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The Influence of Liposome-Encapsulated Prostaglandin E₁ on Hydrogen Peroxide Concentrations in the Exhaled Breath of Patients with the Acute Respiratory Distress Syndrome

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Hydrogen peroxide (H₂O₂) levels are increased in the exhaled breath of patients with the acute respiratory distress syndrome (ARDS). Because liposome-encapsulated prostaglandin E₁ (PGE₁) downregulates the CD11/CD18 receptor of the neutrophil, thereby limiting endothelial adhesion, the use of this drug should decrease the excretion of H₂O₂ in the expiratory condensate of patients with ARDS. Patients >11 yr of age with ARDS (diffuse, patchy infiltrates by chest radiograph; Pao₂/fraction of inspired oxygen [P/F] ratio ≤200 mm Hg; pulmonary capillary wedge pressure ≤18 mm Hg; and the requirement for mechanical ventilation) were randomized to receive placebo (*n* = 14) or escalating doses (0.15–3.6 μg/kg) of liposomal PGE₁ (*n* = 14) every 6 h for up to 7 days. Condensate was collected every morning from the expiratory tubing that was submerged in an ice saltwater bath (–5°C). H₂O₂ levels were measured by using a horseradish peroxidase assay. Other data collected included white

blood cell count and P/F ratios. There was no significant difference in the concentration of H₂O₂ in the expiratory condensate between the liposomal PGE₁ group and the control group either before (0.99 ± 0.52 vs 0.93 ± 0.48 μmol/L) or during treatment (1.04 ± 0.45 vs 0.76 ± 0.25 μmol/L). Liposomal PGE₁ treatment improved the P/F ratio and decreased the white blood cell count over time. Despite its ability to downregulate the CD11/CD18 neutrophil receptor, liposomal PGE₁ did not reduce exhaled H₂O₂ excretion. **Implications:** White blood cells (WBC) are thought to be part of the cause of the acute respiratory distress syndrome, a lung disease. WBC in the lung produce hydrogen peroxide, which is exhaled. Liposomal PGE₁ inhibits WBC function but was found to have no effect in decreasing exhaled hydrogen peroxide in patients with the acute respiratory distress syndrome.

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Neutrophil activation and the release of inflammatory mediators and oxygen radicals are thought to play a prominent role in the pathogenesis of the acute respiratory distress syndrome (ARDS) (1–3). Evidence for this hypothesis is supported by studies of patients with ARDS demonstrating the accumulation of Indium-labeled neutrophils in the pulmonary vasculature (4), increased numbers of neutrophils, large concentrations of elastase, and increased myeloperoxidase activity in bronchoalveolar lavage fluid (3,5,6), and increased excretion of hydrogen peroxide (H₂O₂) in the urine and exhaled breath (7–9).

Prostaglandin E₁ (PGE₁) has a number of immunomodulating effects, including inhibition of neutrophil adherence, chemotaxis, granular enzyme release, and oxygen radical generation *in vitro* (10). These properties make PGE₁ an attractive therapeutic drug for the treatment of patients with ARDS. An early study suggested that an infusion of PGE₁ was effective in the treatment of trauma patients with ARDS (11); however, subsequent studies failed to show any beneficial effect of this treatment (12). In some studies, oxygenation worsened substantially when PGE₁ was used (13).

Liposomes injected IV are opsonized by fibronectin, vitronectin, and the complement component, C3bi (14). Activated neutrophils have more receptors for these molecules. In particular, liposomes that opsonize with C3bi bind to the CD11b/CD18 receptors on neutrophils, which results in increases in intracellular cyclic adenosine monophosphate (cAMP) and downregulation of the CD11b/CD18 receptor (15). Because PGE₁ also increases intracellular cAMP, the net effect

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is to decrease neutrophil activation and adhesion to the endothelial surface. By limiting neutrophil activation and diapedesis, liposomal PGE₁ should decrease neutrophil-mediated lung damage and improve outcome in patients with ARDS.

These observations seemed to be validated by a phase II study evaluating the safety and efficacy of liposome-encapsulated PGE₁ in the treatment of patients with ARDS, which demonstrated that therapy with liposomal PGE₁ was associated with a shorter duration of mechanical ventilation, improved Pao₂/fraction of inspired oxygen (P/F) ratios, and increased lung compliance (16). Consequently, a pivotal phase III multicenter, randomized, controlled, double-blinded trial was undertaken to determine whether liposomal PGE₁ is effective in the treatment of patients with ARDS.

Because liposomal PGE₁ limits neutrophil activation and adhesion, migration of neutrophils into the lung should be impaired, and secondary markers of neutrophil activation, such as the production of exhaled H₂O₂, should be affected. As members of the above-mentioned phase III study, we hypothesized that the use of liposomal PGE₁ should reduce the concentration of H₂O₂ in the exhaled breath compared with placebo. If this phenomenon was proven, it would provide important *in vivo* confirmation of the physiologic effects of liposomal PGE₁ on the human neutrophil and the inflammatory response associated with ARDS.

Methods

Inclusion criteria included patients >11 yr old with ARDS who were expected to survive at least 7 days and who could provide written informed consent. ARDS must have been present for ≤24 h before the initiation of therapy and was defined as: 1) new, bilateral, diffuse, patchy, or homogeneous infiltrates in three or four quadrants on chest radiograph; 2) a P/F ratio ≤200 mm Hg; 3) the absence of cardiogenic pulmonary edema as demonstrated by a pulmonary capillary wedge pressure ≤18 mm Hg; and 4) requirement for mechanical ventilation (to ensure accurate determination of the P/F ratio). Patients were excluded if they demonstrated cardiogenic or neurogenic pulmonary edema; were pregnant or lactating; had severe chronic cardiac, renal (creatinine ≥4 mg/dL), or hepatic (serum bilirubin ≥4 mg/dL) failure; had a history of acute myocardial infarction within 6 wk of study participation; had a neutrophil count <1000 cells/mm³; had participated in an investigational study within 30 days; or had received treatment with an unproven therapy for the current episode of ARDS.

Patients were randomized to receive 1-h infusions of liposomal PGE₁ or placebo every 6 h for 7 days. To

Table 1. Dosing Schedule of Liposomal PGE₁

Day	Dose			
	1	2	3	4
1	0.15	0.15	0.3	0.3
2	0.6	0.6	0.9	0.9
3	1.2	1.2	1.5	1.5
4	1.8	1.8	2.1	2.1
5	2.4	2.4	2.7	2.7
6	3.0	3.0	3.3	3.3
7	3.6	3.6	3.6	3.6

Values are expressed in $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$.
PGE₁ = prostaglandin E₁.

minimize the side effects of hypotension and hypoxia, liposomal PGE₁ was administered in a dose-escalating fashion, starting at $0.15 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and increasing according to the schedule defined in Table 1. Patients treated with placebo received an equivalent volume of 5% dextrose in water.

Each day during therapy, if the patient was tracheally intubated, exhaled breath was collected by connecting airway tubing to the exhalation port of the ventilator and submerging a large segment of the tubing in a rock salt/ice water bath. After 20–30 min, the condensate was collected and frozen at -70°C until the H₂O₂ assay was performed. The temperature in the ice bath was approximately -5°C . Condensate was not collected if the patient was hemodynamically unstable due to drug administration. In addition, the condensate was collected at a fixed time and not in relation to the dosing of the drug.

H₂O₂ was measured by using a spectrophotometric method previously described (7). Briefly, 1 mL of the condensate was added to an equal volume of assay solution containing 1 mM/L 3,3',5,5'-tetramethylbenzidine and 244 U/mL horseradish peroxidase (type VI-A) in 0.2 M potassium citrate buffer (pH 3.95). The reaction proceeded for 30 min at room temperature. The solution was then acidified to pH 1 with sulfuric acid, and the product, 3,3',5,5'-tetramethyl-1,1' diphenylquinone 4,4' diimine (TDD), was measured spectrophotometrically at an absorbance of 450 nm. The absorbance of TDD in the condensate samples was directly proportional to the concentration of H₂O₂ in the solution as determined for the calibration curve obtained with standard titrated amounts of H₂O₂.

Other data collected included the P/F ratio and white blood cell count. Data are expressed as mean \pm SD and were analyzed, where appropriate, by using χ^2 analysis, Student's *t*-tests, or repeated-measures analysis of variance (ANOVA) (Statistica; StatSoft, Tulsa, OK). Normality was confirmed using the Kolmogorov-Smirnov test. For some patients, some daily H₂O₂ concentration data were missing, and repeated-measures ANOVA therefore could not be performed without excluding these

Table 2. Demographics of the Study Population

	Liposomal PGE ₁	Control
Age (yr)	47.4 ± 18	39.9 ± 16
Gender (M/F)	8/6	8/6
Cause of ARDS (direct/indirect)	5/9	6/8
Lung injury score	3.0 (2.25-3.75)	3.25 (2.25-3.75)
APACHE II score	19.0 (9-33)	17.5 (6-43)

Values are mean ± SD or median (range).
ARDS = acute respiratory distress syndrome, PGE₁ = prostaglandin E₁.

patients from the analysis. Therefore, H₂O₂ concentrations were averaged during therapy for each patient, and pretherapy and between-group comparisons were made using ANOVA. The null hypothesis was rejected for *P* < 0.05.

Results

Twenty-eight patients were enrolled in the study. Demographic data appear in Table 2. There were no significant differences between the two groups. There was one significant difference in the mode of ventilation between the two groups: 50% of patients in the liposomal PGE₁ group were initially ventilated by using pressure-control ventilation, whereas all patients in the control group received volume control ventilation at the start of the study (*P* = 0.006 by Fisher's exact test) (Table 3).

There was no significant difference in the H₂O₂ concentrations in exhaled breath between the liposomal PGE₁ group and the control group at baseline (0.99 ± 0.48 vs 0.93 ± 0.52 μmol/L). Mean H₂O₂ values during therapy were 1.04 ± 0.45 and 0.76 ± 0.25 μmol/L, respectively (*P* = not significant) (Figure 1). When controlled for minute volume, H₂O₂ production was no different between the two groups (data not shown). However, P/F ratios increased significantly over time in the liposomal PGE₁ group (Figure 2), and by Day 8, the white blood cell count was significantly lower in the liposomal PGE₁ group (Figure 3).

Discussion

The major finding of this study is that, in patients with ARDS, liposomal PGE₁ was ineffective in reducing H₂O₂ concentrations in the expiratory condensate. This finding is in contrast to an animal study of interleukin-1-induced lung damage, in which liposomal PGE₁ was effective in reducing exhaled H₂O₂ levels (17).

There may be several reasons why liposomal PGE₁ failed to reduce H₂O₂ concentrations in the exhaled breath of patients. Although patients were enrolled

Table 3. Ventilatory Variables

	Liposomal PGE ₁	Control
Mode of ventilation ^a	7:7	14:0*
Tidal volume (mL)	708 ± 168	660 ± 147
Peak inspiratory pressure (cm H ₂ O)	38 ± 6	39 ± 9
Positive end-expiratory pressure (cm H ₂ O)	11 ± 3	11 ± 4
Plateau pressure (cm H ₂ O)	33 ± 5	35 ± 8

Values are mean ± SD.
PGE₁ = prostaglandin E₁.
* *P* < 0.05.

^a Number of patients with volume-control ventilation versus pressure-control ventilation.

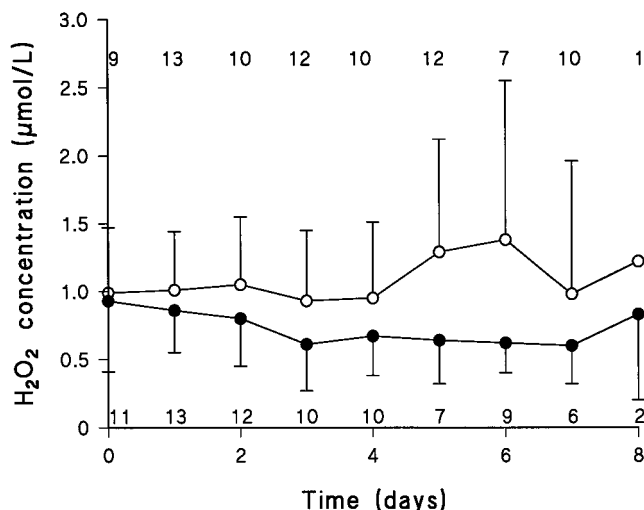


Figure 1. Exhaled breath hydrogen ion concentrations (mean ± SD) as a function of time. ● = control group, ○ = liposomal prostaglandin E₁ group. The numbers above and below each curve signify the number of data points at each time.

within 24 hours of the diagnosis of ARDS, the degree of neutrophil activation and aggregation in the pulmonary vasculature and sequestration in lung parenchyma could have been sufficiently robust to overwhelm any beneficial effect of the prostaglandin therapy. The plasma levels of PGE₁ may not have been high enough to alter important functions of the neutrophil. Tamura et al. (18) demonstrated that concentrations of PGE₁ in the range of 10⁻⁸ to 10⁻⁵ M could attenuate the generation of superoxide anion and release of elastase from primed and activated neutrophils *in vitro* but that doses ≥10⁻⁵ M PGE₁ were required to limit the upregulation of the CD11b/CD18 integrin. In addition to neutrophils, pulmonary macrophages are an important source of oxygen radicals and lysosomal enzymes. Experimental data show that remote organ injury is capable of stimulating alveolar macrophages to release superoxide anion and β-glucuronidase, a lysosomal enzyme (19), effects that would not likely be prevented by liposomal PGE₁.

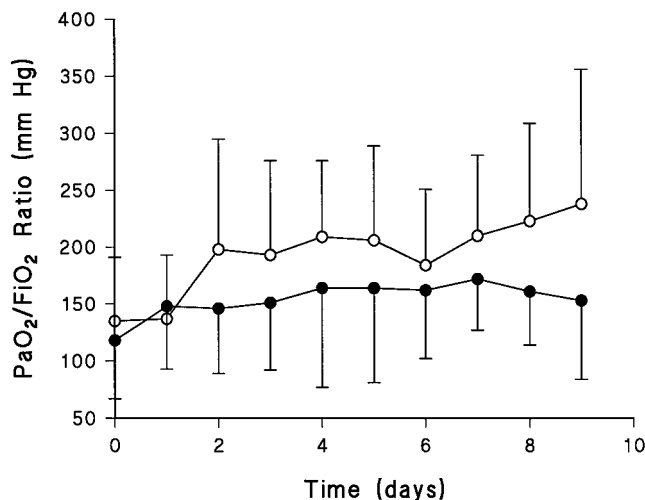


Figure 2. PaO₂/fraction of inspired oxygen (FiO₂) ratio as a function of time. ● = control group, ○ = liposomal prostaglandin E₁ group. The two groups were significantly different from each other (*P* < 0.05).

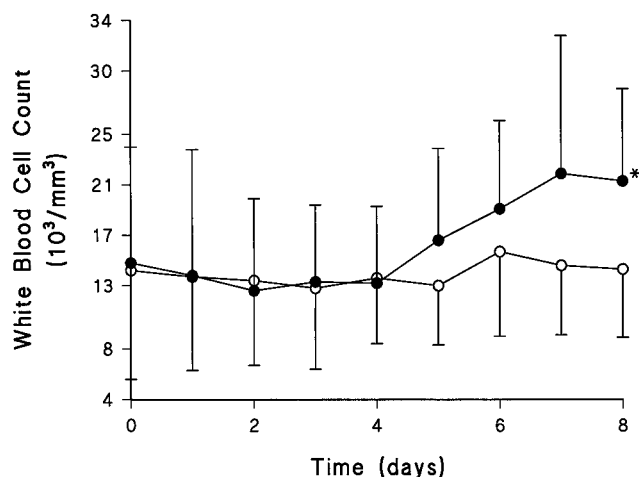


Figure 3. White blood cell count as a function of duration of treatment. ● = control group, ○ = liposomal prostaglandin E₁ group. There was a significant difference between the two groups on Day 8 (*P* < 0.05).

Interference with the CD11b/CD18 integrin could have had the unintended adverse consequence of mitigating any positive effect of PGE₁. Mercer-Jones et al. (20) demonstrated that therapy with anti-CD18 antibodies in an animal model of cecal ligation and puncture resulted in an increase in pulmonary myeloperoxidase activity. Finally, the causes of ARDS are diverse, and the utility of liposomal PGE₁ may be manifest in only a subpopulation of patients with ARDS.

No effort was made to control for the timing of the condensate collection and drug administration. Although the intravascular half-lives of PGE₁ and liposomes are short, the half-life of the effect of liposomal PGE₁ on the neutrophil is estimated to be eight hours (J. Matera-Hinds, The Liposome Company, Princeton,

NJ; personal communication, 1999); therefore, it is unlikely that variation in the timing of the condensate collection had any significant effect on H₂O₂ concentrations.

Exhaled condensate was not collected from critically ill patients without ARDS to serve as a second control. Numerous studies have demonstrated that H₂O₂ levels are increased in the exhaled breath of patients with ARDS. Our results are comparable to those reported by other investigators (9).

Liposomal PGE₁ did have a salutatory effect on the P/F ratio; similar findings were observed in the larger phase III trial (21). This beneficial effect on oxygen loading, taken in conjunction with the negative findings of this and the larger study (21), highlights the problems of investigations into ARDS: therapies that affect oxygenation are unlikely to be of ultimate benefit in the treatment of ARDS. The mortality rate from ARDS has decreased significantly over the past two decades (22). Most studies have shown that patients infrequently die from refractory hypoxemia but more often die from multiple organ dysfunction syndrome or sepsis (23,24). Thus, therapeutic strategies to treat ARDS should focus not only on treatment of the lung injury, but also on prevention of other secondary injury. In addition, the causes of ARDS are diverse, and future studies evaluating therapeutic modalities may have to examine subsets of patients with ARDS that are more homogeneous, such as those with lung injury due to direct causes (e.g., aspiration) or indirect causes (e.g., pancreatitis or sepsis).

The systemic neutrophil count was significantly lower in patients treated with liposomal PGE₁ than placebo. If both liposomes and PGE₁ inhibit neutrophil activation and adhesion, one could expect an increase in neutrophil count. However, PGE₁ suppresses the release of tumor necrosis factor α and interleukin-1 from activated human mononuclear cells (25,26), cytokines that can cause neutrophilia in experimental animals (27) and humans (28). PGE₁ also has an inhibitory effect on normal bone marrow proliferation of granulocyte progenitors (27,29,30) and may be the principal explanation for our observation.

Although this was a blinded, randomized trial, there was a significant difference between the two groups in the mode of mechanical ventilation that was used. All patients in the control group were initially ventilated by volume cycle means, whereas only 50% of these in the liposomal PGE₁ group received this mode of ventilation at the beginning of the study (Table 3). Theoretically, such a discrepancy might have had an important influence on the measurement of H₂O₂ values because the contribution of lung units with large time constants to total H₂O₂ excretion may be different with different inspiratory to expiratory ratios or ventilatory modes. However, we think that this is unlikely for several reasons. First, there was no

difference in baseline H₂O₂ exhaled concentrations between the two groups, although more patients in the liposomal PGE₁ group were supported with pressure-control ventilation at baseline. Second, the expiratory condensate was collected over 20–30 minutes. The impact of lung units with large time constants on H₂O₂ excretion should not have a significant impact with such a long collection time. Although inspiratory to expiratory ratio data were not collected, there were no differences in peak, plateau, or end-expiratory pressures between the groups. Finally, a secondary analysis comparing the effect of the two different modes of ventilation on H₂O₂ excretion failed to discern any effect (data not shown).

In summary, treatment of patients with ARDS with liposomal PGE₁ had no effect on H₂O₂ production in exhaled breath. However, liposomal PGE₁ may be of some benefit in patients with refractory hypoxemia.

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