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Percutaneous absorption of a new antiandrogen included in liposomes or in solution

E. Bernard[a], J.L. Dubois[b], J. Wepierre[a,*]

[a]*Laboratoire de Dermopharmacologie, Faculté de Pharmacie, 5 rue Jean Baptiste Clément, 92290 Châtenay — Malabry, France*

[b]*Laboratoire de Recherche Galénique, Roussel UCLAF, 102 route de Noisy, 93235 Romainville, France*

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Abstract

The percutaneous absorption and the cutaneous distribution of a new antiandrogen, RU 58841, 0.5% in liposomes or in solution were compared after topical application. In vitro percutaneous absorption experiments were performed on human dermatomed skin with Franz diffusion cells. In vivo cutaneous distribution after application on the skin of hairless rat was studied with the method previously described by Schaefer and Stüttgen. The in vitro experiments showed that liposomes considerably reduce the RU 58841 percutaneous absorption compared to solution. After in vivo application, drug content of skin surface, stratum corneum, epidermis and dermis were measured, it demonstrated a slower permeation of the drug and a longer duration in the epidermis and dermis for the liposomes compared with the solution. The distribution profile of solution presented an RU 58841 concentration gradient decreasing from the surface to the deeper layers while an RU 58841 concentration increase between 30 and 150 μm in depth was observed with liposomes showing an accumulation in the sebaceous structures. In conclusion, there is a trend towards a more favourable topical application of liposomes for local effects. They reduce percutaneous absorption, increase accumulation and retention of the drug in the dermis and produce a targeting in the sebaceous structures.

Keywords: Liposome; Antiandrogen; Percutaneous absorption; Cutaneous distribution; Sebaceous gland; Targeting

1. Introduction

Androgens play a major role in skin disorders such as acne, alopecia and hirsutism, they are the steroids involved in stimulation of hair growth and sebaceous gland secretions (Cunliffe and Simpson, 1980). Androgen receptors have been localized in human skin, especially in sebaceous glands and hair follicles (Choudhry et al., 1992). At the present time, some antiandrogens are used by the oral route to treat these skin disorders (Cyproterone Acetate, Flutamide) with undesirable systemic side effects which limit their therapeutic use. RU 58841 is a new non-steroidal antiandrogen which exhibits a strong topical efficacy, in animal models, on sebaceous gland

* Corresponding author.

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activity while producing minor systemic effects (Battmann et al., 1994), androgenetic alopecia and hirsutism (unpublished data). Topical application of RU 58841 is required.

Therapeutic effectiveness certainly depends upon the concentration-time profile of the active agent in the skin layer. The properties of the vehicle used modify the dynamic of this process. In this connection, liposomes usually yield a slow release, a tissue targeting, and a low percutaneous absorption of the drug. In order to test liposomes as a suitable drug delivery system for a RU 58841 topical administration, we prepared a liposomal formulation and a classical solution. We compared these two preparations with RU 58841; percutaneous absorption following in vitro application on human skin and cutaneous distribution after in vivo application on skin of hairless rat. In particular, we compared the amounts in the epidermis and dermis, and drug deposits in the sebaceous structures.

2. Materials and methods

2.1. Materials

RU 58841 (4-[3-(4-hydroxybutyl)-4,4-dimethyl-2,5-dioxo-1-imidazolidinyl]-2-(trifluoromethyl)-benzonitrile) is an original molecule synthetized by Roussel UCLAF. RU 58841 and ^{3}H-RU 58841 were mixed to obtain specific radioactivities of 11.7 kBq/mg and 1.52 MBq/mg. Radiochemical purity was > 95%.

Lipoid E 100-35 (Lipoid KG, Ludwigshafen, Germany), α-tocopherol (Fluka AG, Buchs, Switzerland) and a phosphate buffer, pH 7 (Roussel UCLAF) were used for the liposomes preparation. Ethanol and propylene glycol (Roussel UCLAF) were used for the solution.

2.2. Liposomes formulation

Small unilamellar liposomes (SUV) containing 0.5% weight of ^{3}H-RU 58841 were prepared with Lipoid E 100-35 (egg phosphatidylcholine > 94%) and α-tocopherol in a phosphate buffer at pH 7.4, 0.05 M.

Briefly, powders of ^{3}H-RU 58841, α-tocopherol and Lipoid were mixed at 60°C. Then appropriate volume of phosphate buffer was added at 60°C with a mild agitation. Finally the mixture was homogenized by microfluidisation. The final % weights were 0.5 for RU 58841, 10.76 for Lipoid and 0.11 for α-tocopherol. Molar ratio Lipoid/RU58841 = 10. Liposomes size was measured with a N4 MD Coulter (Coultronics, Margency, France), mean volume diameter was 100 ± 50 nm.

Two liposome formulations were prepared. One formulation was prepared using ^{3}H-RU 58841 with a specific radioactivity of 11.7 kBq/mg, the final concentration for RU 58841 was 4.74 mg/ml of preparation. The second formulation was prepared using ^{3}H-RU 58841 with a specific radioactivity of 1.52 MBq/mg, the final concentration for RU 58841 was 3.7 mg/ml of preparation.

2.3. Solution

Two solutions of ^{3}H-RU 58841 were prepared with ethanol/propylene glycol/water 20/40/40 weight and contained the same specific radioactivities and final concentrations as liposomes.

2.4. Radioactivity assays

Scintillation cocktails used for the samples analyse were Picofluor 40, Soluene 350, Hionic Fluor and Toluene Scintillator (Packard, Rungis, France).

Radioactivity was quantified by liquid scintillation with a Tri-Carb 4530 counter (Packard) and corrected for quenching by the external standard method.

2.5. In vitro percutaneous absorption

The in vitro percutaneous absorption experiments were performed on human skin with Franz diffusion cells (Franz, 1975). Female abdominal skin was obtained from plastic surgery. After removing the subcutaneous fat and trimming to 500 μm of depth (Zimmer™, Dover, Ohio, USA), skin was mounted on cells with a surface area of 1.77 cm^2 and a receiver compartment (6 ml) filled

with sodium chloride (9 g/l) and bovine serum albumin (15 g/l) in distilled water. The receiver fluid was continuously stirred with a teflon-coated magnetic bar and maintained at 37°C. The dosing formulations were applied to the epidermal surface. All experiments were carried out with nonoccluded donor compartments. A minimal of eight cells was used for each formulation. Two experimental series have been realized: one and repeated applications. In order to measure the drug permeation in short periods we tested the highly radiolabelled solution and liposomes. Twenty-five μl of the preparations were applied (90 μg of drug). The receiver fluid was totally removed at 2, 4, 6, 8 and 24 h and replaced with fresh solution. In order to measure the drug permeation after repeated applications, we tested the slightly radiolabelled solution and liposomes. Twenty μl of the preparations (95 μg of drug) was applied once a day over 4 days (total applied = 380 μg of drug). The receiver fluid was totally removed at 24, 48, 72 and 96 h and replaced with fresh solution.

At the end of each study (24 and 96 h), the skin surface was washed to remove residual drug, twice with 200 μl of 0.05% cetrimide and three times with 200 μl of water, wiped with cotton swabs; washing media were then diluted with 30 ml of alcohol. The epidermis was mechanically separated from the dermis. They were both digested with 1 and 3 ml, respectively, of soluene 350 at 60°C overnight.

Assays for measurement of radioactivity of the receiver fluids, epidermis and dermis were carried out after addition of 15 ml of picofluor 40 and hionic fluor, respectively. Radioactivity of washing media was measured by using 1 ml of the dilution mixed with 15 ml of picofluor 40. Results are expressed in μg of RU 58841.

2.6. In vivo cutaneous distribution

^{3}H-RU 58841 cutaneous distribution after in vivo application was determined with the method previously described (Schaefer and Stüttgen, 1976).

Male hairless rats (300–350 g) were anesthetized with sodium pentobarbital (0.1 ml/100 g weight); a metallic protection device was fixed on the back of the animals to prevent contamination by self-licking or scratching. A 5 cm^2 area was delimited with a glass cylinder. Two experimental series were carried out: one and repeated applications of the highly radiolabelled solution and liposomes. One application of 25 μl of preparation (90 μg of drug) and five daily applications (450 μg of drug), with skin washing before each application, were carried out on the 5 cm^2 area. Three animals were used per formulation and per time. Three, 6 and 24 h after one application, and 24 h after five daily applications (120 h), animals were sacrificed by chloroform inhalation, skin was excised, washed as previously described and the horny layer was stripped 15 times with adhesive tape (3M). Then skin samples were frozen at −20°C, three biopsies of each of them were made with a Biopsy punch (Stiefel, Nanterre, France), and slices were cut parallel to the skin surface (10 slices of 20 μm, 10 slices of 40 μm and five slices of 80 μm) with a freezing microtome (Leica Instruments GmbH, Nussloch, Germany). Each strip and each slice was transferred separately into vials and 15 ml of toluene scintillator was added. ^{3}H-RU 58841 content was determined 24 h later. Skin washings were diluted with 20 ml of alcohol and radioactivity was measured by using 1 ml of the dilution mixed with 15 ml of picofluor 40.

3. Results

3.1. In vitro percutaneous absorption

3.1.1. Permeation after one application

Results of percutaneous absorption of RU 58841 after one application of solution and liposomes are presented in Fig. 1.

The solution led to a greater permeation: at 24 h, 6.5 μg of RU 58841 (7.2% of the applied dose) were recovered in the receiver fluid. The flux was relatively low until 6 h, from 0.009 to 0.03 μg/cm^2/h, it then increased to 0.2 μg/cm^2/h at 24 h.

Liposomes produced a remarkably lower permeation: 24 h after the application, 0.35 μg of RU 58841 were recovered in the receiver fluid (0.39% of applied dose). The flux remained constant throughout the experiment (0.01 μg/cm^2/h).

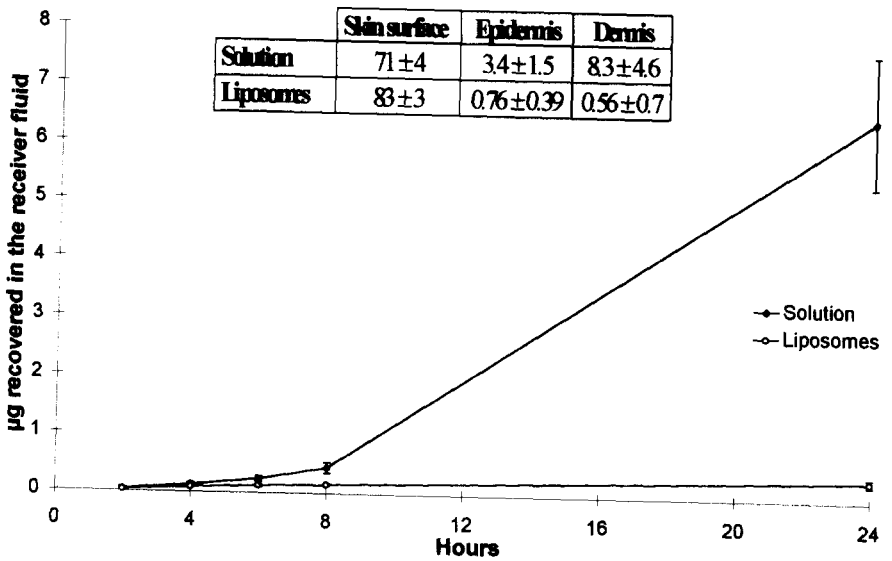


Fig. 1. In vitro RU 58841 percutaneous absorption after one application of solution and liposomes on human skin. The table represents amounts of drug recovered in the different cutaneous layers at 24 h. Results are expressed in μg (mean $\pm$ SEM), $n = 8$. Applied dose was 90 μg.

At 24 h, RU 58841 amounts in skin were significantly higher for the solution than for liposomes (Fig. 1), with 3.4 vs. 0.76 μg in epidermis and 8.3 vs. 0.56 μg in dermis. The residual drug at the skin surface was lower for the solution: washings contained 71 (79% of applied dose) vs. 83 μg (92% of applied dose) with liposomes.

3.1.2. Permeation after repeated applications

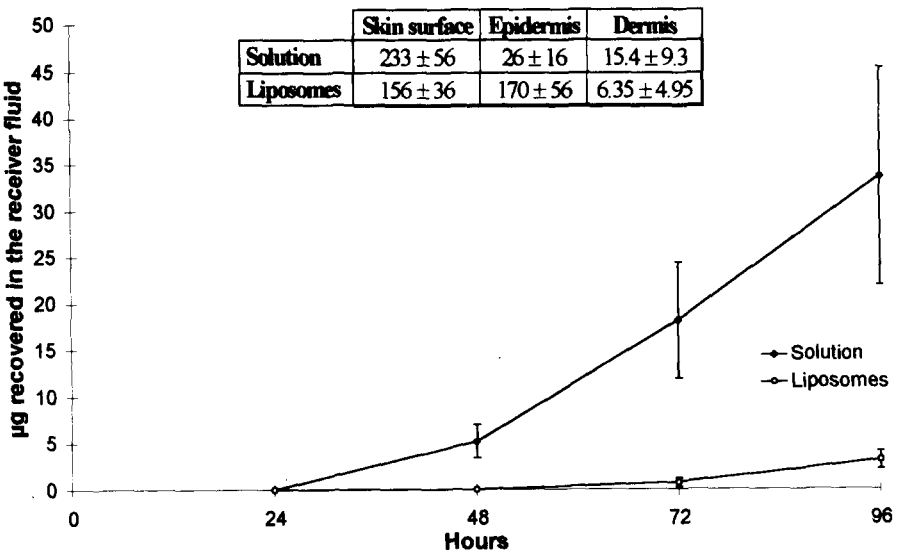
Results of percutaneous absorption of RU 58841 after repeated applications of solution and liposomes are presented in Fig. 2.

As previously, the solution yielded a higher percutaneous absorption compared to liposomes: at 96 h, 33.76 μg (8.9% of total applied dose) of RU 58841 were recovered in the receiver fluid vs. 3.17 μg (0.8%) with liposomes. Amounts present in the receiver fluid were so low with liposomes that no drug was detectable before 48 h with this slightly radiolabelled formulation. Drug amounts detected in the skin were higher with liposomes (Fig. 2), especially in the epidermis where the difference was highly significant (170 vs. 26 μg); 156 μg of RU 58841 (41% of applied dose) were still on the skin surface with liposomes, while 233 μg (61% of applied dose) were still on the skin with solution.

3.2. In vivo cutaneous distribution

3.2.1. Distribution in the different cutaneous layers

After in vivo application of the preparations on hairless rat skin, the drug contents on skin surface, stratum corneum, epidermis and dermis were measured; the results are presented in Table 1. The solution led to a fast drug penetration. At 3 h, only half of the applied dose (40 μg) remained at the skin surface: significant amounts were measured in the stratum corneum (27 μg) and in the epidermis and dermis (2.74 μg). Maximum drug concentration in epidermis and dermis (4.48 μg) was observed 6 h after application. There was also a rapid elimination; at 24 h, little drug remained at the skin surface (12 μg) and in the different skin layers (21.5 and 1.6 μg); 24 h after five daily applications of solution, amounts recovered in the skin were equivalent (19 and 2.28 μg), so repeated applications did not yield a higher accumulation of drug in the skin. Liposomes led to a slow RU 58841 penetration: at 6 h, 61 μg of RU 58841 were still on the skin surface; stratum corneum, epidermis and dermis contained only 6 and 2.4 μg. Maximum drug concentration in the stratum corneum (12 μg) and in the epidermis and dermis



	Skin surface	Epidermis	Dermis
Solution	233 ± 56	26 ± 16	15.4 ± 9.3
Liposomes	156 ± 36	170 ± 56	6.35 ± 4.95

Fig. 2. In vitro RU 58841 percutaneous absorption after four applications of solution and liposomes on human skin. The table represents amounts of drug recovered in the different cutaneous layers at 96 h. Results are expressed in μg (mean ± SEM), $n = 8$. Applied dose was 380 μg.

(3.85 μg) were observed 24 h after application. After five daily applications, an equivalent quantity was recovered in the epidermis and dermis (3.11 μg) but not in the stratum corneum (4.6 μg). As for the solution, repeated applications did not produce a higher accumulation of drug into the skin.

The fastest cutaneous penetration was observed for the solution. Comparison of the epidermis and dermis drug contents showed higher amounts with the solution at shorter times (2.74 and 4.48 μg at 3 and 6 h, respectively, vs. 1.2 and 2.4 μg with liposomes), and higher amounts with liposomes at longer times (3.85 and 3.11 μg at 24 and 24 h after five applications, respectively, vs. 1.6 and 2.28 μg with solution).

3.2.2. Accumulation in the sebaceous glands area

Distribution of ^{3}H-RU 58841 in the epidermis and dermis after in vivo application of solution and liposomes is presented in Fig. 3 and Fig. 4 and Fig. 5.

Solution and liposomes yielded a different RU 58841 cutaneous distribution profile. At 3, 6 and 24 h, the distribution profile of solution presented an RU 58841 concentration gradient decreasing from the surface to the deeper layers (Fig. 3). The RU 58841 distribution profile was similar 24 h after repeated applications (Fig. 5). Three, 6 and 24 h after one application of liposomesn an RU 58841 concentration increase of between 30 and 150 μm in depth was observed (Fig. 4), this indicates an accumulation of the drug. After five daily applications of liposomes the concentration increase disappeared (Fig. 5). RU 58841 concentrations were stable between 30 and 200 μm (from 20 ± 2.7 μg/cm^3 at 30 μm to 15.4 ± 1.7 μg/cm^3 at 200 μm), and were higher than the concentrations obtained with solution (from 15.6 ± 1.65 to 9.4 ± 0.7 μg/cm^3). This shows that liposomes lead to a better fixation of the drug in the area of the dermis.

4. Discussion

The development and secretory activity of the sebaceous glands are under androgen control, particularly of dihydrotestosterone. Actual drugs topically applied to treat acne are benzoyl peroxide, retinoic acid, antibiotics, azelaic acid and others; these compounds are not antiandrogens. RU 58841 is an antiandrogen which presents a

Table 1
RU 58841 penetration in hairless rat skin after in vivo application of solution and liposomes

Skin layer	Formulation	μg^a of RU 58841			
		3 h	6 h	24 h	24 h after 5 applications
Skin surface	Solution	40 ± 4.4	40 ± 15.7	12 ± 0.2	45 ± 2.56
	Liposomes	64 ± 6.4	61 ± 7.8	44 ± 2.5	59 ± 8.8
Stratum corneum	Solution	27 ± 5.2	28 ± 4.3	21.5 ± 2.8	19 ± 2.7
	Liposomes	6 ± 0.8	6 ± 0.7	12 ± 1.4	4.6 ± 1.2
Epidermis/dermis	Solution	2.74 ± 0.4	4.48 ± 1.3	1.6 ± 0.17	2.28 ± 0.16
	Liposomes	1.19 * * ± 0.12	2.4 ± 0.18	3.85 * ± 0.65	3.11 * ± 0.16

[a]Results present the total amounts in μg recovered in the 5 cm^2 of each skin layer (mean ± SEM, $n = 9$). Applied dose was 90 μg.
*Comparison between solution and liposomes by analysis of variance: $*p < 0.05$; $**p < 0.01$)

good activity at the site of sebaceous glands, and low systemic effects on the inner androgeno-dependent organs. In order to administer this drug by the topical route we need a preparation which improves penetration in the skin, especially in sebaceous gland-containing zone, while diminishing systemic absorption. We investigated the cutaneous penetration of RU 58841 in an alcoholic solution and in liposomes. Percutaneous absorptions were compared after in vitro topical application on human skin. Drug levels in the horny layer, epidermis and dermis were compared after in vivo application on hairless rat skin. Since cutaneous blood circulation plays a role in the percutaneous permeation and resorption of drugs, this study was carried out in vivo. Hairless rats were used because of the sebaceous gland density of their skin (about 11% of the cutaneous volume in 1 mm of depth), which allowed us to visualize the possible accumulation of the drug in sebaceous structures.

The in vitro experiments showed that liposomes considerably reduced the RU 58841 percutaneous absorption compared to the solution (by a factor of 19 at 24 h, and a factor of 10 after repeated applications). RU 58841 amounts recovered in the skin after liposomes application were lower than solution at 24 h and higher after repeated applica-

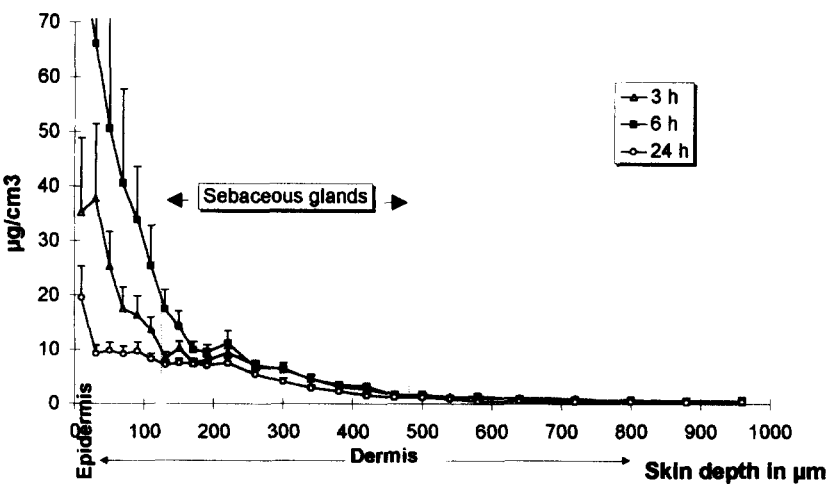


Fig. 3. In vivo distribution in epidermis and dermis of RU 58841 solution, 3, 6 and 24 h after one application on hairless rat skin. Results are expressed in $\mu g/cm^3$ (mean ± SEM), $n = 9$.

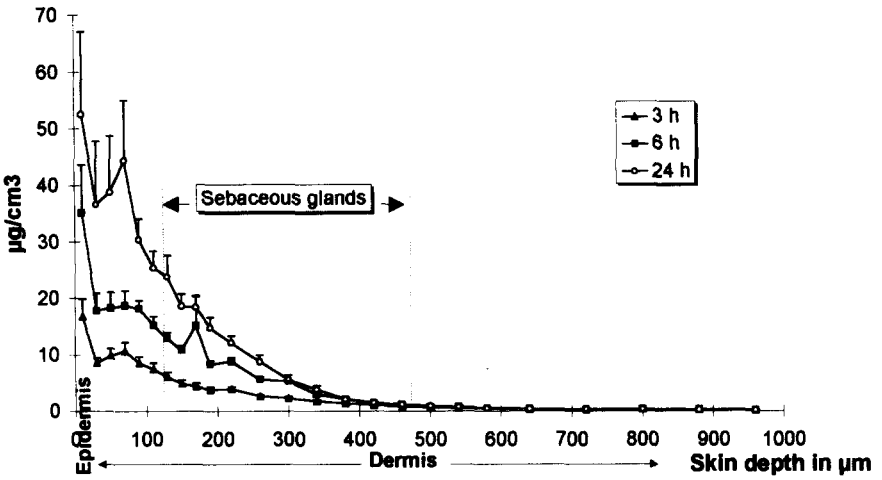


Fig. 4. In vivo distribution in epidermis and dermis of RU 58841 liposomes, 3, 6 and 24 h after one application on hairless rat skin. Results are expressed in $\mu g/cm^3$ (mean $\pm$ SEM), $n = 9$.

tions. So repeated applications of liposomes yielded a better accumulation of drug in the epidermis which certainly allowed a steady diffusion in the dermis, where sebaceous glands are localized. In conclusion, kinetics of permeation observed in in vitro studies clearly demonstrated the retarded delivery of drug by liposomes. Thereby, in terms of drug penetration in the skin, the solution is more efficient in the short term (24 h) while liposomes are more efficient at longer term (96 h). At any time period, inclusion of RU 58841 in liposomes leads to a significantly lower percutaneous absorption.

The in vivo cutaneous distribution studies showed that the highest amounts in epidermis and dermis were obtained with the solution at short times (3 and 6 h) and with liposomes at longer times (24 h after one and five applications). This demonstrated a slower permeation of the drug and a longer duration in the epidermis and dermis for the liposomes compared with the solution. Surprisingly we did not find similar results in the horny layer. We noticed that the kinetics of cutaneous penetration obtained in vivo tallies with the in vitro results: liposomes clearly delayed the RU 58841 permeation compared with solution. RU 58841 would be gradually released by the liposomes. On the other hand, RU 58841 in the solution (containing high levels of ethanol and propylene glycol) is quickly fixed to the stratum corneum after the evaporation of the hydro-alcoholic solution. Moreover ethanol is a very effective skin penetration enhancer and, at high concentrations, it can extract stratum corneum lipids; propylene glycol shows mild accelerant permeation.

The liposomes yielded a different drug distribution profile from the solution. Histological studies (Roguet et al., 1986) have shown that sebaceous glands of hairless rats skin are localized between 195 ± 60 and 397 ± 82 μm (Table 2). So the concentration increase observed between 30 and 150 μm after liposomes application may indicate an accumulation of the drug in the sebaceous ducts and higher part of the glands. The localization of RU 58841 in the sebaceous structures demonstrates the targeting effect of liposomes at any time period. The mechanism of the selective enrichment in the area of the sebaceous structures has not been elucidated. We postulate that liposomes show a good affinity for sebum lipids. But other questions to which there are as yet no clear answers are: why and how the vesicular state influences the interactions of lipids with the skin and the level of drug penetration.

In conclusion, although producing low amounts of drug in the horny layer, there is a trend towards favouring the topical application of liposomes. They reduce percutaneous absorption, prolong duration of the drug in the dermis (per-

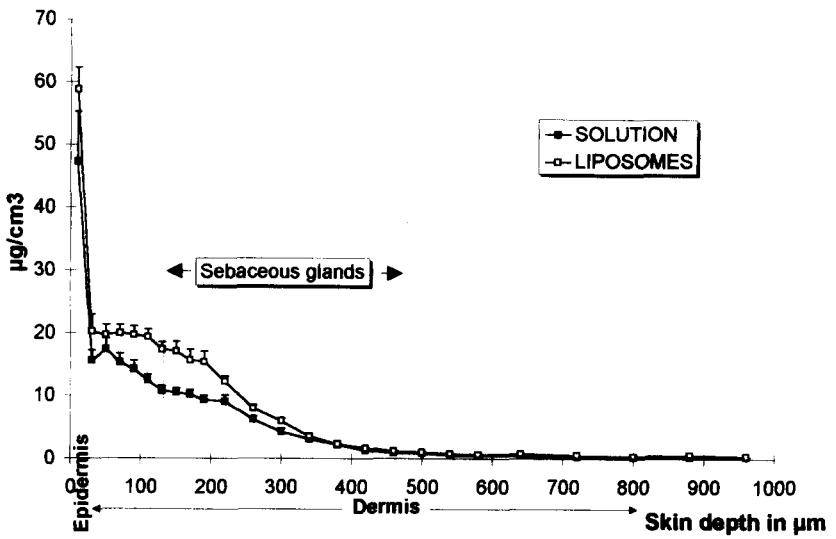


Fig. 5. In vivo distribution in epidermis and dermis of RU 58841 solution and liposomes after five applications on hairless rat skin. Results are expressed in $\mu g/cm^3$ (mean ± SEM), $n = 9$.

Table 2
Histometric parameters of hairless rat skin (back) (Roguet et al., 1986)

Skin layer	Thickness in μm
Epidermis	20.6 ± 6.3
Dermis	796 ± 90
Adipose tissue	258 ± 107
Muscular tissue	330 ± 97
Total thickness of the skin	1383 ± 157
Sebaceous glands localization	
Upper level	195 ± 60
Lower level	397 ± 82

mitting a continuous action, lower number of applications) and produce a targeting of the sebaceous structures.

Another compound topically applied to treat acne is retinoic acid. Studies in which liposomes containing retinoic acid were applied in vitro and in vivo (Mezei, 1992; Masini et al., 1993) have given comparable results: lower percutaneous absorption and skin layers contain higher amounts of retinoic acid after application of liposomes compared with other formulations. Concerning the horny layer, different results have been found with regard to the deposition of drugs: usually liposomes lead to higher amounts of drug and thereby create a drug reservoir in the stratum corneum (Egbaria and Weiner, 1992; Michel et al., 1992; Masini et al., 1993); sometimes no difference is observed (Wohlrab et al., 1992). Liposomes can enhance the passage of drug by sebaceous glands (Lieb et al., 1992) and maintain the drug in the target tissue, but sometimes they do not specifically yield a sebaceous accumulation compared to another formulation (Masini et al., 1993).

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