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Pulmonary Uptake of Liposome-associated α-Tocopherol Following Intratracheal Instillation in Rats

Z. E. SUNTRES, S. R. HEPWORTH AND P. N. SHEK

Operational Medicine Section, Biosciences Division, Defence and Civil Institute of Environmental Medicine, North York, Ontario M3M 3B9, Canada

Abstract—This study examined the uptake and subcellular distribution of α-tocopherol in the lung following intratracheal instillation of liposome-associated α-tocopherol in rats. The liposomal suspension was composed of dipalmitoylphosphatidylcholine (DPPC) and α-tocopherol (molar ratio 7:3), labelled with [^{3}H]α-tocopherol and [^{14}C]cholesterol. Following intratracheal administration of the liposomal preparation (2 mg α-tocopherol/animal), the recovery of [^{3}H]α-tocopherol in the lung was maximal (87% of initial dose) 1 h after treatment; thereafter, α-tocopherol levels remained relatively high (no less than 73% of initial dose) for the rest of the 72-h experimental period. This treatment effect resulted in a 16-fold increase in pulmonary total α-tocopherol concentration 72 h post-instillation. No radioactivity was detected in the blood, liver, kidney, pancreas, spleen and heart of animals during the 72-h experimental period. [^{3}H]α-Tocopherol was recovered largely from cytosolic (45%) and nuclear (36%) fractions of lung and to a lesser extent, from microsomal (11%) and mitochondrial (9%) fractions. Chromatographic analysis of the subcellular fractions revealed that [^{3}H]α-tocopherol was co-eluted with ^{14}C-labelled liposomal lipids. Our in-vitro study, involving the incubation of Fe^{3+}-ADP (a pro-oxidant) with mitochondrial or microsomal fractions isolated from lung tissues of animals treated with liposome-associated α-tocopherol, provided evidence that α-tocopherol levels present in the membranes of these subcellular fractions were sufficient to protect against oxidant-induced lipid peroxidation. α-Tocopherol in the rat lung can be greatly increased by the intratracheal instillation of α-tocopherol entrapped in DPPC-liposomes, suggesting that this liposomal preparation may be used as an effective prophylactic agent against oxidant-induced lung injury.

Pulmonary damage as a result of oxidative stress-mediated mechanisms has been described (Roggli et al 1983; Bend et al 1985; Copper et al 1986). Oxidative stress is a condition characterized by an elevation in the steady-state concentration of reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical. These reactive oxygen species are capable of reacting with cellular lipids, proteins, nucleic acids and carbohydrates leading to changes in the structure and function of cells (Halliwell & Gutteridge 1984; Sies 1985; Boobis et al 1989). In the lung, oxidative stress has been shown to be involved in various biological and pathological conditions including bronchopulmonary dysplasia, emphysema, pulmonary oxygen toxicity (Northway et al 1967; Freeman & Crapo 1982) and tissue injuries caused by exposure to radiation and xenobiotics such as ozone, nitrogen dioxide, paraquat, bleomycin and nitrofurantoin (Roehm et al 1971; Gross 1977; Michelson 1982; Kehrer & Kacew 1985; Suntres & Shek 1992).

The lung, like other organs and tissues, has developed antioxidant cellular defence systems including enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and other endogenous antioxidants such as ascorbic acid, glutathione and vitamin E (White & Repine 1985; Slade et al 1985; Massaro et al 1988). When these cellular defence systems are overwhelmed by the presence of acute oxidative stress, it can lead to cell damage. Strategies for pharmacological modification of oxidant-mediated lung damage have focused on increasing the antioxidant capacity of pulmonary cells (Crapo et al 1977; Turrens et al 1984; Padmanabhan et al 1985; Panus & Freeman 1988; Jurima-Romet et al 1990). Unfortunately, antioxidants such as superoxide dismutase, catalase or glutathione, used in the treatment of oxidant-induced lung damage are known to be without clinical benefit because of their inability to cross cell membrane barriers or because of rapid clearance from the lung (Turrens et al 1984; Padmanabhan et al 1985; Panus & Freeman 1988; Jurima-Romet et al 1990). More recent studies, however, have shown that encapsulation of these antioxidants within liposomes greatly increases their intracellular delivery to specific target cells and enhances their protective effects against intracellular oxidant-mediated damage (Turrens et al 1984; Padmanabhan et al 1985; Panus & Freeman 1988; Jurima-Romet et al 1990).

Liposomes are phospholipid vesicles composed of lipid bilayers enclosing one or more aqueous compartments. Hydrophilic molecules can be encapsulated in the aqueous spaces and lipophilic molecules can be incorporated in the lipid bilayers. Liposomes provide an efficient delivery system because they are biocompatible, biodegradable and relatively nontoxic (Shek & Barber 1986). 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 & Mayhew 1978; Szoka & Papahadjopoulos 1981; Poznansky & Juliano 1984).

Intracellular α-tocopherol, the main constituent of vitamin E, is a major lipid antioxidant that has been shown to protect cells against oxidant-induced damage (Witting 1980; Burton & Ingold 1989; Packer & Landvik 1989; Wender et al 1981). Generally, α-tocopherol is known to function as an antioxidant by its ability to scavenge free radicals and by

Correspondence: P. N. Shek, Defence and Civil Institute of Environmental Medicine, 1133 Sheppard Avenue West, North York, Ontario M3M 3B9, Canada.

stabilizing membranes containing polyunsaturated fatty acids (Witting 1980; Wender et al 1981; Burton & Ingold 1989; Packer & Landvik 1989). Results from in-vivo studies have demonstrated that α-tocopherol, administered in large doses over long periods of time, offers significant protection against oxidant-induced lung damage occurring from exposure to smoke and smog, hyperoxia and certain other oxidants (Roehm et al 1971; Freeman & Crapo 1982; Roggli et al 1983; Knight & Roberts 1985; Bend et al 1985; Copper et al 1986; Packer & Landvik 1989). It has been argued, however, that in an emergency (e.g. paraquat-induced lung damage, bronchopulmonary dysplasia linked to oxygen therapy), where α-tocopherol must be delivered to vulnerable biomembranes of the lung in sufficient quantities and effective rates, α-tocopherol is not an ideal therapeutic antioxidant (Harley et al 1977; Redetzki et al 1980; Phelps 1984). The ineffectiveness of α-tocopherol in protecting lung tissues against oxidant-induced injury is not well understood. The apparent inefficacy of α-tocopherol therapy could be due, at least in part, to the fact that the levels of antioxidant delivered to the lung following dietary manipulation or by injections are not sufficiently high to prevent oxidant injuries (Gallo-Torres 1980; Knight & Roberts 1985).

In the present study, we attempted to deliver α-tocopherol incorporated in liposomes directly to the lung with the aim of attaining high pulmonary concentrations of the antioxidant at a relatively fast delivery rate.

Materials and Methods

Chemicals

Cholesterol and dipalmitoylphosphatidylcholine (DPPC) were purchased from Avanti Polar Lipids (Alabasier, AL, USA). [^{3}H]α-Tocopherol and [^{14}C]cholesterol were obtained from Dupont Canada, Inc. (Mississauga, Ontario, Canada). A Sepharose 4B column was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Animals

Male Sprague-Dawley rats, 220–250 g, were purchased from Charles River Canada, Inc. (St Constant, Quebec, Canada). All animals were housed in stainless-steel cages with free access to pelleted purina laboratory chow and tap water. The animals were exposed to alternate cycles of 12 h light and darkness. Animals used in this research were cared for in accordance with the principles contained in the Guide to the Care and Use of Experimental Animals as prepared by the Canadian Council on Animal Care.

Experimental design

In-vivo study. To examine the distribution of liposome-associated α-tocopherol in rats, animals were treated intratracheally with a single dose of α-tocopherol liposomal suspension as described below and killed 0, 0·5, 1, 2, 8, 24, 48, or 72 h later. The distribution of α-tocopherol in the lungs of treated rats was assessed by measuring [^{3}H]α-tocopherol radioactivity in the whole organ and subcellular fractions.

In-vitro study. To study whether the concentration of α-tocopherol associated with the mitochondrial and microsomal fractions of liposomal α-tocopherol-treated animals was sufficient to prevent oxidant stress-induced injury, the level of Fe^{3+}-ADP-induced lipid peroxidation in mitochondrial or microsomal fraction obtained from the lungs of control and liposomal α-tocopherol-treated animals was compared. Standard incubations were carried out in a 25-mL Erlenmeyer flask with a final volume of 5 mL of incubation mixture containing 1 mg washed mitochondrial or microsomal protein, 10 mM NADPH, 50 mM $MgCl_2$ and 30 mM Tris-HCl buffer, pH 7·4. The mixture was incubated at 37°C with air as the gas phase in a metabolic shaker oscillating at 100 cycles min^{-1}. After a 3 min preincubation period, the reaction was initiated by the addition of Fe^{3+}-ADP to a final concentration of 80 μM Fe^{3+}/0·4 mM ADP in the incubation mixture. Aliquots of the mixture were removed at different times and the reaction was terminated by the addition of ice-cold 10% trichloroacetic acid. The samples were centrifuged at 600 *g* for 10 min and the supernatant fractions were used for the measurement of lipid peroxidation by measuring the formation of thiobarbituric acid reactants (TBAR) as described by Suntres & Lui (1991).

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 both [^{14}C]cholesterol (sp. act. 2·5 mCi $mmol^{-1}$) and [^{3}H]α-tocopherol (sp. act. 5 mCi $mmol^{-1}$) were added as tracers. The lipid mixture was dried in a waterbath at 40°C 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 h. 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, Vancouver, BC, Canada), fitted with 2 stacked polycarbonate filters of 400 nm pore size, using a helium pressure of 100–200 psi. Free α-tocopherol was removed by washing the liposomes twice in 5 mM potassium phosphate buffer, pH 6·5, and pelleting at 110 000 *g* for 1 h at 5°C in a Beckman L8-70 ultracentrifuge. The vesicle size distribution was not affected by ultracentrifugation. Aliquots of the supernatant and pellet were counted for ^{3}H and ^{14}C activity in a Beckman LS-5801 liquid scintillation counter. The α-tocopherol entrapment efficiency was determined to be $0{\cdot}43 \pm 0{\cdot}01$ mol mol^{-1} DPPC ($n = 7$). The liposomes were diluted with 5 mM potassium phosphate buffer, pH 6·5, to give a final α-tocopherol concentration of 2 mg/150 μL suspension. The vesicle-size distribution was determined using a Coulter N4SD particle-size analyser. A unimodal distribution was observed and the mean vesicle diameter was found to be 260 ± 44 nm.

Administration of α-tocopherol-containing liposomes to animals

The endotracheal intubation technique described by Brain et al (1976) was adapted for administering α-tocopherol-containing liposomes. Rats were anaesthetized by an intraperitoneal injection of a mixture of 50 mg kg^{-1} ketamine (Ketalar, Parke-Davis, Scarborough, Ontario, Canada) and

20 mg kg^{-1} xylazine (Rompun, Bayvet, Etobicoke, Ontario, Canada). Once anaesthetized, each animal was placed on a slanted board (20°C from the vertical) and was supported by an elastic band under its upper incisors. A microscope lamp, with its beam directed at the neck area, provided transillumination during procedural operations. By opening the mouth of the animal and depressing the tongue, the larynx can be easily visualized. The liposomal suspension was delivered to the lung via the trachea by a PE-50 polyethylene tubing (6·5 cm) connected to a 25-gauge epidural catheter. The endotracheal tube was introduced into the trachea using gentle pressure. All animals received 150 μL of the liposomal preparation and were kept upright on the slanted board for approximately 3 min to promote the subsequent distribution of the instilled dose. The animals were used later for the harvesting of tissues and fluids as described below.

Preparation of tissues

Rats were anaesthetized with an intraperitoneal injection of sodium phenobarbitone (50 mg kg^{-1}). Heparinized blood was collected by cardiac puncture. Different organs, including the lung, liver, kidney, pancreas, spleen and heart, were removed immediately after decapitation and rinsed with ice-cold 0·9% NaCl to remove excess blood. All subsequent steps were carried out at 0–4°C. Approximately 1 g of finely minced tissue was homogenized with a Brinkman Polytron in a sufficient volume of ice-cold 0·25 M sucrose/5 mM Tris-HCl buffer, pH 6·5, to produce a 20% homogenate. Subcellular fractionation of lung homogenates was achieved by differential centrifugation as described by New et al (1990).

Measurement of radioactivity

Tissue samples and subcellular fractions were digested with 0·5 mL Solvable (DuPont) for 24 h and then bleached with 0·2 mL of 30% H_2O_2. The mixture was subsequently incubated at 25°C for 1 h. After incubation, 10 mL of Formula 989 scintillation fluid (New England Nuclear, DuPont) was added, the samples vortexed, and counted for radioactivity in a Beckman LS 5801 scintillation counter.

Chromatographic analysis

To examine whether [^{3}H]α-tocopherol in the cellular organelles was associated with liposomes, gel filtration of a 0·5 mL sample of the whole homogenate or subcellular fraction at room temperature (21°C) on a calibrated Sepharose 4B column (1·5 × 50 cm) equilibrated with 10 mM potassium phosphate buffer, pH 6·8, was performed. Fractions of 1 mL were collected and analysed for ^{3}H and ^{14}C activity.

Determination of α-tocopherol concentrations

α-Tocopherol concentrations in homogenates and subcellular fractions of lung tissues were determined as described by Suntres & Lui (1991).

Statistical analysis

Results were analysed by one-way analysis of variance and Student's *t*-test (Gad & Weil 1982). The level of significance was taken as $P < 0·05$.

Results

Recovery of α-tocopherol and DPPC in the aqueous dispersion

As shown in Fig. 1, all the DPPC was dispersed in the aqueous solution when the starting ratio of α-tocopherol was less or equal to 30 mol%. At higher starting ratios of α-tocopherol, a rapid decline in the entry of both α-tocopherol and DPPC to the aqueous solution was observed. Similar results have also been reported by other investigators who showed that regardless of the initial ratios used, no more than 30 mol% of α-tocopherol entered the aqueous phase of the reaction mixture (Halks-Miller et al 1985). Therefore, the liposomal suspension prepared for intratracheal instillation in the lungs of rats contained 30 mol% of α-tocopherol. In terms of the extent of entrapment, practically 100% of the α-tocopherol in the reaction mixture was associated with the DPPC lipid. In contrast, the entrapment efficiency of a water-soluble marker, such as glutathione, in similar DPPC liposomes was only about 15% (Jurima-Romet & Shek 1991).

Recovery of [^{3}H]α-tocopherol in the lung

The time-course of the recovery of radioactive α-tocopherol in the lung of rats treated intratracheally with [^{3}H]α-tocopherol liposomes is shown in Fig. 2. Recovery of [^{3}H]α-tocopherol from the lung homogenate was approximately 60% of the initial dose 0·5 h after administration of the liposomal preparation, reaching a maximum (87% of initial dose) 1 h after treatment; thereafter, [^{3}H]α-tocopherol concentrations remained relatively constant for the rest of the experimental period. This treatment effect resulted in an approximate 16-fold increase in pulmonary α-tocopherol concentration 72 h after treatment (Table 1). It is important to note that no radioactivity was detected in the blood, liver, kidney, pancreas, spleen, and heart of treated animals during the 72-h experimental period (data not shown).

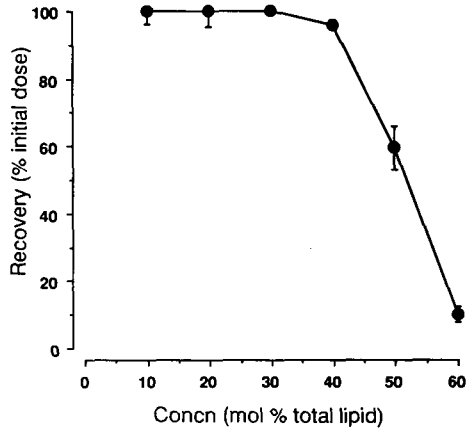

FIG. 1. Recovery of DPPC from an aqueous dispersion of dried lipid mixture containing various initial concentrations of α-tocopherol. Liposomes were prepared from a mixture of DPPC and α-tocopherol at varying molar ratios as described in Materials and Methods. Recovery of DPPC was estimated by determining the percentage of ^{14}C-lipid-marker radioactivity present in the liposomal pellet obtained by ultracentrifugation. Each point represents the mean recovery ± s.e.m. of three separate experiments.

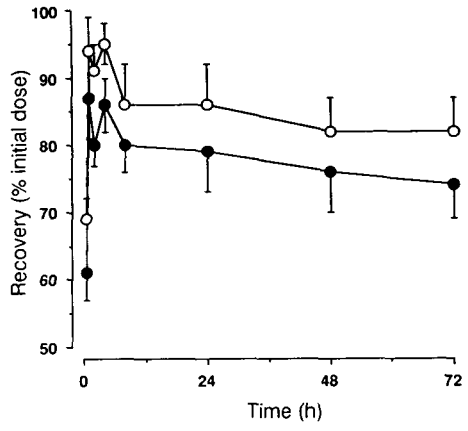

FIG. 2. Recovery of ^{3}H (●) and ^{14}C (○) labels from lung homogenates following the intratracheal instillation of [^{3}H]α-tocopherol incorporated in ^{14}C-labelled liposomes. α-Tocopherol-containing liposomes were prepared as described in Materials and Methods, and lungs of treated animals were removed at various time periods after intratracheal instillation of the liposomal preparation. Each point represents the mean percentage of recovered dose ± s.e.m. of three animals.

Table 1. α-Tocopherol content of homogenate, nuclear, mitochondrial, microsomal and cytosolic fractions isolated from rat lungs.

	μg α-Tocopherol/total fraction mean ± s.e.m. (n = 3)	
Lung fraction	Untreated	Liposomal α-tocopherol-treated
Homogenate	102 ± 12	1620 ± 83
Nuclei	28 ± 6	560 ± 36
Mitochondria	12 ± 2	144 ± 19
Microsome	13 ± 3	208 ± 22
Cytosol	10 ± 2	720 ± 45

Homogenates and subcellular fractions were prepared from lung tissues obtained from animals either untreated or treated intratracheally with an instillation of a single dose of liposomal α-tocopherol 72 h earlier, as described in Materials and Methods. The α-tocopherol present in each lung fraction was extracted with acetone and its total content determined as described by Suntres & Lui (1991).

Distribution of [^{3}H]α-tocopherol in subcellular fractions

Since oxidants are known to exert their toxic effects by damaging cellular organelles, the distribution of α-tocopherol in nuclear, mitochondrial, cytosolic and microsomal fractions was also examined. As shown in Fig. 3, the time-course of the recovery of α-tocopherol in the subcellular fractions was qualitatively similar to that observed for total α-tocopherol in the whole lung. α-Tocopherol was recovered largely from the cytosolic (36–46% of total) and nuclear (36% of total) fractions and to a lesser extent, from the microsomal (11% of total) and mitochondrial (9% of total) fractions. This treatment effect resulted in approximately 20-, 12-, 72- and 16-fold increases in α-tocopherol concentration in the nuclear, mitochondrial, cytosolic and microsomal fractions, respectively, 72 h post-instillation (Table 1).

To examine whether α-tocopherol was associated with liposomes, chromatographic analysis of homogenates as well as nuclear, mitochondrial, cytosolic and microsomal fractions was carried out. A representative chromatogram

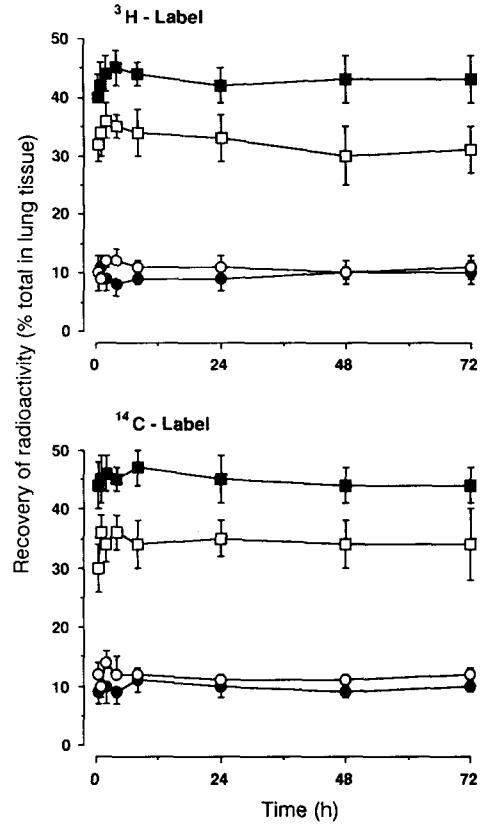

FIG. 3. Recovery of ^{3}H and ^{14}C labels from subcellular fractions isolated from the lung following intratracheal instillation of [^{3}H]α-tocopherol incorporated in ^{14}C-labelled liposomes. Lungs of treated animals were removed, at various time periods after the intratracheal treatment, for the preparation of cytosolic (■), nuclear (□), mitochondrial (●) and microsomal (○) fractions as described in Materials and Methods. Each point in the figure represents the mean percentage of total lung radioactivity ± s.e.m. of three animals.

obtained following the analysis of various subcellular fractions, isolated from lung tissues of rats killed 24 h after intratracheal administration of the α-tocopherol liposomal prepartion, is shown in Fig. 4. Chromatographic analysis of lung homogenate and subcellular fractions revealed that [^{3}H]α-tocopherol was recovered in a single peak co-eluting at the void volume of the column with ^{14}C-radioactivity. The recovery of ^{3}H- and ^{14}C-radioactivity from the homogenate and subcellular samples, isolated from the lungs of animals treated with liposomal α-tocopherol, was approximately 30% of the initial radioactivity with the remaining radioactivity being recovered from the top of the stationary phase of the column. Examination of the chromatographic profile of α-tocopherol liposomes on the Sepharose 4B column revealed the absence of radioactivity in the collected fractions, with all the radioactivity being recovered from the top of the stationary phase of the column (data not shown). These data suggest that suspensions of intact multilamellar liposomes cannot penetrate the column bed, perhaps due to the formation of aggregates.

Comparison of [^{3}H]α-tocopherol and [^{14}C]liposome distribution in the lung

To investigate whether the distribution of [^{3}H]α-tocopherol

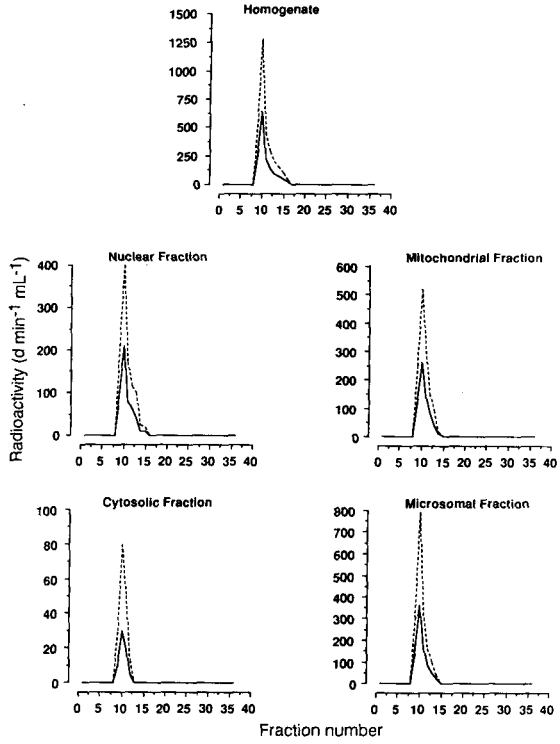

FIG. 4. Chromatographic analysis of lung homogenate and subcellular fractions. Lungs were removed from rats 24 h after the intratracheal instillation of [^{3}H]α-tocopherol incorporated in ^{14}C-labelled liposomes. Homogenates and subcellular fractions were prepared from lung tissues pooled from three animals and were chromatographed on a Sepharose-4B column, equilibrated with 10 mM potassium phosphate buffer, pH 6·8, at room temperature. Fractions (1 mL) were collected at an elution rate of 1 mL min^{-1} and measured for ^{3}H (----) and ^{14}C (——) activity.

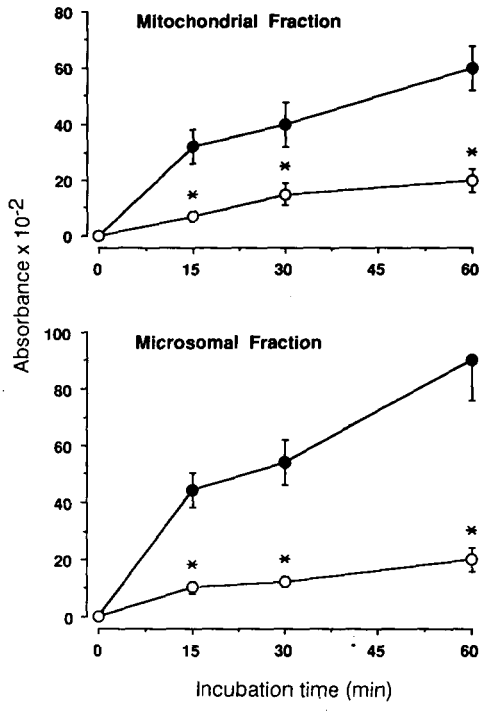

FIG. 5. Fe^{3+}-ADP-induced lipid peroxidation in mitochondrial and microsomal membranes isolated from lungs of untreated (●) or α-tocopherol-liposome-treated (○) animals. In-vitro incubations were carried out as described in Materials and Methods. Each point represents the mean ± s.e.m. of three separate experiments and each asterisk denotes a statistically significant ($P < 0.05$) difference from the corresponding values obtained for the two groups of animals.

in the lung of α-tocopherol-liposome-treated rats was directly related to that of ^{14}C-labelled liposomes, the distribution of ^{3}H- and ^{14}C-radioactivity was compared in homogenate and subcellular fractions. As shown in Figs 2 and 3, the distribution of the two isotopes was similar with a practically constant ^{3}H/^{14}C ratio.

Fe^{3+}-ADP-induced lipid peroxidation in mitochondrial and microsomal fractions

To study whether the concentration of α-tocopherol associated with membranes of mitochondria and microsomes was sufficient to protect the membranes from chemically induced oxidant stress, the following in-vitro experiment was carried out. Mitochondrial or microsomal fractions were isolated from lung tissues of both control and liposomal α-tocopherol-treated animals. The fractions were incubated with 80 μM Fe^{3+}-ADP to promote oxidation, and lipid peroxidation was measured, over time, by following the formation of thiobarbituric acid reactants. It was found that the time-dependent increase in lipid peroxidation of subcellular fractions of the liposomal α-tocopherol-treated animals was significantly lower than that of control animals, suggesting a protective role of α-tocopherol present in the subcellular membranes (Fig. 5).

Discussion

α-Tocopherol is a sparingly soluble antioxidant vitamin. Experiments from our laboratory have shown that free α-tocopherol was too viscous to be delivered directly to the lung, a tissue exposed to oxidants via the respiratory tract or the blood stream (Roggli et al 1983; Bend et al 1985; Copper et al 1986). The results of the present study, however, demonstrated that α-tocopherol can be readily incorporated in liposomes, thereby allowing the delivery of considerable amounts of α-tocopherol to the lung. Liposomes have also been used successfully to facilitate the delivery and enhance the therapeutic efficacy of a number of water-insoluble drugs, including enviroxime (Wyde et al 1988), triamcinolone acetonide (Singh & Mezei 1983) and vitamin K (Yotsuyanagi et al 1988).

It is evident from the results of the present study that DPPC-liposome-associated α-tocopherol may serve as an appropriate formulation for direct administration to the lung. Other α-tocopherol formulations tested in our laboratory revealed that organic solvents, such as ethanol and dimethylsulphoxide, used to solubilize α-tocopherol, were toxic to the lung, and emulsifying agents and detergents, such as polyethylene glycol and Tween 80, caused respiratory failure, possibly by disrupting surface tension of the lung. Results from other studies have also shown that exposure of man or animals to formulations of α-tocopherol, containing emulsifying agents or detergents, is associated with other problems that have limited their use; α-tocopherol is poorly absorbed, it fails to attain sufficient concentrations at the site

of action, and the α-tocopherol formulations tested are toxic to the hepatic, renal and haematopoietic systems, either because of the formulations themselves or the emulsifiers used to solubilize the vitamin for administration (Phelps 1984; Mintz-Hittner 1989). In our study, α-tocopherol was incorporated in liposomes prepared from DPPC. DPPC is known to be the major lipid component of alveolar surfactant, is relatively nontoxic, and has been used in the prevention or treatment of certain lung conditions such as respiratory distress syndrome or in the selective delivery of drugs to the lung and other tissues (Kimelberg & Mayhew 1978; Van Golde et al 1988; Szoka & Papahadjopoulos 1981; Poznansky & Juliano 1984; Shek et al 1990).

It has been previously reported that α-tocopherol, administered to animals by oral or parenteral routes, offers only partial or no protection against oxidant stress-induced lung damage (Harley et al 1977; Redetzki et al 1980; Phelps 1984; Knight & Roberts 1985). The failure of α-tocopherol to completely protect the lung from the injurious action of oxidants may be due, at least in part, to insufficient amounts of the antioxidant accumulating in the membranes of this tissue, a treatment effect attributed to the poor absorption of the antioxidant or to its distribution mainly to tissues other than the lung (Gallo-Torres 1980; Knight & Roberts 1985). The therapeutic efficacy of α-tocopherol, however, in the treatment of oxidant stress-induced lung injury can be improved by the selective delivery of the oxidant to the lung. This can be achieved by the intratracheal instillation of liposome-associated α-tocopherol, which has the distinct advantage of circumventing systemic dilution and minimizing removal by other tissues and organs. Indeed, results of the present study demonstrated that after intratracheal instillation of liposome-entrapped α-tocopherol, 75–85% of ^{3}H was recovered in the lung. In addition, selective delivery of this antioxidant formulation to the lung resulted in a substantial increase in pulmonary total and subcellular α-tocopherol concentrations (Table 1), a treatment effect not observed following oral or parenteral administration. Selective delivery of synthetic and natural surfactants to animals and to premature babies has been used extensively in the treatment of surfactant deficiency state associated with lung immaturity (Brain et al 1976; Morimoto & Adachi 1982; Padmanabhan et al 1985; Shek et al 1990).

To produce a therapeutic effect, a drug must reach its site of action in a concentration sufficient to initiate a response. The concentration achieved, although related to the drug dose administered, also depends on the extent of uptake and the rate of absorption from the site of administration. Previous studies have shown that the recovery of radioactivity from rat lung after parenteral administration of [^{3}H]α-tocopherol was 4·8 and 3·6% of total radioactivity at 0·5 and 48 h, respectively, while only 0·4% of initial α-tocopherol was found in the lung 12 h after intragastric administration (Gallo-Torres 1980). In our study, 60% of radioactivity was recovered 0·5 h after intratracheal administration of liposome-associated [^{3}H]α-tocopherol, while 75–85% of radioactivity was recovered between 1 and 72 h after treatment. These data provide direct evidence substantiating the feasibility of targeting to and retention in the lung of a relatively high dose of α-tocopherol administered in a liposomal formulation. Thus, our α-tocopherol delivery system is potentially useful for the delivery of sufficient antioxidants to the lung, within a short time period, for both prophylactic and therapeutic applications.

The subcellular distribution of liposomal ^{14}C-radiolabel observed in our study was similar to that reported by Zachman & Tsao (1980) who investigated the uptake of intratracheally administered [^{14}C]DPPC liposomes by the lung in rats. It is conceivable, therefore, that the distribution of α-tocopherol in the subcellular fractions of the lung may be attributed to the distribution characteristics of ^{14}C-labelled liposomes or liposomal lipid fragments. This interpretation is consistent with our findings that the $^{3}H/^{14}C$ ratio observed in the subcellular fractions was practically constant throughout the entire experimental period and that the [^{3}H]α-tocopherol radioactivity co-eluted with ^{14}C-labelled lipid marker on chromatographic analysis. Evidence presented elsewhere has shown that lipophilic substances which bind or intercalate in liposomal membranes remain associated with the liposomes (Stamp & Juliano 1978).

There has been evidence to suggest that much of the pathology of animals suffering from vitamin E deficiency or exposed to oxidant stresses is linked to biochemical and subcellular damage by lipid peroxidation (Witting 1980; Burton & Ingold 1989; Packer & Landvik 1989). The results of the present study showed that the concentration of α-tocopherol associated with the microsomal and mitochondrial membranes following the intratracheal instillation of DPPC/α-tocopherol liposomes was sufficient to protect against oxidant-induced membrane lipid peroxidation. The mechanism by which α-tocopherol prevented oxidant-induced lipid peroxidation was not investigated in the present study. However, it is generally accepted that α-tocopherol can exert its antioxidant effect by scavenging free radicals (i.e. singlet oxygen, peroxy radicals) known to be responsible for the initiation or propagation of membrane lipid peroxidation, or by stabilizing cellular membranes (Witting 1980; Burton & Ingold 1989; Packer & Landvik 1989).

The intratracheal instillation of liposome-associated α-tocopherol may be a superior experimental protocol in the treatment of oxidant stress-induced lung damage, particularly that encountered in emergencies. The selective delivery of this nontoxic formulation allows the delivery of appropriate doses of α-tocopherol to the lung accurately, efficiently and rapidly.

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