Oral

NOS produces cyclic octasulfur that enables protection against lipid peroxidation in lipid droplets

Uladzimir Barayeu[1,2], Tsuyoshi Takata[1], Tetsuro Matsunaga[1], Seiryo Ogata[1], Yuka Unno[1], Mike Lange[3], Minkyung Jung[1], Jun Yoshitake[1], Takayuki Shimizu[4], Shinji Masuda[5], Masanobu Morita[1], James A. Olzmann[3], Hozumi Motohashi[6], Takaaki Akaike[1]

[1] Department of Environmental Medicine and Molecular Toxicology, Tohoku University Graduate School of Medicine, Sendai, Japan, [2] Max-Planck-Institut for Polymer research, Mainz, Germany, [3] Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA, [4] Faculty Division of Natural Sciences Research Group of Biological Sciences, Nara Women University, Nara, Japan, [5] Department of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan, [6] Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan

Cyclic octasulfur (S8) is known to be one of the end products and substrates in sulfur respiration of sulfur-oxidizing bacteria. According to endosymbiotic theory, mitochondria have evolved from sulfur bacteria. Following this idea, we have developed a novel mass spectrometry-based method to measure S8 and for the first time, we have detected S8 in the mitochondria of mammalian cell in concentration comparable with bacteria. While bacteria store S8 in sulfur granules, we found large concentrations of lipophilic S8 in lipid droplets in mouse and human adipocytes.

We hypothesized that S8 could be produced by the oxidoreductase associated with lipid droplets. Indeed, we identified eNOS (endothelial NO synthase) associated with lipid droplets in adipocytes. Treatment of recombinant eNOS with a sulfur donor GSSSG and electron donor NADPH resulted in production of S8 and its accumulation in lipid droplets. We have further found inducible and neuronal NOSs for S8 production. Treatment of adipocytes with GSSSG has markedly increased levels of S8 in the lipid droplets.

We propose therefore that S8 in lipid droplets could serve as a reservoir for reactive supersulfides (RSSxH). Supersulfides serve as antioxidants and thus protect cells from lipid peroxidation-driven cell death - ferroptosis. Indeed, depletion of S8 from adipocytes caused lipid oxidation and ferroptosis. In contrast, supplementation with solubilized S8 prevented ferroptosis caused by ferroptosis inducers. Also, in vitro, S8-loaded lipid droplets were resistant to lipid peroxidation.

The present data indicates that S8 could serve as an evolutionarily conserved supersulfide reservoir and thus counteract oxidative stress in cells.

Oral

A new NO-independent immune regulatory role for iNOS via protein-protein interaction with IRG1

Marina Diotallevi[1], Carlos Outeiral[1], Priyanka Patel[2], Thomas Nicol[1], Faseeha Ayaz[1], Daniel Nissley[1], Ganna Krasnoselska[1], Svenja Hester[1], John McVey[2], Roman Fischer[1], Benedikt Kessler[1], Charlotte Deane[1], Raymond Owens[1], Keith Channon[1], Mark Crabtree[2]
[1] University of Oxford, [2] University of Surrey

Itaconate, a TCA cycle-derived metabolite produced by Immunoresponsive Gene 1 (IRG1), is one of the most abundant metabolites in activated immune cells and has pivotal roles in the inflammatory response, metabolic regulation, and redox signalling. We recently discovered that inflamed macrophages lacking inducible Nitric Oxide Synthase (iNOS) (an inducible enzyme generating high levels of nitric oxide (NO) during inflammation) or its cofactor tetrahydrobiopterin (BH4), produced markedly increased amounts of itaconate in comparison with wild-type activated macrophages, by a mechanism independent of IRG1 expression.

To further understand the role of iNOS in mediating itaconate production, and unravel the subsequent immuno-regulatory functions of iNOS, we use bone marrow-derived macrophages cultured from WT, iNOS KO and BH4 KO mice. Following activation with LPS and interferon γ (MLPS/IFNγ), we uncover a strong correlation between the presence of iNOS / NO and a striking decrease in the production of itaconate over time. Using experimental studies in cells, surface plasmon resonance, computational predictions, and molecular dynamics simulations of iNOS and IRG1 molecular interactions, we report a dynamic inhibition of IRG1 by protein-protein interaction between iNOS and IRG1 that is dependent upon specific iNOS conformations, but not on NO generation.

In conclusion, we have discovered a novel fundamental role for iNOS, independent of its NO catalytic activity, in regulating the critical metabolite itaconate. This study places iNOS at the centre of regulating macrophage function and the response to injury, with iNOS effectively acting as a brake to control itaconate production and ultimately macrophage polarization state.

Oral

Nitrite reductase activity of liver derived Xanthine Oxidoreductase maintains cardiovascular homeostasis.

Nicki Dyson[1], Rayomand Khambata[1], Gianmichele Massimo[1], Tipparat Parakaw[1], Krishnaraj Rathod[1], Amrita Ahluwalia[1]
[1] Cardiovascular Medicines & Devices, William Harvey Research Institute, Barts and the London Faculty of Medicine and Dentistry Queen Mary University of London, London

Endothelial dysfunction, characterised by reduced NO bioavailability, is a hallmark of cardiovascular disease (CVD). Nitrite (NO_2^-) reduction to NO can restore endothelial function in CVD. The specific reductase responsible, however, remains unknown. Xanthine oxidoreductase (XOR) acts as a NO_2^- reductase in certain CVD1 but its role in cardiovascular homeostasis is uncertain. Global Xdh KO mice do not survive beyond 4-weeks; therefore, we created a unique hepatocyte specific Xdh KO model as the liver is believed to be the primary source of circulating XOR.

Cardiovascular phenotyping of floxed exon 6 (Xdhfl/fl) or hepatocyte specific Xdhfl/fl albumin Cre+/- (HXOR KO) littermates was conducted utilising echocardiography, non-invasive blood pressure measurement, and ultrasound determined assessment of flow mediated dilation (FMD), and leukocyte rolling and adhesion for assessment of inflammation using intra-vital microscopy. Biochemical analysis of harvested tissues was also performed2.

qPCR and Western blotting confirmed liver specific ablation of XOR and HXOR KO mice also expressed reduced plasma XOR levels. HXOR KO mice vs the WT littermate controls also expressed significantly attenuated liver and plasma NO_2^- reductase activity and platelet cGMP levels. These effects were associated with increased systolic blood pressure, LV remodelling, and impaired LV hemodynamics resembling HFpEF in HXOR KO mice. Enhanced basal leukocyte rolling in HXOR KO mice was associated with increased endothelial CD62P expression and impaired FMD.

Hepatocyte-derived XOR maintains cardiovascular homeostasis due to its role in reducing NO_2^- to NO. Thus, pharmacological inhibition of XOR-dependent NO_2^- reduction may underlie the adverse CV events associated with treatment in patients.

Oral

Erythrocyte-Derived Extracellular Vesicles Induce Endothelial Dysfunction through Arginase-1 and Oxidative Stress in Type 2 Diabetes

Aida Collado[1], Rawan Humoud[1], Eftychia Kontidou[1], Maria Eldh[2,3,4], Jasmin Swaich[1,5], Jiangning Yang[1], Tong Jiao[1], Elena Domingo[6,7], Ali Mahdi[1], John Tengbom[1], Michael Alvarsson[8,9], Susanne Gabrielsson[2,3,4], Per Eriksson[10], Zhichao Zhou[1], John Pernow[1,11]
[1] Division of Cardiology, Department of Medicine Solna, Karolinska University Hospital, Karolinska Institutet, [2] Division of Immunology and Allergy, Department of Medicine Solna, Karolinska University Hospital, Karolinska Institutet, [3] Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden;, [4] Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden, [5] KTH Royal Institute of Technology, [6] Department of Pharmacology, Faculty of Medicine and Odontology, University of Valencia, Spain, [7] Institute of Health Research INCLIVA, University Clinic Hospital of Valencia, Spain, [8] Department of Molecular Medicine and Surgery, Karolinska Institutet;, [9] Center for Diabetes, Academic Specialist Center, Health Care Services Stockholm County, Sweden, [10] Division of Cardiovascular Medicine, Department of Medicine Solna, Karolinska University Hospital, Karolinska Institutet, [11] Department of Cardiology, Karolinska University Hospital, Stockholm, Sweden.

Background: Red blood cells (RBCs) from individuals with type 2 diabetes (T2D-RBCs) induce endothelial dysfunction. However, the mechanism by which RBCs communicate with the vasculature is unknown.

Purpose: This study aimed to test the hypothesis that extracellular vesicles (EVs) secreted by RBCs act as mediators of endothelial dysfunction in T2D.

Methods: EVs released from T2D-RBCs (T2D RBC-EVs) and RBCs from age-matched healthy controls (H RBC-EVs) were isolated and co-incubated with mouse aortas to evaluate endothelium-dependent relaxation. The number of EVs produced, their uptake by endothelial cells, and their arginase-1 content were determined. Functional involvement of EV uptake, arginase, and oxidative stress were investigated using pharmacological interventions and expression analyses.

Results: Despite a lower production of T2D RBC-EVs, their uptake by endothelial cells was greater compared to H RBC-EVs. T2D RBC-EVs significantly impaired endothelium-dependent relaxation, an effect that was attenuated following inhibition of arginase in EVs. Additionally, inhibition of vascular arginase or oxidative stress improved endothelium-dependent relaxation. Arginase-1 was detected in RBC-derived EVs, and levels of arginase-1 and oxidative stress increased in the vasculature following co-incubation with T2D RBC-EVs. These EVs also increased levels of arginase-1 and NADPH oxidase 4 in endothelial cells. An increase in arginase-1 protein was observed even after mRNA silencing.

Conclusions: T2D-RBCs induce endothelial dysfunction through the increased uptake of EVs that transfer arginase-1 from RBCs to the vascular endothelium in T2D, leading to oxidative stress and endothelial dysfunction. These results shed important light on the mechanism underlying vascular injury mediated by RBCs in T2D.

Oral

NO-ferroheme dilates arteries via mobilizing NO moieties from an intracellular NO store in vascular wall

Taiming Liu[1], Arlin Blood[2], Meijuan Zhang[1], Haiyan Ke[3], Amancio de Souza[3], Qian Li[4], Lingchao Zhu[5], Hobe Schroeder[2]
[1] Department of Pediatrics, Division of Neonatology, Loma Linda University School of Medicine, Loma Linda, CA 92354, [2] Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA 92354, [3] Metabolomics Core Facility, University of California, Riverside, [4] Department of Medicine Gregory Fleming James Cystic Fibrosis Research Center University of Alabama at Birmingham, [5] Department of Chemistry University of California

Oral

Assessment of the role of Gasdermin-D on increased oxidative stress and MMP activation in angiotensin II-induced hypertension, and the impact of sodium nitrite treatment on vascular response and protection against oxidative stress and vascular remodeling

Carla Fiama Azevedo Medeiros[1], Macário Arosti Rebelo[1], Rose Inês Matos Batista[1], Sandra Oliveira Conde Tella[1], Anderson Santos Ramos[1], Fernando Queiroz Cunha[1], José Eduardo Tanus dos Santos[1]
[1] University of São Paulo

Introduction: In hypertension, increased oxidative stress and matrix metalloproteinase (MMP) activation lead to endothelial dysfunction. Moreover, inflammation exacerbates organ damage via inflammasome and cytokine activation.

Purpose: The study hypothesizes that gasdermin D (GSDMD) mediates increased oxidative stress and MMP activation in angiotensin II-induced hypertension, contributing to vascular remodeling and endothelial dysfunction. Additionally, sodium nitrite treatment is proposed to reduce these inflammatory factors in hypertension.

Methods: Male C57Bl/6 (WT) and GSDMD knockout (GSDMD-/-) mice, aged 6-10 weeks, were used. Osmotic mini-pumps containing saline or angiotensin II (490 ng/kg/min) were implanted for 28 days to induce hypertension. From the second week onwards, mice were treated with sodium nitrite (15 mg/kg) or vehicle. Blood pressure was measured weekly. Following treatment, plasma NETs, ROS via DHE, and aortic morphology were evaluated.

Results: Both WT and GSDMD-/- mice infused with ANGII showed a progressive increase in systolic blood pressure (SBP), which was not observed in the vehicle groups. Additionally, groups treated simultaneously with nitrite showed reduced blood pressure levels in the second week of treatment. In the WT-ANGII+Vehicle group, there was an elevation in reactive oxygen species (ROS) levels and cross-sectional area of the aorta, both of which were effectively reduced by nitrite treatment (WT-ANGII+Nitrite). However, in the GSDMD-/- ANGII+Vehicle group, protection against ROS elevation and vascular remodeling was observed.

Conclusions: Partial results suggest that nitrite favors cardiovascular homeostasis and that GSDMD may influence vascular response to hypertension, as its absence provides protection against oxidative stress and vascular remodeling.

Oral

Upregulation of the NO cascade through PDE5 inhibitors counteracts the defects in synaptic plasticity and memory in Alzheimer's disease and related dementia

Ottavio Arancio[1], Elisa Zuccarello[1], hong zhang[1], Shi-Xian Deng[1], Jole Fiorito[2], Donald Landry[1]

[1] Columbia University, [2] New York Institute of Technology

Work from our laboratories has demonstrated that elevation of the Alzheimer's disease (AD) proteins beta-amyloid and tau downregulates the nitric oxide cascade with a reduction in i) activity-dependent cGMP levels, ii) cGMP-dependent protein kinase activation, and iii) phosphorylation of the memory-related transcription factor CREB. Thus, increase of cGMP levels through inhibition of the cGMP-degrading enzyme phosphodiesterase-5 (PDE5) improved synaptic plasticity and memory in mouse models of AD and related dementia (ADRD). Moreover, we found that the beneficial effect of different PDE5 inhibitors was not only immediate, but also lasted for a prolonged period beyond the drug administration. Consistent with these data, an increasing number of large population-based cohort studies shows a reduced risk of developing AD in humans exposed to the PDE5 inhibitor sildenafil to counteract erectile dysfunction. Thus, PDE5 inhibition represents a suitable strategy for treating cognitive deficits in AD and ADRD. Given that none of the existing PDE5 inhibitors has been developed to counteract diseases of the CNS and at the same time possesses the selectivity required for chronic administration to an elderly population with comorbid conditions such as AD and ADRD patients, we have started a drug discovery program aimed at finding PDE5 inhibitors that are tailored to be used in these patients. A combination of medicinal chemistry efforts with electrophysiology and behavior expertise has led to the discovery of several PDE5 inhibitors which we are optimizing to obtain an AD and ADRD drug candidate.

Oral

The Na+/I- Symporter is a nitrate transporter in the salivary glands

Gaia Picozzi, Juliane Jurga, John Pernow, Mattias Carlström, Hugo Zeberg, Eddie Weitzberg, Rickard Ågren, Jon O. Lundberg

The entero-salivary circulation of nitrate (NO3-) describes absorption of dietary nitrate in the gut, the active uptake of circulating nitrate by the salivary glands and its concentration in saliva. This in vivo recycling of nitrate is a crucial part of the nitrate-nitrite-nitric oxide (NO) pathway, an important alternative pathway for maintaining NO signaling in mammals. Approximately 25% of circulating nitrate is taken up by the salivary glands, resulting in salivary levels that are 10–20 fold higher than those in plasma. The specific mechanisms of nitrate uptake and the transporters involved are not entirely clear although Sialin (encoded by the gene SLC17A5) has been suggested to play a major role. Interestingly, studies from the 50s indicate that nitrate transport in the salivary glands occurs in competition with iodide (I-) which prompted us to explore the role of the Sodium/Iodide Symporter (NIS) in this process.

Starting with a database analysis looking at mRNA and protein levels, it was revealed that the gene SLC5A5 (encoding for the NIS protein) is in fact expressed at higher levels than SLC17A5 in the salivary glands. Next, we decided to test SLC5A5 in the Xenopus Expression System and identified an anion influx, evoked by a NO3- perfusion solution indicating nitrate transport. We also observed increased levels of NO3- in SLC5A5-injected oocytes after incubation with NO3-. Finally, to test the competition between nitrate and iodide in vivo, we collected saliva samples from patients receiving high doses of intravenous iodine (I2) contrast media, a procedure known to generate considerable levels of iodide (I-). We observed a marked decrease in salivary nitrate following the administration of contrast medium, indicating competition for salivary transport. Overall, these findings suggest that the Na+/I- Symporter is mediating uptake of nitrate in the salivary glands and concentration in saliva.

Oral

Breaking the NO Code: Denitrosylases and Tumor Growth

Giuseppe Filomeni

Nitric oxide (NO) production in the tumor microenvironment is a common element in cancer. S-nitrosylation, an enzymatically regulated post-translational modification of cysteines by NO, is emerging as a key transduction mechanism that sustains tumorigenesis.

Here we show the oncogenic effects induced by the loss (or hypo-expression) of two denitrosylases, i.e., S-nitrosoglutathione reductase (ADH5/GSNOR) and SNO-CoA reductase (SCoR/AKR1A1). Both indirectly turn off the signal induced by protein S-nitrosylation by removing two different low-molecular-weight nitrosothiols, GSNO and SNO-CoA, respectively. In silico analyses revealed that GSNOR and AKR1A1 are hypo-expressed in human malignancies. Using multiple tumor models, we demonstrate that excessive S-nitrosylation due to GSNOR deficiency sustains phospho-activation of focal adhesion kinase 1 (FAK1), thus enhancing the aggressiveness of cancer. On the other hand, we provide compelling evidence that AKR1A1 downregulation causes significant metabolic rewiring, which results in a higher antioxidant response and the development of a more invasive and chemoresistant phenotype.

Altogether, these findings advance our understanding of the oncogenic role of S-nitrosylation, argue for denitrosylases acting as a novel class of tumor suppressors, and point to their loss as a therapeutically exploitable vulnerability in cancer.

Oral

Inhibition of Salivary Carbonic Anhydrase VI (Gustin) by Acetazolamide but not Chlortalidone Reveals a Novel Mechanism for Regulating the (Dietary/Endogenous) Nitrate-Nitrite-NO Pathway and Blood Pressure in Healthy Volunteers

Andrew Webb[1,2], Kevin O'Gallagher[1], Satnam Lidder[1], Jeremy Au-Yeung[1], Fayez Aldhufairi[1], Taran Deol[1], Lily-Ann Wills-Gray[1], Craig Ferguson[1], Scott Downham[1], Chimedtseren Odongerel[1], Vivien Nebo[1], Perry Maskell[1]
[1] King's College London, [2] Guy's & St. Thomas' NHS Foundation Trust

Background: The renal carbonic anhydrases (CAs) have been implicated in nitrate (NO_3^-) and nitrite (NO_2^-) excretion. Acetazolamide and chlortalidone are both CA-inhibiting diuretics. However, acetazolamide lacks consistent blood pressure (BP)-lowering activity, albeit beneficial in decompensated heart failure.

Purpose: to determine whether acetazolamide, which also inhibits salivary CA-VI/gustin (unlike chlortalidone), perturbs oral bioactivation of nitrate to nitrite and BP-lowering.

Methods: We conducted three acute randomised, placebo-controlled, cross-over studies (38 volunteers, 7h/visit), of chlortalidone (50 mg), acetazolamide (500 mg) and ±acetazolamide ±chlorhexidine mouthwash (3 visits) alongside a nitrate load (8 mmol; inorganic nitrate capsules, or beetroot juice shot (70 ml)).

Results: Chlortalidone and acetazolamide induced similar diureses (~77 ml/h, ~80 ml/h, respectively, $P<0.0001$) but inhibited nitrate excretion (both $P=0.006$). Chlortalidone lacked effect on plasma or salivary [nitrate] or [nitrite] but lowered systolic BP (SBP) $P=0.01$. By contrast, acetazolamide decreased salivary nitrite production by 21% ($P=0.003$), and plasma [nitrite] by 25% ($P<0.0001$), and lacked a BP-lowering effect. Acetazolamide increased salivary pH ($P=0.001$), via bicarbonate secretion ($P=0.0002$). Mouthwash blocked salivary nitrate reduction to nitrite (by 94% ($P<0.0001$)), enabling isolation of acetazolamide's effects on nitrate secretion, which it elevated by 57% ($P=0.025$).

Conclusions: Inhibition of salivary CA-VI/gustin by acetazolamide, but not chlortalidone, enhances salivary nitrate secretion, but inhibits nitrate reduction to nitrite, diminishing circulating [nitrite], counteracting diuretic-induced BP-lowering. This identifies (i) CA-VI as a key regulator of the dietary nitrate-nitrite-NO pathway and (ii) a mechanism for acetazolamide's limited BP-lowering effects.

Oral

Nitrite-dependent NO synthesis in mitochondria by sulfite oxidase

Titus Gehling[1], Günter Schwarz[1], Daniel Bender[1], Alexander Kaczmarek[1]
[1] Institute of Biochemistry, University of Cologne

Background: Under hypoxic conditions NO levels are dependent on the reductive nitrate-nitrite-NO pathway, which itself relies on metal cofactor-dependent proteins. Among these, molybdenum cofactor (Moco)-dependent enzyme sulfite oxidase (SOX) was identified to reduce nitrite to NO (1). SOX is localized to the intermembrane space (IMS) of mitochondria and consists of an N-terminal heme domain linked via a tether domain to the catalytic molybdenum cofactor domain.

Purpose: We aimed to further uncover the mechanism of nitrite reduction by SOX, thus providing a molecular understanding towards the physiological relevance of SOX in nitrite-dependent NO signaling.

Methods: To decipher the reaction mechanism of SO-dependent nitrite reduction we purified recombinant human SOX and performed steady state enzyme kinetics. To investigate the physiological relevance, we measured various parameters of mitochondrial respiration using HEK as well as MEF cells.

Results: We have shown that sulfite is a competitive inhibitor of nitrite reduction. Furthermore, the reaction is favored at acidic conditions, likely to be present in the

IMS (2). Furthermore, by measuring downstream metabolite cyclic GMP, we revealed SOX as a major source of cellular NO (3). Nitrite-dependent cGMP formation was even increased under hypoxic conditions. Given that cytochrome c serves as the final electron acceptor, an impact on mitochondrial respiration was proposed. We could underline this hypothesis with experimental evidence of a SOX- and nitrite-dependent inhibition of mitochondrial respiration.

Conclusion: Taken together, we gained new insights into the mechanism of nitrite reduction by SOX and its physiological relevance.

Oral

Fatty acid nitroalkenes induce anti-inflammatory function by metabolic reprogramming of BV2 microglial cells

Changjiang Guo[1], Elena Abramova[1], Bruce Freeman[2], Andrew Gow[1]
[1] Rutgers University, [2] University of Pittsburgh

Background: Microglial activation is a critical step in neuroinflammation and involves the production of high flux NO via iNOS. Purpose: examine the anti-inflammatory potential of fatty acid nitroalkenes OA-NO_2 in microglial cells.

Methods: Metabolism of BV2 cells was analyzed by SEAHORSE eflux analyzer. Pro-inflammatory mediators were determined by real-time PCR or Western blot assay. S-nitrosylated proteins were determined by Biotin-switch assay.

Results: Incubation of BV-2 cells, a model of microglia, with LPS leads to production of NO and inhibition of mitochondrial spare respiratory capacity (SRC) via S-nitrosylation of complex IV and V. Direct inhibition of iNOS with 1400W reduces the loss of SRC seen with LPS treatment. Nitroalkenes represent a biologically relevant form of NO-derived biomolecule that can reduce inflammation. We found that pre-treatment of BV-2 cells with the nitroalkene, nitrooleic acid (OA-NO_2), abrogated LPS-mediated loss of SRC and the nitrosylation of complex IV and V. However, acute incubation of LPS treated BV-2 cells with OA-NO_2 did not affect the loss of SRC, implying that it does not directly inhibit iNOS function. Pretreatment with OA-NO_2 inhibited LPS-mediated increases in gene expression of pro-inflammatory mediators IL -1beta, IL-6, CCL2, Ptgs2 and NOS2. Cellular signaling events p38, IkB, NF-kB H3K9, HADC2 are involved in OA-NO_2 mediated inhibition of activated microglia.

Conclusions: Our data reveal that OA-NO_2 has anti-inflammatory activity in combination with correction of mitochondrial function in activated microglial cells. Since OA-NO_2 can cross the blood-brain barrier of the central nervous system, OA-NO_2 has potential as a therapeutic strategy for neuro-inflammation associated disorders.

Poster

Nitric oxide releasing graphene nanomaterials for cardiovascular applications

Tanveer Tabish[1]
[1] University of Oxford

Background: For more than a century, nitric oxide (NO) donating formulations including organic nitrates/nitrites remains a mainstay of cardiovascular pharmacology. These donors primarily produce NO systemically, however it is desirable to deliver the right amount of NO to a right location at the right time. Purpose: To achieve these aims, we developed several strategies aimed at generating or releasing NO in living systems showing that graphene could either generate NO endogenously by catalytic decomposition of endogenous NO substrates or can store/release NO.

Methods: we describe the design and characterisation of a novel NO delivery system via the reaction of acidified sodium nitrite with thiol groups introduced by cysteamine conjugation to graphene, thereby generating S-nitrosated graphene.

Results: An NO electrode, chemiluminescence and electron paramagnetic resonance spectroscopy were used to measure NO released from graphene which was sustained at >5 × x10-10 mol cm-2min -1 for 3 hours, which is comparable to production by healthy endothelium (cf, 0.5 – 4 x10-10 mol cm-2min-1). Single-cell Raman micro-spectroscopy showed that vascular endothelial and smooth muscle cells (SMCs) took up graphene, with intracellular NO release detected via fluorescent NO-specific probe. Graphene had a dose-dependent effect to promote proliferation in endothelial cells and to inhibit growth in SMCs, which was associated with cGMP release indicating intracellular activation of canonical NO signalling. Chemiluminescence detected negligible production of toxic N-nitrosamines.

Conclusions: This talk will present our recent findings on developing graphene as a NO delivery vehicle, thereby highlighting the potential of graphene for the treatment of cardiovascular diseases.

Poster

The crosstalk between nitric oxide and mTOR signaling in autism spectrum disorder.

Shashank Kumar Ojha[1], Manish Kumar Tripathi[1], Maryam Kartawy[1], Wajeha Hamoudi[1], Haitham Amal*[1]
[1] The Hebrew University of Jerusalem

Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder associated with numerous behavioral deficits. The prevalence rate of ASD is increasing every year i.e., 1/36 children from the latest epidemiological study, but still no drugs are available. For the first time, we have reported a dramatic increase in nitric oxide (NO) levels in ASD mouse models and clinical samples.

Purpose: To investigate the role of NO in ASD and decipher the underlying molecular mechanism.

Methodology: We conducted a multidisciplinary study (proteomics, biochemistry, behavioral) to investigate the role of NO in ASD. Shank3 and Cntnap2 ASD mouse models were used in the study. We also used human iPSC and human plasma to test our hypothesis.

Results: High levels of nitrosative stress biomarkers are found in both ASD mouse models. Pharmacological intervention with a neuronal NO synthase inhibitor in both models led to a reversal of the molecular, synaptic, and behavioral ASD-associated phenotypes. Clinically, we found a significant increase in nitrosative stress biomarkers in the plasma of low-functioning ASD patients. An increase in NO levels caused S-Nitrosylation of TSC-2 in Shank3 and Cntnap2 models, which led to loss of function of TSC-2 and to mTOR pathway over-activation. Pharmacological inhibition of nNOS prevented overactivation of mTOR signaling pathways and prevented autistic phenotype.

Conclusion: This work reveals that NO plays a significant role in ASD. It showed a novel cross talk between NO and mTOR signaling. Finally, it suggests novel therapeutic drug targets for the disorder.

Poster

Intermittent breathing of 300 parts-per million nitric oxide (iNO300) reduces Pseudomonas aeruginosa lung colonization in a swine model of pneumonia

Binglan Yu[1], Dario Winterton[1], Talisa Buehl[1], Stefano Cenci[1], Mohamed Okda[1], Kyle Medeiros[1], Gamonmas Ekkapat[1], Giovanni Bruno[1], Roberta Santiago[1], Hatus Wanderley[1], Valentina Giammatteo[1], Alice Nova[1], Cristina Mietto[1], Stefano Spina[1], Bijan Safaee[1], Mary Delaney[2], Ryan Carroll[1], Fumito Ichinose[1], Angela Shih[1], Lynn Bry[2], Lorenzo Berra[1]

[1] Massachusetts General Hospital, [2] Brigham & Women's Hospital

Background/Introduction:Pseud omonas aeruginosa (P.A.) is a common multi-drug resistance pathogen that can cause hospital-acquired pneumonia. Accumulating evidence suggests that, at high doses, nitric oxide (NO) acts as an antimicrobial. Our preclinical and early clinical work demonstrates promising antimicrobial effects of inhaled high-dose NO (up to 300 ppm).

Purpose: To test whether inhaled high-dose NO (300 ppm, iNO300) is safe and effective for treating pigs with P. aeruginosa pneumonia.

Methods: Bacterial pneumonia was induced by colonizing P.A. in bronchus of Yorkshire pigs. Pigs were randomly assigned into four groups: (1) without P.A. challenge and without iNO300 (PA-/iNO-, N=3), (2) without P.A. challenge but with iNO300 (PA-/iNO+, N=6), (3) with P.A. challenge but without iNO300 (PA+/iNO-, N=6), and (4) with P.A. challenge and iNO300 (PA+/iNO+, N=6). All animals were mechanically ventilated for 72 hrs. Vital signs, microbiology and histology of lung tissues, and NO metabolites were measured.

Results: Breathing iNO300 for 30 min/session, 6 times/day for 72 hours was safe with average methemoglobin levels at the end of each session at 7.9±3.1%and the inspired NO2 at 3.9±0.5 ppm. In PA+/iNO+ group, iNO300 produced 2-log reductions in P.A. counts in the infected lung, improved oxygenation, lung compliance, and gross findings on autopsy when compared to PA+/iNO- group. Lung histology revealed that iNO300 reduced severity of acute bronchopneumonia in PA+/iNO+ group. NO metabolites were increased in lung tissue, plasma, and urine by breathing iNO300.

Conclusions: Intermittent breathing iNO300 was safe and reduced P. aeruginosa lung colonization and acute bronchopneumonia in mechanically ventilated pigs.

Poster

NOSH-aspirin (NBS-1120) attenuates motor defects and dopaminergic neuron degeneration in a rat model of Parkinson's disease: Modulation of MAPKs signaling pathway

Khosrow Kashfi[1], Bita Bita Firouzan[2], Rasoul Ghasemi[2]
[1] City University of New York School of Medicine, NY USA, [2] Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Parkinson's disease (PD) involves the progressive loss of dopaminergic neurons in the nigrostriatal pathway, leading to motor and non-motor symptoms. Chronic neuroinflammation is believed to play a significant role in its pathogenesis. NOSH-aspirin, a novel derivative of aspirin that releases nitric oxide (NO) and hydrogen sulfide (H2S), has shown potent anti-inflammatory effects.

Purpose: This study assessed the neuroprotective effects of NOSH-aspirin against neurotoxicity induced by 6-hydroxy-dopamine (6-OHDA) in a PD animal model.

Methods: Male Wistar rats (N=8 per group) were used. Groups: Control (sham/vehicle), 6-OHDA (20 μg/rat, right medial forebrain bundle), 6-OHDA + NOSH-aspirin (25 or 100 mg/kg), 6-OHDA + aspirin (38 or 100 mg/kg), NOSH-aspirin (100 mg/kg) starting 3 days post-6-OHDA. Treatment duration was 11 days, starting 24 hours post 6-OHDA administration. Evaluations: Behavioral assessments (Rotarod treadmill, Beam walking, Open field, apomorphine-turning behavior), histological analysis, tyrosine hydroxylase (TH)-positive cell immunohistochemistry, and molecular evaluations via Western blot. Results: NOSH-aspirin significantly improved motor impairments in the PD model rats compared to the untreated 6-OHDA group. NOSH-aspirin preserved TH+ neurons in the nigrostriatal pathway, indicating protection against dopaminergic neuron loss. Treatment with NOSH-aspirin led to a notable reduction in the phosphorylated levels of MAPKs protein family members, including JNK, P38, and ERK, suggesting a reduction in inflammatory signaling pathways.

Conclusions: NOSH-aspirin demonstrates significant neuroprotective effects in a PD animal model, likely through its anti-inflammatory properties and ability to mitigate MAPK-related neuroinflammation. These findings suggest that NOSH-aspirin could be a promising therapeutic candidate for neuroinflammation-associated neurodegenerative disorders like Parkinson's disease.

Poster

Hemin Conjugates as Potential Nitric Oxide-Scavengers with Anti-Cancer Molecular Actions

Amir Abdo[1], Sharon Glynn[1,2], Pau Farràs[1,3], Abhay Pandit[1]
[1] CÚRAM, SFI Research Centre for Medical Devices, University of Galway, [2] Discipline of Pathology, Lambe Institute for Translational Research, School of Medicine, University of Galway, [3] School of Biological and Chemical Sciences, Ryan Institute, University of Galway

Background/Introduction: Triple-negative breast cancer (TNBC) is aggressive with limited treatments and poor prognoses. Nitric Oxide (NO) promotes tumor growth and spread by regulating angiogenesis and oncogenic pathways. Inhibiting NO synthesis through NO synthases (NOS) is a potential TNBC treatment strategy, though current NOS inhibitors have limitations.

Purpose: The study aimed to: (1) synthesize various hemin conjugates and test their NO-scavenging capabilities; (2) evaluate their cytotoxicity and concentration-dependent NO-scavenging ability in vitro; and (3) investigate the downstream effects of NO-scavenging.

Methods: Several hemin conjugates were synthesized and evaluated for NO-binding capacity using luminescence and electrochemical methods. Their cytotoxicity was tested on TNBC cell lines, and intracellular NO levels were monitored over 24 hours. The study also examined the impact of NO-scavenging on cell migration and invasion, and analyzed downstream effects and various protein markers.

Results and discussions: The NO-binding affinities of various concentrations of hemin and hemin conjugates were assessed using metabolic activity assays. Intracellular NO detection showed inhibition of DAF-FM-DA fluorescence in MDA-MB-231 cells treated with DETA-NONOate, with the different compounds displaying varying NO-scavenging activity. Additionally, the increased migration of MDA-MB-231 cells due to DETA-NONOate was inhibited by the action of these compounds.

Conclusions: This study is the first to develop a series of hemin and hemin conjugates as NO-scavenging compounds with the ability to inhibit breast cancer cell migration, tumor angiogenesis, and modulate specific inflammatory cytokines. Despite variations in solution stability and reactivity towards NO, these compounds show potential as anti-angiogenic therapeutics for treating triple-negative breast cancer.

Poster

Oral nitrite treatment promotes nitric oxide metabolites accumulation in the liver and increases its antioxidant capacity: major relevance of gastric pH.

Alessandra Barros[1], Sandra Conde-Tella[1], Ana Karolina Lima-Silva[1], Paula Ovideo Payão[2], Alceu A. Jordão-Junior[2], Jose Eduardo Tanus-Santos[1,2]
[1] State University of Campinas, [2] University of São Paulo

Background: Treament with both nitrate and nitrite has been associated with protection against many disease conditions. These nitric oxide (NO) metabolites accumulate in the tissues and counteract pathophysiological mechanisms, which may depend on gastric pH.

Purpose: Here we examined the accumulation of nitrite, nitrate, S-nitrosothiols (RSNO) and mercury-resitant (RNNO) species after 14 days of 15 mg/kg nitrite treament by gavage. The relevance of gastric pH on nitrite treatment effects was also examined.

Methods: The concentrations of NO-related species were determined by ozone-based chemiluminescence methods in the plasma, liver, heart and brain. Total antioxidant capacity and gluthathione (GSH) concentrations were determined by spectrophotocolorimetric methods. Omeprazol (a gastric proton pump inhibitor) was used to examine the relevance of gastric pH.

Results: Nitrite increased NO metabolites concentrations particularly in the liver (and the heart), especially RSNO and RNNO. This effect was associated with major increases in liver (but not in the heart and in the brain) antioxidant capacity. In parallel with these results, nitrite treatment increased GSH concentrations only in the liver. While omeprazol did not affect NO metabolites accumulation, it prevented the effects of nitrite on liver antioxidant capacity and GSH concentrations.

Conclusion: Our results strongly suggest that oral nitrite treatment enhances liver (but not other organs) NO metabolites accumulation and antioxidant capacity by mechanisms promoting GSH accumulation. Increasing gastric pH with omeprazole prevents oral nitrite-induced effects. Our results suggest that oral nitrite may protect the liver against diseases with pathophysiological mechanisms involving liver pro-oxidant stress and that omeprazole prevents this protection.

Poster

Arginine Therapy Improves Mitochondrial Function and Oxidative Stress in Children with Sickle Cell Disease (SCD) Hospitalized with Pain: A Randomized Controlled Trial

Claudia R. Morris[1,2], Nitya Bakshi[3], Scott Gillespie[2], Lou ann Brown[2], Frank Harris[2], Dunia Hatabah[2], Rawan Korman[2], Kirshma Khemani[1,2], Chris A. Rees[1,2], Mark R. Griffiths[1,2], Loretta Reyes[1,2], Polly Kumari[2], Shiva Sruti[4], Carlton Dampier[1,2]
[1] Children's Healthcare of Atlanta, Atlanta, GA , [2] Emory University School of Medicine, Department of Pediatrics, Atlanta, GA , [3] Yale School of Medicine, New Haven, CT, [4] University of Pittsburgh, Pittsburgh, PA

Background: Pain is the leading cause of emergency department (ED) visits and hospitalization in patients with SCD. During SCD-pain, patients develop an acute arginine deficiency. Since arginine therapy is opioid-sparing in SCD we performed a phase-2 randomized control trial of intravenous-arginine in children with SCD age 3-21years hospitalized for pain requiring intravenous-opioids.

Methods:Subjects were randomized into 1 of 3 arms: 100 mg/kg/dose intravenous-arginine, 200 mg/kg loading-dose followed by 100 mg/kg/dose or placebo 3x/day. Demographics, total parenteral opioid (TPO) use, time-to-crisis-resolution (time-of-study-drug-delivery-to-last-intravenous-opioid in hours), pain scores, and mitochondrial function/oxidative stress (protein-carbonyls) were obtained before treatment and at discharge. Primary outcome measure was TPO use.

Results:We randomized 108 patients, 36 per arm (mean age 12.6±3.8years, 48% male). Placebo-group required 45% higher TPO and experienced >15hours longer mean time-to-crisis-resolution compared to combined arginine groups when adjusted for hydroxyurea use, continuous age, and sex. Among children <17years, placebo-arm required 80% more TPO versus combined arginine groups (p=0.075). Mitochondrial Complex-V activity was higher (p=0.02), and protein-carbonyl levels were lower (p=0.003) at presentation in patients on Hydroxyurea. Notably a significant, dose-dependent increase in mitochondrial Complex-IV/-V activity occurred in both arginine arms, with no change in the placebo-group (p<0.001); protein-carbonyl levels in platelet-rich plasma decreased in both arginine groups (p<0.001), suggesting a decrease in oxidative stress that increased in the placebo-group (p=0.02). Greatest mitochondrial improvement occurred with arginine loading-dose.

Conclusion:Arginine therapy significantly increases mitochondrial activity and decreases oxidative stress in children with SCD-pain, with a clinically relevant opioid-sparing trend.

Poster

Arginine Beyond Nitric Oxide (NO): Kyotorphin and Implications for Pain

Rawan Korman[1], Dunia Hatabah[1], Lou ann Brown[1], Frank Harris[1], Nitya Bakhsi[2], Chris A. Rees[1,3], Mark Griffiths[1,3], Carlton Dampier[1,3], Claudia R. Morris[1,3]

[1] Emory University School of Medicine, Department of Pediatrics, Atlanta, GA , [2] Yale School of Medicine, New Haven, CT , [3] Children's Healthcare of Atlanta, Atlanta, GA

Background:Low arginine bioavailability is associated with sickle cell disease (SCD) mortality and morbidity, including acute pain severity. Multiple phase-2 trials support safety and efficacy of arginine therapy in children with SCD-pain. As the obligate substrate for NO production, arginine's mechanism-of-action may be partly linked to an increase in NO production. Kyotorphin is an endogenous opioid-like analgesic composed of the amino-acids tyrosine and arginine. Association between arginine supplementation as a kyotorphin precursor in SCD is unknown.

Methods: We performed a pharmacokinetics study evaluating intravenous-arginine therapy on plasma arginine and kyotorphin concentration in children 7-21years hospitalized with SCD-pain, randomized to receive one of 3 dosing arms of intravenous-arginine. Plasma arginine, kyotorphin, and NO metabolites (NOx) were measured. Mean±SD, paired t-tests, and Pearson-correlation analyses between groups were performed where appropriate.

Results: Thirteen patients (13±3years, 56% male, 88% Hb-SS, 85% on hydroxyurea) were enrolled. Arginine and kyotorphin levels peaked between 60-90minutes after arginine infusion in all arms ($p<0.01$), with no differences in peak concentration across arms noted. Rise in kyotorphin levels correlated strongly with changes in plasma arginine levels ($r=0.72, p<0.0001$). Plasma NOx levels increased (Tmax=1-2 hours; mean %change 156 ±313%, $p=0.02$). Day2 pain scores significantly and inversely correlated with peak kyotorphin levels on Day1($r=0.71, p=0.05$).

Conclusions:Intravenous-arginine therapy increased plasma arginine concentration 2-5X above baseline peaking within 1-hour of infusion. We report for the first time the impact of intravenous-arginine on kyotorphin levels in SCD. This may represent a novel opioid-sparing mechanism-of-action of arginine with implications for pain syndromes beyond SCD.

Poster

The G6PD A- variant is not associated with pulmonary hypertension phenotypes

Qinzi Xu[1], Mouna Ouchari[1], Akueba Bruce[1], Ling Wang[2], Mark Gladwin[1], Elizabeth Rochon[1]

[1] University of Maryland, Baltimore, [2] University of Iowa

Background: Intravascular hemolysis decreases nitric oxide (NO) bioavailability and generates reactive oxygen species (ROS) resulting in vascular dysfunction. The pulmonary vasculature is highly sensitive to NO signaling and hemolysis is linked to pulmonary hypertension (PH), a condition characterized by narrow, hyperproliferative pulmonary vasculature, elevated pulmonary pressures and consequent right heart dysfunction. In RBCs, the pentose phosphate pathway is responsible for generating reduced NADPH, the electron carrier required for glutathione reduction, a major RBC antioxidant. G6PD is the rate limiting enzyme in this pathway and individuals deficient for G6PD experience intravascular hemolysis resulting from a decreased capacity to buffer ROS.

Purpose: Determine if mice harboring the humanized G6PD A- mutation have increased propensity to develop pulmonary hypertension.

Methods: G6PD A- SNP mice were exposed to hypoxia for 3 weeks or injected twice weekly with primaquine, an antimalaria that induces oxidative hemolysis in G6PD-deficient individuals. Pulmonary pressures were measured by right heart catheterization and Fulton index was determined as a measure of right heart remodeling.

Results: G6PD A- mice had normal pulmonary pressures in normoxic conditions and experienced increased pulmonary pressures equivalent to WT siblings following hypoxia. Following primaquine exposure, G6PD A- mice did not have elevated pulmonary pressures or increased Fulton index.

Conclusions: G6PD A- mice do not have a heightened susceptibility to developing PH following hypoxia or repeated incidences of mild intravascular hemolysis. While some hemolytic anemias are plagued by pathological pulmonary vascular remodeling, these observations are in line with a paucity of data associating G6PD deficiency with PH.

Poster

Loss of Sec14l4 renders red cells more susceptible to oxidative stress and storage hemolysis

Mouna Ouchari[1], Akueba Bruce[1], Ling Wang[2], Mark Gladwin[1], Elizabeth Rochon[1]

[1] University of Maryland, Baltimore, [2] University of Iowa

Background: Intravascular hemolysis decreases nitric oxide (NO) bioavailability via scavenging by cell-free hemoglobin/heme and reduced NO production, attributed to Arginase-1 depletion of L-Arginine, the obligate substrate of NOS. Sickle cell disease (SCD) is characterized by severe chronic hemolysis, leading to excess cell-free hemoglobin which promotes oxidative damage. Genetic modifiers of red blood cell (RBC) function contribute to variation in SCD phenotype severity and steady-state hemolysis. Single nucleotide polymorphisms (SNPs) in Sec14l4, a gene of undetermined function, was identified in a genome-wide association study as heritable mediators of oxidative stress in red cells.

Purpose: Determine the phenotypic consequences of Sec14l4 on RBC integrity.

Methods: Whole body SNP and knockout mouse models were generated using CRISPR/Cas9. In vitro hemolysis assays were performed on human recall donors and mouse SNP or knockout models to evaluate fresh and stored RBC stability with and without oxidative stressors.

Results: Mice with the humanized SNP had decreased Sec14l4 expression. Decreased Sec14l4 has no effect on hematopoietic differentiation, complete blood count and reticulocytes. Sec14l4 KO red cells had increased hemolysis following storage and oxidative stress exposure. Donors homozygous for Sec14l4 SNPs saw trends towards increased in vitro hemolysis and increased echinocyte formation following cold storage.

Conclusions: RBCs with decreased Sec14l4 expression are more likely to lyse during cold storage. While Sec14l4 does not influence intravascular hemolysis at baseline, it likely plays a role in RBC oxidative detoxification. Given the increased oxidative load experienced by SCD RBCs, loss of Sec14l4 may further exacerbate chronic hemolysis and worsen symptom severity.

Poster

Exploring the role of Nitric Oxide (NO) priming in increasing medicinally important secondary metabolites production in plants

Ankita Sehrawat[1]
[1] Maitreyi College, University of Delhi

NO regulates various processes of plant growth and development, including seed dormancy, root growth, flowering, fruit ripening and senescence via post-translational modifications (PTMs) such as S-nitrosyltion and protein tyrosine nitration. Priming is an immunity-like response by prior stimulation. Prominent function of “NO priming” in providing resistance from abiotic and biotic stress is known. However, little information is available regarding the role of “NO priming” in enhancing medicinally important secondary metabolites compounds such as glucosinolates (rich in Brassica sp.), flavonoids, carotenoids, phenolics, alkaloids, ginseng, taxol, and catharanthine. As the natural yield of secondary metabolites by plants is very low, to meet the commercial requirement, there is a great need to understand the mechanism of action of NO in regulating therapeutically important compounds in plants.

Poster

The role of nitric oxide-mediated glutamatergic alterations in the Shank3 Δ4-22 mouse model of autism

Shelly Ginzburg[1], Haitham Amal[1]

[1] The Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy, Ein Karem

Background: SHANK3 gene is mutated in 1% of autism spectrum disorder (ASD) cases. SHANK3 codes for a major scaffolding protein in glutamatergic neurons having central role in post-synaptic density structure and stability. The Shank3Δ4□22 mouse model exhibits the major behavioral phenotype of ASD and is used in this work. Previously we showed that nitric oxide (NO) signaling is impaired in ASD mouse models and patients carrying the mutation, however the mechanism causing excessive NO is unknown.

Purpose: Our goal is to decipher the mechanism causing the NO overproduction. We hypothesize that a mutation in SHANK3 gene causes imbalance in the glutamatergic system which could potentially lead to the ASD-related behavioral deficits.

Methods: We preformed biochemical and pharmacological experiments using Shank3 mouse model and SH-SY5Y cell line to test our hypothesis.

Results: We discovered dysregulation of the NMDA and AMPA receptors expression in brain of mutant mice. Selective nNOS inhibitor, reversed the glutamatergic alterations in the mutant mice. To understand how exactly NO affects different proteins, we focused on PSD-95 protein, which may be affected by NO levels. Immunoprecipitation assay showed that in the KO mice, there is over-ubiquitination of PSD-95, reversed by nNOS inhibition, suggesting that NO causes poly-ubiquitination and excessive protein degradation. Using SH-SY5Y cells with SHANK3 deletion, we showed that a selective antagonist to specific NMDA receptors inhibits nNOS activity and NO production.

Conclusion: we deciphered novel crosstalk mechanism between NO and the glutamatergic system, which may lead to the discovery of novel drug targets for ASD.

Poster

Lactobacillus in the oral microbiome blunts the conversion of nitrate to nitrite

Stephen Baker[1], Daniel Kim-Shapiro[1], Jesse Gao[1], Hariom Yadav[2]
[1] Wake Forest University, [2] University of South Florida

Background: Nitric Oxide is a vital signaling molecule that aids in vasodilation, reduces platelet activation, enhances angiogenesis, and protects against ischemic reperfusion injury. Nitrite therapeutics, derived from nitrate-rich foods like leafy greens and beet juice, promote NO production through a cycle involving oral bacteria and the salivary glands.

Purpose: To determine the effect lactobacillus, a healthy gut bacterium typically found in yogurt, has on the conversion of nitrate to nitrite in the oral microbiome.

Methods: Lactobacillus (paracasei, rhamnosus, reuteri, and plantarum) from stocks are grown overnight in MRS broth at 37 ºC in both aerobic and anaerobic environments. The following morning, nitrate is added to fresh donor saliva samples and cocultured in vitro with either lactobacillus, the supernatant from freshly grown lactobacillus, or MRS broth alone at 37 ºC. After one hour of incubation, samples are diluted in 100% methanol and centrifuged for 10 minutes before measuring nitrite and nitrate levels using a NOx analyzer.

Results: The presence of lactobacillus bacteria or supernatant severely blunted the conversion of nitrate to nitrite under both aerobic (>96% inhibition) and anaerobic (>98% inhibition) conditions. In contrast, a significant amount of nitrate is reduced in the presence of MRS broth alone under both aerobic (>35% reduced) and anaerobic (>90% reduced) conditions.

Conclusions: We have determined that the presence of lactobacillus or its supernatant when cocultured with salvia in vitro severely blunts the conversion of nitrate to nitrite. Ongoing work is aimed at determining what is excreted by the bacteria that is blocking this conversion.

Poster

Maternal exercise on fetal heart development during pregestational diabetes: Role of eNOS

Qingping Feng, Ryleigh van Neck, Xiangru Lu

Background: Pregestational diabetes (PGD) increases the risk of congenital heart defects (CHD) by more than five-fold. Maternal exercise enhances endothelial nitric oxide synthase (eNOS) function, which benefits developing embryos, however the causal relationship has not been established. We hypothesize that eNOS mediates the protective effects of maternal exercise in reducing the incidence of CHDs in fetuses exposed to PGD.

Methods: Adult eNOS+/- female mice with diabetes induced by streptozotocin were bred with healthy males, then placed in a cage with or without a running wheel (exercise). Fetuses were collected on embryonic day 18.5 and genotyped. Heart morphology was assessed for CHDs.

Results: Maternal exercise lowered mortality rate and litter size to control levels in offspring of diabetic eNOS+/- dams ($p<0.05$). PGD eNOS+/- female in mice induced CHDs including atrial septal defects, ventricular septal defects, atrioventricular septal defects, bicuspid aortic valve, and double outlet right ventricle in E18.5 fetuses. Additionally, craniofacial defects such as exencephaly and cleft lip were also seen in the fetuses. Notably, maternal exercise did not reduce the incidence of CHD or craniofacial defects in offspring of eNOS+/- females with diabetes when compared to control. Offspring genotype (wildtype or eNOS+/-) had no significant impact on CHD incidence.

Conclusion: Haploinsufficiency of eNOS does mitigate the benefits of maternal exercise on fetal heart development during pregestational diabetes in mice.

Poster

The role of Cytoglobin in cardiac development

Adam Clark[1], Akueba Bruce[1], Paola Corti[1]

[1] University of Maryland School of Medicine

Introduction: Cytoglobin is a heme-containing globin protein, but unlike other globins cytoglobin does not appear to facilitate oxygen transport. Rather, cytoglobin is postulated to regulate nitric oxide (NO) bioavailability, potentially working in concert with nitric oxide synthase to upregulate NO production. The role of cytoglobin as a NO regulator is supported by observations of reduced NO and cyclic guanosine monophosphate levels in the zebrafish cytoglobin knockout (cygb2801a). Intriguingly, cygb2801a also displays cardiac laterality abnormalities rescuable by NO administration. These observations suggest cytoglobin regulated NO signaling is an important contributor to vertebrate cardiac development.

Purpose: This study aims to gain greater understanding of the nuanced network of NO mediated signaling events required for proper heart formation, with the greater goal of contributing to improvements in the prevention of congenital heart defects.

Methods

Mutants and transgenics

The zebrafish cytoglobin knockout mutant (cygb2801a); generated by CRISPR/Cas9. The zebrafish transgenic line tg(myl7:gfp)

Microscopy

Leica FC165, Zeiss LS7, Nikon W1

Results

1. Cytoglobin expression is high in heart valves of zebrafish, mouse, and human.
2. Cygb2801a embryos have decreased NO signaling and disrupted heart laterality.
3. Cygb2801a larvae have decreased ventricle size and stroke volume, independent of heart laterality.
4. NO administration can rescue cardiac laterality defects and reduced stroke volume.
5. Cygb2801a larvae have disrupted expression of cardiomyocyte specific markers and altered heart morphology.

Conclusions: Cytoglobin knockout in zebrafish results in a disruption of NO signaling events during embryonic development that lead to defects in heart structure, morphology, and function.

Poster

Dietary nitrate and nitrite intake and the risk of gastric and colorectal cancers in a Swedish adult population – A population-based cohort study

Ana Rodrigues[1], Jon Lundberg[2], Eddie Weitzberg[2], Agneta Åkesson [1], Emilie Helte[1]
[1] Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [2] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Background: Dietary nitrate and nitrite can through endogenous nitrosation give rise to carcinogenic N-nitroso compounds, potentially affecting the risk of gastrointestinal cancer. The epidemiological evidence is limited.

Objective: This population-based prospective cohort study investigated the association between nitrate and nitrite intake and the incidence of colorectal (CRC) and gastric cancer (GC) in Swedish middle-aged to elderly women and men.

Methods: Using a food frequency questionnaire combined with food content combined with drinking water monitoring data, total dietary exposure to nitrate and nitrite was assessed in 82,031 men and women in two population-based cohorts. Participants were categorised into sex-specific quintiles of nitrate and nitrite exposure and incident CRC and GC cases were ascertained using the Swedish Cancer Register.

Results: During an average follow-up time of 19 years, 3,015 cases (1,562,857 person-years) and 401 cases (1,578,365 person-years) of CRC and GC were ascertained, respectively. No associations between dietary nitrate intake and cancer risk were observed. A statistically non-significant tendency towards increased GC, but not CRC risk was observed with increasing dietary nitrite intake in both men and women. At the 4th quintile of nitrite exposure, women with vitamin C consumption below the median had a significant increased GC risk (HR=2.23; 95% CI: 1.08, 4.59).

Conclusion: While the findings suggest that nitrate intake does not influence any of the cancer forms studied, the results for nitrite intake was more uncertain concerning the GC risk. Especially the potential effects of low antioxidant intake together with nitrite exposure needs further evaluation.

Poster

Variations in Blood Methemoglobin Concentration with Different Nitric Oxide Delivery Techniques as a Marker of Nitric Oxide Absorption

Stefano Cenci[1,2], Kyle J. Medeiros[1,2], Regina Villalobos[1,2], Giovanni Bruno[1,2], Binglan Yu[1,2], Tilo Winkler[1,2], Roberto Nardini[1], Edward Bittner[1,2], Fumito Ichinose[1,2], Lorenzo Berra[1,2]

[1] Massachusetts General Hospital, [2] Harvard Medical School

Background: Inhalation of Nitric oxide (NO) gas is a selective pulmonary vasodilator. Recently, NO gas has been used to enrich the bloodstream with NO metabolites to target extrapulmonary benefits, such as reducing postoperative kidney and liver dysfunction. During NO breathing, clinicians monitor the production of methemoglobin (MetHb) in the blood, which is unable to carry oxygen.

Purpose: We evaluated MetHb as a potential marker of NO absorption into the blood when delivered with different methods.

Methods: This is a sub-analysis of a single-center, randomized, controlled trial. Patients undergoing cardiac surgery were delivered NO to the blood with the cardiopulmonary bypass (CPB) oxygenator. Afterwards, NO was administered to the lungs with mechanical ventilation and nasal cannulas after extubation.

Results: 105 patients received 80 ppm of NO for 24 hours. Mechanical ventilation resulted in higher maximum MetHb levels compared to CPB and to nasal cannulas (median 2.7 vs 1 vs 1.7%, p-value < 0.005). The rate of MetHb increase was higher when NO was delivered with mechanical ventilation compared to CPB (p-value < 0.05). The estimated NO delivery rate was higher during mechanical ventilation compared to CPB (26.40 vs 9.77 μmoles/min, p-value = 0.001).

Conclusion: This study shows that different methods of NO delivery affect MetHb levels and are associated with different NO delivery rates and systemic absorption. Future studies targeting the extrapulmonary benefits of NO therapy should evaluate MetHb monitoring as a marker of NO delivery in the blood.

Poster

Relationship between nitric oxide biomarkers and physiological outcomes following dietary nitrate supplementation in humans

Chenguang Wei, Anni Vanhatalo[1], Matthew Black[1], Jamie Blackwell[1], Raghini Rajaram[1], Stefan Kadach[1], Andrew Jones[1]
[1] University of Exeter

Background: Plasma nitrite (NO_2^-) is the most oft-used biomarker of nitric oxide (NO) bioavailability, however, other NO biomarkers might also contribute to the physiological benefits of dietary nitrate (NO_3^-) ingestion.

Purpose: To investigate the relationships between various NO biomarkers (NO_3^-, NO_2^-, S-nitrosothiols (RSNOs)) in different blood compartments and skeletal muscle and the physiological effects of dietary NO_3^- ingestion.

Methods: Using a randomized, double-blind, crossover design, 12 participants consumed NO_3^--rich beetroot juice (BR) (~12.8 mmol NO_3^-) and NO_3^--depleted placebo beetroot juice (PL) acutely and then chronically for two weeks. Biological samples were collected, resting blood pressure (BP) was assessed, and 10 maximal voluntary isometric knee extensions were performed at 2.5-3.5 hours following supplementation on day 1 and day 14.

Results: Diastolic BP was significantly lower in BR (-2 ± 3 mmHg, P=0.03) following acute supplementation, while the rate of torque development (RTD) was significantly greater in BR at 0-30 ms (39 ± 57 N.m.s-1, P=0.03) and 0-50 ms (79 ± 99 N.m.s-1, P=0.02) than PL following two weeks supplementation. Greater whole blood [RSNOs] was correlated with lower diastolic BP (r=-0.68, P=0.02) following acute supplementation, while greater skeletal muscle [NO_3^-] was correlated with greater RTD at 0-30 ms (r=0.64, P=0.03) in BR than PL following chronic supplementation.

Conclusion: [RSNOs] in blood, and [NO_3^-] in skeletal muscle, are relevant NO biomarkers which are related to the BP reduction and the enhanced muscle contractility following dietary NO_3^- ingestion in humans.

Poster

Omeprazole blunts oral nitrite treatment-induced increases in plasma nitrosylated species and attenuation of cardiac MMP-2 activity and hypertrophy in hypertensive rats

Thaís Ribeiro Vitorino [1], Rose Inês Matos Batista[2], Sandra de Oliveira Conde Tella[1,2], Ana Karoline Lima da Silva[1], Elen Rizzi[3], José Eduardo Tanus dos Santos[1,2]

[1] Department of Pharmacology, Faculty of Medical Sciences, University of Campinas, UNICAMP, Brazil, [2] Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, USP, Brazil, [3] Unit of Biotechnology, University of Ribeirao Preto, UNAERP, Brazil

Introduction: Oral nitrite administration may result in beneficial effects in hypertension attributable to increased nitric oxide (NO) concentrations. Omeprazole treatment may affect NO metabolites tissue accumulation and modify oral nitrite-induced effects.

Purpose: To examine whether treatment with oral nitrite attenuates cardiac MMP-2 activity and hypertrophy in spontaneously hypertensive rats (SHR) and whether co-treatment with omeprazole affects these responses.

Methods: SHR and Wistar rats were treated with sodium nitrite (15 mg/kg p.o.) or vehicle and omeprazole (3.4 mg/kg i.p.) or vehicle for 6 weeks and systolic blood pressure (SBP) was assessed. Cardiac hypertrophy was assessed by cardiomyocyte diameter. MMP-2 activity was assessed by in situ zymography. The concentrations of NO-related species were determined in plasma and in the heart by ozone-based chemiluminescence techniques.

Results: Nitrite reduced SBP in SHR whereas co-treatment with omeprazole prevented this effect and increased gastric pH. Nitrite treatment increased plasma and cardiac nitrite, nitrate, and nitrosylated species (RxNO) concentrations in SHR and Wistar rats. Co-treatment with omeprazole tended to attenuate nitrite-induced increases in plasma RxNO concentrations in SHR. Nitrite treatment reduced cardiac MMP-2 activity and cardiomyocyte diameter in SHR, and co-treatment with omeprazole prevented these effects.

Conclusions: Our results suggest that nitrite treatment decreased cardiac MMP-2 activity and hypertrophy found in SHR. This effect was attenuated by omeprazole and associated with lower plasma RxNO concentrations. Together, these results show that omeprazole may attenuate oral nitrite induced increases in RxNO concentrations and prevent beneficial cardiovascular effects associated with oral nitrite treatment.

Poster

Improving the validity of biochemical assays of nitric oxide biomarkers in human skeletal muscle tissue samples

Raghini Rajaram [1], Matthew Black[1], ChenGuang Wei[1], Paul Winyard[1], Anni Vanhatalo[1], Andrew Jones[1]
[1] University of Exeter

Background: Valid measurements of nitrate and nitrite concentrations in human skeletal muscle are fundamental to further the research on the role of human skeletal muscle in NO homeostasis. PURPOSE: To investigate the effects of saline wash and processing with nitrite-preserving stop solution – to remove the effects of haemoglobin from blood surrounding the tissue – on human skeletal muscle nitrate and nitrite concentrations.

Methods: Skeletal muscle tissue was collected from 25 healthy adults and processed with and without saline wash, and with and without nitrite-preserving stop solution.

Results: Tissue samples washed in saline had significantly lower nitrate concentrations and lower inter-individual variability (45 ± 23 nmol/g) compared to tissue samples not washed in saline (104 ± 52 nmol/g), with no significant correlation ($r = -0.09$, $p > 0.05$) and high systematic bias (Bias = - 58.70, SD = 48.72) between the concentrations measured by the two methods. The nitrite concentrations were not significantly different between tissue samples washed with saline (1.94 ± 0.90 nmol/g) and samples not washed in saline (1.55 ± 0.86 nmol/g), with no significant correlation ($r = 0.45$, $p > 0.05$) and minimal systematic bias (Bias = 0.39, SD = 0.85) between the concentrations measured by the two methods. Addition of nitrite-preserving stop solution did not cause significant differences in concentrations of nitrate or nitrite in these samples.

Conclusion: Saline wash could improve the validity of nitrate and nitrite concentrations measured in human skeletal muscle.

Poster

Nitric oxide-driven metabolic and functional regulation in classically activated macrophages

Nicholas Arp[1,2,3], Uzziah Urquiza[1,4], Steve John[1,3], Jing Fan[1,3,4,5]
[1] Morgridge Institute for Research, [2] University of Wisconsin School of Medicine and Public Health Medical Scientist Training Program, [3] University of Wisconsin-Madison Cellular and Molecular Biology Program, [4] University of Wisconsin-Madison Integrated Program in Biochemistry, [5] University of Wisconsin-Madison Department of Nutritional Sciences

Macrophages, innate immune cells important for inflammatory responses, rewire their metabolic pathways to support their immune functions. Macrophages exposed to pathogen associated molecular patterns, such as lipopolysaccharide, and pro-inflammatory cytokines, such as interferon-gamma, expression of inducible nitric oxide synthase (iNOS), which converts arginine to citrulline and nitric oxide (NO), is induced. NO production is important for classically activated macrophages. In addition to its role in direct pathogen killing, NO is known to have a myriad of intracellular effects through mechanisms involved in cellular signaling, gene expression, and protein post-translation modifications. Key knowledge gaps remain regarding the molecular targets of NO, mechanisms of its regulation, and its specific impacts on the metabolic and functional rewiring. Through metabolic profiling of macrophages with or without iNOS, we identified a NO-dependent post translational modification driving the inhibition of the alpha-ketoacid dehydrogenase complexes: pyruvate dehydrogenase complex (PDHC), oxoglutarate dehydrogenase complex (OGDC), and branched chain amino acid dehydrogenase complex (BCKDC). Such NO-driven inhibition is critical for the metabolic and functional transition from early to late stage in classical activation. Furthermore, we investigated the mechanisms controlling the NO-mediated metabolic and functional changes, the maintenance of these changes within appropriate physiologic range, and the reversibility of NO-mediated effects. Enzymes modulating low molecular weight thiol containing compounds are known to play key roles in the regulation of NO. Therefore, current work using unbiased metabolomics, genetic perturbation, stable isotopic tracing, and biochemical assays seeks to uncover the role of these enzymes in regulating this NO-driven dynamic metabolic and functional rewiring.

Poster

Red blood cell (RBC) eNOS couples RBC phenotype to O2 gradients during circulatory transit

Stephen Rogers[1], Zohreh Safari[1], Mary Brummet[1], Paisley Maloney[1], Jacqueline Nguyen[1], Sarah Enjati [1], Aishwarya Anand[1], Emioluwa Odunaiya[1], Qihong Wang[1], Tobi Rowden[1], Daniel Bennett[1], Giuliano Scarcelli[2], Yogeshwari Sanjayrao[2], Angelo D'Alessandro[3], Aaron Issaian[3], Monika Dzieciatkowska[3], Fabia Gamboni[3], Miriam Cortese Krott[4], Brant Isaksson[5], Luke Dunaway[5], Allan Doctor[1]

[1] University of Maryland School of Medicine, Baltimore, MD, USA, [2] University of Maryland College Park, MD, USA, [3] University of Colorado Denver – Anschutz Medical Campus, Aurora, CO, USA, [4] Heinrich-Heine-University of D sseldorf, Germany, [5] University of Virginia, Charlottesville, VA, USA

Background: During circulatory transit, red blood cells (RBCs) respond to oxygen (O2) gradients to efficiently maintain O2 delivery homeostasis. This is achieved via O2 responsive regulation of energy metabolism, blood rheology (deformability), HbO2 capture/release (Bohr effect), and blood flow (via release of vasoactive compounds) – all of which can be modulated by nitric oxide (NO). Here, we explore the role of RBC endothelial nitric oxide synthase (eNOS) derived NO in regulating these essential homeostatic responses.

Purpose: Define regulation of RBC features relevant to O2 delivery homeostasis by RBC eNOS.

Methods: Wild type (C57BL/6J), global eNOS(-/-), RBC specific eNOS(-/-) mice, and/or heparinized whole blood/washed RBCs from these mice were analyzed. We assessed; (1) glucose metabolic flux (metabolomics), (2) RBC cytoskeletal morphology (3D imaging), (3) RBC deformability (Brillouin microscopy), (4) HbO2 affinity/Bohr effect (tonometer with dissolved gas analysis and Hb co-oximetry), (5) the hypoxic vasodilatory reflex (vascular ring bioassay), and (6) exercise tolerance (treadmill).

Results: We observed eNOS mediated O2 responsive regulation of (1) RBC metabolic poise, with significantly reduced HMP flux in the global eNOS(-/-) mice. Furthermore, global eNOS(-/-) mice demonstrated (2) impaired RBC cytoskeletal assembly/disassembly, resulting in (3) reduced RBC deformability, (4) enhanced HbO2 affinity and reduced Bohr effect, in addition to (5) a blunted hypoxic vasodilatory reflex. The culmination of these findings was significantly impaired (6) exercise tolerance in RBC specific eNOS(-/-) mice.

Conclusions: This work identifies RBC eNOS as a key regulatory element in O2 delivery homeostasis, by coupling RBC phenotype to O2 gradients during circulatory transit.

Poster

Intraarterial infusions of the novel ultra-fast-releasing nitric oxide donor nitrosooxypropanol (PDNO) to augment organ blood flow

Kristofer F. Nilsson[1,2], Anna Stene Hurtsén[1,2], Madelene Lindkvist[1], Magnus Grenegård[1], Måns Edström[3]
[1] School of Medical Sciences, Örebro University, Örebro, Sweden,[2] Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine and Health, Örebro University, Örebro, Sweden,[3] Department of Anesthesiology and Intensive Care, Faculty of Medicine and Health, ÖrebroUniversity, Örebro, Sweden

Background: Intraarterial administration of a nitric oxide (NO)-donor with short half-life may limit the effects to the targeted organ and minimize systemic side effects. Nitrosooxypropanol (PDNO) is a novel ultra-fast-releasing organic nitrite NO donor with a short, but unknown, half-life.

Purpose: To compare intraarterial and intravenous infusions of PDNO regarding organ blood flow and systemic effects, and to estimate the half-life of PDNO.

Methods: Intraarterial infusions of PDNO (0.01-3000 nmol/kg/min) in common carotid artery (CCA), common femoral artery (CFA), and superior mesenteric artery (SMA) were compared to intravenously administered PDNO (3-100 nmol kg/min) in anesthetized pigs (n=14). Regional blood flow, mean arterial pressure (MAP), and end-tidal nitric oxide (ETNO) were measured.

Results: Compared to baseline, CFA and CCA blood flow increased from 1 nmol/kg/min ($p<0.05$), and SMA blood flow increased from 30 nmol/kg/min ($p<0.05$), during the respective intraarterial infusions. Intravenous infusions did not affect organ blood flow ($p>0.05$). MAP decreased from 10 nmol/kg/min during intravenous infusions, and from 30, 100 and 3000 nmol/kg/min during CCA, CFA and SMA infusions, respectively. The dose equivalents for ETNO increase comparing intravenous administration (reference) and CFA, CCA, and SMA infusions were 2.7, 2.9, and 47, respectively. The in vivo half-life of PDNO was 3.0-6.1 s.

Conclusions: Organ-directed intraarterial infusions of PDNO increased organ-specific blood flow dose-dependently, with minor systemic effects at effective doses, probably due to the very short half-life. Next, intraarterial infusions of PDNO in disease models with compromised organ blood flow will be investigated.

Poster

Red blood cell (RBC) endothelial nitric oxide synthase (eNOS) is activated by O2 gradients during circulatory transit

Zohreh Safari [1], Stephen C Rogers[1], Mary Brummet[1], Qihong Wang[1], Tobi Rowden[1], Shilpa Dilip Kumar[2], Joseph Mauban[2], Thomas Blanpied[2], Angelo D'Alessandro[3], Brant Isakson[3], Monika Dzieciatkowska[3], Miriam Cortese Krott[4], Allan Doctor[1]

[1] Department of Pediatrics & Center for Blood Oxygen Transport and Hemostasis , University of Maryland School of Medicine, USA, [2] University of Maryland School of Medicine, USA, [3] Department of Biochemistry and Molecular Genetics at the University of Colorado Denver, USA, [4] Heinrich Heine University of D sseldorf , D sseldorf, Germany.

Background: Mature RBCs harbor functional eNOS; however, the mechanism of eNOS activation is undefined. Given that RBC phenotype features essential to O2 delivery homeostasis are regulated by bioavailability of nitric oxide (NO) equivalents as a function of hemoglobin (Hb) oxygen(O2) saturation, we investigated the relationship between circulatory O2 gradients and RBC eNOS activation.

Purpose: Determine mechanistic links between O2 gradients and RBC eNOS activation.

Methods: Human, wild-type (C57BL/6J), and global eNOS(-/-) murine RBCs were subjected to oxygenation/deoxygenation, with/without pre-treatment(s) and imaged (routine and super-resolution microscopy). Protein interactions were determined using a proximity ligation assay (Duolink) and RBC eNOS phosphorylation by in-cell western blot. Intracellular RBC calcium and NO levels were quantified via fluorescence (calcium probe Fluo-3 and NO probe DAF-FM). PBZyn tagged S-nitrosylated proteins were analyzed via mass spectrometry.

Results: RBC deoxygenation resulted in Piezo1 activation, calcium entry, with eNOS, PKCα, and protein 4.1 migration to the membrane in proximity to Band3. Calmodulin interaction and phosphorylation by PKCα caused eNOS activation, which was dependent on calcium and Hb conformational transition. Additionally, we also observed a significantly different S-nitrosylation pattern in human, wild-type, and global eNOS(-/-) RBCs under oxygenated and deoxygenated conditions, suggesting that regulation of RBC phenotype by eNOS occurs, at least in part, through reversable S-nitrosylation of key regulatory proteins.

Conclusion: Upon circulatory transit, RBC deoxygenation triggers eNOS activation, which is directly linked to: Hb conformational transition, Piezo1-mediated calcium flux, eNOS migration to the B3 metabolon (assembling with calmodulin and PKCα), which results in S-nitrosylation of RBC proteins.

Poster

Two novel dinitrate ester bearing piperazinyl-purine analogues (MK177 and MK222) elicit nitric oxide dependent and independent effects on the cardiovascular system

Magnus Grenegård[1], Kristofer Nilsson[1], Karin Fransén[1], Madelene Lindkvist[1], Marianne Haug[1], Josef Jakobsson[1], Eva Lindström[2], Petter Hedlund[2], Maria Zervou[3], Theano Fotopoulou[3], Maria Koufaki[3]
[1] Örebro University,[2] Linköping University, [3] National Hellenic Reserach Foundation

Introduction: mononitrate ester bearing 6-piperazinyl-purine analogues (MK drugs) are cardioprotective in myocardial infarction animal models and act as inhibitors of Janus kinase (JAK) and Rho-associated kinase (ROCK). Despite the presence of a nitrate ester moiety, MK drugs do not release nitric oxide (NO). To further improve the therapeutic potential, we synthesized two dinitrate ester bearing analogues and characterized these drugs (denoted MK177 and MK222) in various experimental models.

Methods: We utilize organic chemistry platforms to synthesis, purify and characterize MK177 and MK222. Furthermore, several cell-free, cellular and tissue assays as well as animal models were utilized to elucidate drug activities in vitro and in vivo.

Result: In anesthetized piglets, intravenous infusion of MK177 produced “nitroglycerin-like” effects on vital parameters (e.g. reduction of mean arterial blood pressure). Analysis of exhaled air confirmed release of NO. Both MK177 and MK222 caused relaxation of iliac arteries in vitro, and this effect was mediated by NO/cyclic GMP signalling. In suspensions of isolated platelets, MK177 and MK222 exhibited antiplatelet activity by targeting ROCK in a NO-independent manner. Conversely, the drugs did not cause NO/cyclic GMP-dependent phosphorylation of vasodilator-stimulated phosphoprotein. In LPS-stimulated monocyte-like THP-1 cells, MK222 induced a significant inhibition of monocyte-chemoattractant molecule (MCP)-1 release. In comparison, the anti-inflammatory activity of MK177 was more modest.

Conclusion: We have successfully developed two bifunctional piperazinyl-purine analogues. These drugs act by both NO-dependent and NO-independent mechanisms and induce significant vasorelaxant, antiplatelet and anti-inflammatory effects. We suggest that MK177 and MK222 might have promising therapeutic potential in ischemic disease.

Poster

Elucidating antiplatelet activity of the novel nitric oxide-donor Nitrosooxypropanol (PDNO)

Magnus Grenegård[1], Kristofer Nilsson[1]
[1] Örebro University

Introduction: The novel nitric oxide (NO)-donor Nitrosooxypropanol (PDNO) is an ultra-fast vasodilator and has been therapeutically evaluated in several cardiovascular diseases. The direct impact of PDNO on blood platelets has not yet been explored. Considering physiology and pharmacology, platelets are one of the most NO-sensitive cell types, and via the NO/cyclic GMP signaling pathway, pronounced antiadhesive and antiaggregatory effects are induced. It is however noteworthy that platelets respond weakly to clinically used NO-donors.

Methods: Effects of PDNO on human platelet aggregation and secretion were thoroughly investigated using residual platelet counting, impedance aggregometry in whole blood, light transmission aggregometry in platelet-rich plasma (PRP) and aliquots of isolated platelets. Molecular evidence of NO/cyclic GMP signaling was investigated by measuring cytosolic calcium mobilization and ser239-specific phosphorylated vasodilator-stimulated phosphoprotein (VASP).

Results: PDNO inhibited platelet activation in a concentration-dependent manner. The IC50-value was 3-8 μM depending on specific experimental conditions and methods used. The inhibitory effect of PDNO was equally pronounced using collagen, ADP or thrombin receptor hexapeptide agonist (SFLLRN) as stimulus of platelets. Pharmacological comparison revealed that PDNO was far more potent than the clinically used NO-donor glyceryltrinitrate (nitroglycerin). Measurements of cytosolic calcium and VASP phosphorylation confirmed activation of the NO/cyclic GMP signaling pathway. Interestingly, the effect of PDNO was surprisingly long-lasting, and even after wash-out, a significant antiplatelet activity was observed.

Conclusions: The novel NO-donor PDNO elicits significant platelet inhibitory capacity and has on that account, antithrombotic therapeutic potential. The exact mechanism underlying the long-acting effect of PDNO is presently unknown.

Poster

Lipid Droplets are Essential NO Carriers in Red Light Assisted Exocytosis of Vasodilatory Substances from the Endothelium

Dorothee Weihrauch, Agnes Keszler, Eva Aranda, Grant Broeckel, Brian Lindemer, Guihua Brown[1], Nicole Lohr[1]
[1] University of Alabama at Birmingham

Low level light therapy is a targeted non-invasive intervention that can be employed to alleviate serious cardiovascular conditions. When enzymatic production of critical vasodilator nitric oxide is compromised, red light application is a way to increase NO bioavailability locally and efficiently from various precursor molecules. In earlier works we established that 670 nm light dilates ex vivo murine blood vessels via exerting S-nitrosothiol and free NO comprising vasodilatory vesicles from the endothelium. We hypothesized that lipid droplets (LD), intracellular storages of triacylglycerols and sterol esters surrounded with a phospholipid monolayer, harbor a hydrophobic environment for NO and maintain its transport through the cellular endomembrane system. By using bovine aortic endothelial cell model, we investigated the LD traffic to Multi Vesicular Bodies (MVB), the destination of secretory materials. Expression of Caveolin-1 transmembrane protein which regulates LD metabolism in the endothelium was amplified after irradiation. Increased overlapping between NO marker DAF-2DA and LD marker LipidSpot in light exposed cells was found with immunocytochemistry. Overlap between LipidSpot and MVB-specific marker LBPA, and between DAF-2DA and LBPA was also enhanced after light treatment. Electron microscopy showed larger MVBs after light exposure, while flow cytometry data displayed more abundant presence of NO in extracellular vesicles isolated from irradiated cells. We captured the travel of LDs during and right after irradiation with live imaging using BODIPY fluorescent dye. Our results suggest that immediately after 2 min irradiation the red-light dependent exocytosis has already started, and increased levels of critical vasodilatory substances were assessed in MVB.

Poster

Red blood cells induce endothelial dysfunction in patients with ST-elevation myocardial infarction and elevated C-reactive protein

John Tengbom[1], Rawan Humoud[1], Eftychia Kontidou[1], Tong Jiao[1], Jiangning Yang[1], Ulf Hedin[2,3], Zhichao Zhou [1], Juliana Jurga[4], Aida Collado[1], Ali Mahdi[1,4], John Pernow[1,4]

[1] Division of Cardiology, Department of Medicine Solna, Karolinska Institutet, Karolinska Unviersity Hospital, Stockholm, Sweden., [2] Department of Vascular Surgery, Karolinska University Hospital, Stockholm, Sweden., [3] Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden., [4] Department of Cardiology, Karolinska University Hospital, Stockholm, Sweden.

Background: Red blood cells (RBCs) modulate cardiovascular function through nitric oxide bioactivity regulated by arginase. It is unknown how RBCs regulate vascular function in ST-elevation myocardial infarction (STEMI) and how this is influenced by inflammation.

Purpose: To investigate the effect of RBCs on endothelial function in patients with STEMI and whether their modulatory effect is associated with inflammation.

Methods: Blood samples were collected from patients with STEMI and 21 age-matched controls. Patients were grouped based on absence or presence of systemic inflammation defined as C-reactive protein (CRP) levels <2 mg/L and 2 mg/L (n=17 and n=20, respectively) at admission. RBCs were incubated with rat aortas for 18h with subsequent evaluation of endothelium-dependent and -independent relaxations.

Results: RBCs collected from patients with STEMI and elevated CRP impaired endothelium-dependent relaxation compared to RBCs from patients with STEMI and low CRP and healthy controls. Inhibition of arginase in RBCs or in the aortas prevented the development of endothelial dysfunction induced by RBCs from patients with STEMI and elevated CRP. Inhibition of reactive oxygen species (ROS) in the aorta also improved endothelial function. RBCs from patients with STEMI and elevated CRP had increased ROS compared to RBCs with low CRP and healthy controls. Arginase 1 and the oxidative stress marker 4-HNE were upregulated in the aorta.

Conclusion: RBCs from patients with STEMI and low-grade inflammation induce endothelial dysfunction by a mechanism dependent on arginase 1 and reactive oxygen species, suggesting the RBC as a potential therapeutical target in patients with STEMI and low-grade inflammation.

Poster

ATP9A in erythrocytes: a regulator of extracellular vesicle release and endothelial dysfunction in type 2 diabetes?

Rawan Humoud, Eftychia Kontidou, Otto Bergman, Maria Eldh, Per Eriksson, Susanne Gabrielsson, John Pernow, Aida Collado, Zhichao Zhou

Background: Red blood cells (RBCs) from individuals with type 2 diabetes (T2D-RBCs) induce endothelial dysfunction. However, the underlying mechanisms are not fully understood.

Purpose: To explore the role of ATP9A in T2D-RBCs in endothelial dysfunction with a focus on RBC-derived extracellular vesicles (EVs).

Methods: Bulk RNA-sequencing was conducted on human RBCs to analyze their transcriptomic profiles. EVs were isolated with the ExoEasy commercial kit, and their concentrations were measured by nanoparticle tracking analysis. EVs were incubated with mouse aortic rings to evaluate endothelium-dependent relaxation (EDR) with a wire myograph. Loss of function was achieved with GapmeR injection in db/db mice in vivo. Immunofluorescence (IF) and qPCR were used for expression analysis.

Results: ATP9A was identified as one of the most upregulated genes in T2D-RBCs compared to RBCs from healthy subjects (H-RBCs). This was further confirmed by qPCR and IF in both human and mouse RBCs. Previous studies demonstrated that ATP9A negatively regulates EV release in other cell types. Indeed, T2D-RBCs released fewer EVs (12.7±3.5*10^8) compared to H-RBCs (31.7±7.0*10^8), which also impaired EDR. RBCs isolated from db/db mice injected with GapmeR, that significantly reduced ATP9A protein expression levels in RBCs, exacerbated EDR compared to the vehicle group.

Conclusion: Upregulated ATP9A in T2D-RBCs is associated with lower concentrations of released EVs. EVs from T2D-RBCs induce endothelial dysfunction, which is further impaired by ATP9A inhibition. Future studies are needed to establish the regulatory mechanisms of ATP9A for EV release and possible compensatory mechanisms for endothelial dysfunction in T2D.

Poster

Inactivation of cystathionine γ-lyase by L- but not by D-CysNO

Yasuo Watanabe[1], Shoma Araki[1], Shingo Kasamatsu[2], Hideshi Ihara[2], Hidehiko Nakagawa[3], Takaaki Akaike[4], Yukihiro Tsuchiya[1]
[1] Showa Pharmaceutical University, [2] Osaka Prefecture University, [3] Nagoya City University, [4] Tohoku University

Background/Introduction: Cystathionine γ-lyase (CSE) is pyridoxal phosphate (PLP)-dependent enzyme responsible for the biosynthesis of cysteine from cystathionine in the final step of the transsulfuration pathway. CSE also plays an important role in the production of cysteine polysulfides (Cys-S-(S)n-H), identified as supersulfides, via metabolizing cystine under the pathological conditions. Interestingly, CSE itself has been identified as cysteine-based redox enzyme through S-nitrosylation at Cys136/171 residues.

Purpose: The present study aims to explore the mechanisms by which cysteine-based redox switches could regulate CSE supersulfides producing activity from cystine by S-nitrosyl L-cysteine (L-CysNO), but not by D-CysNO.

Methods: The CSE activity was analyzed using cystine as a substrate. These methods are based on fluorescence detection of supersulfides from cystine by sulfane sulfur probe 4 (SSP4).

Results: In vitro Incubation of CSE with L-CysNO resulted in a dose-dependent inhibition of supersulfides production which was NOT cancelled by reducing agent, dithiothreitol. Interestingly, the activity of CSE was not altered by pre-incubation with another NO donor such as S-nitrosoglutathione (GSNO) or D-CysNO, but being inhibited with co-incubation with cysteine. Furthermore, when PLP is eliminated from CSE/ L-CysNO premix, L-CysNO could not inhibit CSE activity, assuming that CSE metabolize L-CysNO and the metabolites inactivate the enzyme activity. Indeed, we detected thionitrous acid (HSNO) and pyruvate as primary products of CSE/ L-CysNO.

Conclusion: Thus, we identify L-CysNO as a substrate of CSE and its metabolites as inhibitors of the enzyme via novel irreversible modification at Cys136/171 residues.

Poster

Arginase 1 in erythrocytes is a critical modulator of cardioprotective signalling by nitric oxide and soluble guanylyl cyclase

Jiangning Yang[1], Jiangning Yang[1], Yahor Tratsiakovich[1], Tong Jiao[1], Ali Mahdi[1], John Tengbom[1], Aida Collado[1], Zhichao Zhou [1], John Pernow[1]
[1] Department of Medicine, Karolinska Institutet

Background: Arginase is regulating cardioprotective nitric oxide (NO) bioactivity competing with NO synthase (NOS) for their common substrate L-arginine. Erythrocytes are known to contain high levels of arginase that may reduce export of NO bioactivity.

Aim: To determine the role of arginase 1 in erythrocytes for cardiac protection.

Methods: A tie2 cre-flox mouse model, in which arginase 1 was deleted (Arg 1-KO) in hematopoietic and endothelial cells, was studied using in vivo ischemia-reperfusion model performed by left anterior descending coronary artery ligation. To determine the specific role of erythrocytes, erythrocytes from Arg 1-KO and wild type (WT) mice were given to isolated hearts from WT mice at the onset of global ischemia. After 40 min ischemia, the recovery of left ventricular developed pressure (LVDP) during 60 min reperfusion was recorded as an indicator of cardiac functional recovery.

Results: Following in vivo ischemia-reperfusion, infarct size was smaller in Arg 1-KO mice than in WT mice, which was abolished by the NOS inhibitor L-NMMA. In in vivo ischemia-reperfusion, there was no difference in LVDP between Arg 1-KO and WT hearts. When erythrocytes from Arg 1-KO mice were administrated to isolated WT mouse hearts, the post-ischemic recovery of LVDP was significantly improved compared to hearts given erythrocytes from WT mice, which was abolished by pre-incubation of the erythrocytes with the NOS inhibitor L-NAME or the inhibitor of soluble guanylyl cyclase (sGC), ODQ.

Conclusion: Arginase 1 in erythrocyte plays an important role in regulating cardioprotection mediated via the NO-sGC pathway.

Poster

Chronological aging regulated by supersulfides in yeast

SUNGHYEON YOON[1,2], Akira Nishimura[3], Tomoaki Ida[1], Minkyung Jung[1], Masanobu Morita[1], Tetsuro Matsunaga[1,4], Hiroshi Takagi[3], Hozumi Motohashi[5], Takaaki Akaike[1]

[1] Department of Environmental Medicine and Molecular Toxicology, Tohoku University Graduate School of Medicine, Sedai, Japan, [2] Department of Pharmacology, Showa Pharmaceutical University, Tokyo, Japan, [3] Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan, [4] Epidemiology and Molecular Pathophysiology of Infectious Diseases, Akita University, Akita, Japan, [5] Department of Molecular and Chemical Life Sciences, Tohoku University, Sedai, Japan

Background/Introduction: We have discovered that supersulfides, a class of sulfur compounds produced ubiquitously in various organisms, including primitive unicellular organisms, prokaryotes, eukaryotes, plants, and mammals, play a crucial role in numerous physiological functions. These versatile sulfur compounds, found in cells and tissues at sub-millimolar to millimolar levels, are now recognized as universal bioactive metabolites. Supersulfides engage in polysulfidation, a process vital for protecting protein thiol residues against irreversible oxidative modifications. This mechanism safeguards protein functionality in challenging oxidative environments, a key factor in the aging process.

Purpose/Method: We identified that the cysteinyl-tRNA synthetase (CARS) catalyzes a pyridoxal phosphate (PLP)-dependent biosynthesis of CysSSH, a supersulfide highly conserved across organisms. To explore CysSSH's function, we engineered mutant yeasts of CARS, which decreased the synthesis of CysSSH using a precise CRISPR/Cas9 system targeting the PLP-binding site. We quantified the active supersulfides in these mutants using the LC-ESI-MS/MS technique and compared their lifespan with wild-type yeast to understand the impact of CysSSH on aging.

Results/Conclusion: The results of this study have far-reaching implications. CARS mutant yeast, which exhibited decreased production of supersulfides, including CysSSH, also showed a significant reduction in chronological aging compared to the wild type. This study uncovers the complex relationship between CARS-mediated supersulfide generation, the CARS-mediated supersulfide oxidation process, and their collective impact on mitochondrial energy metabolism. Importantly, the decrease in aging was rescued by treatment with Na2S2, a supersulfide donor, suggesting the significant role of supersulfides produced by CARS in regulating yeast longevity.

Poster

Cystathionine γ-lyase self-inactivates by supersulfidation during cystine metabolism

Yukihiro Tsuchiya[1], Shoma Araki[1], Tsuyoshi Takata[2], Katsuhiko Ono[3], Tomohiro Sawa[3], Shingo Kasamatsu[4], Hideshi Ihara[4], Yoshito Kumagai[5], Takaaki Akaike[2], Yasuo Watanabe[1]

[1] Showa Pharmaceutical University, [2] Tohoku University, [3] Kumamoto University, [4] Osaka Metropolitan University, [5] Kyushu University

Background/Introduction: Cystathionine γ-lyase (CSE) is an enzyme responsible for the biosynthesis of cysteine from cystathionine in the final step of the transsulfuration pathway. It also has β-lyase activity toward cystine, generating cysteine persulfide (Cys-SSH), identified as supersulfides. The reactivity of supersulfides is involved in the catalytic activity of particular proteins via protein supersulfidation, the formation of -S-(S)n-H on their reactive cysteine residues. The Cys136/171 of CSE have been proposed to be redox-sensitive residues.

Purpose: We investigated whether CSE supersulfidation occurs at Cys136/171 during cystine metabolism.

Methods: The CSE β-lyase activity was analyzed using β-chloro-L-alanine (β-CA) or cystine as a substrate.

Results: The C136V and C136/171V CSE mutants exhibited higher β-lyase activity when cystine, but not β-CA, was used as the substrate. Meanwhile, the cysteine-producing γ-lyase activity of this mutant was equivalent to that of the wild-type enzyme. Transfection of wild-type CSE into COS-7 cells resulted in increased intracellular supersulfides production, which was significantly increased when C136V or C136/171V CSE mutants were transfected. In vitro incubation of CSE with CSE-enzymatically synthesized supersulfides resulted in the inhibition of supersulfides production. In contrast, C136V and C136/171V CSE mutants proved resistant to inhibition. A biotin-polyethylene glycol-conjugated maleimide capture assay revealed that CSE supersulfidation occurs at Cys136/171 during cystine metabolism.

Conclusion: We propose that CSE self-inactivates by supersulfidation at Cys136 during its β-lyase activity of cystine. Thus, the supersulfidation of CSE at Cys136 functions to down-regulate supersulfides synthesis by the enzyme.

Poster

Supersulfides protect lethal bacterial infection by enhancing bacterial killing functions of neutrophils

Azizur Rahman[1], Tianli Zhang[2], Touya Toyomoto[1], Hiroyasu Tsutsuki[1], Takaaki Akaike[3], Tomohiro Sawa[1]
[1] Kumamoto University, [2] Akita University, [3] Tohoku University

Supersulfides are endogenously occurring molecular species that have catenated sulfur chains in their structures. Because of their unique chemical properties, supersulfides contribute to the diverse biological functions such as antioxidant responses, anti-inflammatory responses, signal transduction, anti-aging, and respiratory energy metabolism (Br J Pharmacol 2023). It has recently been reported that supersulfides exhibit a potent host defense actions against virus infections (Nat Commun 2023). In this study, we studied the effects of supersulfides in bacterial infection using Staphylococcus aureus (S. aureus), a gram-positive bacterium. We used a lethal animal model in which mice were infected intraperitoneally with 7 x 107 bacterium. When these mice were intravenously administered the supersulfide donor NAC-S2 (Cell Chem Biol 2019) twice, 30 minutes and 3 hours after infection, the survival period was significantly extended compared to the untreated group. Neutrophils collected from NAC-S2 treated mice showed stronger bacterial killing compared to those obtained from untreated mice. In addition, intracellular glutathione (GSH) and its persulfide (GSSH) in neutrophils from mice pre-treated with NAC-S2 were significantly higher than neutrophils from untreated mice. On the other hand, when human PBL cells differentiated into neutrophil-like cells in vitro were infected with S. aureus, intracellular GSH and GSSH were significantly decreased compared to uninfected cells. These results indicate that neutrophils with increased intracellular supersulfide (e.g., GSSH) acquire stronger bactericidal ability. Further study is warranted to clarify the molecular basis of this enhanced bacterial killing to develop a new therapeutic approach based on supersulfide.

Poster

Inorganic Nitrate Supplements attenuates Acute Kidney Injury in mice with Western Diet

Huirong Han, Xiaojie Wang

Introduction: Acute kidney injury (AKI), the most common threat to hospitalized patient, is highly associated with overweight and metabolic disorder which are caused by the western diet as a major contributor. Dietary nitrate has been proven to be able to reverse several features of the metabolic syndrome. However, the mechanism of the impact of nitrate on AKI under the western diet remains unclear.

Purpose: To evaluate the effects of inorganic nitrate supplements on the development of ischemia/reperfusion (IR) injury induced AKI under the influence of the western diet, and its molecular mechanisms.

Methods: Eight-week-old C57BL/6 male mice, exposed to the western diet with/out nitrate supplements for 15 weeks, followed by unilateral kidney ischemia (45 mins) operation, with 7 days reperfusion.

Results: Firstly, the western diet did not directly cause renal dysfunction, but it increased fat content, fasting glucose and impaired glucose clearance, which were all attenuated by nitrate supplements. Secondly, IR operation induced renal dysfunction was ameliorated by nitrate supplements. Mechanistically, podocytes exposed to palmitate and treated with nitrite exhibited SREBP-1A expression resulted in the lipid synthesis process interruption; in glomerular endothelial cells nitrite reversed the dampen viability; and in kidney cortex, nitrate decreased the Caspase-3 expression and restored the COX IV level.

Conclusion: Inorganic nitrate supplements restore the metabolic disorder and protect the kidney function from acute kidney injury under the western diet, partly by interrupting lipid synthesis process, dampening the apoptosis and recovering the mitochondrial function.

Poster

Effects of clozapine, risperidone, and sodium nitroprusside on glutamatergic transmission in the hippocampus

Kent Jardemark[1], Oscar Jungholm[1], Joseph Bruton[1], Monica Marcus[1]
[1] Karolinska Institutet

Evidence indicates that schizophrenia is a neurodevelopmental disorder, including an interplay between genetics and the environment, leading to different stages of the illness, i.e. the premorbid, prodromal, progressive, and residual phases. In the prodromal phase, or in early stages of schizophrenia, an increased intrinsic activity in the hippocampus has been shown, During the prodromal stage, this hypermetabolism in the hippocampus CA1 region, with increased extracellular glutamate, may lead to atrophy of this brain region, which may contribute to the development of psychosis and hippocampal-related cognitive dysfunction. Thus, it is important with an early pharmacology-based treatment of the patients, optimally in the prodromal phase, since longer duration of psychotic periods has been shown to be associated to lower rates of symptom remission of the disease.

Here we have examined the ability of co-administration of sodium nitroprusside to modulate the effects of clozapine or risperidone on hippocampal glutamate receptor-mediated transmission, by using an extracellular electrophysiological recording technique in slices from rats.

Our results indicate that sodium nitroprusside (SNP) has the capability of inhibit the glutamatergic transmission in the CA1 region of the hippocampus. SNP also inhibited facilitating effects of both clozapine and risperidone on the glutmatatergic transmission in this brain region. These results indicate that SNP may be used as pharmacological treatment in the prodromal phase of schizophrenia, in order to downregulated the glutamate-induced atrophy of hippocampus, and that the combination of SNP and clozapine or risperidone would be useful for treatment of the early stages of the disease.

Poster

Breath analysis for supersulfide metabolome and disease-specific profiling

Seiryo Ogata[1], Seji Asamitsu [1,2], Masanobu Morita[1], Tetsuro Matsunaga[1], Yohei Ozawa[2], Takashi Kamei[2], Fan-Yan Wei[3], Hozumi Motohashi[4], Takaaki Akaike[1]

[1] Department of Environmental Medicine and Molecular Toxicology, Tohoku University Graduate School of Medicine, [2] Department of Surgery, Tohoku University Graduate School of Medicine, [3] Department of Modomics Biology and Medicine, Institute of Development, Aging and Cancer, Tohoku University, [4] Department of Medical Biochemistry, Tohoku University Graduate School of Medicine

Background & Purpose: Since the exhaled breath condensate (EBC) is obtained in a non-contacting and non-invasive manner, the "breath omics" is receiving great attention as a novel disease control approach. Our research group has developed a high precision technique for supersulfide metabolome and discovered supersulfides increased in the EBC of some chronic respiratory syndromes. The current study aims to evaluate the usefulness of supersulfide metabolome for new biomarker finding in various diseases including COVID-19 and cancer.

Methods & Results: We first collected EBC from COVID-19 patients and healthy controls, and performed the supersulfide metabolome analysis. The levels of supersulfides such as HSSH and HSSSH were significantly elevated in COVID-19. Also, we compared the levels of supersulfides in EBC from the esophageal cancer patients with that of healthy controls. We found the increase of CysSSH and decrease of HSSH in EBC collected from the esophageal cancer patients. Furthermore, this breath omics was applicable to other cancers and various diseases such as irritable bowel syndrome, which thus exhibit disease-specific sulfur metabolome profiles.

Conclusion: Herein, we clarified that the sulfur metabolites in EBC could be a good biomarker for COVID-19 and esophageal cancer as well as other many diseases. Thus, the supersulfide profiling via breath omics may represent the fingerprint characteristic of distinctive diseases, allowing us to non-invasively assess the health and disease conditions, which will thereby help us to develop a brand-new clinical diagnostic technology based on the breath omics for various supersulfide metabolites.

Poster

Cyclo-octa-sulfur contributes to energy metabolism in mitochondria

Tetsuro Matsuanga[1,2], Uladzimir Barayeu[2], Masanobu Morita[2], Seiryo Ogata[2], Minkyung Jung[2], Tianli Zhang[1], Tsuyoshi Takata[2], Michito Yoshizawa[3], Hozumi Motohashi[4], Takaaki Akaike[2]

[1] Center for Integrated Control, Epidemiology and Molecular Pathophysiology of Infectious Diseases, Akita University, [2] Department of Environmental Medicine and Molecular Toxicology, Tohoku University Graduate School of Medicine, [3] Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, [4] Department of Medical Biochemistry, Tohoku University Graduate School of Medicine

Reactive persulfides such as cysteine persulfide play an important role in various biological redox reactions and signaling. While sulfur-based respiration is known as the most primitive prototype of energy metabolism, their significance in eukaryotes, especially in higher animals, remain to be clarified. Therefore, this study focuses on the molecular mechanism underlying sulfur-dependent energy metabolism in mammals, with specific attention to cyclo-octa-sulfur, S8. Mass spectrometry employing a polyaromatic capsule that effectively captures and detects S8 revealed that huge amounts of S8 are produced in mammalian mitochondria as well as in the sulfur bacteria Allochromatium vinosum and Rhodobacter capsulatus. The S8 production was remarkably reduced in R. capsulatus that is deficient in sulfide:quinone oxidoreductase (SQR). Additionally, we have developed a single mitochondria imaging system, which allows us to precisely quantify the membrane potential formation. Thus, the mitochondrial membrane potential formation of mouse embryonic fibroblasts increased by the addition of sulfur donors (NaHS and Na2S2) in an SQR-dependent manner. Notably, the same membrane potential of HEK293T cells was significantly abolished immediately when the cells were treated with the aromatic capsule. These results suggest that cyclo-octa-sulfur, S8, is produced and accumulated abundantly in mitochondria, which maintains and promotes the energy metabolism in mammals.

Poster

Erythroid specific knock out of soluble guanylate cyclase leads to disrupted bone marrow erythropoiesis and splenomegaly in mice

Sophia K Heuser[1], Anthea LoBue[1], Junjie Li [1], Zhxin Li [1], Ron-Patrick Cadeddu[2], Luke Dunaway[3], Doris Koesling[4], Corinna Strupp[2], Jon O. Lundberg[5], Brant Isakson[3], Evanthia Mergia[4], Ulrich Germing[2], Miriam M. Cortese-Krott[1,5,6]

[1] Myocardial Infarction Research Laboratory, Department of Cardiology, Pulmonology, and Angiology, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany,[2] Department of Hematology, Oncology and Clinical Immunology, Medical Faculty, Heinrich-Heine-University, Düsseldorf, German, [3] Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine, Charlottesville, VA, USA, [4] Department of Pharmacology, Ruhr-University Bochum, Bochum, Germany, [5] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden, [6] CARID, Cardiovascular Research Institute Düsseldorf

Background/Introduction: Nitric oxide plays a central role in cellular differentiation and survival. It was shown by us and others that erythroid cells and adult red blood cells (RBCs) carry a functional eNOS/ sGC protein kinase G pathway and may play a role in erythroid differentiation and/or RBC survival. However, the functional significance of red cell sGC in vivo is still unknown.

Purpose: In this study, we investigated the role of red cell sGC on erythroid cell differentiation in vivo.

Methods: RBC-specific sGC-KO mice were generated by crossing GC 1flox/flox mice with erythroid-specific Cre-mice. Cell-specific gene targeting and loss of sGC in RBCs were analyzed by testing for DNA recombination, and mRNA expression and transmission electron microscopy. We analyzed blood count, and the levels of plasma transferrin, ferritin, hemoglobin and erythropoietin. Erythropoiesis in bone marrow and spleen were determined by Pappenheim-staining, colony forming unit assay and subpopulations were characterized by flow cytometric analysis.

Results: We found that RBC sGC KO mice lack sGC expression in RBCs. Interestingly, we found decreased erythropoietic activity in the bone marrow but preserved erythropoietin levels in plasma. Furthermore, we found splenomegaly and presence of erythroid precursors in the spleen, which was accompanied by a preserved blood count as compared to WT mice demonstrating stress erythropoiesis.

Conclusion: Taken together, lack of red cell sGC leads to disrupted erythropoiesis in the bone marrow, leading to a compensatory stress erythropoiesis in the spleen and splenomegaly. Therefore, red cell sGC regulates erythropoiesis in the bone marrow.

Poster

Endothelial cell eNOS regulates sodium excretion but not glomerular filtration rate in the kidney as determined in cell-specific eNOS KO mice

Anthea LoBue[1], Denada Arifaj [2], Sophia K. Heuser[1], Junjie Li [1], Drielle D. Guimaraes Braga[3], Zhengbing Zhuge [3], Annika Olsson[3], Carina Nihlén [3], Eddie Weitzberg[3], Jon O. Lundberg[3], Mattias Carlström [3], Johannes Stegbauer[2,4], Miriam M. Cortese-Krott[1,3,4]
[1] Myocardial Infarction Research Laboratory, Department of Cardiology, Pulmunology and Angiology, Medical Faculty, Heinrich Heine University, Düsseldorf,[2] Department of Nephrology, Medical Faculty, Heinrich Heine University, Düsseldorf, [3] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden, [4] CARID, Cardiovascular Research Institute Düsseldorf

Background: The kidney contributes to blood pressure control by water and sodium handling. The nitric oxide (NO)/soluble guanylate cyclase (sGC) signalling is a crucial regulator of medullary blood flow and natriuresis through vasculo-tubular crosstalk involving multiple cell types including endothelial cells (EC), epithelial cells, pericytes and perhaps red blood cells (RBC), which also express eNOS.

Purpose: The aim of the study is to identify the role of eNOS in ECs and RBCs to control renal function.

Methods: Global eNOS knock out (KO), EC eNOS KO/knock in (KI) and RBC eNOS KO/KI mice were characterized for eNOS expression in the kidney, blood pressure and kidney function as assessed in vivo by analysing glomerular filtration rate (GFR), sodium excretion in urine in basal condition and after AngII infusion and ex vivo by isolated perfused kidney.

Results: Global eNOS KO mice showed decreased GFR before and after AngII treatment as compared to WT mice. A further decrease was also observed with aging contrary to WT mice. EC eNOS KO mice showed a preserved baseline GFR but lack of AngII-induced compensatory increase in GFR. EC eNOS KO mice showed a decreased sodium excretion at baseline and after sodium challenge with/without AngII. In RBC eNOS KO mice the GFR and sodium excretion were preserved at baseline and in response to AngII infusion. Reactivation of eNOS in RBC or EC does not recover GFR in the global eNOS KO mice.

Conclusion: EC eNOS does not modulate GFR but regulates sodium excretion in vivo in mice.

Poster

Expression of soluble Guanylate Cyclase (sGC) and its ability to form a functional sGC Heterodimer can be critical factors for sGC-based therapies in PAH

Mamta Sumi[1], Blair Tupta[1], Kevin Song[1], Lori Mavrakis[1], Suzy Comhair[1], Dennis Stuehr[1], Arnab Ghosh[1]

[1] Cleveland Clinic Foundation

Background: Pulmonary hypertension is a complex disorder with diverse origins and pathologies, resulting in increased pressure in lung arteries. Key features include abnormal cell growth, disrupted signaling like NO-sGC pathway, and vascular remodeling. These lead to rising pulmonary pressure, resistance, and eventual heart failure.

Purpose: We aimed to explore the underpinnings of a dysfunctional NO-sGC signaling pathway in pulmonary arterial hypertension (PAH).

Methods: Western blots, immunoprecipitation, and Immunofluorescence assays were used for protein expression and protein-protein interaction. Endothelial and pulmonary arterial smooth muscle cells (PASMCs) transwell co-culture assays were performed to establish proof-of-concepts. cGMP was estimated by ELISA and NO measurements were made by an ozone based chemiluminescent assay.

Results: We found low expression of sGC, a poor sGCα1β1 heterodimer correlated with low expression of its facilitator chaperon, hsp90. Treating PASMCs 16h with low micromolar doses of a slow-release NO donor like DETANONOate reinstated the sGCα1β1 heterodimer restoring its NO-heme-dependent activity. Doing transwell co-culture of HEK cells stably expressing eNOS or activated control/PAH PAECs with control/PAH PASMCs in various combinations restored the sGC heterodimer and its heme-dependent activity, suggesting that PAECs from PAH can also generate NO. Additionally, a uniform expression was observed of globins (Hbα/β, Mb) in PASMCs/PAECs in PAH or controls, which are impediments to vasodilation as they can scavenge the eNOS-generated NO.

Conclusion: Our studies suggest that low doses of NO along with sGC stimulators (BAY 41-2272) as a potential drug for PAH subjects as this can activate the sGC despite the presence of the globins.

Poster

A Novel Role of 5-Methyl-(6S)-tetrahydrofolate in Mediating Endothelial Cell Tetrahydrobiopterin in Pregnancy and Implications for Gestational Hypertension

Surawee Chuaiphichai

Folate intake during pregnancy is essential for fetal development and maternal health. However, the specific effect of folic acid (FA) and 5-methyl-(6S)-tetrahydrofolate (5-MTHF) on the prevention and treatment of hypertensive disorders of pregnancy remains unclear. We investigated whether FA and 5-MTHF have different effects on endothelial cell tetrahydrobiopterin (BH4) metabolism in pregnancy,and possible consequences for endothelial nitric oxide (NO) generation, maternal blood pressure (BP) and fetal growth.

We analysed the maternal BP in pregnant wild-type (Gch1fl/fl) and Gch1fl/flTie2cre mice treated with either FA or 5-MTHF starting at before pregnancy, mid-pregnancy or late pregnancy. BH4, superoxide, and NO bioavailability were determined in mouse and human models of endothelial cell BH4 deficiency by HPLC.

In vitro studies in mouse and human endothelial cells showed that treatment with 5-MTHF, but not FA, elevated BH4 levels,reduced superoxide production and increased NO synthase (NOS) activity. In primary endothelial cells isolated from women with hypertensive pregnancies, exposure to 5-MTHF,but not FA, restored the reduction in BH4 levels and NOS activity. In vivo studies in mice revealed that oral treatment with 5-MTHF, but not FA, prevented, and treated, hypertension in pregnancy,when administered either prior to or during pregnancy,respectively, and normalised placental and fetal growth restriction if administered from mid-gestation onwards.

Collectively, these studies identify a critical role for 5-MTHF in endothelial cell function in pregnancy, related to endothelial cell BH4 availability and NOS activity. Thus, 5-MTHF represents a novel therapeutic agent that may potentially improve endothelial function in hypertensive disorders of pregnancy by targeting endothelial cell BH4.

Poster

Exogenous and Diet-Derived Formation of Dinitrosyl Iron Complexes (DNIC) Prevents Cardiometabolic Dysfunctions Induced by a Western Diet in Mice

Miho Shimari[1], Andrei Kleschyov[1], Tomas Schiffer[1], Gianluigi Pironti[1], Gaia Picozzi[1], Carina Nihlén [1], Eddie Weitzberg[1,2], Jon Lundberg[1], Mattias Carlström [3]

[1] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. , [2] Department of Perioperative Medicine and Intensive Care, Karolinska University Hospital, Stockholm, Sweden, [3] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Rationale: Nitric oxide (NO) has been recognised as a significant free radical in many physiological activities in the body, and reduced NO signalling is associated with various pathophysiological processes. Given its reactive feature, there have been a number of NO-related metabolites and derivatives suggested as “secure” storage of NO. Among such molecules, dinitrosyl iron complexes (DNIC), comprised of NO, thiol and nonheme iron, has been described as one of the largest cellular pools of NO bioactivity. In recent unpublished studies, we found that endogenous DNIC formation from dietary inorganic nitrate and iron largely depends on the gut microbiota, and that produced DNIC are found systemically, particularly in the liver. Yet, the role of DNICs’ functions in health and disease and its contribution to NO dynamics and consequences are unexplored. In this study, we aimed to explore the therapeutic value of dietary DNIC supplementation, or inorganic nitrate+nonheme iron, in a mouse model of cardiometabolic disease induced by chronic Western diet (WD) in combination with the NO synthase inhibitor, L-NAME.

Methods: Male C57BL/6J mice were divided into 4 experimental groups (n=10 each); 1) Regular chow and drinking water, 2) WD+L-NAME, 3) WD +L-NAME+DNIC (5 mM), and 4) WD+L-NAME+nitrate+iron (10 mM). All pharmacological agents were administered via the drinking water. The mice were treated for 8 weeks with food and water provided ad libitum. Metabolic and cardiovascular parameters were measured in vivo at the end of the study period. Ex vivo functional studies were performed upon euthanization, followed by biochemical analyses and histological evaluation.

Results: Supplementation with both DNIC and nitrate+iron attenuated the development of several cardiometabolic dysfunctions associated with this model (e.g., hypertension, cardiac hypertrophy, obesity, hyperglycemia and reduced glucose clearance.

Conclusions: Gut microbiota is a significant source of DNICs found in blood and tissues of mice. Exogenous DNIC, or stimulation of endogenous DNIC formation by dietary nitrate and iron, may have preventive and therapeutic effects in cardiometabolic disease.

Poster

New Insights into the Anti-Inflammatory Effects of Cationic Polymers through iNOS Inhibition

Houman Alimoradi[1,2], Amin Shavandi[2], Anita Fallah[1], Christine Delporte[1]
[1] Laboratory of Pathophysiological and Nutritional Biochemistry (LPNB), Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium , [2] 3BIO - BioMatter Lab, École interfacultaire de Bioingénieurs (EIB), Université Libre de Bruxelles, Brussels, Belgium

Background: Cationic polymers, such as polyamines, chitosan, and chitosan oligosaccharides, exhibit significant anti-inflammatory activity in experimental arthritis models. However, the mechanisms underlying these effects are not yet fully understood. Inducible nitric oxide synthase (iNOS) is a key regulator of the immune response and inflammation, and its inhibition has shown protective effects in various inflammatory conditions.

Methods: We investigated the effects of chitosan oligosaccharide, water-soluble chitosan, polyethyleneimine, poly-L-lysine, and polyamine on iNOS enzymatic activity, NO generation, and apoptosis induced by TNF-α in salivary gland cells and fibroblast cells.

Results: We report an unexpected role for polyamines and other cationic polymers in directly antagonizing iNOS activity through a mechanism that is independent of the classical key-lock paradigm. We also confirm that these cationic materials display anti-inflammatory activity in cells stimulated by TNF-α, at least in part, through the inhibition of iNOS.

Conclusion: Polyamines act as iNOS antagonists. Our data provide new insights into the anti-inflammatory mechanisms of cationic polymers and their potential therapeutic applications in inflammatory diseases.

Poster

Arginase role in limiting NO production in diabetes

Alia Shatanawi[1]
[1] The University of Jordan

Cardiovascular complications of diabetes are a leading cause of morbidity and mortality. Vascular endothelial dysfunction (VED) is strongly implicated in the pathogenesis of diabetic vascular complications. Impaired endothelial cell (EC) production of nitric oxide (NO) is a main characteristic of VED. In ECs NO is produced by endothelial nitric oxide synthase enzyme (eNOS), by utilizing L-arginine. Arginase in ECs also uses L-arginine as a substrate to produce urea and ornithine. Recently arginase upregulation has been shown to play a role in vascular dysfunction in diabetes by limiting L-arginine bioavailability to eNOS and limiting NO production. Our research have identified the role of arginase in diabete-induced vascular dysfunction through limiting NO production. Additionally, arginase activity in type 2 diabetic patients is shown . Arginase activity was elevated in type 2 diabetic patients as compared to age-matched healthy volunteers. Levels of arginase activity has a positive correlation with HbA1c levels in diabetic patients (R2=0.8 Pearson r=0.87). Cell studies also agreed with these findings as high glucose (25 mmol/L, 72 hrs) treatment to ECs resulted in a 66% increase in arginase activity. This increase in arginase activity was concomitant with a 27% drop in NO produced by EC. Inhibitor of arginase (ABH 100 umol/L) restored NO level to normal. Collectively our results indicate that diabetic conditions cause an elevation of arginase activity which can limit EC production of NO and thus impair vasorelaxation.

Arginase can be regarded as a novel marker for the vascular complications of diabetes. Drugs targeting arginase or its signaling pathway may show benefits in delaying or preventing these vascular complications of the disease. Endothelial dysfunction leading to decreased blood flow is strongly implicated in the complications of diabetes.