



ELSEVIER

International Journal of Pharmaceutics 103 (1994) R1–R5

**international
journal of
pharmaceutics**

Rapid Communication

The influence of *in vivo* treatment of skin with liposomes
on the topical absorption of a hydrophilic
and a hydrophobic drug *in vitro*

J. du Plessis^{a,*}, N. Weiner^b and D.G. Müller^a

^a Department of Pharmaceutics, Potchefstroom University for CHE, Potchefstroom, South Africa, ^b College of Pharmacy, University of Michigan, MI, USA

(Received 27 July 1993; Modified version received 3 November 1993; Accepted 11 November 1993)

Abstract

Hairless mice were treated *in vivo* with empty phospholipid and 'skin lipid' liposomes, respectively. The deposition of a hydrophobic drug (hydrocortisone) and a hydrophilic drug (inulin) was investigated in both treated and untreated skin. It was found that drugs should be encapsulated in liposomes or at least be administered together with the lipid, to achieve targeted drug delivery. Pretreatment of the skin with liposomes did not show the advantage of liposomally encapsulated drugs.

Key words: Topical delivery; Liposome; Hydrophilic drug; Hydrophobic drug

The treatment of many dermatological diseases by topical application of drugs is expected to be more efficient if significant concentrations of the drug can be retained in the epidermis. The ability of the stratum corneum to act as a reservoir for drug transport through the skin has been demonstrated by Rougier et al. (1983). A study by Egbaria and Weiner (1991) showed that topically applied liposomal formulations may be an effective delivery system for the treatment of skin diseases. Since these liposomal formulations provide sustained enhanced drug levels in strata as-

sociated with blood and lymph supplies, they have the capacity to deliver sufficient quantities of drug into the skin. Such delivery should also reduce the incidence of undesirable side-effects arising from systemic administration and enhanced systemic absorption of drug after topical application with permeation enhancers which irreversibly disrupt stratum corneum bilayer structures.

Egbaria and Weiner (1991) found that systemic percutaneous absorption of hydrocortisone from liposomes was reduced when compared to solutions. However, a hydrophilic drug (inulin) showed an increase in percutaneous absorption when a liposomal formulation was compared to an aqueous solution. Egbaria and Weiner (1991) sug-

* Corresponding author.

gested that a molecular mixing of the liposomal bilayers and those of the stratum corneum takes place.

Ritschel et al. (1991) proposed a mechanism by which an intracutaneous depot is formed by using the sorption promoter Transcutol® as solvent in topical drug delivery. The stratum corneum is a heterogeneous structure consisting of protein bricks and lipid mortar (Elias, 1983). According to Elias et al. (1981) and Williams and Elias (1987), the intercellular lipids are important in controlling the percutaneous absorption. They found that Transcutol® caused swelling of intercellular spaces and accumulation of foreign material outside the cell membrane. It seems as if Transcutol® causes swelling of the intercellular lipids without altering the multiple bilayer structure of the stratum corneum. These swollen lipids may cause accumulation of the drug and thereby form an intracutaneous depot. In the case of liposomes the phospholipids may mix with the intercellular lipids and thereby cause the same swelling effect to form an intracutaneous depot. However, further evaluation including electron-microscopy studies is necessary to confirm this hypothesis.

Because the mechanism by which liposomes deliver drugs topically is still unknown, it was thought that by treating the skin *in vivo* with empty liposomes, the interaction (if any) of the liposomes and the skin would be shown.

This study describes the effect that *in vivo* treatment of hairless mice with empty liposomes has on the deposition of a hydrophobic drug (hydrocortisone) and a hydrophilic drug (inulin) *in vitro*, in an attempt to explain the mechanism of liposomal interaction with the skin.

[¹⁴C]Inulin and [³H]hydrocortisone were obtained from Amersham (U.K.). Cholesterol (CH), cholesteryl sulphate (CS), bovine brain ceramides (CM), palmitic acid (PA) and HEPES free acid were obtained from Sigma (St. Louis, MO). HEPES free acid has a buffer capacity of 6.8–8.2 and a pK_a of 7.5 at 25°C. Egg lecithin (PC) was obtained from Avanti Polar Lipids (Birmingham, AL) and α -tocopherol (α -T) from Eastman Kodak (Rochester, NY). All other chemicals were of analytical grade and the water used was double-

distilled, deionized and filtered with a Milli-Q system (Millipore Corp., Bedford, MS).

Empty large unilamellar vesicles (DRV) containing PC/CH/CS at a molar ratio of 1:0.5:0.1 and CM/CH/PA/CS at a weight ratio of 4:2.5:2.5:1 were prepared by a modification of the method reported by Kirby and Gregoriadis (1984). Appropriate amounts of the various lipids were dissolved in chloroform/methanol (2:1; v/v) in a round-bottomed flask, and the solvents were removed using a roto-evaporator under vacuum at 40°C. The flask containing the film was dried overnight in a desiccator to remove residual solvent. An appropriate aliquot of 0.05 M HEPES buffer was then added and the mixture was hydrated at 45°C for about 40 min. Intermittent vortexing was required for complete hydration. The resultant dispersion was then dehydrated at 50°C under vacuum, using the roto-evaporator. When the liposomal suspension became very viscous, an amount of water, equivalent to that removed, was reintroduced into the viscous suspension. The rehydrated liposomes were allowed to equilibrate for about 45 min at 45°C. DRV liposomes containing hydrocortisone (HC) 5 mg/ml and inulin 5 mg/ml, respectively, were prepared. HC was incorporated in the lipid bilayers and inulin in the water phase. This was achieved by including the HC in the lipid components when preparing the liposomes and by dissolving the inulin in the water phase (buffer). The liposomes were prepared to have a final total lipid concentration of 25 mg/ml. 1% of α -T was added to the lipid phase of all liposomes.

12 nude mice were anesthetized with sodium pentobarbital (60 mg/kg *i.p.*) and 2 cm² of the dorsal skin of six nude mice were treated with 1 ml of empty phospholipid liposomes (PC/CH/CS) and six were treated with 'skin lipid' liposomes (CM/CH/PA/CS) twice daily for 7 days. The rationale for the use of phospholipid and skin lipid liposomes was to determine if different liposomes might have different effects on skin. A third group of mice were left untreated with liposomes and the diffusion of HC solution, liposomal HC, inulin solution and liposomal inulin was determined as controls to evaluate the influence of the *in vitro* treatment of skin with lipo-

somes on the diffusion of HC and inulin solutions.

Full thickness hairless mouse skin was excised from fresh carcasses of treated and untreated mice, respectively, and subcutaneous fat was carefully removed using a scalpel. The skin sections were mounted on Franz diffusion cells with nominal surface areas of 2 cm² and receiver compartments with 7 ml capacities (Crown Glass, Somerville, NJ). The epidermal side of the skin was exposed to ambient conditions while the dermal side was bathed by a 0.05 M isotonic Hepes buffer. The receiver solution was stirred continuously, using a small teflon covered magnet. Care was exercised to remove any air bubbles between the underside of the skin and solution in the receiver compartment. The temperature of the receiver was maintained at 37°C. Following mounting of the section of skin, 200 µl (5 mg/ml) of the test formulation was applied to the skin. A minimum of three cells were used for each formulation, using sections of skin from different skin specimens for each formulation. All experiments were carried out with non-occluded donor compartments. After 24 h, the experiments were stopped and the diffusion set-up was dismantled for assay of radiolabelled lipids.

Upon dismantling, the donor compartment of the cell was carefully rinsed five times with 0.5 ml buffer, after which the skin was removed. The skin too, was rinsed twice with 3 ml of buffer. The validation of this procedure showed that the washing procedure was found to be sufficient to remove more than 99% of the total dose after application to skin. All washings were collected and assayed for radiolabel.

A common procedure for the determination of phospholipid levels in the stratum corneum involves stripping of the skin. A skin patch was mounted on a board and a piece of adhesive tape (Scotch Magic Tape, 810, 3M Commercial Office Supply Division, St. Paul, MN), 1.9 cm wide and about 6 cm long, was used to strip the skin. The tape was of sufficient size to cover the full area of the skin which was in contact with the formulation. The stripping procedure was repeated until the stratum corneum was completely removed, as determined by transepidermal water loss and vis-

ual observation (Tsai et al., 1991). It was determined that nine such strippings were necessary for mouse skin to ensure removal of the stratum corneum. Each strip was analyzed separately for radiolabelled drug. The remaining skin, as well as the receiver compartment solution, were also assayed for drug content.

Assays of the donor, skin rinses and receiver solutions were carried out after addition of about 15 ml of the scintillation fluid Ecolite + (ICN Biomedical, Inc., Irvine, CA) to every sample. The tape strippings and remaining skin were assayed by placing each sample in a combustion-cone in a tissue oxidizer (Model 306 Packard oxidizer, Packard Instrument Co., Downers Grove, IL). The separated radionuclides were assayed using a scintillation counter.

Table 1 shows the degree of deposition of hydrocortisone and inulin from solutions and liposomal formulations into the various strata (surface stratum corneum, deeper stratum corneum and deeper skin strata) of hairless mouse skin 24 h after *in vitro* topical application of the formulations. The amount of drug adhering to the stratum corneum surface was defined as that determined by analysis of the first two tape strippings. The amount of the lipids penetrating the deeper stratum corneum was defined as that determined by the analysis of tape strippings 3–9 for hairless mouse skin, while the amount of drug penetrating the deeper skin strata was defined as that determined by analysis of the remainder of the full thickness skin. A mass balance of more than 96% was achieved after the donor compartment and the skin rinses were accounted for.

This study, together with others (Weiner et al., 1989; Egbaria and Weiner, 1990; Egbaria et al., 1990), once again demonstrates the superior efficiency of liposomal drug formulations in allowing larger drug reservoirs in the stratum corneum and epidermis than those obtained by using solutions.

The topical delivery of a lipophilic drug in a suitable vehicle usually results in a high concentration of drug in the receiver cell as was found with HC (Table 1). The liposomal encapsulation of the lipophilic drug, hydrocortisone, showed a 10% decrease in the amount of drug found in the

Table 1
The amount (expressed as % of dose) of hydrocortisone and inulin found in the various strata of in vivo treated (liposomes) and untreated nude mice skin after application of the drugs in solution or in liposomal formulations

Compartment	Untreated skin				Liposomal treated skin			
	Liposomal HC		Liposomal inulin		PC/CH/CS		CM/CH/PA/CS	
	HC solution	Inulin solution	HC solution	Inulin solution	HC solution	Inulin solution	HC solution	Inulin solution
Donor	57.4 ± 0.7	27.1 ± 3.7	26.6 ± 3.0	27.9 ± 3.1	67.6 ± 7.0	98.3 ± 1.1	66.6 ± 0.6	93.4 ± 4.4
Surface stratum Corneum	12.8 ± 2.3	49.0 ± 1.8	62.6 ± 2.1	55.8 ± 1.6	8.4 ± 3.3	0.8 ± 0.0	10.2 ± 4.8	5.4 ± 2.8
Deeper Stratum Corneum	7.3 ± 1.7	20.9 ± 0.5	9.4 ± 1.7	14.4 ± 2.1	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
Deeper Skin Strata	1.9 ± 0.2	1.1 ± 0.5	0.4 ± 0.0	1.2 ± 0.0	1.5 ± 0.3	0.2 ± 0.0	2.2 ± 0.6	0.6 ± 0.2
Receiver	20.6 ± 1.1	2.0 ± 0.3	0.9 ± 0.2	0.7 ± 0.0	22.2 ± 5.2	0.5 ± 0.1	20.6 ± 2.5	0.3 ± 0.0

^a All values were corrected to 100%.

receiver cell compared to that when delivered as a solution. A depot effect is thus formed in the stratum corneum from which sustained release into the deeper skin strata can take place. The reduced percutaneous absorption of HC from liposomal formulations compared to solutions (Table 1) may also be an advantage wherever high systemic absorption of the drug is undesirable due to harmful side-effects.

Surprisingly, the *in vivo* treatment of hairless mice with empty liposomes before *in vitro* diffusion studies was shown to have no advanced drug absorption for either hydrophilic and lipophilic drugs into the deeper stratum corneum and the deeper skin strata. The amount of hydrocortisone in the receiver cells found were even higher (22.2% compared to 20.6%) than the amount delivered in a solution.

Inulin is a very hydrophilic drug. Topical delivery of hydrophilic drugs usually creates problems due to their inability to penetrate the stratum corneum. However, it was found that by encapsulation of inulin into liposomes, the amount of drug delivered into the deeper stratum corneum was increased by 53%, while the amount of drug into the deeper skin strata was improved by 300% (Table 1). Diffusion studies with an aqueous solution of inulin, using *in vivo* liposomal treated skin, resulted in very small amounts of inulin delivered into the skin. Even when compared to the amounts of drug delivered with an aqueous solution into untreated skin, very low concentrations of drug was found. The hypothesis that some interaction between the liposomes and stratum corneum takes place, could thus not be illustrated in this study. Skin lipid liposomes and phospholipid liposomes both resulted in the same effect. The fact that in both cases more than 93% of the dose was found in the donor cells showed that very little partitioning of the hydrophilic drug into the liposomal treated skin took place which probably was to be expected.

It is therefore clear that drugs should be encapsulated in liposomes or at least be administered together with the lipid to achieve targeted drug delivery. Further studies, including electron

microscopy, are necessary to show the interaction of liposomes and the stratum corneum. From this study it could be concluded that the use of liposomes is an effective delivery system for the treatment of skin diseases, however, the drug should be administered encapsulated in the liposomes. Pre-treatment of the skin with liposomes, regardless of their composition, did not show the advantages observed with liposomal encapsulated drugs.

References

- Egbaria, K. and Weiner, N., Liposomes as a topical drug delivery system. *Adv. Drug Del. Rev.*, 5 (1990) 287–300.
- Egbaria, K. and Weiner, N., Topical application of liposomal preparations. *Cosmet. Toilet.*, 106 (1991) 79–93, Mar.
- Egbaria, K., Ramachandran, C. and Weiner, N., Topical delivery of ciclosporin: evaluation of various formulations using *in vitro* diffusion studies in hairless mouse skin. *Skin Pharmacol.*, 3 (1990) 21–28.
- Elias, P.M., Epidermal lipids, barrier function and desquamation. *J. Invest. Dermatol.*, 80 (Suppl.) (1983) 44–49.
- Elias, P.M., Cooper, F.R., Kore, A. and Brown, B.F., Percutaneous transport in relation to stratum corneum structure and lipid composition. *J. Invest. Dermatol.*, 76 (1981) 297–301.
- Kirby, C.J. and Gregoriadis, G., A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions. In Gregoriadis, G. (Ed.), *Liposome Technology*, Vol. I, CRC Press, Boca Raton, FL, 1984, pp. 19–27.
- Ritschel, W.A., Panchagnula, R., Stemmer, K. and Ashraf, M., Development of an intracutaneous depot for drugs. *Skin Pharmacol.*, 4 (1991) 235–245.
- Rougier, A., Dupuis, D., Lotte, C., Roguet, R. and Schaefer, H., *In vivo* correlation between stratum corneum reservoir function and percutaneous absorption. *J. Invest. Dermatol.*, 81 (1983) 275–278.
- Tsai, J.C., Cappel, M.J., Weiner, N.D., Flynn, G.L. and Ferry, J., Solvent effects on the harvesting of stratum corneum from hairless mouse skin through adhesive tape stripping *in vitro*. *Int. J. Pharm.*, 68 (1991) 127–133.
- Weiner, N., Williams, N., Ramachandran, C., Flynn, G., Shipman, C. and Birch, G., Topical delivery of liposomally encapsulated interferon in a cutaneous herpes guinea pig model. *Antimicrob. Agents Chemother.*, 33 (1989) 1217–1221.
- Williams, M.L. and Elias, P.M., The extracellular matrix of stratum corneum: role of lipids in normal and pathological function. *CRC Crit. Rev. Ther. Drug Carrier Syst.*, 3 (1987) 95–122.