spray mass spectrometry (CBS-MS).

CBS-MS is one of the solid-phase microextraction methods where probe is sword-like blade coated with chosen sorbent particles. In CBS-MS sampling is conducted similarly to the classical SPME protocol (sampling device is introduced into studied biological material). However, desorption, introduction of extract do the mass spectrometer, and ionization is combined. The probe is positioned in an interface mounted on the mass spectrometer inlet and a drop of desorption solvent is applied on the coated surface. After a few seconds of desorption high voltage is applied to the blade to ionize the compounds and enable their analysis in mass spectrometer.

CBS-MS was widely tested in biomedical applications. For instance, this technology was applied in the quantitative analysis of immunosuppressive drugs, benzodiazepines, and opioids in both, urine and plasma. Moreover, CBS-MS has been tested in pilot studies based on the metabolomic differentiation of different brain tumor types. It was possible to distinguish benign meningiomas and malignant gliomas. Additionally, meningiomas were characterized by lower heterogeneity than gliomas which could be explained by the significantly lower malignancy of these lesions. It was also presented that CBS-MS technology could be a useful tool in the quantitative analysis of a wide range of endogenous analytes, e.g. carnitine.

To sum up, coated blade spray mass spectrometry can be useful in a variety of biomedical studies due to its simplicity and fast analysis. Moreover, its characteristics indicate future applications in on-site as well as in intra-surgical settings.

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TARGETED METABOLOMICS ANALYSIS OF AQUEOUS HUMOR IN PATIENTS UNDERGOING SIMULTANEOUS BILATERAL CATARACT SURGERY

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Background and aims: Although eyes are paired organs, numerous anatomic asymmetries have been described, even in ophthalmically healthy patients. Aqueous humor (AH) is a transparent fluid that fills the anterior and posterior chambers of the eyeball. The aim of our research was to assess the symmetry of concentrations of certain metabolites belonging to various classes in human aqueous humor.

Material and methods: Twenty-three patients qualified for simultaneous bilateral cataract surgery were included in the study. Quantitative analysis of 188 metabolites was performed using commercial AbsoluteIDQ® p180 kit. Liquid chromatography coupled with tandem mass spectrometry was used to perform measurements following the protocol and methodology provided with the kit.

Results: In total, sixty-seven metabolites fulfilled the inclusion criteria. The concentrations the majority of AH metabolites from both eyes of the same patient were not significantly different. The exception was taurine ($p = 0.037$), the concentration of which appeared higher in the right eyes. In contrast, a positive inter-eye correlation was noted between most of the metabolites. However, this correlation was not significant for acylcarnitines (C5:1 and C10:2) and glycerophospholipids (PC aa C32:3, PC aa C40:2 and PC aa C40:5).

Conclusion: With a few exceptions, one eye was determined to be representative of the other eye in terms of the concentrations of analyzed metabolites.

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METABOLIC FINGERPRINTING OF VITREOUS HUMOR FROM PATIENTS WITH EPIRETINAL MEMBRANE OR MACULAR HOLE

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Vitreous humor (VH) is the largest structure of the eye. Since the VH does not have blood vessels, it is nourished by the vessels of the retina and ciliary body. For this reason, in the case of retinal diseases, the composition of the VH may change. The question of how it is changing and the cause of it remains open. An attempt to answer this question may be the examination of the molecular composition of the VH of patients with a given eye disease.

VH can be collected only postmortem or during a vitrectomy procedure. Therefore, it is very difficult to find a control group. Currently, samples from patients with the epiretinal membrane (ERM) or the macular hole (MH) are considered as one control group, despite the different etiology of these diseases. The aim of this study was to compare the metabolome of VH from patients with these two diseases.

Vitreous samples collected from 27 patients (14 with ERM and 13 with MH) were obtained during vitrectomy surgery. Samples were analyzed by LC-QTOF-MS using reversed-phase and HILIC chromatographies.

The conducted study showed slight differences (statistically insignificant after the correction of the *p*-value) in the composition of small molecule compounds between VH from patients with the epiretinal membrane and the macular hole. Before the *p*-value correction, the compounds differentiating VH from patients with both diseases were: octanoylcarnitine, which level was 64% higher in the VH of ERM patients compared to the VH of MH patients, and pipecolic acid, which level was 83% lower in the VH of ERM patients. Elevated levels of octanoylcarnitine may be associated with disturbed β-oxidation of fatty acids in the mitochondria, specifically, with a deficiency of medium-chain acyl-CoA dehydrogenase. Octanoylcarnitine is likely excreted into the VH from myofibroblast cells located in the epiretinal membrane being formed. Pipecolic acid, in turn, can be associated with the kynurenine pathway due to common enzymes with the pipecoline acid pathway in lysine metabolism. There are also reports that it is involved in synaptic transmission or its modulation in GABA synapses. So far, no association of pipecolic acid with retinal center diseases has been found.

The obtained results do not indicate significant differences in the vitreous metabolome of the epiretinal membrane or the macular hole. These illnesses can be treated as one control group.

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METABOLIC PROFILES OF SAMPLES FROM PATIENTS WITH AND WITHOUT ATRIAL FIBRILLATION BASED ON GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Cardiovascular diseases (CVDs) are the leading cause of death worldwide. Despite the increased awareness of society and the increase in the effectiveness of cardiac prevention, CVDs are still a major medical problem consuming considerable financial resources for hospitalization and patient treatment. Atrial fibrillation (AF) is the most common type of arrhythmia, and the incidence and prevalence of AF are increasing globally. Due to the non-specific symptoms, it is often hard to diagnose, which is even more relevant as about one-third of the total population is asymptomatic. Moreover, AF is closely associated with a high risk of ischemic stroke or other thromboembolic events, leading to significant patient morbidity and mortality. However, effective diagnostics and therapeutic approaches are still limited, and the primary method of diagnosing AF is based on electrocardiography (ECG). Recent advances in high-throughput technologies, including metabolomics, will accelerate our understanding of AF. The untargeted metabolomic approach allows studying the unique chemical signature specific cellular processes leave behind. As a global in-scope analysis, metabolomics provides insights into the disease mechanisms and has the potential to generate novel noninvasive diagnostic tests. We deliver the results from an untargeted study designed to explore the molecular signature and disturbed metabolic pathways of arterial fibrillation. Untargeted metabolomics analysis based on gas chromatography-mass spectrometry was performed on plasma samples obtained from patients suffering from arterial fibrillation originating from valvular heart diseases. A cohort of 81 patients was enrolled on the study and divided into a control group without and with observed AF (persistent and paroxysmal). Standard operating procedures have been applied for the metabolite extraction where the samples followed two-step chemical conversion (methoxymation & silylation) and analysis DB5–MS column, GC system, Agilent Technologies 8860 coupled to a mass spectrometer with triple-Axis detector (Agilent Technologies 5977B). Quality control and quality assurance procedure were applied in each step of the workflow to identify and control any source of potential unwanted variation. Spectral deconvolution with Agilent Unknown Analysis software (Ver. B.10.1.Agilent Technologies) was used to extract the acquired data. Assignment of the target ion and the qualifiers, entire batch pre-processing and manual inspection of the data, including peak area and RT integration, was performed with Agilent MassHunter Quantitative Analysis (Ver. B.10.1. Agilent Technologies). Compound identification was performed with the target metabolite Fiehn GC-MS Metabolomics RTL (Retention Time Locked) and the NIST (National Institute of Standards and Technology) mass spectra library. The data matrix was subjected to appropriate univariate tests (parametric or nonparametric) and multivariate statistical analyses, providing a list of significant metabolites. Our data indicate the metabolic pathways altered in patients with atrial fibrillation and could provide deeper insights into the mechanisms of the disease. It may allow for further research for potential specific indicators of pathophysiological conditions for AF early onset and have potential value for improved patient stratification.

BENEFICIAL EFFECTS OF NICOTINAMIDE RIBOSIDE ON NAD+ METABOLOME AND CARDIAC FUNCTION IN HEART FAILURE RATS

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Nicotinamide adenine dinucleotide (NAD+) plays a pivotal role in energy production, oxidative stress control and DNA repair. NAD+ pool tends to decline with ageing, obesity, and hypertension, thus NAD+ precursors might demonstrate a protective effect in diverse pathologies including heart failure. The main purpose of this study was to examine the effects of nicotinamide riboside (NR) on NAD+ related metabolic pattern and to investigate the functional effect in the heart of Spontaneously Hypertensive Rats (SHR) that progressed into heart failure.

To establish the optimal dose, male WKY rats (n = 3) were injected intraperitoneally with NR (250, 350 or 500 mg/kg body weight). The blood was collected before injection and after 6 and 24 h when also hearts were collected. To assess the metabolic and functional effect of 7-day administration of the optimal dose of NR, the erythrocyte and heart concentrations of NAD+ and nicotinamide metabolites and cardiac function were measured in the WKY and SHR, in the treated and untreated groups (n = 8 in each group). Echocardiography was performed using a Vinno6 system equipped with a 21 MHz linear probe. The concentration of nicotinamide (NA) and metabolites: N-methylnicotinamide (MNA), N-methyl-2-pyridone-5-carboxamide (Met-2PY), N-methyl-4-pyridone-3-carboxamide (Met4PY), 4-pyridone-3-carboxamide ribonucleoside (4PYR) were measured by LC/MS. The blood and heart concentrations of NR and NAD+ were measured by RP-HPLC. Results are presented as mean ±SEM and p-value <0.05 (unpaired t-test) was considered significant.

After a single injection, NR persisted longest in blood at a dose of 500 mg/kg: 14.8 and 1.6 µM after 6 and 24 h, respectively, and effectively increased the NAD+ concentration in whole blood (from 544 to 650 µM) and heart (from 2 to 14 nmol/mg). Hence, a 500 mg/kg NR dose was selected for further studies. Such 7-day NR treatment significantly elevated NAD+ concentration in the whole blood (715 ±34 vs. 1143 ±40 µM, $p < 0.01$) and in the heart (2.0 ±0.8 vs. 6.1 ±1.4 nmol/mg) in treated vs. non-treated WKY rats. Moreover, significant increases in Met2PY, Met4PY and MNA concentrations were observed after NR treatment in both WKY (0.32 ±0.06 vs. 5.06 ±0.9 µM, $p < 0.001$, 1.66 ±0.4 vs. 6.46 ±1.5 µM, $p < 0.05$, 0.2 ±0.04 vs. 4.80 ±1.4 µM, $p < 0.05$, respectively) and SHR (0.14 ±0.02 vs. 1.5 ±0.2 µM, $p < 0.001$, 0.11 ±0.02 vs. 0.41 ±0.07 µM, $p < 0.01$, 1.3 ±0.02 vs. 14.4 ±2.8 µM, $p < 0.001$, respectively). Seven days of NR treatment enhanced EF and fractional shortening in the SHR rats vs. the non-treated group ($p < 0.01$).

These studies showed that NR at a daily dose of 500 mg/kg effectively increased NAD+ concentration in the erythrocytes and in the heart, but this beneficial change was associated with complex alterations in NAD+ catabolites concentrations. These metabolic changes were associated with the improvement of cardiac function in hypertension-based heart failure.

ALTERATIONS OF NICOTINAMIDE RELATED METABOLIC PATTERN IN HYPERTENSION AND HEART FAILURE

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Nicotinamide (NA) and its derivatives are involved in cellular energy metabolism and regulation. Alteration in nicotinamide metabolism are linked to diverse pathologies including endothelial dysfunction (ECD) and cancers. To address potential importance of these changes in hypertension and heart failure we examined the blood concentrations of nicotinamide and its metabolites in a Spontaneously Hypertensive Rats (SHR), and established model of hypertension and its progression into heart failure.

Concentrations of nicotinamide (NA) and its metabolites, including: 1-Methylnicotinamide (MNA), N-methyl--2-pyridone-5-carboxamide (Met2PY), N-methyl-4-pyridone-3-carboxamide (Met4PY) and 4-pyridone-3--carboxamide ribonucleoside (4PYR) were measured by liquid chromatography-mass spectrometry (LC-MS/MS) in plasma obtained from venous blood taken from 6-month-old SHR rats (n = 10) with advanced arterial hypertension and initial signs of heart failure and Wistar Kyoto rats (WKY) as a control group (n = 10). Transthoracic echocardiography of the systolic and diastolic function of the heart was performed using Vinno6 ultrasound system with a 21 MHz linear transducer. To measure blood pressure parameters the CODA tail-cuff system was used. Paired Student t-test was used for comparisons between two groups. Results are presented as mean ±SEM.

We observed advanced systolic and diastolic hypertension in SHR rats compared to control WKY rats group. Echocardiographic parameters showed a significant deterioration of the systolic function of the heart in SHR rats-deteriorated Ejection Fraction (EF) and Fractional Shortening (FS). We observed an increase in plasma MNA concentration from 2.189 ±0.281 to 0.481 ±0.056 µmol/L ($p < 0.0001$) and in 4PYR from 0.079 ±0.006 to 0.051 ±0.007 µmol/L ($p < 0.01$) in SHR vs. WKY rats. In turn, the plasma concentrations of breakdown products of MNA: Met2PY and Met4PY decreased in SHR rats vs. WKY (0.068 ±0.010 to 0.116 ±0.015 µmol/L for Met2PY $p < 0.05$ and 0.052 ±0.003 to 1.016 ±0.107 µmol/L for Met4PY $p < 0.0001$).

The results indicate that hypertension progressing into heart failure is associated with altered metabolism of NA with increased plasma concentrations of its initial breakdown metabolites but decreased concentration of products of its further methylation.

METABOLOMIC ANALYSIS OF A PSEUDOMONAS-RESISTANT VERSUS A SUSCEPTIBLE ARABIDOPSIS ACCESSION

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Accessions of one plant species may show significantly different levels of susceptibility to stresses. The *Arabidopsis thaliana* accessions Col-0 and C24 differ significantly in their resistance to the pathogen *Pseudomonas syringae* pv. *tomato* (Pst). To help unravel the underlying mechanisms contributing to this naturally occurring variance in resistance to Pst, we analyzed changes in transcripts and compounds from primary and secondary metabolism of Col-0 and C24 at different time points after infection with Pst. Our results show that the differences in the resistance of Col-0 and C24 mainly involve mechanisms of salicylic-acid-dependent systemic acquired resistance, while responses of jasmonic-acid-dependent mechanisms are shared between the two accessions. In addition, arginine metabolism, and differential activity of the biosynthesis pathways of aliphatic glucosinolates and indole glucosinolates may also contribute to the resistance. Thus, this study highlights the difference in the defense response strategies utilized by different genotypes.

ASSESSING THE PENETRATION OF RED CABBAGE AND ARONIA ANTHOCYANINS AND THEIR PHASE II METABOLITES THROUGH THE BLOOD-CSF BARRIER: A SHEEP MODEL STUDY

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Anthocyanins are a class of flavonoids that are found in a wide variety of fruits and vegetables. They have been the subject of recent research due to their potential neuroprotective effects. Studies have shown that anthocyanins may have the ability to improve cognitive function, reduce inflammation, and protect against neurodegenerative diseases such as Alzheimer's and Parkinson's. These beneficial effects are thought to be due to the antioxidant and anti-inflammatory properties of anthocyanins. Despite promising research, more studies are needed to fully understand the mechanisms by which anthocyanins protect against neurodegeneration and to determine their potential as a treatment for these diseases.

Therefore, the aim of this study was to investigate the ability of anthocyanins and their phase II metabolites to penetrate the blood-cerebrospinal fluid barrier in sheep. The study analyzed changes in the profile and concentration of these compounds in physiological fluids (blood plasma, urine, and cerebrospinal fluid (CSF)) of sheep after intraruminal administration of chokeberry (1), and red cabbage (2), and after intravenous administration a mixture of cyanidin 3-galactoside and cyanidin 3-diglucoside 5-glucoside (3).

Our study show that chokeberry and red cabbage are a rich source of anthocyanins. The basic structure of anthocyanins found in these two plant is cyanidin in the glycoside form. The main cyanidin derivative found in chokeberry is 3-galactoside of cyanidin, while the basic structure of red cabbage anthocyanins is 3-diglucoside-5-glucoside of cyanidin. What is more, the study has shown that chokeberry and red cabbage anthocyanins are absorbed and appear in the physiological fluids of these animals in their native form and in the form of their phase II metabolites (glucuronated, methylated, sulfated and combined derivatives). We observed that changes in the profile and concentration of anthocyanins and their phase II metabolites in the CSF result from fluctuations in the concentrations of these pigments in the blood, their different ability to penetrate the blood-CSF barrier, and metabolic changes in the cells of the CSF blood barrier during penetration.

In conclusion, chokeberry and red cabbage are unique matrices that enable the study of the relationship between the presence of various glycosidic substituents and the occurrence of mono – and diacylation in anthocyanin structures and the processes that occur in the body of sheep after administrating the preparation of these plants. Research has shown that these anthocyanins are absorbed and penetrate the blood-CSF barrier in sheep, and that the blood-CSF barrier is selective, depending on the type and number of substituents and the size of the molecule. The accumulation of native anthocyanins and their phase II metabolites within the CSF may potentially exert a positive influence on the processes transpiring within the central nervous system environment, owing to the strong antioxidant properties of these compounds. This is particularly important as the central nervous system is highly susceptible to oxidative damage.

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY AS A TOOL FOR TRACKING ROASTING-INDUCED CHANGES IN THE VOLATILOME OF COLD-PRESSED RAPESEED OIL

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The aim of this study was to track changes in the volatilome of cold-pressed oil and press cakes obtained from roasted seeds to understand pathways of volatile organic compound (VOC) formation caused by thermal processing. Comprehensive two-dimensional gas chromatography-time of flight mass spectrometry was used for the analysis of VOCs in cold-pressed oils and corresponding press cakes obtained after roasting of seeds at 140 and 180 °C before pressing. After roasting, the formation of volatile products of Maillard reaction increased, which is typical for thermal processing. Moreover, levels of the products of thermal oxidation of fatty acids, such as aldehydes and ketones, increased with the increasing temperature of roasting. Among sulphur-containing compounds, contents of the products and intermediates of methionine Strecker degradation increased significantly with the increasing temperature of roasting. Degradation of glucosinolates to nitriles occurred after thermal treatment. The results of this study confirmed that seed roasting before cold pressing has a significant effect on the volatilomes of oil and press cake obtained from rapeseed. Notably, the significant changes were observed between the two studied temperatures and the profile of VOCs of oil obtained after roasting at 140 °C was more similar to control oil than roasted at 180 °C.

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EFFECT OF DNA METHYLATION INHIBITOR – ZEBULARINE ON THE *SALIX PURPUREA* L. METABOLOME

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DNA methylation is one of the major regulatory systems in gene expression and is known as an epigenetic mechanism in living organisms. The transfer of the methyl group to cytosine is able to modify the activity of DNA segments, that results in expression silencing. The whole level of DNA methylation oscillates between 6 and 30% in higher plants, depending on tissue as well as on development stage, and has an important role in the plant tolerance of several stress factors [1]. Hence, the expression of several genes involved in biosynthetic pathways are also silenced by DNA-methylation [2]. This process causes inhibition of metabolic biosynthesis and decreases their accumulation. However, some compounds like zebularine or 5-azacytidine work as inhibitors of DNA-methylation and unblock the expression of genes. This strategy is used as a universal tool for epigenomic research and has been applied by us to demonstrate the effect of methylation on accumulation of metabolites in tissues of *Salix purpurea* (purple willow).

S. purpurea is an important source of biomass for energy production, a source of medicine and a tool for soil remediation. Willow accumulates many groups of metabolites, including primary (aminoacids, carbohydrates, organic acids, etc.) and secondary metabolites (phenylpropanoids, benozoates, terpenes, etc.). According to salicin occurrence, this plant is used as an anti-inflammatory drug [3]. The inhibition of DNA methylation could indicate the groups of metabolites and metabolic pathways under epigenetic control in purple willow. In the experiment, zebularine was applied as an anti-methylation agent on a *S. purpurea* plant cultivated in *in vitro* culture. The organs (internodes, roots) and tissues (xylem and bark) were collected one and two months after zebularine application. Plant tissues were homogenized and extracted using 80% methanol. The metabolite profiling was performed using the UPLC-HESI-QExactive-MS system in both ion modes. Collected data (FullMS-ddMS2) was processed using MSDIAL software, filtrated using the MSCleanR package and annotated using MSFinder software.

The multivariant and univariant analysis shows the differences between control and treated samples in all types of organs and tissues. The obtained results suggest that zebularine effectively changes the metabolomic profiles, including several secondary metabolites. However, future analysis like total methylation level, RNA-Seq, WGBS-Seq and their integration with metabolomics data may show the exact impact of the DNA-methylation on the metabolic pathways.

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BACTERIAL METABOLITE ISOLATION AND QUANTIFICATION FROM CLINICAL *E. COLI* CELL CULTURE – CHALLENGES IN SAMPLE PREPARATION OF UTI AND UROSEPSIS STRAINS

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Sepsis is one of the causes of morbidity and mortality in hospitals. It is estimated that about a quarter of all sepsis cases is due to urinary tract infection (UTI). Prompt administration of the appropriate drug (antibiotic) targeting the correct causative agent (bacterium) increases the chance of patient survival. The Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine established a sepsis council and in 2016 a new definition of sepsis was published, calling the guidelines SEPSIS-3. According to the current definition, "sepsis is a life-threatening organ dysfunction caused by an abnormal (deregulated) response of the body to infection (bacterial, fungal, viral)." It is estimated that there are about 49 million cases of sepsis per year, with a mortality rate exceeding 23%, which is 20% of all deaths worldwide. Due to its widespread prevalence, high mortality and costs related to both treatment and subsequent consequences of sepsis, it is both a medical and economic problem on a global scale. Bacterial infections, especially those related to the urinary tract, are the most common cause of sepsis. *Escherichia coli* is most often responsible for urosepsis, accounting for as much as 52% of cases, followed by bacteria belonging to *Proteus spp*. *Enterobacter spp*., *Klebsiella spp*., and *P. aeruginosa*.

Isolation of metabolites form cell culture (which could cause the urinary tract infection or urosepsis) is challenging: (i) the generation time of bacteria is around 20 minutes (all handling should be done in less than 20 minutes), (ii) the shock in temperature or environment will have impact on metabolite levels for *E. coli* clinical strain culture (clinical strains have different growth conditions, temperature oxygenation and others), (iii) the cell growth condition very often requires the full medium like urine. In literature we can find, that the first step when isolating the bacteria should be the metabolism quenching, this should stop the immediate changes during liquid handling. Nevertheless, the problem raises when we want to analyze intra- and internal-metabolites. With use of minimal medium, we have prepared over 100 cell cultures form different clinical *E. coli* strains, which were centrifugated (10 min, room temperature, 4000 × g) to obtain the cell pellet and supernatant. The cell pellet metabolic activity was quenched by using cold methanol solution and vortexed. Then the supernatant and inactivated pellet were freeze dried, the metabolites were extracted by methanol/ethanol (1/1, v,v) extraction and centrifugated. Resulted supernatants were concentrated and resuspended in (0.1% formic acid, H_2O, Acetonitrile – 2% solution). The resulting metabolite solution was then suspected to LC-MS analysis with use of nanoLC (Dionex Ultimate 3000, Thermo Fisher Scientific) equipped with 0.075 × 5 mm C18 precolumn and 0.075 × 100 analytical PepMap Aclaim column). The LC apparatus was connected with ESI-qTOF (Sciex 6600+, apparatus set for Information depended analysis).

Obtained data were analyzed against NIST and All-in-one Sciex database to identify the metabolites, then the MultiQuant Software was used to quantify them. The statistical analysis was performed with the help of MetaboAnalyst and Mathematica software. We have identified around 500 metabolites, from which more than 100 were statistically significant among two groups UTI and urosepsis.

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IDENTIFICATION OF BIOSURFACTANTS PRODUCED IN MICROBIAL FUEL CELLS USING LC-MS/MS METHOD

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Microbial fuel cell (MFC) is one of the most common bioelectrochemical system that converts energy from organic compounds into electrical energy. This process is possible due to the activity of microorganisms called electricigens, which carry out the oxidation reactions in the anodic compartment and thus, generate electrons and protons. As we have demonstrated in our previous works, some bacteria are also capable of synthesizing biosurfactants that facilitate the degradation of hydrophobic compounds within MFC system.

The aim of this study was to identify the biosurfactants produced by previously enriched, mixed microbial consortia in microbial fuel cells, operating under current-producing conditions. The anolyte consisted of mineral salt medium with waste vegetable oil as a carbon source. The production of biosurfactants was assessed by measuring surface tension over time, evaluating foaming ability and emulsification index. Biosurfactants were extracted from the medium using Solid Phase Extraction (SPE) approach. Then, obtained extracts were analyzed using LC-MS/MS technique. As a result, ten different chemical compounds were identified. They represent a mixture of mono – and dirhamnolipids, which belong to the glycolipid group of biosurfactants. Therefore, the SPE approach coupled with LC-MS/MS analysis provided a satisfactory results for characterize biosurfactants produced in microbial fuel cells. The results of the analysis provided the confirmation the presence of biosurfactants in the anode chamber and their chemical composition. It is therefore possible to produce rhamnolipids under current-generating conditions by using waste vegetable oil as a fuel in microbial fuel cells.

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METABOLIC RESPONSE OF *ACINETOBACTER BAUMANNII* FOR APDT MULTIPLE PHOTOSENSITIZATION

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Acinetobacter baumannii is a saprophytic bacterium, widely distributed in the natural environment (water, soil, sewage and skin of humans and animals). Today this coccobacillus is one of the major pathogens responsible for causing nosocomial infections, especially in intensive care units (ICUs) worldwide. Infections with these bacteria in hospital settings result in a mortality of 26%, which increases to as much as 43% in intensive care units5. It has been confirmed that *A. baumannii* is resistant to many classes of antibiotics and disinfectants due to chromosome-mediated genetic elements and may persist for a long time under harsh conditions.

One of the methods of effective pathogen inactivation that has gained great interest in many medical communities is antimicrobial photodynamic therapy (aPDT). This treatment has a significant advantage over existing antimicrobial therapies as it is faster compared to other antimicrobials, equally effective in killing both multidrug resistant and non-resistant pathogens, and eliminates the secreted virulence factors. One of the important advantages of this antimicrobial technique is that it can be applied topically to avoid systemic side effects.

^{1}H NMR analysis allowed the identification of metabolites that are affected by oxidative stress induced by antimicrobial photodynamic therapy. All assignments were verified using the following databases (KEGG Pathways, PubChem). For 35 identified metabolites, statistical and multivariate data analyses were performed. VIP scores were calculated for each compound based on NMR signal relative intensities to assess whether the compound was more/less present in the samples after aPDI. VIP scores indicate the relative influence of the corresponding metabolites on the discrimination between the two conditions: (1) before and after sub-lethal phototherapies, (2) after ten and fifteen sub-lethal aPDTs; (3) before and after lethal phototherapies, (4) after ten and fifteen lethal aPDTs.

Most of the identified VIP metabolites were amino acids, their derivatives or precursors. The multivariate data analysis clearly showed the natural grouping of samples.

Understanding the metabolic changes induced by multiple photosensitization of *A. baumannii* and the cell defense systems remains an open question, requiring further analysis of complex metabolic pathways, also at the level of gene expression.

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METABOLOMICS IN CLINICAL PERSPECTIVE – ANALYSIS OF GASTROINTESTINAL STROMAL TUMOUR SAMPLES

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Imatinib is a well-established first-line drug for treating most gastrointestinal stromal tumours (GIST). However, GISTs acquire secondary resistance during therapy. Multi-omics approaches provide an integrated perspective to empower the development of personalised treatments through a better understanding of functional biology underlying the disease and molecular-driven selection of the best-targeted individualised therapy. In this study, we applied integrative metabolomic and transcriptomic analyses to elucidate tumour biochemical processes affected by imatinib treatment.

A GIST xenograft mouse model was used in the study, including ten mice treated with imatinib and ten non-treated controls. Metabolites in tumour extracts were analysed using gas chromatography coupled with mass spectrometry (GC-MS). RNA sequencing was also performed on the sample subset

Metabolomic analysis revealed 21 differentiating metabolites, whereas next-generation RNA sequencing data analysis resulted in 531 differentially expressed genes. Imatinib significantly changed the profile of metabolites associated mainly with purine and pyrimidine metabolism, butanoate metabolism, alanine, aspartate, and glutamate metabolism. The related changes in transcriptomic profiles included genes involved in kinase activity and immune responses and supported its impact on the purine biosynthesis pathway.

Our multi-omics study confirmed previously known pathways involved in imatinib anticancer activity and correlated imatinib-relevant downregulation of expression of purine biosynthesis pathway genes with the reduction of respectful metabolites. Furthermore, considering the importance of the purine biosynthesis pathway for cancer proliferation, we identified a potentially novel mechanism for the anti-tumour activity of imatinib. Based on the results, we hypothesise metabolic modulations aiming at reducing purine and pyrimidine pool may ensure higher imatinib efficacy or re-sensitise imatinib-resistant tumours.

METABOLOMIC PROFILING OF SERUM EXOSOMES IN CANCER PATIENTS TREATED BY RADIOTHERAPY

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Here we were looking for molecular components related to the progression of cancer and the response to treatment, with particular emphasis on the participation of exosomes modulating these processes. We applied an MS-based metabolomic approach to reveal exosome components in serum samples from patients diagnosed with head and neck cancer and locally advanced rectal cancer treated with ionizing radiation. We have shown that the metabolomic profile of exosomes isolated from serum in relation to whole serum is different in head and neck cancer after radiotherapy. Moreover, we revealed that the molecular composition of exosomes isolated from serum can be used to predict the response to neoadjuvant radiotherapy in rectal cancer.

This study was supported by the National Science Centre, Poland, Grant 2017/26/D/NZ2/00964.

URINE LIPID MEDIATOR METABOLITES ASSOCIATES WITH ENDOMETRIAL CANCER

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Rationale: Endometrial cancer (EC) is one of the most common cancers in the female reproductive system with 400 000 cases diagnosed world-wide in 2020 (Global Cancer Observatory). Available EC diagnostic procedures include intrauterine ultrasound, endometrial biopsy, and hysteroscopy. However, no available EC screening tests are based on measurement of non-invasive diagnostic markers (e.g., found in blood or urine samples).

Aim: Our study aimed to quantify 17 urinary eicosanoid metabolites from arachidonic acid by LC-MS/MS in healthy and EC women to find discriminatory metabolites which potentially may serve as new non-invasive biomarkers of EC.

From the chosen panel of quantified lipid mediators some are well characterized as potent inflammatory mediators and others have demonstrated bioactivity in various cancers, such as prostaglandins, thromboxanes and leukotrienes, but also from the less known isoprostanes. To the best of our knowledge, this is the first study reporting the levels of a broad panel of urinary metabolites in EC patients.

Methods: The study was performed on urine samples collected from 55 EC patients and 52 healthy volunteers (HV), age range 29–89 years. Lipid mediators were extracted by solid phase extraction (ABN Evolute, 30 mg, Biotage, Uppsala) in 96-well positive pressure format. Redissolved extracts were filtered and injected onto a Xevo TQ-XS triple quadrupole mass spectrometer (Waters, Milford, MA) operated in negative electrospray ionization mode (ESI). Lipid concentrations were normalized against specific gravity (UG-α; Atago Co., Ltd.). Non-parametric Mann–Whitney U-test was applied, and p-values were FDR-corrected.

Results: We found statistically significant differences in concentrations of 11 urinary metabolites comparing HV vs EC. The majority of lipids were increased in EC, with the greatest elevation observed for 13,14-dihydro-15-ketoPGF$_{2\alpha}$ metabolite from the PGF$_{2\alpha}$ pathway (311% change, *p*-value = 2.94E-10) and for 8-iso-PGF$_{2\alpha}$ from isoprostanes pathway (149% change, *p*-value = 3.60E-7). Two out of three metabolites from the PGD$_2$ pathway were not different between the groups. Interestingly, LTE$_4$ was increased by 62% (*p*-value = 2.14E-03) in EC group compared to HV group.

Conclusion: The observed increase in several lipid mediator concentrations in EC patients highlight the importance of arachidonic acid metabolism in cancer and suggest prostaglandins and leukotrienes as new potential non-invasive diagnostic markers of endometrial cancer. These results encourage further research to better understand association between their excretion levels and disease state and/or disease progression.

4D-MULTIOMICS – NEXT DIMENSION BRINGS RESEARCH TO A NEW LEVEL

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Over the last years, metabolomics has continued to grow rapidly and is considered a vital approach in envisaging and elucidating complex phenotypes in systems biology area. The advantage of metabolomics compared to other omics applications such as proteomics and transcriptomics is that these later omics only consider the intermediate steps in the central dogma pathway.

Small molecule analyses in metabolomics, applied research and pharma often face similar challenges. Analysis of complex samples for detection, identification, and semi-quantification of known and unknown analytes across a wide mass and dynamic range. Recent advances in high resolution LC-MS in combination with trapped ion mobility separation (LC-TIMS-MS) address the needs for rapid and confident identifications. TIMS capabilities to separate coeluting isobaric and isomeric analytes and collisional cross sections (CCS) can increase confidence in compound identification. The TIMS enabled PASEF provides extensive MS/MS precursor coverage in single injections.

Here I present the principle of timsTOF, PASEF technology multiomics workflow for data acquisition and processing, CCS values reproducibility, high dynamic range due to the clean MS/MS data, CCS-Predict Pro as a powerful tool for identification of unknown metabolites among other.

PHOSPHOCHOLINES OXIDATION IN PATIENTS WITH NON-SMALL-CELL LUNG CANCER

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Without a doubt, cancer is one of the greatest health problems and challenges facing medicine today, with lung cancer being one of the most frequently diagnosed. The high number of new cases, lack of effective diagnosis, and difficult treatment underscore the need for investigating metabolic changes in the development and progression of lung cancer.

Previous studies, including our own, have indicated profound changes in the lipid profile of non-small cell lung cancer (NSCLC) patients. Given that oxidative stress plays a critical role in the development of lung cancer, we chose to investigate changes in oxidized phosphocholines (oxPCs). Although historically, oxPCs were believed to be toxic by-products of oxidation, recent research has revealed them to be essential signalling molecules with multiple, often pleiotropic functions.

We conducted an extensive, multi-level metabolomics analysis of plasma and lung tissue samples, including 250 NSCLC patients and 50 controls with chronic obstructive pulmonary disease (COPD). Four types of LC-MS analyses were performed. Additionally, NSCLC patients were grouped into subtypes of adenocarcinoma (ADC) and squamous cell carcinoma (SCC).

Our analysis found that the predominant forms of oxPCs present in plasma samples were long-chain oxPCs (LCh-oxPCs), while short-chain oxPCs (SCh-oxPCs) were primarily found in lung tissue samples. There was an overall increase in the level of oxPCs in both plasma and tissue samples, but the differences were more pronounced in plasma samples. Exploration of the level of oxPCs in various parts of the tumour revealed their accumulation in the central part, probably caused by non-enzymatic ROS-induced oxidation as a result of poor blood vessel formation within the tumour. Additionally, the observed increase was higher in ADC patients than in SCC patients. Further exploration of oxPC levels in ADC and SCC patients revealed an intriguing link between the subtypes of NSCLC and inflammation and oxidation, which appeared to differ between the subtypes. Our findings provide new insights and underscore the need for further research in this area.

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GAS CHROMATOGRAPHY MASS SPECTROMETRY-BASED UNTARGETED METABOLOMICS OF PLASMA FROM PAPILLARY THYROID CANCER PATIENTS

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Papillary thyroid cancer (PTC) is the most common pathological type of thyroid cancer worldwide, accounting for 80–85% of all cases. PTC is diagnosed based on histological examination; however, in some cases, this tool is insufficient. Thus, additional predictors/methods are needed for a more accurate diagnosis. Numerous studies have shown that metabolomics approach may be helpful in understanding pathogenesis of cancer. To gain knowledge about the metabolic changes leading to the development of PTC, we conducted a study using gas chromatography coupled with mass spectrometry (GC-MS) to identify altered metabolites in plasma samples from patients with PTC.

Plasma profiles of 78 patients with PTC and 43 healthy individuals were compared. A total of 78 annotated metabolites were subjected to statistical analysis. Univariate statistic (Mann–Whitney U test) and receiver operating characteristic (ROC) analysis were applied, using MetaboAnalyst 5.0. In total, 16 metabolites involved in various biochemical pathways, etc. amino acid metabolisms, glyoxylate and dicarboxylate metabolism and metabolites related to the tricarboxylic acid cycle were found statistically significant. Interestingly, obtained results showed an increase in the intensity of palmitic, glyceric and glutamic acid (fold change (FC) in the range of 1.25–3.57) and decreased intensity of pyruvic, threonic and hippuric acid (FC range 0.02–0.58) in patients with PTC. Finally, to evaluate the potential of significant metabolites to serve as biomarkers indicating PTC development, ROC curves were obtained. Seven metabolites showed the best discriminatory power to distinguish PTC patients from healthy controls (AUC = 0.995; Cl = 0.985-1).

This preliminary work contributes to existing knowledge of PTC metabolism by providing evidence on a distinctive metabolic profile of plasma of PTC patients. To confirm a diagnostic value of obtained results future targeted metabolomics studies are needed. The proposed panel of metabolites could be helpful in the diagnosis of PTC.

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EFFECT OF ZINC AND POLYPHENOLS CO-SUPPLEMENTATION ON THE DEVELOPMENT OF BREAST CANCER AND URINARY LIPIDOMIC PROFILE IN RATS

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The aim of the study was to evaluate the effect of selected polyphenolic compounds: epicatechin, apigenin and naringenin, administered separately or in combination with zinc (Zn), on the growth and development of the neoplastic process induced by 7,12-dimethylbenz[a]anthracene (DMBA) in rats. The impact of supplementation with the above-mentioned compounds on urinary lipid profile in rats with mammary cancer was also assessed.

Female Spraque-Dawley rats divided into 7 groups were used in the study: animals without supplementation and supplemented with apigenin, epicatechin and naringenin separately or in combination with zinc. To induce the mammary cancer, rats were treated with DMBA. Lipidome was analyzed using ESI-FT-ICR-MS (ultra-high resolution Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry with an electrospray ion source, SolariX 2xR 7T, Bruker). The collected mass spectra were analyzed in the MetaboScape 5.0 software (Bruker) using the T-Rex 2D algorithm (MRMS single spectra) and the identified compounds were assigned to specific signaling pathways using LipidMaps and the KEGG database.

Based on the obtained results it can be said that supplementation of the animals with naringenin inhibits the development and progression of neoplastic process in rats treated with 7,12-dimethylbenzanthracene. Neoplastic tumors were found in only 2 of 8 rats (incidence: 25%) and were considered by at most grade 1 malignancy. The first palpable tumors in the group of animals receiving naringenin appeared at week two-three weeks later when comparing to other groups. The combination of zinc with flavonoids (apigenin, epicatechin and naringenin) seems to stimulate the process of carcinogenesis. Coming to urinary lipid profile, in untargeted lipidomic analysis in group supplemented with apigenin we observed only 1 up-regulated lipid compound, and 53 down-regulated in comparison to control group. While for group supplemented with apigenin and zinc – 15 up-regulated and 10 down-regulated lipid compounds comparing to standard. In group supplemented with epicatechin we observed 26 up-regulated lipid compound, and 23 down-regulated in comparison to control group. While for group supplemented with epicatechin and zinc – 22 up-regulated and 56 down-regulated lipid compounds comparing to standard. In case of naringenin we identified 15 up-regulated and 45 down-regulated lipid compounds comparing to standard. Whereas in group supplemented with naringenin and zinc: 10 up-regulated and 45 down-regulated lipids in comparison to control group.

In conclusion, supplementation of rats with selected flavonoids administered separately or in combination with Zn has impact on the development of neoplasms and altered the urinary lipid profile of rats with breast cancer. For sure further studies are more than welcome in this field.

METABOLOMIC RESEARCH PLATFORMS AND SOLUTIONS FROM SHIMADZU. FROM RAPID AND REPRODUCIBLE BLOOD PLASMA COLLECTION TO MULTI-OMICS HRAM/IMS PLATFORM

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Recent years in the biological sciences are not characterized by a lack of ready-made applications served by equipment vendors. It's the opposite situation. Scientists are literally flooded with too many application notes. However, there is a lack of reliable and complete packages of ready-made methods and workflows in the field of metabolomics, which are offered by Shimadzu, e.g. method package for reactive sulfur profiling, for lipid mediators, phospholipids, bile acids, primary metabolites etc.

With high speed data acquisition capability we can do more with our data analysis, acquiring high mass accuracy MS and MS/MS with fast cycle times. By bringing together a high sensitivity QTOF capable of acquiring up to 200 spectra per second without a marked loss of ion transmission we can acquire DIA mass scans at high scan speeds (typically 20 msecs. for a DIA-MS/MS mass scan) for a MS/MS mass range of m/z 50-1000.

Modern HRAM spectrometers equipped with an optical microscope and MALDI source with the possibility of tissue imaging (IMS) allow to go to the unknown level of metabolomics research. Using IMScope QT from Shimadzu it is possible, e.g., to find molecules unique to NASH (Non-Alcoholic Steatohepatitis) tissue. Using statistical methods (IMAGEREVEAL MS) and comparing the average spectra of the ROIs, it is possible to find molecules that are causing the differences between the ROIs. In the next step finding molecules with similar distributions to the stained image and finally creating an MS image showing the concentration distribution of the target molecule.

METABOLOMIC STATUS OF BY2 CELLS ADAPTED TO LONG-TERM OSMOTIC STRESS

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Over the coming decades, water shortages and osmotic stress will likely have growing adverse effects on plant cell functioning. Surprisingly, despite the response of plant cells to abiotic stress conditions being well studied, we know little about the molecular rearrangements that allow cells to adapt to chronic adverse conditions.

Here we present data on tobacco BY2 suspension cells adapted to osmotic stress in 2006: a perfect and unique model for investigating the molecular background of adaptation processes. The adapted BY2 cells are smaller than those living under normal conditions, similar to many plants living under chronic stress. It has been postulated that this decrease in mass and size is a "cost" of the stress response; plant cells that cannot overcome this cost, die, whereas those that can, develop a state of "new molecular homeostasis". Despite this assumption being widely accepted, the molecular basis for the smaller size of plant cells adapted to long-term stress remains unclear.

We utilized an untargeted GC/MS analysis to study four BY2 lines adapted to various stress conditions (450 mM mannitol/sorbitol and 190 mM NaCl/KCl) and controls. The high-throughput metabolome study was supplemented with mitochondria structure analysis and NGS mRNA study focused on mitEC changes as well as with biochemical examination of MDA, radical levels, etc.

The abundance of numerous core metabolites was unchanged in adapted cells compared to controls suggesting the "stable state" of essential cellular molecular pathways. Nevertheless, the clear signals in cells' adaptation to osmotic stress were visible with significantly up-regulated proline or sorbitol. Moreover, some signs of the different molecular states of years-adapted cells compared to known data on shorter stress exposition were found. For example, the increased level of MDA was not correlated with radical up-regulation, which suggests the existence of some unknown defense mechanisms of cells exposed to years-long stress. The number of differently abundant metabolites was higher in BY2 lines adapted to salt (osmotic) stress compared to Mannitol/sorbitol exposed BY2.

The source of energy for the biosynthesis of stress-related compounds remained not fully recognized in adapted cell lines, as the energy-related genes/metabolites levels were generally unchanged in adapted all BY2 lines. However, signals of increased networking of mitochondria were found.

As a conclusion it is stated that increased levels of compounds enabled cells to function in higher osmoticum found in adapted cells coexisting with symptoms of new molecular homeostasis, in the form of a relatively unchanged core metabolic pathway, together with signals of unknown protective mechanisms of plant cells.

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MODIFIED GLUCOSE AS A SENSOR TO TRACK THE METABOLISM OF INDIVIDUAL LIVING ENDOTHELIAL CELLS – OBSERVATION OF THE "RAMAN SPECTROSCOPIC SIGNATURE OF LIFE"

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Endothelial cells (ECs) play a significant role in a healthy and dysfunctional state, the latter is correlated with a range of lifestyle diseases, such as cardiovascular disorders or some cancers. Therefore, knowledge of the dysfunctional state, cellular metabolism, and especially glucose metabolism, is important from the point of view of studying disease mechanisms. The metabolism and glucose uptake may reflect the physiopathological conditions and cell activity correlated with energy metabolism. Our method of choice for studying changes at the subcellular level is Raman microscopy to investigate the metabolism and tracking of glucose uptake. The study was focused on a modified glucose analogue, 3-O-propargyl-D-glucose (3-OPG), which gives a characteristic and intense Raman band at 2124 cm^{-1} in both normal and inflamed ECs. So, the alkyne-labeled glucose, 3-OPG, was used as a sensor to track its accumulation and then metabolism in live and fixed ECs by employing two spectroscopic techniques, i.e., spontaneous Raman and stimulated Raman scattering microscopies. The results indicate that 3-OPG is suitable as a sensor for fixed and live cells in normal as well as in inflammatory conditions to follow the glucose metabolism, by analyzing the Raman band of 1602 cm^{-1} attributed to the glucose metabolites, and the 2124 cm^{-1} Raman band of glucose accumulation. Additionally, we have shown that glucose metabolism and its uptake are slowed down in inflammation. Gaining further knowledge about metabolic patterns in the endothelium, and especially in the various pathologies affecting ECs, should open up valuable opportunities for entirely new medical treatments.

This work was supported by a grant from the National Science Center Poland (NCN) (OPUS15 no. UMO-2018/29/B/ST4/00335 to Malgorzata Baranska) and by the Visibility&Mobility module under the program "Excellence Initiative – Research University" at the Jagiellonian University in Krakow.

LOCAL TRANSLATION IN THE SYNAPSE – PROTEOMIC ANALYSIS

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Synapses are the regions of the neuron that enable the transmission and propagation of action potentials at the cost of high energy consumption and elevated demand for mitochondrial ATP production. The rapid changes in local energetic requirements at dendritic spines imply mitochondria's role in maintaining their homeostasis. Using isobaric (TMT, iTRAQ) and label-free (LFQ) quantification approach proteomic analysis supported with complementary experimental approaches, we show that an essential pool of mitochondrial proteins is locally produced at the synapse, indicating that mitochondrial protein biogenesis takes place locally to maintain functional mitochondria in axons and dendrites. Furthermore, we show that stimulation of synaptoneurosomes induces the local synthesis of mitochondrial proteins that are transported to the mitochondria and incorporated into the protein supercomplexes of the respiratory chain. Importantly, in a mouse model of fragile X syndrome, *Fmr1* KO mice, a common disease associated with dysregulation of synaptic protein synthesis, we observed altered morphology and respiration rates of synaptic mitochondria. That indicates that the local production of mitochondrial proteins plays an essential role in synaptic functions. We also used surface labeling of synaptoneurosomes to confirm it receptors are recruited to the synaptic membrane.

METABOLOMICS ANALYSIS OF THE MITOCHONDRIAL METABOLISM AFTER HYPOXIA AND HIF KNOCKDOWN

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Aerobic eukaryotes require a steady oxygen (O_2) supply to sustain the production of adenosine triphosphate (ATP) in the mitochondrial respiration chain. Consecutively, ATP plays an important role as a carrier of energy for the numerous biochemical reactions of the cell metabolism. Evolutionarily, however, aerobic eukaryotes have adapted their cell metabolism to overcome periods of hypoxia. An example of such an adaptive mechanism in human cells would be the hypoxia-inducible factors (HIFs) [1].

This experiment aimed to examine how the mitochondrial metabolomic fingerprint changes after exposure of human umbilical vein endothelial cells (HUVEC) to hypoxic conditions. In addition to that, small interfering RNAs (siRNAs) were used to knock down the hypoxia-inducible factor 1 (HIF1) and the endothelial PAS domain protein 1 (EPAS1) genes to see how the mitochondrial metabolic fingerprint is affected.

HUVEC cells were transfected using Lipofectamine for both the single and double knockdowns. After 24 hours, the cells were put into a CO_2/O_2 incubator and cultured for another 24 hours at 0.9% O_2. The study involved the use of two control samples which were incubated under either hypoxic or normoxic conditions. A commercial isolation kit was used to isolate the mitochondria from the cell pellet. The mitochondrial pellet was dissolved in methanol for analysis.

The mitochondrial samples were analyzed via HPLC-ESI-TOF-MS. First, the molecular feature extraction was performed in Agilent MassHunter Profinder 10.0, followed by filtration in RStudio and multivariate analysis in SIMCA 16. Next, the putative annotation of the features was performed in CEU mass mediator 3.0, and lastly, the metabolomic differences between the conditions are discussed.

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ANALYSIS OF CHANGES IN THE CELLULAR METABOLOME DURING OXIDATIVE STRESS AND THE STUDY OF THE ANTI-INFLAMMATORY PROPERTIES OF CURCUMIN AND METFORMIN

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Chronic inflammation is a consequence of long-term acute inflammation in the body and may lead to the induction of chronic diseases, i.e., obesity, diabetes, neurodegenerative diseases. One of the reasons for this condition may be the accumulation of excessive amounts of free radicals. Under physiological conditions, this state is favorable because it accelerates many positive for human body processes, eg., wound healing and bone fusion. Unfortunately, free radicals also have a negative role in the body that can be generated in situ and/or come from the environment external. cell cultures are particularly useful for studying the effects of oxidative stress on cells. Cells incubated with pro-oxidant compounds constitute great models of inflammation that are triggered by persistent oxidative stress in the cell. Such models help explain the role and pathogenesis of oxidative stress in many diseases, e.g., in neurodegenerative disorders of the brain, rheumatoid arthritis.

Thanks to metabolomic studies, it is possible to monitor changes in metabolism, for example after administration of therapeutics or induction of oxidative stress. Using the appropriate metabolic tools such as nuclear magnetic resonance (NMR) and mass spectrometry (MS), it is possible to observe the concentrations of individual metabolites, which allows us to assess the condition of the cell and detection of pathologies in the cells. The purpose of the research is to register change occurring in cells both during ongoing oxidative stress and at the moment effects on the cell with potential antioxidant therapeutics.

The purpose of this study was to explore the antioxidative role of curcumin and metformin with a description of their antioxidative potential. The obtained results confirmed the main hypothesis for both substances. Metformin and curcumin decreased the number of free radicals generated in cells under oxidative stress and contributed to an increase in cell viability. For this purpose, MTT and DCF-DA assays were performed. Metabolomic studies investigated the effect of different concentrations of hydrogen peroxide on HEK-293 cells. The research was based on the analysis of the medium during cell culture without and with the addition of hydrogen peroxide. Changes in the metabolome were observed in the NMR spectra during oxidative stress in cells. There is a noticeable increase in the relative concentration of metabolites such as isobutyrate, acetate, lactate, hydroxyvalerate. The greatest changes are observed at the highest concentrations of hydrogen peroxide, such as 3.19 mM and 37.8 mM.

IMPUTOMICS: IMPUTATION OF MISSING VALUES FOR "OMICS" DATA

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Background: Missing data is a significant issue in mass-spectrometry-based untargeted metabolomics. Proper handling of this type of data is essential, as they have profound implications for downstream statistical analyses and lead to misleading or uninterpretable inferences.

In metabolomics data we can highlight three types of missing values, missing not at random (MNAR), missing at random (MAR), and missing completely at random (MCAR).

There are various methods to deal with this problem including, zero, half minimum, mean, median, random forest, singular value decomposition, k-nearest neighbors, multiple imputation by chained equations, and quantile regression imputation of left-censored data. However, these algorithms vary in time of calculation and accuracy of the imputation of missing values, depending on the type of data we provided.

Methods: We have collected, re-implemented and provided with a standardized API 36 imputation methods that are used in metabolomics studies. We benchmarked these methods considering the accuracy of the imputation and the time needed to perform the computations.

Results: Our results showed that the fastest and most accurate method is softimpute. Moreover, we have found out that the imbalance between the number of samples and metabolites negatively affects the speed of PCA-based methods.

We plan to share our tool as the Imputomics R package and a Shiny web server.

A COMPARISON OF DIFFERENT MACHINE-LEARNING TECHNIQUES FOR THE SELECTION OF A PANEL OF METABOLITES ALLOWING EARLY DETECTION OF BRAIN TUMORS

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Metabolomics combined with machine learning methods (MLMs), is a powerful tool for searching novel diagnostic panels. This study was intended to use targeted plasma metabolomics and advanced MLMs to develop strategies for diagnosing brain tumors. Measurement of 188 metabolites was performed on plasma samples collected from 95 patients with gliomas (grade I–IV), 70 with meningioma, and 71 healthy individuals as a control group. Four predictive models to diagnose glioma were prepared using 10 MLMs and a conventional approach. Based on the cross-validation results of the created models, the F1-scores were calculated, then obtained values were compared. Subsequently, the best algorithm was applied to perform five comparisons involving gliomas, meningiomas, and controls. The best results were obtained using the newly developed hybrid evolutionary heterogeneous decision tree (EvoHDTree) algorithm, which was validated using Leave-One-Out Cross-Validation, resulting in an F1-score for all comparisons in the range of 0.476–0.948. Brain tumor diagnostic panels were constructed with unique metabolites, which reduces the likelihood of misdiagnosis. This study proposes a novel interdisciplinary method for brain tumor diagnosis based on metabolomics and EvoHDTree, exhibiting significant predictive coefficients.

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