Hyperoxia, HMGB1, and Ventilator-Associated Pneumonia

Reducing Risk by Practicing What We Teach

Ventilator-associated pneumonia (VAP), occurring more than 48 to 72 hours after endotracheal intubation, accounts for 25% of all ICU bacterial infections (1). VAP develops from aspiration of oropharyngeal pathogens, bacteria leakage around the endotracheal tube cuff, or colonization of the endotracheal tube with bacteria and biofilm, with embolization to distal lung (1). Because it greatly increases patient mortality, ICU length of stay, and cost of hospitalization, VAP has been identified by the Centers for Medicare and Medicaid Services (CMS) as a preventable hospital-acquired condition (HAC), for which CMS would consider denying reimbursement (2). The adverse impact of VAP on patient outcomes and cost of care has prompted widespread adoption of preventative practices known as “ventilator bundles,” including semi-recumbent patient positioning to prevent aspiration, continuous aspiration of secretions pooling above the endotracheal tube cuff, antibacterial silver coating of endotracheal tubes to prevent biofilm formation, oral decontamination with antibacterial chlorhexidine swabbing, use of noninvasive mask ventilation whenever possible, daily withdrawal from sedation and assessment of patient readiness to be liberated from mechanical ventilation, and even broad-spectrum oral antibiotics to decontaminate the oropharynx and upper gastrointestinal tract (1, 3). Almost all bundle measures are designed to reduce inoculation of the lung with infecting bacteria. Despite these measures, VAP continues to occur at a rate of 2-11 per 1,000 mechanical ventilation days, depending upon the type of ICU (4).

This issue of the *Journal* (pp. 280–287) presents a novel potential explanation for the occurrence of bacterial pneumonias in ventilated patients (5). Most ventilated patients are exposed to higher than normal concentrations of inspired oxygen. Hyperoxia has been previously shown to impair alveolar macrophage phagocytic activity (6–8) and increase mortality in animals after bacterial inoculation of the airway (6, 7, 9–11), with deleterious effects even at 40 to 65% oxygen (7, 8, 10), levels generally considered safe by clinicians (12). In the study presented by Patel and coworkers (5), exposure of mice to hyperoxia led within 72 hours to a time-dependent dramatic increase in airway levels of high mobility group box-1 (HMGB1), a nuclear transcription factor that is also exported by phagocytes as a proinflammatory “alarmin” cytokine (13). Alarmins orchestrate specific inflammatory and immune responses in the lung. Hyperoxic exposure for 72 hours also resulted in dramatically reduced bacterial clearance from the lung and increased mortality when mice were nasally challenged with *Pseudomonas aeruginosa*. Almost 70% of mice died within the first 4 hours of bacterial challenge, suggesting that mortality occurred from “injury” rather than infection, and was caused by entry into the circulation of inflammatory mediators normally compartmentalized within the lung. Compared with control antibodies, pretreatment of hyperoxic, *P. aeruginosa*-challenged mice with a blocking

monoclonal antibody against HMGB1 reduced lung bacterial counts, lung injury, and lung neutrophil influx. Anti-HMGB1 also improved phagocytic activity of lung leukocytes obtained by bronchoalveolar lavage (BAL) from hyperoxic animals. Exposure of RAW 264.7 macrophages to hyperoxia in culture likewise increased macrophage HMGB1 secretion and impaired phagocytic function. In a recent publication, this group showed that HMGB1 inhibits macrophage phagocytic function through a Toll-like receptor-4 (TLR4)-dependent mechanism (14). Hyperoxia impairs macrophage phagocytosis by inducing disorganization of the actin cytoskeleton (6, 7), important for bacterial internalization during phagocytosis. Hyperoxia has been previously shown to induce macrophage secretion of HMGB1 (15), and HMGB1 is known in other cells to produce deleterious effects on the actin-cytoskeleton through ligation of TLR4 (16). Furthermore, HMGB1 is substantially elevated in BAL after several days of mechanical ventilation of patients without pneumonia, and in patients with VAP, HMGB1 levels are similarly increased in BAL compared with patients without VAP, and comparably elevated between infected and contralateral lungs (17). Together, these data suggest that hyperoxia stimulates redox-dependent alveolar macrophage secretion of HMGB1, which feeds back upon the macrophage in a TLR4-dependent, autocrine manner to produce cytoskeletal changes that impair macrophage phagocytic function. This mechanism implies that VAP occurs not only from the bypassing of upper airway antibacterial mechanical defenses with intubation, but also from an oxygen-mediated acquired defect of innate immunity.

Physicians are taught to decrease oxygen to the lowest concentration needed for saturation of hemoglobin (arterial oxygen saturation ~ 90–92%). However, in practice we physicians often ignore this principle. In a Dutch academic ICU, the fraction of inspired oxygen (FiO_2) was decreased only 25% of the time when arterial oxygen partial pressure was greater than 120 mm Hg (12). Two recent studies in mechanically ventilated patients have found associations between high FiO_2 and mortality (18) and between excessive FiO_2 and longer time on the ventilator and ICU stay (19). These findings combined with the mechanism presented by Patel and colleagues (5) suggest that more careful control of FiO_2 might reduce VAP if added to “ventilator bundles.” Although they were not intubated, leaving upper airway defenses against bacterial aspiration intact, normal humans exposed to 100% oxygen for up to 110 hours did not spontaneously develop pneumonia (reviewed in Ref. 20). Therefore, not all will agree with compulsive oxygen titration. For this reason, a multi-center trial may be needed to determine if more careful oxygen titration can decrease VAP. The Adult Respiratory Distress Study Network (ARDSNet) is winding down activity, but such a study could be an ideal project for this group’s successor.

Many patients with acute lung injury will still need high FiO_2 . In this group, the mechanism presented by Patel and coworkers (5) suggests that blocking secretion or activity of HMGB1 might offer protection from VAP. Neutralizing anti-HMGB1 antibodies would seem expensive, but antioxidants can reduce macrophage HMGB1 secretion (7). Alternately, both heparin and its

low anticoagulant derivatives can block HMGB1 either directly (21) or by binding to its receptors (22), and inhaled heparin significantly reduced days on the ventilator in a recent Phase 2 human trial (23). Therefore, the mechanism for VAP offered in this issue (5) is immediately translatable through protocol-driven modulation of FiO_2 , or with several presently available strategies for human therapeutics development, providing we remember that lung host defense operates in a delicate balance. Any effort to modulate host response must be carefully targeted and titrated.

Author disclosures are available with the text of this article at www.atsjournals.org.

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DOI: 10.1165/rcmb.2013-0020ED