Photoinstability of S-Nitrosothiols during Sampling of Whole Blood: A Likely Source of Error and Variability in S-Nitrosothiol Measurements

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BACKGROUND: The determination of reference intervals for the concentration of total S-nitrosothiols (RSNOs) in whole blood is a highly controversial topic, likely because of the inherent instability of these species. Most currently available techniques to quantify RSNOs in blood require considerable sample handling and multiple pretreatment steps during which light exposure is difficult to completely eliminate. We investigated the effect of brief light exposure on the stability of RSNO species in blood during the initial sampling process.

METHODS: A novel amperometric RSNO sensor, based on an immobilized organoselenium catalyst at the distal tip of an electrochemical nitric oxide detector, was used to determine RSNO species in diluted whole blood without centrifugation or pretreatment. Porcine blood was collected into aluminum foil–wrapped syringes and without centrifugation. Porcine blood was collected into aluminum foil–wrapped syringes and one with the tubing exposed to ambient room light. The two blood samples were collected from the same animal—one with the butterfly needle tubing wrapped in aluminum foil and one with the tubing exposed to ambient room light. The RSNO concentrations in these sequential blood samples were determined by a standard addition procedure.

RESULTS: Eight sets of measurements were made in 6 animals. Samples exposed to light yielded RSNO concentrations only 23.6% (7.2%) (mean (SD)) of the RSNO concentrations determined in samples that were shielded from light and obtained from the same animals.

CONCLUSIONS: These results suggest significant photoinstability of RSNOs in whole blood and indicate the critical importance of proper light protection during sampling and processing of blood samples for the accurate determinations of endogenous RSNO concentrations.
tor total RSNOs in whole blood samples (initially employing animal models), we found that even normal sampling of the venous blood via a conventional butterfly type needle/tubing/syringe assembly can dramatically alter the observed response of the sensor toward the endogenous RSNO species that are present (unpublished data). We initially thought that the inherent absorbance of blood would be high enough to protect the endogenous RSNO species from photodecomposition (via 550–600 nm light, which causes homolytic cleavage of the S-N bond (5)), but we have discovered that this is not the case.

To prove that photodecomposition of the RSNO species in blood can occur during normal blood collection, we measured RSNOs in whole blood by drawing animal blood through a butterfly needle/tubing either with or without protection from light (via an aluminum foil covering of the plastic tubing that connects the needle with the syringe). Blood samples from pigs were collected by venipuncture in the abdomen via a butterfly needle (23G, Abbott) into a 5 mL heparinized syringe (5 U/mL final heparin) wrapped in aluminum foil. To evaluate the effect of light exposure on the stability of RSNO species in blood, another blood sample was drawn from the same animal with the butterfly tubing also wrapped with aluminum foil. During typical blood collection, it normally takes <15 s for blood to pass through the 12-inch length of plastic tubing. The blood RSNO concentrations were determined immediately after collection via a dual RSNO/NO sensor setup (9), using a standard addition technique. Within 1 min of blood draw, 5 mL of fresh animal blood was injected into an amber chamber thermostatted at 34 °C, containing 10 mL PBS (10 mol/L phosphate, pH 7.4), 100 mol/L glutathione, and 0.5 mmol/L EDTA (to chelate trace metal ions), under an N2 atmosphere in which the RSNO and NO sensors were placed. The signal from the NO sensor (without catalyst) was used to correct for any low concentrations of other components in blood (e.g., ammonia) that may elicit a slight electroactive interference with both the RSNO and NO sensors (typically a very minor correction). After the amperometric responses from blood samples reached steady state, an aliquot of GSNO calibrator was added to calibrate the sensor response in situ.

Fig. 1 shows typical sensor response patterns to blood collected using the covered and exposed sampling devices from the same animal, followed by the standard addition of 3 μmol/L GSNO to the diluted whole blood sample. It can be seen that exposure to light, though brief, substantially decreases the detected RSNO concentration from 1.45 μmol/L in the covered blood sample (whole blood concentration, not plasma phase concentration, which can be reported if values are corrected for hematocrit) to 0.40 μmol/L in the exposed sample (based on converting the steady-state response in current units to RSNO concentration units). The sensor response to the subsequent addition of the GSNO standard is reproducible between the 2 runs. Further, we confirmed the reproducibility of the sensor’s response to endogenous RSNOs in a separate experiment by consecutively measuring the RSNO concentration during a 90 min period in 4 fully covered/protected blood samples from another animal. The measured RSNO concentrations were consistent, with a mean (SD) of 3.36 (0.32) μmol/L. Thus, the only plausible explanation for the large decrease in initial response obtained immediately after injecting blood that was not protected from ambient light is the photodecomposition of a significant fraction of the RSNO species present. A total of 8 experiments of this type were performed using 6 animals. When the RSNO concentrations determined in samples collected with the protected butterfly tubing were normalized to 100%, the RSNO concentrations measured using the exposed tubing were only 23.6% (7.2%) [mean (SD)] of the values obtained when the protected tubing device was employed for collection. These findings clearly suggest that exposure to light, even if it is brief, has a dramatic impact on the concentration of total LMW RSNOs in blood samples. Using the new sensor technology we have observed similar results in preliminary testing for RSNO concentrations in human whole blood samples (data not shown).

S-Nitrosothiols exhibit ultraviolet/visible absorbance maxima at 330–350 nm and 550–600 nm (5). Despite a nearly 100-fold higher molar absorptivity at
the lower wavelength, results of previous studies sug-
uggest that photodecomposition of RSNOs is primarily
the result of light absorption in the 550–600 nm region
(10). The light effect can be expected to be much
greater for plasma samples, given the lack of any shield-
ing from light offered by the presence of red blood cells.
Hence, this light effect may explain the wide discrep-
ancy of mean reference range values reported in the
literature, because additional photoinduced losses can
occur during the extra sample preparation steps (e.g.,
preparing plasma) that are required for all other RSNO
detection methods. Indeed, some researchers have
found LMW RSNO species to be “undetectable” in
plasma samples (11).

Our data highlight the critical importance of even
the initial sample-handling step in RSNO determina-
tions. Furthermore, the findings described here regard-
ig RSNO instability under normal blood collection
conditions may also have profound consequence for
the interpretation of results of various in vitro blood
clotting and other coagulation tests that rely on inher-
ent platelet functionality in the blood sample. Because
it is known that RSNOs are potent inhibitors of platelet
aggregation (8), if a significant fraction of endogenous
RSNO species is lost during collection and handling of
blood samples drawn for such in vitro testing, one may
conclude that results obtained will not necessarily be a
true indictor of in vivo platelet functionality in the
presence of the in vivo concentrations of RSNO spe-
cies. It would therefore be interesting to assess whether
shielding blood samples from light during all collection
and handling steps significantly alters results obtained
for such in vitro coagulation tests.

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