Anesthesiology 2010; 113:4–6

Hydrogen Sulfide in Lung Injury

Therapeutic Hope from a Toxic Gas?

COULD a toxic gas treat injured lungs in critically ill patients? Maybe. In this issue of the journal, Faller *et al.*[1] provide evidence that hydrogen sulfide is beneficial in an animal model, raising the question: might it help patients? To understand the importance of these findings,[1] we need to understand the context. Acute respiratory distress syndrome is common, often lethal, and consumes enormous resources. Mechanical ventilation is the mainstay of supportive therapy, allowing adequate oxygenation and assisting with the increased work of breathing. Although such mechanical ventilation is acutely lifesaving, it has an attributable mortality and morbidity caused by lung injury that is initiated by cyclic distension of lung tissue.

This problem, ventilator-associated lung injury, has been the subject of immense progress for the past 40 yr. Since the original *in vivo* model was described by Webb and Tierney in 1976, there have been several landmark mechanistic insights. One of the first such insights was the recognition of the obligatory role of neutrophils in ventilator-induced lung injury,[2] confirming an inflammatory basis for the condition, and one of the most recent major insights describes some of the fundamental cell signaling events that propagate many forms of acute lung injury.[3]

Clinical practice has also evolved. That high tidal volumes should be avoided was recognized in several case series from the 1980s that described improved outcomes with lowered tidal volumes in premature neonates[4] and in adults with status asthmaticus[5] or lung injury.[6] Ten years ago, an important prospective trial confirmed that tidal volume makes a critical difference in patients with acute respiratory distress syndrome: mortality was less in patients randomized to lower *versus* higher tidal volumes.[7]

Since then, clinical trial groups have expended extensive effort in testing approaches to treatment—beyond lowering tidal volumes—that the clinician could apply to populations of patients with acute respiratory distress syndrome and expect to improve outcome. Such approaches (*e.g.*, prone positioning, increased positive end-expiratory pressure, surfactant, or inhaled nitric oxide) have certainly advanced our knowledge in the field, but they have not provided the clinician with new therapies that will necessarily improve their patients' outcome.

Whether progress in clinical trials seems swift or stalled, future advances will always depend on discovering new mechanisms that are amenable to clinical testing. Such insights might come from physiologic or molecular studies, and although molecular mechanisms are most distant from the minds of the bedside clinician, they might ultimately offer the brightest hope for testable "candidate" therapies. Faller *et al.*[1] offer early signs of one such candidate.

Hydrogen sulfide, a potent toxin, is a gaseous mediator that has created great excitement as a therapy for preserving organ function—and life—during suspended animation in *in vivo* models.[8] Faller *et al.*[1] hypothesized that because ventilator-induced lung injury involved inflammatory and apoptotic pathways, suspending such processes might decrease the injurious effects of ventilation with high tidal volume.

In this important study—the first of its kind in the field—low concentrations of inhaled hydrogen sulfide (80 ppm) attenuated the key indices of lung injury in the anesthetized *in vivo* mouse.[1] The injury was induced solely by high tidal volume without the confounding effects of previous injury, and in addition to reducing the histologic evidence of lung injury, hydrogen sulfide inhibited several inflammatory pathways and decreased neutrophil activation, apoptosis, and heme-oxygenase expression. A separate series of experiments confirmed that although both hydrogen sulfide and hypothermia protect against ventilator-induced lung injury and hydrogen sulfide does induce hypothermia, the protective effects of hydrogen sulfide were demonstrable when hypothermia was already induced. Thus, hydrogen sulfide was protective over and above the protective effects of hypothermia. This is potentially important because hypothermia from a therapy such as hydrogen sulfide might not result in larger animals (*e.g.*, humans), and in situations where hypothermia exists, hydrogen sulfide might be expected to have additional benefit.

Although the biologic effects of hydrogen sulfide have been described in a variety of *in vivo* models, the molecular mechanisms by which it elicits these responses implicate a

Accepted for publication February 4, 2010. The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

◆ This Editorial View accompanies the following article: Faller S, Ryter SW, Choi AMK, Loop T, Schmidt R, Hoetzel A: Inhaled hydrogen sulfide protects against ventilator-induced lung injury. ANESTHESIOLOGY 2010; 113:104–15.

Table 1. Major Categories of "Positive" and "Negative" Actions of Hydrogen Sulfide (H_2S)

	Mitochondrial	Vasoactive	Inflammatory
Positive effects	Low H_2S concentration Mechanism: partial inhibition of cytochrome *c* oxidase 1. Hypometabolic state 2. Hypothermia 3. Suspended animation	Vasodilatation Mechanisms: 1. Endothelial K-ATPase: channel activation 2. Endothelial ACE: inhibition Mechanisms potentiated where O_2 concentration is low	Antiinflammatory effects Mechanisms: 1. ↓ Neutrophil adhesion because of the activation of endothelial K-ATPase channels 2. ROS scavenging, especially peroxynitrite 3. ↑ Hemoxygenase-1 (releases CO [antiapoptotic] and bilirubin and Fe^{3+} [antioxidants]) 4. Inhibition of MAPKs and NFκB (context dependent)
Negative effects	High H_2S concentration Mechanism: total inhibition of cytochrome *c* oxidase 1. Free radical generation 2. Glutathione depletion 3. Apoptosis 4. "Poisoning" of the electron transport chain	Vasoconstriction Mechanisms: 1. NO interactions: formation of inactive nitrosothiols reduces individual effects of H_2S and NO 2. O_2 interactions: more H_2S is required to achieve vasodilation at higher O_2 concentrations; vasoconstriction results at high O_2:H_2S ratios Mechanisms potentiated where O_2 concentration is high	Proinflammatory effects Mechanism: 1. Activation of MAPKs and NFκB (context dependent)

ACE = angiotensin-converting enzyme; K-ATPase = potassium-adenosine triphosphate-ase; MAPKs = mitogen-activated protein kinases; NFκB = nuclear factor κB; ROS = reactive oxygen species.

wide array of molecules (table 1), yet remain incompletely understood.[9] Major targets clearly include the inhibition of mitochondrial cytochrome *c* oxidase and activation of endothelial cell K^+-ATPase channels, but the downstream effects of both pathways are highly context-dependent. For example, moderate inhibition of cytochrome *c* oxidase at low hydrogen sulfide concentrations induces the "suspended animation" (*i.e.*, hypometabolism, hypothermia) that has been associated with protection. However, higher hydrogen sulfide concentrations are clearly toxic because of the block in cellular respiration akin to cyanide poisoning. The vasodilator effects cited in protection against myocardial and ischemia–reperfusion injury primarily result from the activation of endothelial K^+-ATPase channels and can be enhanced *via* hydrogen sulfide interactions with nitric oxide and angiotensin-converting enzyme; yet, these vasodilator effects may actually switch to vasoconstriction depending on the concentrations of oxygen or hydrogen sulfide (table 1). For example, chemical reactions between hydrogen sulfide and nitric oxide can produce inactive nitrosothiols, negating the relaxant effect of either compound (fig. 1). In addition, higher oxygen concentrations increase the EC_{50} for hydrogen sulfide–induced vasorelaxation, and high ratios of oxygen to hydrogen sulfide cause vasoconstriction; this may be of particular importance in the lung, where oxygen concentrations are higher than in the systemic circulation.

Hydrogen sulfide also functions as a scavenger of reactive oxygen species, particularly of peroxynitrites, directly reducing oxidative stress damage. In this context, hydrogen sulfide is also associated with the up-regulation of the protective gene heme-oxygenase 1, conferring additional protection. Diminished oxidative stress may explain the inhibition of nuclear factor κB elicited by hydrogen sulfide in lipopolysaccharide-stimulated macrophages. Yet, data on hydrogen sulfide effects on intracellular signaling are also context-dependent, with both positive and negative effects reported not only on nuclear factor κB but

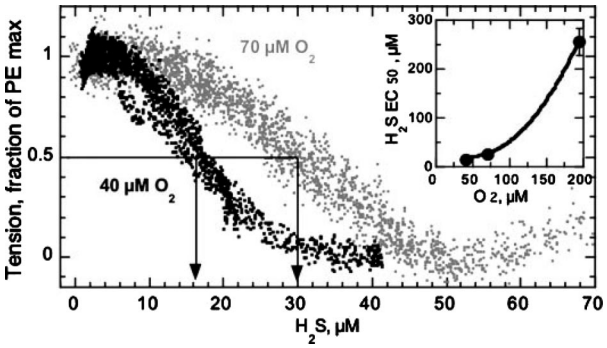

Fig. 1. Aortic tension at 40 (*black symbols*) and 70 μM (*gray symbols*) O_2. Effective hydrogen sulfide (H_2S) concentrations for EC_{50} at 40 and 70 μM are shown using *arrows*. *Inset:* H_2S EC_{50} values of aorta as a function of O_2 concentration. Reproduced with permission from: Koenitzer JR, Isbell GS, Patel HD, Benavides GL, Dickinson DA, Patel RP, Darley-Usmar VM, Lancaster JR Jr, Doeller JE, Kraus DW: Hydrogen sulfide mediates vasoactivity in an O_2-dependent manner. Am J Physiol Heart Circl Physiol 2007; 292:1953–60.

also on other critical cell signaling pathways (*e.g.*, mitogen-activated protein kinase and phosphatidylinositol-3-kinase/Akt). These contrasting effects may explain why hydrogen sulfide has been reported to yield either proinflammatory or antiinflammatory effects in a variety of different contexts. If all that was not complicated enough, by scavenging nitric oxide, hydrogen sulfide may cause potentially harmful vasoconstriction or platelet aggregation.

Faller *et al.*[1] have demonstrated antiinflammatory and antiapoptotic effects of hydrogen sulfide in ventilator-induced lung injury that are independent of two of the known mechanisms of action of hydrogen sulfide (*i.e.*, hypothermia or increased heme-oxygenase 1). However, the potential roles of cytochrome *c* oxidase, angiotensin-converting enzyme inhibition, radical scavenging, and cell signaling pathways such as nuclear factor κB and mitogen-activated protein kinase remain to be explored. Understanding the factors that tip the balance among the many pathways impacted by hydrogen sulfide will be important in developing hydrogen sulfide–related pharmacologic agents, particularly in light of paradoxical proinflammatory and antiinflammatory effects.

How might investigators use this work to advance the field? As with all excellent basic research, the specificity and reproducibility of the model provide maximum confidence about the observed mechanisms but restrict the extrapolation to other situations. Although many inflammatory pathways were attenuated in parallel with the protection against lung injury in the current study, only by individually perturbing specific pathways can we know whether such effects explain—or simply reflect—the lung protection afforded by hydrogen sulfide. Although there are many plausible candidate pathways, a promising one is the K^+-ATPase channel, which is known to be activated by hydrogen sulfide. If the actions of hydrogen sulfide were inhibited by glibenclamide (a well-characterized K^+-ATPase blocker that can be used *in vivo*) in this context, this would suggest that the activation of such channels is a key mechanism of protection as opposed to a nonspecific effect. Finally, it will be important to understand the effects of hydrogen sulfide on organ systems other than lungs.

In conclusion, ventilator-associated lung injury is an important iatrogenic illness that complicates the care—and worsens the outcome—of critically ill patients. Hydrogen sulfide, a toxic gas, protects against such injury in an experimental setting by mechanisms that are not yet understood. The work of Faller *et al.*[1] sets the stage for exploring how this "new" therapy works in this context. By understanding these mechanisms, we may eventually improve outcomes for ventilated patients and, in the process, learn lessons from a toxic gas that apply to other disease states.

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