Oral

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

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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

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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.

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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

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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

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Confidential

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

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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

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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

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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

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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

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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-NO2 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-NO2), 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-NO2 did not affect the loss of SRC, implying that it does not directly inhibit iNOS function. Pretreatment with OA-NO2 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-NO2 mediated inhibition of activated microglia.

Conclusions: Our data reveal that OA-NO2 has anti-inflammatory activity in combination with correction of mitochondrial function in activated microglial cells. Since OA-NO2 can cross the blood-brain barrier of the central nervous system, OA-NO2 has potential as a therapeutic strategy for neuro-inflammation associated disorders.

Poster

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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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.