Influence of liposomal encapsulation on the penetration of retinoic acid through human skin in vitro

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Accepted May 21, 1994.

Synopsis

The penetration of liposome-encapsulated trans-retinoic acid (t-RA) through the different strata of the skin was investigated using in vitro diffusion experiments on human cadaver skin. Simple phospholipid liposomes and a more complex pH-sensitive preparation containing 0.05% t-RA were applied to isolated human stratum corneum, isolated human epidermis, and dermatomed human skin, along with one of two non-liposomal controls. The control solutions were either 0.05% t-RA in ethanol (for small-dose experiments) or saturated solutions of t-RA in Transcutol®/water mixtures (for large-dose experiments and some small-dose experiments). Skin-specific interactions were differentiated from solution thermodynamic effects by repeating some experiments using a silicone rubber membrane. The influence of dose volume and occlusion was investigated. The results showed no evidence of enhanced epidermal penetration, nor of decreased percutaneous absorption from liposomal t-RA vs unencapsulated controls under realistic dose conditions. However, under one set of exaggerated dose conditions (consisting of a large, non-occluded dose of t-RA in PC liposomes), there was a suggestion that diffusion of t-RA across the lower skin layers may have been retarded by the liposomal components.

INTRODUCTION

Over the past fifteen years liposomes have been claimed to provide topical delivery benefits for both systemically active and locally active drugs. For systemic drugs, workers have reported increased percutaneous absorption from either liposomal dispersions (1–2) or other related phospholipid formulations (3–5), leading to higher systemic levels for a given topical dose. For topical drugs, a commonly cited benefit is increased levels of active in the skin (including, in many cases, the lower epidermis and dermis), combined with decreased percutaneous absorption (6–13). This implies reduced drug clearance by the capillary bed. Other investigators have found no effects of liposomes on topical delivery (14). These findings have been reviewed by Egbaria and Weiner (15). Trans-retinoic acid (t-RA, also called tretinoin or vitamin A acid), a widely used anti-acne medication, is one of the drugs for which the skin localization benefit has been claimed (6,7,16). Given the side effects associated with systemic retinoid therapy (17),
such a change in delivery pattern would be expected to widen the safety margin associated with topical use.

Somewhat awkward for liposome proponents is the lack of a coherent explanation of how liposomes might increase drug concentrations in skin while reducing systemic absorption. The phenomenon implies that liposome components penetrate through the stratum corneum and affect drug clearance from the underlying tissues. Yet, careful investigations have failed to show appreciable concentrations of either intact liposomes or of liposome components (phosphatidylcholine or cholesterol) in lower skin layers following topical applications (6,14,18–20), and to our knowledge, no effects of liposomes on cutaneous blood flow have been reported. Thus, the mechanism for the liposome effect remains a mystery, and one is led to examine the methods by which the unexpected results were derived.

Most of the evidence for increased drug concentrations in skin following liposomal application has been obtained from skin stripping experiments. This technique, while useful for estimating drug levels in the stratum corneum, does not clearly differentiate between the stratum corneum and the underlying skin layers. We reasoned that a properly controlled technique that measured delivery rates through increasingly thick layers of skin should be able to identify formulations leading to drug accumulation in the lower skin layers. Either the input rate (flux through the stratum corneum) must be higher or the output rate (flux through the whole tissue) must be lower in order for accumulation to occur. With this in mind, we studied the flux of liposomally encapsulated t-RA vs unencapsulated controls through human stratum corneum, heat-separated human epidermis, and dermatomed human cadaver skin. We studied two liposome systems—a simple soy phosphatidylcholine system and a more complex, four-component system claimed to deliver enzymes through skin (21,22)—and compared their performance against an ethanolic vehicle and a saturated t-RA solution in transcutol/water. We also tested the influence of dosing conditions (dose volume, occlusion) on the penetration results and used an inert membrane to identify purely thermodynamic effects. This allowed us to determine whether or not a specific skin/liposome interaction was taking place.

MATERIALS AND METHODS

MATERIALS

Soya phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were obtained from Avanti Polar Lipids (Alabaster, AL). Cholesteryl hemisuccinate (CHEMS), oleic acid (OA), α-tocopherol, all trans-retinoic acid (t-RA), [11, 12-3H(N)]-retinoic acid ([3H]t-RA, specific activity 49.30 Ci/mmol), bovine pancreas trypsin, type II-S soybean trypsin inhibitor, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), Dulbecco's phosphate-buffered saline (PBS), and EDTA (disodium salt) were purchased from Sigma (St. Louis, MO). Transcutol (diethylene glycol monoethyl ether) was obtained from Gattefosse (Elmsford, NY). Tritiated water (specific activity 1.6 μCi/ml) was obtained from Dupont/NEN (Boston, MA). All other chemicals were of analytical grade.
METHODS

Preparation and characterization of liposomes

Preparation. Liposomes were prepared by a combination of Bangham's film hydration method (23) and the extrusion technique described by Nayar et al. (24). First, \( t \)-RA, trace amounts of \( [^3 \text{H}]t \)-RA, and the lipids in chloroform solutions were mixed together in the appropriate ratio and 1% of \( \alpha \)-tocopherol (based on total lipid weight) was added as an antioxidant. For PC/t-RA liposomes, the ratio was 16 \( \mu \text{mol PC} \), 4 \( \mu \text{mol } t \)-RA, and 50 \( \mu \text{Ci }[^3 \text{H}]t \)-RA for the low-dose studies, and 16 \( \mu \text{mol PC} \), 4 \( \mu \text{mol } t \)-RA, and 1.2 \( \mu \text{Ci }[^3 \text{H}]t \)-RA for the high-dose studies; for PC/PE/OA/CHEMS/t-RA liposomes, it was 4 \( \mu \text{mol PC} \), 4 \( \mu \text{mol PE} \), 2 \( \mu \text{mol OA} \), 10 \( \mu \text{mol CHEMS} \), 4 \( \mu \text{mol } t \)-RA, and 50 \( \mu \text{Ci }[^3 \text{H}]t \)-RA for the low-dose studies, and 4 \( \mu \text{mol PC} \), 4 \( \mu \text{mol PE} \), 2 \( \mu \text{mol OA} \), 10 \( \mu \text{mol CHEMS} \), 4 \( \mu \text{mol } t \)-RA, and 1.2 \( \mu \text{Ci }[^3 \text{H}]t \)-RA for the high-dose studies. The lipid-drug mixture was deposited as a thin film in a round-bottom flask by roto-evaporating the chloroform (Rotavapor RE 111®, Buchi, Switzerland) under a gentle nitrogen stream. Vacuum was applied for one hour to ensure total removal of trace solvents. The film was then hydrated at 40°C for one hour with HEPES buffer (20 mM HEPES, 150 mM NaCl, 0.1 mM EDTA). HEPES buffer, pH 7.4, was used for the PC liposomes and HEPES buffer, pH 8.0, for the PC/PE/OA/CHEMS liposomes. After hydration was complete, the preparation was sonicated for 30 min (Ultramet III sonic cleaner, Buehler Ltd., Evanston, IL) and five freeze-thaw cycles were performed (5 min, dry ice/40°C waterbath). When necessary, the pH of the PC/PE/OA/CHEMS dispersion was adjusted back to 8.0 with 1 N NaOH.

The resulting large multilamellar vesicle dispersion was then transferred into a stainless steel extrusion device (The Extruder™, Lipex Biomembranes, Vancouver, BC), and unilamellar liposomes were generated by forcing the preparation through two stacked polycarbonate filters (Nuclepore Corp., Pleasanton, CA) of defined pore size (400 nm, 200 nm, 100 nm, or 50 nm in diameter). Nitrogen pressures up to 2800 kPa (400 psi) were used, and all extrusions (ten passes for each preparation) were performed at room temperature. After extrusion was complete, the volume of preparation was adjusted with buffer so that the final suspension contained 0.05% \( [^3 \text{H}]t \)-RA. For the low-dose studies, \( [^3 \text{H}]t \)-RA specific activity in the final preparation was 12.5 \( \mu \text{Ci/\mu mol} \), and its radiochemical concentration was 21 \( \text{Ci/ml} \). For the high-dose studies, \( [^3 \text{H}]t \)-RA specific activity was 0.3 \( \mu \text{Ci/\mu mol} \), and its radiochemical concentration was 0.5 \( \mu \text{Ci/ml} \). The final lipid concentration was about 6 mg/ml in both cases. The liposome preparations were stored at 4°C under nitrogen before use.

Size determination. We determined the particle size distribution of the extruded liposomal systems by quasi-elastic light scattering (QELS) using a Malvern 4700c submicron particle size analyzer (Malvern Instruments, Southborough, MA) equipped with a 60 mW helium-neon laser at an excitation wavelength of 633 nm. The temperature was set at 25°C and measurements were taken at 90 degrees.

In vitro skin permeation studies

Preparation of control formulations. For the low-dose studies, we prepared an alcoholic \( [^3 \text{H}]t \)-RA formulation (50 \( \mu \text{Ci/ml} \), 12.5 \( \mu \text{Ci/\mu mol} \)) by spiking a 0.05% ethanolic solution of \( t \)-RA with trace amounts of radioactive \( t \)-RA. Alcohol has been shown to be an efficient delivery vehicle for \( t \)-RA (25) and is used in commercial topical preparations.
of t-RA like Retin-A™ and Aberel® (16). The control vehicle for the high-dose studies and one set of low-dose studies (Figure 3) consisted of a mixture of transcutol and water, 68:32 v:v. This cosolvent system yields a very nearly saturated solution of t-RA at the 0.05% w:v level. In order to ensure that this formulation did yield nearly maximum thermodynamic activity of t-RA, we also included a 0.10% t-RA suspension in transcutol/water 68:32 in some experiments.

Preparation of human skin samples. Split-thickness human cadaver skin, dermatomed to a thickness of 250 µm, was obtained from the Ohio Valley Skin & Tissue Center (Vernon Place, Cincinnati, OH). The skin was stored frozen at -80°C in a 10% glycerol solution. Before use, it was rapidly thawed and thoroughly rinsed with distilled water.

Isolated sheets of epidermis were prepared from the dermatomed skin samples using the heat separation method (2-min immersion in 60°C water) (26). Isolated epidermal sheets were mounted on the diffusion cells immediately after preparation.

Stratum corneum sheets were prepared from isolated epidermis by trypsin digestion (26). The epidermal sheets were incubated overnight at 37°C, dermal side down, on filter paper saturated with 0.01% trypsin solution. The stratum corneum sheets were then rinsed with a 0.007% trypsin inhibitor solution, followed by distilled water. They were mounted on 20-mm drain discs (Nuclepore Corp., Pleasanton, CA) for easy handling, and stored dry at -20°C in a dessicator until needed.

The integrity of all tissue samples was assessed using the ³H₂O permeation method described by Franz and Lehman (27). The samples yielding a water flux greater than 1.2 mg/cm² were discarded unless otherwise noted. For comparison, the barrier integrity of some dermatomed skin samples was compromised by adhesive tape-stripping (about nine strips with 3M cellophane tape). Example results from that test are shown in Table I. Dermatomed skin, isolated epidermis, and isolated stratum corneum yielded equivalent water permeation rates, comparable to the values found by Franz and Lehman (27). Tape-stripped skin, as expected, gave a higher water flux.

Care was taken to use skin from a single donor for all types of samples in a particular experiment. Nevertheless, since quality variability among packs of skin from a single donor was sometimes experienced, the water flux geometric mean of each set of samples is presented in the figure legends below. Note that the data in Table I were obtained from a single pack.

Diffusion studies. All samples were mounted on modified Franz cells with a nominal surface area of 0.79 cm² (28). The receptor solution was Dulbecco’s phosphate-buffered

<table>
<thead>
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<th>Preparations</th>
<th>³H₂O penetration, mg/cm²</th>
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<tbody>
<tr>
<td></td>
<td>Geometric mean</td>
</tr>
<tr>
<td>Dermatomed skin</td>
<td>0.27</td>
</tr>
<tr>
<td>Isolated epidermis</td>
<td>0.27</td>
</tr>
<tr>
<td>Isolated stratum corneum</td>
<td>0.21</td>
</tr>
<tr>
<td>Tape-stripped skin</td>
<td>1.34</td>
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saline, pH 7.4, containing 0.02% (w:v) sodium azide to retard microbial growth. The receptor solutions were stirred and maintained at 37°C in thermostatted aluminum blocks, yielding a skin surface temperature of 30–32°C. To improve sensitivity, we ranked the tissue samples in order of increasing water permeability, and applied each formulation to at least seven samples using a random complete block design (29). Most of the diffusion studies employed small, non-occluded topical doses (3.8 μl, 4.8 mg/cm², herein called “small dose”), similar to those achieved by application of a skin cream or moisturizer. Other studies employed much higher doses (160 μl, 200 mg/cm², herein called “large dose”) to provide a comparison with the work of other investigators (10–13). To identify purely thermodynamic effects in these studies, the formulations were also applied to a silicone rubber membrane (Silastic® membrane, Dow Corning). The membrane thickness was 410 ± 19 μm (mean ± SD of eight determinations).

Receptor solutions were collected for radiochemical assay after elapsed times of 2, 4, 7, and 24 hours, and the cells refilled with fresh buffer. Samples were analyzed for ³H activity by liquid scintillation counting (Packard Model 1900 TR), using a maximum counting time of 5 min per sample.

Statistical analysis. Results were reported as either cumulative amount or cumulative percent-of-dose of t-RA penetrated at each time point. Statistical comparisons were made using PROC GLM in SAS Vers. 6.06 (SAS Institute Inc., Cary, NC). To compare data from different treatments applied to the same type of tissue, we performed a two-way analysis of variance on logarithmically transformed data, blocking on water permeability (29). To compare penetration through the different skin preparations, we performed a one-way analysis of variance on the log-transformed data. The potential for liposome-induced accumulation of t-RA in the lower layers of the skin was assessed in two ways. The first was to directly compare the penetration rates of liposomal t-RA through skin samples of varying thickness. This method was used when all skin samples from a donor had comparable water permeabilities (Figures 1, 2). The second method, used for samples having different water permeabilities (Figures 3–5), was to form the ratio between the amount of t-RA penetrated through stratum corneum to that through dermatomed skin. A significantly higher value (Student’s t-test) of this ratio for liposome-encapsulated active versus non-encapsulated active was taken as evidence for liposome-induced accumulation of t-RA in the skin.

RESULTS

LIPOSOME SIZE DISTRIBUTION

The size distribution modes for four different systems—PC, PC/t-RA, PC/PE/OA/CHEMS, and PC/PE/OA/CHEMS/t-RA—are reported in Table II. For the empty PC liposomes, the mode of the number distribution was about half the pore size used for extrusion. These results are somewhat smaller than those obtained by Mayer et al. (24), but they are consistent with their conclusion that different size distributions can be obtained by using different pore sizes. The distribution modes for the empty PC/PE/OA/CHEMS liposomes were lower (about one fourth of the pore diameter instead of one half). However, there was still a strong positive correlation between distribution mode and pore size. Incorporation of t-RA into these systems totally changed the size distri-
Table II
Influence of Liposome Composition and Filter Pore Diameter on Particle Size Distribution

<table>
<thead>
<tr>
<th>Liposome composition</th>
<th>Filter pore diameter (nm)</th>
<th>Distribution mode (nm)</th>
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<tbody>
<tr>
<td>PC</td>
<td>No extrusion</td>
<td>&gt;1000</td>
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<tr>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>26</td>
</tr>
<tr>
<td>PC/t-RAa 5:1 mole ratio</td>
<td>No extrusion</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>152</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>50</td>
<td>36b</td>
</tr>
<tr>
<td>PC/PE/OA/CHEMS</td>
<td>No extrusion</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>2:2:1:5 mole ratio</td>
<td>400</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>48</td>
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<tr>
<td></td>
<td>100</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>13</td>
</tr>
<tr>
<td>PC/PE/OA/CHEMS/t-RAa 2:2:1:5:2 mole ratio</td>
<td>No extrusion</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>92</td>
</tr>
</tbody>
</table>

a Corresponds to a final t-RA concentration of 0.05% w/v.
b Bimodal distribution.

Extrusion pressures were 3–4 times higher than those of the empty systems. For the PC/t-RA liposomes, extrusion through pores below 400 nm in diameter yielded samples exhibiting bimodal intensity distributions (first mode around 15 nm, second mode in the liposome range). Since it is not possible to achieve a liposome diameter below about 20 nm due to steric constraints, we suspected that these systems also contained micellar structures. The size distributions of the extruded systems were unchanged after two weeks; however, after one week the non-extruded sample had flocculated. For the PC/PE/OA/CHEMS/t-RA liposome preparations, no micellar structures were detected, but regardless of the filter used for extrusion, the distribution modes were all about 80 nm. Although a detailed investigation of the effects of t-RA on liposome stability was beyond the scope of our investigation, it seems likely that the explanation may involve an increased propensity to form H-II phases or other non-bilayer phases (30).

We concluded that the size distribution of liposomes prepared by the extrusion technique cannot always be linked to the filter pore diameter. Furthermore, incorporation of a lipophilic permeant like t-RA into the formulation can significantly alter liposome structure. Thus, we chose to extrude our liposome preparations through filters with 400-nm pores and to routinely size them prior to use.

IN VITRO SKIN PERMEATION STUDIES

Effect of liposome size on penetration. The penetration of $[^3]$H]t-RA from the PC/PE/OA/CHEMS/t-RA liposomes in Table II was measured through dermatomed skin (data not shown). The three extruded preparations gave equivalent t-RA penetration. The unex-
truded preparation yielded somewhat lower penetration in one test, somewhat higher penetration in another; in each case the differences observed were modest (<25%). Since the particle size of the unextruded preparation was much greater than that of the extruded preparations, we concluded that size and lamellarity of the vesicles were not critical to the penetration of t-RA. This agrees with the findings of Du Plessis et al. for other lipophilic compounds (11). Nevertheless, we continued to extrude the liposome preparations for stability and reproducibility purposes.

**Small-dose diffusion studies.** Figure 1 shows the results obtained when small doses of t-RA encapsulated in PC liposomes were applied to the skin samples whose water permeability was shown in Table I. The cumulative percent of t-RA dose penetrated at all time points studied was equivalent for all of the non-damaged skin preparations (stratum corneum, epidermis, dermatomed skin). Significantly higher penetration was obtained from the samples with damaged stratum corneum. It is apparent from this comparison that the primary diffusion barrier for liposomally encapsulated t-RA in skin is the stratum corneum.

Figure 2 shows the results obtained from a similar test employing 0.05% t-RA in PC/PE/OA/CHEMS liposomes. Although small differences in penetration rate through the stratum corneum and dermatomed skin were observed through 7 h post-dose, there

![Figure 1. In vitro penetration of t-RA encapsulated in PC liposomes through different skin preparations (geometric mean ± SE). A small (4.8 mg/cm²), non-occluded dose of a 0.05% t-RA formulation was applied to each tissue sample at time zero. ☐, tape-stripped skin (n = 4); ▲, isolated stratum corneum (n = 8); △, isolated epidermis (n = 6); ●, dermatomed skin (n = 7). Error bars not pictured are smaller than the size of the symbol.](image-url)
was no significant difference in t-RA penetration between the two substrates at 24 h (Figure 2a).

Furthermore, in a follow-up study, a 0.05% ethanolic solution of t-RA yielded equivalent penetration through dermatomed skin as the PC/PE/OA/CHEMS liposome system (Figure 2b). The 24 h t-RA penetration values were consistent with the results obtained by Lehman and Franz for solvent-deposited RA (25). We thus found no evidence of appreciable effects of liposomal encapsulation on t-RA delivery through or into the skin in these studies.

Figure 3 shows the results of additional tests in which small doses of t-RA in either PC-liposomes or the transcutol/water control solution were applied to various mem-
Influence of liposomal encapsulation

Figure 3. Penetration of small (4.8 mg/cm² of a 0.05% solution), non-occluded doses of t-RA through (a) silicone rubber membrane; (b) dermatomed skin (²H₂O flux = 1.29 mg/cm²); and (c) isolated stratum corneum (²H₂O flux = 0.79 mg/cm²). ▲, PC-liposome vehicle; ●, transcutol/water vehicle (geometric mean ± SE, n = 5–8).

Penetration of t-RA from the two formulations was essentially the same whether the testing was carried out on a synthetic membrane (Figure 3a), dermatomed skin (Figure 3b), or isolated stratum corneum (Figure 3c). Furthermore, t-RA penetration through the dermatomed skin samples was actually slightly higher than through the stratum corneum samples, reinforcing the findings from Figure 1 that the lower skin layers do not contribute appreciably to the diffusion barrier for t-RA. Again, there was no evidence for significant effects of liposomal encapsulation on t-RA delivery through or into the skin based either on a direct comparison of penetration values for liposomal and control formulations or on a comparison of the penetration ratios (Figure 3c/Figure 3b) as described in the Experimental section.

Large-dose diffusion studies. Since a number of studies showing liposome effects on topical
delivery have employed much larger dose volumes than those in Figures 1–3, we repeated the comparisons from Figure 3 under high-dose conditions. The results are shown in Figure 4 (large, occluded dose) and Figure 5 (large, non-occluded dose). Figure 4a demonstrates that 0.05% t-RA in the PC-liposome formulation and 0.05% t-RA in transcutol/water 68:32 have equal thermodynamic activity, since they both yielded the same diffusion rate for t-RA through an inert membrane. Additional diffusion experiments with 0.10% t-RA suspension in the transcutol/water vehicle (data not shown) showed that this activity was near maximal. However, on dermatomed skin (Fig. 4b) and isolated stratum corneum (Figure 4c), the transcutol/water formulation yielded 50–100% greater t-RA penetration than did PC liposomes. This is evidence for a mild permeability-enhancing effect of transcutol on skin, as suggested by the work from other

Figure 4. Penetration of high (200 mg/cm² of a 0.05% solution), occluded doses of t-RA through (a) silicone rubber membrane; (b) dermatomed skin ($^3$H₂O flux = 3.94 mg/cm²); and (c) isolated stratum corneum ($^3$H₂O flux = 1.07 mg/cm²). The symbols and formulations are the same as in Figure 3 (geometric mean ± SE, n = 7–8).
laboratories (31). Note, however, that dermatomed skin and isolated stratum corneum again yield nearly equivalent penetration rates for either control or liposomal t-RA, in agreement with the findings from the small-dose studies. A comparison of penetration ratios (Figure 4c/Figure 4b) gave no evidence for accumulation of liposomal t-RA in the skin.

Figure 5 shows the results from a similar set of large-dose studies in which evaporation of the donor solution was allowed to occur. In this case, PC liposomes yielded higher t-RA penetration through the inert membrane than did transcutol/water (Figure 5a). This most likely reflects the decreased thermodynamic activity of t-RA in the transcutol/water formulation, as the more volatile water component evaporated during the study.

![Graphs showing penetration of t-RA](image)

**Figure 5.** Penetration of high, non-occluded doses of t-RA through (a) silicone rubber membrane; (b) dermatomed skin (\(^{3}H_2O\) flux = 1.08 mg/cm\(^2\)); and (c) isolated stratum corneum (\(^{3}H_2O\) flux = 0.98 mg/cm\(^2\)). The symbols and formulations are the same as in Figures 3 and 4 (geometric mean ± SE, n = 6–7).
Note that the opposite pattern was seen on dermatomed skin (Figure 5b) and isolated stratum corneum (Figure 5c), providing further evidence for an enhancing effect of transcutol.

Relative to the occluded-dose experiments in Figure 4, t-RA penetration from the transcutol/water solution was depressed in approximately the same proportion as seen with the inert membrane. However, t-RA penetration from the PC liposomes was depressed to an even greater extent, resulting in substantially lower t-RA delivery from the liposomes than from the transcutol/water. There was a suggestion in these experiments that t-RA penetration from PC liposomes was higher through stratum corneum (Figure 5c) than through dermatomed skin (Figure 5b). The penetration ratios (Figure 5c/Figure 5b) at 7 and 24 h for the PC liposome formulation were significantly greater than those for the transcutol/water formulation. The results of this analysis, as well as comparable ratios for Figures 3 and 4, are shown in Table III. A summary of the experimental conditions and penetration results is presented in Table IV.

DISCUSSION

In this paper we have investigated two delivery phenomena attributed to topical liposomes: increased delivery of compounds through the stratum corneum and localization of these compounds in the lower skin layers, with an associated decrease in systemic absorption. The first of these phenomena was studied directly, by comparing the penetration rate of liposomally encapsulated t-RA with that of solvent-deposited t-RA and with that of a saturated solution of t-RA in a simple solvent system. The second phenomenon was studied indirectly, by comparing the penetration rates of liposomal t-RA through skin preparations of varying thickness. We found no evidence for enhanced penetration of t-RA through the stratum corneum by these liposome systems vs encapsulated control formulations. This was shown for PC liposomes under both small-dose (Figure 3) and large-dose (Figures 4, 5) conditions and for PC/PE/OA/CHEMS liposomes in the small-dose case (Figure 2). The latter system was chosen due to its reported ability to deliver large-molecular-weight enzyme across the stratum corneum (21,22). While one cannot rule out the possibility that there may be liposome compositions that would enhance the delivery of t-RA, we could not find such an effect with two quite different systems. Moreover, our results are in agreement with the majority of recent studies employing other penetrants, liposome compositions, and dose condi-

| Table III |
| Penetration Ratio Analysis of Liposome-Induced Accumulation of t-RA in the Skin |

<table>
<thead>
<tr>
<th>Figure</th>
<th>Time</th>
<th>Liposomes</th>
<th>Control</th>
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<tbody>
<tr>
<td>3</td>
<td>24 h</td>
<td>0.42 ± 0.15</td>
<td>0.60 ± 0.21</td>
</tr>
<tr>
<td>4</td>
<td>24 h</td>
<td>0.84 ± 0.48</td>
<td>0.68 ± 0.20</td>
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<tr>
<td>5</td>
<td>7 h</td>
<td>1.72 ± 0.17*</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>1.75 ± 0.14*</td>
<td>0.88 ± 0.07</td>
</tr>
</tbody>
</table>

*a Ratio of the cumulative amount of t-RA penetrated through stratum corneum to that through dermatomed skin at time t (mean ± S.E., n = 5–7).

b Transcutol/water 68:32 v:v.

* Significant difference from control, p < 0.01.
<table>
<thead>
<tr>
<th>Liposome Type</th>
<th>Figure 1</th>
<th>Figure 2</th>
<th>Figure 3</th>
<th>Figure 4</th>
<th>Figure 5</th>
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<tr>
<td>Vehicle comparison</td>
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<td>Liposomes vs ethanol</td>
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<td>No</td>
</tr>
<tr>
<td>Increased penetration for liposomes?</td>
<td>—</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Evidence of retention of liposomal t-RA into the lower layers of the skin?</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* All vehicles contain 0.05% t-RA.
tions: liposomes do not, as a rule, enhance skin penetration relative to conventional topical formulations (6,7,10,12).

Having established that these liposome systems do not enhance t-RA penetration across the stratum corneum, we can reformulate the second liposome delivery question as follows: Does liposomal encapsulation significantly retard the delivery of t-RA across the skin relative to the amount transported across the stratum corneum? An affirmative response to this question would support the contention that liposomes can increase drug concentrations in the skin while decreasing systemic absorption. Two recent studies employing t-RA have led to such a conclusion (6,7).

Our studies do not support this position. In three of the five conditions studied (Figures 1, 3, 4), the penetration rate of liposomal t-RA across dermatomed skin was either equal to or slightly greater than the penetration rate across stratum corneum. In the fourth study (Figure 2a), although the situation was reversed, the differences seen were small and were not significant after 7 h post-dose. Moreover, liposomal delivery of t-RA across dermatomed skin was greater than or equal to that from the solvent-deposited t-RA in this study (Figure 2b). The final study (Figure 5; large, non-occluded dose conditions) does present the possibility of an accumulation of t-RA in the lower skin layers from the PC liposome formulation. In this study, the penetration rate of liposomal t-RA across the stratum corneum was 4.0 nmol/cm²/24 hr, whereas that across dermatomed skin was only 2.3 nmol/cm²/24 hr, giving a penetration ratio of 1.75 (Table III). The transcutol/water control solution yielded no such difference (penetration ratio of 0.88). Using a simple diffusion model, one can show that the 1.7 nmol/cm²/24 hr difference between these two rates values, 4.0 and 2.3 nmol/cm²/24 hr, represents many times the average steady-state concentration of freely diffusing t-RA in the lower skin layers. This could clearly represent a pharmacologically important accumulation of t-RA. Why would this effect develop under large, non-occluded dose conditions, but not under other circumstances? One difference that stands out is the high lipid concentrations achieved under these conditions. Whereas the starting lipid concentrations in all studies was about 6 mg/ml, evaporation of the water from the large, non-occluded doses could easily have led to lipid concentrations 10-20 times that value after several hours. We offer the possibility that with sufficiently high PC concentrations at the skin surface, enough PC may diffuse across the stratum corneum to complex a significant amount of t-RA in the lower skin layers. This possibility seems worthy of further study.

This particular set of conditions, however, is not realistic from the point of view of cosmetic formulations. Realistic application levels of moisturizers and other topical creams and lotions tend to be in the 1–2 mg/cm² range, similar to the small-dose conditions in our in vitro studies. Lipid concentrations are constrained to a few percent or less by both aesthetics and cost. We chose to study the effects of large doses of liposomes as well as small ones in order to compare our findings with those of others (10–13). It seems possible from our work that liposomes do retard the diffusion of t-RA across the lower skin layers under exaggerated dose conditions. But, based on our findings, it is unlikely that this effect would persist under realistic dose conditions.

CONCLUSION

Incorporation of trans-retinoic acid into either simple PC liposomes or a complex,
four-component liposome system did not increase its penetration rate across human stratum corneum relative to unencapsulated controls. Furthermore, under most (four of five) of the conditions studied, liposomes also failed to significantly reduce the delivery rate of t-RA across dermatomed human skin relative to that across stratum corneum; hence, there was no evidence for liposome-induced accumulation of t-RA in the lower skin layers in these studies. Although there was evidence for accumulation of t-RA in the skin under one set of conditions (consisting of a large, non-occluded dose of t-RA in PC liposomes), these conditions do not correspond to a practical dose regimen for a topical skin care product.

REFERENCES


