

## Nitric Oxide As a Bactericidal Agent: Is the Cure Worse Than the Disease?

Wouldn't it be nice to turn a knob on a ventilator, and eliminate bacterial infection in the lung in just a few magical hours? McMullin et al, from the University of British Columbia, hypothesize that such an idea might not be that fanciful.<sup>1</sup> These researchers exposed bacterial isolates in a specially designed incubator to a high concentration (200 ppm) of nitric oxide (NO) gas and found that the bacteria were killed within 2–4 hours of exposure. But does this mean that NO should be considered as a bactericidal agent in clinical situations?

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Multiple studies have documented that NO is important for the clearance of bacteria from the lung.<sup>2–4</sup> NO may produce bacterial killing by several mechanisms. It may bind to iron or thiol groups on the proteins and inactivate enzymes responsible for replication. NO also binds with the superoxide radical  $O_2^-$ , to form peroxynitrite ( $OONO^-$ ).<sup>5</sup> This species is a strong oxidant and catalyzes membrane lipid peroxidation and formation of nitrotyrosine residues in proteins. NO will also react with oxygen to form toxic species such as  $NO_2$  and  $N_2O_3$ . All these mechanisms may cause harm to both bacteria and host cells.

Even if high concentrations of NO are effectively bactericidal, is the cure worse than the disease? What of the toxicity of NO to patients? At concentrations below 80 ppm, NO breathing appears to be well tolerated. Adverse effects and complications of NO inhalation in controlled trials appear to be no different from breathing a placebo gas mixture.<sup>6</sup> High concentrations of NO, on the other hand, may have deleterious effects, including acute pulmonary injury, methemoglobinemia, asphyxia, and death.<sup>7–9</sup> High levels of intracellular NO have been reported to cause DNA damage and mutations in human cell preparations.<sup>10</sup> The United States Occupational Safety and Health Administration has set a time-weighted average exposure limit of 25 ppm for NO when breathed for 8 h/d in the workplace.<sup>11</sup>

As for  $NO_2$ , occupational safety and health standards limit the  $NO_2$  exposure of workers to 5 ppm.<sup>11</sup> Adverse effects, however, may be caused by exposures below that limit. Inhaled  $NO_2$  reacts with water within the lungs to produce nitric acid and undergoes irreversible reactive ab-

sorption by pulmonary epithelial lining fluid.<sup>12</sup> High levels ( $> 10$  ppm) of inhaled  $NO_2$  have produced pulmonary edema, hemorrhage, changes in the surface tension of surfactant, reduced alveolar numbers, and death in experimental animals.<sup>8,13,14</sup> At inhaled concentrations as low as 2 ppm, alveolar cell hyperplasia, altered surfactant hysteresis, changes in the epithelium of the terminal bronchioles, and loss of epithelial cilia have been reported.<sup>15,16</sup> In humans, 2.3 ppm  $NO_2$  increased alveolar permeability<sup>17</sup> and at inhaled  $NO_2$  concentrations of  $< 2$  ppm airway responsiveness may be increased.<sup>18–20</sup> These studies are concerning in that NO exposure in the study by McMullin et al was associated with  $NO_2$  concentrations as high as 18 ppm.

Nitrosylation of iron-containing enzymes and iron-sulfur proteins may alter the function of important proteins, such as surfactant and hemoglobin. In the case of hemoglobin, NO binding forms nitrosyl Fe(II) hemoglobin.<sup>21</sup> The heme iron of the hemoglobin subsequently is oxidized from  $Fe^{+2}$  to  $Fe^{+3}$  to form methemoglobin. While the formation of methemoglobin during exposure to clinical concentrations ( $< 40$  ppm) of NO is low, patients with decreased methemoglobin reductase activity may develop important methemoglobinemia if exposed to high concentrations of NO.

Might NO have a bactericidal role in clinical situations? The evidence is far from complete. McMullin et al studied the effects of NO under highly artificial conditions. The bacteria were suspended in saline, not buried within the pulmonary parenchyma and surrounded by macrophages and associated inflammatory mediators. A high concentration of NO was required, which was far greater than the 80 ppm maximum dose approved by the United States Food and Drug Administration. Concentrations much greater than 200 ppm might be necessary to achieve sufficiently high concentration of NO in atelectatic or consolidated areas of the lung. Also, high concentrations of  $NO_2$ , up to 18 ppm, were present. Lastly, and most importantly, the effect of 200 ppm NO on host pulmonary tissues remains to be defined, but is likely to be detrimental.

Nevertheless, inhaled administration of traditional antibacterial agents has been shown to be an effective method of treating some pulmonary infections.<sup>22</sup> Might we be able to deliver “antibiotic-strength” concentrations of NO to

the lung while minimizing toxicity to host tissue? Macrophages express their bactericidal activity in part by generating high local concentrations of NO. What is tantalizing about the research of McMullin et al is the possibility of creating an “artificial macrophage” to deliver NO. Such a therapy would consist of a drug or nano-delivery device specifically designed to deliver a sufficiently high concentration of NO directly to infected tissue without harming normal areas.<sup>23</sup> One might precisely tailor the cure to the disease. Conceivably, such directed therapies someday could be useful in treating infections that are highly resistant to our current antibiotic regimens.

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