Simple and Reliable Measurement of Nitric Oxide Metabolites in Plasma

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The importance of nitric oxide spurred the development of assays to monitor its production (1). The site of nitric oxide production was initially determined by the detection of NOS isoforms. The activity of NOS was first determined by use of enzyme histochemical assays and after the development of antibodies by immunohistochemical methods, although the development and use of antibodies proved to be challenging (2).

Nitric oxide is a short-lived radical and readily reacts with small scavenger molecules (e.g., uric acid, glutathione) and macromolecules (proteins, DNA) or is oxidized into nitrite and nitrate. Therefore, the detection of the radical itself proved very difficult, and only labor-intensive, sophisticated spin-trapping methods measured actual production of the nitric oxide radical. Assays for the stable reaction products of nitric oxide were also developed. Nitric oxide radical readily reacts with reactive oxygen species such as superoxide anions to yield peroxynitrite, an extremely reactive and toxic compound that is able to nitrosylate tyrosine residues in proteins, leading to the formation of modified proteins with changed or inhibited function. This nitrotyrosine modification can be detected using specific antibodies.

We were interested in nitric oxide’s role in the liver during systemic inflammatory conditions (endotoxemia, a model of sepsis) and in acute rejection after liver transplantation (3,4). More specifically, we were interested in the effects of NOS inhibitors on liver function in these conditions (3). To assess the effect of NOS inhibitors, we needed a reliable method to measure nitric oxide metabolites. We opted for an assay based on the Griess reagent, which is specific for nitrite, and we validated this assay for use with plasma samples. Our main findings were: (a) nitric oxide was predominantly oxidized into nitrite and nitrate, therefore it was useless to apply the Griess assay without prior reduction of nitrate to nitrite using nitrate reductase; (b) plasma and serum samples needed to be deproteinized to eliminate artifacts; and (c) the assay proved to be reliable and reproducible, and the recovery of nitrate and nitrite from plasma by use of our Griess-based assay was approximately 90%. We subsequently modified this assay for other body fluids such as urine and sputum (5). In addition to the Griess assay, HPLC methods were developed to measure nitrite and nitrate. The disadvantage of methods measuring

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2 This article has been cited 370 times since publication.

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stable end products is that actual nitric oxide production from NOS cannot be determined. Methods have also been developed that use stable isotopes to measure nitrite and nitrate derived from nitric oxide. These methods are, however, rather labor-intensive and complicated.

Although the interest in nitric oxide may have somewhat diminished over the last decade, methods to measure nitric oxide (metabolites) are still used extensively.

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**References**