Alveolar Epithelial Fluid Clearance Mechanisms Are Intact After Moderate Hyperoxic Lung Injury In Rats*

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The capacity of the alveolar epithelial barrier to remove excess alveolar fluid from the airspaces of the lung was studied in an experimental model of moderate hyperoxic lung injury. Rats were exposed to 100% oxygen for 40 h in an exposure chamber and compared with control animals exposed to room air. Extravascular lung water was calculated gravimetrically. Alveolar and lung liquid clearance were studied over 1 h by instillation of a 5% albumin solution with 1.5 μCi of 125I-labeled albumin (6 mL/kg into both lungs). The concentration of both the unlabeled and labeled albumin was used to calculate alveolar liquid clearance. Hyperoxic rats developed pulmonary edema, with a 33% increase in extravascular lung water to 5.3±0.1 g of water per gram of dry lung, compared with 4.0±0.2 g of water per gram of dry lung in control rats (p<0.05). This degree of edema was associated with a significant increase in the alveolar-arterial oxygen difference (241±61 vs 124±14 mm Hg in control animals exposed to room air, p<0.05). Despite this moderate degree of lung injury, alveolar fluid clearance was normal (30±3%) compared with control rats (33±6%). Furthermore, the hyperoxic injured rats responded normally to an exogenous β-adrenergic agonist (terbutaline, 10−4 mol/L) with a 67% increase in the rate of alveolar liquid clearance (50±5%). Thus, in the setting of moderate hyperoxic lung injury, the alveolar epithelial barrier is still capable of removing fluid at a normal rate and responding to β-adrenergic agonist treatment. These experimental results have potential clinical implications for patients with acute lung injury.

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**Key words:** alveolar epithelial barrier; hyperoxia; pulmonary edema; terbutaline

**Abbreviations:** ANOVA=analysis of variance; mRNA=messenger RNA; MnSOD=manganese superoxide dismutase; P(A-a)O2=alveolar-arterial oxygen pressure difference; SOD=superoxide dismutase

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Hyperoxia has been widely used to study the effects of oxidant injury to the lungs *in vivo*. Oxygen toxicity primarily affects the lung, causing damage to lung endothelial cells, thereby increasing pulmonary vascular permeability to protein, resulting primarily in lung interstitial edema. Animal studies have indicated that different animal species have different sensitivities to high levels of oxygen.

In adult rats, exposure to continuous >95% oxygen has been shown to result in death within 60 to 72 h with physiologic markers of lung injury, such as pulmonary edema or pleural effusions apparent by 48 to 60 h. The mechanism of oxidant damage probably involves generation of reactive oxygen metabolites, such as hydrogen peroxide, superoxide anion radicals, singlet oxygen, peroxynitrite, and hydroxyl radical.

The alveolar epithelial barrier plays an essential role in regulating fluid and protein balance under both normal and abnormal conditions. Several studies have investigated the effects of reactive oxygen species on sodium transport across the alveolar epithelium, by using either isolated lung or isolated alveolar epithelial type II cell preparations. In earlier studies, oxygen-derived free radicals were reported to reduce Na,K-ATPase activity in isolated, ventilated, and perfused rat lungs and in cultured rat alveolar type II cells and to decrease *in vitro* alveolar type II cell active sodium transport. Re-
ently, Olivera et al\textsuperscript{11} reported a significant reduction in active sodium transport and lung liquid clearance immediately after 100% oxygen exposure for 64 h, using both an isolated-perfused rat lung and isolated alveolar epithelial type II cell models. In contrast, exposure of rats to \textgreater 95% oxygen for 60 h has been shown to increase sodium transport across the alveolar epithelium of isolated perfused lungs\textsuperscript{12} as well as upregulate the activity of Na,K-ATPases in the intact rat lung.\textsuperscript{13} Also, a sublethal 85% oxygen exposure for 7 days increased active sodium transport and lung liquid clearance, partly due to upregulation of alveolar epithelial Na,K-ATPases in an isolated perfused rat lung model.\textsuperscript{14} Also, an upregulation of alveolar type II cell conductive pathways with low affinity to amiloride and an increase of sodium transport have been reported with rats exposed under the same conditions.\textsuperscript{15} However, the capacity of the alveolar epithelium to transport excess alveolar fluid under basal and stimulated conditions (with \beta-adrenergic agonist therapy) has not been comprehensively evaluated in an \textit{in vivo} model of moderate hyperoxic lung injury.

In the present study, adult rats were exposed to 100% oxygen for 40 h before alveolar and lung liquid clearance were measured. We anticipated that pulmonary edema would occur after exposure to 100% oxygen for 40 h. Therefore, the first objective was to evaluate the effect of the hyperoxic exposure on extravascular lung water accumulation. The second objective was to determine whether the alveolar epithelial barrier was still sufficiently functional to remove some of the excess alveolar fluid by sodium-dependent transport. Therefore, we studied basal clearance and the effect of amiloride (an inhibitor of apical sodium uptake) on alveolar liquid clearance in the hyperoxic injured rats. Also, since several \textit{in vivo} studies from our laboratory have demonstrated the ability of exogenous \beta-adrenergic agonists to increase alveolar fluid clearance in several species,\textsuperscript{6,18} including the human lung,\textsuperscript{19} the third objective was to study the effect of an exogenous \beta-adrenergic agonist (terbutaline) on alveolar liquid clearance in the hyperoxic injured rats.

\textbf{Materials and Methods}

\textit{Animal Selection and Overall Design, and Oxygen Exposure}

A total of 40 male Wistarrats (300 to 400 g) were studied. The rats were divided randomly into two series: series 1 (n=21) rats were exposed to room air; series 2 (n=19) rats were exposed to hyperoxia for 40 h.

\textit{Exposure of Rats to Hyperoxia:} The rats were placed in groups of three in an acrylic resin (Lucite) chamber (40×15×15 cm\textsuperscript{3}) that was flushed with 100% oxygen at 15 L/min for 5 min and then maintained at 8 L/min thereafter. The concentration of oxygen in the chamber was monitored using an oxygen analyzer (model OA150; Servocom; Crawshay, UK) and was \textgreater 99% at all times. Carbon dioxide was trapped by sodium hydroxide stored in the chamber. The rats were given free access to water and diet. The exposure was stopped after 40 h. Then, either quantification of pulmonary edema was done gravimetrically or alveolar and lung liquid clearances were studied.

\textbf{General Protocol}

\textbf{Surgical Preparation and Ventilation:} Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). The rats were ventilated through a tracheostomy using a constant-volume small animal ventilator (Harvard Apparatus; Millis, Mass) with a fraction of inspired oxygen of 1.0, a peak airway pressure of 8 to 10 cm H\textsubscript{2}O, and positive end-expiratory pressure of 3 cm H\textsubscript{2}O, as we have done in previous rat studies.\textsuperscript{16,20} The respiratory rate (40 to 60 breaths/min) was adjusted to maintain the arterial P\textsubscript{CO\textsubscript{2}} between 35 and 40 mm Hg. Pancuronium bromide (0.5 mg/kg intraperitoneally) was given for neuromuscular blockade. A right carotid arterial line was inserted to monitor systemic BP and to obtain blood samples. Because hypothermia can reduce the rate of alveolar liquid clearance,\textsuperscript{21} body temperature was kept constant at 38°C by placing the animals on a temperature-controlled pad with a rectal thermometer (homeothermic blanket system; Harvard Apparatus).

\textbf{Preparation and Instillation of 5% Albumin Solution:} The procedures for preparation and instillation of the 5% bovine albumin Ringer’s lactate solution were the same as we have previously published.\textsuperscript{16,20} We added 1.5 mg of anhydrous Evans blue to bind to the albumin\textsuperscript{22} to confirm at postmortem the location of the instillate in the lungs. Also, 1.5 \mu Ci of \textsuperscript{125}I-labeled human serum albumin (CIS BIO International; Sacay, France) was added to the instilled solution as the alveolar protein tracer. Before the 5% albumin solution was instilled, a sample of the instillate was saved for total protein measurements, radioactivity counts, and water to dry weight ratio measurements.

\textbf{General Protocol:} For all rat studies, the general protocol was followed. After surgery, a 30-min baseline of stable systemic BP was required. Arterial blood gas values were measured before alveolar liquid instillation. The instillate (6 mL/kg of the 5% bovine albumin solution with 1.5 \mu Ci \textsuperscript{125}I-labeled human albumin) was delivered into both lungs over 2 min, using a syringe and a silicone elastomer (Silastic) tubing. Blood samples were then obtained for arterial blood gas measurement before and 30 min after the instillation. The alveolar protein tracer was used to measure liquid clearance from the distal air spaces and to calculate the residual \textsuperscript{125}I-albumin in the lung after 1 h.

The rats were studied for 1 h. Then, the rats were exsanguinated and the lungs were removed through a midline sternotomy. A sample of alveolar fluid (0.2 to 0.3 mL final alveolar fluid) was aspirated using a 3-mL syringe and a silicone elastomer (Silastic) tubing that was passed into a wedged position in both lungs. Total protein concentration and radioactivity of the alveolar samples were measured. It has been reported previously that concentration of native protein in liquid sampled by a catheter wedged into the distal airways was the same as in a directly obtained alveolar micropuncture sample.\textsuperscript{14} Alveolar liquid clearance from the distal airspaces was calculated by the concentration of the instilled unlabeled albumin and instilled labeled \textsuperscript{125}I-albumin for the different groups, as we have done before.\textsuperscript{20} Both lungs were homogenized and extravascular lung water was measured gravimetrically by calculating the wet-to-dry weight ratio (grams H\textsubscript{2}O per grams dry lung).\textsuperscript{23} Alveolar and lung liquid clearances were calculated for the different groups (described later). Lung liquid
clearance was calculated as the difference in water-to-dry ratio of the instilled lungs and additional control lungs from rats without fluid instillation.

**Specific Protocols**

**Series 1, Control Studies** (n=21): The rats were housed in an acrylic resin (Lactite) chamber flushed with room air for 40 h. Three different experiments were done in series 1.

First, we studied alveolar and lung liquid clearance after instillation of the 5% albumin solution over 1 h (n=11). On the day of the experiment, six rats were surgically prepared. After the baseline period, 6 mL/kg of a 5% albumin solution with $^{125}$I-albumin was instilled into both lungs. Then, the variables described in the general protocol were measured. The remaining five rats were ventilated for 1 h without instillation of fluid. The lungs of these rats were removed and processed for gravimetric determination of extravascular lung water.

Second, to determine the effect of a sodium transport inhibitor on basal alveolar and lung liquid clearance, we used amiloride (10$^{-3}$ mol/L) added to the instilled albumin solution (n=5). But because amiloride is 50% bound to protein, the effective concentration declines by approximately 10-fold over 2 h. The effective concentration of amiloride was approximately 10$^{-4}$ mol/L.

Third, to determine the effect of a β-adrenergic agonist on alveolar and lung liquid clearance, we added terbutaline (10$^{-4}$ mol/L) to the instilled protein solution (n=5).

**Series 2, Hyperoxic Studies** (n=19): The rats were studied after exposure to 100% oxygen for 40 h. Three different experiments were done in series 2.

First, to determine the effect of hyperoxic exposure on alveolar and lung liquid clearance, 11 rats were studied. Six of the 11 rats were instilled with 5% albumin solution as described for the control experiments. The five remaining rats were used to determine the extravascular lung water.

Second, to determine if hyperoxia alters amiloride-sensitive sodium channels, we added amiloride (10$^{-3}$ mol/L) to the instillate (n=4).

Third, to determine the effect of an exogenous β-adrenergic agonist on alveolar and lung liquid clearance in the hyperoxic rats, we administered terbutaline (10$^{-4}$ mol/L) mixed in the 5% albumin solution and measured the variables described in the general protocol (n=4).

**Measurements**

**Hemodynamics, Airway Pressure, and Arterial Blood Gases:** Systemic BP and airway pressures were monitored continuously on a polygraph (Grass model 7D; Grass Instrument Co, Quincy, Mass) using calibrated pressure transducers; arterial blood gas values and pH were measured before and 30 min after instillation of the 5% albumin solution. The alveolar-arterial oxygen pressure difference (P(A-a)O$_2$) was calculated as follows:

$$P(A-a)O_2 = PAO_2 - PaO_2$$

In equation 1, PAO$_2$ is alveolar oxygen pressure and PaO$_2$ is arterial oxygen pressure expressed in mm Hg.

**Lang Fluid Balance**

**Extravascular Lung Water:** Our method for determination of extravascular lung water has been described previously in detail. In brief, the lungs were homogenized with an equal weight of distilled water; extravascular lung water was determined by measuring the water-to-dry weight ratio. The excess lung water in the experimental lungs was calculated as the difference between the water-to-dry weight ratios of the instilled lungs (5% albumin solution) and the lungs from uninstilled rats times the dry weight of the lungs from the instilled rats. The dry weight of the instilled lungs was corrected for the dry weight of the protein in the instillate remaining in the lungs at the end of the experiment. To determine the mass of protein remaining in the lung, the dry weight of the instillate was multiplied by the fraction of $^{125}$I-albumin in the lung. This value was then subtracted from the total dry weight of the lungs:

$$E = \frac{W_i}{(D_i-P)} - \frac{W_o}{D_o} \times (D_i-P)$$

where E is the excess water in the experimental lungs, W and D are the water and dry weights of the uninstilled lungs (c) and of the 5% albumin-instilled lungs (e), and P is the weight of the protein instilled into the lungs multiplied by the fraction of $^{125}$I-albumin left in the lungs.

**Lung Liquid Clearance:** Lung liquid clearance was determined by first calculating the excess water in the lungs as described by equation 2 above. Thus, lung liquid clearance was the difference between the instilled volume and the excess lung water remaining in the lungs 1 h after the 5% albumin instillation, divided by the instilled volume.

**Alveolar Liquid Clearance:** The concentration of the instilled unlabeled 5% albumin and the instilled labeled $^{125}$I-albumin over 1 h was used to measure fluid clearance from the distal airspaces, as we have done before. In these experiments, we determined the rate of fluid clearance by measuring the increase in the final alveolar fluid protein concentration compared with the initial instilled alveolar protein concentration. Protein concentrations were measured by the biuret method.

**Statistics**

All data are summarized and presented as means±SD. Statistical comparisons were made with Student’s unpaired t test when two groups were compared and with one-way analysis of variance (ANOVA) followed by a post hoc multiple comparison test when more than two groups were compared as described in table and figure legends. Statistical significance was defined as p<0.05.

**RESULTS**

**The Effect of Oxygen Exposure on Systemic Arterial Pressure, P(A-a)O$_2$, and Extravascular Lung Water**

The exposure to 100% oxygen for 40 h resulted in moderate systemic arterial hypotension and a significant increase in the P(A-a)O$_2$ (Table 1). The hemodynamic changes were accompanied by the development of pulmonary edema as indicated by the 33% increase in the water-to-dry weight ratio compared with control room air-exposed rats (Fig 1).

**The Effect of Oxygen Exposure on Alveolar and Lung Liquid Clearance**

The concentration of the instilled unlabeled 5% albumin solution and instilled labeled $^{125}$I-albumin over 1 h was used to measure alveolar liquid clearance. There was no difference in alveolar liquid clearance in rats with hyperoxic exposure compared...
FIGURE 1. Extravascular lung water content of the noninstilled lung is shown for rats exposed to room air (control animals; n=5) and for rats exposed to 100% oxygen for 40 h (oxygen; n=5). The extravascular lung water content of hyperoxic-exposed rats was significantly increased compared to that measured in control rats. Asterisk indicates p<0.05 compared to control animals (Student’s unpaired t test).

with the control rats exposed only to room air (Table 2 and Fig 2). Total lung liquid clearance in hyperoxic rats was similar to control rats also (Fig 3).

When amiloride (10⁻³ mol/L) was added to the instilled protein solution, basal alveolar and lung liquid clearance were significantly reduced by approximately 50% in both room air and hyperoxic rats (Table 2 and Figs 2 and 3).

The addition of terbutaline (10⁻⁴ mol/L) to the instilled protein solution resulted in a marked increase in alveolar and lung liquid clearance in the hyperoxic rats (Table 2 and Figs 2 and 3). There was no significant difference in the magnitude of the terbutaline-stimulated alveolar or lung liquid clearance in rats with hyperoxic exposure compared with room air-exposed rats (Table 2 and Fig 3).

**Discussion**

The overall objective of these experiments was to study the function of the alveolar epithelial barrier in acute lung injury from moderate hyperoxia. This model is particularly relevant to clinical syndromes such as the acute respiratory distress syndrome in which hyperoxic treatment is often necessary as a therapeutic modality to support arterial oxygenation, as well as in infant respiratory distress syndrome in which hyperoxia may contribute to lung injury. More severe hyperoxic lung injury has already been studied. For example, Olivera et al have reported that active sodium transport and lung liquid clearance were reduced by approximately 45% after exposure to 100% oxygen for 64 h. However, these previous studies of hyperoxic lung injury have not focused on

<table>
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<th>Table 1—Baseline Arterial Oxygenation and Systemic BP after 40 h of Hyperoxic Exposure (100% Oxygen) Before the Instillation of 5% Albumin Solution Into Both Lungs in Anesthetized, Ventilated Rats *</th>
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<td><strong>Experimental Condition</strong></td>
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<td>Room air exposure</td>
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<td>Hyperoxic exposure</td>
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*Data are means±SD.
†p<0.05 compared with room air-exposed rats (Student’s unpaired t test).

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<th>Table 2—Effect of 100% Oxygen for 40 h on Alveolar and Lung Liquid Clearance Over 1 h in Anesthetized, Ventilated Rats *</th>
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<td><strong>Experimental Condition</strong></td>
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<td>Room air exposure</td>
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*Data are means±SD.
†p<0.05 compared with initial protein concentration (Student’s paired t test).
‡p<0.05 compared with albumin alone (ANOVA).

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the role of basal and stimulated alveolar epithelial fluid transport in lung fluid balance after moderate hyperoxic lung injury.

In the present study, we exposed the rats to 100% oxygen for 40 h to achieve a moderate hyperoxic lung injury model. The primary purpose of these experiments was to evaluate the capacity of the alveolar epithelium to respond to β-adrenergic agonist treatment under pathologic conditions. There are two major new findings in this study. First, the transport properties of the alveolar epithelium are intact even after 40 h of 100% oxygen exposure. The amiloride data demonstrated that most of the transport or clearance of alveolar fluid depended on sodium uptake by alveolar epithelial cells. Furthermore, the alveolar epithelium can respond to terbutaline ($10^{-4}$ mol/L) with a significant increase in alveolar fluid transport, indicating that even in the presence of moderate lung injury, the net capacity of alveolar epithelium to remove excess alveolar fluid was intact. This finding is particularly interesting because Bui et al recently reported that rats will survive 100% hyperoxia after 48 h if they are returned to room air, which is what the results of our index of alveolar epithelial function would predict.

To provide reliable morphologic studies to complement our in vivo functional data, quantitative morphometric histologic study would be needed. Several other investigators, such as Crapo et al, have published detailed morphologic studies of hyperoxic lung injury in rats. During the first 24 to 72 h after exposure to 100% oxygen, most animal species do not demonstrate significant morphologic changes in the alveolar septa. The earliest morphologic changes observed in the lung in response to hyperoxic stress involve subtle changes in endothelial cell structure, which primarily result in pericapillary fluid accumulation (interstitial lung edema). In the present study, hyperoxic rats developed a moderate increase in extravascular lung water (5.3±0.1 g water per gram of dry lung), consistent primarily with interstitial edema formation. In addition, the

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**Figure 2.** The effects of amiloride and terbutaline on alveolar liquid clearance in room air-exposed (n=16) and hyperoxic-exposed rats (n=14) are shown. There was no significant difference in alveolar liquid clearance in rats with hyperoxic exposure compared to room air exposure. In addition, amiloride reduced the rate of active sodium transport by approximately 50% in both room air-exposed and hyperoxic-exposed rats. Also, alveolar liquid clearance increased to 42±7% for room air-exposed rats and to 50±10% for hyperoxic-exposed rats, after terbutaline stimulation. Asterisk indicates p<0.05 vs control rats; dagger, p<0.05 vs hyperoxia (ANOVA).

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**Tables and Figures**

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<th>Group</th>
<th>Treatment</th>
<th>Liquid Clearance (%)</th>
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<tr>
<td>Controls</td>
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<td>30±5</td>
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<tr>
<td>Amiloride (10^{-3} mol/L)</td>
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<tr>
<td>Terbutaline (10^{-4} mol/L)</td>
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<td>40±6</td>
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<tr>
<td>Hyperoxia</td>
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<td>35±7</td>
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<td>42±7</td>
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<tr>
<td>Hyperoxia + Terbutaline (10^{-4} mol/L)</td>
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<td>50±10</td>
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**Graph**

![Graph showing alveolar liquid clearance](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21747/)
P(A-a)O₂ in the hyperoxic rats was only modestly increased compared with control rats (Table 1). Finally, the terbutaline data indicated that the transport of alveolar fluid by alveolar epithelium can be stimulated by β-adrenergic treatment, as under normal conditions (Figs 2 and 3). Therefore, it is unlikely that the epithelium was structurally damaged.

Also, in these studies, there was no measure of passive solute flux across the alveolar epithelial barrier as we and other investigators have used in some studies. However, we did not think these measurements were needed in this in vivo model because the concentration of labeled and unlabeled protein provides an accurate index of net alveolar fluid clearance, a measurement that includes both active and passive flux. Even in the clinical setting of acute lung injury, the sequential concentration of protein in the airspaces is a reliable index of net alveolar fluid clearance that is associated with a decrease in edema in the chest radiograph and better oxygenation.

The time course of functional repair of the alveolar epithelium after hyperoxic injury has been studied by Palazzo et al. They exposed hamsters to >95% oxygen for 4.5 days (equivalent to a 60-h exposure for rats) and returned them to room air. The alveolar epithelial permeability was measured for two hydrophilic solutes, sucrose (342 Da) and dextran 20 (20,000 Da), in isolated perfused lungs. Significant mortality was reported when the epithelial solute permeability was high compared to when the permeability had returned to control levels after recovery in room air. Therefore, the contribution of alveolar epithelial barrier during both acute lung injury and recovery is important. Interestingly, in a clinical study from our group, we found that 40% of patients with acute lung injury and increased permeability pulmonary edema were capable of removing some of their excess alveolar fluid within the first 12 h after injury. This finding indicated that the epithelial barrier was functional and intact, at least to some degree. Also, experimental studies from our laboratory indicated that the alveolar epithelial bar-

![Graph showing lung liquid clearance (% of instilled liquid) for different treatments.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21747/)

**Figure 3.** The effects of amiloride and terbutaline on lung liquid clearance in room air-exposed \((n=16)\) and hyperoxic-exposed rats \((n=14)\) are shown. Lung liquid clearance in hyperoxic-exposed rats was similar to room air-exposed rats. Amiloride inhibited lung liquid clearance equally in both oxygen-exposed and room air-exposed rats. Moreover, terbutaline stimulated lung liquid clearance at equal rates in both groups of rats. The effects of amiloride and terbutaline in the hyperoxic rats were similar to the effects in rats exposed to room air alone. The good correlation in alveolar liquid clearance (Fig 2) and lung liquid clearance provides evidence of the reliability and internal consistency of these two measurements. Asterisk indicates \(p<0.05\) vs control rats; dagger, \(p<0.05\) vs hyperoxia (ANOVA).
rrier is more resistant to injury than the lung endothelial barrier.29,31,32 The net capacity of the alveolar epithelial barrier to remove excess alveolar fluid may be useful as a marker of recovery from acute lung injury.29 Some injury may be present, but overall the alveolar epithelium must be sufficiently intact with a functional sodium transport system.

The present experimental studies indicate that even in the presence of moderate hyperoxic lung injury, the net capacity of the alveolar epithelium to remove excess alveolar fluid was preserved. In addition, we found that instillation of terbutaline into the airspaces increased alveolar fluid clearance in these hyperoxic rats. The capacity for active sodium transport across the alveolar epithelium of rats exposed to sublethal oxidant stress already has been reported to play a vital role in resolving pulmonary edema. For example, Nici et al13 demonstrated that there was an increase in total lung messenger RNA (mRNA) expression of the α1- and β-subunits of Na,K-ATPase beginning at 36 h and increasing another threefold to fourfold at 60 h of hyperoxia compared with unexposed rats. Also, Carter et al12 studied lungs from rats acutely exposed in vivo to >95% oxygen for 60 h immediately post-exposure and during recovery in room air. They found two populations of animals: those with higher epithelial injury and those with lower epithelial injury. In addition, they reported an increase of Na,K-ATPase mRNA and protein levels after in vitro hyperoxic exposure of rat alveolar epithelial type II cells.

In contrast, Olivera et al11 reported that active sodium transport and lung liquid clearance were reduced by 45% immediately after 64-h oxygen exposure but clearance increased after 7 days of recovery in room air. These investigators suggested that the decrease of Na,K-ATPase activity during the acute exudative phase of hyperoxia might be the result of oxidative stress and/or cell damage, as others have suggested.7 Clearly, the degree of injury and the capacity for recovery are a function of the level of hyperoxia (85% vs 100%) as well as the duration of exposure (40 to 64 h). Our studies were done in the presence of moderate hyperoxic injury (40 h at 100% oxygen).

Survival during oxidant stress depends on the magnitude of the oxidant load and the ability of the animals to mount appropriate defenses. Catalase, superoxide dismutase (SOD), and the enzymes of the glutathione redox cycle are primary intracellular antioxidant defense mechanisms. These antioxidant enzymes eliminate oxygen radicals and hydroperoxides that may subsequently oxidize crucial cellular structures.33,34 Chang et al35 reported that rats exposed to 85% oxygen can survive subsequent exposures to 100% oxygen. These investigators suggested that the development of this tolerance to oxygen toxicity was associated with an increase of total rat lung manganese superoxide dismutase (MnSOD) in association with specific inductions of MnSOD protein expression in the mitochondria of alveolar type II epithelial cells and interstitial fibroblasts as well the proliferation of these cells. Interestingly, cellular proliferation of alveolar epithelial cells and especially the induction of cyclin-dependent protein kinase activity (cyclins A and D and p34cdc2 kinase) was the key event mediating the proliferative response during recovery from short-term hyperoxic lung injury in adult rats.26

It is well established that the development of oxygen tolerance depends on a number of factors, including induction of antioxidant enzymes, which decrease the intracellular and extracellular steady-state concentrations of reactive oxygen species and higher levels of pulmonary surfactant in the alveolar hypophase,36,37 which delays the onset of alveolar collapse. An additional protective mechanism induced by preexposure of rats to 85% oxygen followed by 100% oxygen has been reported to be an upregulation of the “α-subunit of the rat epithelial Na+ channel” (osNaC) mRNA and the number of amiloride-sensitive channels and the quantity of sodium transport transported by each channel.38

In the experiments in this study, there was no change in the net capacity of the alveolar epithelial barrier to transport sodium and fluid, because terbutaline increased alveolar liquid clearance (50±5% of instilled volume) to the same level as in the control rats not exposed to hyperoxia (Fig 2). These results indicate that the epithelial barrier can function well after the development of acute lung injury from moderate hyperoxia. Since the capacity of the alveolar epithelium to remove excess alveolar fluid may be intact, even in the presence of moderate lung injury, this finding suggests that the injury may be confined primarily to the endothelium or injury to the alveolar barrier may not be uniform. An inward leak of fluid and protein into the airspaces in one location may be compensated by clearance of excess alveolar fluid from other locations in the distal airspaces. This study provides further support to the data currently available regarding the protective effect of alveolar epithelial sodium transport and alveolar liquid clearance during hyperoxic lung injury. Recently, Lasnier et al39 reported that rats exposed to >95% O2 for 60 h (severe hypoxic injury model) responded to terbutaline at a rate similar to control rats exposed to room air. Therefore, these experiments suggest that β-adrenergic agonist therapy might have some value for hastening the resolution of alveolar edema in patients with acute lung
injury, providing that the injury to the endothelial and epithelial barrier is not too severe.

REFERENCES


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