Understanding the role of NOS-3 in ventilator-induced lung injury: don’t take NO for an answer

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Although nitric oxide synthases (NOSs) were first described in 1989 (12), these complex enzymes are still not fully understood, and their role in human lung disease remains unclear. Three NOS isoforms have been described in humans: neuronal or nNOS (NOS-1), inducible or iNOS (NOS-2), and endothelial or eNOS (NOS-3). All isoforms of NOS are modular enzymes with a reductase domain and an oxygenase domain. When electron transfer between the reductase and oxygenase domains is coupled, synthesis of nitric oxide (NO) and L-citrulline from the substrate L-arginine is catalyzed via coupling of L-arginine oxidation with O₂ reduction. Dimerization of NOS monomers is required for this NO synthesis and occurs in the presence of heme protein. The NOS product NO is a ubiquitous signaling molecule that regulates vascular tone and blood flow, leukocyte adhesion, platelet aggregation, and mitochondrial oxygen consumption (9).

There are several conditions under which the tightly linked reductase and oxygenase functions of NOS can become uncoupled, inhibiting NO production. Under uncoupled conditions, NOS preferentially catalyzes the reduction of molecular oxygen to form superoxide ion. One well-described factor leading to NOS uncoupling is substrate deficiency (5). L-Arginine is the only known substrate for NO synthesis by NOS. Although not an essential amino acid, L-arginine can become conditionally essential in situations of metabolic stress such as sepsis. L-Arginine can be synthesized in vivo from the urea cycle intermediate product L-citrulline (also generated by NOS), by argininosuccinate synthase, and argininosuccinate lyase. Factors that lead to reductions in citrulline/arginine availability include ischemia reperfusion and physiological stress (3, 13, 18). A second factor that can lead to uncoupling of NOS is insufficiency of the NOS cofactor tetrahydrobiopterin (BH₄), which is normally bound to the oxygenase domain (22). Factors that can lead to reductions in BH₄ include oxidative stress (8) and ischemia reperfusion (22). Reactive oxygen species can also react with NO to form peroxynitrite. Peroxynitrite can, in and of itself, lead to uncoupling of NOS. Another factor that can contribute to NOS uncoupling is endogenous production of asymmetric dimethyl arginine (ADMA), a competitive inhibitor of NOS that has been shown to cause uncoupling of NOS-3 (2, 17). Chronic uncoupling of NOS has been implicated in several human diseases including diabetes (9), hypertension (9), and diastolic dysfunction (16).

The role of NOS in ventilator-induced lung injury is not well understood, and data from experimental models have provided conflicting results. Frank and colleagues (6) reported that ventilator-induced lung injury (VILI) in rats was associated with induction of NOS-2. Inhibition of NOS-2 in this model ameliorated VILI. Peng and colleagues (14) reported that in a mouse model of VILI using NOS-2−/− mice, NOS-2 deficiency was protective. Peng and colleagues also studied NOS-3−/− mice (15). Contrary to their findings in NOS-2−/− mice, NOS 3−/− mice were more susceptible to VILI. In another model (19), NOS-3 overexpression protected mice from VILI. However, Schmidt and colleagues (15a) found that NOS-3 deficiency was protective in a VILI model in isolated perfused mouse lungs. One possible explanation for the contradictory findings in both animal and human studies is that the presence or absence of NOS is not the only determinant of the degree of VILI. The coupling state of NOS may also be a major determinant of the degree of injury because the coupling state determines the relative amount of NO vs. superoxide production by the NOS enzymes. The coupling state of NOS has not been directly studied in VILI.

In this issue, Vaporidi and colleagues (21) explored the role of NOS-3 in an experimental model of VILI in mice. Wild-type and NOS-3-deficient mice were ventilated with a high-tidal volume (40 ml/kg) for 4 h. In wild-type mice, the high-tidal volume ventilation produced acute VILI with decreased lung compliance, increased bronchoalveolar lavage protein and proinflammatory cytokine levels, and evidence of oxidative stress. VILI was markedly attenuated in NOS-3-deficient mice. Treating NOS-3-deficient mice with NO during high-tidal volume ventilation did not augment VILI, suggesting that the protective effects of NOS-3 deficiency were not mediated through decreases in NO production. In wild-type mice, oxidative stress was markedly enhanced in vivo by high-tidal volume ventilation, and lung extracts from wild-type mice had high levels of superoxide production that could be inhibited by the NOS inhibitor L-NAME. By contrast, L-NAME did not affect superoxide production in NOS-3-deficient mice subjected to high-tidal volume ventilation. Together, these findings suggest that superoxide production and not NO production is the predominant role for NOS-3 in the pathogenesis of VILI in this model, thus providing in vivo evidence for uncoupling of NOS as a mechanism for oxidative stress in VILI. In support of this, treatment of wild-type mice with the NOS-3 cofactor BH₄ and the antioxidant ascorbic acid led to a reduction in VILI, presumably via enhanced coupling of NOS-3. The major limitation of this study was the use of a supraphysiological tidal volume to induce VILI, limiting the potential clinical relevance of the findings. A second limitation is the lack of direct measurements of NOS cofactor or NOS substrate levels. Although supplementation of BH₄ in the wild-type animals reduced indices of VILI, it is not known whether BH₄ levels were actually depleted by high-tidal volume ventilation.
Whether high-tidal volume ventilation also reduced endogenous l-arginine levels was also not determined. Nevertheless, the findings are intriguing since they provide in vivo evidence, albeit indirect, that NOS uncoupling could be a major pathogenic mechanism in VILI.

The findings of Vaporidi et al. (21) highlight the importance of evaluation of the coupling state of NOS in studies of the NOS enzymes in both experimental and human lung disease. Furthermore, these findings shed some light on why the various prior studies of NOS deletion, overexpression, and inhibition have not produced consistent findings in studies of VILI. It appears that when evaluating the NOS pathway, it is not sufficient to measure NOS gene and protein levels; the relative production of NO and superoxide as indices of NOS coupling must also be considered. Since NOS inhibitors inhibit both NO and superoxide production by NOS, any protective (or harmful) effects of NOS inhibitors must also be considered in light of the coupling state of NOS. Furthermore, effects of NOS inhibitors cannot be definitively attributed to reductions in NO synthesis if NOS is uncoupled, since NOS inhibition will also decrease superoxide production.

What do these findings tell us about prevention and treatment of VILI in humans? In a large randomized controlled trial of lower- vs. higher-tidal volume ventilation, urine levels of the NO products nitrate and nitrite were higher in survivors than non-survivors and rose more between enrollment and day 3 in patients treated with a low-tidal volume protective ventilatory strategy compared with patients treated with a higher-tidal volume ventilatory strategy (11). These data suggest that preserved NO production is an important marker of better outcomes in acute lung injury that is improved with a protective ventilator strategy. Preserved NO production in this study could be a marker of less endothelial injury. Alternatively, preserved NO production might be a marker of less uncoupling of NOS with less superoxide production. Together with the current study by Vaporidi and colleagues (21), an intriguing hypothesis emerges that therapies that target maintenance of coupling of NOS might be beneficial in VILI and in acute lung injury in general. Several potential strategies are possible. Restoration of substrate levels via arginine or citrulline supplementation is one potential therapy. Substrate deficiency of l-arginine and its precursor l-citrulline has been reported in critical illness. For example, in adults with severe sepsis, levels of citrulline and arginine were very low, and lower levels were associated with development of acute lung injury and acute respiratory distress syndrome (10). In critically ill children, plasma levels of citrulline and l-arginine were also low and were associated with the severity of inflammation (20). Children at risk for postoperative pulmonary hypertension after cardiopulmonary bypass, low plasma l-arginine levels are a risk factor for postoperative pulmonary hypertension (3), and citrulline supplementation is currently being studied as a potential therapy (1, 4). BH4 supplementation is another potential target that is currently being studied in vascular diseases including systemic hypertension, peripheral arterial disease, coronary artery disease, pulmonary arterial hypertension, and sickle cell disease (7).

In summary, we are just beginning to unravel the complexities of the NOS family of enzymes and their role in lung disease. The findings of Vaporidi and colleagues (21) emphasize the potential importance of NOS uncoupling in VILI and acute lung injury and provide us with a new lens through which to interpret prior and future studies of NOS and its products in both experimental and human lung disease.

REFERENCES


