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PROTECTIVE EFFECT OF LIPOSOME-ASSOCIATED α-TOCOPHEROL AGAINST PARAQUAT-INDUCED ACUTE LUNG TOXICITY

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Abstract—The present study was undertaken to investigate whether α-tocopherol, entrapped in liposomes and delivered directly to the lung, could protect against paraquat-induced lung damage in the rat. Plain liposomes (composed of dipalmitoylphosphatidylcholine, DPPC) or DPPC/α-tocopherol liposomes were administered intratracheally to animals 24 hr prior to an intraperitoneal injection of paraquat (20 mg/kg); rats were killed 24 or 48 hr after paraquat treatment. Results of this study showed that lungs of animals treated with paraquat were damaged extensively as evidenced by an increase in lung weight and a significant reduction in lung angiotensin-converting enzyme (ACE) activity and cytochrome P450 concentration. Furthermore, paraquat treatment resulted in a significant decrease in reduced glutathione (GSH) concentrations and a marked elevation in microsomal lipid peroxidation levels as measured by the formation of diene conjugates. Pretreatment of rats with DPPC liposomes alone did not alter significantly the paraquat-induced changes of all parameters examined. On the other hand, pretreatment of rats with DPPC/α-tocopherol liposomes 24 hr prior to paraquat challenge resulted in a significant increase in pulmonary α-tocopherol concentrations and antagonized paraquat-induced changes in lipid peroxidation, GSH/GSSG ratio, lung ACE activity and cytochrome P450 concentrations. Results of this study suggested that α-tocopherol, delivered directly to the lung in a liposomal formulation 24 hr prior to paraquat administration, confers protection against paraquat-induced lung damage.

Paraquat (1,1-dimethyl-4,4 bipyridylium dichloride) is a broad-spectrum bipyridylium herbicide, known to cause lethal toxicity in humans and animals [1, 2]. In the past two decades, hundreds of cases of accidental or suicidal fatalities from paraquat poisoning have been reported [1, 3]. Paraquat, irrespective of its route of entry into biological systems, whether oral, parenteral, dermal, or by inhalation, is known to preferentially damage the lung. The pulmonary damage is manifested by edema, hemorrhage, interstitial inflammation and proliferation of bronchial epithelium [1, 2, 4]. In most instances, death is caused by pulmonary impairment and characteristically occurs several days to 3 weeks after exposure to paraquat [1–3].

The pulmonary toxicity of paraquat is well documented, but the exact mechanism(s) by which paraquat causes cell damage is not well understood. It is generally agreed that a cyclic single electron reduction/oxidation (redox cycling) of paraquat is a critical mechanistic event, resulting in the generation of reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical. Some investigators have suggested that paraquat produces its toxic effects by inducing membrane lipid peroxidation mediated by superoxide anion. Others have argued that redox cycling of paraquat in biological systems causes depletion of cellular reducing equivalents necessary for normal cellular functions, such as cellular energy metabolism and antioxidant defence mechanisms [1–4].

The effective management of paraquat poisoning is well known to be rather difficult. Although a wide range of treatments, such as induced vomiting, gastric lavage, adsorption of the herbicide, hemodialysis, peritoneal dialysis, immunosuppressive therapy and several other pharmacological interventions can be implemented [1, 5–7], unfortunately they have been shown to be generally ineffective and the actual fatality rate has been reported to be relatively high [1, 7]. Recognizing the fact that paraquat is a strong redox agent and contributes to the formation of reactive oxygen species, attempts have been made to explore pharmacological strategies which may reduce the formation of these reactive oxygen species and/or prevent their toxic effects. Accordingly, antioxidants such as superoxide dismutase, catalase, reduced glutathione (GSH)†, ascorbic acid and vitamin E have been tested in paraquat-exposed humans and animals as a pharmacological treatment [7–10]. Results from these studies have shown that these antioxidants are not able to eliminate the *in vivo* toxic actions of paraquat, an observation perhaps attributable to their inability to cross cell membrane barriers and/or their rapid clearance from cells [11–13]. Recent studies, however, have demonstrated that the encapsulation of antioxidants

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† Abbreviations: ACE, angiotensin-converting enzyme; DPPC, dipalmitoylphosphatidylcholine; GSH, reduced glutathione; and GSSG, oxidized glutathione.

in liposomes may promote their therapeutic potential against oxidant-induced lung damage, presumably by the abilities of liposomes to facilitate the intracellular uptake of macromolecules and to extend the half-lives of the same macromolecules [11–15].

α-Tocopherol, the main constituent of vitamin E, is known to be an important component of biological membranes, contributing to membrane stabilization and functioning as a free radical scavenger to prevent lipid peroxidation by quenching singlet molecular oxygen [16, 17]. α-Tocopherol has antioxidant properties and has been investigated in a number of pathological conditions including arthritis, cancer, ageing and cataracts [18]. For the treatment of acute paraquat-induced lung damage, α-tocopherol has been shown by most investigators not to be an ideal therapeutic agent [9, 19, 20]. It may be possible, however, that the level of α-tocopherol reaching the lung tissue, following its oral or parenteral administration, is relatively low. The agent is slowly absorbed and widely distributed, with low concentrations being achieved in the lung [21].

The present study was undertaken to investigate whether a liposomal formulation of α-tocopherol, delivered directly to the lung by intratracheal instillation, would protect against paraquat-induced lung injury in rats. Liposomes were used as the carrier system for α-tocopherol delivery, because α-tocopherol is extremely insoluble and in its free form is too viscous for intratracheal administration. Our attempts and those of others to solubilize the antioxidant using different solvents, such as Tween 80, propylene glycol and dimethyl sulfoxide, proved not to be useful because the resulting preparations were found to be toxic for lung and other tissues [22, 23]. Our results of a separate study (accepted for publication) have also shown that the intratracheal administration of liposome-associated α-tocopherol can result in a substantial increase (16-fold) of the antioxidant delivered to the lung. In this report, the protective effect of dipalmitoylphosphatidylcholine (DPPC)/α-tocopherol liposomes against paraquat-induced lung toxicity was assessed biochemically by measuring membrane lipid peroxidation levels, angiotensin-converting enzyme (ACE) activities, and glutathione and cytochrome P450 concentrations.

MATERIALS AND METHODS

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

Animals. Male Sprague–Dawley rats (approximate body weight 220–250 g) were purchased from Charles River Canada Inc. (St. Constant, Quebec). 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 research were cared for in accordance with guidelines recommended by the Canadian Council on Animal Care in the *Guide to the Care and Use of Experimental Animals.*

Preparation of liposome-associated α-tocopherol. Liposome-associated α-tocopherol was prepared from a mixture of DPPC 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°, under a stream of helium, to a thin film coating the interior surface of a 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 ten times with an extruder (Lipex Biomolecules, Vancouver, BC) through two stacked polycarbonate filters of 400-nm pore size, under a helium pressure of about 150 lb/in^2. The extruded vesicles displayed a more homogenous size distribution. Free α-tocopherol was removed by washing the liposomes twice in 5 mM potassium phosphate buffer, pH 6.5, and pelleting at 110,000 *g* for 1 hr at 5° in a Beckman L8-70 ultracentrifuge. The vesicle size distribution was not affected by ultracentrifugation. The liposomes were diluted with 5 mM potassium phosphate buffer, pH 6.5, to yield a final α-tocopherol concentration of 2 mg/150 μL suspension.

Treatment of animals. DPPC/α-tocopherol liposomes (2 mg α-tocopherol/animal) or DPPC liposomes alone were intratracheally instilled into the lungs of rats as described by Brain *et al.* [24]. Twenty-four hours after the administration of liposomal preparations, the animals were injected intraperitoneally with a single dose of paraquat dichloride (20 mg/kg) to induce pulmonary toxicity. Injections were administered between 8:00 and 9:00 a.m. Paraquat dichloride was dissolved in physiological saline and prepared shortly before use. Control animals received an equivalent volume of the vehicle solution.

Experimental design. To investigate whether α-tocopherol, instilled intratracheally into the lung, can confer protection against the acute pulmonary toxic effects of paraquat, rats pretreated 24 hr earlier with DPPC/α-tocopherol liposomes were given a single dose of paraquat and killed 0, 24 or 48 hr later. The protective effect of DPPC/α-tocopherol against paraquat-induced lung damage was assessed biochemically by measuring the activity of ACE, lipid peroxidation, and GSH and microsomal cytochrome P450 concentrations.

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, the 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 9000 *g* for 10 min in a refrigerated Sorvall RC-5B centrifuge. The post-mitochondrial supernatant was decanted and recentrifuged at 105,000 *g* for 60 min

in a refrigerated Beckman L8-70 ultracentrifuge to obtain the cytosolic and microsomal fractions. For the measurement of lipid peroxidation, lung tissue homogenates were prepared as described above except that the homogenizing medium contained 3 mM EDTA.

Enzyme measurements. The activity of ACE was determined using the Sigma Diagnostic procedure described by Suntres and Shek [25]. One unit of ACE activity was defined as the amount of enzyme which catalyzed the formation of 1 μmol furylacrylloylphenylalanine/min at 37°.

Protein determinations. Protein determinations were estimated by the method of Lowry *et al.* [26], using crystalline bovine serum albumin as the standard.

Determination of lipid peroxidation. Microsomes obtained from lung tissues of treated and control animals were assayed for the presence of lipid conjugated dienes as described by Suntres and Shek [25]. Briefly, 1 mL of microsomal suspension (10.1 ± 1.9 μg protein/mL) was added to 5 mL of chloroform:methanol (2:1) mixture, mixed, and centrifuged at 3000 rpm for 5 min. The lower layer of chloroform was transferred to a test tube and dried in a water-bath (40°) under a stream of argon. The resultant lipid residue was dissolved in 1.5 mL of cyclohexane, and the absorbance was determined at 243 nm.

Determination of reduced and total glutathione concentrations. Reduced (GSH) and total [GSH and oxidized glutathione (GSSG)] glutathione, more precisely non-protein sulfhydryl, concentrations in lung homogenates were determined as described by Suntres and Shek [25]. Briefly, the tissue was homogenized in 20% (w/v) trichloroacetic acid and centrifuged at 10,000 rpm for 20 min in a refrigerated Sorvall RC-5B centrifuge. To determine GSH concentration in the tissue, an aliquot of the deproteinized supernatant fraction was added to 2 mL of 0.3 M Na_2HPO_4 solution followed by the addition of 0.5 mL of 0.04%, 5,5-dithiobis-[2-nitrobenzoic acid] dissolved in 10% sodium citrate. The absorbance at 412 nm was measured immediately after mixing and the GSH values were determined by extrapolation from a standard curve. To determine total glutathione concentration, an aliquot of the deproteinized supernatant fraction was mixed with 1 mL of 5% sodium borohydride ($NaBH_4$), a reducing agent, and then incubated at 45° for 60 min. The mixture was neutralized with 0.5 mL of 2.7 N HCl and the resulting sulfhydryl groups of GSH were assayed as described above.

Determination of tissue cytochrome P450 *concentration.* Microsomal cytochrome P450 concentration was determined by measuring the differential carbon monoxide-binding spectra of reduced cytochrome P450 [27].

Statistical analysis. Data from control, liposome-treated and α-tocopherol/liposome-treated animal groups were analyzed 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 control groups [28]. The level of significance was accepted at $P < 0.05$.

RESULTS

Whole body and wet lung weights. Treatment of control animals with paraquat (20 mg/kg) resulted in a significant decrease of about 17% in body weight 48 hr post-treatment (Table 1). A similar decrease in body weight was also observed in each of the two other groups of rats pretreated with DPPC liposomes and DPPC/α-tocopherol liposomes, respectively, 24 hr prior to the paraquat challenge. Lung weights of control animals treated with paraquat were increased significantly by 43%, 48 hr after treatment (Table 2). Pretreatment of animals with DPPC liposomes or DPPC/α-tocopherol liposomes failed to alter the paraquat-induced increase in lung weight.

Lung ACE activity and cytochrome P450 *concentrations.* Since ACE, localized primarily in capillary endothelial cells, has been used as a marker of lung injury [29, 30], the effect of paraquat on the activities of this enzyme in the lung was determined. As shown in Fig. 1A, paraquat treatment produced a very significant decrease (32% at 24 hr and 42% at 48 hr) in lung ACE activity among control animals. The reduction of ACE activity in the lungs of rats treated with DPPC liposomes was practically identical to that of control animals challenged with

Table 1. Effects of liposome and α-tocopherol-liposome pretreatment on paraquat-induced changes in body weight*

Pretreatment	PQ challenge†	Body weight (g)	
		24 hr	48 hr
Saline	−	302 ± 12	305 ± 10
Saline	+	283 ± 10	254 ± 16‡
DPPC liposomes	+	279 ± 13	273 ± 11‡
DPPC/α-Tocopherol liposomes	+	276 ± 16	254 ± 14‡

* Animals (N = 5 per group) were pretreated as indicated, 24 hr prior to paraquat challenge. Values are means ± SEM.

† Paraquat (PQ) was administered to rats intraperitoneally at a dose of 20 mg/kg and animals were killed 24 or 48 hr later.

‡ Significantly different ($P < 0.05$) from the value of control animals, pretreated with saline but without paraquat challenge.

Table 2. Effects of liposome and α-tocopherol-liposome pretreatment on paraquat-induced changes in lung weight*

Pretreatment	PQ challenge†	Lung weight (g) 24 hr	Lung weight (g) 48 hr
Saline	−	1.24 ± 0.21	1.26 ± 0.12
Saline	+	1.26 ± 0.13	1.80 ± 0.20‡
DPPC liposomes	+	1.39 ± 0.18	1.84 ± 0.18‡
DPPC/α-Tocopherol liposomes	+	1.35 ± 0.14	1.79 ± 0.22‡

* Animals (N = 5 per group) were pretreated as indicated, 24 hr prior to paraquat challenge. Values are means ± SEM.

† Paraquat (PQ) was administered to rats intraperitoneally at a dose of 20 mg/kg and animals were killed 24 or 48 hr later.

‡ Significantly different ($P < 0.05$) from the value of control animals, pretreated with saline but without paraquat challenge.

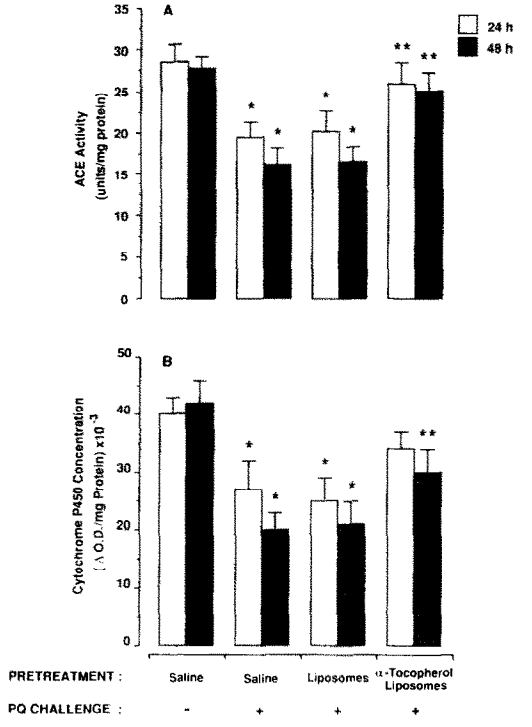

Fig. 1. Effect of pretreatment with liposomal preparations on paraquat-induced changes in ACE activity (A) and cytochrome P450 concentration (B). Animals were instilled intratracheally with either saline, DPPC liposomes or DPPC/α-tocopherol liposomes, and 24 hr later they were all challenged intraperitoneally with paraquat (PQ) at a dose of 20 mg/kg. Control animals were also pretreated intratracheally with saline but they were not challenged with PQ. Each vertical bar represents the mean ± SEM of five animals. Each single asterisk (*) indicates a significant difference ($P < 0.05$) between the mean and the corresponding mean value of control animals pretreated with saline, but not challenged with PQ. Each double asterisk (**) indicates a significant difference ($P < 0.05$) between the mean and the corresponding time-matched mean value of saline-pretreated and PQ-challenged animals.

paraquat. In contrast, pretreatment of rats with DPPC/α-tocopherol liposomes was found to provide substantial protection of the lung in terms of a significant reduction of the paraquat-induced decrease in ACE activity (9% at 24 hr and 10% at 48 hr) when compared to non-paraquat-treated rats.

Paraquat-induced lung damage in control rats was also accompanied by reduced cytochrome P450 concentrations (Fig. 1B). The reduction was evidenced by a significant decrease of the enzyme activity by 34 and 52%, respectively, 24 and 48 hr after paraquat administration. Although pretreatment of rats with DPPC liposomes did not alter the paraquat-induced decrease in cytochrome P450 concentrations, the administration of DPPC/α-tocopherol liposomes 24 hr prior to paraquat injection resulted in a partial protective effect.

Lipid peroxidation. It has been postulated that pulmonary toxicity induced by paraquat is due to membrane lipid peroxidation [1, 4]. Therefore, in this study, the extent of microsomal lipid peroxidation in the lung was measured. It can be seen from Fig. 2 that paraquat produced a time-dependent increase in lipid peroxidation levels, as measured by the formation of diene conjugates, in the lungs of control animals. Pretreatment of rats with DPPC liposomes 24 hr prior to paraquat challenge did not alter significantly the paraquat-induced lipid peroxidation. On the other hand, pretreatment of rats with DPPC/α-tocopherol liposomes prevented the paraquat-induced lipid peroxidation.

Lung GSH and GSSG concentrations. Since GSH is known to play an important role in protecting cells from oxidant-induced tissue injury, levels of GSH as well as GSSG in the lung were measured in this study. As shown in Fig. 3, lung GSH levels of paraquat-treated rats were significantly lower than those of control animals, both 24 and 48 hr post-treatment. A very similar decrease in GSH levels was also observed in rats pretreated with DPPC liposomes 24 hr prior to paraquat injection. In contrast, no significant decrease in GSH concentration was observed in the lungs of animals pretreated with DPPC/α-tocopherol liposomes. Among all the animals examined in this study, any observed decrease in GSH concentration was

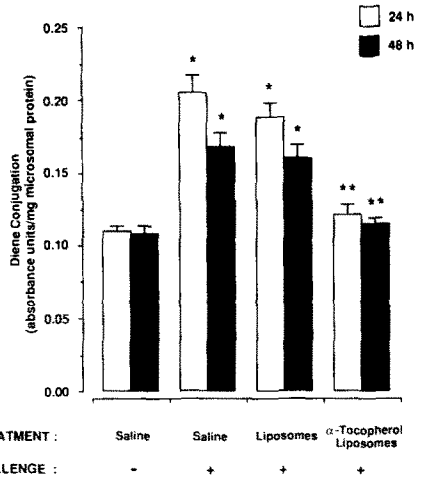

Fig. 2. Lipid peroxidation levels, as measured by the formation of diene conjugates, in lungs of paraquat-challenged rats pretreated with liposomal preparations. Animals were instilled intratracheally with either saline, DPPC liposomes or DPPC/α-tocopherol liposomes and 24 hr later, they were all challenged intraperitoneally with paraquat (PQ) at a dose of 20 mg/kg. Control animals were also pretreated intratracheally with saline but they were not challenged with PQ. Each vertical bar represents the mean ± SEM of five animals. Each single asterisk (*) indicates a significant difference ($P < 0.05$) between diene-conjugate level and the corresponding mean level of control animals pretreated with saline, but not challenged with PQ. Each double asterisk (**) indicates a significant difference ($P < 0.05$) between mean diene-conjugate level and the corresponding time-matched value of saline-pretreated and PQ-challenged animals.

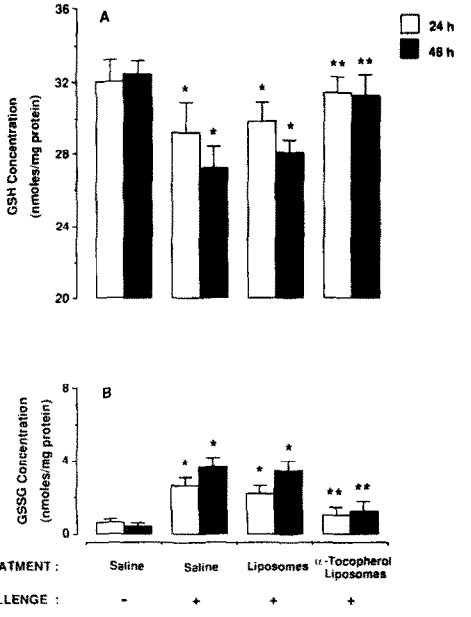

Fig. 3. Effect of pretreatment with liposomal preparations on paraquat-induced changes in pulmonary GSH (A) and GSSG (B) concentrations. Animals were instilled intratracheally with either saline, DPPC liposomes or DPPC/α-tocopherol liposomes and 24 hr later, they were all challenged intraperitoneally with paraquat (PQ) at a dose of 20 mg/kg. Control animals were also pretreated intratracheally with saline but they were not challenged with PQ. Each vertical bar represents the mean ± SEM of five animals. Each single asterisk (*) indicates a significant difference ($P < 0.05$) between the glutathione concentration and the corresponding mean concentration of control animals pretreated with saline, but not challenged with PQ. Each double asterisk (**) indicates a significant difference ($P < 0.05$) between mean glutathione concentration and the corresponding time-matched value of saline-pretreated and PQ-challenged animals.

invariably accompanied by a concomitant increase in GSSG concentration. These data appear to demonstrate that glutathione was oxidized following paraquat treatment and the administration of DPPC/α-tocopherol liposomes provided protection against paraquat-induced GSH oxidation.

DISCUSSION

Results of the present study demonstrated that the intratracheal administration of liposome-associated α-tocopherol directly to the lung conferred protection against paraquat-induced lung damage. Some studies have provided evidence that α-tocopherol plays an important role in modulating paraquat-induced cellular injury [31, 32]. It has been reported that vitamin E deficiency increases mortality and worsens histologic lung damage in rats exposed to paraquat [31]. Also, potentiation of acute paraquat toxicity by vitamin E deficiency can be reversed by subsequent administration of vitamin E [31]. Results from *in vitro* studies have also shown that α-tocopherol antagonizes paraquat-induced toxicity in hepatocytes [32]. It is apparent from this and other studies that cellular α-tocopherol levels in mammalian cells can modulate paraquat toxicity, allowing cells with high levels of the antioxidant to be more resistant to paraquat insult.

Numerous *in vivo* studies have shown that orally or parenterally administered α-tocopherol to animals fails to ameliorate lung injury produced by paraquat [5, 9, 20]. In contrast, results of this study provided evidence to substantiate a beneficial effect of liposome-associated α-tocopherol in alleviating paraquat-induced lung damage. It is known that protection against oxidant-induced toxicity by α-tocopherol is dependent on its incorporation into membranes and the extent of this protection is related to the quantity of α-tocopherol present in the membranes. The apparent difference in α-tocopherol effect between our study and those of others may well be due to a difference in α-tocopherol concentration delivered to the site of protection, the lung. Typical results from studies examining the uptake and distribution of α-tocopherol have shown that the amount of antioxidant recovered from the lungs of animals 24 hr after oral or parenteral administration was 13 or 36 μg/g lung tissue, respectively [21, 33]. Our present results showed that the intratracheal administration of liposome-associated α-tocopherol can achieve a

substantially higher α-tocopherol level in the lung, approximately 1 mg/g lung weight. Based on these observations, it may be concluded that the augmentation of pulmonary cellular defence and protection against paraquat-induced lung injury are dependent, at least in part, on the route of administration and the formulation of α-tocopherol used.

To elucidate the mechanisms of how α-tocopherol confers protection against paraquat-induced lung damage, it is important to first consider the mechanisms whereby paraquat exerts its pulmonary toxicity. The results of this study demonstrated a significant increase in lipid peroxidation of microsomal membranes isolated from the lungs of paraquat-treated animals. This observation is consistent with other reports which have suggested that lipid peroxidation is a mechanism by which paraquat produces toxicity *in vivo* [1, 4, 34]. Furthermore, *in vitro* studies have shown that paraquat undergoes cyclic oxidation and reduction reactions, resulting in the generation of superoxide anion. This anion can directly peroxidize cell membrane lipids to produce lipid hydroperoxides which simultaneously decompose, in the presence of a trace amount of transition metal ions, to lipid free radicals, thus initiating the chain reaction process of lipid peroxidation [1, 4, 35]. Peroxidation of membrane lipids as a mechanism of oxidant-induced tissue damage has been reviewed by several investigators [16, 17, 35].

Considering the evidence presented in this study that paraquat induced membrane lipid peroxidation and the fact that lipid peroxidation is a major mechanism of oxidant-induced tissue damage [35], it is reasonable to suggest that the antagonistic action of α-tocopherol on membrane lipid peroxidation is responsible, at least partly, for the protection of the lung from paraquat toxicity. Other studies have suggested that α-tocopherol may exert its antioxidant effects by serving as a structural membrane component rendering membrane polyunsaturated fatty acids more resistant to peroxidation or by acting as a free radical scavenger [16–18]. The exact mechanisms whereby α-tocopherol suppresses paraquat-induced membrane lipid peroxidation have not been established. Results of the present study, however, support the hypothesis that α-tocopherol can prevent the formation and/or accumulation of lipid peroxides, products of peroxidized membranes known to participate in the initiation and propagation stages of lipid peroxidation [16, 17, 35]. This interpretation of our results is consistent with the finding that α-tocopherol completely protected against paraquat-induced oxidation of GSH, which serves as a substrate in the GSH peroxidase/GSSG reductase system to detoxify lipid peroxides. The role of GSH in protecting cells from peroxidative damage induced by several xenobiotics has been reported by many investigators [35, 36].

It can be argued that the protective effects observed following administration of DPPC/α-tocopherol liposomes may be attributed to the DPPC component of the liposome and not to α-tocopherol. There is evidence to suggest that paraquat induces its toxic effects in the lung, at least in part, by depleting the surfactant present in Type II alveolar cells [37, 38]. In contrast, results of the present study suggested that depletion of lung surfactant may not be a major mechanism of paraquat-induced lung damage, because treatment of rats with plain liposomes composed of DPPC, major component of surfactant, 24 hr prior to paraquat administration did not protect against lung damage. Moreover, the lack of paraquat-mediated effect on pulmonary phospholipid concentration and on the rate of incorporation of [^{3}H]palmitic acid into dipalmitoyl lecitin (phosphatidylcholine) [39] also suggests that any lung surfactant changes are unlikely to be the initial biochemical defect leading to pathologic changes of paraquat poisoning.

One of the most common manifestations of pulmonary paraquat toxicity is the development of lung edema, but the mechanisms of the induction of lung edema are not understood. The results of the present study, however, appeared to demonstrate that paraquat-induced edema is not associated with cellular injury caused by membrane lipid peroxidation. This conclusion is supported by the finding that administration of DPPC/α-tocopherol liposomes in paraquat-treated animals failed to modify the pulmonary edema condition. The lack of protection by α-tocopherol against paraquat-induced edema could perhaps be explained by the fact that DPPC/α-tocopherol liposomes failed to reach the target cells involved in the formation of edema. This interpretation is consistent with our finding that α-tocopherol exerted a partial protective effect against paraquat-induced decreases in microsomal cytochrome P450 concentration. Cytochrome P450 has been shown to be localized largely in type II and Clara cells [40]; damage to type II cells has been implicated in the production of pulmonary edema [41]. The results of the present study do not exclude the possibility that various cell types of the lung may differ in their sensitivity to the cytotoxic effects of paraquat, irrespective of the α-tocopherol accumulated in these cells. Further studies will be required to elucidate this possibility. It is apparent, however, that the protective effect of α-tocopherol on the activity of ACE, mostly localized in endothelial cells [29, 30], perhaps reflects the likelihood that paraquat-induced edema is not due to the direct damage of pulmonary capillary endothelial cells.

The reason for the lack of protective effect of α-tocopherol against paraquat-induced decrease in body weight is not known at the present time. Previous studies have shown that changes in body weight correspond well with biochemical and morphological changes seen in lungs of rats [42]. The results of the present study suggested that the paraquat-induced pulmonary biochemical changes are not solely responsible for body weight loss and other mechanisms may also be responsible. Other studies have shown that paraquat-treated animals are hypoactive and indifferent to food and water intake after a latency time of a few hours in the absence of any respiratory difficulties. It has also been shown that administration of single large doses of paraquat (25–100 mg/kg) to rats resulted in severe neurological disturbances 1–2 hr after intraperitoneal injection [43].

In summary, the results of the present study showed that DPPC/α-tocopherol liposomes administered to rats intratracheally antagonized some of the toxic effects of paraquat. It is apparent that intratracheal instillation of α-tocopherol or other antioxidants may be preferable to other routes of administration for immediate augmentation of the lung antioxidant system required in emergency situations such as paraquat poisoning. In addition, incorporation of antioxidants within liposomes can increase their intracellular delivery to lung cells and enhance their protective effects against intracellular-oxygen mediated damage.

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REFERENCES

1. Pasi A, *The Toxicity of Paraquat, Diquat and Morfamquat.* Hans Huber Publishers, Bern, 1978.
2. Heath D and Smith P, The pathology of the lung in paraquat poisoning. In: *Biochemical Mechanisms of Paraquat Toxicity* (Ed. Autor AP), pp. 39–51. Academic Press, New York, 1977.
3. Vale JA, Meredith TJ and Buckley BM, Paraquat poisoning: Clinical features and immediate general management. *Hum Toxicol* **6**: 41–47, 1987.
4. Bus JS and Gibson JE, Paraquat: Model for oxidant-initiated toxicity. *Environ Health Perspect* **55**: 37–46, 1984.
5. Meredith TJ and Vale JA, Treatment of paraquat poisoning in man: Methods to prevent absorption. *Hum Toxicol* **6**: 49–55, 1987.
6. Proudfoot AT, Prescott LF and Jarvie DR, Haemodialysis for paraquat poisoning. *Hum Toxicol* **6**: 69–74, 1987.
7. Bateman DN, Pharmacological treatments of paraquat poisoning. *Hum Toxicol* **6**: 57–61, 1987.
8. Autor PN, Reduction of paraquat toxicity by superoxide dismutase. *Life Sci* **14**: 1309–1319, 1974.
9. Anonymous, Effects of vitamin E and selenium on the toxicity of paraquat. *Nutr Rev* **42**: 260–262, 1984.
10. Matkovits B, Barabas K, Szabo L and Berencsi G, *In vivo* study of the mechanism of protective effects of ascorbic acid and reduced glutathione in paraquat poisoning. *Gen Pharmacol* **11**: 455–461, 1980.
11. Padmanabhan RV, Gudapathy R, Liener IE, Schwartz BA and Hoidal JR, Protection against pulmonary oxygen toxicity in rats by the intratracheal administration of liposome-encapsulated superoxide dismutase or catalase. *Am Rev Respir Dis* **132**: 164–167, 1985.
12. Jurima-Romet M, Barber RF, Demeester J and Shek PN, Distribution studies of liposome-encapsulated glutathione administered to the lung. *Int J Pharm* **63**: 227–235, 1990.
13. Turrens JF, Crapo JD and Freeman BA, Protection against oxygen toxicity by the intravenous injection of liposome-entrapped catalase and superoxide dismutase. *J Clin Invest* **73**: 87–95, 1984.
14. Poznansky MJ and Juliano RL, Biological approaches to the controlled delivery of drugs: A critical review. *Pharmacol Rev* **36**: 277–336, 1984.
15. Kimelberg HK and Mayhew EG, Properties and biological effects of liposomes and their uses in pharmacology and toxicology. *CRC Crit Rev Toxicol* **96**: 25–79, 1978.
16. Witting LA, Vitamin E and lipid antioxidants in free-radical-initiated reactions. In: *Free Radicals in Biology* (Ed. Pryor WA), Vol. IV, pp. 295–319. Academic Press, New York, 1980.
17. Diplock AT, The role of vitamin E in biological membranes. In: *Biology of Vitamin E* (CIBA Foundation Symposium 101), pp. 45–55. Pitman Books, London, 1983.
18. Packer L and Landvik S, Vitamin E: Introduction to biochemistry and health. *Ann NY Acad Sci* **570**: 1–6, 1989.
19. Harley JB, Grinspan S and Root RK, Paraquat suicide in a young woman: Results of therapy directed against the superoxide radical. *Yale J Biol Med* **50**: 481–488, 1977.
20. Redetzki HM, Wood CD and Grafton WD, Vitamin E and paraquat poisoning. *Vet Hum Toxicol* **22**: 395–397, 1980.
21. Gallo-Torres HE, Transport and metabolism. In: *Vitamin E: A Comprehensive Treatise* (Ed. Machlin LJ), pp. 193–267. Marcel Dekker, New York, 1980.
22. Alade SL, Brown RE and Paquet A, Polysorbate 80 and E-ferol toxicity. *Pediatrics* **77**: 593–597, 1986.
23. Finer NN, Peters KL, Hayek Z and Merket CL, Vitamin E and necrotizing enterocolitis. *Pediatrics* **73**: 387–393, 1984.
24. Brain ID, Knudson DE, Sorokin SP and Davis MA, Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. *Environ Res* **11**: 13–33, 1976.
25. Suntres Z and Shek PN, Nitrofurantoin-induced pulmonary toxicity: *In vivo* evidence for oxidative stress-mediated mechanisms. *Biochem Pharmacol* **43**: 1127–1135, 1992.
26. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
27. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
28. Gad SC and Weil CS, Statistics for toxicologists. In: *Principles and Methods of Toxicology* (Ed. Hayes AW), pp. 273–319. Raven Press, New York, 1982.
29. 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.
30. Hollinger MA, Giri SN, Patwell S, Zuckerman JE, Gorin A and Parsons G, Effect of acute lung injury on angiotensin converting enzyme in serum, lung lavage, and effusate. *Am Rev Respir Dis* **121**: 373–376, 1980.
31. Block ER, Potentiation of acute paraquat toxicity by vitamin E deficiency. *Lung* **156**: 195–203, 1979.
32. Watanabe E, Shiki Y, Morisaki N, Sato Y and Yoshida S, Cytotoxic effects of paraquat and inhibition of them by vitamin E. *Biochim Biophys Acta* **883**: 420–425, 1986.
33. Knight ME and Roberts RJ, 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, 1987.
34. Yasaka T, Okudaira K, Fujito H, Matsumoto J, Ohya I and Miyamoto Y, Further studies of lipid peroxidation in human paraquat poisoning. *Arch Intern Med* **146**: 681–685, 1986.
35. Girotti AW, Mechanisms of lipid peroxidation. *J Free Radic Biol Med* **1**: 87–95, 1985.
36. Reed DJ and Fariss MW, Glutathione depletion and susceptibility. *Pharmacol Rev* **36**: 25S–33S, 1984.
37. Manktelow BW, The loss of pulmonary surfactant in paraquat poisoning: A model for the study of the respiratory distress syndrome. *Br J Exp Pathol* **48**: 366–369, 1967.

38. Robertson B, Enhorning G, Ivemark B and Modee J, Experimental respiratory distress induced by paraquat. *J Pathol* **103**: 239–244, 1971.
39. Fletcher K and Wyatt I, The action of paraquat on the incorporation of palmitic acid into dipalmitoyl lecithin in mouse lungs. *Br J Exp Pathol* **53**: 225–230, 1972.
40. Bend JR, Serabjit-Singh CJ and Philpot RM, The pulmonary uptake, accumulation and metabolism of xenobiotics. *Annu Rev Pharmacol Toxicol* **25**: 97–125, 1985.
41. Keeling PL, Pratt IS, Aldridge WN and Smith LL, The enhancement of paraquat toxicity in rats by 85% oxygen: Lethality and cell-specific lung damage. *Br J Exp Pathol* **62**: 643–653, 1981.
42. Ogata T and Manabe S, Correlation between lipid peroxidation and morphological manifestation of paraquat-induced lung injury in rats. *Arch Toxicol* **64**: 7–13, 1990.
43. Smith P, Heath D and Kay JM, The pathogenesis and structure of paraquat-induced pulmonary fibrosis in rats. *J Pathol* **114**: 57–67, 1973.