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TREATMENT OF LPS-INDUCED TISSUE INJURY: ROLE OF LIPOSOMAL ANTIOXIDANTS

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ABSTRACT—Tissue inury is a common occurrence in multiple organ failure, a possible clinical complication of Gram-negative bacterial sepsis. Gram-negative bacteria, in part through lipopolysaccharide (LPS), tumor necrosis factor, and other cytokines, activate neutrophils to increase oxygen consumption and produce reactive oxygen species (ROS). ROS have been suggested to play a critical role in the pathogenesis of multiple organ failure. Accordingly, we hypothesized that the susceptibility of tissues to ROS can be reduced by augmenting the antioxidant status of the affected tissues. Rats were challenged intravenously with LPS (*Escherichia coli*: 0111:B4) at a dose of 1 mg/kg body weight, and 0, 2, 4, or 6 h later were treated intravenously with plain liposomes or α-tocopherol liposomes (20 mg α-tocopherol/kg body weight); treated rats were then killed 24 h after LPS challenge. Animals challenged with LPS were extensively damaged in the liver, as evidenced by an increase in plasma alanine aminotransferase and aspartate aminotransferase activities, and also in the lung, as indicated by a decrease in pulmonary angiotensin-converting enzyme and alkaline phosphatase activities. The injection of LPS also resulted in increased myeloperoxidase activities in the two organs, suggestive of activation of the inflammatory response. Within the pulmonary and hepatic organs of LPS-challenged animals, the involvement of oxidative stress mechanisms was evident, because a significant decrease in reduced glutathione and an increase in lipid peroxidation were observed. In contrast, the administration of α-tocopherol liposomes in the post-LPS-challenge period resulted in a significant alleviation of both lung and liver injuries, evidenced by a general reversal of the altered biochemical indices toward normal among treated animals. The therapeutic effect was found to be greater when liposomal α-tocopherol treatment was given earlier during the development of injury. Plain liposomes administered immediately after LPS injection also protected hepatic and pulmonary tissues from injuries. However, unlike α-tocopherol liposomes, plain liposomes did not confer any beneficial effect when administered at later timepoints post-LPS injection. These data suggest that α-tocopherol, administered in a liposomal form, may serve as a potentially effective pharmacological agent in the treatment of LPS-induced tissue injuries.

INTRODUCTION

Multiple organ failure is a common clinical complication of Gram-negative bacterial sepsis. The pathophysiological changes observed in Gram-negative bacterial sepsis resemble those often following lipopolysaccharide (LPS) administration. Lipopolysaccharides are normal components of the cell wall of Gram-negative bacteria and have been recognized for many years as key risk factors in the development of septic shock syndrome. The incidence of septic shock has increased progressively over the past few decades and, despite advances in antimicrobial therapy and critical care medicine, the mortality rate remains relatively high (1–5).

In studies examining the disposition of intravenously administered LPS, it has been demonstrated that the liver is the most important and predominant organ of entrapment, followed in importance by the spleen and lung (6, 7). Despite the ability of the liver to detoxify LPS, marked morphological and biochemical alterations occur in hepatic tissues exposed to LPS. Patients and experimental animals dying from septic shock display alterations in Kupffer cells, formation of fibrin thrombi, neutrophil infiltration in liver sinusoids, and zonal hepatic necrosis (6, 7). Another common and frequently lethal complication of sepsis syndrome is the adult respiratory distress syndrome, characterized by severe hypoxemia, diffused pulmonary infiltrations, reduction in lung compliance, increase in pulmonary artery pressure, and pulmonary resistance (7–9). It has been estimated that 18–42% of patients with Gram-negative infections will develop adult respiratory distress syndrome with a mortality rate of approximately 50%.

Considerable evidence has implicated neutrophils and macrophages as playing a critical role in the pathogenesis of LPS-induced tissue injury. Some of the observations that have identified neutrophils as the culprit of LPS-induced tissue injury are as follows: 1) neutrophil counts in clinical and experimental sepsis are usually increased in the hepatic, pulmonary, and other tissues; 2) depletion of circulating neutrophils with polyclonal antibodies attenuates corresponding liver and lung injuries; and 3) inhibition of toxic neutrophil products ameliorates both hepatic and pulmonary lesions (1–4, 7–9).

Stimulated neutrophils release a plethora of mediators that can destroy normal cells. Among these agents are proteins such as lysozyme, peroxidases, and elastase as well as reactive

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oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical, and hypohalous acid. Among these mediators, reactive oxygen species have stimulated considerable interest in recent years as a major contributor of LPS-induced tissue injury. Reactive oxygen species are known to injure tissues through peroxidation of membrane lipids, breakage of DNA strands, alteration of amino acids, and disruption of cellular metabolism (1–4, 7–11).

Evidence supporting oxidative stress as a contributing mechanism of tissue injury associated with septic shock arises from studies that have demonstrated that pretreatment of animals with agents known to augment the cellular antioxidant defense system can attenuate the shock state, including hypotension, tachycardia, tissue edema, and lipid peroxidation (9, 12–16). However, the use of antioxidants in the treatment of sepsis remains controversial and results from studies examining the effectiveness of antioxidants during the development of septic shock have been disappointing. These results are not too surprising because most antioxidants are short-lived, rapidly eliminated from tissues, and unable to gain access to the cell interior, conditions that reduce the efficacy of exogenously administered antioxidants (17–20). The failure of successful antioxidant therapy indicates a need for the development of formulations that could enhance the delivery and retention of antioxidants in target tissues.

Liposomes are artificially prepared phospholipid vesicles with amphipathic features. Hydrophilic molecules can be encapsulated in the aqueous spaces and lipophilic molecules can be incorporated in the lipid bilayers. Liposomes are considered to be an acceptable drug delivery system because they are biocompatible, biodegradable, and relatively nontoxic. Thus, considerable efforts have been focused on the use of liposomes for delivering drugs, proteins, and potentially therapeutic molecules to specific sites of action. With respect to treating oxidant-induced tissue injuries, it has been demonstrated that the encapsulation of antioxidants in liposomes promotes their therapeutic efficacy, presumably by liposomes facilitating the intracellular uptake and extending the half-lives of the encapsulated antioxidants (17–19, 21, 22).

The present study was undertaken to investigate whether liposome-associated α-tocopherol could attenuate LPS-induced liver and lung injuries. It is known that α-tocopherol, a lipophilic antioxidant, protects against oxidant-induced tissue injury by inhibiting membrane lipid peroxidation and lipid peroxide formation, by scavenging singlet oxygen and other reactive oxygen species, and by exerting a stabilizing effect on membranes (23–25). However, α-tocopherol in its free form is too viscous for parenteral administration and emulsifiers used to solubilize the antioxidant are generally found to be toxic to tissues (26). In contrast, the extremely insoluble α-tocopherol can be readily incorporated in liposomes, thereby facilitating its administration and delivery to the body (26). In this report, we examined the potential protective effect of liposome-associated α-tocopherol against LPS-induced damage by measuring changes in appropriate enzyme markers of hepatic and pulmonary injuries. In addition, the tissue content of reduced glutathione (GSH) and the extent of lipid peroxidation in the liver and the lung were also measured to determine the possible benefit of the liposomal antioxidant treatment.

MATERIALS AND METHODS

Chemicals

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

Animals

Male Sprague-Dawley rats (approximate body weight 220–250 g) were purchased from Charles River Canada, Inc. (St. Constant, Quebec, Canada). All animals were housed in stainless-steel cages with free access to pelleted purina laboratory chow and tap water. The animals were kept at room temperature (22–24°C) and were exposed to alternate cycles of 12 h 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.

Preparation of liposome-associated α-tocopherol

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 v/v) 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 h. The dried lipid was hydrated with 1.0 mL of 5 mM potassium phosphate buffer, pH 6.5, containing 3 mM EDTA, and then vortexed to form multilamellar vesicles. The multilamellar vesicles were extruded (10 times) with an extruder (Lipex Biomolecules, Vancouver, BC) through two stacked polycarbonate filters of 400 nm pore size using a helium pressure of 100–200 lb/in^2. 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 h at 5°C in a Beckman L8–70 ultracentrifuge. Liposomal vesicle size was determined with the use of a Coulter N4SD particle-size analyzer and was found to have a mean diameter of 376 ± 35 nm.

Preparation of LPS-liposome mixture

The LPS-liposome mixture was prepared by mixing 5 mg of LPS with 442.9 mg of plain liposomes at room temperature. The mixture was intravenously administered to animals at a dose of 1 mg LPS/kg body weight.

Treatment of animals

Lipopolysaccharide (1 mg/kg body weight) was intravenously administered to animals via the tail vein. At various times (0, 2, 4, or 6 h) after LPS administration, animals were injected intravenously with a single dose of α-tocopherol liposomes (20 mg α-tocopherol/kg body weight) or plain liposomes, and were killed 24 h after LPS administration. Injections with LPS were carried out between 8:00 a.m. and 9:00 a.m. and LPS was dissolved in saline shortly before use. Control animals received an equivalent volume of saline.

Experimental design

To investigate whether α-tocopherol can attenuate LPS-induced tissue injury, rats challenged with LPS were treated at different times (0, 2, 4, or 6 h) with a single dose of α-tocopherol liposomes or plain liposomes, and livers and lungs of treated animals were harvested. The effectiveness of α-tocopherol liposomes against LPS-induced liver and lung injury was assessed biochemically by measuring the following: 1) plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes activities, indicative of liver injury (27); 2) alkaline phosphatase (AKP) and angiotensin-converting enzymes (ACE) activities, indicative of lung injury (28, 29); 3) myeloperoxidase (MPO) activity, indicative of an inflammatory response (30); and 4) tissue lipid peroxidation and reduced GSH levels, both indicators of oxidative stress (31, 32), in the tissues of control and treated animals.

Tissue preparation

Blood samples were collected from animals by cardiac puncture under light halothane anesthesia. The collected blood was centrifuged immediately and plasma was stored at −70°C and used within 48 h for the determination of enzyme activities. Livers and 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 0–4°C. Following rinsing, the organs were quickly weighed and finely minced. Approximately 1 g of tissue sample was homogenized with a Brinkmann Polytron 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

Activities of plasma AST and ALT were determined by the method of Reitman and Frankel (33) with a Sigma Diagnostic Kit (No. 505) and enzyme activities were expressed as Sigma Frankel U/mL of plasma. The activity of MPO in sonicated whole organ 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 (30). The activities of ACE and AKP were determined as previously described by Suntres and Shek (34). Protein determinations were estimated by the method of Lowry et al. (35).

Determination of lipid peroxidation

Liver and lung homogenates from treated and control animals were assayed for the presence of thiobarbituric acid reactants as described by Suntres and Lui (36).

Determination of reduced glutathione concentrations in tissues

Reduced glutathione, more precisely nonprotein sulfydryl, concentrations in hepatic and pulmonary homogenates was determined as described by Suntres and Shek (34). Briefly, the tissue was homogenized in 20% (w/v) TCA and centrifuged at 10,000 rpm for 20 min in a refrigerated Sorval RC-5B centrifuge. An aliquot of the supernatant fraction in .3 M phosphate buffer was treated with 5,5-dithiobis-[2-nitrobenzoic acid] (NbS_2) and the absorbance at 412 nm was measured.

Statistical analysis

Data from control and experimental animals treated with plain liposomes or liposomal α-tocopherol were evaluated by one way analysis of variance (ANOVA). If the F values were significant, the unpaired two-tailed Student's *t* test was used to compare the treated and the control groups (37). The level of significance was accepted at $p < .05$.

RESULTS

Organ wet weight

The intravenous injection of LPS in rats resulted in a relatively large increase in lung weight (71%) compared with that of saline-injected animals. This lung weight increase was significantly reduced to 24–37% in LPS-injected animals subsequently treated with α-tocopherol liposomes (Fig. 1). On the other hand, treatment of LPS-injected animals with plain liposomes did not reduce the lung weight increase. A significant protective effect, however, was observed when plain liposomes were administered immediately after LPS injection (Table 1). In contrast to the LPS-induced increase in lung weight, a similar injection of LPS, with or without subsequent treatment with α-tocopherol liposomes or plain liposomes, was found to have no significant effect on the liver weight of all treated animals.

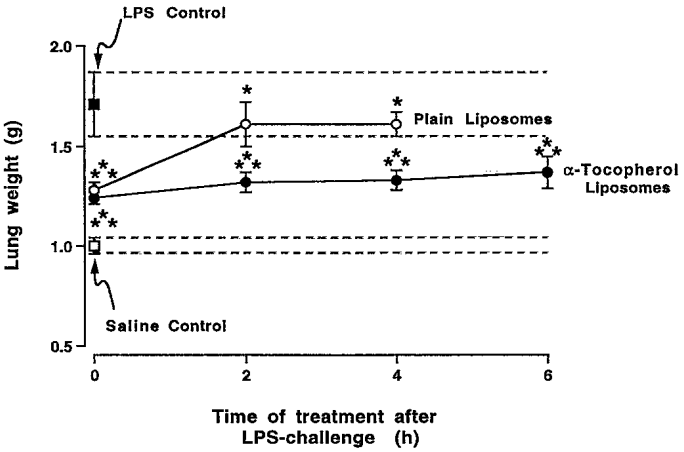

FIG. 1. **Effects of α-tocopherol liposomes or plain liposomes on LPS-induced changes in wet lung weight.** Animals were challenged with LPS (1 mg/kg body weight, i.v.) and 0, 2, 4, or 6 h later were treated with liposomal α-tocopherol (20 mg/kg body weight, i.v.); treated animals were killed 24 h post-LPS challenge. Each data point represents the mean ± SE of five animals. *significantly different from corresponding values obtained from non-LPS-challenged and saline-treated animals, $p < .05$; **significantly different from corresponding values obtained from LPS-challenged and saline-treated animals, $p < .05$.

Enzyme markers of organ injury

AST and ALT in liver injury—The measurement of hepatic enzymes appearing in the blood has been employed as a reliable indicator for the assessment of hepatotoxicity (27). Plasma AST and ALT activities in LPS-challenged animals were substantially elevated by about 4- and 23-fold, respectively, when compared with that of saline-injected animals (Fig. 2). Treatment with liposomal α-tocopherol significantly decreased plasma AST and ALT in LPS-challenged animals. The extent of protection appeared more prominent when the antioxidant treatment was administered at earlier timepoints during the development of injury. The highest protection was observed when the liposomal antioxidant treatment was given immediately after the LPS challenge. In this regard, the administration of plain liposomes immediately after LPS injection also conferred similar protection to α-tocopherol liposomes (Table 1), but not at later timepoints (data not shown).

ACE and AKP in lung injury—ACE and AKP have been shown to be good injury indicators of pulmonary endothelial and epithelial type II cells, respectively (28, 29). Indeed, animals challenged with LPS produced a significant decrease in pulmonary ACE and AKP activities (Fig. 2). These injury-induced decreases in enzyme activities, however, were attenuated by treatment with liposomal α-tocopherol and the extent of attenuation appeared to be dependent on the time of treatment post-LPS challenge. Similar to observations in the liver, the administration of plain liposomes immediately after LPS injection also protected the lung from injury-associated release of ACE and AKP (Table 1). However, unlike α-tocopherol liposomes, plain liposomes did not confer any beneficial effect when administered at later timepoints (data not shown).

MPO in neutrophil infiltration—The infiltration of neutrophils and other inflammatory cells has been shown to contribute to LPS-induced tissue injuries via phagocyte activation and

TABLE 1. **Comparison between plain liposomes and α-tocopherol liposomes in protecting against LPS-induced injuries**

		Immediate treatment post-LPS administration		
	Normal baseline	Saline	Plain liposomes	α-Tocopherol liposomes
LPS	−	+	+	+
Lung Weight (g)	1.00 ± .04	1.71 ± .16*	1.28 ± .04	1.24 ± .03
Plasma AST (SF U/mL)	72.26 ± 3.96	358.54 ± 13.83*	146.08 ± 5.99‡	155.63 ± 10.12*‡
Plasma ALT (SF U/mL)	15.82 ± .71	383.60 ± 8.78*	99.58 ± 3.51*‡	103.87 ± 11.47*‡
Lung ACE (U/mg protein)	32.13 ± 1.40	16.47 ± .70*	28.96 ± 1.61‡	29.88 ± .67‡
Lung AKP (nmol pNP/min/mg protein)	101.84 ± 2.76	58.33 ± 1.11*	105.66 ± 3.89‡	96.37 ± 1.78‡
Liver MPO (OD_{450}/mg protein × 10)	.50 ± .07	1.08 ± .06*	.61 ± .08‡	.70 ± .06‡
Lung MPO (OD_{450}/mg protein × 10)	1.40 ± .10	2.70 ± .30*	1.70 ± .20‡	1.80 ± .20‡
Liver TBAR (OD_{535} nm × 10^{-1})	1.19 ± .06	3.50 ± .35*	1.44 ± .13*‡	1.50 ± .20*‡
Lung TBAR (OD_{535} nm × 10^{-1})	.45 ± .02	1.98 ± .18*	.71 ± .05*‡	.74 ± .08*‡
Liver GSH (μmol/g)	6.61 ± .12	4.08 ± .35*	6.36 ± .18‡	6.32 ± .22‡
Lung GSH (μmol/g)	1.39 ± .04	.56 ± .06*	1.32 ± .03‡	1.33 ± .08‡

*Significantly different from normal baseline values, $p < .05$.
‡Significantly different from values obtained from LPS-injected and saline-treated animals, $p < .05$.

associated oxidative stress-mediated mechanisms (1–4, 7–11). In the present study, the infiltration of phagocytes in tissues was assessed by measuring the activity of MPO, an enzyme primarily localized in neutrophils (30). As shown in Fig. 2, MPO activities of LPS-challenged animals were significantly elevated in the liver and lung by 104% and 80%, respectively, suggestive of neutrophil infiltration in both organs. However, a smaller increase in MPO activity was observed in LPS-challenged animals treated immediately with α-tocopherol liposomes (Fig. 2) or plain liposomes (Table 1). A delay in liposomal treatment by 2, 4, or 6 h post-LPS challenge was found ineffective in attenuating the LPS-induced increase in MPO activity.

Lipid peroxidation and GSH levels

Lipid peroxidation of membrane lipids has been implicated as a possible mechanism of acute oxidative stress-induced lethal injury (31, 32). In this study, animals challenged with LPS produced a significant increase in lipid peroxidation levels in both hepatic and pulmonary tissue homogenates, as measured by the formation of thiobarbituric acid reactants (Fig. 3). The administration of α-tocopherol liposomes, as late as 6 h post LPS-challenge, significantly reduced the extent of lipid peroxidation in liver and lung tissues (Fig. 3). Plain liposomes, if administered immediately after LPS injection, was also found to be effective in reducing lipid peroxidation (Table 1). A similar administration of plain liposomes at 2–6 h post LPS-challenge, however, was found ineffective (data not shown).

Since reduced GSH is known to play an important role in protecting cells from oxidant-induced tissue injury (31, 32), GSH levels of liver and lung tissues were also measured. LPS injection resulted in a significant reduction of GSH levels in the liver and lung by 40 and 60%, respectively (Fig. 3). The administration of α-tocopherol liposomes or plain liposomes immediately after LPS injection prevented subsequent GSH depletion to more or less the same extent. The administration of α-tocopherol liposomes 2–6 h after LPS resulted in less and less protection, while plain liposomes were totally nonprotective.

Toxicity of LPS premixed with liposomes

To ascertain whether the protective effects observed following the co-administration of liposomal formulations and LPS were due to the capacity of liposomes in masking the toxic effects of LPS, animals were injected with a mixture of LPS and liposomes. The intravenous injection of such a mixture failed to induce any alterations in liver and lung weights nor changes in oxidative-stress and enzyme markers of hepatic and pulmonary injuries, except plasma AST and ALT activities were moderately elevated by about 24% and 44%, respectively, as compared with a profound elevation of the respective enzymes by 3.6 and 23.9 times in LPS-challenged and saline-treated animals.

DISCUSSION

Results of the present study demonstrated that liver and lung injuries observed following the administration of LPS can be attenuated by treatment with liposomal α-tocopherol. The therapeutic effect is evidenced by a significant reduction in LPS-induced changes in wet lung weight, suggestive of a reduction in lung edema, and a reversal of changes in different enzyme markers of liver and lung injuries. Liposomal α-tocopherol treatment also conferred a reinforcement of the antioxidant defense system as indicated by a reduction in membrane lipid peroxidation and less depletion of GSH in the hepatic and pulmonary organs.

Among other mechanisms of LPS-induced organ injuries, oxidative stress has been suggested as an underlying destructive factor (7–16). This suggestion is supported by results of this study where LPS administration resulted in a significant increase in lipid peroxidation and reduction in GSH levels. These detrimental oxidative effects, however, can be antagonized by liposomal α-tocopherol treatment. Our results are in agreement with those reported by other investigators who observed that administration of antioxidants to animals, either before or at the time of onset of injury prevented LPS-induced tissue injuries (1–4, 7–16).

Neutrophils have been implicated as playing an important role in the pathogenesis of LPS-induced tissue injury. It is

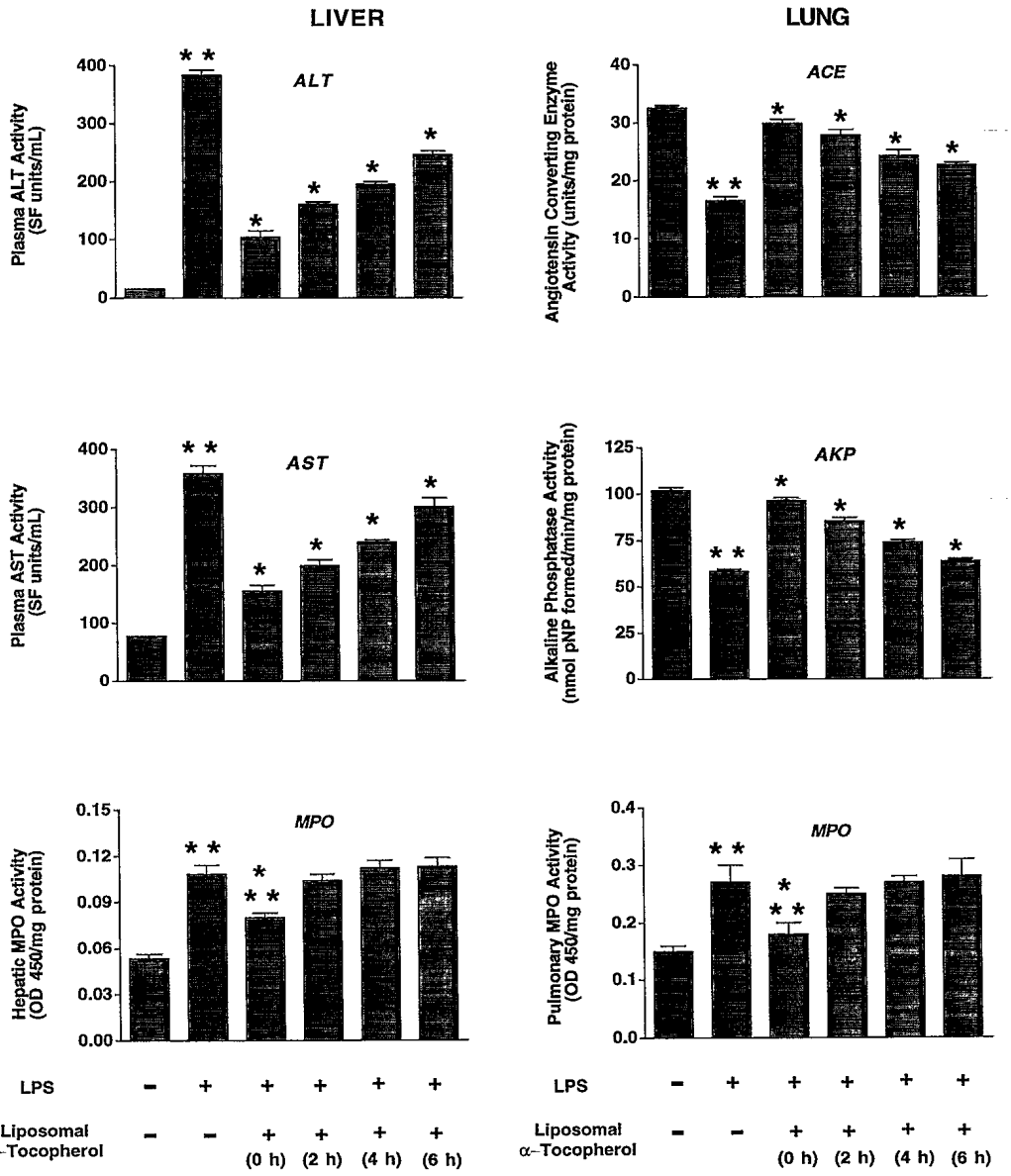

FIG. 2. **Effects of liposomal α-tocopherol treatment on LPS-induced changes in plasma ALT and AST; hepatic MPO; and pulmonary ACE, AKP, and MPO activities.** Animals were challenged with LPS (1 mg/kg body weight, i.v.) and 0, 2, 4, or 6 h later were treated with liposomal α-tocopherol (20 mg/kg body weight, i.v.); treated animals were killed 24 h post-LPS challenge. Each data point represents the mean ± SE of five animals. *significantly different from corresponding values obtained from LPS-challenged and saline-treated animals, $p < .05$. **significantly different from corresponding values obtained from non-LPS-challenged and saline-treated animals, $p < .05$.

known that the depletion of circulating neutrophils by polyclonal antibodies to neutrophils, or the attenuation of the toxic effects of neutrophil products, such as reactive oxygen species and proteases, can reduce the extent of LPS-induced tissue injury (38–40). In the present study, liposomal α-tocopherol failed to prevent neutrophil recruitment in the liver and lung as indicated by a marked increase in MPO activity in these organ tissues. This failure may be explained by the inability of the antioxidant to prevent the production of chemotactic mediators that promote cellular infiltration (41–43).

Although α-tocopherol failed to downregulate the accumulation of neutrophils in the tissues, the antioxidant could have reduced or prevented the toxic effects of reactive oxygen species released by neutrophils. Reactive oxygen species can cause serious tissue damage by peroxidation of membrane lipids and depletion of reducing equivalents such as GSH. Our present results have provided direct evidence substantiating that indeed, LPS administration can induce increased lipid peroxidation and reduced GSH levels and furthermore, both oxidative imbalances can be minimized or abrogated by the administration of liposomal α-tocopherol. It is not known, however, whether the protection conferred by liposomal α-tocopherol was due to its ability to scavenge reactive oxygen species or its ability to modulate the phagocytic activities. It has been demonstrated that α-tocopherol, in addition to its antioxidant properties, has weak anti-inflammatory actions; α-tocopherol has also been shown to inhibit the generation of reactive oxygen species from granulocytes activated by certain stimuli (44–46).

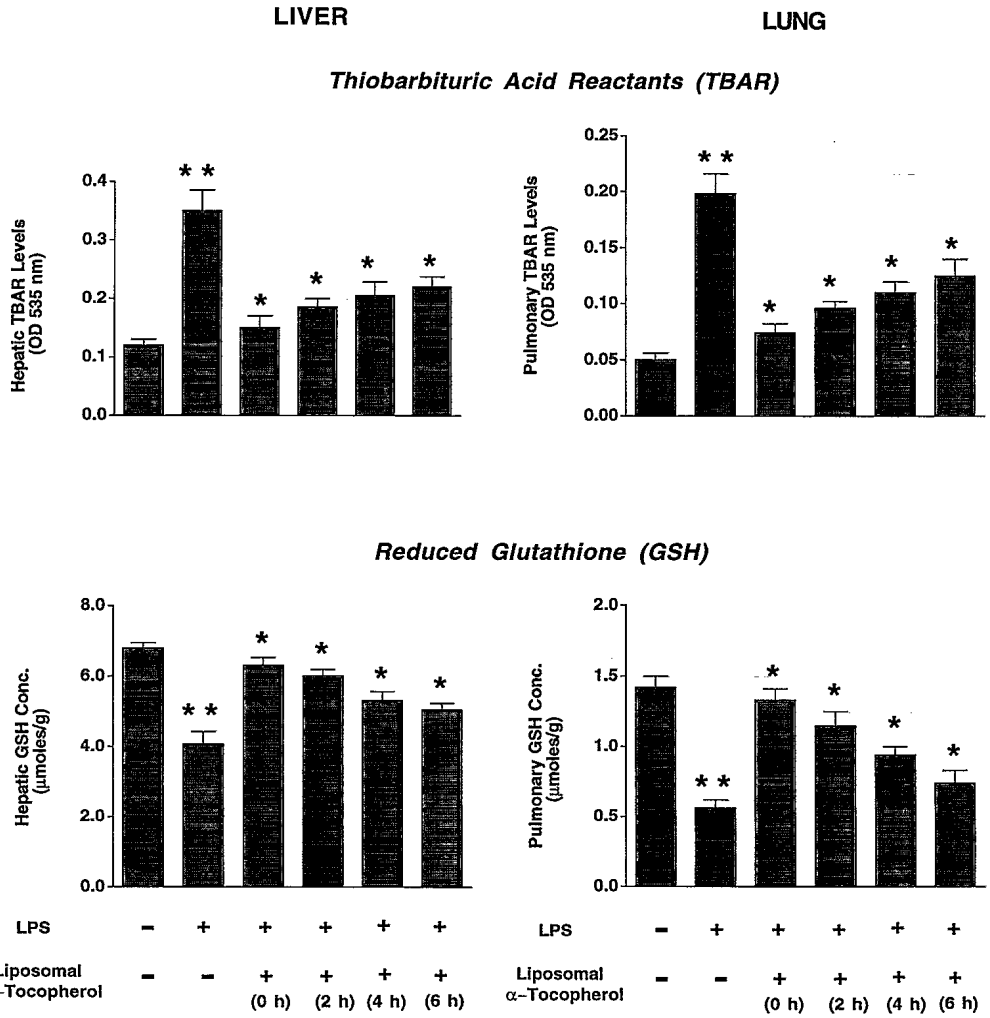

FIG. 3. **Effects of liposomal α-tocopherol treatment on LPS-induced changes in hepatic and pulmonary lipid peroxidation and GSH levels.** Animals were challenged with LPS (1 mg/kg body weight, i.v.) and 0, 2, 4, or 6 h later were treated with liposomal α-tocopherol (20 mg/kg body weight, i.v.); animals were sacrificed 24 h post-LPS challenge. Each data point represents the mean ± SE of five animals. *significantly different from corresponding values obtained from LPS-challenged and saline-treated animals, $p < .05$. **significantly different from corresponding values obtained from non-LPS-challenged and saline-treated animals, $p < .05$.

Results from other studies have demonstrated that antioxidants such as superoxide dismutase, catalase, and *N*-acetylcysteine are effective in protecting against LPS-induced pulmonary edema (8, 12, 15, 47, 48). In light of these observations, it is apparent that LPS-induced edema is mediated by reactive oxygen species known to damage cellular membranes. α-Tocopherol, a lipophilic antioxidant localized in cellular membranes, is known to be effective in protecting against the common pathway of these reactive oxygen species, namely membrane damage. This known α-tocopherol effect is consistent with results of this study, in which liposomal α-tocopherol was found to be effective in lessening LPS-induced lipid peroxidation and preserving the membrane integrity of endothelial and alveolar cells, as indicated by a significant reversal of depressed ACE and AKP activities. Injury to epithelial and endothelial cells can cause transudation into the alveoli, resulting in the formation of edema. Thus, a reduction in pulmonary endothelial and epithelial cell damage by liposomal α-tocopherol may partly explain the significantly reduced elevation in lung weight of LPS-challenged animals, suggestive of an attenuation of the resultant lung edema.

Our results demonstrated that the immediate treatment of LPS-challenged animals with α-tocopherol liposomes or plain liposomes is equally effective in conferring a protective effect against LPS-induced toxicities. It can be hypothesized that the treatment effect is most likely due to the capacity of liposomes in binding and sequestering LPS, thus limiting the availability of active LPS in the circulation and its distribution in tissues. Indeed lipid A, the active domain of the LPS molecule, has been shown to bind to the phospholipid bilayer of liposomes and this binding effectively masks the biological activity of LPS (49–51). This "masking hypothesis" is also supported by our results demonstrating that the administration of LPS premixed with plain liposomes conferred little or no toxicity.

Unlike α-tocopherol liposomes, plain liposomes were ineffective in counteracting LPS-induced toxic effects when administered 2–6 h post LPS-challenge. In contrast, α-tocopherol liposomes mediated a significant therapeutic effect even when

they were administered as late as 6 h after LPS injection. This treatment effect, however, appeared time dependent, namely the therapeutic efficacy of liposomal α-tocopherol gradually decreased at later times post LPS-challenge. Since plain liposomes administered at similar later timepoints had no beneficial effect, the therapeutic benefit of α-tocopherol liposomes is most likely attributable to the antioxidant moiety of the liposomal preparation.

The failure of liposomal α-tocopherol to completely neutralize LPS-induced tissue injuries may be partly due to the inability of the antioxidant to scavenge all available reactive oxygen species. An additional explanation could be that tissue injuries observed following LPS challenge are induced not only by reactive oxygen species, but also by other granulocyte-released factors such as proteases and lysosomal enzymes, known to be capable of destroying key structural elements of connective tissues (7–12). Furthermore, it is now accepted that oxidants and proteases may enhance each other's damaging effects. For example, leukocytic proenzymes are activated by reactive oxygen species and oxidatively modified proteins are preferred targets of certain proteases (7–12).

The therapeutic efficacy of liposomal α-tocopherol in ameliorating LPS-induced hepatic and pulmonary injuries implicates the potential benefit of this antioxidant formulation for treating clinical conditions in which LPS is a pathogenic element, as in certain cases of sepsis and septic complications. However, since the development of septic shock is known to involve a rather complex cascade of pathophysiological events, no single pharmacological treatment will represent the "magic bullet" for successful intervention. It is conceivable that a combination of treatments will be required that may include, but are not limited to the following: 1) elimination of bacteria with antibiotics; 2) neutralization of released bacterial toxins by anti-toxin reagents; 3) inhibition of the inflammatory response by anti-inflammatory agents; and 4) protection against deleterious mediators, such as reactive oxygen species, proteases, and TNFα by appropriate counteracting agents.

The key advantage of employing the liposomal delivery system is that liposomes are virtually non-toxic and can be administered in a fairly high dose safely, enabling the delivery of a meaningful therapeutic dosage of an entrapped drug to the target site (17–19, 21, 22, 26, 52–54). In the case of sepsis and LPS-induced septic shock, the respiratory system is most vulnerable to failure because of the development of the shock-lung condition. Results of this study have demonstrated the superior efficacy of a liposomal antioxidant in counteracting LPS-induced oxidant-mediated injuries. Since liposomal formulations are amenable to rapid delivery of entrapped therapeutic agents to the lung via the intratracheal or intravenous route, further development of liposome-mediated drug delivery systems merits serious considerations.

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