cytoprotective effect is independent of nitrite reduction and involves PKA activation. Thus, we evaluated the mechanism by which nitrite activates PKA and regulates mitochondrial dynamics. We observe that nitrite activates PKA and increases basal and maximal mitochondrial respiration under normoxia. Moreover, nitrite increased complex IV activity and the phosphorylation of COXIV at serine 58, an effect mediated by PKA. We show that nitrite increases cellular cAMP levels in cardiomyocytes leading to PKA activation. This cAMP increase is due to the inhibition of a mitochondrially localized phosphodiesterase activity. Further, nitrite upregulates the A-kinase anchoring protein, the AKAP121 which localizes PKA to the mitochondrial membrane. In conclusion, our results demonstrate that nitrite can be a versatile signaling molecule, not only by inducing protein nitration and nitrosylation but also through modulating protein expression and phosphorylation. Further, these data contribute to the understanding of the mechanism by which nitrite selectively regulates mitochondrial function and metabolism as well as expands the therapeutic potential of nitrite in preventing and treating cardiovascular diseases.

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The Effect of ProliNONOate on Fibrin Clot Polymerization

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When a break in the blood vessel wall occurs, a clot is formed to prevent extensive blood loss. The supporting framework of the clot structure is formed by fibrin fibers. Fibrin fibers are formed from the glycoprotein fibrinogen by a process that is catalyzed by thrombin. Additional covalent bonds are formed by transglutaminase, FXIIIa, after polymerization.
We investigated the changes in the fibrin clots due to the addition of NO donor ProliNONOate to whole clots, fibrinogen only, thrombin only and FXIIIa only. Structural and mechanical properties were measured using various techniques including Confocal Microscopy, Atomic Force Microscopy (AFM), spectrophotometry, and protein gel electrophoresis.
The addition of 5 μM ProliNONOate significantly altered fibrin clot densities and fibrin fiber diameters as determined by confocal microscopy and AFM. Clots formed in the presences of ProliNONOate were less dense, were composed of smaller fibers and had larger pores. Changes in clot properties were seen when ProliNONOate was added to all constituents during clot polymerization or to thrombin prior to polymerization. 5 μM ProliNONOate added to fibrinogen or FXIII prior to polymerization did not alter clot properties. Extensibility of individual fibrin fibers were not significantly altered by the addition of ProliNONOate. However, using gel electrophoresis techniques and spectrophotometry, we observed that the addition of NO to thrombin decreased the rate of clot formation and crosslinking. Our data suggests the addition of ProliNONOate under oxygenated conditions changes the activity of thrombin which in turn alters fibrin clot properties.
Future steps plan to investigate thrombin nitrosylation in the presence of ProliNONOate and transnitrosation by GSNO.

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Development of Blue-Light-Controllable Nitric Oxide Releaser and Its Biological Application

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Nitric oxide (NO) is biologically synthesized in human body by nitric oxide synthase and plays roles of key mediator. Due to its instability, NO donors have been developed as chemical tools for biological research of NO or candidates as chemotherapeutic drugs. Among NO donors, light controllable NO donors, which can control NO release by light, are expected as controllable chemical tools or agents for photodynamic therapies.
Because of these advantages, we designed a visible light controllable NO donor, named as NOBL-1. By means of ESR spin trapping method, NO release induced by 470–500 nm light was observed. To assess the light control of NOBL-1, NO detection by DAR-4M, a fluorogenic NO probe, was conducted *in vitro* and *in cells*. Finally, we achieved vasodilation control of rat aorta strip with NOBL-1 and the triggering light in highly temporal manner. NOBL-1 is expected to be a useful chemical tool for NO research, and it may also have potential for phototherapy.
As a conclusion, we synthesized a novel visible light controllable NO donor and succeeded to observe light controllable release of NO *in vitro*, *in cell* and *ex vivo*. This compound is controllable by visible light, so that it would be expected to be a useful chemical tool for biological NO research.
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Liver-Specific Deletion of Xanthine Dehydrogenase ($Xdh^{-/-}$) Validates in Vivo XOR-Catalyzed Nitrite Reductase Activity

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The capacity of molybdopterin enzymes to reduce nitrite (NO_2^-) to nitric oxide ($^{\bullet}NO$) has become increasingly appreciated as an alternative source of $^{\bullet}NO$ under hypoxic/inflammatory conditions. This is evidenced by in vivo studies demonstrating diminution of beneficial outcomes attributable to NO_2^- upon pre- or co-treatment with xanthine oxidoreductase (XOR) inhibitors. However, there is substantive concern regarding potential off-target effects of currently utilized XOR inhibitors including allo/oxypurinol, febuxostat and Na-tungstate. This is notably true when regarding the potential impact on purine catabolic pathways that alter levels of adenosine, a potent signaling agent in the vasculature. As such, we attempted to validate results obtained with these pharmacologic agents by subjecting our recently developed liver-specific conditional xdh knockout model to analysis of XOR-dependent NO_2^- reduction to $^{\bullet}NO$. Using the cre/lox system in a C57Blk/6j background we generated $xdh^{fl/wt}$ mice which were bred with mice expressing cre recombinase in hepatocytes (B6.FVB(129)-Tg(Alb1-cre)1Dlr/J). The resultant liver-specific $xdh^{-/-}$ knockout demonstrates 96% diminution of XOR activity in the absence of deleterious phenotypic issues and without altering XOR activity in the lung, heart, skeletal muscle, kidney and adipose tissue. Livers from $xdh^{-/-}$, $xdh^{flox/flox}$ and wild-type mice

were harvested and homogenates were analyzed for NO_2^- reductase activity using enhanced chemiluminescence. When compared to $xdh^{flox/flox}$ and wild-type littermates, $xdh^{-/-}$ livers demonstrated 61% less total NO_2^- reductase activity. This value is similar to liver homogenates treated with febuxostat (Uloric®) (57 ± 6%) or allopurinol (54 ± 8%) and/or when livers from mice treated with febuxostat (47 ± 11%) were analyzed. Nitrite reductase activity was reduced to near baseline when homogenates treated with CO + febuxostat suggesting that heme-catalyzed reductive mechanisms were responsible for the proportion of •NO formed independent of XOR. Combined, these data serve to validate a major contributory role for XOR-catalyzed reduction of NO_2^- to •NO as well as unveil a significant new tool for evaluating XOR-derived products in numerous disease states.

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Thionitrosyl Binuclear Iron Complexes - a New Class of Agents for Anti-Aging Therapy

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Aging is associated with the dysfunctions in vascular smooth muscle cells, neurotransmission, renal and immune systems, to name just a few, many of which stems from decline in reliability of the regulatory functions of nitric oxide radical (NO). Our approach to the creation of a new class of agents for anti-aging therapy is based on the design of the novel nitric oxide donors, i.e. – Fe-thionitrosyl complexes which are considered as long-living reservoirs and transport agents of NO in living cells. It was previously shown that such the complexes demonstrate anti-cancer properties [Sanina & Aldoshin, 2011]. Here, we present the evidence of the geroprotective effects of the synthetic NO-complex, $Na_2[Fe_2(S_2O_3)_2(NO)_4]\cdot 4H_2O$ (FeSNO). Wistar male rats, the age of which was 24 months at the beginning of the experiment, were intraperitoneally administered with FeSNO, 5 mg/kg of mass in 1.0 ml of physiological solution (experimental animals) or with 1.0 ml of physiological solution (control animals) daily for 14 days. It was found that the rats administered with FeSNO had extended survival, 20-30 % more than the control animals. Furthermore, the adult rats of age of 7-8 months were subjected to the acute irradiation with 5 Gy dose of X-rays after which the irradiated animals were intraperitoneally administered daily for 30 days with FeSNO, 5 mg/kg of mass in 1.0 ml of physiological solution (experimental animals) or with 1.0 ml of physiological solution (control animals). Thereafter, the levels of NO-metabolites, NO_2 and NO_3, along with Hb, HbA1c, glucose, cholesterol and lipoproteins of high density were determined in blood of the irradiated animals. Besides, the important indicators of oxidative stress, i.e. – the levels of MDA and activities of antioxidant enzymes, SOD, catalase, glutathione peroxidase and glutathione reductase, were measured in liver, heart and brain tissues of the animals of both groups, the experimental and the control ones. It has been revealed that the administration of FeSNO after the single-dose irradiation prevents the negative impacts, inherent for post-radiation accelerated aging, in blood and tissues of the irradiated animals. Thus, the nitrosyl complexes of iron with the functionalized sulfur-containing ligands hold considerable promise as the potential agents for anti-aging medicine.

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Nitrite Prevents Iron Mediated Damage to Mitochondria Upon Hypoxia

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Nitrite protects various organs from ischemia-reperfusion injury by ameliorating mitochondrial dysfunction. Here we provide evidence that this protection is due to the inhibition of iron-mediated oxidative reactions caused by the release of iron ions upon hypoxia. We show in a model of isolated rat liver mitochondria that upon hypoxia mitochondria reduce nitrite to nitric oxide (NO) in amounts sufficient to inactivate redox active iron ions by formation of inactive dinitrosyl iron complexes (DNIC). The scavenging of iron ions in turn prevented the oxidative modification of the outer mitochondrial membrane and the release of cytochrome c during reoxygenation. This action of nitrite protected mitochondrial function. The formation of DNIC with nitrite-derived NO could also be confirmed in a ischemia/reperfusion model in liver tissue. Our data suggest that the formation of DNIC is a key mechanism of nitrite-mediated cytoprotection.

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Sulfomics and Nitromics: Characterizations of S- and N-Based Speciation in Reactions of *S*-nitroso-glutathione (GSNO) with Hydrogen Sulfide (H_2S)

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Several recent reports suggest that HNO may be produced endogenously by reaction of Hydrogen Sulfide (H_2S) and *S*-nitrosoglutathione (GSNO). This hypothesis was tested using deoxy myoglobin (deoxy-Mb) to trap the expected HNO released from the target reaction, which should generate the stable HNO adduct of Mb, HNO-Mb, under anaerobic conditions. Under numerous experimental conditions, the sole globin product was NO-Mb as characterized by absorbance, EPR, and NMR spectroscopies. Additional studies with alternative biological reductants such as ascorbic acid, dithiothreitol, glutathione, and dithionite also yielded NO-Mb as the sole globin product; however, reduction by $NaBH_4$ generated HNO-Mb in high yield. Production of N_2O is unchanged by the presence of Mb,

$$\text{GSNO} + H_2S \rightarrow \begin{matrix} \nearrow SNO^- + SSNO^- + S_2O_3^{-2} \\ GSS_nG + GSS_{n-1}H + GSS_{n-1}NH_2 + GSS_{n-1}O_xH \\ \searrow NO + N_2O + NH_2OH + NH_4OH \end{matrix}$$

$$n \geq 1 \;\&\; x = 1\text{-}3$$

discounting the intermediacy of either NO or HNO in its formation. Quantitative GC-MS analyses of reactions of $GS^{15}NO$ with H_2S showed that the main reaction product was ^{15}NO, with $^{15}N_2$ produced ca. 10-fold greater than $^{15}N_2O$. Taken together, these studies strongly discount the presence of free HNO in the reactions of GSNO with H_2S, but imply alternative reduced species generate the observed N-based gases. The protein products of the reaction of H_2S and GSNO at varied