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TITLE

PROPHYLAXIS AGAINST LIPOPOLYSACCHARIDE-INDUCED ACUTE LUNG INJURY BY ALPHA-TOCOPHEROL LIPOSOMES

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Prophylaxis against lipopolysaccharide-induced acute lung injury by α-tocopherol liposomes

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Objective: To investigate whether intravenously administered liposomal α-tocopherol can protect the lung from the injurious action of *Escherichia coli* lipopolysaccharide (LPS).

Design: Prospective, randomized animal study.

Setting: Government research laboratory.

Subjects: Twenty adult male Sprague-Dawley rats.

Interventions: Animals were intravenously pretreated with α-tocopherol liposomes (20 mg α-tocopherol/kg body weight), plain liposomes, or saline. Twenty-four hours later, pretreated animals were challenged with an intravenous injection of LPS (*E. coli* 0111:B4, 1 mg/kg body weight), and killed 2 hrs after LPS challenge.

Measurements and Main Results: Challenge of saline-pretreated animals with LPS resulted in lung injuries as evidenced by an increase in wet lung weight and a reduction in pulmonary angiotensin converting enzyme (25%) and alkaline phosphatase (28%), injury markers of lung endothelial and epithelial type II cells, respectively. Also, LPS administration resulted in an increase in pulmonary myeloperoxidase and protease activities, indicative of a neutrophilic inflammatory response. Pretreatment of animals with liposomal α-tocopherol significantly attenuated the LPS-induced edematous lung weight response, and reduced the extent of injuries to the pulmonary endothelial and epithelial cells, demonstrated by a significantly smaller reduction in the corresponding enzyme marker activities.

Conclusion: These results suggest that augmentation of the pulmonary antioxidant status can ameliorate LPS-induced lung injuries mediated by oxidative stress mechanisms. (Crit Care Med 1998; 26:723–729)

KEY WORDS: liposomes; α-tocopherol; vitamin E; lung injury; lipopolysaccharide; endotoxin; inflammation; neutrophils; oxidative stress; antioxidant

Gram-negative sepsis remains an important cause of morbidity and mortality in septic and endotoxemic patients. The array of pathophysiologic features that accompany Gram-negative bacterial sepsis appear to be qualitatively similar to those encountered after a lipopolysaccharide (LPS) insult. LPS, a component of the cell wall of Gram-negative bacteria, is responsible for initiating a series of highly complex cascading events leading to tissue injury. A common and frequent lethal complication observed in sepsis or septic shock is the acute respiratory distress syndrome (ARDS), which is characterized by severe hypoxemia, diffuse infiltration of the lung, reduction in respiratory compliance, increase in pulmonary arterial pressure, and pulmonary resistance (1–5).

Traditionally, mechanical ventilation with end-expiratory pressure, extracorporeal membrane oxygenation, fluid and vasopressor administration, and antibiotic and corticosteroid treatment have been used as supportive measures in ARDS therapy with no significant improvement in mortality. Current research is being directed toward improving the prognosis and critical care support of ARDS patients by exploiting strategies based on the current understanding of the biological mechanisms of ARDS at the molecular level. Prevention or amelioration of the progression of injury would allow remodeling of lung architecture and eventually recovery of respiratory function (1–5).

It is generally accepted that inflammatory cells, particularly neutrophils, are important mediators of the injury process induced by LPS (2, 4–7). The suggestion that neutrophils play a pivotal role in LPS-induced lung injury is largely based on the following lines of evidence: a) animals and humans with sepsis- or LPS-induced ARDS have increased neutrophil infiltrations in their lungs; b) the depletion of circulating neutrophils ameliorates LPS-induced lung injury in experimental animals; and c) an inhibition of toxic neutrophil products prevents the LPS-induced lung injury. Stimulated neutrophils release a collection of inflammatory mediators including, for example, cytokines, reactive oxygen species, proteolytic enzymes, and metabolic products of lipid metabolism (8–10). Of these mediators, reactive oxygen species have stimulated considerable interest in recent years as a major mechanism of LPS-induced acute lung injury. Reactive oxygen species are involved in the tissue damaging process through various mechanisms (11, 12).

Recognizing the fact that reactive oxygen species play an important role in the development of LPS-induced acute lung injury, the therapeutic treatment of ARDS with agents that augment the pulmonary antioxidant status appears promising. So far, investigators have been examining the

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potential efficacy of agents known to increase the cellular glutathione (GSH) concentrations, augment antioxidant enzyme systems, chelate iron and other molecules which enhance oxygen radical toxicity, and inhibit xanthine oxidase. Currently, results from both clinical and experimental studies have shown that the administration of antioxidants appears to be useful as an additional therapy to endotoxic shock (5, 10, 13, 14).

Oxidative stress is a known mechanism of injury in LPS-induced acute lung toxicity (13). The present study was undertaken to investigate whether α-tocopherol, delivered as a liposomal preparation, can ameliorate the LPS-induced acute lung injury. The rationale for choosing α-tocopherol was based on the fact that α-tocopherol is a very strong antioxidant, which should be effective in counteracting the destructive action of reactive oxygen species released during acute lung injury induced by LPS. α-Tocopherol, the main constituent of vitamin E, is known to be an important component of biological membranes by functioning as a free radical scavenger to prevent lipid peroxidation; by quenching singlet molecular oxygen; and by contributing to membrane stabilization (15, 16). α-Tocopherol was incorporated in liposomes because in its free form, the antioxidant is too viscous for administration and emulsifiers used to solubilize the antioxidant are generally highly toxic to tissues (17, 18). In contrast, the extremely insoluble α-tocopherol can be readily incorporated in liposomes, thereby facilitating its administration and delivery to the body (17).

The prophylactic effect of α-tocopherol liposomes against LPS-induced lung injury was assessed biochemically by measuring a) the activities of angiotensin-converting enzyme (ACE) and alkaline phosphatase (AKP), established markers of endothelial cell and alveolar type II cell integrity, respectively; b) the levels of GSH and lipid peroxidation, both markers of oxidative stress; and c) the pulmonary activities of myeloperoxidase and proteases as indicators of the inflammatory response.

MATERIALS AND METHODS

Chemicals. Lipopolysaccharide (*E. coli* LPS 0111:B4) and α-tocopherol were purchased from Sigma Chemical (St. Louis, MO). Dipalmitoylphosphatidylcholine was obtained from Avanti Polar Lipids (Alabaster, AL). All other chemicals were obtained from Sigma Chemical or BDH (Toronto, ON, Canada).

Animals. Male Sprague-Dawley rats, weighing ~225 to 250 g, were purchased from Charles River Canada (St. Constant, PQ, Canada). Animals were housed in stainless-steel cages with free access to pelleted laboratory chow and tap water. The animals were kept at room temperature (22° to 24°C) and were exposed to alternate cycles of 12 hrs light and darkness. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Canadian Council on Animal Care, and the experimental protocol on animal use was approved by the Institute's Animal Care Committee.

Preparation of α-Tocopherol Liposomes. The α-tocopherol liposomes were prepared from a mixture of dipalmitoylphosphatidylcholine and α-tocopherol in a 7:3 molar ratio. The lipids were dissolved in chloroform/methanol (2:1 volume to volume) and the lipid mixture was dried in a water bath at 40°C under a stream of helium to a thin film coating the interior surface of the glass vessel. Any traces of solvent were removed by placing the vessel under vacuum for at least 1 hr. The dried lipid was hydrated with 1.0 mL of 5 mM potassium phosphate buffer, pH 6.5, containing 3 mM ethylenediaminetetraacetic acid (EDTA), and then vortexed to form multilamellar vesicles. The multilamellar vesicles were extruded (ten times) with an extruder (Lipex Biomolecules, Vancouver, BC) through two stacked polycarbonate filters of 400 nm pore size using a helium pressure of 100 to 200 lbs/inch^2 (7 to 14 kg/cm^2). The extrusion process favors a more homogeneous size distribution. Free α-tocopherol was removed by washing the liposomes twice in 5 mM potassium phosphate buffer, pH 6.5, and pelleting at 110,000 *g* for 1 hr at 5°C in an ultracentrifuge (L8-70, Beckman Instruments, Irvine, CA). Liposomal vesicle size was determined with the use of a particle-size analyzer (N4SD, Coulter Corporation, Miami, FL) and was found to have a mean diameter of 389 ± 35 (SD) nm.

Treatment of Animals. α-Tocopherol liposomes (20 mg α-tocopherol/kg body weight), plain liposomes, or saline were administered intravenously via the tail vein of rats. Twenty-four hours later, rats were injected intravenously with a single dose of LPS (1 mg/kg body weight) to induce acute lung injury. The chosen dose was based on a titration in which 1 mg/kg was found to be sufficient to produce a consistent and reproducible injury at 2 hrs post-LPS, in the context of the injury markers used in this study. Injections were administered between 0800 and 0900 hrs. LPS was dissolved in saline and prepared shortly before use. Control animals received an equivalent volume of saline.

Experimental Design. To investigate whether α-tocopherol liposomes would ameliorate the LPS-induced lung injury, rats pretreated with α-tocopherol liposomes, plain liposomes, or saline were challenged with a single dose of LPS and killed 2 hrs later. The protective effect of α-tocopherol liposomes against LPS-induced lung injury was assessed biochemically by measuring the pulmonary activities of ACE, AKP, myeloperoxidase (MPO), proteases, diene conjugates, and reduced glutathione.

Tissue Preparation. Blood samples were collected from animals by cardiac puncture in EDTA-containing syringes under anesthesia. The collected blood was centrifuged immediately and the isolated plasma was stored at –70°C. Lungs were removed from animals immediately after decapitation and rinsed with ice-cold saline to remove excess blood. All subsequent steps were carried out at 0° to 4°C. After rinsing, lungs were quickly weighed and finely minced. Approximately 1 g of lung sample was homogenized with a homogenizer (Polytron PT1200C, Brinkmann Instruments, Mississauga, ON, Canada) in a sufficient volume of ice-cold 50 mM potassium phosphate buffer, pH 7.4, to produce a 20% homogenate. For the measurement of lipid peroxidation, homogenates were prepared as described previously, except the homogenizing medium contained 3 mM EDTA.

Enzyme Measurements. The activities of ACE, AKP, and MPO were determined as previously described by Suntres and Shek (19). One unit of ACE activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol furylacrylloylphenylalanine/min at 37°C. One unit

of AKP activity was defined as the amount of enzyme that catalyzed the formation of 1 nmol p-nitrophenol/min at 37°C. The activity of MPO in sonicated whole lung homogenates was determined by following the changes in optical density resulting from the decomposition of hydrogen peroxide in the presence of tetramethylbenzidine and was expressed as changes in absorbance measured at 450 nm/min. The protease concentration in pulmonary homogenates was assayed by a QuantiCleave Protease assay kit (Pierce Chemical, Rockford, IL), which is based on the cleavage of succinylated casein by proteases in the sample. Protein determinations were estimated by the method of Lowry et al (20).

Determination of Lipid Peroxidation. Homogenates from treated and control animals were assayed for the presence of lipid conjugated dienes as described by Suntres and Shek (19).

Determination of Reduced Glutathione Concentrations in the Lung. Reduced GSH, more precisely nonprotein sulphydryl, concentrations in pulmonary homogenates was determined as described by Suntres and Shek (19). The tissue was homogenized in 20% (weight to volume) trichloroacetic acid and centrifuged at 10,000 rpm for 20 mins in a refrigerated centrifuge (Sorval RC-5B, DuPont Instruments, Wilmington, DE). An aliquot of the supernatant fraction in 0.3 M phosphate buffer was treated with 5,5-dithiobis-[2-nitrobenzoic acid] and the absorbance at 412 nm was measured.

Statistical Analysis. Data from control and treated groups were evaluated by one-way analysis of variance with a Student-Newman-Keuls' test of multiple comparisons (21). The level of significance was accepted at $p < .05$.

RESULTS

Wet Lung Weight. The effects of intravenously administered LPS on lung weights of animals pretreated with saline, plain liposomes, and α-tocopherol liposomes are shown in Table 1. Lung weights of saline-pretreated animals were significantly increased by 38% 2 hrs after LPS challenge. Pretreatment of animals with plain liposomes did not alter the LPS-induced increase in lung weight. On the other hand, lung weights of LPS-challenged animals pretreated with α-tocopherol liposomes were significantly lower than those pretreated with saline.

Angiotensin Converting Enzyme and Alkaline Phosphatase Activities. Since ACE and AKP have been used as injury markers of pulmonary endothelial and epithelial type II cells, respectively (22, 23), the effects of LPS on these enzyme activities in lung homogenates of animals pretreated with saline, plain liposomes, or α-tocopherol liposomes were measured. As shown in Figure 1, the challenge with LPS produced a significant reduction in ACE (25% decrease) and AKP (28% decrease) activities in lung homogenates of saline-pretreated animals. Unlike pretreatment with plain liposomes which failed to reduce the LPS effect, pretreatment of animals with α-tocopherol liposomes significantly attenuated the LPS-induced decrease in ACE and AKP activities.

MPO Activity and Protease Concentration. The lung injury observed after LPS challenge is associated with the infiltration and activation of neutrophils. In the present study, the

Table 1. Effect of antioxidant pretreatment on lipopolysaccharide (LPS)-induced changes in wet lung weight (mean ± SEM)

Pretreatment[a]	LPS Challenge	Wet Lung Weight (g)
Saline	–	1.00 ± 0.05
Saline	+	1.38 ± 0.01[b]
Plain liposomes	+	1.37 ± 0.08[b]
α-Tocopherol liposomes	+	1.18 ± 0.04[b,c]

[a]Animals were pretreated as described in the Materials and Methods section; [b]significantly different ($p < .05$) from the value of saline-pretreated animals without lipopolysaccharide (LPS) challenge; [c]significantly different ($p < .05$) from the value of saline-pretreated animals challenged with LPS.

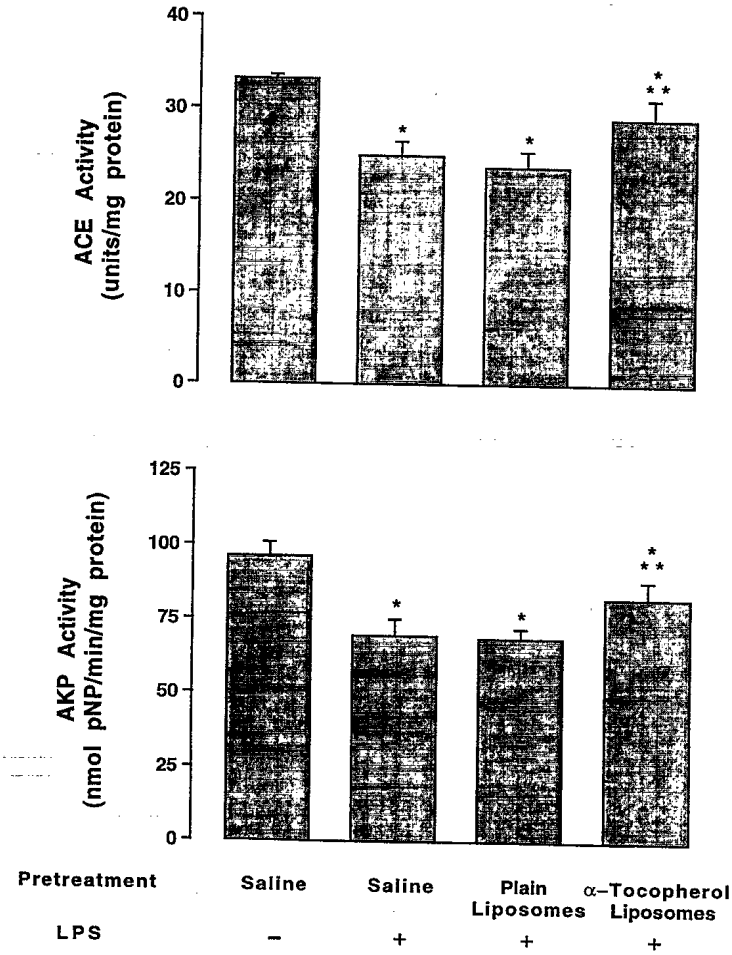

Figure 1. Effects of various pretreatments on lipopolysaccharide (*LPS*)-induced changes in angiotensin-coverting enzyme (*ACE*) and alkaline phosphatase (*AKP*) activities in the lung. Each *vertical bar* represents the mean ± SEM of five animals. *Significantly different ($p < .05$) from the corresponding value obtained from saline-pretreated animals challenged with LPS; **significantly different ($p < .05$) from the corresponding value obtained from saline-pretreated animals challenged with LPS. *pNP*, para-nitrophenol.

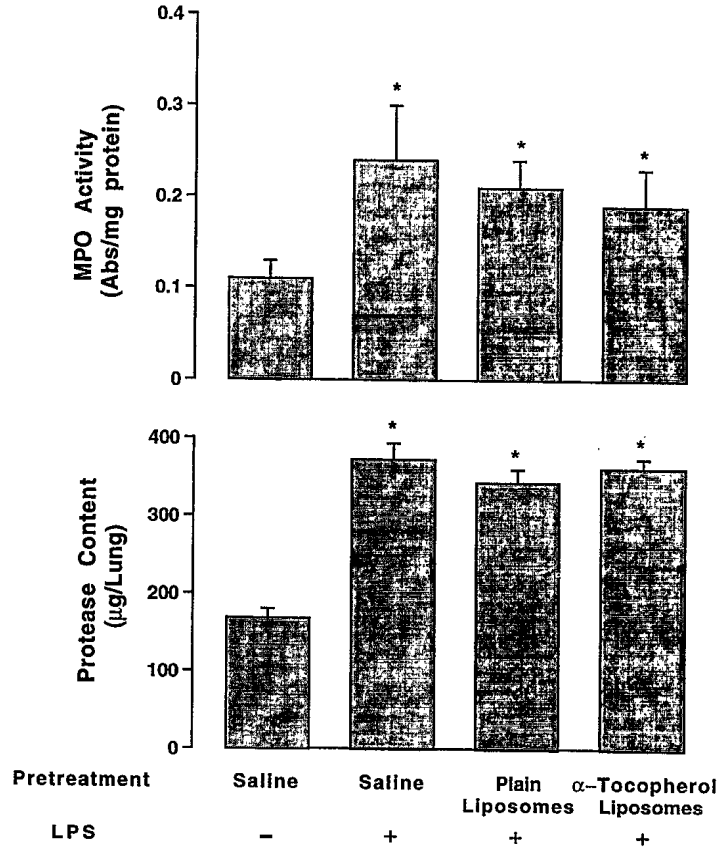

Figure 2. Effects of various pretreatments on lipopolysaccharide (*LPS*)-induced changes in myeloperoxidase (*MPO*) activity and protease content in the lung. Each *vertical bar* represents the mean ± SEM of five animals. *Significantly different ($p < .05$) from the corresponding value obtained from saline-pretreated animals without LPS challenge.

infiltration of neutrophils in the lungs of LPS-challenged animals was assessed by measuring the activity of MPO, an enzyme localized primarily in neutrophils (24). As shown in Figure 2 (*top*), the MPO activity in saline-pretreated animals was significantly increased by 118%, after LPS administration, suggestive of neutrophil infiltration in the lung. A very similar increase in MPO activity was also observed in LPS-challenged rats pretreated with plain liposomes or α-tocopherol liposomes.

Neutrophils are known to injure normal tissues by releasing proteases (8–10). The injection of LPS was found to induce a significant increase of protease content in the lungs of endotoxemic animals (Fig. 2, *bottom*). Pretreatment of LPS-challenged animals with plain or α-tocopherol liposomes failed to reduce the increased pulmonary protease concentrations.

Lipid Peroxidation and GSH Content. The level of lipid peroxidation has been used as an indicator of oxidative stress (11, 25). Injection of animals with LPS produced a significant increase in lipid peroxidation in pulmonary homogenates obtained from saline-pretreated animals, as measured by the formation of diene conjugates (Fig. 3, *top*). Pretreatment of rats with plain liposomes 24 hrs before LPS administration did not significantly alter the LPS-induced lipid peroxidation. On the other hand, pretreatment of rats with α-tocopherol liposomes partially protected against the LPS-induced lipid peroxidation.

Since GSH is known to play an important role in protecting cells from oxidant-induced tissue injury (11, 25), reduced glutathione concentrations in the lung were also measured. The results presented in Figure 3 (*bottom*) demonstrated that lung GSH concentrations of LPS-injected animals were significantly decreased by 25% compared with that of control animals. Similarly, a significant decrease in GSH concentrations was also observed in lungs of rats pretreated with plain liposomes 24 hrs before LPS challenge. On the other hand, pretreatment with α-tocopherol liposomes partially restored the LPS-induced GSH depression.

DISCUSSION

The precise mechanisms by which LPS induces acute lung injury are not clearly understood. Reactive oxygen species, however, have been implicated as key mediators of LPS-induced tissue injuries (2, 4, 5, 7, 10, 13, 14). A number of studies examining the effectiveness of antioxidants in attenuating LPS-induced pulmonary toxicities support the notion that oxidative stress is the major culprit (5, 10, 13, 14). In the present study, our demonstration that the injection of LPS caused an increase in lipid peroxidation and a reduction in GSH, both sensitive indicators of oxidative stress, reaffirms the involvement of reactive oxygen species. The pretreatment of animals with an antioxidant such as liposomal α-tocopherol was found effective in partially reversing these parameters of oxidative stress and also in alleviating some aspects of LPS-induced lung injuries. The increase in wet lung weight, suggestive of lung edema, and decreases in lung ACE and AKP activities, indicative of pulmonary endothelial and epithelial cell damage, respectively, were significantly alleviated by pretreatment with liposomal α-tocopherol. These results strongly suggest the potential usefulness of liposomal α-tocopherol as a prophylactic agent in ameliorating LPS-induced lung toxicities.

In this study, the assessment of lung injury status was determined using indirect methods. Although it has been shown that a reduction in the pulmonary content of the indicator enzyme markers, AKP and ACE, correlates well with damages in alveolar epithelial cells and pulmonary endothelial cells, respectively (22, 23), direct and specific damage of each cell type remains to be established. A more specific indicator of endothelial injury, for example, is the determination of extravascular accumulation of radioactively labeled albumin administered intravenously. Nevertheless, the enzyme markers used in this study served as a useful

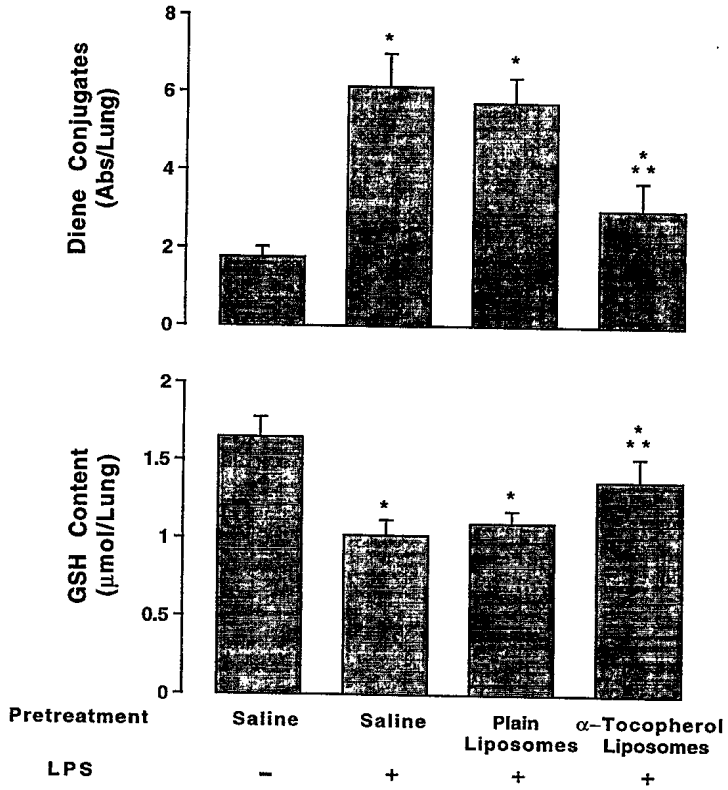

Figure 3. Changes in lipid peroxidation and glutathione (*GSH*) concentrations in the lungs of lipopolysaccharide (*LPS*)-injected animals receiving various pretreatments. Each *vertical bar* represents the mean ± SEM of five animals. *Significantly different ($p < .05$) from the corresponding value obtained from saline-pretreated animals without LPS challenge; **significantly different ($p < .05$) from the corresponding value obtained from saline-pretreated animals challenged with LPS. *Abs*, absorbance at 243 nm.

Our results demonstrated that the administration of liposomal α-tocopherol did not reduce increased lung myeloperoxidase activities and pulmonary protease contents, suggesting that no reduction in neutrophil infiltration or neutrophil activation occurs in the lungs of animals challenged with lipopolysaccharide.

indication of the general state of cell damage in the lung.

Pulmonary edema, normally a result of increased lung microvascular permeability, is thought to be a crucial abnormality in ARDS in humans and experimental animals. Results presented in this and other studies (2–5, 7) have demonstrated that lungs from animals, challenged with LPS, show pulmonary endothelial and epithelial injuries (2–5, 7). Injury to endothelial and epithelial cells which act as the air-blood barrier can cause transudation into the alveoli, causing pulmonary edema. Although the exact mechanisms of LPS-induced edema are not clear, the ability of exogenously administered antioxidants in lessening edema strongly suggests that this treatment effect is mediated by reducing oxidative stress in conditions such as endotoxemia and sepsis (5, 10, 13, 14). It is possible that α-tocopherol reduces edema formation by preserving the integrity of pulmonary endothelial and epithelial cells, as evidenced by a higher retention of ACE and AKP activities in the lungs of animals pretreated with liposomal α-tocopherol.

LPS-induced lung injury has been shown largely to be attributed to the recruitement and participation of neutrophils (2, 4–7). Our results demonstrated that the administration of liposomal α-tocopherol did not reduce increased lung myeloperoxidase activities and pulmonary protease contents, suggesting that no reduction in neutrophil infiltration or neutrophil activation occurs in the lungs of animals challenged with LPS. Pretreatment with the liposomal antioxidant, however, appeared to confer a significant beneficial effect in reducing the extent of pulmonary endothelial and epithelial cell damage, as suggested by the partial reversal of LPS-induced reduction in ACE and AKP activities, respectively. The apparent paradox of an improved preservation of endothelial and epithelial cell integrity in the absence of any curtailment of neutrophilic infiltration and activation suggests another mechanism other than simply reducing the number of neutrophils recruited to the lung. α-Tocopherol is known to stabilize and strengthen cell membranes (26), and it is conveivable that pretreatment of animals with liposomal α-tocopherol reduces the susceptibility of target cells to neutophilic oxidative insults. Very recently, a reduction in lung lipid alterations and lung leak, without decreasing neutrophil infiltration, has also been observed in rats challenged intratracheally with interleukin-1, but pretreated with lisofylline, an inhibitor which reduces the production of unsaturated phosphatidic acid species (27). Our finding is consistent with the notion that liposomal α-tocopherol protects the lung intrinsically against oxidative stress.

In addition to releasing proteolytic enzymes, neutrophils are also known to release reactive oxygen species which can damage cells via different mechanisms. The beneficial effects of liposomal α-tocopherol observed in this study may be partly attributable to the ability of the antioxidant to suppress the toxic effects of reactive oxygen species, increasingly recognized as the final mediators of tissue injury in inflammation. It has been demonstrated that α-tocopherol, in addition to its antioxidant properties, can also inhibit

the generation of reactive oxygen species from granulocytes activated by inflammatory stimuli (28–30). In this present study, pretreatment of animals with α-tocopherol liposomes failed to completely counteract the LPS-induced lung injury. One possible explanation is that α-tocopherol is unable to protect against the toxic effects of all available reactive oxygen species. An alternate explanation is that granulocytes may exert their injurious effects by mechanisms independent of oxidative stress (7–10).

Membrane lipid peroxidation occurs when reactive oxygen species react with polyunsaturated fatty acids and has been considered as a major mechanism of cell damage (11, 25). This process alters the structure and function of cellular membranes, including those of lung vascular endothelial cells. In our study, pretreatment of animals with α-tocopherol liposomes resulted in a partial protection against LPS-induced initial stages of lipid peroxidation, as evidenced by a reduction in the formation of diene conjugates. The failure of plain liposomes to confer any protection against the same LPS insult suggests that the prophylactic effect of α-tocopherol liposomes was due to the antioxidant component of the formulation. Although the exact mechanisms whereby α-tocopherol suppresses the LPS-induced lipid peroxidation are not known, it is known, however, that α-tocopherol can prevent the formation and/or accumulation of lipid peroxides, products of peroxidized membranes (11, 25). This interpretation is consistent with the observation that α-tocopherol liposomes significantly attenuated LPS-induced oxidation of GSH, which serves as a substrate in the GSH peroxidase/GSH reductase pathway to detoxify lipid peroxides. Thus, α-tocopherol liposomes are effective in attenuating lipid peroxidation in the respiratory system possibly by decreasing the extent of GSH oxidation.

In the present study, the assessment of lung injury was based on examining the injury status at a single time-point. Therefore, our results cannot substantiate a long-term beneficial effect of the treatment, and the administration of liposomal α-tocopherol may simply be shifting the time-course of the injury. However, in another study where paraquat, a very strong oxidant, was used to challenge rats pretreated with a similar preparation of liposomal α-tocopherol, the pretreatment significantly reduced the lung injury, in the context of the injury markers ACE and AKP, for several days, thereby promoting recovery (31).

Clinical studies have demonstrated that a reduction of α-tocopherol concentrations in ARDS patients correlates with an increase in their susceptibility to oxidant-stress mediated tissue injury (32–34). Some investigators have suggested that the concentrations of the antioxidant should be monitored in critically ill patients for replacement therapy considerations to avoid antioxidant insufficiency and to maximize protection against oxidative injury (32–34). Replacement therapy with α-tocopherol may facilitate a faster recovery of the critically ill patient. The results of this study suggested that the liposomal α-tocopherol formulation may be of significant value as a replacement therapy in ARDS because, unlike other α-tocopherol preparations, our formulation does not require activation in the gastrointestinal tract or an exposure period of several days as in the case of antioxidant esters, the form most commonly used in supplements.

The use of liposomes as useful vectors for mediating intracellular delivery of biologically active substances has been recognized for some time. Liposomes are considered an acceptable and superior drug delivery system because they are biocompatible, biodegradable, and relatively nontoxic (35). With respect to treating oxidant-induced tissue injuries, it has been demonstrated that the entrapment of antioxidants in liposomes promotes their therapeutic potential against oxidant-induced lung injury, presumably by liposomes facilitating the intracellular uptake and extending the half-lives of the encapsulated antioxidants (36–38). Recognizing the fact that the development of ARDS is multifactorial, the most effective treatment strategy may involve the simultaneous administration of a number of agents with diverse actions. The potential efficacy of liposomal α-tocopherol in treating ARDS and associated complications has not been established and should be considered for future studies.

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