Role of Circulating S-Nitrosothiols in Control of Blood Pressure

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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). The observation of abnormal SNO levels in numerous pathophysiological states 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. Although such altered SNO levels may simply mirror NO production (eg, induction of inducible NO synthase SNOs), they may also reflect changes specific to SNO biosynthesis and metabolism. Indeed, mice lacking a SNO-metabolizing enzyme are profoundly hypotensive under anesthesia. Thus, blood pressure is evidently regulated by both synthesis and turnover of SNOs. In this issue of Hypertension, Gandley et al 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 Cys393 thiol on T to R structural transition (oxygenation) of the hemoglobin tetramer. SNO-Hb associates with the red blood cell (RBC) membrane via an interaction with the cytoplasmic domain of anion-exchanger 1 protein (CDAE1, Band 3); on deoxygenation (R – T) transfer of the NO group from SNO-Hb to a cysteine thiol within CDAE1 supports export of RBC vasodilatory activity. SNO-Hb thus serves as an O2 sensor and O2-dependent transducer of NO bioactivity. In contrast, it appears that rather than transducing a specific signal, albumin operates as a buffer to maintain NO homeostasis. S-nitrosylation of albumin occurs at Cys347 via reactions—with NO or nitrosothiols—that are favored by design: specifically, both hydrophobic pockets in albumin (NO/O2 coupling) and bound copper (NO/metal redox coupling) may serve to generate nitrosylating species. Gandley et al make the case that the buffering function of SNO-albumin is impaired in preeclamptic patients, where the thiol of albumin acts as a sink for NO and thus, raises blood pressure. Nudler and colleagues have previously demonstrated that albumin can raise blood pressure independently of its oncotic effects: Redistribution of NO, from the tissues into the hydrophobic core of the protein, subserves S-nitrosylation and lowers the steady-state level of vasodilatory NO within the vascular smooth muscle.

Accumulating evidence strongly suggests a role of SNO-albumin in mitigating cardiovascular risk. Elevated (>2 μmol/L) concentrations of plasma SNOs (of which SNO-albumin is the major constituent) portend adverse cardiovascular outcomes in patients with end-stage renal disease and correlate with elevated blood pressures. Plasma SNOs are also elevated in patients with hypercholesterolemia. Increases in SNO are correlated with an increase in plasma ceruloplasmin, a protein that is known to support SNO synthesis, and with a decline in ascorbate, a compound known to promote SNO degradation. Gandley et al propose compelling evidence that SNO throughput, not level, is the key measure of NO bioactivity in plasma. They observe elevated levels of SNO-albumin in preeclamptic versus normal pregnancy plasma (7 μmol/L versus 2 μmol/L, respectively) and link reduced levels of ascorbate in preeclampsia to impaired SNO-albumin-dependent vasorelaxation. Notably, SNO-albumin is elevated in preeclampsia despite factors that might be assumed to abrogate the formation of nitrosothiols, including a well-characterized oxidative stress and deficit of endothelial-derived NO.)

Excessive sequestration of SNOs also occurs in patients with type I diabetes mellitus. In this case, however, hemoglobin is the culprit. Accumulation of SNO-Hb in diabetes has been attributed to glycosylation of Hb, which preferentially stabilizes the R structure, thereby impairing NO group release. Reduced vasodilation by diabetic RBCs has been implicated in the microvascular flow impairment associated with the disease. Hypoxic vasodilatory activity of RBCs is also impaired in other disorders of heart, lung, and blood, consistent with the idea that RBCs regulate blood flow to maximize O2 delivery. Accumulations of SNO-Hb can also raise blood pressure. This effect, however, occurs through some central action rather than through peripheral vasoconstriction. Comparable levels of plasma hemoglobin (micromolar) have no pressor effects, despite the frequent claims to the contrary.
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 \(\sim 10 \mu\text{mol/L}\) (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\(_x\) 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 ensures prevents access to others. Disruption of cellular and organelar 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.\(^{31}\) 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 preeclamptic 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,\(^{36}\) 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

10. Stubauer G, Giuffre A, Sarti P. Mechanism of S-nitrosothiols and Blood Pressure


34. Lipton AJ, Johnson MA, Macdonald T, Lieberman MW, Gozal D, Gaston Foster et al. S-Nitrosothiols and Blood Pressure

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