Profiles of Cardioprotection: Integrated Proteomic and Metabonomic Study of the Effects of Nitrite Treatment on the Heart

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Introduction:

Nitrite, stable and abundant *in vivo*, was thought for decades to be biologically inert at physiological concentrations. Formed endogenously through oxidation of nitric oxide (a ubiquitous signaling molecule known to elicit broad biological effects) and derived extensively from dietary sources, it has very recently proven to be a signaling molecule in its own right, affecting soluble guanylyl cyclase and cytochrome P450 activities, heat shock protein 70 and heme oxygenase-1 expression[1], and protecting against cardiac ischemia-reperfusion injury[2]. Based on this recent evidence, we sought to characterize the impact of changes in systemic nitrite availability on the cardiac proteome, using 2D gel-based separation followed by MALDI-TOF MS and peptide mass fingerprint (PMF) analyses, then correlate these data to metabonomic studies.

Methods:

Male Wistar rats administered a single intraperitoneal injection of sodium nitrite (0.1, 1 and 10 mg/kg) or saline were anesthetized 24 hours later, perfused free of blood, and their cardiac tissue was harvested. Immediately after homogenization. The concentrations of NO-related metabolites were determined by gas phase chemiluminescence and HPLC. Homogenates were clarified by low speed centrifugation (1,500x*g*), then separated into crude mitochondria and post-mitochondrial cytoplasmic supernatant by high speed centrifugation (15,000x*g*). Samples were denatured directly in IEF buffer and subjected to 2D-PAGE analysis, followed by Coomassie or silver staining. Protein spots were imaged and quantitated using PDQuest™ software, excised and subjected to in-gel trypsin digestion. Peptides were eluted, de-salted and analyzed by MALDI-TOF MS. Spectra were analyzed with MoverZ™ and PMF analysis was conducted using MASCOT, searching against the rat proteome.

Results and Discussion:

Using 2D gel electrophoresis, we have created extensive 2D protein reference maps of cardiac proteomes of control Wistar rats and those systemically administered physiologic and therapeutic levels of nitrite. We have mapped both whole cardiac homogenate as well as purified mitochondria and post-mitochondrial cytoplasmic supernatant, confirming the identity of hundreds of isolated protein spots through in-gel digestion followed by MALDI-TOF MS and PMF analyses. Quantitative comparative analyses have revealed significant changes in cardiac protein expression upon treatment with nitrite. These changes consisted of both up- and down-regulation of steady-state protein levels, as well as alterations in protein post-translational modifications and have included proteins involved in energy metabolism, redox balance, chaperone activity, cell structure, contractility, and proteins previously implicated in the metabolism of nitric oxide, among others. Additionally, we have measured metabonomic changes and changes to the cellular redox status and we have correlated the results to the proteomics data. Specifically, we have detected short-term spikes in the levels of *S*-nitroso, *N*-nitroso, and heme-nitroso species in the tissues, as well as a large and persistent increase in the ratio of oxidized to reduced ascorbate.The results obtained using this combined proteomics/ metabonomics approach indicate specific changes in expression of cellular stress response proteins triggered by nitrite that may confer resistance to further oxidative insults. Our data suggest that nitrite, due to its stability *in vivo*, may be able to act in a long-range endocrine fashion to establish this protective tone in the heart. Our integrated proteomic and metabonomic approach is a step toward elucidating the scope and mechanism of cardioprotection and the potential activity of nitrite, a biological compound with newly discovered importance in human health and medicine. Our data may have direct and immediate implications for

current experimental therapeutic uses of nitrite and provokes a reassessment of the impact of daily dietary intake of nitrite.

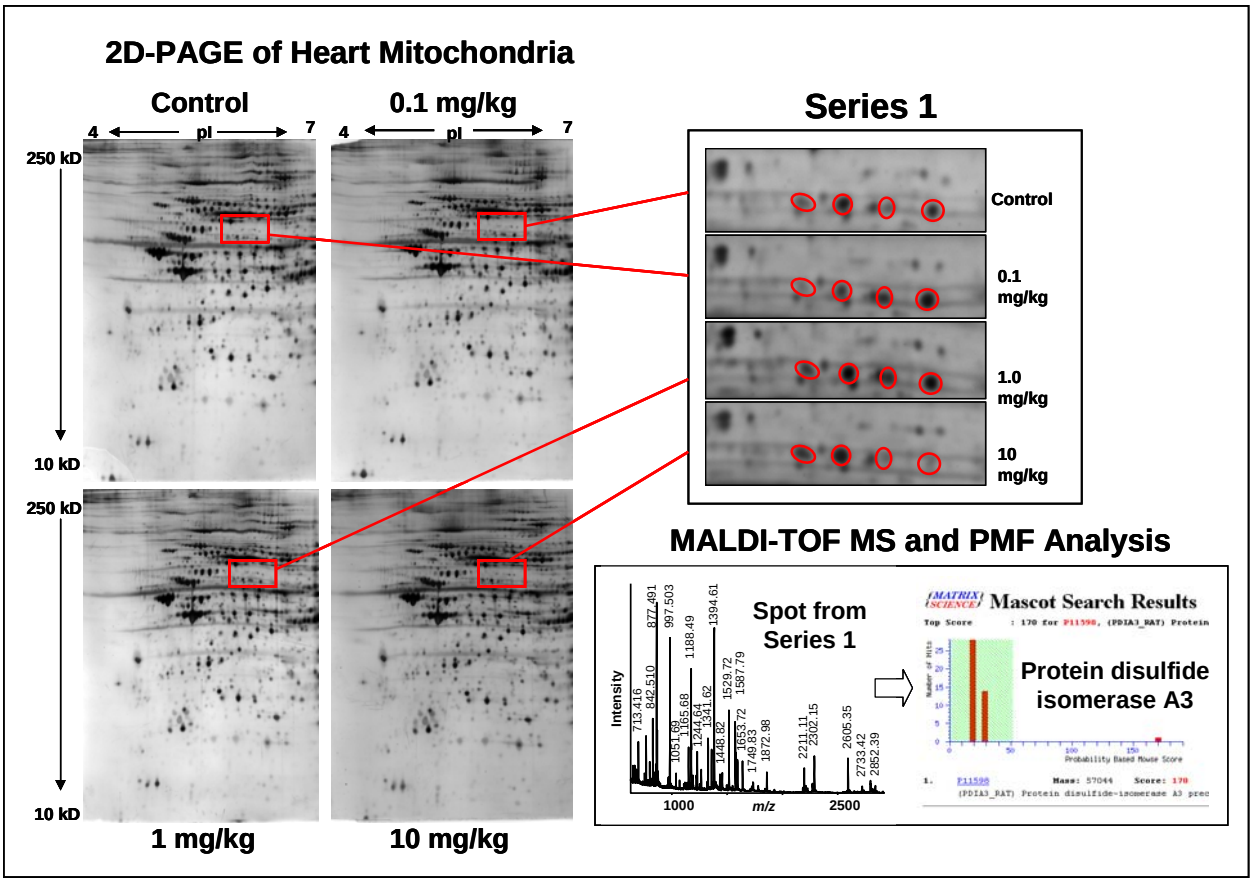

Figure 1. 2D-PAGE analysis of crude cardiac mitochondria isolated from nitrite-administered Wistar rats, including spot identification by MALDI-TOF MS and peptide mass fingerprinting. Left panels, silver stain 2D gel images of pooled mitochondrial samples isolated from rats 24 hours after administration of 0, 0.1, 1.0, and 10 mg/kg sodium nitrite. Upper right panels, enlargements of a small region of the gels displaying a differentially expressed train of spots (circled) all yielding the same protein ID. Lower right panels, an example of a MALDI-TOF MS of peptides derived from one of the spots and the protein ID, PDIA3, that it yielded (with a Mowse score of 170) by PMF using Mascot™ to search against the rat proteome.

References:
[1]Bryan, N. S., B. O. Fernandez, et al. (2005). "Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues." *Nat Chem Biol* **1**(5): 290-7.
[2]Duranski, M. R., J. J. Greer, et al. (2005). "Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver." *J Clin Invest* **115**(5): 1232-40.

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