NO solutions?

Commentary

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The saga of the explosive growth in understanding the physiological role of nitric oxide (NO) that followed its identification in 1986 as the "endothelium-derived relaxing factor" of smooth muscle, has been told many times (1). For much of the decade-and-a-half since the initial identification of NO as a ubiquitous biological effector molecule, it has seemed likely that this knowledge would lead quickly to robust therapeutic applications. Surprisingly, this has not happened — yet another example of the difficult nature of translational research.

In December 1999 the US Food and Drug Administration (FDA) approved inhalational administration of NO "for the treatment of term and near-term (> 34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation" (2). This approval for NO as a pulmonary vasodilator for neonates built on intensive laboratory and clinical study of the effects of inhaled NO on cardiopulmonary processes in animals, as well as in children and adults with a variety of lung pathologies (3). Although there have been many indications that this treatment could be beneficial for patients with these syndromes, clinical benefit has been shown only in the two trials (the NINOS study and the CINRGI study) that led to the limited 1999 FDA approval. Perhaps it is time to re-evaluate some of the assumptions that have focused so much of the pharmacological effort up to now on inhalation uses of NO and primarily on diseases of the lung.

Limitations of inhaled NO

NO inhalation therapy has been based on impressive observations of reduced pulmonary artery pressures, improved ventilation/perfusion matching, and increased oxygenation (3) when NO is administered. The usual protocol involves inhalation of the gas at between 5 and 40 parts per million (ppm) for hours or a few days, under high-flow conditions designed to minimize reaction of NO with oxygen and the consequent formation of the relatively toxic NO_2. Excessive methemoglobin production — in which oxyhemoglobin is oxidized by NO, yielding nitrate — limits the chronic application of high-dose (>40 ppm) NO inhalation therapy. The lack of large systemic cardiovascular effects, such as changes in blood pressure or heart rate, is attributed to this destruction by oxyhemoglobin of the residual bioactive NO that enters the pulmonary circulation after transit of the alveoli and their associated blood vessels.

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The apparent dominance of the hemoglobin reaction has markedly discouraged the exploration of alternative modes of delivering bioactive NO, especially for the purpose of altering blood flow in other organs, but also for any of the other potentially beneficial effects of NO, ranging from inhibition of platelet aggregation to inhibiting pathogens (4). It has also been assumed that NO's effective half-life in biological fluids would be limited by its reaction with oxygen (with formation of nitrite) but that its lifespan would be much shorter in hemoglobin-rich blood. Oxidative reaction of NO with hemoglobin does indeed largely limit the effects of inhaled NO to the lung vasculature. However, we (5) and others (6) have reported that peripheral vascular effects of high-concentration exogenous NO can be observed when local endothelial NO synthesis is blocked, suggesting that at least a portion of the introduced NO survives for long enough to reach remote tissues.

Action at a distance?

In this issue of *JCI*, Rassaf and colleagues (7) report a very original and important human experimental protocol that has allowed them to observe such effects. They infused an aqueous saturated NO solution in 0.9% saline, prepared with careful removal of oxygen, into the brachial arteries of groups of normal volunteers. Bolus infusions of 0.75 to 6 μmol of NO led to very rapid dilatations of the radial artery, a conduit vessel, and somewhat slower (>20 seconds) increases of forearm blood flow resistance vessels. The vasodilation observed was similar in magnitude to that achieved by infusion of acetylcholine and bradykinin, which work by causing local endothelial NO generation. NO infusions led not only to the expected large increases in plasma nitrite and nitrate, but also to smaller increases in plasma *S*-nitrosothiol species, as measured by a chemiluminescence assay.

To establish the pharmacodynamics of these compounds, Rassaf et al. infused *S*-nitrosoglutathione (not

S-nitrosoalbumin, which is presumed to be the major plasma *S*-nitrosothiol species), into three subjects and observed the dilatory effect in conduit and resistance arteries. They observed delayed vasodilation with this compound, which is not consistent with the immediate vasodilation found following NO solution infusion but which could explain the longer-term effects that they have noted. Thus, Rassaf et al. argue that the infusions of NO solutions work over physiologically relevant times and distances by two distinct pathways: the immediate effects of bioactive NO gas itself and the delayed effects of plasma *S*-nitrosothiol compounds.

These very interesting and somewhat unexpected results clearly suggest a new pharmacological route for delivering NO to patients' tissues. More immediately, however, infusions of NO solutions provide tools for understanding the complex processes that the small quantities of NO, physiologically produced in normal endothelia, undergo within the circulatory system. These questions ultimately focus on whether blood tends to limit the bioactivity of NO to the vascular beds in which it is synthesized or whether the circulation — normally, or pathologically, or following the pharmacologic administration of NO or NO donors — can distribute NO from one vascular bed to another.

Physiological lifetime of NO gas

In principle, the effective lifetime of NO in the blood is limited by its interactions with erythrocytic hemoglobin and the red cell membrane, and with the oxygen or other constituents of the plasma. The extremely rapid destruction of NO in hemoglobin solutions (8) — an effect believed to cause the hypertensive and other deleterious effects of stroma-free hemoglobin blood substitutes — initially suggested that the hemoglobin in red cells would rapidly destroy NO. However, it is now understood that, in flowing blood, this degradative reaction is markedly attenuated by several mechanisms. First, because of the properties of the erythrocyte membrane, entry of NO into red cells occurs at a rate up to three orders of magnitude slower than would be expected from simple diffusion (9, 10). In addition, in vessels with rapid blood flow, the endothelial surface is in contact with a layer of about 2–4 μm of virtually cell-free plasma (11), with the red cells concentrated more axially, thus allowing a fraction of the blood's free NO to persist for some time without encountering a high concentration of hemoglobin. Indeed, within this red cell–free zone of laminar flowing blood, NO may have a surprisingly long half-life.

If blood oxygen is in the range of 150–250 μM and NO concentration is about 180 μM, as we calculate should have occurred in some of the protocols used by Rassaf et al., the effective half-life of NO would be on the order of several seconds. Such values correspond well to the initial kinetics of vasodilation observed. Since physiological NO concentrations are much lower than those achieved in the present study, the effective chemical lifetime of NO in such a plasma layer has been calculated to be 100–500 seconds (12), clearly within the range in which NO can be transported in a bioactive form from its site of synthesis to other tissues. These rates and lifetimes will be substantially affected, however, by the hydrophobicity of the reacting medium and by reactions with other plasma constituents. Indeed the results of Rassaf et al. raise the possibility that our own previous observations (5) of a systemic vascular effect of NO inhalation might be due (at least in part) to residual NO gas itself, a mechanism we had not previously considered.

Protein modification by NO

As mentioned above, NO that enters the arterial red cells reacts primarily with oxyhemoglobin to form methemoglobin and nitrate, the latter increase measured by Rassaf et al. Small amounts of nitrosylhemoglobin may also form through a reaction with deoxyhemoglobin (13). It now seems that if *S*-nitrosation reactions occur in the red cell, they result primarily in modifications of membrane proteins (14) and little or no *S*-nitrosation of hemoglobin occurs due to the predominance of NO-heme reactions (ref. 13; and M.T. Gladwin et al., unpublished observations).

According to the present study, *S*-nitrosation of albumin appears to be the major, potentially reversible reaction observed with plasma after NO gas infusions. However, only about 0.1% of the applied NO can be detected in a form resulting from *S*-nitrosation, as compared with other oxidative chemistries under these conditions. These values for levels of *S*-nitrosoalbumin, before and after NO administration, are much lower than those originally reported (15). Thus, *S*-nitrosation reactions overall appear to be extremely limited under basal physiology. However, more work of this type with human subjects is needed to define further these reactions and the biological activity of their products — work that will be quite relevant to understanding the metabolism of endogenously produced NO.

Prospects of NO delivery in solution

Although Rassaf et al. have tested a moderate range of NO concentrations, clearly much further work on dosages and duration of administration needs to be done to establish potency with respect to various biological response parameters and safety. Further, intravenous infusions of NO may have transport paths and hemodynamic effects different from those of arterial infusions, given the lack of laminar flow and relatively high levels of deoxyhemoglobin in the veins. Studies of the potential systemic effects of infusions are needed to see if these solutions can be used therapeutically, for example for conditions such as cerebral vasospasm that require rapid vasodilation (16). The hemodynamic effects of NO solutions will need to be compared in detail with those of NO donor compounds like sodium nitroprusside that are already in widespread clinical use.

Clearly, this new protocol has the potential to inform us greatly about the physiological reactions of NO and will likely resolve many of the major controversies, stemming from extrapolations of in vitro data to physiological conditions in human beings, that have plagued this field. Such studies may also resolve the complex problems of which chemical species can deliver significant amounts of NO under both physiological and pathophysiological conditions and the multifold mechanisms by which blood can either destroy or transport NO. Beyond the insights it provides into these compelling questions, the present work (7) hints at new approaches to NO therapeutics for the many diseases that derive from an absolute or relative lack of this highly potent gas (17).

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