Role of Circulating S-Nitrosothiols in Control of Blood Pressure

Matthew W. Foster, John R. Pawloski, David J. Singel, Jonathan S. Stamler

The biological effects of nitric oxide (NO) are in large part mediated by S-nitrosylation of peptides and proteins to produce bioactive S-nitrosothiols (SNOs).1–3 The observation of abnormal SNO levels in numerous pathophysiological states2 suggests that dysregulation of SNO homeostasis may contribute to disease pathogenesis. For example, the hypotension of human sepsis is accompanied by increases in circulating levels of vasodilatory SNOs.3 Although such altered SNO levels may simply mirror NO production (eg, induction of inducible NO synthase in sepsis), they may also reflect changes specific to SNO biosynthesis and metabolism. Indeed, mice lacking a SNO-metabolizing enzyme are profoundly hypotensive under anesthesia.3 Thus, blood pressure is evidently regulated by both synthesis and turnover of SNOs. In this issue of Hypertension, Gandley et al4 extend this paradigm by proposing that a defect in SNO turnover contributes to the hypertension of preeclampsia.

In the blood, S-nitrosoalbumin (SNO-albumin) and S-nitrosohemoglobin (SNO-Hb) constitute the major conduits for circulating NO bioactivity. Although both SNOs may influence blood pressure, they operate within distinct signaling circuits. SNO-Hb can be viewed as a principal regulator of SNO homeostasis, adaptively modulating NO chemistry to control NO bioactivity. SNO-Hb is formed by transfer of NO from heme-iron to Cys of SNO homeostasis.5 As such, SNO-Hb may simply reflect altered NO metabolism. Indeed, mice lacking a SNO-metabolizing enzyme are profoundly hypotensive under anesthesia.3 Thus, blood pressure is evidently governed by both synthesis and turnover of SNOs. In this issue of Hypertension, Gandley et al4 extend this paradigm by proposing that a defect in SNO turnover contributes to the hypertension of preeclampsia.

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Gandley et al\(^4\) add much to address the perpetuated controversy over the concentration of plasma SNOs. They not only report a new set of measurements that are complementary to and consistent with previous work,\(^{21}\) but also provide a confirmatory measure of activity. Levels of plasma SNOs ranging from 10 nmol/L to \(\sim 10\) \(\mu\)mol/L (this study) in the literature reflect differences in sample preparation and handling, choice of standards, and detection methods.\(^{22}\) Mass spectrometry of human plasma shows SNO-albumin levels in the 200 nmol/L range,\(^{23,24}\) establishing a lower limit of normal. The idea published by Gladwin\(^{25}\) (and Feelisch\(^{26}\)) using the method of Gladwin) that SNOs constitute a negligible to undetectable fraction of bioactive NO, species in plasma and blood should be viewed with skepticism. The discrepancy between multiple methods,\(^{16,22}\) most notably the direct mass spectroscopic approach\(^{23,24}\) and the method of Gladwin,\(^{25,27}\) indicates that the latter is inappropriate for use in complex biological samples. In our experience, the use of acid, thiol blocking agents, and sulfanilamide (to remove contaminating nitrite), destabilizes some SNO (and iron nitrosyl Hb) species, whereas the precipitation that ensues prevents access to others. Disruption of cellular and organellar integrity may also destabilize SNO-proteins by providing unfettered access to various effectors of SNO stability (eg, Ca\(^{2+}\), Mg\(^{2+}\), other proteins).\(^{28}\) Finally, the Gladwin assay does not properly differentiate SNO-Hb from iron nitrosyl Hb or nitrite.\(^{16,22}\) Indeed, measurements of iron nitrosyl Hb in rodents\(^29\) by the method of Gladwin are also incompatible with the direct EPR measurements by Kirima et al.\(^{30}\)

In addition to quantitative measurements using the biotin switch assay\(^4\) and 4,5-diaminofluorescein–based detection,\(^{21}\) Kagan, Gandley, and coworkers also directly measure the liberation of both NO and NO-dependent bioactivity from plasma samples (in healthy controls versus preeclamptic patients) by reaction with exogenous Cu\(^{2+}\) and ascorbate.\(^3\) In this assay, ascorbate functions to reduce Cu\(^{2+}\) to Cu\(^{+}\), which in turn reduces nitrosothiol to thiolate and nitric oxide. Taken together, the results of these different methods make a persuasive case for a buildup of SNO in preeclampsia. Nevertheless, we offer a word of caution. Whereas the authors infer from their assays differences in amounts of SNO, some measurements may instead reflect differential reactivity of the various SNOs\(^{22}\) (eg, in health versus disease). Thus, for example, the Cu/ascorbate redox reaction may be favored in preeclamptic versus normal pregnancy plasma and thus yield apparent differences from similar amounts of SNO-albumin. Indeed, Kagan et al have shown that the ascorbate-oxidizing activity of Cu\(^{2+}\) (with formation of Cu\(^{+}\)) is greater in preeclampsia than in normal plasma.\(^{31}\) They suggest that this phenomenon is because of an increased fatty acid content of albumin in preeclampsia, as fatty acids modulate both copper-binding to albumin and Cys34 reactivity,\(^{31,32}\) and thus may potentiate Cu/ascorbate-dependent decomposition of SNO-albumin.\(^4\)

The challenge remains to determine the molecular basis for altered SNO-albumin levels in preeclampsia. Plasma ascorbate is 50% lower in preeclampsia versus normals. Gandley et al\(^4\) argue for a defect in ascorbate/Cu-mediated release of NO. They find that in the (patho)physiological range of plasma ascorbate concentrations, the rate of NO release from SNO-albumin depends linearly on ascorbate, suggesting that the lower levels of ascorbate in preeclampsia could account for the increase in SNO-albumin. Because these experiments required exogenous copper, identification of the endogenous source of copper would advance the case for physiological relevance. Alternatively, SNO-albumin denitrosylation may be regulated via transnitrosylation with glutathione (GSH) or cysteine, which readily accept NO\(^+\) from SNO-proteins. In preeclampsia, plasma GSH levels are low,\(^{33}\) most likely because of oxidative stress. Loss of plasma glutathione could also have profound effects on the NO-dependent vasodilatory activity of RBCs, as GSH may facilitate export of SNOs from RBCs.\(^6,34\) Reactive oxygen species (eg, superoxide, O\(_2^-\)), which are also increased in preeclampsia, have been found to potentiate albumin S-nitrosylation,\(^35\) providing another possible mechanism for elevated SNOs. Overall, we favor the explanation that redox state influences the equilibria between thiols/nitrosothiols that convey NO bioactivity, rather than the view ascorbate, per se, regulates the amounts of free NO delivered to the vessel wall.

Further investigation is needed to address the mechanistic basis and relevance of alterations in SNO-protein levels in disease and to identify therapeutic leads. It would be interesting to know if the preeclamptic state can be controlled through administration of NO or SNO or perhaps even thiols, and whether endogenous SNO-albumin or SNO-hemoglobin transduces the therapeutic effects of these compounds. Finally, ascorbate itself has been shown to improve endothelial dysfunction in atherosclerosis,\(^35\) hypercholesterolemia, and preeclampsia.\(^36\) If acute or chronic treatment with ascorbate were to produce a decrease in SNO-albumin commensurate with a fall in blood pressure, then a role for SNO-albumin in control of blood pressure and for ascorbate in modulating its bioactivity would be confirmed.

References


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