Physiological Levels of S-Nitrosothiols in Human Plasma

To the Editor:

The hypothesis that endothelial-derived relaxing factor (EDRF) is nitric oxide (NO) has stimulated a wealth of research into the significance of this novel, intriguing molecule. Given its short life, many forms of storage and targeting have been postulated: among these, a pool of derivatives of NO (RSNOs) covalently bound to SH groups of proteins and low molecular weight thiols (e.g., glutathione) has been identified in various biological systems. The importance of RSNOs results from the very similar biological actions exhibited by both NO and RSNOs in vivo as well as in vitro.

S-nitroso derivatives of GSH, cysteine, hemoglobin, BSA, and many other protein or nonprotein thiols are potent, fast-acting vasodilators, as well as strong inhibitors of platelet aggregation, like NO itself. Moreover, several nitrosovasodilators, such as nitrosoglycerin, have been reported to function through the formation of S-nitrosothiol intermediates.

S-nitrosothiols in biological samples have been measured by a wide spectrum of different techniques. However, in our opinion, most of these techniques are not devoid of artifacts, and the investigation of endogenous S-nitrosothiol levels, as stated by Neil Hogg, “is hampered by methodological concerns” (page 1482). For example, the original report of 7 μmol/L for S-nitrosothiols in human plasma has been more recently downgraded to very low levels.2–5

A recent study by Tyurin et al6 showed that the S-nitrosoalbumin level (mostly S-nitroso albumin) was 9.2 ± 1.6 μmol/L in normal human plasma and 11.1 ± 2.9 and 9.4 ± 1.5 μmol/L in pregnant and preeclamptic pregnant women, respectively. In particular, these differences were ascribed to the diminished level of antioxidants (e.g., ascobic acid in preeclampsia). This, together with another recent study7 (250 and 520 nmol/L in healthy subjects and subjects with hypercholesterolemia, respectively), was the first report on differences in S-nitrosothiol levels in human plasma correlated with some pathological status.

We think that an accurate revision of the methods used is of fundamental importance before any speculation can be made on the role of S-nitrosothiols in different pathophysiological conditions. The statement of Tyurin et al.6 “We detected low micromolar concentrations of S-nitrosothiols in normal plasma samples; these concentrations were well within the range previously reported by several investigators in human plasma” (page 1214) is surely true. However, differences were ascribed to the diminished level of antioxidants (e.g., ascobic acid in preeclampsia), and this was disputed by all recent studies.

We assume that the detection of RSNOs in biological fluids, including plasma, may be associated with many difficulties that can lead to artifacts. In our experience, the relative instability of RSNOs, trans-nitrosation reactions, artificial formation of RSNOs during sample manipulation, and the coiritation of other NOx species together with RSNOs must be carefully evaluated. Other difficulties result from the many different methods that have been introduced and validated for the titration of RSNOs. Reports on RSNO concentration in human plasma are relatively few, and we have recently applied a modification of the most widely used methods to evaluate the normal plasma levels of endogenous S-nitrosothiols (authors’ unpublished results, 2001). Our results suggest that RSNOs in human plasma are at low nanomolar concentrations.

The main problem we encountered was mainly due to the low concentration of RSNOs. Consequently, it is fundamentally important to avoid the NOx contamination that normally occurs in all reagents and water used for the assays (and also from the air). In this sense, all reagents and water were of the highest purity grade available, they were also degassed, and all solutions were always fresh. The reagents were also processed through preparative HPLC to obtain a reagent of the highest grade of purity. Under these conditions, results obtained by all methods used gave convergent values. By spectrophotometry (with Griess reagent), we assessed that RSNO levels are less than 200 nmol/L (which represent the detection limit of the technique); with the aid of HPLC and with either sulfanilamide/NED (Griess reagent) or DAN (fluorescent detection), we found that RSNO concentration is close to 30 to 40 nmol/L. Our results were, therefore, in agreement with recent reports4,5 and in contrast with those assessed by Tyurin et al.6

The reason that Tyurin and colleagues found such a high concentration of RSNOs is probably due to the use of photolysis to free NO from RSNO. This method has been shown to be nonspecific for S-nitrosothiols, also causing NO release from compounds such as nitrite, nitrosamine, and dinitrosyliron complexes.

RSNOs may have many biological functions; the evaluation and the study of their concentration variation under different pathophysiological conditions, as well as after administration of different drugs, could be of fundamental importance. However, a previous careful analysis of the techniques used for RSNO determination must precede every attempt.

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