S-Nitrosothiols in Blood: Does Photosensitivity Explain a 4-Order-of-Magnitude Concentration Range?

To the Editor:

S-Nitrosothiols such as S-nitroso glutathione (GSNO) are nitric oxide derivates with potent biological actions. There is disagreement regarding the blood concentrations of nitrosothiols, which are reported to range between nondetectable and approximately 10 000 nmol/L (1). Problems and pitfalls in the analysis of nitrosothiols have been attributed to their wide concentration range in biological samples (1). Many of these analytical problems arise from the chemical nature of S-nitrosothiols and are difficult to control, but other problems may be avoidable (1).

Wu and coworkers recently reported that the photoinstability of S-nitrosothiols during sampling of whole blood may be a likely source of error and variation in S-nitrosothiol measurement (2). In general, we think that the findings of this study (2) are incorrect and suffer from analytical and methodologic shortcomings, as are discussed below.

1. SELECTIVITY
Wu and coworkers assumed that their electrochemical method was selective for S-nitrosothiols and that any signal obtained was attributable to S-nitrosothiols (2). They reported that the ammonium/ammonia system may interfere with S-nitrosothiol analysis by this method (3) but did not report on a quantitative basis the potential contributions by NH₃, NH₃CH₃, NH(CH₃)₂, and other volatile, labile, and readily oxidizable substances present in porcine blood. For instance, carbamino compounds are abundant in blood and spontaneously decompose to NH₃ and CO₂. Additionally, the authors did not report experiments that demonstrated the selectivity of this method for S-nitrosothiols [e.g., the use of HgCl₂ (1)]. Given the potential nonselectivity of the method and the lack of additional experiments, we do not agree with the authors’ conclusion that “… the only plausible explanation … is the photodecomposition . . . “ (2).

2. INSUFFICIENT EXPERIMENTS ON PHOTOSTABILITY OF S-NITROSOTHIOLS
Wu and coworkers (2) provided no solid and convincing evidence for the photoinstability of endogenous S-nitrosothiols, although they may have been constrained by the “Brief Communication” format. The following are additional experiments they should have included: (a) unequivocal demonstration of the high photoinstability of endogenous and externally added S-nitrosothiols, which would have excluded other possibilities for S-nitrosothiol degradation; and (b) measurements in plasma and erythrocytes. S-Nitrosothiols are indeed sensitive to light; however, other published studies (1, 4) have not reported endogenous S-nitrosothiols to be as profoundly sensitive to light as proposed by Wu et al. (2). Our own experiments show that externally added GSNO to be equally stable in rat blood, whether “exposed” to daylight or protected from it (Fig. 1). This experiment also demonstrates, in contrast with what Wu and coworkers have stated (2), that N-ethylnmaleimide does not induce NO liberation from GSNO.

3. INCORRECT CALCULATION
In our opinion, Wu et al. did not correctly calculate the sensor signals from “covered” and “exposed” blood (2). If one refers to Fig. 1 in their report, it is easily observed that the initially constant current changes markedly with addition of blood to the chamber in which the electrode is placed and drops to levels clearly below the starting level. The greatest difficulty in assessing such current signals quantitatively is how to set the correct reference level. The baseline current level before blood addition cannot be used as the reference value for the signal resulting from blood addition.

By setting the baseline reference levels to values close to the nadir of the signals observed after blood addition, our calculation of the traces shown in Fig. 1 of the report by Wu et al. (2) (available from us upon request) indicates that the differences between “exposed” and “covered” blood are much less than the authors claim (2). The prolonged and marked changes in signal caused by addition of the blood render accurate quantitative analysis impossible. Moreover, the signal for the GSNO calibrator (3 μmol/L) added to “exposed” blood is clearly lower than the signal produced by the GSNO calibrator (3 μmol/L) added to “covered” blood. Interestingly, the ratios of the signals from putative endogenous S-nitrosothiols to those of the calibrator GSNO in “exposed” and “covered” blood differ insignificantly.

4. COMPARISON WITH DATA FROM THE LITERATURE
It is very strange that about 75% of the S-nitrosothiols in blood disappeared in the study of Wu et al. (2) within only about 15 s of blood sampling. This finding suggests that not protecting blood from light during sampling (the common practice of most scientists in this area, including those who have reported micromole-per-liter concentrations of S-nitrosothiols) would yield extremely low S-nitrosothiol concentrations; however, this is reportedly not the case (1). Other analytical and preanalytical factors are more likely to be responsible for the great discrepancies in reported S-nitrosothiol concentrations in blood (1).

5. PHYSIOLOGICAL CONSIDERATIONS AND CONSEQUENCES
In our opinion, GSNO concentrations in the blood of the order of 3 μmol/L, as the authors claimed to have detected (2), would be not compatible with health in mammals and humans.

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given the potent biological activity of GSNO (1).

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References


Ranieri Rossi1*
Dimitrios Tsikas2*

1 Department of Evolutionary Biology
Laboratory of Pharmacology and Toxicology
University of Siena
Siena, Italy
2 Institute of Clinical Pharmacology
Hannover Medical School
Hannover, Germany

* Address correspondence to these authors at:
Dr. Ranieri Rossi
Department of Evolutionary Biology
Laboratory of Pharmacology and Toxicology
University of Siena
Via A. Moro 4
53100 Siena, Italy
Fax 39-0577234476
E-mail ranieri@unisi.it
Dr. Dimitrios Tsikas
Institute of Clinical Pharmacology
Hannover Medical School
Carl-Neuberg-Strasse 1
30625 Hannover, Germany
Fax 49-511-532-2750
E-mail tsikas.dimitros@mh-hannover.de

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