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Evaluation of a Liposome System for the Delivery of Desferrioxamine to Lungs in Rats

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Abstract—Liposomes with various lipid composition and sizes, prepared by two different techniques were evaluated for their potential to deliver desferrioxamine to lungs as a treatment against oxidative lung damage. Multilamellar vesicles (MLV) and reverse evaporation vesicles were prepared out of a lipid mixture containing dipalmitoyl phosphatidylcholine, stearyl amine, cholesterol and vitamin E. The administration of desferrioxamine-encapsulated liposomes to rats by the intravenous route at a dose of 100 mg kg^{-1}, significantly prolonged the presence of desferrioxamine in all the tested organs when compared with the administration of free desferrioxamine. The injection of reverse evaporation vesicles extruded through a 2 μm polycarbonate membrane exhibited a longer residence time of the desferrioxamine and of liposomal vitamin E in lungs compared with the other types of liposomes tested. The examination of liposome components in the bronchoalveolar lavage fluid (BALF) and the alveolar macrophages recovered from BALF revealed that about 7×10^{-3}% of the administered desferrioxamine dose was recovered by this technique at 3 and 17 h after liposome administration. This high residual concentration in the alveolar space confirms the hypothesis that liposomes can be delivered to the lung tissue when encapsulated in alveolar macrophages.

Administration of liposome-encapsulated drugs lead to prolonged life time of the drug in the body as well as preferential liposome uptake by certain cells compared with that of the free drug.

Iron ions are involved in the oxidative damage of various tissues due to their catalytic action in the Haber–Weiss reaction (Haber & Weiss 1934), according to which the very cytotoxic hydroxyl radicals ($OH^{\cdot}$) are believed to be produced from superoxide (O_2^-) and hydrogen peroxide (H_2O_2):

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + Fe^{3+}$$

The iron chelator desferrioxamine, commercially available under the name of Desferal (desferrioxamine B methanesulphate), has been successfully used for the treatment of iron overdose and thalassaemia (Hershko & Weatherall 1988). The very high and specific affinity of desferrioxamine for Fe^{3+} preventing its reduction to Fe^{2+} made desferrioxamine suitable for the in-vitro and in-vivo study of iron-dependent oxygen reactions. It has been shown that desferrioxamine is a powerful inhibitor of $OH^{\cdot}$ formation and, as a result, of lipid peroxidation (Gutteridge et al 1979; Halliwell 1985; Hershko & Weatherall 1988).

Oxidative damage to lungs is caused by hyperoxia and by various environmental and occupational pollutants such as NO_2 and ozone (Menzel 1976). The use of desferrioxamine in the treatment of paraquat-induced oxidative lung injury has been investigated, but the reported results were contradictory (Kohen & Chevion 1985; Osheroff et al 1985; Van Asbeck et al 1989; Hoffer et al 1992). As the half-life of desferrioxamine in the body is short (about 7 min in man), it is to be expected that if delivery to the lungs is improved by transport via liposomes, desferrioxamine must act as an antioxidant at the site of injury. An attempt to protect against oxidative lung damage by means of liposome treatment was undertaken by Freeman et al (1983) and Turrens et al (1984), who showed that liposome-entrapped superoxide dismutase and catalase have a protective effect on cultured cells as well as on experimental animals exposed to hyperoxia.

Poste et al (1982) demonstrated that, due to their size, liposomes have a limited ability to diffuse from the blood capillaries into undamaged lung tissue. Nevertheless, they might penetrate into the lung tissue via alveolar macrophages, apparently after phagocytosis. As oxidative lung damage is characterized by an increased number of phagocytes (Hampson et al 1989), the transport of liposomes to the lungs via these cells might be enhanced.

The objective of the present work was to investigate desferrioxamine delivery to lungs by intravenous administration of desferrioxamine-liposomes. For this purpose, two different types of liposomes, multilamellar vesicles (MLVs) and reverse evaporation vesicles (REVs) containing vitamin E were prepared, and the time-dependent distribution of desferrioxamine and of vitamin E in various organs was evaluated.

Materials and Methods

Chemicals

All organic solvents and those used in HPLC were of analytical and HPLC grade, respectively, purchased from Frutarom, Israel. Desferrioxamine was a gift from Ciba-Geigy, Basel, Switzerland. Osmium tetroxide was purchased

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from Johnson Matthey, Materials Technology, UK, and Spurr's low viscosity media (hard) from Bio Rad, Microscience, Hertfordshire, UK. All other chemicals used were purchased from Sigma Chemical Co., St Louis, MO.

Preparation of liposomes

The preparation of MLVs was based on the method of Bangham et al (1965); the REVs were prepared according to the method of Szoka & Papahadjopoulos (1978). Both types of liposomes were made up of a lipid mixture containing dipalmitoyl phosphatidylcholine, stearylamine, vitamin E and cholesterol. For MLVs, the lipid mixture was dissolved in chloroform and evaporated to dryness in a glass flask. An aqueous solution of 100 mg mL^{-1} desferrioxamine was then swirled until complete dissolution. For REVs, the lipid mixture was dissolved in chloroform to which various concentrations of aqueous desferrioxamine solution (chloroform:aqueous solution, 3:1) were added. The two phases were vortexed and then sonicated at room temperature (21°C) for 5–10 min in a bath-type sonicator. Liposomes were obtained by evaporating the organic phase under vacuum at 42°C in a rotary evaporator. The resulting liposomal suspension, heated to 40°C, was extruded either through a 2-μm, or through a 2-μm, 0·6-μm and final 0·2 μm Nucleopore polycarbonate membrane. The non-liposomal desferrioxamine was removed by two consecutive ultracentrifugations in a Beckmann L7-55 ultracentrifuge at 200 000 *g* and 4°C for 1 h. The concentration of desferrioxamine in liposomes was calculated from the total amount of desferrioxamine in the liposomal cake, whose volume was considered equivalent to the captured volume. As the liposomal desferrioxamine concentration remained unchanged until their injection and was close to the concentration in the formulation mixture, one can assume that most of the desferrioxamine injected was encapsulated in liposomes.

Transmission electron microscopy of liposomes

Liposomes were fixed for 1 h in 0·1 M cacodilate buffer containing 2% glutaraldehyde and 8% tannic acid at pH 7·3. Subsequently they were rinsed with buffer and fixed with 1% buffered osmium tetroxide for 1 h, then stained en bloc with 1% uranyl acetate. After dehydration with graded series of ethanol solutions, they were embedded in Spurr's low viscosity medium, cut and stained with Reynold's lead citrate for 1 min. The samples were examined in a Zeiss EM 9A transmission electron microscope.

Animal experiments

Female Sprague-Dawley rats, 180–250 g, were used. All the animal experiments received the approval of the Ethics Committee of the Technion Medical School. The rats were injected intravenously (via the tail) with 4 mL kg^{-1} liposome suspension in saline containing 100 mg kg^{-1} desferrioxamine or with an equivalent solution of free desferrioxamine.

Since the desferrioxamine dose was kept constant, the animals given 2-μm-extruded MLVs received 140 mg kg^{-1} total lipid, of which 1·84 mg kg^{-1} was vitamin E, while animals given 2-μm-extruded REVs received 104 mg kg^{-1} total lipid, of which 4·4 mg kg^{-1} was vitamin E. Animals given 0·2-μm-extruded MLVs or REVs received 253 or 157 mg kg^{-1} total lipid, and 3·33 or 6·64 mg kg^{-1} vitamin E, respectively. At various time intervals after the liposome injection (up to 40 h), the animals were killed by excess anaesthesia with ether and the lungs, liver and spleen were quickly removed. Blood was withdrawn from the heart.

Bronchoalveolar lavage (BAL)

BAL was performed on sodium pentobarbitone (80 mg kg^{-1}) anaesthetized animals by three consecutive lavages, each with 6 mL saline, prewarmed to 37°C. The collected fluid was centrifuged at 1200 rev min^{-1} at 4°C for 10 min. For the determination of [^{3}H]cholesterol and of desferrioxamine in the cell-free lavage (BALF), the supernatant was concentrated by evaporation in a Speed-Vac centrifuge (SC-100 Savant). The residue was reconstituted in 0·5 mL water and treated as an organ homogenate. The cell pellet was dissolved in 1 M NaOH to a final volume of 0·5 mL which was further treated as an organ homogenate.

Analytical methods

Analysis of desferrioxamine. The lungs, liver, and spleen were homogenized (Kinematica) under cooling in a 10 mM phosphate-buffered saline (PBS) solution containing 0·2% Triton X-100. Deproteinization was carried out with 10% sulphosalicylic acid (v/v) and desferrioxamine was determined in the supernatant. For the determination of liposome desferrioxamine (non-biological samples) only a liposome disruption step in 0·2% Triton X-100 was carried out. To a 1-mL sample, 5 μL 25 mg mL^{-1} $FeCl_3$ solution in methanol was added and the mixture was injected into an HPLC (LKB) equipped with a 20 μL loop, according to a modified Van der Horst et al (1986) technique, using a Lichrosorb RP8 column (250 × 4). The mobile phase consisted of 15% acetonitrile in 20 mM phosphate buffer and 2 mM EDTA (pH 6·2); the flow rate was 1 mL min^{-1}. The detector (Jasco Uvidec 100V) was set at 430 nm.

Analysis of vitamin E. A modified Pacht et al (1986) method was used. Briefly, 0·1 mL saturated KOH was added to 0·1 g tissue homogenized in 2 mL methanol, containing 1% vitamin C. The mixture was heated at 70°C for 30 min and after cooling it was extracted with 3·5 mL hexane. For non-biological samples such as liposome suspensions, the homogenization and heating steps were omitted. After hexane evaporation under nitrogen at 25°C, the lipid residue was redissolved in methanol and injected onto an 100RP-C18 Lichrosphere column (250 × 4) equipped with a 20 μL loop injector. The flow rate was 1·5 mL min^{-1}, the mobile phase consisted of 87% methanol, 10% *n*-butanol and 3% water and the detector was set at 295 nm.

Determination of [^{3}H]cholesterol in blood and tissues. For this purpose, 0·1–0·2 g tissue was solubilized in 1 mL Soluene-350 (Packard); 19 mL of Pico-fluor (Packard) was added to each sample and counted in a liquid scintillation counter (LKB).

Statistical evaluation of results

The statistical analysis of data was carried out using the unpaired Student's *t*-test.

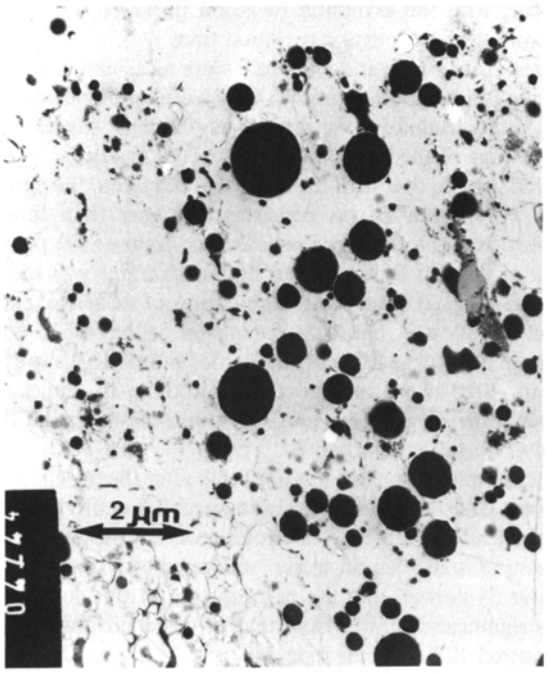

FIG. 1. Transmission electron photomicrograph of a typical reversed evaporation vesicle extruded through a 2-μm membrane.

Results

Evaluation of liposome formulations

A typical transmission electron micrograph of REVs extruded through a 2-μm membrane is shown in Fig. 1. As can be seen, the liposomes were not of a uniform size, but the largest ones did not exceed the pore diameter of the membrane through which they were extruded.

The effect of various formulations and preparation techniques on liposomal desferrioxamine concentration and aqueous volume captured per amount of lipid (capture volume mol^{-1}), is given in Table 1. The capture volume obtained after extrusion through a 0·2-μm membrane is lower than that of liposomes extruded through 2-μm membrane. For MLVs extruded through 0·2 μm, the highest capture volume was observed in liposomes prepared from a formulation containing 7% by weight stearylamine. As expected, REVs had a higher capture volume than MLVs.

The capture volume of REVs made in 100 mg mL^{-1} desferrioxamine was higher than that of REVs made in 60 mg mL^{-1} desferrioxamine, but their desferrioxamine concentration was lower. This indicates that REVs made in 100 mg mL^{-1} desferrioxamine swell during washes in saline. A similar trend was observed in MLVs prepared without cholesterol, which emphasizes the role of cholesterol in stabilizing liposomal membranes.

The vitamin E incorporated in MLVs and REVs was evaluated for liposomes prepared from identical lipid mixtures with varying concentrations of cholesterol. The results given in Fig. 2 show that, when the proportion of cholesterol in the formulation was increased, the vitamin E content of both types of liposomes decreases. The effect is more pronounced for the MLVs than for REVs. Thus, in REVs prepared from 29% cholesterol, the vitamin E content was about three times that in MLVs.

In-vivo evaluation of various types of liposomes

MLVs and REVs with the highest liposomal desferrioxamine concentration and two different sizes (extruded through 2 or 0·2 μm) were selected for in-vivo studies. The selected liposomes were prepared from a lipid mixture containing 57% dipalmitoyl phosphatidylcholine, 29% cholesterol, 7% stearylamine and 7% vitamin E (by weight). MLVs were prepared in 100 mg mL^{-1} desferrioxamine and REVs in 60 mg mL^{-1} desferrioxamine.

The in-vivo evaluation was carried out by the determination of the percent dose of desferrioxamine in various organs after the administration of liposomes and of free (non-encapsulated) desferrioxamine, all of them at a dose of 100 mg kg^{-1}. The results (Table 2) show that the administration of liposome-encapsulated desferrioxamine results in tissue desferrioxamine levels considerably higher than those obtained from the administration of the free drug.

From the percent dose, given in Table 2, and of vitamin E, given in Table 3 in various organs, it is evident that the uptake of all evaluated liposomes was higher in liver and spleen than in the lungs. This feature, well documented in

Table 1. Effect of liposomal type, size and formulation on liposomal desferrioxamine concentration and liposomal capture volume.

Membrane pore size (μm)	Formulation				Liposomal desferrioxamine (mg mL^{-1})	Capture volume (mL μmol^{-1})
	Dipalmitoyl phosphatidylcholine (%)	Cholesterol (%)	Stearylamine (%)	Desferrioxamine (mg mL^{-1})		
Multilamellar vesicles						
2·0	74·4	9·3	9·3	100	87·5 ± 11·0	2·27 ± 0·21
0·2	74·4	9·3	9·3	100	83·1 ± 9·1	1·32 ± 0·13
0·2	79·7	9·3	4·0	100	72·3 ± 14·8	1·01 ± 0·15
0·2	76·7	9·3	7·0	100	82·4 ± 6·2	1·32 ± 0·07
0·2	86·0	0	7·0	100	56·4 ± 4·4	1·65 ± 0·09
0·2	57·0	29·0	7·0	100	90·0 ± 3·8	1·41 ± 0·07
Reverse evaporation vesicles						
2·0	57·0	29·0	7·0	100	35·9 ± 2·1	5·26 ± 0·46
2·0	57·0	29·0	7·0	60	59·4 ± 2·9	3·04 ± 0·13
0·2	57·0	29·0	7·0	60	58.7 ± 3·1	2·04 ± 0·22

Results are the mean ± s.d. of 3–6 separate liposomal preparations.

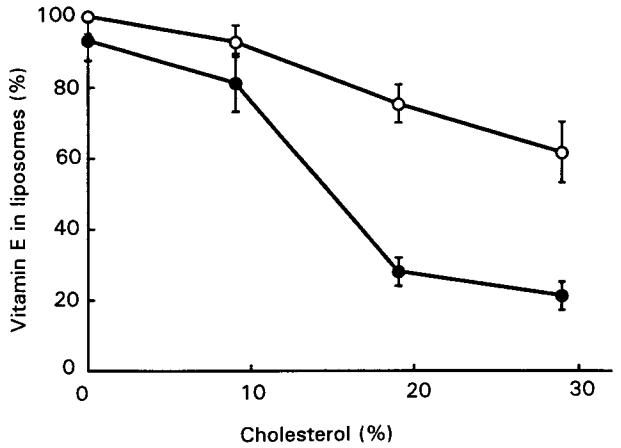

FIG. 2. The effect of cholesterol used in formulation on vitamin E in liposomes. The lipid formulation mixture contained: 7% stearylamine, 7% vitamin E, 86% dipalmitoyl phosphatidylcholine and cholesterol. The liposome suspension was extruded through a 2-μm membrane. ● Multilamellar vesicles, ○ reverse evaporation vesicles.

the literature, may be explained by the rapid removal of liposomes from the blood by the phagocytic cells of the reticulo-endothelial system. To study the disposition of liposomal vitamin E, it is necessary to subtract from the measured amount the average concentration of endogenous vitamin E (shown in Table 4) in the given organ.

One can see from Table 2 that the percent dose of desferrioxamine in the lungs is higher for REVs extruded through a 2-μm membrane than for other liposomes (statistically different when measured 1 and 3 h after liposome administration). In other organs, the distribution of liposomes extruded through 0·2- and 2-μm membranes was similar, with the exception of blood in which the smaller liposomes had a longer circulation time.

Desferrioxamine and vitamin E were measured in various organs as a function of time up to 40 h after a single injection of 2-μm-extruded REVs; the results obtained, expressed as percentage of the injected dose, are given in Fig. 3.

One can see that with the exception of spleen, the percent dose of vitamin E was consistently higher than that of desferrioxamine. The largest difference between the percent dose of vitamin E and that of desferrioxamine was seen in plasma (Fig. 2). The rapid elimination of desferrioxamine from blood was basically completed within 15 h, while vitamin E returned to normal (endogenous) levels only at about 40 h after the liposome injection. In lungs, the elimination of desferrioxamine is also more rapid than that of liposomal vitamin E.

In the spleen, starting at about 10 h after the injection, the percent dose of desferrioxamine exceeded that of vitamin E. The variation with time of cholesterol, another lipid component of liposomes, in spleen, measured by [^{3}H]cholesterol (Table 5) showed that the percent dose of this component was significantly lower than that of desferrioxamine when measured 18 h after the injection.

The desferrioxamine levels in various organs obtained after multiple intravenous liposome injections, given every 24 h at a dose of 100 mg kg^{-1} desferrioxamine are shown in Table 6. One can see that in blood, the levels were rapidly falling to low levels (2–3 μg mL^{-1}) after each injection. In the lungs too, a rapid decrease was observed, which indicated no accumulation as a result of repeated injections. In liver and spleen, the levels increased after each injection, which shows that under the tested conditions these organs were not liposome-saturated.

Table 2. Desferrioxamine (% dose) in tissues after the intravenous administration of 100 mg kg^{-1} unencapsulated and encapsulated desferrioxamine in liposomes.

Desferrioxamine	Time (h)	Blood[a]	Lungs	Liver	Spleen
Free	1	0·37 ± 0·19	0·05 ± 0·03	0·19 ± 0·09	0·003 ± 0·002
	3	0·08 ± 0·07	0·01 ± 0·01	0·02 ± 0·01	not detectable
Multilamellar vesicles					
2 μm	1	3·79 ± 2.0	2·13 ± 0·30	18·6 ± 1·5	14·0 ± 5·4
	3	0·09 ± 0·05	0·85 ± 0·34	20·5 ± 3·6	15·3 ± 4·8
0·2 μm	1	14·9 ± 3·1	1·68 ± 0·20	13·2 ± 2·5	18·8 ± 3·5
	3	7·8 ± 2·3	0·80 ± 0·10	16·8 ± 2·3	15·7 ± 2·2
Reverse evaporation vesicles					
2 μm	1	5·2 ± 0·5	3·4 ± 0·2	19·8 ± 2·6	15·3 ± 1·9
	3	3·4 ± 0·2	2·1 ± 0·2	23·1 ± 0·4	13·8 ± 2·7
0·2 μm	1	13·2 ± 2·5	1·75 ± 0·67	16·0 ± 1·2	15·9 ± 0·1
	3	5·1 ± 1·2	0·5 ± 0·21	16·1 ± 0·5	17·4 ± 4·8

Table 3. Liposomal vitamin E (% dose) in tissues, after a single intravenous injection of liposomes containing 100 mg kg^{-1} encapsulated desferrioxamine.

Liposome type	Time (h)	Blood	Lungs	Liver	Spleen
Multilamellar vesicles	1	8·5 ± 4·8	8·2 ± 1·2	30·7 ± 11·8	19·0 ± 3·7
	3	4·0 ± 1·7	3·3 ± 1·9	35·3 ± 4·5	20·1 ± 10·2
Reverse evaporation vesicles	1	33·4 ± 6·8	5·2 ± 0·7	36·1 ± 6·7	24·1 ± 0·1
	3	4·6 ± 0·6	2·9 ± 0·5	34·9 ± 2·3	19·9 ± 0·3

All liposomes were extruded through a 2-μm polycarbonate membrane. Results are the mean ± s.d. of 4–6 animals.

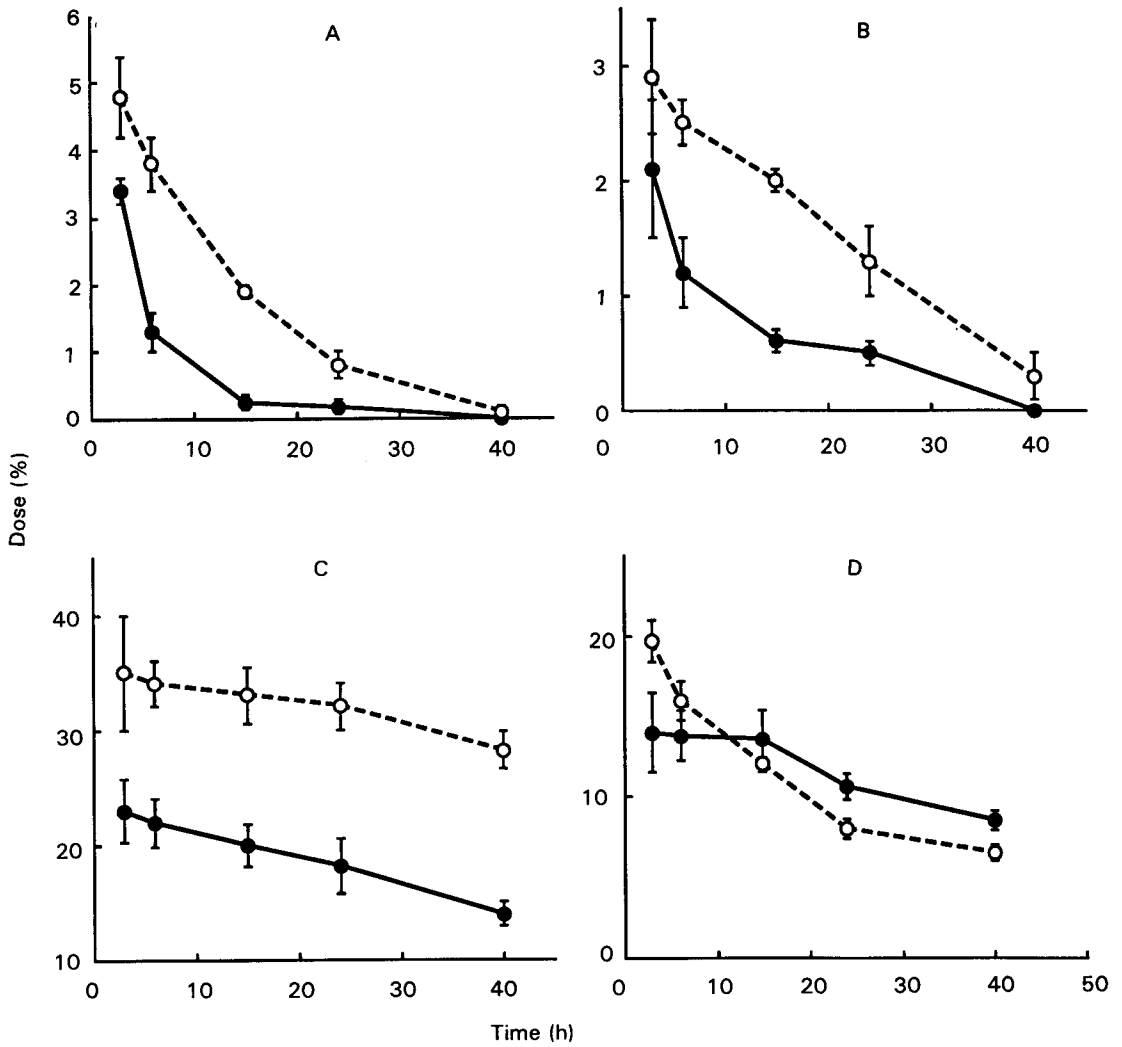

FIG. 3. Desferrioxamine and vitamin E in tissues after a single intravenous injection of liposome-encapsulated desferrioxamine in reverse evaporation vesicles, extruded through a 2-μm membrane, at a dose of 100 mg kg^{-1}. A, Blood; B, lungs; C, liver; D, spleen. ● Desferrioxamine, ○ vitamin E. Mean ± s.d. for 4–9 animals.

Liposomal components in BALF and alveolar macrophages

The desferrioxamine content (in μg and percentage dose) of the alveolar macrophages isolated from the bronchoalveolar lavage, as well as that of the cell free lavage (BALF) at 3 and 18 h after a single liposome injection of 100 mg kg^{-1} desferrioxamine are given in Table 7. The percentage dose of [^{3}H]cholesterol as a lipophilic liposomal component is also shown in this table. It can be seen that the desferrioxamine content in BALF was higher than that in alveolar macrophages. In macrophages, the percentage dose of liposomal [^{3}H]cholesterol was higher than that of desferrioxamine. The percentage dose of [^{3}H]cholesterol in macrophages and BALF increased between 3 and 18 h, whereas that of desferrioxamine remained unchanged.

Table 4. Endogenous levels of vitamin E (μg (g tissue)$^{-1}$) in tissues of control rats.

Plasma	6·88 ± 1·14
Lungs	50·2 ± 0·29
Liver	35·4 ± 0·49
Spleen	59·2 ± 0·80

Results are the mean ± s.d. for nine control animals.

Discussion

In the present research we found (Table 1) that the extrusion of the liposomes through progressively decreasing pore diameter membranes lowered the liposomal capture volume. This finding disagrees with the results of Olson et al (1979) who demonstrated a reverse effect when the extrusion was carried out in a solution of high lipid concentration. The discrepancy cannot be explained by lipid losses during the preparation process, since the losses obtained (12·5–24%, depending on formulation and extrusion type), determined by measurements of inorganic phosphate, cholesterol and vitamin E in liposomes (results

not shown), were low and cannot account for the significant decrease of capture volume. The only possible explanation for this behaviour would be an interaction between desferrioxamine and the liposomal membrane, leading to the formation of liposomes with lower capture volume.

Increasing the percent cholesterol in the formulation resulted in liposomes with lower vitamin E (Fig. 2). The significant difference between the vitamin E content of MLVs and of REVs is based on the method of preparation: in the case of MLVs the inclusion of vitamin E is a consequence of the thermodynamically attained equilibrium, while for REVs the vitamin E was forced into the liposomes during the evaporation of the organic phase. This oversaturation of REVs with vitamin E is evident in the in-vivo experiments; the percentage dose of blood vitamin E measured 1 h after injection is much higher for REVs than for MLVs. Considering the fact that the percentage dose of vitamin E in the liver was higher than that of [^{3}H]cholesterol, one might assume that vitamin E was redistributed shortly after injection from circulating liposomes to the liver.

As expected, due to the rapid elimination of non-liposome-associated desferrioxamine from the body, the percentage dose of desferrioxamine in blood, lungs and liver was lower than that of vitamin E. This trend was reversed in the spleen, where the percentage dose of desferrioxamine was higher than that of vitamin E and of [^{3}H]cholesterol, when measured a few hours after the liposome injection. A possible explanation of this finding is that after the internalization of liposomes in reticulo-endothelial cells which are abundant in the spleen, desferrioxamine leaks from liposomes and binds the available iron in which these cells are rich. In this way, desferrioxamine is retained in the spleen much longer than the lipophilic liposomal components vitamin E and cholesterol.

The rapid elimination of desferrioxamine from lungs after a single or repeated liposome injection is in agreement with other studies, in which it was shown that in this organ liposomes are predominantly entrapped in blood capillaries and the amount of liposomes which can be deposited is limited (Poste 1983). Presumably, due to their high flexi-

Table 5. Liposomal cholesterol, vitamin E and desferrioxamine (% dose) in tissues after a single intravenous liposome injection containing encapsulated desferrioxamine (100 mg kg^{-1}).

Liposomal component	Time (h)	Lung	Liver	Spleen
[^{3}H]Cholesterol	3	3·0 ± 0·3	17·7 ± 1·5	22·6 ± 0·32
	18	1·4 ± 0·4	12·3 ± 3·9	11·8 ± 0·47
Desferrioxamine	3	2·1 ± 0·6	23·1 ± 0·4	13·8 ± 2·7
	18	0·5 ± 0·2	19·2 ± 2·6	13·5 ± 1·4
Vitamin E	3	2·9 ± 0·5	34·9 ± 2·3	19·7 ± 3·6
	18	1·6 ± 0·2	33·2 ± 5·4	11·2 ± 0·4

Reverse evaporation vesicles were extruded through a 2-μm membrane. Results are the mean ± s.d. of 4–6 animals.

Table 6. Desferrioxamine in blood and tissues after consecutive intravenous injections of desferrioxamine (100 mg kg^{-1}) encapsulated in liposomes.

Injection number	Time[a] (h)	Blood (μg mL^{-1})	Lungs (mg g^{-1})	Liver (mg g^{-1})	Spleen (mg g^{-1})
1	6	48·0 ± 0·6	0·42 ± 0·03	0·99 ± 0·18	6·1 ± 0·9
	24	2·9 ± 0·1	0·14 ± 0·02	0·92 ± 0·09	5·3 ± 0·7
2	6	8·4 ± 0·2	0·43 ± 0·04	1·52 ± 0·10	10·0 ± 0·6
	24	2·0 ± 0·5	0·09 ± 0·03	1·30 ± 0·11	8·5 ± 0·5
3	6	5·2 ± 0·7	0·32 ± 0·03	2·28 ± 0·23	14·4 ± 1·2
	24	1·8 ± 0·2	0·05 ± 0·02	1·91 ± 0·10	11·3 ± 0·3

[a] Time after each consecutive liposomal injection given every 24 h. Desferrioxamine was administered in 2-μm extruded reverse evaporation vesicles. Results are the mean ± s.d. of three animals.

Table 7. Liposomal cholesterol and desferrioxamine recovered BALF and alveolar macrophages (AM) after a single liposome injection.

Time (h)	[^{3}H]Cholesterol (% dose)		Desferrioxamine (% dose)		Desferrioxamine (μg/rat)	
	AM	BALF	AM	BALF	AM	BALF
3	0·0028 ± 0·0014	0·0078 ± 0·0014	0·00077 ± 0·00028	0·0060 ± 0·0040	0·20 ± 0·05	1·64 ± 0·88
18	0·037 ± 0·0165	0·063 ± 0·0127	0·00064 ± 0·00037	0·0061 ± 0·0024	0·16 ± 0·11	1·57 ± 0·69

Reverse evaporation vesicles were extruded through a 2-μm membrane. Results are the mean ± s.d. of 4–6 animals which received desferrioxamine (100 mg kg^{-1}) liposomes by intravenous injection.

bility, related to fewer lamellae, REVs extruded through 2 μm can penetrate deeper and be more effectively entrapped then a comparable MLV.

It has previously been shown that the penetration of liposomes into the alveolar space is facilitated by their endocytosis by alveolar macrophages (Poste et al 1982; Poste 1983). In the present work, the amount of desferrioxamine transported to lungs via alveolar macrophages was quantitatively evaluated. Since the number of macrophages recovered from BAL was about $2-5 \times 10^6$ cells per rat and their volume, as reported by Haies et al (1981) was about 0·7 μL/10^6 cells, the total volume of macrophages recovered from BAL was 1·4–3·5 μL. Considering this cellular volume, one could calculate that the intracellular desferrioxamine concentration in macrophages was in the range of 50 to 150 μg mL^{-1} (about 0·2 μg desferrioxamine/rat), which indicates that a significant amount of desferrioxamine was delivered to the lung extravascular space by macrophages. The desferrioxamine recovered from BALF was even higher; about 1·6 μg/rat. This desferrioxamine could either be released from macrophages or delivered by diffusion from leaky liposomes entrapped in lung vascular capillaries. The first possibility is supported by the fact that the percentage dose of [^{3}H]cholesterol was higher than the percentage dose of desferrioxamines in macrophages indicating a leaking of desferrioxamine from macrophages. As the lung weight of the rats used in these experiments was about 1 g, the total alveolar fluid in which the macrophages are suspended is much less than 1 mL. Thus, the desferrioxamine concentration in the alveolar space, 18 h after the liposome injection, was much higher than that measured at that time in blood (2–3 μg mL^{-1}).

The increase in [^{3}H]cholesterol in macrophages measured at 3 and 18 h after liposome administration, suggests that more liposomes were transported to the lung via macrophages. As the desferrioxamine levels in macrophages and in BALF were relatively independent of time, it is possible that desferrioxamine leaked from macrophages and returned by diffusion to blood circulation or entered other cells.

The relatively high concentrations of desferrioxamine in the vicinity of alveolar cells which are usually the target of oxidative damage might be of significant therapeutic importance.

Intratracheal instillation of liposome encapsulated drugs offers a direct route to deliver large quantities of therapeutic agents to lungs. In the past, the liposome-encapsulated superoxide dismutase and catalase delivered by such routes (Padmanabhan et al 1985) had a protective action against pulmonary oxygen toxicity in rats. Recently, Suntres et al (1993) demonstrated that high and long-lasting levels of vitamin E in rat lungs are produced by intratracheal instillation of liposomes containing vitamin E, suggesting that this technique can be used as an effective prophylactic treatment against oxidant-induced lung injury. The intratracheal instillation results in discomfort to the patient and the uniformity of the drug distribution by this technique in the lungs is questionable. We have recently investigated the delivery of liposome-encapsulated drugs by aerosol inhalation, a technique which has lately gained attention (Taylor & Newton 1992). Our preliminary results indicate that desferrioxamine-containing liposomes are not stable during nebulization and a significant amount of encapsulated hydrophilic liposome content is lost. Nevertheless, this technique is promising for liposome-encapsulated lipophilic drugs, such as vitamin E, where the drug remains embedded in the phospholipid core of the liposome.

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