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Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro

Gamal M. M. El Maghraby, Adrian C. Williams and Brian W. Barry

Abstract

The potential use of ultradeformable and standard liposomes as skin drug delivery systems was investigated in-vitro. An improved experimental design gave a good measure for skin deposition of drug. This avoided the contamination that can occur due to incomplete washing of the donor before direct determination of the amount of drug in the skin. The design used aqueous ethanolic receptor which is believed to diffuse into skin, disrupting deposited liposomes (if any) and thus releasing both bound and free drug. The receptor fluid was refined by testing different concentrations of ethanol. The applied dose was also optimized. Using the improved design and the optimum dose, an ultradeformable formulation was compared with four traditional liposomes for skin delivery of 5-fluorouracil (5-FU). The best receptor was 50% aqueous ethanol and the optimum dose was 20 μL. The ultradeformable formulation was superior to standard liposomes in the skin delivery of 5-FU. Of the traditional liposomes, the non-rigid preparation was the best. However, stabilization of the liposome membrane with cholesterol abolished the benefit of this non-rigid preparation. It was concluded that ultradeformable vesicles are promising agents for skin delivery of drugs.

Introduction

The strategy of using liposomes to overcome the barrier nature of the skin and control drug delivery has gained interest in the last two decades (Mezei & Gulasekharam 1980, 1982; Mezei 1992; Touitou et al 1994). A new type of lipid vesicle (Transfersome) has been reported to penetrate intact skin and travel deep enough to be distributed throughout the body via lymphatic and blood circulation, after open application to partly hydrated skin (Cevc & Blume 1992; Cevc et al 1995). It might be thought that the absence of a lymphatic system and blood could hinder the in-vitro evaluation of these vesicles. However, for vesicles to reach the lymphatic system and blood circulation, they must penetrate the dead stratum corneum (SC) and then the viable epidermis. Thus in-vitro investigations should be possible provided that an epidermal membrane is used with a receptor fluid that ensures sink conditions. In agreement with this concept, it was recently reported that when sodium cholate-containing deformable phospholipid vesicles carrying ciclosporin A were applied to freshly excised non-hydrated mouse skin, the permeation and deposition of ciclosporin A improved. Vesicles only increased skin deposition when applied to fully hydrated mouse skin (Guo et al 2000).

We therefore began our in-vitro investigations by employing a protocol of open application to partially hydrated human epidermis or SC and using 5-fluorouracil (5-FU) as a model drug, with a sodium cholate-containing highly deformable

Drug Delivery Group, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP, UK

Gamal M. M. El Maghraby, Adrian C. Williams, Brian W. Barry

Correspondence: B. W. Barry, School of Pharmacy, University of Bradford, Bradford, BD7 1DP, UK.

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Table 1 Formulation code, lipid composition, type and drug entrapment efficiency expressed as percentage of the initial drug added.

Code	Lipid composition	Type	Entrapment efficiency (%)
UFU	PC/sodium cholate (84:16, w/w)	Ultradeformable	8.8 (3.0)
T1	Pure PC	Non-rigid	9.2 (3.4)
T2	PC/cholesterol (1:1, molar ratio)	Membrane-stabilized	9.6 (3.7)
T3	Pure DPPC	Rigid	9.2 (3.1)
T4	DPPC/cholesterol (2:1, molar ratio)	Rigid	9.3 (3.2)

Values in brackets are s.d., n = 3. PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine

(ultradeformable) formulation (El Maghraby et al 2000a). The study used an aqueous receptor and determined the cumulative amount of drug penetrating for 36 h. Then the remaining donor was removed by washing and the amount of drug deposited in the skin was determined after dissolving the tissue. The work demonstrated that deformable vesicles were promising vectors for skin drug delivery. The results suggested improved skin deposition, with little effect on the penetration of the drug, after application of the ultradeformable vesicles to epidermis or SC, compared with saturated aqueous solution of the drug. However, the values measured for skin deposition were even higher than the entrapment efficiency of the drug in liposomes. This finding may have arisen from incomplete removal of the donor during the five times washing protocol, suggesting that an improvement was needed in the experimental design. This refinement could be the use of a receptor fluid that would extract the deposited drug from skin and thus avoid interference from incomplete washing of the donor before determination of the amount in the skin.

Accordingly, the aim of this study was to optimize the experimental design with regard to the receptor fluid and the applied dose and to compare ultradeformable vesicles with standard liposomes for the delivery of 5-FU in-vitro. The optimization process used sodium cholate ultradeformable liposomes. The formulation details are given in Table 1.

Materials and Methods

Materials

5-(6-^{3}H)-Fluorouracil was obtained from DuPont NEN, Germany. 5-FU (purity 99%), phosphatidylcholine (PC) from soybeans (99%), dipalmitoylphosphatidylcholine (DPPC), sodium cholate (99%), cholesterol and Sephadex G50 medium were purchased from Sigma Chemical Company (UK). Scintillation fluid, OptiPhase 'HiSafe' 3, was obtained from LKB Scintillation Products Ltd (UK). All chemicals were used without further purification. Water was double-distilled.

Preparation of lipid vesicles

Vesicles were prepared by an extrusion method (El Maghraby et al 2000a). Briefly, lipid mixtures were dissolved in ethanol, except for cholesterol or DPPC-containing formulations which were solubilized in chloroform/ethanol, 2:1 (v/v). Organic solvents were removed by rotary evaporation above the lipid transition temperatures (ambient temperature for ultradeformable and pure PC vesicles, or 50°C for cholesterol or DPPC-containing formulations); solvent traces were removed from the deposited lipid films under vacuum overnight. Lipid films were hydrated with saturated radiolabelled aqueous 5-FU by rotation at 60 rev min^{-1} for 1 h at the corresponding temperatures. The resultant hydrated vesicles were swollen for 2 h at room temperature before manual extrusion 10 times through sandwiches of 200- and 100-nm polycarbonate membranes. The lipid concentration was 5% (w/v). These procedures produced vesicles with a size of 129 ± 27 nm as determined by photon correlation spectroscopy. In skin permeation studies, liposomes were applied without separation of the non-entrapped drug (i.e. suspended in saturated solution to minimize loss of entrapped drug).

Determination of entrapment efficiency

Entrapment efficiency was calculated after separation of the non-entrapped drug using the mini-column centrifugation method (Fry et al 1978; New 1990). Sephadex G50 (10 g) was swollen in distilled water (120 mL) at room temperature for at least 5 h and stored at 4°C. To prepare the mini-columns, Whatman GF/B filter pads

were inserted in the bottom of the barrels of 2.5-cm^3 syringes which were then filled with gel. Excess water was centrifuged off at 3000 rev min^{-1} for 3 min, and 500 μL of the liposome suspensions were added dropwise to the center of the column, followed by centrifugation as before. To the mini-column, 625 μL distilled water was added and centrifugation repeated. Liposomes (depending on their type and size) can be recovered from the first or the first and second stages of centrifugation (New 1990). In this study, two stages were necessary to recover the vesicles. The amount of drug entrapped in the vesicles was determined by liquid scintillation counting after correction for the dilution factor. The entrapment efficiency was calculated as a percentage of the initial drug added. This method was able to separate all the free drug as evidenced by the absence of any drug in the centrifugate in the two stages when performing the separation process after application of saturated drug solution instead of liposomes.

Drug release from liposomes

Liposome suspensions in double-distilled water or saturated 5-FU solution were incubated at 32°C, 500-μL samples were taken at different times and the free drug was separated by the mini-column centrifugation as above. The percentage drug released was then calculated indirectly from the percentage drug entrapped, taking the amount entrapped at time zero as the initial amount (100 % entrapped and 0 % released).

Preparation of skin samples

Mid-line abdominal Caucasian postmortem skin samples were obtained from 19 donors (5 male) average age 77.5 ± 7.5 years. The samples were flattened and stored in vacuum-sealed double polythene bags at -20°C (Harrison et al 1984). Epidermal membranes were prepared by a heat separation technique (Kligman & Christophers 1963); fat and connective tissue were removed, the skin was heated for 45 s in a water bath at 60°C, after which the epidermis was gently teased off the underlying dermis.

To obtain partially hydrated membranes, an open hydration protocol was used (El Maghraby et al 1999, 2000b). This procedure maintains a transepidermal hydration gradient which has been proposed as generating the driving force for the Transfersome skin penetration (Cevc & Blume 1992). The membranes were thus floated with the SC side uppermost on 0.002 % aqueous sodium azide and the upper surface was left open to the atmosphere for 24 h. Membranes were equilibrated for a further 12 h on diffusion cells with receptor fluid flowing beneath the membrane and the skin surface left opened to the atmosphere.

Skin penetration studies

An automated diffusion apparatus with cells having flow-through receptor compartments and a diffusional area of 0.126 cm^2 was used (Akhtar et al 1984). The membranes were mounted with the SC surface uppermost; the system held the skin surface at 32°C. Sink conditions were maintained by pumping heated degassed aqueous ethanolic receptor solution (as specified in the corresponding section) through the receptor compartments at 2 mL h^{-1}. These penetration studies involved open application of 5-FU in liposomes or as saturated aqueous solution (control). A dose of 5 μL was used initially, and, thereafter, the determined optimum dose was applied. Receptor samples were collected at time intervals for 36 h and the cumulative amounts of drug delivered were calculated. The data are presented as the amount penetrated after 12 and 36 h. The Student's *t*-test was used as a test for significance throughout.

Results and Discussion

Entrapment efficiency

The entrapment efficiency is expressed as fraction of drug incorporated into liposomes relative to the total amount used (Montengero et al 1996; Kim et al 1998). Liposome formulations encapsulating 5-FU included one ultradeformable preparation (UFU) containing PC, sodium cholate (84 : 16, w/w) and with 7 % (v/v) ethanol in the final formulation and four traditional formulations (T1–T4; Table 1). Results of entrapment efficiency are given in Table 1.

5-FU, having a low partition coefficient, mainly localized in the liposome aqueous domain. Accordingly, entrapment efficiency depends on the liquid volume entrapped. Table 1 reveals similar entrapment efficiencies for different formulations, as expected for unilamellar vesicles with similar diameters.

Drug release from liposomes

Initially, sodium cholate-containing ultradeformable vesicles, UFU, (Table 1) were prepared and non-entrapped 5-FU was separated. Liposomes were then suspended in distilled water; Figure 1 illustrates the release results. There was an initial rapid release of 5-FU, after which the data plateau; > 80 % of entrapped drug was lost (see also Simmons & Kramer 1977).

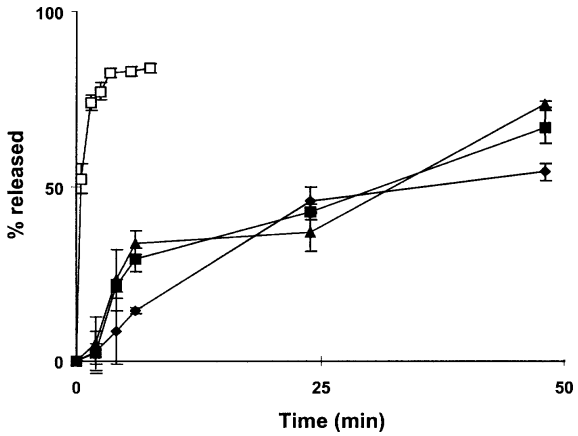

Figure 1 Release of 5-fluorouracil from liposomes. □, Sodium cholate ultradeformable vesicles (UFU) suspended in water; ■, UFU suspended in saturated drug solution; ▲, pure PC vesicles (T1) suspended in saturated drug solution; ◆, PC/cholesterol (T2) liposomes suspended in saturated drug solution.

Kinetics were first-order only at the early stage. The plateau may be due to free and entrapped drug reaching an equilibrium. Accordingly, it was decided not to separate the non-entrapped drug and liposomes were used suspended in saturated drug solution in further studies.

Release of 5-FU from UFU, pure PC (T1) and PC/cholesterol, 1:1 molar ratio (T2) liposome formulations suspended in saturated drug solution was investigated (Figure 1).

Suspending UFU vesicles in drug solution significantly reduced the loss of 5-FU. 5-FU release from liposomes depends on their lipid composition (Elorza et al 1993); cholesterol reduced the drug release from PC (liquid state lipids) (Crommelin et al 1994). Our study showed no significant differences between UFU and T1, but incorporation of cholesterol in PC vesicles significantly ($P < 0.05$) reduced 5-FU release after 48 h (T1 vs T2).

Optimization of receptor fluid for measurement of skin delivery of 5-FU from liposomes

A previous investigation employed open application to partially hydrated human skin with 5-FU in a sodium cholate-containing ultradeformable formulation using an aqueous receptor. The protocol determined the amount of drug deposited (after washing the donor compartment) by dissolving the skin (El Maghraby et al 2000a). Deposition values, however, were even higher than drug entrapment efficiency in liposomes. Presumably, some of the liposomes in the donor attached to SC, resisting washing. Thus, the experimental design needed improvement.

Receptor fluid containing 50% aqueous ethanol was used to study the effect of penetration enhancers on permeation of lipophilic tetrahydrocannabinol compared with hydrophilic 5-FU, to achieve sink conditions (Touitou & Fabin 1988). Ethanol solution was similarly used with estradiol (Goodman & Barry 1988) and for evaluating liposomal delivery of retinoic acid (Montengero et al 1996).

Accordingly, we investigated aqueous ethanol as a receptor. To determine the lowest effective concentration, an approximate solubility study was performed, whereby 10 parts liposomes (UFU) were added to 90 parts ethanol in water (10, 20, 30, 40 and 50%, v/v). Solutions of 30–50% ethanol visually solubilized liposomes. However, when 20 parts liposomes were added to 80 parts of different concentrations of ethanol, only 50% (v/v) ethanol dissolved the liposomes. Accordingly, 50% (v/v) ethanol in 0.002% sodium azide solution was selected as the receptor. Based on the approximate solubility study, we considered that the ethanolic receptor will diffuse into the epidermis, disrupt the structure of any deposited liposomes and thus release free and liposome-bound drug. This design could give a more accurate comparative measure for the delivery of 5-FU into the membrane, compared with determination of drug in skin using a washing protocol. Thus, the problem of possible failure of the washing procedure to remove liposomes adhering to the SC surface was avoided.

The study was designed to monitor delivery of 5-FU using aqueous or ethanolic receptor; 5 μL of UFU or saturated aqueous drug solution was applied as the donor. Data are given in Table 2.

Ethanol/water co-solvents can increase the permeation of a wide range of drugs through human or animal skin. Other authors have used ethanol either in the donor side (Berner et al 1989; Kurihara-Bergstrom et al 1990; Hatanaka et al 1993; Obata et al 1993) or both in the donor and receptor compartments (Liu et al 1991; Megrab et al 1995). Our study used 50% aqueous ethanol in the receptor compartment only. The use of the ethanolic receptor on one side of skin results in a solvent gradient in the skin. Using ethanol in the donor is expected to have the greatest effect on the SC side (the main barrier). However, the effect will be lower if using the solvent in the receptor only. As expected with our design, for saturated aqueous 5-FU (control), the use of an ethanolic receptor increased the amount permeated through human epidermis by 2.7- and 2.3-fold (after 12 and 36 h, respectively), compared with aqueous recep-

Table 2 Amount of 5-fluorouracil (μg cm^{-2}) penetrated through human epidermis after its open application (5 μL) as ultradeformable vesicles (UFU) and saturated aqueous solution (control), using aqueous and 50% ethanolic receptors.

	Aqueous receptor		Ethanolic receptor	
	UFU	Control	UFU	Control
After 12 h	0.790 (0.31, n = 4)	0.733 (0.14, n = 6)	19.5 (4.9, n = 6)	2.01 (0.61, n = 4)
After 36 h	2.12 (0.64, n = 4)	1.66 (0.28, n = 6)	35.1 (9.4, n = 6)	3.88 (1.5, n = 4)

Values in brackets are s.e.m. and number of replicates, respectively.

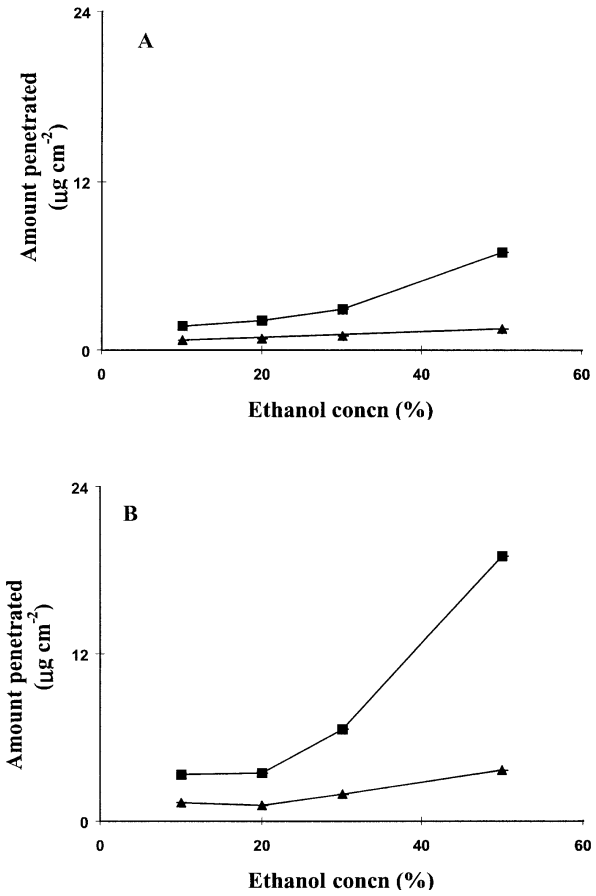

Figure 2 Amount of 5-fluorouracil penetrated through human epidermal membrane after 12 (A) and 36 (B) h of its application in ultradeformable vesicles (■) or saturated aqueous solution (▲), using receptors containing different concentrations of ethanol in aqueous sodium azide.

tor. Although this increase was not significant ($P > 0.05$) (Table 2), it was not neglected when investigating UFU penetration, as the saturated aqueous solution was used as control.

Using an ethanolic receptor, the amount of 5-FU penetrated through epidermis from the ultradeformable vesicle was significantly higher ($P = 0.017$ and 0.022 for 12 and 36 h, respectively) than that delivered using an aqueous receptor (Table 2).

Based on this study, the use of an ethanolic receptor appears to be an improvement in the experimental design compared with a direct determination of the amount of drug in the skin. However, a question remained regarding the selection of 50% ethanol on the basis of the simple solubility study and whether it was possible to optimize this selection further.

To answer this, we studied the effect of different concentrations of ethanol in the receptor fluid on the penetration of estradiol from lipid vesicles and saturated solution. This experiment attempted to achieve an ethanol concentration in the receptor $< 50\%$, but with similar efficiency in disrupting any liposomes deposited in skin. Accordingly, we investigated the effects of 10, 20, 30, and 50% (v/v) ethanol/aqueous sodium azide (0.002%, w/v) as receptor fluids on the transepidermal delivery of 5-FU from saturated solution and from ultradeformable liposomes (UFU), using open application of a 5-μL dose. The experiment involved two runs: one used saturated 5-FU solution as the test donor and the other investigated the lipid vesicles. The amounts of drug penetrated after 12 and 36 h were determined (Figure 2).

For saturated aqueous solution, raising the ethanol concentration showed a trend of increasing the amount of drug penetrated (Figure 2). This trend was linear with ethanol concentration (10–50%) after 12 h and was linear over the range 20–50% ethanol after 36 h (Figure 2). This increase was generally not significant ($P > 0.05$ for 10 vs 50%). Similar effects were reported for 5-FU even when ethanol was used in the donor solution (Hatanaka et al 1993). It seems that ethanol up to 50% (v/v) applied to one side of the membrane only marginally alters the pathway of hydrophilic drugs

permeating through human skin, compared with its partitioning effect on lipophilic drugs.

For ultradeformable vesicles (UFU), increasing ethanol concentration (10–30 %, v/v) gradually enhanced 5-FU penetration, but not significantly. However, at 50 % (v/v), drug delivery increased abruptly ($P < 0.02$ for 50 vs 10 % and 50 vs 20 %; $P < 0.05$ for 50 vs 30 %).

The trend of an initial gradual rise in drug penetration from liposomes with increased ethanol concentration (from 10 to 30 %), followed by the abrupt promotion at 50 % ethanol, indicates that 50 % (v/v) ethanol in the receptor is the most effective. This marked inflection is due to the increase in the efficiency for disrupting deposited liposomes. This result is supported by the approximate solubility study. Also, comparing the trends obtained for saturated solution with that for UFU (Figure 2), the abrupt increase with 50 % (v/v) ethanol only occurred with UFU. This indicates that the sudden increase was not due to the usual penetration enhancing effect of ethanol, but arises from vesicle disruption. This observation suggests further that ultradeformable vesicles penetrate into epidermis, and if 5-FU was deposited only as free drug, an ethanolic receptor would not be needed to release it. However, these studies did not provide clear evidence to support the hypothesis of intact vesicles penetrating through skin rather than simply entering and remaining within the SC (Cevc & Blume 1992).

Based on these data, 50 % (v/v) ethanol/aqueous sodium azide solution (0.002 %, w/v) was selected as the optimum receptor for further studies.

Optimization of applied dose for in-vitro skin delivery of 5-FU from liposomes

Transfersomes have been reported to deliver corticosteroids into and through skin in a dose-dependent manner (Cevc et al 1997). Therefore, we studied the effect of applied dose of ultradeformable liposomes on epidermal penetration of 5-FU in-vitro. The investigation involved open application of 10, 20 and 40 μL liposomes, with 50 % (v/v) ethanol/aqueous sodium azide as receptor fluid. As liposomes were suspended in saturated drug solution, we needed to avoid the effect of different volumes and different contents of free drug. Thus, all applied doses were adjusted to a final volume of 40 μL using saturated solution. Expressing the doses as amount of total lipids applied per unit area of skin, these were equivalent to 4, 8 and 16 mg cm^{-2}, respectively. Saturated aqueous solution of 5-FU (40 μL) was the control. As the dose volume was relatively high, evaporation was accelerated by streaming ambient air above the cells. This procedure dried the skin surface, as required for optimal delivery of Transfersomes (Cevc & Blume 1992).

The amounts of drug penetrated after 12 and 36 h were calculated (Table 3). Figure 3 shows the profiles of 5-FU penetration through human epidermis, the rate plots, and the percentage drug penetrated (after 36 h) obtained after application of different doses of liposomes (the value for the 5-μL dose equivalent to 2 mg lipids cm^{-2} skin was obtained from Table 2).

Penetration profiles and rate plots (Figure 3) showed typical low-dose behaviour with most of the delivered amounts being recovered in the first 12 h.

The UFU liposomes at all doses significantly increased drug penetration compared with saturated solution (Table 3).

Different doses of liposomes delivered different amounts of drug through human epidermis. Increasing the dose from 4 to 8 mg cm^{-2} (10 to 20 μL) almost doubled the delivery (Table 3). However, increasing from 8 to 16 mg cm^{-2} (20 to 40 μL) did not change the amount (difference within the range of experimental error). Expressing the total amount penetrated as a percentage of the total applied dose (Figure 3c) indicated

Table 3 Amount of 5-fluorouracil penetrated (μg cm^{-2}) through human epidermis after open application of different doses of ultradeformable vesicles (UFU) using 50 % ethanolic receptor.

	Applied dose			
	4 mg cm^{-2}	8 mg cm^{-2}	16 mg cm^{-2}	Control
After 12 h	222 (65, n = 6)	425 (73, n = 6)	374 (89, n = 6)	32.2 (16, n = 6)
After 36 h	231 (66, n = 6)	437 (76, n = 6)	392 (92, n = 6)	37.4 (17, n = 6)

Saturated aqueous solution was used as control. Values between brackets are s.e.m. and number of replicates, respectively.

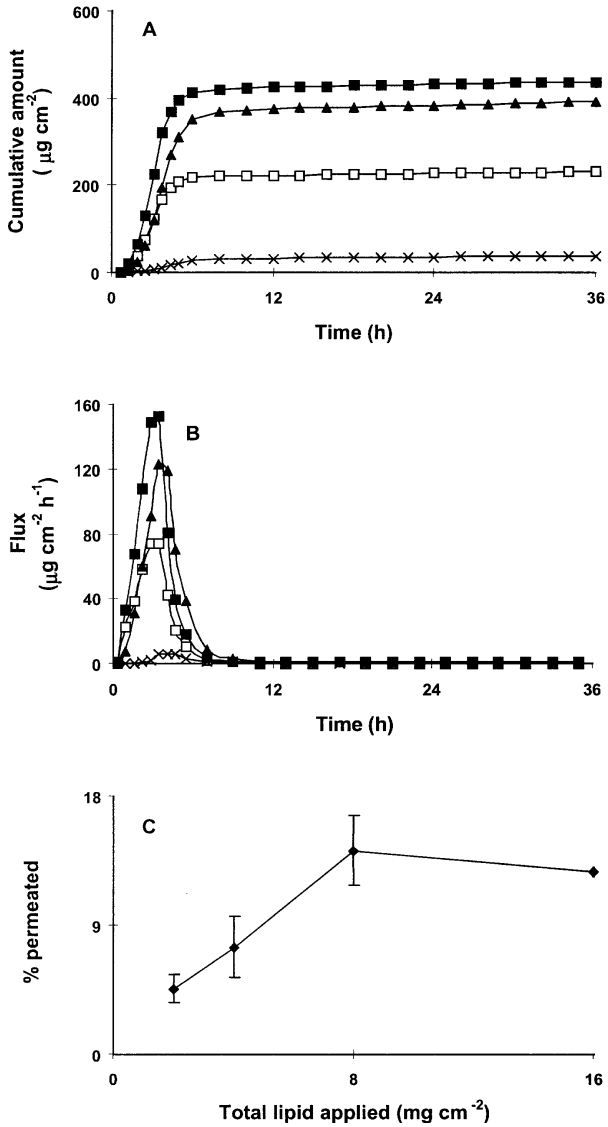

Figure 3 Penetrated profiles (A) and rate plots (B) of 5-fluorouracil through human epidermis obtained after application of 4 (□), 8 (■), 16 (▲) mg cm^{-2} liposomes or 40 μL saturated aqueous control (×). Error bars have been omitted for clarity. Total percent of drug penetrated as a function of applied dose of liposomes expressed as mg lipids cm^{-2} skin (C). The value for 2 mg cm^{-2} dose was calculated from the equivalent 5-μL dose (Table 2).

that UFU delivered 5-FU in a dose-dependent saturable process. Thus, there was an optimum dose at 8 mg cm^{-2} (20 μL liposomes dispersed as 50 mg mL^{-1} suspension).

The percentage of drug penetrated was higher than the drug entrapment efficiency of liposomes. This strongly suggested that liposome components may have altered the skin structure, thus enhancing 5-FU transmission through human epidermis.

From this study a 20-μL dose of liposomes (dispersed as 50 mg mL^{-1}) was selected as the optimum dose for the remainder of the work.

Ultradeformable vesicles vs standard liposomes for in-vitro skin delivery of 5-FU

Improved skin deposition and reduced systemic concentration and side-effects of triamcinolone acetonide were reported after topical application in traditional liposomes of DPPC and cholesterol, compared with conventional dosage forms (Mezei & Gulasekharam 1980, 1982). Liposomes prepared from soya phospholipids were used as percutaneous drug carriers (Artman et al 1990a, b). PC, cholesterol and phosphatidylserine liposomes increased skin deposition of inulin compared with aqueous solution (Egbaria & Weiner 1992). Depending on lipid composition, traditional liposomes have been reported to localize drug in skin strata and in a few cases increased transdermal delivery has been claimed (Mezei 1992). Traditional liposomes of PC and cholesterol improved skin deposition of hydrophilic caffeine (Touitou et al 1994). When non-rigid traditional vesicles were used, the percutaneous absorption of inulin increased, but liposomes comprising SC lipids produced a localized effect in the skin (Fresta & Puglisi 1996). These reports demonstrated the potential use of traditional liposomes for skin delivery of hydrophilic drugs. Accordingly, we compared the skin delivery of 5-FU from ultradeformable vesicles with that from traditional liposomes.

Tested formulations included ultradeformable vesicles and a range of traditional liposomes (Table 1). The ultradeformable vesicles comprised phosphatidylcholine (PC) mixed with sodium cholate and contained 7% (v/v) ethanol in the final formulation. Traditional formulations included pure PC liposomes (T1) as the prototype of non-rigid liposomes (low T_m), PC mixed with the membrane stabilizer, cholesterol (T2), pure DPPC (T3, rigid vesicles, high T_m) and DPPC mixed with cholesterol (T4, rigid liposomes).

The study employed an open application of a 20-μL dose (optimum dose) and used a 50% (v/v) ethanolic receptor. Saturated aqueous solution of 5-FU was the control. The amounts of drug penetrated after 12 and 36 h are given in Table 4. When comparing different runs, values relative to the corresponding controls were compared to counteract inherent skin variability.

As expected from the previous work, encapsulation of 5-FU into ultradeformable vesicles significantly increased drug delivery through human epidermis using ethanolic receptor (Table 4) compared with saturated

Table 4 Amount of drug penetrated through human epidermis after 12 and 36 h of open application of 20 μL of liposome formulations or saturated aqueous solution (control) and relative amount compared to control, using 50% ethanolic receptor.

Formulation	Amount permeated (μg cm^{-2}) and relative amount			
	Amount (12 h)	Relative amount	Amount (36 h)	Relative amount
UFU	32.1 (8.9, n = 6)	10.2	41.3 (8.4, n = 6)	8.21
T1	8.05 (1.9, n = 7)	2.55	12.3 (2.3, n = 7)	2.45
T2	3.8 (1.4, n = 8)	1.20	6.62 (2.5, n = 8)	1.32
T3	7.9 (4.3, n = 6)	0.77	30.7 (7.7, n = 6)	2.18
T4	19.0 (10, n = 8)	1.84	28.8 (10.7, n = 8)	2.04
C1	3.16 (0.84, n = 7)		5.03 (1.2, n = 7)	
C2	10.3 (3.7, n = 8)		14.1 (4.3, n = 8)	

Values between brackets are s.e.m. and number of replicates, respectively; C1 = control for UFU, T1 and T2 formulations; C2 = control for T3 and T4 liposomes. Formulation details are given in Table 1.

aqueous control ($P < 0.01$). Most of the drug was recovered in the first 12 h.

For traditional formulations, non-rigid vesicles (pure PC; T1) significantly increased penetration by 2.5-fold ($P < 0.05$). In contrast, the membrane-stabilized formulation (T2) delivered similar amounts compared with control. Encapsulation of 5-FU in rigid formulations (T3 and T4) showed a trend of doubling the total amount penetrated, but this was not significant ($P > 0.05$).

To compare individual formulations, relative values were used (Table 4). Ultradeformable vesicles produced the highest relative value; they were significantly better than any of the traditional formulations ($P = 0.011$–0.026) (the Student's t-test was done on the amounts when comparing UFU vs T1 or T2 and on the relative values in case of UFU vs T3 or T4, because UFU, T3 and T4 were tested in different runs).

The only successful traditional formulation was the non-rigid pure PC vesicles (T1), but its delivering efficiency was at most only 30% of that of UFU. Incorporation of cholesterol (T2), a membrane stabilizer, abolished the effect obtained with pure PC liposomes. This finding may suggest that cholesterol could have inhibited or reduced vesicle breakdown with subsequent monomeric transfer of PC. Thus PC, cholesterol vesicles produced no enhancing effect. This may suggest that monomeric transfer of PC is responsible for the enhancing effect produced in general by pure PC vesicles.

Conclusions

To assess drug deposition in skin after a penetration experiment using liposomes, the use of an aqueous ethanolic receptor to release deposited drug was better than determining the amount in skin tissue (because of failure of the washing process). The ultradeformable formulation is a promising drug delivery system, although results suggested only improved skin deposition as an aqueous ethanolic receptor was required to liberate drug from skin. The non-rigid traditional formulation was better than membrane-stabilized or rigid formulations in the delivery of 5-FU to the epidermis.

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