Biochemical Pharmacology, Vol. 59, pp. 1155–1161, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(99)00411-6

Prophylaxis Against Lipopolysaccharide-Induced Lung Injuries by Liposome-Entrapped Dexamethasone in Rats

Zacharias E. Suntres and Pang N. Shek*

Biomedical Sciences Section, Defence and Civil Institute of Environmental Medicine, Toronto, Ontario, M3M 3B9, Canada

ABSTRACT. Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, stimulates phagocytes to generate metabolites that play an important role in the pathogenesis of acute lung injury. In this study, the prophylactic effect of liposome-entrapped dexamethasone (L-DEX) was evaluated in an animal acute lung injury model. Rats were pretreated intratracheally with L-DEX or dexamethasone phosphate (DEX) at a dose of 800 μg dexamethasone/kg body weight; 1 hr later, pretreated animals were challenged i.v. with LPS (*Escherichia coli* 0111:B4, 1 mg/kg body weight) and killed 24 hr later. Challenge of saline-pretreated animals with LPS resulted in lung injury, as evidenced by increases in wet lung weight and decreases in lung angiotensin-converting enzyme and alkaline phosphatase activities, injury markers of pulmonary capillary endothelial and alveolar type II epithelial cells, respectively. Also, LPS injection resulted in significant increases in plasma phospholipase A_2, thromboxane B_2, and leukotriene B_4 concentrations. The LPS challenge also increased pulmonary myeloperoxidase and elastase activities as well as chloramine concentrations, suggestive of neutrophil infiltration and activation of the inflammatory response. Pretreatment of animals with L-DEX was significantly more effective than pretreatment with the free drug in reducing lung inflammation and other lung injuries, as indicated by the appropriate injury markers used in this study. Our results suggested that the pulmonary delivery of liposome-entrapped anti-inflammatory drugs such as dexamethasone improves prophylactic efficacy in counteracting LPS-induced lung injury. BIOCHEM PHARMACOL **59**;9:1155–1161, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. liposomes; dexamethasone; acute lung injury; lipopolysaccharide; sepsis; inflammation

Challenge of experimental animals or humans with LPS† induces an array of pathophysiological changes that resemble those often accompanying Gram-negative bacterial sepsis [1–4]. A common and frequent lethal complication of sepsis is ARDS. ARDS is associated with diffuse lung microvascular injury and is characterized by severe hypoxemia, diffuse infiltration of the lung, reduction in compliance, and increased pulmonary resistance [3–5].

Neutrophils and other inflammatory cells play a crucial role in the pathogenesis of LPS-induced acute lung injury [2, 4, 6, 7]. Several lines of evidence now suggest that stimulated neutrophils release a plethora of mediators including, but certainly not limited to, reactive oxygen species, proteolytic enzymes, and products of lipid metabolism. All of these mediators have engendered a considerable interest in recent years for their possible roles in contributing to the mechanisms of LPS-induced acute lung injury [2, 4, 6, 7]. Accordingly, we hypothesized that the susceptibility of the lung to phagocyte-mediated release of toxic metabolites should be reduced by preventing their infiltration and activation with glucocorticoids.

Glucocorticoids are used extensively in the treatment of pulmonary inflammatory disorders; however, their beneficial effects have been counterbalanced by their systemic side-effects [8, 9]. This is of great concern, particularly for patients who are in need of high doses of the anti-inflammatory agents. Thus, it is considered desirable to develop glucocorticoid preparations efficacious at the target site, but with weak systemic side-effects. Currently, we have developed an alternative delivery system, by entrapment in liposomes, for the direct administration of glucocorticoids to the lung with minimal adverse side-effects [10]. Liposomes are artificially prepared phospholipid vesicles where 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 non-toxic [11].

We have demonstrated previously that the retention of

* Corresponding author: Zacharias E. Suntres, Ph.D., Defence and Civil Institute of Environmental Medicine, 1133 Sheppard Avenue West, Toronto, Ontario, M3M 3B9, Canada. Tel. (416) 635-2150; FAX (416) 635-2104; E-mail: Zach.Suntres@dciem.dnd.ca

† *Abbreviations*: LPS, lipopolysaccharide; ARDS, adult respiratory distress syndrome; L-DEX, liposome-entrapped dexamethasone; DEX, dexamethasone phosphate; ACE, angiotensin-converting enzyme; AKP, alkaline phosphatase; MPO, myeloperoxidase; and DPPC, dipalmitoylphosphatidylcholine.

Received 28 June 1999; accepted 20 September 1999.

dexamethasone delivered directly to the lung as a liposomal formulation can be prolonged significantly and the systemic side-effects reduced significantly [10]. The present study was conducted to examine the effectiveness of the liposomal formulation in a rodent model of acute lung injury. Accordingly, the protective effect of L-DEX against LPS-induced lung injury was assessed biochemically by measuring: (i) ACE and AKP activities as markers of pulmonary capillary endothelial cell and alveolar type II epithelial cell integrity, respectively; (ii) MPO and elastase activities as well as chloramine concentrations in lung homogenates as markers of the inflammatory response; and (iii) several proinflammatory mediators (phospholipase A_2 activity, concentrations of the eicosanoids leukotriene B_4 and thromboxane B_2 in plasma, and lung histamine concentrations) as a measure of the extent of the pro-inflammatory response.

MATERIALS AND METHODS

Chemicals

LPS (*Escherichia coli* 0111:B4), DEX, and dexamethasone were purchased from the Sigma Chemical Co. DPPC was obtained from Avanti Polar Lipids. All other chemicals were obtained from Sigma and BDH.

Animals

Male Sprague–Dawley rats (approximate body weight 220–250 g) were purchased from Charles River Canada, Inc. 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° and were exposed to alternate cycles of 12 hr 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 for treating animals was approved by our institutional animal care committee.

Preparation of L-DEX

L-DEX was prepared from a mixture of DPPC and dexamethasone in a 9:1 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° 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 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) through two stacked polycarbonate filters of 400 nm pore size using a helium pressure of 100–200 lb/in^2. Non-entrapped dexamethasone 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° in a Beckman L8–70 ultracentrifuge. The loading capacity of liposomes was found to be 50.4 μg dexamethasone/mg DPPC, and the size of the liposomal preparation was 231 nm (± 32 SEM) as determined by a Coulter N4SD particle-size analyzer (Coulter Electronics of Canada). The liposomes were diluted with 5 mM potassium phosphate buffer, pH 6.5, to give a final dexamethasone concentration of 200 μg/150 μL suspension.

Treatment of Animals

Saline, plain liposomes, L-DEX, or DEX (800 μg dexamethasone/kg body weight in a volume of 150 μL/animal) was instilled intratracheally into the lungs of rats as previously described [10]. One hour later, rats were injected i.v. via the tail vein with a single dose of *E. coli* LPS (1 mg/kg body weight), and were killed 24 hr later. Pretreatments were carried out between 8:00 and 9:00 a.m. LPS was dissolved in saline shortly before use. Control animals received an equivalent volume of saline.

Experimental Design

To investigate whether dexamethasone would ameliorate the LPS-induced lung injury, rats pretreated with DEX, L-DEX, plain liposomes, or saline were challenged with a single dose of LPS and killed 24 hr later. In experiments conducted to investigate the time- and dose-dependent responses to LPS-induced lung injury, it was shown that i.v. administration of 1 mg/kg of LPS (*E. coli* 0111:B4) produced a pronounced lung injury 24 hr post-challenge with no apparent mortality (unpublished observation). The prophylactic effect of dexamethasone against LPS-induced lung injury was assessed biochemically by measuring the activities of ACE and AKP in lung homogenates, injury markers of pulmonary endothelial and alveolar type II epithelial cells, respectively. Also, the infiltration and activation of neutrophils in the lung were assessed by measuring the activities of MPO and elastase as well as chloramine concentration in lung homogenates. In addition, several proinflammatory mediators (phospholipase A_2 activity and concentrations of the eicosanoids leukotriene B_4 and thromboxane B_2 in plasma) were also measured as indicators of the extent of the pro-inflammatory response.

Plasma 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°.

Lung Tissue Preparation

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°. Following rinsing, lungs were weighed quickly and minced finely. Approximately 1 g of lung 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.

Enzyme Measurements

Activities of ACE and AKP were determined by methods described by Suntres and Shek [12]. The activity of MPO in sonicated whole lung homogenates was estimated by using a specific enzyme-linked immunosorbent assay kit (R&D Systems). Elastase activity was estimated by measuring the rate of hydrolysis of the 4-nitroanilide MeO-Suc-Ala-Ala-Pro-Met-NA as previously described [13]. The phospholipase A_2 concentration was estimated by using a specific enzyme-linked immunosorbent assay kit (Boehringer Mannheim Canada) according to the manufacturer's directions [14]. Protein determinations were estimated by the method of Lowry *et al.* [15].

Measurement of Thromboxane B_2 and Leukotriene B_4

Measurement of plasma thromboxane A_2 or leukotriene B_4 was carried out by using a specific enzyme-linked immunosorbent assay kit (R&D Systems) according to the manufacturer's directions.

Determination of Chloramine Concentration

Chloramine concentrations in pulmonary homogenates were determined by colorimetric measurement of the triiodide ion formed by the oxidation of potassium iodide [16].

Statistical Analysis

Data from control and experimental animals pretreated with saline, empty liposomes, L-DEX, and DEX and challenged with LPS were evaluated by one-way ANOVA with a Newman–Keuls test of multiple comparisons [17]. The level of significance was accepted at $P < 0.05$.

RESULTS

Wet Lung Weight Changes

Wet lung weights of saline-pretreated animals were increased significantly (49%) 24 hr post-LPS challenge (Fig. 1). The wet lung weight of LPS-challenged animals pretreated with plain liposomes was not significantly different from the saline-pretreated group. Pretreatment of animals with L-DEX was more effective than pretreatment with DEX in reducing the LPS-induced changes in wet lung weight (16 vs 27%, respectively).

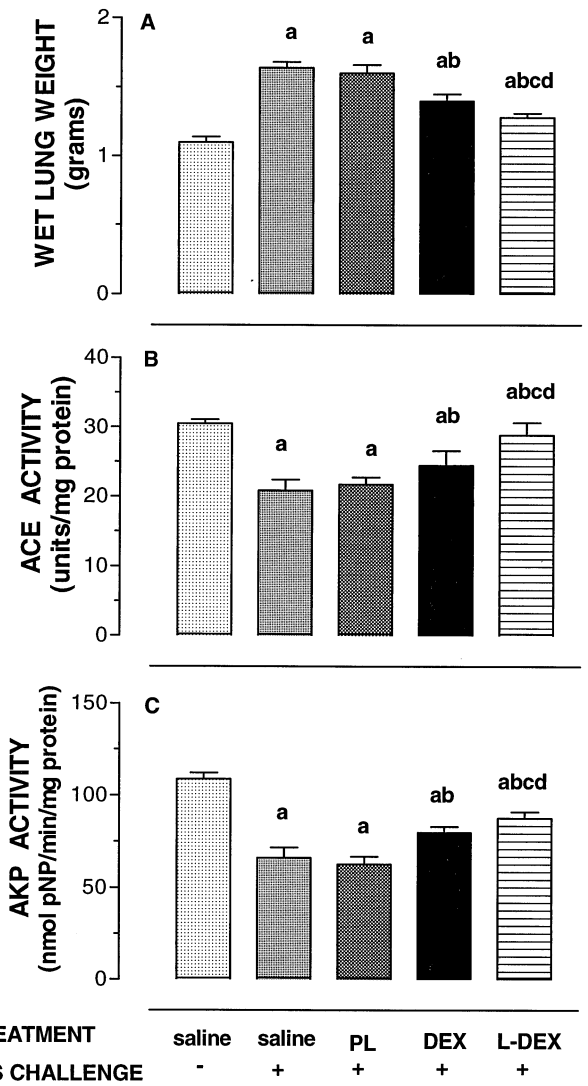

FIG. 1. Effect of pretreatment with L-DEX, DEX, or plain liposomes (PL) on LPS-induced changes in wet lung weight (A), and in ACE (B) and AKP (C) activities. L-DEX, DEX (800 μg dexamethasone/kg), or PL were instilled intratracheally into the lungs of rats, and 1 hr later animals were challenged with LPS (1 mg/kg, i.v.); animals were killed 24 hr later. Each data point represents the mean ± SEM of 5 animals. Key: (a) denotes a significant difference ($P < 0.05$) from the mean value of the control group pretreated with saline without LPS challenge; (b) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with saline and challenged with LPS; (c) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with DEX and challenged with LPS; and (d) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with PL and challenged with LPS.

Changes in Pulmonary ACE and AKP Activities

Pulmonary activities of ACE (localized in capillary endothelial cells) and AKP (localized primarily in alveolar type II epithelial cells) have been used as markers of lung injury [18, 19]. Challenge of saline-pretreated animals with LPS resulted in significant decreases in pulmonary ACE and AKP activities (32 and 39%, respectively) (Fig. 1). The

LPS-induced changes in pulmonary enzyme activities of rats pretreated with plain liposomes were similar to those observed in the saline-pretreated rats. Pretreatment of animals with DEX or L-DEX conferred protection against LPS-induced changes in ACE and AKP activities, and the protective effect of L-DEX (5 and 18% decrease, respectively) was significantly better than that of DEX (20 and 28% decrease, respectively).

Changes in Pulmonary MPO, Elastase, and Chloramine Levels

In the present study, infiltration and activation of neutrophils in the lungs of treated animals were assessed by measuring the activities of MPO [20, 21] and elastase [22] as well as the chloramine concentration [20, 23] (long-lived oxidants produced by neutrophils). Challenge of animals with LPS resulted in a 4-fold increase in pulmonary MPO activity, suggestive of neutrophil infiltration (Fig. 2). Also, LPS challenge of saline-pretreated rats resulted in significant increases in pulmonary elastase activities (5.3-fold) and chloramine concentrations (3-fold), suggestive of phagocyte activation. Pretreatment of animals with plain liposomes did not alter the LPS-induced inflammatory responses. On the other hand, pretreatment of animals with L-DEX was significantly more effective than pretreatment with DEX in suppressing the LPS-induced increases in MPO activity (55 vs 40%, respectively), elastase activity (68 vs 51%, respectively), and chloramine concentration (50 vs 28%, respectively) in the lung.

Plasma Concentrations of Phospholipase A_2, Leukotriene B_4, and Thromboxane B_2

Phospholipase A_2 is involved in inflammatory processes such as the liberation of free arachidonic acid from membranes for the biosynthesis of eicosanoids such as thromboxane A_2 and leukotriene B_4 [24–26]. In the present study, LPS challenge resulted in significant increases in plasma phospholipase A_2 activity (4-fold) as well as concentrations of thromboxane B_2 (6.5-fold), a stable metabolite of thromboxane A_2, and leukotriene B_4 (27-fold) (Fig. 3). Pretreatment of animals with either saline or plain liposomes did not alter the LPS-induced increases in these parameters. Pretreatment of animals with L-DEX was significantly more effective than pretreatment with DEX in suppressing the LPS-induced increases in plasma phospholipase A_2 (62 vs 45%, respectively), thromboxane B_2 (76 vs 64%, respectively), and leukotriene B_4 (76 vs 64%, respectively) concentrations.

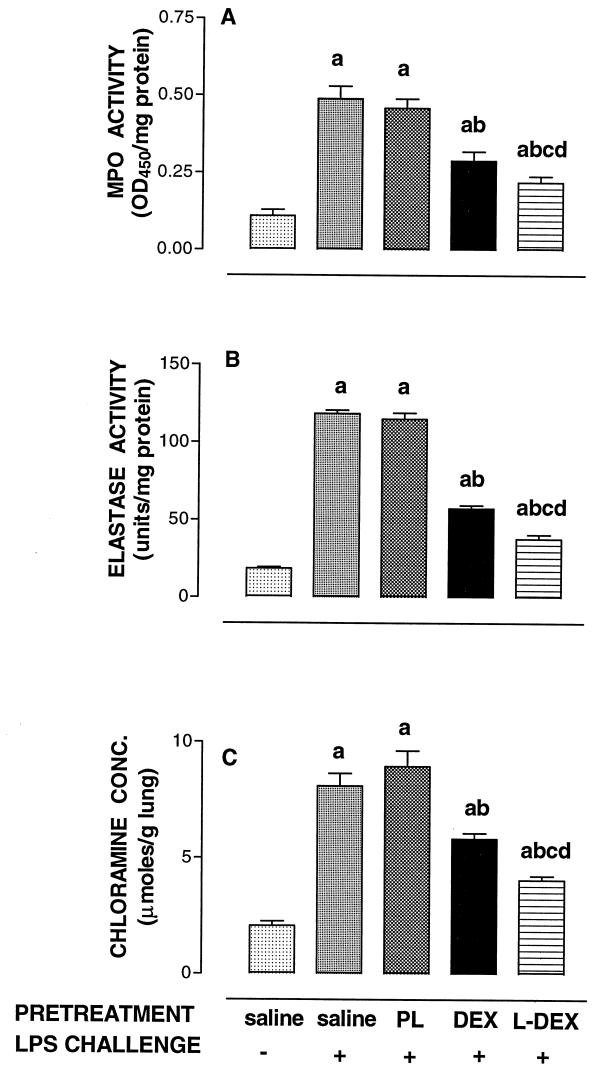

FIG. 2. Effect of pretreatment with L-DEX, DEX, or plain liposomes (PL) on LPS-induced changes in MPO activity (A), elastase activity (B), and chloramine concentration (C). L-DEX, DEX (800 μg dexamethasone/kg), or PL were instilled intratracheally into the lungs of rats, and 1 hr later animals were challenged with LPS (1 mg/kg, i.v.); animals were killed 24 hr later. Each data point represents the mean ± SEM of 5 animals. Key: (a) denotes a significant difference ($P < 0.05$) from the mean value of the control group pretreated with saline without LPS challenge; (b) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with saline and challenged with LPS; (c) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with DEX and challenged with LPS; and (d) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with PL and challenged with LPS.

DISCUSSION

Evidence presented in this study clearly demonstrated that L-DEX delivered directly to the lung was more effective than DEX in conferring protection against LPS-induced acute lung injury. More precisely, the liposomal drug formulation was more effective than the free drug in ameliorating the LPS-induced changes in wet lung weight (indicative of edema), and ACE and AKP activities (indicative of capillary endothelial and alveolar type II epithelial cell integrity, respectively). Also, the liposomal drug formulation was more effective in suppressing the LPS-induced inflammatory response, assessed by the measure-

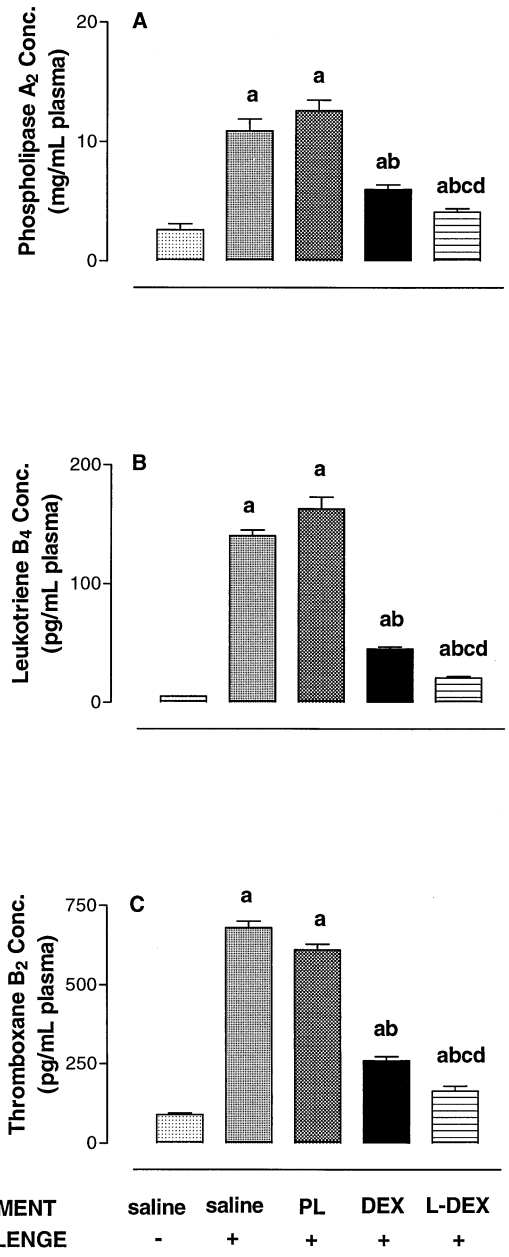

FIG. 3. Effect of pretreatment with L-DEX, DEX, or plain liposomes (PL) on LPS-induced changes in phospholipase A_2 (A), leukotriene B_4 (B), and thromboxane B_2 (C) concentrations. L-DEX, DEX (800 μg dexamethasone/kg), or PL were instilled intratracheally into the lungs of rats, and 1 hr later animals were challenged with LPS (1 mg/kg, i.v.); animals were killed 24 hr later. Each data point represents the mean ± SEM of 5 animals. Key: (a) denotes a significant difference ($P < 0.05$) from the mean value of the control group pretreated with saline without LPS challenge; (b) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with saline and challenged with LPS; (c) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with DEX and challenged with LPS; and (d) denotes a significant difference ($P < 0.05$) from the mean value of the group of animals pretreated with PL and challenged with LPS.

ment of pulmonary activities of MPO and elastase as well as chloramine and eicosanoid concentrations. Other investigators have reported that treatment of arthritis in experimental models [27] or humans [28] with a liposome preparation containing corticosteroids resulted in a significant suppression of inflammation, whereas treatment with an equivalent amount of the free drug alone was not as effective. The findings from our study and the other studies [27, 28] support the use of liposomes as a drug delivery system for glucocorticoids in the treatment of inflammatory disorders.

Previous studies from our laboratory have shown that the intratracheal instillation of L-DEX directly to the lung results in significantly higher levels of the drug in the lung tissues [10]. In addition, the liposomal formulation was found more effective than the free drug in inducing a less severe leukopenia, presumably by a slow, sustained release of the drug into the systemic circulation [10]. Accordingly, the improved anti-inflammatory activity of L-DEX observed in this study may be attributable to a prolonged retention of the drug in the lung, which serves as a depot for continuous drug release. Similar results have been reported by other investigators, who have employed liposomes as a means in altering the pharmacokinetics of drugs, prolonging their residence time within airways, and achieving sustained plasma levels [11]. However, liposomes have also been shown to facilitate intracellular drug delivery, and, therefore, our results cannot rule out the possibility that liposomal dexamethasone may also contribute to promoting cellular expression at the transcriptional or translational level.

The rationale for the use of glucocorticoids, such as dexamethasone, in the treatment of inflammatory lung injuries stems from their abilities to suppress the migration of phagocytes to the site of injury as well as to inhibit some responses of phagocytes to activating stimuli [24, 29]. It has been recognized that acute lung injury following administration of LPS is associated with infiltration and activation of phagocytes in the lung [30–32]. Phagocytes are known to play a major role in lung injury by releasing oxidants and proteases that damage the pulmonary endothelial and epithelial cells, thus disrupting the alveolar–capillary unit [30–32]. Indeed, pretreatment with DEX attenuated the sequestration of neutrophils in the lung as assessed by the measurement of MPO in these tissues. The improved prophylactic effect conferred by dexamethasone administered as a liposomal formulation appears to be related to its ability to further suppress the sequestration and activation of neutrophils in the lung tissue (Fig. 2). The additional reduction in elastase activity and chloramine levels in the lungs of L-DEX-pretreated animals appears to support the enhanced anti-inflammatory effectiveness of L-DEX. It is worth noting that the administration of plain liposomes, without any entrapped drug, conferred no prophylactic efficacy against LPS-induced lung injury, strongly suggesting that the lipid carriers themselves were ineffective in alleviating the pulmonary complications, but they effec-

tively served as a vehicle system for the delivery of the entrapped dexamethasone.

Another benefit of the liposomal formulation is that liposomes are normally taken up by macrophages and other inflammatory cells, known to play an important role in the pathogenesis of lung injury [33, 34]. The intracellular uptake of dexamethasone is known to impair the chemotactic ability of phagocytes and suppress the release of toxic metabolites (proteases and reactive oxygen species) from these cells. A successful reduction by liposomal dexamethasone in toxic metabolite release from phagocytes may contribute to a reduction in tissue damage associated with inflammation. This cell-selective intracellular transfer of drug may also explain the superiority of the liposomal formulation, and it substantiates the use of liposomes as a drug carrier in the treatment of inflammatory disorders.

Many changes found in the lungs of LPS-treated animals could also be attributable to the products of arachidonic acid metabolism, such as thromboxanes and leukotrienes [3, 5, 35, 36]. Thromboxanes and leukotrienes are released in large quantities in acute lung injury models [3, 5, 35, 36]. These factors can act as potent chemotactic agents for neutrophils, by altering the function of airway and vascular smooth muscle and increasing vascular permeability [24–26]. For instance, thromboxane is a potent vasoconstrictor and may interact with neutrophils to accentuate cell aggregation. Leukotriene B_4 has chemotactic activity for phagocytes and can contribute to acute lung injury by increasing phagocytic cell recruitment [24–26]. In view of these observations, it is evident that lowering the levels of arachidonic acid products may be effective in ameliorating acute lung injury. Glucocorticoids such as dexamethasone have been used effectively in lowering these metabolites by inhibiting the activity of phospholipase A_2, an enzyme that catalyzes the breakdown of phospholipids to arachidonic acid [24–26]. The results of this study demonstrated that dexamethasone administered as a free drug or as a liposomal formulation could reduce LPS-induced acute lung injury, perhaps attributable to a suppression of the release of the eicosanoid acid metabolites via an inhibitory effect on phospholipase A_2 (Fig. 3). The suppressive action of the liposomal dexamethasone preparation was far greater than that of the free drug. This elevated suppressive effect may well be related to a liposome-mediated dexamethasone availability that we have demonstrated previously [10] rather than to a change in the mechanism of drug action.

In summary, the results of the present study indicated that the use of a liposomal anti-inflammatory preparation is far more effective than the free drug in counteracting inflammation-induced lung injury. Therefore, liposomal entrapment offers an alternative means of administering dexamethasone for patients at risk of developing ARDS secondary to sepsis, burns, or pulmonary inflammatory disorders, such as asthma.

References

1. Fink MP and Heard SO, Laboratory models of sepsis and septic shock. *J Surg Res* **49**: 186–196, 1990.
2. Hewett JA and Roth RA, Hepatic and extrahepatic pathology of bacterial lipopolysaccharides. *Pharmacol Rev* **45**: 381–411, 1993.
3. Repine JE, Scientific perspectives on adult respiratory distress syndrome. *Lancet* **339**: 466–472, 1992.
4. Glaucer MP, Zanetti G, Baumgartner J-D and Cohen J, Septic shock: Pathogenesis. *Lancet* **338**: 732–739, 1991.
5. Fulkerson WJ, MacIntyre N, Stamler J and Crapo JD, Pathogenesis and treatment of the adult respiratory distress syndrome. *Arch Intern Med* **156**: 29–38, 1996.
6. Rackow EC and Astiz ME, Mechanisms and management of septic shock. *Circ Shock* **9**: 219–237, 1993.
7. Weiss SJ, Tissue destruction by neutrophils. *N Engl J Med* **320**: 365–379, 1989.
8. Barnes PJ and Pedersen S, Efficacy and safety of inhaled corticosteroids in asthma. *Am Rev Respir Dis* **148**: S1–S26, 1993.
9. Check WA and Kaliner MA, Pharmacology and pharmacokinetics of topical corticosteroid derivatives used in asthma therapy. *Am Rev Respir Dis* **141**: S44–S51, 1990.
10. Suntres ZE and Shek PN, Liposomes promote pulmonary glucocorticoid delivery. *J Drug Target* **6**: 175–182, 1998.
11. Taylor KMG and Farr SJ, Liposomes for drug delivery to the respiratory tract. *Drug Dev Ind Pharm* **19**: 123–142, 1993.
12. Suntres ZE and Shek PN, Alleviation of paraquat-induced lung injury by pretreatment with bifunctional liposomes containing α-tocopherol and glutathione. *Biochem Pharmacol* **52**: 1515–1520, 1996.
13. Nakajima K, Powers JC, Ashe BM and Zimmerman M, Mapping the extended substrate binding of cathepsin G and human leukocyte elastase. Studies with peptide substrates related to the α_1-protease inhibitor reactive site. *J Biol Chem* **254**: 4027–4032, 1979.
14. Takayama K, Kudo I, Hara S, Murakami M, Matsuta K, Miyamoto T and Inoue K, Monoclonal antibodies against human synovial phospholipase A_2. *Biochem Biophys Res Commun* **167**: 1309–1315, 1990.
15. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
16. Witko-Sarsat V, Delacourt C, Rabier D, Bardet J, Nguyen AT and Descamps-Latscha B, Neutrophil-derived long-lived oxidants in cystic fibrosis sputum. *Am J Respir Crit Care Med* **152**: 1910–1916, 1995.
17. Gad SC and Weil CS, Statistics for toxicologists. In: *Principles and Methods of Toxicology* (Ed. Hayes AW), pp. 221–274. Raven Press, New York, 1994.
18. Lazo JS, Lynch TJ and McCallister J, Bleomycin inhibition of angiotensin converting enzyme activity from serum, lungs, and cultured pulmonary artery endothelial cells. *Am Rev Respir Dis* **134**: 73–78, 1986.
19. Boudreau J and Nadeau D, Lung hydrolases in paraquat poisoning: Early response of alkaline phosphatase. *J Toxicol Environ Health* **22**: 329–340, 1987.
20. Schraufstatter IU and Cochrane CG, Oxidants: Types, sources, and mechanisms of injury. In: *Lung Injury* (Eds. Crystal RG and West JB), pp. 43–50. Raven Press, New York, 1991.
21. Bradley PP, Priebat DA, Christensen RD and Rothstein G, Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* **78**: 206–209, 1982.
22. Hubbard RC, Brantly ML and Crystal RG, Proteases. In: *Lung*

Injury (Eds. Crystal RG and West JB), pp. 3–13. Raven Press, New York, 1991.
23. Grisham MB, Jefferson MM, Melton DF and Thomas EL, Chlorination of endogenous amines by isolated neutrophils. *J Biol Chem* **259**: 10404–10413, 1984.
24. Barnes PJ, Mechanisms of action of glucocorticoids in asthma. *Am J Respir Crit Care Med* **154**: S21–S27, 1996.
25. Williams KM, Day RO and Breit SN, Biochemical actions and clinical pharmacology of anti-inflammatory drugs. *Adv Drug Res* **24**: 121–198, 1993.
26. Vane J and Botting R, Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB J* **1**: 89–96, 1987.
27. Lopez-Garcia F, Vasquez-Auton JM, Gil F, Latoore R, Moreno F, Villalain J and Gomez-Fernandez JC, Intra-articular therapy of experimental arthritis with a derivative of triamcinolone acetonide incorporated in liposomes. *J Pharm Pharmacol* **45**: 576–578, 1993.
28. de Silva M, Hazleman BL, Page Thomas DP and Wraight P, Liposomes in arthritis: A new approach. *Lancet* **1**: 1320–1322, 1979.
29. Williams TJ and Yarwood H, Effect of glucocorticoids on microvascular permeability. *Am Rev Respir Dis* **141**: S39–S43, 1990.
30. Bone RC, The pathogenesis of sepsis. *Ann Intern Med* **115**: 457–469, 1991.
31. Brigham KL and Meyrick B, Endotoxin and the lung. *Am Rev Respir Dis* **133**: 913–927, 1986.
32. Sharar SR, Winn RK and Harlan JM, Endotoxin-induced interactions of inflammatory cells within the lungs. In: *Endotoxin and the Lungs* (Ed. Brigham KL), pp. 229–265. Marcel Dekker, New York, 1994.
33. Schreier H, Gonzalez-Rothi RJ and Stencenko AA, Pulmonary delivery of liposomes. *J Control Rel* **24**: 209–223, 1993.
34. Taylor KMG and Newton JM, Liposomes for controlled delivery of drugs to the lung. *Thorax* **47**: 257–259, 1992.
35. Henderson W, Eicosanoids and lung inflammation. *Am Rev Respir Dis* **135**: 1176–1185, 1987.
36. Stephenson A, Lonigoo J, Hyers T, Webster RO and Fowler AA, Increased concentration of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. *Am Rev Respir Dis* **138**: 714–719, 1988.