Power of Place: Intravascular Superoxide Dismutase for Prevention of Acute Respiratory Distress Syndrome

The Earth formed ~4.5 billion years ago, but its atmosphere was largely devoid of oxygen until 2 billion years later, when concentrations increased dramatically (1). This increase in oxygen was followed by an explosion in biodiversity and included the evolution of multicellular organisms that depended on oxygen for energy production and survival (2). At virtually the same time, adaptations to deal with superoxide and other reactive oxygen species (ROS) also evolved. Many of these adaptations remain highly conserved among today's species, including the superoxide dismutase (SOD) enzymes (3) that catalyze the breakdown of superoxide into hydrogen peroxide. Accordingly, their function is absolutely essential for normal development, maintenance of homeostasis, and prevention of disease. Because oxidant damage is a well-recognized contributor to acute respiratory distress syndrome (ARDS) and septic shock, strategies that enhance SOD function pose a seemingly attractive therapeutic strategy. In this issue of the *Journal*, Tanaka and colleagues (pp. 179–190) test the efficacy of lecithinized SOD for preventing organ damage in mouse models of sepsis and ARDS (4).

The lecithinized SOD used by Tanaka and colleagues contains a human Cu/Zn-SOD complexed with phosphatidylcholine (PC) derivatives to form PC-SOD, a compound that exhibits enhanced stability in plasma and improved tissue affinity (5). Using the murine cecal ligation and puncture (CLP) model of sepsis, the authors convincingly demonstrate that administration of intravenous PC-SOD before surgery improved survival, diminished systemic inflammation, attenuated kidney injury, and reduced vascular permeability in the kidneys and liver. Notably, these beneficial effects were not seen when the PC-SOD was administered intratracheally. Salutary effects of intravenous PC-SOD were also demonstrated in ventilator-induced lung injury and intratracheal LPS models of ARDS. In both models, pretreatment with PC-SOD attenuated pulmonary capillary leak and histologically assessed injury. Moreover, in the LPS model, mice treated with intravenous PC-SOD had reduced inflammatory cytokine and leukocyte levels in alveolar lavage fluid and attenuated capillary leak in the liver. Intratracheal dosing of PC-SOD was not tested. Finally, to demonstrate that the beneficial effects of PC-SOD were mediated by ROS, the authors used *in vivo* imaging with the luminol-based chemiluminescent probe L-012. Treatment with PC-SOD reduced uptake of the probe in the abdomens of mice after CLP and in the lungs after intratracheal LPS.

Taken as a whole, the results of this study support a role for targeted administration of PC-SOD in the treatment of sepsis and ARDS. However, several critical questions remain unanswered, including ones regarding the optimal timing of therapy, potential differences between direct and indirect causes of lung injury, the cellular compartments targeted by PC-SOD, and the precise mechanisms by which redox balance is altered. In the context of clinical therapeutics, the timing of administration is perhaps the most critical. At the heart of the matter is whether PC-SOD is only effective as a preventative agent or whether it may provide benefit after the onset of illness. This issue is clearly highlighted by the group's mortality studies in the CLP model, in which pretreatment with PC-SOD led to striking improvements in survival, but delay of treatment by a mere hour provided no statistically significant benefit. Because many patients arrive in the emergency room or intensive care unit after the onset of sepsis and/or lung injury, the therapeutic window must be more clearly defined.

In addition to understanding when PC-SOD might be administered in relationship to the onset of disease, it is also essential to determine which patient populations might derive the greatest benefit. One potential group might be chronic alcoholics, in whom impaired glutathione synthesis leads to enhanced oxidative stress. Such individuals are at increased risk for the development of ARDS and suffer increased mortality (6, 7). Tobacco smoke also induces oxidative stress in the lungs, and smokers are also increased risk of developing ARDS (8–10). Individuals with genetic polymorphisms that affect redox balance make up a third group, in which case both gain and loss of function need to be considered. For example, in one study, patients with ARDS with polymorphisms in glutathione-S-transferase appeared to derive significant benefits from N-acetylcysteine infusion, whereas control subjects did not (11). Conversely, in another study, a block of single-nucleotide polymorphisms in extracellular superoxide dismutase (EC-SOD) was associated with improved outcomes in individuals with infection-related ARDS (12). One could speculate that the first group would benefit from PC-SOD and the second group would not because of baseline high vascular SOD levels. These observations support the need to define at-risk populations and provide a rationale for new therapeutic approaches.

The authors note that a nebulized form of PC-SOD has been developed for treatment of idiopathic pulmonary fibrosis and that this may decrease systemic toxicity. This raises interesting questions about the optimal route of administration for patients with direct versus indirect causes of lung injury. In this context, it must be noted that although intratracheally administered PC-SOD did not appear to be beneficial in the CLP model of sepsis, the intravenous route was. Unfortunately, intratracheal dosing of PC-SOD was not tested in the ventilator-induced lung injury and LPS models. However, the authors have previously demonstrated efficacy of both inhaled and intravenous PC-SOD for reducing bleomycin-induced fibrosis (13, 14) and elastase-induced emphysema (15, 16), models in which disease is largely lung-limited. Accordingly, it is possible that intravenous administration of PC-SOD may be optimal for indirect forms of lung injury, whereas inhaled dosing may be preferable for direct forms.

Three isoforms of SOD exist in mammals: cytosolic Cu/Zn-SOD (SOD1), mitochondrial Mn SOD (SOD2), and

extracellular EC-SOD (SOD3). Each has a distinct cellular distribution and thus modulates redox-sensitive pathways in different cellular compartments. The authors' prior work carefully documented the ability of PC-SOD to augment SOD in the circulation and within lysosomes (5), and similarly, they show an increase in plasma SOD activity with PC-SOD treatment in the current manuscript. Plasma SOD activity is attributable to EC-SOD, except potentially in the setting of hemolysis with release of red blood cell Cu/Zn-SOD. Ergo, intravenously administered PC-SOD is most likely restoring or augmenting endogenous extracellular SOD (17, 18). In this context, it would also be highly interesting to know whether PC-SOD increased lung or alveolar SOD activity in the different ARDS models.

Although EC-SOD is normally protein bound, it can be released into fluids after proteolytic cleavage of the EC-SOD heparin-binding domain. Similar release also occurs in humans with a polymorphism in the EC-SOD heparin-binding domain that results in lower heparin-binding affinity. Using mice expressing this human R213G SNP, our group recently demonstrated that elevated plasma EC-SOD levels were present at baseline and that this protected against intratracheal bleomycin-induced lung injury (19). These results are consistent with the study by Tanaka and colleagues, as both studies suggested the importance of plasma SOD activity in protecting against initiation of lung injury (4).

Tanaka and colleagues carefully measured a number of relevant endpoints to establish the protective effects of PC-SOD in the different models of ARDS (4). However, more work is needed to better understand the role of disrupted redox-dependent signaling on the pathogenesis of disease. It is important to emphasize that SOD catalyzes the degradation of superoxide to hydrogen peroxide or O_2, rather than acting as a general "ROS scavenger." Thus, it will decrease superoxide and, depending on local environment, increase NO bioavailability, decrease peroxynitrite formation, and increase hydrogen peroxide. Hydrogen peroxide is a potent signaling molecule under some circumstances, but can also lead to toxic hydroxyl radicals through Fenton chemistry, depending on its concentration and the local availability of other antioxidant enzymes, including catalase and glutathione peroxidase. The *in vivo* studies using L012 are exciting, as they begin to address site-specific changes in the oxidant environment. The probe does not specifically determine the specific reactive species, as this sensitive tool has been previously reported to detect neutrophil-dependent superoxide as well as SOD inhibitable signals generated from peroxidase and hydrogen peroxide (20). It is also unclear what specific redox-regulated pathways are interrupted by PC-SOD. Overall, these new data provide further rationale to continue to delineate the compartment in which the inflammation and ROS production is initiated and to design antioxidant therapies that may overcome problems observed in other studies that arise from broad, nontargeted approaches (21).

In summary, building on the foundation of previous work, Tanaka and colleagues now show that administration of lecithinized SOD provides organ protection in a series of mouse models of ARDS. Although a number of questions still remain, it is exciting to postulate that the strategy of targeting specific compartments with selective antioxidant enzymes may have the potential to overcome some of the barriers observed in the disappointing human studies testing redox/antioxidant strategies in ARDS or sepsis. ■

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