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Research Papers

Effect of liposomes on percutaneous penetration of lipophilic materials

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Summary

Liposomes were prepared of two lipophilic drugs, dl-α-tocopherol nicotinate (TN) and the anti-inflammatory substance, L44O. In the case of dl-α-tocopherol nicotinate liposomes, oleoyl-hydrolyzed animal protein (OHAP) was added in order to control the vesicle size. The skin penetration ability of both drugs from liposomal gels into human stratum corneum was determined in vivo by a stripping method and compared with conventional galenical formulations. The anti-inflammatory effect of the released L44O was examined in the ear edema model in mice. The penetration tests showed significantly higher absorption rates for both drugs after application of the liposomal preparations in comparison to other topical formulations. However, only a slight dependence of the extent of drug permeation into the stratum corneum on liposome diameter was observed. The pharmacological effect of L44O was investigated in the mouse ear edema test (Young et al., *J. Invest. Dermatol.*, 80 (1982) 48–52; and *J. Invest. Dermatol.*, 82 (1984) 367–371). No difference was found between the liposome preparation and an o/w base, whereas a w/o base yielded only a 50% reduction in edema.

Introduction

Topical application of liposome preparations has been shown to enhance or actually enable the penetration of vesicle-bound drugs into human skin in comparison to other galenical formulations (Bonnekoh and Mahrle, 1990). Although the mechanism of enhancement of this effect is not clear, it does appear to be strongly dependent on liposomal parameters, such as the number of lamellae, surface charge and particle size (Artmann et al., 1990).

Topical application of tocopherol nicotinate (TN), the nicotinic acid ester of vitamin E, increases blood circulation in the epidermal microcapillaries as compared to the traditional nicotinic acid esters but does not exhibit the undesirable side effects of the latter, such as erythema, burning, itching and flush (Vogel et al., 1987).

L44O (2-(*t*-butyl)-4-cyclohexylphenylnicotinate *N*-oxide) gives rise to in vitro inhibition of 5-lipoxygenase and cyclooxygenase activity, i.e., development of inflammation (Michel, 1990b; per-

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sonal communication). This agent exerts bacteriostatic effects on the growth of *Staphylococcus aureus* and *Propionibacterium acnes* (Schäfer, 1990; personal communication). The dermal application of L44O has been reported to exhibit anti-inflammatory activity (Michel, 1990a; personal communication), which is probably due to interruption of the arachidonic acid cascade, as demonstrated in vitro.

However, both substances are highly lipophilic (Table 1) and have only a poor ability for penetration into the skin. The extent of skin permeation cannot be increased in the usual manner by the use of galenical formulations. The degree of release of the substances from ointments and creams is extremely low as a result of the high partition coefficients. In ethanolic solutions, they may crystallize on the skin whilst the ethanol evaporates.

Therefore, liposomal preparations were considered to be much more suitable formulations due to their ability to mediate the penetration of therapeutic amounts of poorly permeable lipophilic drugs into the skin, and capability of sustained drug release (Bonnekoh and Mahrle, 1990).

Materials and Methods

Chemicals

Tocopherol nicotinate (TN) was obtained from Nutrilo (Cuxhafen, Germany); L44O was synthesized and supplied by Merz & Co (Frankfurt, Germany); pure soybean lecithin (PL 90) was purchased from Nattermann (Cologne, Germany); oleoyl-hydrolyzed animal protein (OHAP; Dehymuls E®, Cetiol V® and Lanette 16®) was obtained from Henel (Düsseidorf, Germany); Tween 60® and Span 60® were obtained from Atlas Chemie (Essen, Germany); Miglyol 812® was purchased from Dynamit Nobel (Troisdorf, Germany); phenoxyethanol came from Riedel de Haen (Seelze, Germany); Carbopol 940® was supplied by Goodrich (Neuss, Germany) and Tris was from E. Merck (Darmstadt, Germany). Arachidonic acid (AA) and butylhydroxytoluene were obtained from Sigma (Deisenhofen, Germany), Eucerinum anhydricum® being from Beiersdorf (Hamburg, Germany). All chemicals were of the purest grade available.

Preparation of liposomes

TN liposomes The liposomes were prepared by melting a mixture of soy lecithin, OHAP, TN, butylhydroxytoluene, phenoxethol and ethanol on a water bath at moderate temperature. The addition of buffer solution pH 6.5 formed a multilamellar vesicle (MLV) solution which was subsequently homogenized under high pressure (15 000 psi) by passage through about 25 cycles on a Nanojet® (Nanojet Engineering, Düsseldorf, Germany). The resulting small unilamellar vesicle (SUV) dispersion (GP application, 1991) was used to prepare a 1% Carbopol® gel in the usual manner. The lipid concentration in this hydrogel was 100 mg/g.

L44O liposomes The lipids were dispersed into a suitable volume of phosphate-buffered saline (PBS) pH 6.5, already containing the preservative. To the resulting MLV dispersion an ethanolic solution of L44O was admixed using an Ultraturrax® (IKA, Stauffen, Germany), and the drug-containing MLVs were reduced in size and homogenized by means of a French Pressure Cell® (Amicon, U.S.A.). After addition of a mixture of Carbopol 940®/Tris to the resulting SUV dispersion and completion of swelling, the liposomal gel was ready for use (Table 2).

Preparation of other formulations

Wool alcohol ointment Wool alcohol ointment DAB 9 (Eucerin® anhydricum) has the following composition: wool wax alcohols, 6.0%; cetylstearyl alcohol, 0.5%; vaseline, 93.5%. TN containing ointment (2%) was prepared by melting the lipid phase and added drug at about 50°C. The mixture was then stirred during cooling.

o/w and w/o base containing 1% L44O The preparations were carried out in accordance with common pharmaceutical rules and the various compositions are listed in Table 2. The lipid phase was melted at about 70°C. L44O and the aqueous phase, which was also heated to 70°C, were admixed. The mixtures were stirred throughout the cooling period (Table 2).

TABLE 1

Physical properties of dl-α-tocopherol nicotinate and L44O

Substance	dl-α-Tocopherol nicotinate	L44O
Molecular weight	535.82	353.46
Melting point	38°C	176.2°C
Solubility	– freely soluble in acetone, ether, chloroform, ethanol – insoluble in water – miscible with oils and fats	– almost insoluble in water (< 0.1%) – slightly soluble in ethanol – freely soluble in chloroform
Partition coefficient	26.3	
Stability	– practically stable to heat and oxygen – affected only by strong acids and alkalis – gradually darkens on exposure to light	– hydrolyzed in aqueous medium to substituted phenol and nicotinic acid *N*-oxide

Characterization of liposomes

The liposomes as well as the gel preparations were characterized on the basis of the most important physical parameters of the vesicles such as size, size distribution and number of lamellae. Particle diameter and size distribution were assessed by proton correlation spectroscopy using an Autosizer IIc® (Malvern, Herrsching, Germany). These parameters as well as vesicle shape and lamellarity were additionally determined by freeze-fracture electron microscopy (Sternberg et al., 1989).

TABLE 2

Difference between the total accumulated amount absorbed of L44O from all test preparations and the amount, assessed at a depth of 1 μm in the stratum corneum

Liposomal gel (lipid content: 10%)	w/o preparation (lipid content: 36.6%)	o/w preparation (lipid content: 29%)
PBS + preservative	Dehymuls E	Miglyol 812
PL/OHAP (ratio 8:2)	Cetiol V	Lanette 16
Ethanol	Miglyol 812	Tween 60
C940/Tris	Vaselinum alb.	Span 60
L44O (1%)	Phenoxethol	Phenoxethol
	aqua pur.	aqua pur.
	L44O (1%)	L44O (1%)

For chemical characterization, the liposomes were separated from dissolved material or non-vesicular aggregates using a photometrically controlled Sephadex® G-50 medium column. Liposome fractions were analysed for bilayer-bound drug content and lecithin concentration by performing TLC after disruption of vesicles by treatment with a sufficient amount of *n*-propanol (Mentrup, 1988).

The conditions for TN analysis were as follows: TLC plate, silica gel 60 F_{254} (E. Merck, Darmstadt, Germany); solvent: cyclohexane/ether/acetone/acetic acid (60:40:2:1); location: water/sulphuric acid/methanol (85:10:5), 5 min at 110°C, examination under visible light (620 nm) (Barwitzki, 1991).

For lecithin/lysolecithin analysis the conditions used were the following: HPLC plate, silica gel 60 F_{254} (E. Merck, Darmstadt, Germany); solvent: chloroform/methanol/acetic acid/water (100:60:25:10); location: 0.2 g $MnCl_2 \cdot 2H_2O$ in water/methanol/sulphuric acid (30:30:2), 5 min at 160°C, examination under visible light (420 nm) (Barwitzki, 1991).

The conditions for the analysis of L44O were: TLC plate, silica gel 60 F_{254} (E. Merck, Darmstadt, Germany); developing eluent: *n*-hexane/ether/acetic acid 4:4:2; detection: fluorescence quenching, 280 nm.

In order to confirm the results of the above measurement, the content of non-incorporated L44O was also determined after ultracentrifugation (Ultrafiltration units: Centricon 100®, Amicon, Beverly, U.S.A.) from the liposome-free solution by TLC.

To assess the maximal possible rate of inclusion of L44O for SUVs with a lipid composition of PL 90/OHAP (ratio 8:2) and a lipid content of 100 mg/ml (= 10% of the total), a series of SUV dispersions with increasing content of L44O (1, 2, 3% = 10, 20, 30% in relation to the lipid content) were prepared. In the case of the 2 and 3% L44O-containing preparations, precipitation and crystallization of L44O occurred during preparation. After the complete separation of insoluble compounds by centrifugation and filtration (0.22 μm), the amount of drug remaining solubilized in the liposomes was determined.

Penetration studies

The penetration behaviour was investigated in vivo according to a modification of the stripping method originally described by Pinkus (1951), Zesch et al. (1973) and Bredthauer (1990). According to this method, the TN formulations and L44O preparations were applied onto areas of 33.6 and 45.6 cm^2, respectively, of the proximal area of each forearm under an occlusive dressing (Okklufol®, Lohmann, Neuwied, Germany). In order to avoid intra- and interindividual differences, liposome gels and the formulations for comparison were examined in a crossover fashion on the same forearm. After 2 h, the residual preparations were completely removed. Thereafter, stratum corneum layers were completely stripped off using adhesive tape (Tesa® 4202, Beiersdorf, Hamburg, Germany) under defined conditions (pressure: 10 times with a 1 kg roller; rapid stripping off; same investigator). These samples were accurately weighed before and after stripping, the resulting layer thickness being calculated according to the following equation:

$$T = d/ap$$

where T represents the thickness of stratum corneum removed (in μm), d is the difference in strip weight after and before stripping (in μg), a denotes the area of the strip (μm^2) and p is the density of the stratum corneum (1×10^3 $\mu g/1 \times 10^{12}$ μm^3).

Each strip was then immersed in 3 ml ethyl acetate to dissolve the adhering TN or L44O. Drug content was evaluated by performing TLC under the following conditions: HPLC plate, silica gel 60 F_{254} (E. Merck, Darmstadt, Germany); (TN) solvent: cyclohexane/ether (75:30); examination under UV light (230 nm); (L44O) solvent: *n*-hexane/ether/acetic acid (4:4:2); detection: fluorescence quenching, 280 nm. Glue components of the adhesive tape did not interfere with the determinations.

Arachidonic acid-induced ear edema

Male NMRI mice (Charles River Breeding Laboratories, Sulzfeld, Germany), fed on a standard laboratory diet and allowed tap water ad libitum, were used (28–35 g body weight). The mice were anaesthetized with urethane (15%, 0.1 ml/10 g mouse i.p.). For edema induction, 4 mg arachidonic acid (AA) was applied to the inside of the left ear in 6.5 μl of 96% ethanol. The right ear was treated with ethanol alone and served as reference. The test compounds were applied topically in a volume of 25 μl to the inside of both ears 30 min before edema induction. Using a warming pad, the body temperature of the anaesthetized animals was maintained constant during the whole experiment. 60 min after edema induction, the mice were killed by cervical dislocation. Discs of 8 mm diameter were cut from both ears and weighed immediately. Edema was measured as the difference in wet weight of the tissue discs between both ears of each animal.

Statistics The mean and standard deviation (SD) of the wet weight of tissue discs from both ears were calculated as well as those of the difference in wet weight of the two ears. Inhibition of edema formation was expressed as percent vs the unpretreated control. Using the unpaired t-test (two groups t-test, two-tailed) at a significance level of $\alpha = 0.05$, the means of the differences for the control and drug-treated groups were compared.

Results and Discussion

Properties of TN liposomes

Due to TN being highly lipophilic, it underwent integration into the lipid bilayer. Consequently, the vesicle diameter grew with increasing TN content (Fig. 1). However, irrespective of TN content, the size of all these liposomes could be reduced by addition of OHAP (Fig. 2). For example, the diameter of 25 mg/ml TN-containing liposomes (102.4 ± 23.9 nm) could be halved (49.1 ± 13.9 nm) by replacing 25% (25 mg/ml) of the lecithin by the same amount of OHAP. Through such fractional substitution of lecithin with OHAP, it was possible to regulate the size of 2.5% TN-containing liposomes in the range between 39 and 102 nm.

These liposomes and the corresponding gels exhibited no changes in either liposome size or size distribution when stored at room temperature for over 6 months (Fig. 3). Additionally, in these preparations, neither drug nor lecithin showed any decrease in concentration (Fig. 4). A decrease in these components was frequently found to occur with SUVs and was also observed with OHAP-free liposomes in our studies. Hence, it can be concluded that OHAF exerts a stabilizing effect.

The content of lysolecithin remained almost constant in all cases.

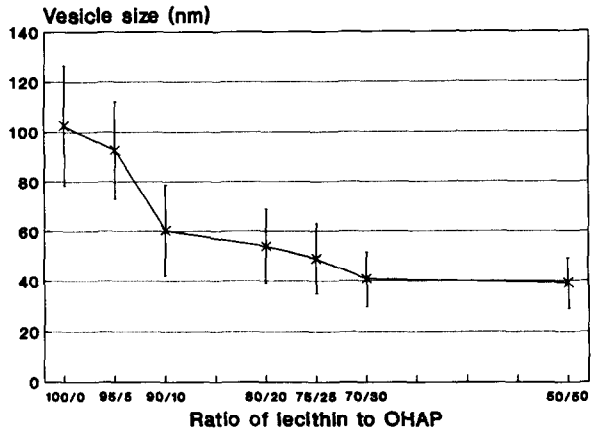

Fig. 2. Dependence of vesicle size on lecithin/OHAP ratio (TN/lipid ratio: 0.25).

Properties of liposomes containing L44O

Liposomes containing 1% of L44O were visualized in the gel by means of transmission electron microscopy (TEM) using the freeze-fracture technique (Sternberg, Schiller Universität, Jena, Germany). The TEM micrograph shows a homogeneous population of small vesicles (< 100 nm). Liposomes of size between 100 and 200 nm were occasionally observed (Fig. 5). The average size of the liposomes was calculated after measuring the diameter of all the types of vesicles located on an area of 1 cm^2, performed on three different squares selected at random. The mean size ± SD was calculated to be 36.4 ± 12.6 nm, taking into

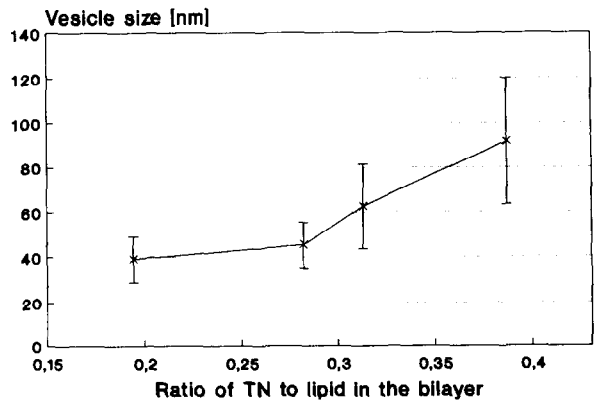

Fig. 1. Dependence of vesicle size on TN/lipid ratio in the bilayer. Concentration of both lecithin and OHAP: 50 mg/ml.

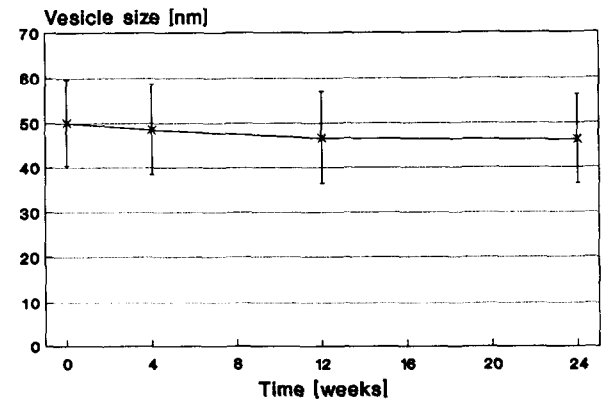

Fig. 3. Stability of vesicle size of TN liposomes in gel preparation (TN/lipid ratio: 0.25).

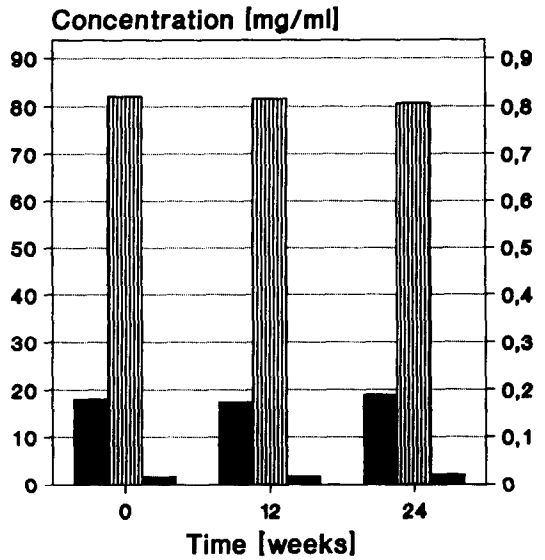

Fig. 4. Chemical stability of TN liposome gel preparation. Bars (from left to right): [TN], [lecithin], [lysolecithin].

account the magnification of the microscope (×50 625). The particle size of the liposomes was evaluated after dilution and centrifugation of the gel base by laser light scattering (= LLS, Autosizer IIc®, Malvern, U.S.A.). A size of 22.6 ± 3.7 nm was initially determined. As can be seen in Fig. 6, the size of the vesicles showed no significant increase during storage of the liposome gel at 21°C. Determination of the vesicle size in the gel formulation by LLS after 3 months storage, performed under TEM, yielded a value of 28.8 ± 3.5 nm.

The L44O content of the liposomal gel was found to be 1.03%. 95.9% of the substance used was incorporated in the liposomes, as shown by gel filtration of the SUV dispersion. After ultracentrifugation, no traces of the agent were detectable in the liposome-free fraction. Both results confirm that incorporation of L44O into the liposomal membranes was complete. This was expected, based on its hydrophobicity. The maximum amount of L44O incorporated using liposomal preparations with a lipid content of 100 mg/ml and a lipid composition of PL 90/OHAP in a ratio 8:2 was evaluated to be, on average, 11.2 mg/ml L44O. This corresponds to a liposomal preparation containing maximally 1.12% L44O.

In vivo penetration experiments

Tocopherol nicotinate In in vivo experiments the liposome gel was compared to the standard formulation of the German Pharmacopeia DAB 9, the wool alcohol ointment (Eucerin® anhydricum). The amount of drug penetrating into the stratum corneum layers varied among individuals. Therefore, the experiments were designed as a crossover trial using the same forearm of each volunteer. In Fig. 7 the results obtained on one volunteer are shown as example. Altogether, the plots of the amount penetrating vs depth of penetration run approximately parallel with a 2.7-fold higher total amount absorbed with the liposome preparation in comparison to the wool alcohol formulation (SD = 1.1; $n = 6$). The maximal observable depth of drug penetration with the liposome preparation was 8.3 μm (SD = 2.3; $n = 6$) as compared to 5.1 μm (SD = 2.9; $n = 6$) with the ointment.

Using the same stripping technique and experimental design, it could be demonstrated that SUVs (50.6 ± 8.6 nm; freeze-fracture electron micrograph; Fig. 8) were superior to MLVs (242.4 ± 98.8 nm; Fig. 9) with regard to drug penetration into stratum corneum. In this case, SUVs showed a 1.5-fold (SD = 0.2; $n = 6$) enhancement in the amount of drug absorbed vs MLVs (representative data from one volunteer; Fig. 10). However, no differences in the maximal depth of drug penetration were found between the different types of liposomes.

The effect of an occlusive dressing on drug penetration into the stratum corneum is illustrated in Fig. 11 (typical data from one volunteer). Here, the penetration behavior of TN after 2 h without occlusion is compared to absorption under occlusive conditions for 0.5 and 2 h, respectively (in each case $n = 3$). Occlusive conditions for 2 h yielded a much higher degree of saturation of the stratum corneum with TN up to a depth of 2 μm. Furthermore, absorption of drug extended into deeper layers. With the SUV liposome preparations, a total penetration enhancement factor (F) of 1.4-fold was observed for both

Fig. 5. TEM freeze-fracture micrograph of 1% L44O-containing SUVs in a gel formulation (magnification, ×50 625; bar, 100 nm).

the amount of TN absorbed and the depth of penetration by TN. The penetration enhancement due to occlusion was dependent on the time of treatment.

L44O Inter-individual differences in penetration occurred, as demonstrated by the wide range of SD values determined during the in vivo measurements. This was due to the different types of skin of the volunteers. The concentrations of L44O determined per strip at a definite depth of the stratum corneum were extrapolated to the amount of drug penetrating into the skin scaled in 1 μm steps. The means ± SD were calculated for each 1 μm interval in depth for each investigation.

The penetration of L44O out of the liposomal gel into the stratum corneum at a depth of 9 μm ($n = 6$) was 2.6-fold greater than that out of the o/w preparation (Fig. 12). In total 13.7 ± 4.5% of the applied dose of liposomal L44O was found up to a depth of 9 μm whereas only 5.2 ± 1.8% of the substance from the o/w preparation penetrated to the same depth of the skin.

In a second experiment, the liposomal gel was compared to a w/o preparation. In this experiment performed with three volunteers, a 3.2-fold greater amount of L44O was found to be absorbed after application of the liposomes (Fig. 13). In total 6.6 ± 2.4% of the applied drug was found at a depth of 9 μm in the stratum corneum vs only 2.1 ± 0.7% with the w/o preparation.

A third experiment was performed in order to

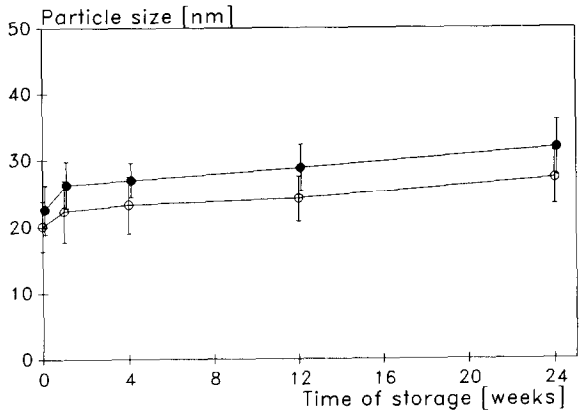

Fig. 6. Particle size of liposomes containing 1% L44O as a dispersion (○——○) and as a gel formulation (●——●). Lipid used: pure soybean lecithin/OHAP (8:2); 100 mg/ml; storage temperature, 21°C.

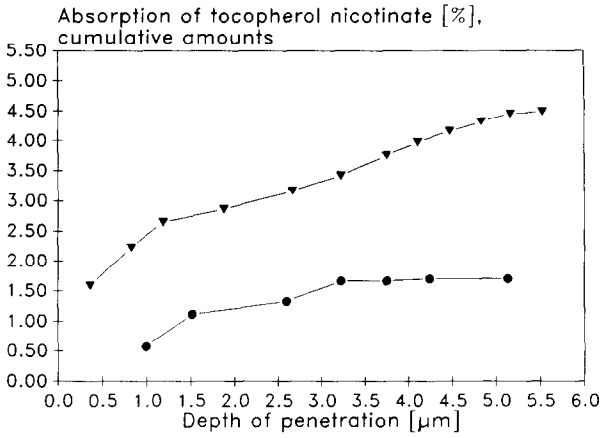

Fig. 7. Absorption of TN under occlusive conditions: liposome gel (▼——▼) vs Eucerin® anhydricum ointment (representative data from one volunteer). Enhancement factor, $F = 3.0$, liposome/Eucerin® anhydricum ointment. Percentage related to the amount of TN applied (0.35 mg/cm^2); liposome gel and Eucerin® anhydricum ointment: TN content 2%.

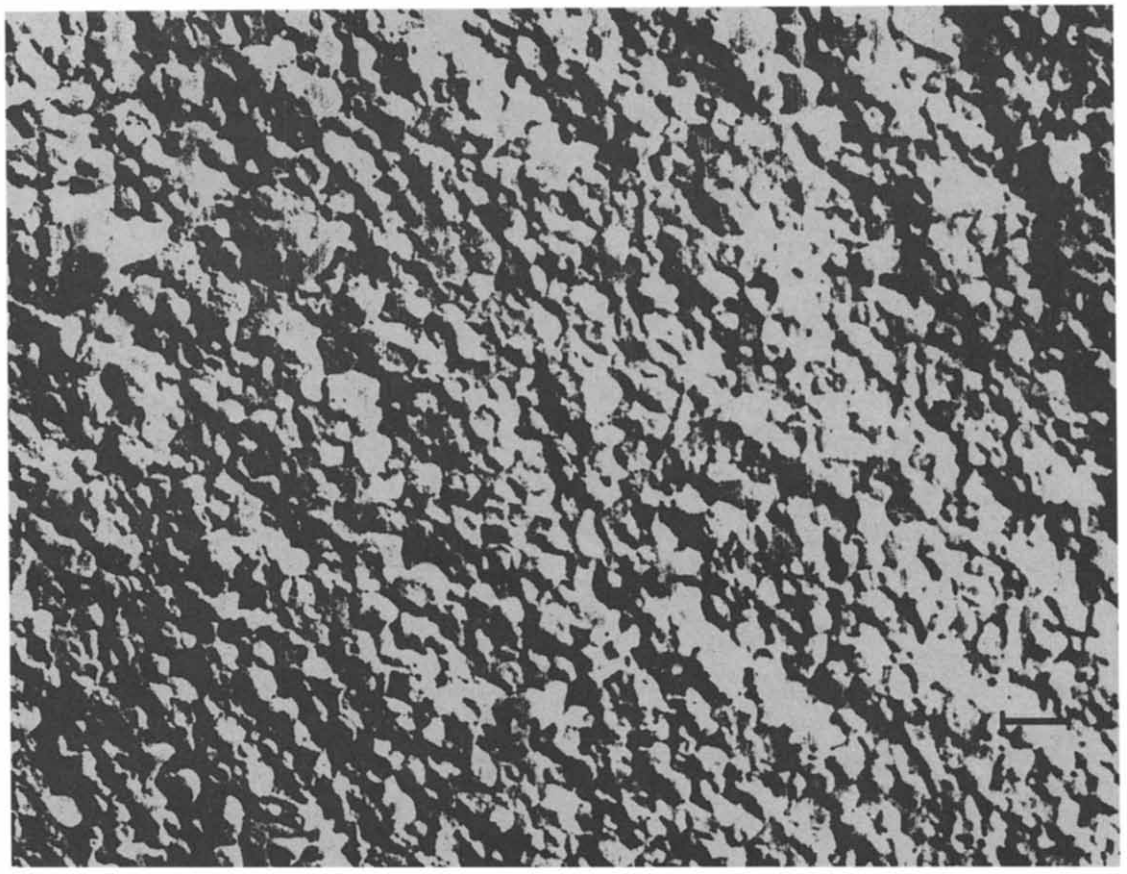

Fig. 8. TN SUVs (bar, 100 nm).

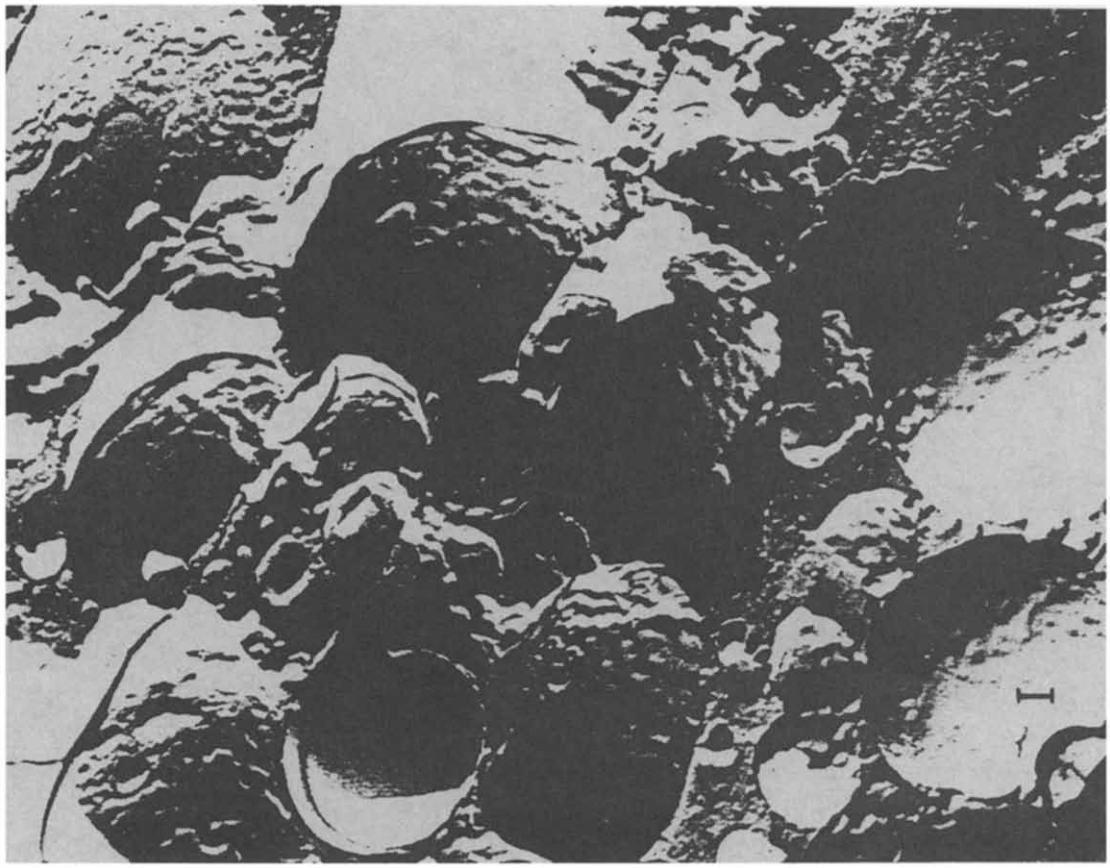

Fig. 9. TN MLVs (bar, 100 nm).

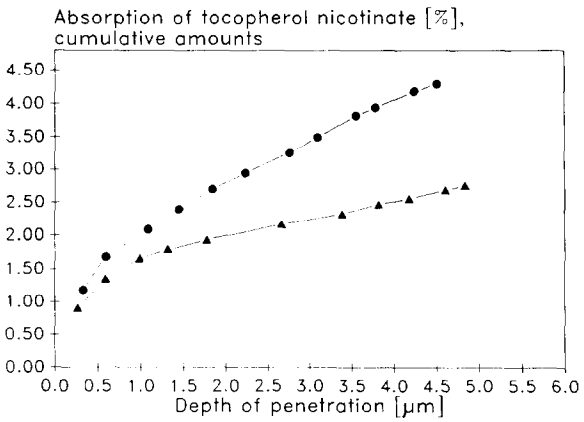

Fig. 10. Absorption of TN under occlusive conditions: SUV-liposome gel (●———●) vs MLV-liposome gel (▲———▲) (representative data from one volunteer). Enhancement factor, $F = 1.6$, SUV-liposome/MLV-liposome. Percentage related to the amount of TN applied (0.35 mg/cm^2); SUV- and MLV-liposome gel: TN content 2%.

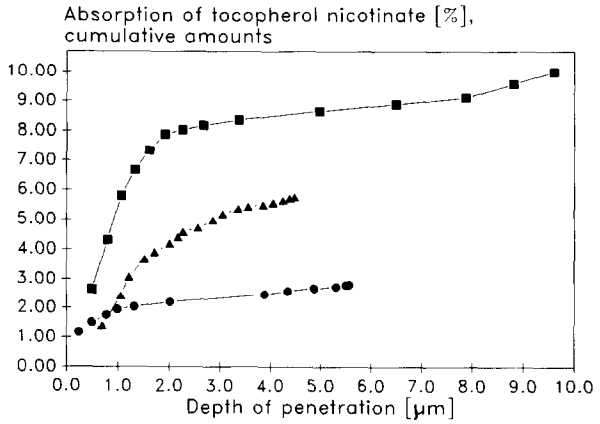

Fig. 11. Absorption of TN after application of liposome gel: non-occlusion vs occlusion (representative data from one volunteer). (■———■) Occlusion, 2 h; (▲———▲) non-occlusion, 2 h; (●———●) occlusion, 0.5 h. Percentage related to the amount of TN applied (0.35 mg/cm^2); liposome gel: TN content 2%.

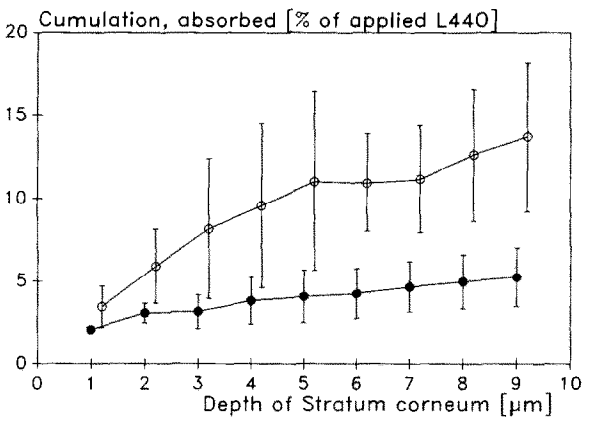

Fig. 12. Penetration of L44O into stratum corneum: comparison of liposome gel (○———○) vs o/w preparation (●———●). Dose, 5 mg; area, 45.6 cm^2; occlusive conditions for 2 h; $n = 6$ volunteers.

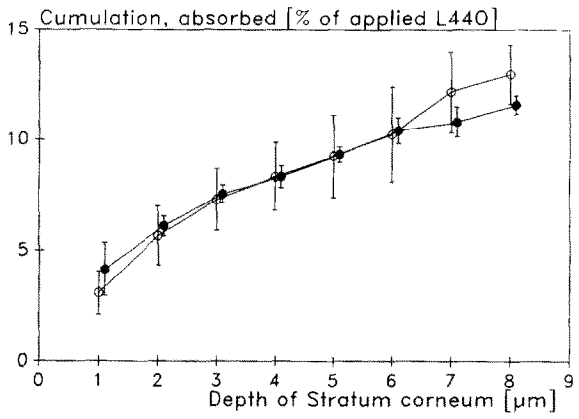

Fig. 14. Penetration of L44O into stratum corneum: comparison of SUV-liposome gel (○———○) vs MLV-liposome gel (●———●). Dose, 5 mg; area, 45.6 cm^2; occlusive conditions for 2 h; $n = 4$ volunteers; particle size: (SUV), 22.6 nm; (MLV), 122.9 nm.

compare the penetration behaviour of L44O out of an MLV liposome gel, which had not been homogenized by French Press® treatment during preparation, with that of the SUV liposome gel used in the previous investigations. The mean particle size of the drug-containing MLVs in the gel was 122.9 nm. No significant differences were found between these preparations ($n = 4$ volunteers, Fig. 14). With the 1% drug-containing SUV liposome gel, $13.0 \pm 1.4\%$ of the applied L44O penetrated to a depth of 8 µm in the stratum corneum. With the non-homogenized MLV liposome gel, penetration amounted to $11.6 \pm 0.4\%$ (factor: 1.12). Both penetration plots, determined for SUVs as well as for MLVs, were nearly congruent.

The differences between the total accumulated amount absorbed for each test preparation and the amount of drug absorbed in the first 1 µm of the stratum corneum again demonstrated the su-

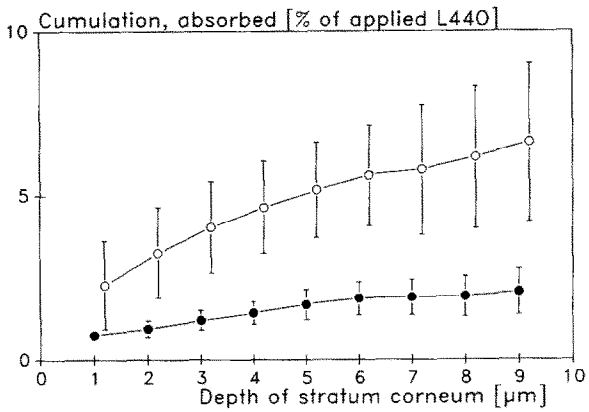

Fig. 13. Penetration of L44O into stratum corneum: comparison of liposome gel (○———○) vs w/o preparation (●———●). Dose, 5 mg; area, 45.6 cm^2; occlusive conditions for 2 h; $n = 3$ volunteers.

TABLE 3

Composition of the L44O preparations (values expressed as percentages)

Preparation	1 µm depth (% of applied dose)	Σ penetrated (% of applied dose)	Difference Σ/1 µm
o/w	2.1	5.2	3.2
SUV (compared to o/w)	3.5	13.7	10.2
w/o	0.8	2.1	1.3
SUV (compared to w/o)	2.3	6.6	4.3
MLV	4.2	11.6	7.4
SUV (compared to MLV)	3.1	13.0	9.9

periority of all liposomal preparations over the conventional formulations (o/w and w/o, Table 3).

The differences in total amount of penetrated drug with the SUV preparation between the three sets of experiments referred to above, i.e., 13.7, 6.6 and 13.0 (Table 3), may be explained on the basis of inter-individual differences between the skin of the volunteers selected at random. As can be seen in Table 3, the amount of drug at a depth of 1 μm in the stratum corneum is greater for the liposomal preparations than for the conventional types. The question of whether L44O-loaded liposomes penetrate the skin in unchanged form or act only as a penetration enhancer is currently under investigation and has been discussed in a number of studies (Abraham and Downing, 1990; Bouwstra et al., 1991).

Pharmacological effect of L44O preparations

In eight mice/group, the liposomal gel containing L44O reduced AA-induced edema significantly by 45% vs control. An ethanolic solution containing 1% L44O yielded only 25% reduction (Fig. 15).

Comparison of all three preparations containing 1% L44O and the three bases without drug was performed on groups of mice, each group

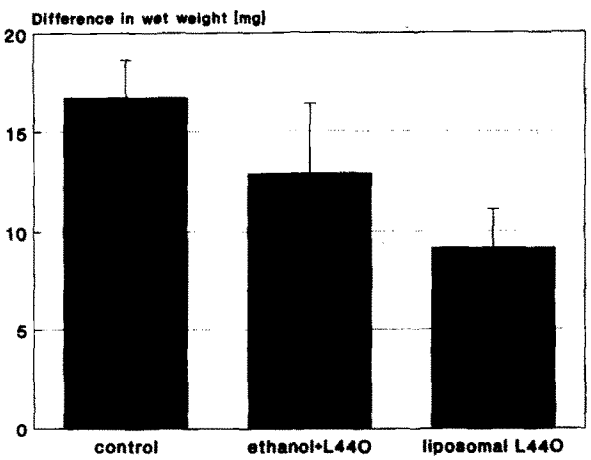

Fig. 15. Arachidonic acid-induced ear edema in mice. Topical application of 1% L44O as ethanolic solution and liposome gel. Dose, 25 μl/ear; $n = 8$/dose (mean $\pm$ SD; * $p < 0.05$ vs control). Contact time: 30 min before edema induction.

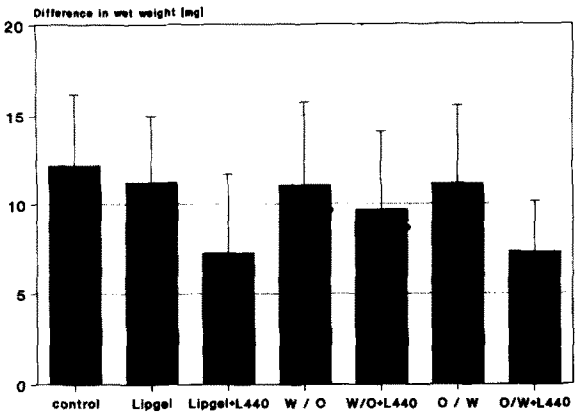

Fig. 16. Arachidonic acid-induced ear edema in mice. Comparison of liposome gel, w/o and o/w preparations. Dose, 25 μl/ear; $n = 18$/dose (mean $\pm$ SD; * $p < 0.05$ vs control). Contact time: 30 min before edema induction.

comprising 18 mice. All blank bases produced an 8% reduction in edema. 20% reduction in edema was determined for the drug-containing w/o preparation, whereas significant anti-inflammatory effects (40 and 44%) were observed for the o/w preparation and the liposomal gel (Fig. 16).

Fig. 16 demonstrates that the effect of the o/w preparation is similar to that of the liposome gel. The reduction in edema of 44 and 45% achieved for the gel in independently performed determinations (Figs 15 and 16) reflects the good reproducibility of the results.

The 8% effect of the blank bases is probably due to the film built up by the preparations on the ears after application. Thus, contact of AA with the skin and hence the development of inflammation are diminished.

The edema reduction appears to depend on the lipid content (Table 2) of the test preparations. The preparation with a higher lipid content (w/o ointment) was found to produce a smaller extent of edema reduction (Fig. 16). The lower degree of edema reduction (20%) and also the diminished release of L44O may be explained on the basis of the hydrophobicity of the agent, which has a higher affinity for the lipid phase of the vehicle than for the skin. On the other hand, a similar relatively low degree of reduction in edema of 25% was obtained after application of

an ethanolic solution of L44O (Fig. 15), despite ethanol being known to act as a penetration enhancer. Thus far, this rather contradictory result cannot be explained.

It is tempting to speculate on the mechanism of the overall enhancement of skin penetration of the two lipid drugs, TN and L44O, after delivery with liposomes. In contrast to other colloidal carriers and carrier systems (Kreuter et al., 1983; Cappel and Kreuter 1991a,b), the thermodynamic activity of these drugs in liposomes appears to play a minor role. Kreuter et al. (1981) reported a significantly ($2p < 0.01$) lower permeability of another lipophilic drug, cholesterol, in this case through a silastic membrane following delivery via multilamellar liposomes in comparison to SUVs. This could be interpreted as the result of the drug having a potentially higher thermodynamic activity in the SUV preparation. In the present studies, however, no major differences between the larger MLVs (242 or 123 nm) and smaller SUVs (51 or 23 nm) were detectable. This observation, in contrast with the findings of Kreuter et al. (1981) using an inert membrane, fails to resolve the question posed above concerning whether intact liposomes penetrate into the skin. It may be possible that membrane fragments or fully dissociated liposomal lipids act as penetration enhancers either by interacting with the skin, thereby diminishing its barrier function, or by exhibiting a solvent drag effect. Microanatomical studies are necessary to elucidate the actual mechanism of enhancement of skin penetration of these drugs by liposomes.

Conclusion

The addition of OHAP enables size reduction and size control of TN liposomes. Sizes between 39 and 102 nm were reproducibly achievable with this compound. In addition, OHAP stabilizes the liposomes and prevents the release of TN and lecithin during storage.

The penetration studies indicate that liposome formulations can enhance the penetration of TN and L44O into the skin in comparison with conventional ointment or cream formulations. However, there is only a comparatively weak dependence between the skin penetration of liposome-entrapped drug and the size of such liposomes. For tocopheryl nicotinate, only a 1.5-fold greater amount of drug penetrated with the small (50.8 ± 11.2 nm) SUVs as compared to the much larger MLVs (242.4 ± 98.8 nm). For L44O, penetration from SUVs (22.6 nm) and MLVs (122.9 nm) was almost equal in extent.

Significant pharmacological effects of L44O liposomes and the o/w preparation were observed in the mouse edema test, whereas in the case of the w/o formulation the anti-inflammatory effect of the drug was diminished.

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