Image Cover Sheet

CLASSIFICATION	SYSTEM NUMBER
UNCLASSIFIED	151942

TITLE

LIPOSOMAL ALPHA-TOCOPHEROL ALLEVIATES THE PROGRESSION OF PARQUAT-INDUCED LUNG DAMAGE

System Number:

Patron Number:

Requester:

Notes:

DSIS Use only:

Deliver to: FF

Journal of Drug Targeting, 1995, Vol. 2, pp. 493–500
Reprints available directly from the publisher
Photocopying permitted by license only

Liposomal α-Tocopherol Alleviates the Progression of Paraquat-induced Lung Damage

Z. E. SUNTRES[1] and P. N. SHEK[1,2]*

[1]*Operational Medicine Division, Defence and Civil Institute of Environmental Medicine, North York, Ontario M3M 3B9, Canada*
[2]*Department of Clinical Biochemistry, Faculty of Medicine, University of Toronto, Toronto, Ontario M5G 1L5, Canada*

(Received July 28, 1994; Accepted September 29, 1994)

The present study was carried out to investigate the efficacy of liposome-associated α-tocopherol in treating pulmonary damage caused by paraquat exposure. α-Tocopherol liposomes (8 mg α-tocopherol/kg body weight) or plain liposomes were intratracheally instilled into the lungs of rats 24 h after paraquat treatment (20 mg/kg, ip); treated animals were killed 8, 24 or 48 h after administration of the liposomal preparations. Lungs of animals exposed to paraquat were extensively damaged as evidenced by an increase in lung weight and decreases in pulmonary angiotensin converting enzyme and alkaline phosphatase activities. Also, paraquat treatment resulted in a significant reduction in glutathione (GSH) concentration in the lung and an elevation in microsomal lipid peroxidation levels, as measured by the formation of diene conjugates. Treatment of paraquat-injected rats with plain liposomes did not significantly alter paraquat-induced changes of all parameters examined. On the other hand, treatment of rats with α-tocopherol liposomes, 24 h after paraquat administration, resulted in a significant increase in pulmonary α-tocopherol concentrations as well as a reduction in paraquat-induced changes in lipid peroxidation, GSH concentration, and lung angiotensin converting enzyme and alkaline phosphatase activities. The results of the present study suggest that α-tocopherol, administered directly to the lung in a liposomal form, may serve as a potentially effective pharmacological agent in the treatment of paraquat-induced lung injury.

KEYWORDS: Liposome, drug delivery, lung, antioxidant, α-tocopherol, paraquat

INTRODUCTION

Paraquat, a wide-spectrum herbicide, is known to preferentially cause lung damage, manifested by edema, haemorrhage, interstitial inflammation and proliferation of the bronchial epithelium (Heath and Smith, 1977; Pasi, 1978; Bus and Gibson, 1984; Vale et al., 1987). Although the precise mechanism whereby paraquat induces lung injury is not well understood, it is generally accepted that generation of reactive oxygen species propagated by a cyclic single electron reduction/oxidation of paraquat, is a critical mechanistic event. Some investigators have suggested that paraquat produces its toxic effects primarily by inducing membrane lipid peroxidation mediated by the superoxide anion (Heath and Smith, 1977; Pasi, 1978; Bus and Gibson, 1984; Vale et al., 1987).

Ingestion of even a small amount of paraquat is often associated with a fatal outcome. The major cause of death in paraquat poisoning is respiratory failure due to an oxidative insult to the alveolar epithelium with subsequent obliterating fibrosis (Heath and Smith, 1977; Pasi, 1978; Bus and Gibson, 1984; Vale et al., 1987). Since there is no true pharmacological antagonist for paraquat and there are no chelating agents capable of binding the herbicide in the blood or other tissues, innovations in the management of paraquat poisoning have been directed towards the modification of the toxicokinetics of the herbicide by either decreasing its absorption or enhancing its elimination. Such approaches are intended to prevent the accumulation of paraquat in tissues and include procedures such as induced emesis or diarrhoea, gastric lavage, administration of oral absorbents, hemodialysis, and hemoperfusion (Bateman, 1987; Meredith and Vale, 1987; Proudfoot et al., 1987; Bismuth et al., 1990).

Another therapeutic approach to paraquat poisoning is to alleviate the paraquat-mediated oxidant injury by procedures which may reduce the formation of reactive oxygen species to prevent their toxic effects. Antioxidants such as superoxide dismutase, catalase, GSH, ascorbic acid, and vita-

*Corresponding author.

min E have been used in treating paraquat-exposed humans and animals with little or no success (Autor, 1974; Harley et al., 1977; Matkovits et al., 1980; Redetzki et al., 1980; Anyonymous, 1984; Bateman, 1987; Bismuth et al., 1990). The failure of antioxidants to modify the toxicodynamics of paraquat perhaps may be attributed to their inability to cross cell membrane barriers and/or their rapid clearance from cells (Turrens et al., 1984; Padmanabhan et al., 1985; Jurima-Romet et al., 1990). Recent studies, however, have demonstrated that the encapsulation of antioxidants in liposomes improves their therapeutic potential against oxidant-induced lung damage, because presumably liposomes facilitate intracellular delivery and prolong the retention time of entrapped agents inside the cell (Kimelberg and Mayhew, 1978; Poznansky and Juliano, 1984; Turrens et al., 1984; Padmanabhan et al., 1985; Jurima-Romet et al., 1990).

Liposomes are artificial microvesicles composed of phospholipid membranes alternating with aqueous compartments. Water-soluble compounds can be entrapped in the aqueous spaces and lipid-soluble compounds in the lipid bilayers of liposomes. Currently, liposomes have been used to provide a superior delivery system because they are biocompatible, biodegradeable and relatively non-toxic. As a drug delivery system, liposomes can significantly alter the pharmacokinetics and pharmacodynamics of entrapped drugs, for example, by enhancing drug uptake; delaying rapid drug clearance; and, reducing drug toxicity (Kimelberg and Mayhew, 1978; Poznansky and Juliano, 1984; Ostro, 1987; Shek et al., 1990; Schreier et al., 1993).

We have recently shown that pretreatment of rats with liposome-associated α-tocopherol can elevate the α-tocopherol content of pulmonary tissues by 16-fold (Suntres et al., 1993) and the same pretreatment also provides an excellent prophylactic effect against paraquat-induced lung injury (Suntres et al. 1992). However, the effectiveness of this formulation as a therapeutic agent for treating paraquat poisoning has not been examined. 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 (Witting, 1980; Burton and Ingold, 1989). The present study was undertaken to investigate whether liposome-associated α-tocopherol, administered intratracheally to the lungs in rats, could alleviate the progression of paraquat-induced acute lung injury. The efficacy of treatment in reducing pulmonary injury was monitored by measuring the change in enzyme activities associated with specific cell damage in the lung, angiotensin converting enzyme and alkaline phosphatase being used as indicator enzymes for pulmonary endothelial cell and alveolar type II cell damage, respectively. Changes in GSH levels and the extent of lipid peroxidation in the lung were also measured.

MATERIALS AND METHODS

Chemicals

Paraquat dichloride salt and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Dipalmitoylphosphatidylcholine (DPPC) was obtained from Avanti Polar Lipids (Alabaster, AL). All other chemicals were obtained from either Sigma Chemical Co. (St. Louis, MO) or BDH (Toronto, Ont.).

Animals

Male Sprague-Dawley rats (approximate body weight 220–250 g) were purchased from Charles River Canada, Inc. (St. Constant, Que.). 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 contained in the Guide to the Care and Use of Experimental Animals as prepared by the Canadian Council on Animal Care, and the experimental protocol was approved by the DCIEM Animal Care Committee.

Preparation of liposome-associated α-tocopherol

Liposome-associated α-tocopherol was prepared by dissolving DPPC and α-tocopherol, in a 7:3 molar ratio, in chloroform:methanol (2:1 v/v). 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 a glass vessel. The residual trace of solvent was removed by placing the vessel under vacuum for at least 1 h. The dried lipid was hydrated at 50°C with 1.0 mL of 5 mM potassium phosphate

buffer, pH 6.5, containing 3 mM EDTA, and then vortexed to form multilamellar vesicles where the highly hydrophobic α-tocopherol would partition predominantly in the phospholipid bilayers. The multilamellar vesicles were extruded (10 times) with an extruder (Lipex Biomolecules, Vancouver, B.C.) through doubly stacked Nucleopore filters of 400-nm pore size using a helium pressure of 100–200 psi. The extruded liposomes, primarily oligolamellar vesicles (Jousma et al., 1987; Hope et al., 1993), was washed twice by pelleting at 110 000 g for 1 h at 5°C in a Beckman L8–70 ultracentrifuge. The liposomes were diluted in phosphate buffer to a final α-tocopherol concentration of 2 mg/150 μL suspension. Liposomal vesicle size was determined with the use of a Coulter N4SD particle-size analyzer and was found to have a mean diameter of 385 ± 36 nm.

Treatment of animals

Paraquat dichloride (20 mg/kg, body weight) was injected intraperitoneally to rats. Twenty four hours after the administration of paraquat, the animals were anaesthetized with 50 mg/kg ketamine and 8 mg/kg xylazine. Each anaesthetized animal was intratracheally instilled with 150 μL of α-tocopherol liposomes (8 mg α-tocopherol in 31.8 mg DPPC/kg) or of an equivalent amount of plain liposomes, as described by Brain et al. (1976). This method of instillation has been shown to mediate a rather uniform distribution in the left lung (consisting of a single large lobe) but a less even distribution in the right lung (consisting of four smaller lobes) of the rat (Shek et al., 1994). Injections were administered between 0800–0900 hours. Paraquat dichloride was dissolved in saline and prepared shortly before use. Control animals received an equivalent volume of the vehicle solution.

Experimental design

To investigate whether the intratracheal instillation of liposomal α-tocopherol could alleviate the progression of pulmonary damage induced by paraquat, rats exposed to paraquat 24 h earlier were treated with α-tocopherol liposomes and killed 8, 24 or 48 h later. The antagonistic action of α-tocopherol liposomes on paraquat-induced lung injury was assessed biochemically by measuring the activities of angiotensin converting enzyme (ACE) and alkaline phosphatase (AKP), lipid peroxidation and GSH concentration in the 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° C. Following rinsing, lungs were quickly weighed and finely minced. 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. The homogenate was centrifuged at 9,000 *g* for 10 min in a refrigerated Sorvall RC-5B centrifuge. The post-mitochondrial supernatant was decanted and re-centrifuged at 105 000 *g* for 60 min in a refrigerated Beckman ultracentrifuge to obtain the cytosolic and microsomal fractions. For the measurement of lipid peroxidation, homogenates were prepared as described above except the homogenizing medium contained 3 mM ethylenediaminetetraacetic acid (EDTA).

Biochemical Analyses

Enzyme measurements

The activity of angiotensin converting enzyme (ACE) was determined using the Sigma Diagnostic procedure as described by Suntres et al. (1992). One unit of ACE activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol furylacrylloylphenylalanine per min at 37°C. Alkaline phosphatase activity was determined at 37°C and pH 9.95 as described previously. One enzymatic unit was defined as the amount of enzyme that catalyzed the formation of 1 nmol of p-nitrophenol per min (Boudreau and Nadeau, 1987). Protein determinations were estimated by the method of Lowry et al. (1951), using crystalline bovine serum albumin (BSA) as the standard.

Determination of lipid peroxidation

Microsomes from treated and control animals were assayed for the presence of lipid conjugated dienes as described by Suntres et al. (1992).

Determination of tissue reduced glutathione concentrations

Reduced glutathione, more precisely non-protein sulphydryl, concentration in pulmonary homogenates was determined as described by Suntres et al (1992).

Statistical Analysis

Data from control and treated groups 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 (Gad and Weil, 1982). The level of significance was accepted at $p < 0.05$..

RESULTS

Body and lung weights

The effect of intraperitoneally administered paraquat on body and lung weights of animals is shown in Figure 1. Exposure of animals to paraquat (20 mg/kg, ip) resulted in a significant reduction in body weight. Treatment of rats with liposomes or α-tocopherol liposomes 24 h after paraquat administration did not significantly alter the paraquat-induced reduction in body weight. Lung weights of control animals were significantly increased 32 and 48 h after paraquat administration (by 17% and 31%, respectively); thereafter, lung weights returned to control values. Treatment of animals with liposomes alone or α-tocopherol liposomes did not significantly alter the paraquat-induced changes in lung weights.

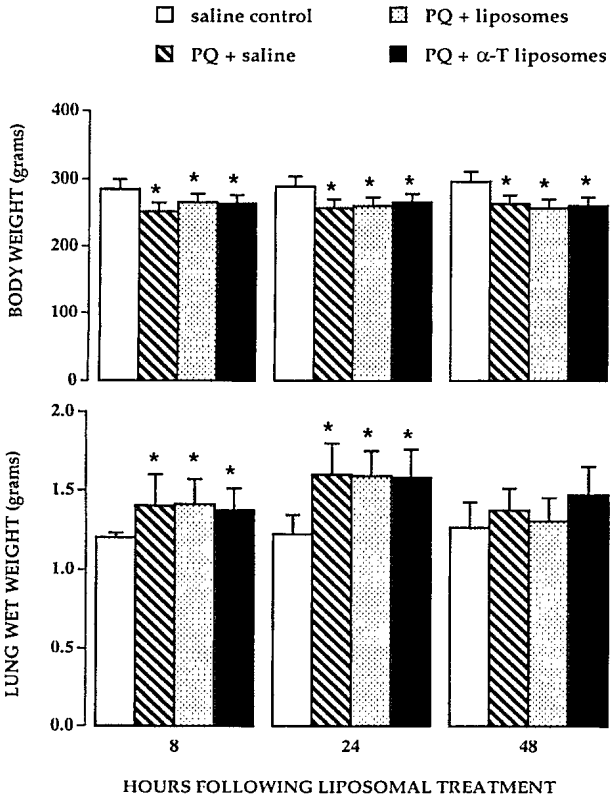

FIGURE 1. The effect of liposome or α-tocopherol liposome treatment on paraquat-induced changes in body weight (upper panel) and wet lung weight (lower panel). Liposomes or α-tocopherol (α-T) liposomes were intratracheally instilled into the lungs of rats 24 h after exposure to paraquat (PQ, 20 mg/kg, ip) as described in Materials and Methods. Each data point represents the mean ± SEM of 5 animals, and each asterisk denotes a statistically significant ($p < 0.05$) difference compared with the mean value of the saline control group.

Pulmonary enzyme activities

Since activities of angiotensin converting enzyme (ACE) and alkaline phosphatase (AKP), enzymes localized primarily in capillary endothelial cells and alveolar type II epithelial cells, respectively, have been used as markers of lung injury (Lazo et al., 1986; Boudreau and Nadeau, 1987), the effect of paraquat on the activities of these enzymes in lung tissue was also measured. As shown in Figure 2, paraquat administration produced a significant decrease in ACE (by 31%, 41% and 50% at 8, 24 and 48 h after intratracheal instillation of saline, respectively) and AKP (by 24% and 33% at 24 and 48 h after intratracheal instillation of saline, respectively) activities in the lungs of control animals. The reduction in ACE and AKP activities in the lungs of paraquat-exposed animals treated with liposomes were similar to those observed in the paraquat-exposed control rats. In contrast, a decrease in the rate of paraquat-induced reduction in enzyme activities was observed in the lungs of animals treated with α-tocopherol liposomes. More precisely, the paraquat-induced reduction in ACE activity was 25%, 30% and 32% at 8, 24 and 48 h, respectively, and in AKP activity was 18% and 23% at 24 and 48 h, respectively, after the intratracheal instillation of α-tocopherol liposomes.

Lipid peroxidation and GSH concentration

It has been postulated that pulmonary toxicity induced by paraquat is due to membrane lipid peroxidation (Heath and Smith, 1977; Pasi, 1978; Bus and Gibson, 1984). Therefore, in the present study, the levels of lipid peroxidation in microsomes of saline- and α-tocopherol-treated animals were also measured. As shown in Figure 3, paraquat produced a time-dependent increase in lipid peroxidation levels in microsomal fractions isolated from lungs of control animals as measured by the forma-

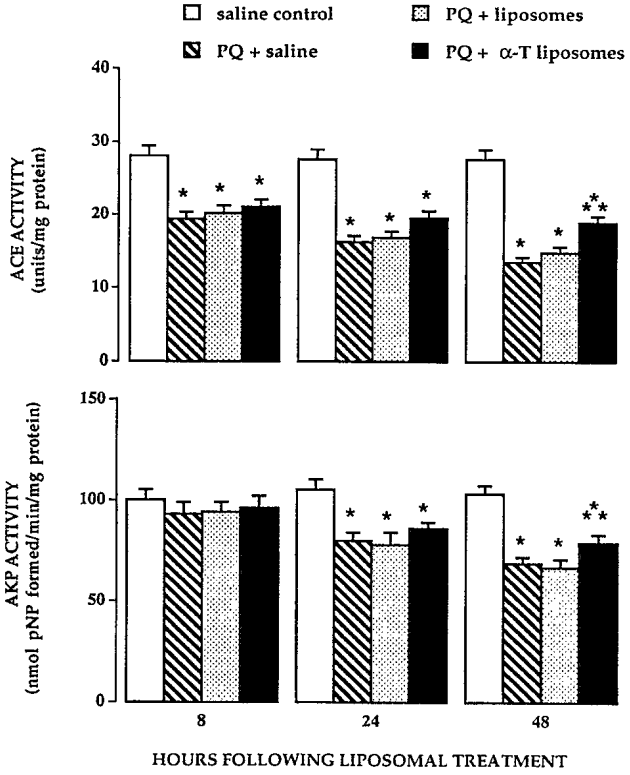

FIGURE 2. The effect of liposome or α-tocopherol liposome treatment on paraquat-induced changes in angiotensin converting enzyme (ACE, upper panel) and alkaline phosphatase (AKP, lower panel) activities. Liposomes or α-tocopherol (α-T) liposomes were intratracheally instilled into the lungs of rats 24 h after exposure to paraquat (PQ, 20 mg/kg, ip) as described in Materials and Methods. Each data point represents the mean ± SEM of 5 animals. Each single asterisk (*) denotes a significant difference ($p < 0.05$) compared with the mean value of the control group injected with saline; a double asterisk (**) denotes a significant difference ($p < 0.05$) compared with the mean value of the group of animals injected with either paraquat and saline or paraquat and liposomes.

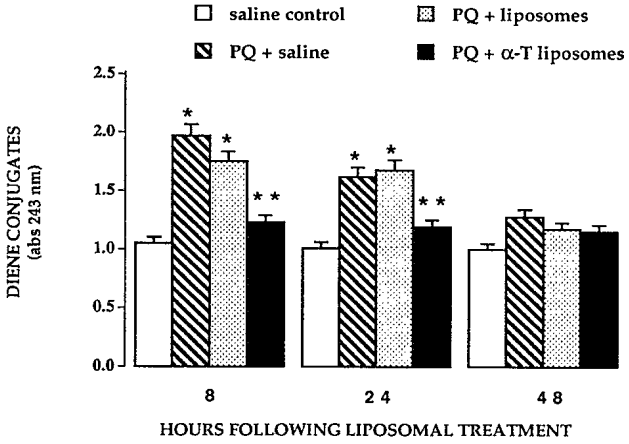

FIGURE 3. Paraquat-induced microsomal lipid peroxidation in rat lungs. Liposomes or α-tocopherol (α-T) liposomes were intratracheally instilled into the lungs of rats 24 h after exposure to paraquat (PQ, 20 mg/kg, ip) as described in Materials and Methods. Each data point represents the mean ± SEM of 5 animals. Each single asterisk (*) denotes a significant difference ($p < 0.05$) compared with the mean value of the control group injected with saline; a double asterisk (**) denotes a significant difference ($p < 0.05$) compared with the mean value of the group of animals injected with either paraquat and saline or paraquat and liposomes.

tion of diene conjugates. Treatment of rats with liposomes alone 24 h after administration of paraquat did not significantly alter the paraquat-induced membrane lipid peroxidation. On the other hand, treatment of rats with α-tocopherol liposomes abolished the progression of paraquat-induced lipid peroxidation.

Since GSH is known to play an important role in protecting cells from oxidant-induced tissue injury (Reed and Fariss, 1984), levels of glutathione in the lungs of animals were also measured. The results presented in Figure 4 revealed that GSH levels in lungs of animals exposed to paraquat were significantly decreased in a time-dependent manner. Similarly, a comparable reduction in glutathione levels was observed in animals treated with liposomes 24 h after paraquat administration. In contrast, the GSH levels in lungs of rats treated with α-tocopherol liposomes were higher than those observed in paraquat-treated controls.

DISCUSSION

Therapy of paraquat-induced lung injury with antioxidants has been the subject of research for some time but so far, it has remained ineffective. It has been reported that intrapulmonary administration of superoxide dismutase into the lungs of patients did not ameliorate the symptoms of paraquat poisoning. Manipulation of dietary ascorbate or selenium levels failed to produce a significant alteration in the toxicity of paraquat. Also, administration of deferoxamine to rats seemed to increase paraquat toxicity with an apparent aggravation of pulmonary fibrosis in surviving animals, while acute treatment with vitamin E in normal rats did not protect against acute paraquat poisoning (Matkovits et al., 1980; Anonymous, 1984; Bateman, 1987; Bismuth et al., 1990). The failure of these antioxidants in suppressing the paraquat-induced toxicity cannot be explained at the present time;

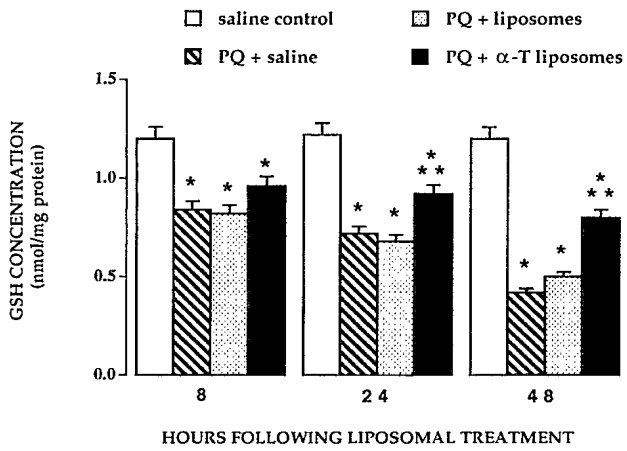

FIGURE 4. Paraquat-induced oxidation of GSH in rat lungs. Liposomes or α-tocopherol (α-T) liposomes were intratracheally instilled into the lungs of rats 24 h after exposure to paraquat (PQ, 20 mg/kg, ip) as described in Materials and Methods. Each data point represents the mean ± SEM of 5 animals. Each single asterisk (*) denotes a significant difference ($p < 0.05$) compared with the mean value of the control group injected with saline; a double asterisk (**) denotes a significant difference ($p < 0.05$) compared with the mean value of the group of animals injected with either paraquat and saline or paraquat and liposomes.

however, part of the explanation could be due to their inability to cross membrane barriers, their rapid clearance from cells, or their poor uptake by target tissues (Turrens et al., 1984; Padmanabhan et al., 1985; Jurima-Romet et al., 1990).

In the present study, direct delivery of liposome-associated α-tocopherol to the lungs of animals was found effective in lessening the lung injury induced by paraquat. This observation was evidenced by the effectiveness of the administered α-tocopherol in retarding the paraquat-induced membrane lipid peroxidation; in suppressing the rate of decrease in ACE and AKP enzyme activities; and, in reducing GSH oxidation. Delivery of liposome-encapsulated antioxidants to biological systems has been shown to enhance their therapeutic potential against oxidant-induced tissue injury by facilitating the transfer of liposomal agents inside the cell and also by extending their intracellular residence time (Kimelberg and Mayhew, 1978; Posnansky and Juliano, 1984; Turrens et al., 1984; Padmanabhan et al., 1985; Ostro, 1987; Jurima-Romet et al., 1990; Shek et al., 1990; Schreier et al., 1993). Indeed, intratracheally administered liposomal α-tocopherol has been shown to be associated with subcellular fractions (Suntres et al., 1993) and the half-life of the liposomal antioxidant in the lung can be as long as 6 days (unpublished data).

In contrast to the results observed in this study, it has been demonstrated that α-tocopherol administered by the oral or parenteral routes to animals failed to ameliorate paraquat-induced lung injuries (Redetzki et al., 1980; Anonymous, 1984; Meredith and Vale, 1987). To produce a therapeutic effect, a drug must reach its site of action in a sufficient concentration. The inconsistencies observed among the different studies may be primarily due to a difference in the α-tocopherol concentrations attained in the lung. The confounding treatment effects could be explained by differences in the route of administration and the dosage form of α-tocopherol used. It has been shown that the levels of the antioxidant accumulated in the lung of rats after oral or parenteral administration is low (13–36 μg/g lung), a treatment effect possibly attributable to the slow absorption of the antioxidant and also its predominantly extrapulmonary distribution (Gallo-Torres, 1980; Knight and Roberts, 1985). In our study, the intratracheal instillation of liposomal α-tocopherol resulted in a much higher level of the antioxidant delivered to the lung (approximately 1 mg/g lung tissue). It is reasonable to expect that the attainment of such a high level of α-tocopherol in the lung should be sufficient to augment pulmonary cellular defence and protect against paraquat-induced cellular injury.

The exact mechanism(s) by which α-tocopherol inhibits some of the toxic effects of paraquat are not entirely clear. However, considering the evidence presented in this and other studies, namely that paraquat induces membrane lipid peroxidation and lipid peroxidation is a major mechanism of oxidant-induced tissue damage (Girotti, 1985), it is likely that α-tocopherol confers protection by suppressing membrane lipid peroxidation (Figure 3). The ability of α-tocopherol to retard the formation and/or accumulation of lipid peroxides, products of peroxidized membranes known to participate in the initiation and propagation stages of lipid peroxidation (Girotti, 1985), is also consistent with our finding that α-tocopherol suppressed the paraquat-induced oxidation of GSH, which serves as a substrate in the GSH peroxidase/GSH reductase system to detoxify lipid peroxides. Results from other studies have suggested that α-tocopherol may exert its antioxidant effects by acting as a free radical scavenger or by serving as a structural component in membranes, rendering membrane poly-unsaturated fatty acids more resistant to peroxidation (Witting, 1980; Burton and Ingold, 1989).

It has been shown that paraquat can injure surfactant-producing alveolar type II epithelial cells and capillary endothelial cells (Smith and Heath, 1976; Boudreau and Nadeau, 1987; Bus and Gibson, 1984), which act as the air-blood barrier. Damage to the air-blood barrier and impairment of surfactant production in the lung can cause transudation into the alveoli, causing pulmonary edema and collapse of the fine airways (VanGolde et al, 1988). In our study, administration of α-tocopherol liposomes or liposomes alone failed to modify the paraquat-induced edema. The lack of protective effect by α-tocopherol against paraquat-induced lung edema cannot be ascertained at the present time, but other investigators have suggested that paraquat-induced edema may be due to mechanisms other than direct damage of pulmonary capillary endothelial cells (Gardiner, 1972; Smith and Heath, 1976; Suntres et al, 1992). This suggestion corroborates with the results of this study where pulmonary edema persisted despite apparent reduction in endothelial and type II epithelial cell damage (Figure 2). Although the paraquat-induced lung injury was not monitored for an extended period of time in this study, it is reasonable to expect that α-tocopherol liposomes may promote the alveolar type II epithelial cells and endothelial cells to proliferate to normalcy at faster rates, thus allowing a faster recovery of the tissue.

The most important prognostic factor in paraquat poisoning is the plasma concentration of the herbicide and symptoms of poisoning usually become evident 24 h following paraquat ingestion, when aggressive management of the poisoning must be undertaken to prevent a possibly fatal outcome (Bismuth et al, 1982). The type of therapy and time interval between ingestion of paraquat and initiation of therapy are critical determinants that have to be appraised in order to offer a favourable outcome. Since the toxic effects of paraquat in the lung are more obvious, and in most cases responsible for the fatal outcome, interventions directed towards preventing the accumulation of paraquat in the pulmonary cells have been employed (Bateman, 1987; Bismuth et al., 1990). However, studies have shown that successful treatment of paraquat poisoning does not depend on the modification of toxicokinetics; secondary biochemical changes, such as membrane lipid peroxidation and depletion in reducing equivalents, are probably responsible for its delayed pulmonary toxicity and fatal outcome. In this study, the administration of α-tocopherol as a liposomal formulation 24 h after paraquat exposure, suppressed the progression of pulmonary damage, suggesting that α-tocopherol liposomes may be of beneficial significance in the management of paraquat-induced lung damage.

In summary, results of the present study have shown that liposomal α-tocopherol, administered to rats intratracheally, significantly improves the recovery of the lung from paraquat-induced toxic effects. This study strongly suggests that intratracheal instillation of α-tocopherol or other antioxidants may well be the preferred route of administration for achieving immediate augmentation of the lung antioxidant system, required in emergency situations such as paraquat poisoning. In addition, the incorporation of antioxidants within liposomes may also increase their intracellular delivery to lung cells and enhance their protective effects against intracellular oxygen mediated damage.

ACKNOWLEDGEMENTS

The authors wish to thank Shelley Hepworth for technical assistance and Tom Brown for graphic preparations.

References

Anonymous (1984) Effects of vitamin E and selenium on the toxicity of paraquat. *Nutr. Rev.* **42**, 260–262.

Autor, P.N. (1974) Reduction of paraquat toxicity by superoxide dismutase. *Life Sci.* **14**, 1309–1319.

Bateman, D.N. (1987) Pharmacological treatments of paraquat poisoning. *Hum. Toxicol.* **6**, 57–62.

Bismuth, C., Garnier, R., Baud, F.F., Muszynski, J., Keyes, C. (1990) Paraquat poisoning. An overview of the current status. *Drug Safety* **5**, 243–251.

Boudreau, J., Nadeau, D. (1987) Lung hydrolases in paraquat poisoning: early response of alkaline phosphatase. *J. Toxicol. Environm. Health* **22**, 329–340.

Brain, I.D., Knudson, D.E., Sorokin, S.P., Davis, M.A. (1976) Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. *Environ. Res.* **11**, 13–33.

Burton, G.W., Ingold, K.U. (1989) Vitamin E as an in vitro and in vivo antioxidant. *Ann. NY Acad. Sci.* **570**, 7–22.

Bus, J.S., Gibson, J.E. (1984) Paraquat: Model for oxidant-initiated toxicity. *Environm. Health Perspect.* **55**, 37–46.

Bismuth, Ç., Garnier, R., Dally, S., Fournier, P.E., Scherrmann, J.M. (1982) Prognosis and treatment of paraquat poisoning: a review of 28 cases. *J. Toxicol. Clin. Toxicol.* **19**, 461–474.

Gad, S.C., Weil, C.S. (1982) Statistics for toxicologists. In *Principles and Methods of Toxicology*. A.W. Hayes (ed). Raven Press: New York, pp. 273–319.

Gallo-Torres, H.E. (1980) Transport and metabolism. In *Vitamin E: A comprehensive treatise*. L.J. Machlin (ed). Mercell Dekker: New York, pp 193–267.

Gardiner, A.J.S. (1972) Pulmonary oedema in paraquat poisoning. *Thorax*, **27**, 132–135.

Girotti, A.W. (1985) Mechanisms of lipid peroxidation. *J. Free Rad. Biol. Med.* **1**, 87–95.

Harley, J.B., Grinspan, S., Root, R.K. (1977) Paraquat suicide in a young woman: results of therapy directed against the superoxide radical. *Yale J. Biol. Med.* **50**, 481–488.

Heath, D., Smith, P. (1977) The pathology of the lung in paraquat poisoning. In *Biochemical Mechanisms of Paraquat Toxicity*. A.P. Autor (ed) Academic Press: New York, pp. 39–51.

Hope, M.J., Nayar, R., Mayer, L.D., Cullis, P.R. (1993) Reduction of liposome size and preparation of unilamellar vesicles by extrusion techniques. In *Liposome Technology: Liposomes Preparation and Related Techniques*, Vol. I. G. Gregoriadis (ed). CRC Press: Boca Raton, pp. 123–139.

Jousma, H., Talsma, H., Spies, F., Joosten, J.G.H., Junginger, H.E., Crommelin, D.J.A. (1987) Characterization of liposomes. The influence of extrusion of multilamellar vesicles through polycarbonate membrane on particle size, particle size distribution and number of bilayers. *Int. J. Pharmaceut.* **35**, 263–274.

Jurima-Romet, M., Barber, R.F., Demeester, J., Shek, P.N. (1990) Distribution studies of liposome-encapsulated glutathione administered to the lung. *Int. J. Pharmaceut.* **63**, 227–235.

Kimelberg, H.K., Mayhew, E.G. (1978) Properties and biological effects of liposomes and their uses in pharmacology and toxicology. *CRC Crit. Rev. Toxicol.* **96**, 25–79.

Knight, M.E., Roberts, R.J. (1985) Tissue vitamin E levels in newborn rabbits after pharmacologic dosing: influence of dose, dosage form and route of administration. *Dev. Pharmacol. Ther.* **8**, 96–106.

Lazo, J.S., Lynch, T.J., McCollister, J. (1986) Bleomycin inhibition of angiotensin converting enzyme activity from serum, lungs and cultured pulmonary artery endothelial cells. *Am. Rev. Resp. Dis.* **134**, 73–78.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Matkovits, B., Barabas, K., Szabo, L., Berencsi, G. (1980) In vivo study of the mechanism of protective effects of ascorbic acid and reduced glutathione in paraquat poisoning. *Gen. Pharmacol.* **11**, 455–461.

Meredith, T.J., Vale, J.A. (1987) Treatment of paraquat poisoning in man: methods to prevent absorption. *Hum. Toxicol.* **6**, 49–55.

Ostro, M.J. (1987) Liposomes. *Sci. Am.* **256**, 103–111.

Padmanabhan, R.V., Gudapaty, R., Liener, I.E., Schwartz, B.A., Hoidal, J.R. (1985) Protection against pulmonary oxygen toxicity in rats by the intratracheal administration of liposome-encapsulated superoxide dismutase or catalase. *Am. Rev. Resp. Dis.* **132**, 164–167.

Pasi, A. (1978) *The toxicity of paraquat, diquat and morfamquat*. Hans Huber Publishers: Bern.

Poznansky, M.J., Juliano, R.L. (1984) Biological approaches to the controlled delivery of drugs: a critical review. *Pharmacol. Rev.* **36**, 277–336.

Proudfoot, A.T., Prescott, L.F., Jarvie, D.R. (1987). Haemodialysis for paraquat poisoning. *Hum. Toxicol* **6**, 69–74.

Redetzki, H.M. Wood, C.D., Grafton, W.D. (1980) Vitamin E and paraquat poisoning. *Vet. Hum. Toxicol.* **22**, 395–397.

Reed, D.J., Fariss, M.W. (1984) Glutathione depletion and susceptibility. *Pharmacol. Rev.* **36**, 25S–33S.

Schreier, H., Gonzalez-Rothi, R.J., Stecenko, A.A. (1993) Pulmonary delivery of liposomes. *J. Controlled Release* **24**, 209–223.

Shek, P.N., Barber, R.F., Jurima-Romet, M. (1990) Liposomes for pulmonary applications. *Modern Med. Canada* **45**, 233–239.

Shek, P.N., Suntres, Z.E., Brooks, J.I. (1994) Liposomes in pulmonary applications: physicochemical considerations, pulmonary distribution and antioxidant delivery. *J. Drug Targeting* (in press).

Smith, P., Heath, D. (1976) Paraquat. *CRC Crit. Rev. Toxicol.* **4**, 411–445.

Suntres, Z.E., Hepworth, S.R., Shek, P.N. (1992) Protective effect of liposome-associated α-tocopherol against paraquat-induced acute lung toxicity. *Biochem. Pharmacol.* **44**, 1811–1818.

Suntres, Z.E., Hepworth, S.R., Shek, P.N. (1993) Pulmonary uptake of liposome-associated α-tocopherol following intratracheal instillation in rats. *J. Pharm. Pharmacol.* **45**, 514–520.

Turrens, J.F., Crapo, J.D., Freeman, B.A. (1984) Protection against oxygen toxicity by the intravenous injection of liposome-entrapped catalase and superoxide dismutase. *J. Clin. Inv.* **73**, 87–95.

Vale, J.A., Meredith, T.J., Buckley, B.M. (1987) Paraquat poisoning: clinical features and immediate general management. *Hum. Toxicol.* **6**, 41–47.

VanGolde, L.M.G., Batenburg, J.J., Robertson, B. (1988) The pulmonary surfactant system: biochemical aspects and functional significance. *Physiol. Rev.* **2**, 374–455.

Witting, L.A. (1980) Vitamin E and lipid antioxidants in free-radical-initiated reactions. In *Free Radicals in Biology*, Vol. IV, W.A. Pryor (ed), Academic Press: New York, pp. 295–319.