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Effect of charge and lipid concentration on in-vivo percutaneous absorption of methyl nicotinate from liposomal vesicles

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Abstract

We have investigated the influence of charge and lipid concentration on the in-vivo percutaneous absorption of a model compound, methyl nicotinate (MN), from liposomal vesicles. MN-loaded liposomes were produced by the reverse-phase evaporation method (REV) using different concentrations of phosphatidyl choline (PC), in association with surfactants such as dioctadecyl dimethyl ammonium bromide ($DDAB_{18}$) and dicetyl phosphate (DCP), which impart a positive or negative charge to the systems, respectively. The liposomal suspensions were then processed to hydrogels and used to study in-vivo the MN permeation profile. MN was chosen as the model compound since it was capable of causing cutaneous erythema, the intensity and duration of which was proportional to the amount entering the living epidermis over time. The extent of the erythema was monitored by reflectance spectrophotometry, a non-invasive technique. In-vivo findings showed an interesting MN delayed release, which was proportional to the amount of phospholipids in each liposomal formulation. Furthermore, it could be noted that the erythematous effect was more prolonged when MN was delivered from neutral or negatively-charged liposomal forms.

Introduction

Liposomes, obtained from a variety of natural and synthetic phospholipids, are considered as interesting vehicles to administer active substances to the skin (Kirjavainen et al 1999; El Maghraby et al 2001). Not only do these vehicles enhance drug penetration into the skin with the slow release of the drug, but decrease the clearance of the drug by minimizing its absorption into the systemic circulation (Glavas-Dodov et al 2002). They may be used as a solubilization matrix, as a local depot for sustained release of dermally-active compounds, as permeation enhancers, or as a rate-limiting membrane barrier for the modulation of systemic absorption of drugs via the skin (Schreier & Bouwstra 1994; Fang et al 2001).

The mechanism by which liposomes deliver drugs into the skin has been studied widely. Different studies have reported that conventional liposomes are not able to penetrate intact into the skin, while it has been well accepted that liposomal material (e.g. lipid bilayer constituents) can interact with skin components (intracellular lipid domain) producing a drug 'reservoir' in the stratum corneum (Du Plessis et al 1994; Bonina et al 1995; Puglia et al 2004). Recently, we evaluated this interaction (Puglia et al 2004) regarding the in-vivo percutaneous absorption of indometacin-loaded liposomes. In particular, an interesting sustained release of the drug toward skin layers was observed, suggesting the hypothesis of the slow release of the drug from a depot within the stratum corneum originating from a mix of liposomal phospholipids and physiological intercellular lipids.

Many researchers have outlined that drug release from liposome formulations can be controlled by varying the size and the composition of lipids (Verma et al 2003) and modifying the surface charge of liposomes (Katahira et al 1999; Ogiso et al 2001; Manosroi et al 2002). In particular, the use of charged liposomes has been generally

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recommended, since the adsorption of liposomes onto the skin is due to physical (electrostatic, hydrophobic, etc.) forces (Hofland et al 1995).

Manosroi et al (2004) studied in-vitro the transdermal absorption of amphotericin B from a liposome formulation through rat skin, evaluating the effect of charged lipids (dicetyl phosphate or stearylamine) on the drug permeation profile. The results outlined that the drug entrapped in charged liposomes showed sustained skin absorption. Katahira et al (1999) prepared rhodamine B-loaded liposomes and studied in-vitro the effect of positive and negative components incorporated in the liposomal bilayer on topical delivery of the drug. It was found that skin permeability of rhodamine B enclosed in positively-charged liposomes was higher compared with the use of negatively-charged liposomes. More recently, Ogiso et al (2001) demonstrated in-vitro and in-vivo the capability of negatively-charged liposomes to increase the permeation rate of different model drugs through rat skin.

Notwithstanding this copious literature, until now we have not found in-vivo data about the effect of the surface charge of liposomes on the percutaneous absorption of active compounds through human skin. Therefore, in this study we have evaluated the effect of liposomes on the in-vivo percutaneous absorption of the model compound methyl nicotinate (MN). In particular, the influence of the lipid concentration and the presence of an electric charge in the liposomal vesicles were studied. To this aim different concentrations of phosphatidyl choline (PC) (comprising between 0.05 and 0.1% w/v) in association with surfactants imparting a positive or negative charge (dioctadecyl dimethyl ammonium bromide ($DDAB_{18}$) and dicetyl phosphate (DCP), respectively) were used.

MN was chosen as the model compound due to its rapid absorption by the horny layer and once applied onto human skin causes an erythema, the intensity and duration of which is proportional to the quantity of the substance that has entered the living epidermis over time (Bonina et al 1995). MN-induced erythema was monitored using reflectance spectrophotometry, an instrumental non-invasive technique.

Materials and Methods

Materials

The soybean lecithin (90% phosphatidyl choline; PC) used for production of liposomes was obtained from Epikuron 200 (Lucas Meyer, Hamburg, Germany). Methyl nicotinate (MN), dioctadecyl dimethyl ammonium bromide ($DDAB_{18}$) and dicetyl phosphate (DCP) were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland), Carbopol 934P (CTFA: Carbomer) was obtained from BFGoodrich (Cleveland, OH, USA).

Preparation of liposomes

Liposomes were prepared by a reverse-phase evaporation method and successive extrusion through polycarbonate filters (Szoka & Papahadjoupoolos 1978; Bonina et al 1995). Typically, 2 g PC or PC eventually plus the ionic surfactant were dissolved in diethyl ether and placed in a 100-mL round-bottomed flask. The mixture was vacuum-dried under nitrogen using a rotary evaporator to form a lipid film. Positively- and negatively-charged liposomes were obtained by using $DDAB_{18}$ (0.190 g) or DCP (0.164 g), respectively (molar ratio between PC and surfactant of 9:1, mol/mol). The resultant dried film was dissolved in 40 mL diethyl ether, and to this solution was added 100 mg MN in 10 mL isotonic phosphate buffer pH 7.4. The two-phase system was then sonicated at 0°C for 10 min. The ether was removed at room temperature by rotary evaporation under reduced pressure, giving a turbid liposome suspension. After preparation, the liposomal suspensions were subjected to successive extrusion through polycarbonate filters (200-nm pore size) to obtain homogeneously sized vesicles (Bonina et al 1995). The liposome suspensions were characterized by a phospholipid and MN concentration of 0.10, 0.15 or 0.20 and 1% (w/v), respectively. In addition blank liposomal suspensions with a PC concentration of 0.2% w/v were prepared.

Physical characterization of liposomes

After production and extrusion, liposomes were characterized by freeze-fracture electron microscopy analyses and by photon correlation spectroscopy (PCS). Electron microscopy analyses were performed by the freeze fracturing technique (Hofland et al 1995). For the electron microscopy analysis a Philips EM 301 (Philips Electron Optics, Mahwah, NJ) was employed at 100 kV. All images were registered digitally by a low dose TV rate camera system (Dage, MTI, SIT 66) combined and processed with a digital imaging processing system.

Submicron particle size analysis was performed using a Zetasizer 3000 PCS (Malvern Instruments, Malvern, UK) equipped with a 5 mW helium neon laser with a wavelength output of 633 nm. Glassware was cleaned of dust by washing with detergent and rinsing twice with Water for Injections. Measurements were made at 25°C at an angle of 90°. Data were interpreted using the method of cumulants (Koppel 1972).

The electrophoretic mobility of neutral, cationic and anionic liposomes with a 100 mg mL^{-1} PC concentration was measured at room temperature by means of a Zetasizer 3000 PCS (Malvern Instruments, Malvern, UK). Briefly, almost 15 mL liposome dispersion in 1 mM saline solution was injected into a glass capillary cell. Under constant voltage, liposomes migrated across the capillary being focused with a 5 mW helium neon laser. The zeta potential (mV) was automatically calculated from the electrophoretic mobility based on the Smolukowski formula (Masoliver & Llosa 1990).

Preparation of hydrophilic gels

Liposome suspensions were further processed to hydrogel to obtain topical formulations having the desired semisolid consistency. The gels were prepared with a weighed amount (1.5% w/w) of Carbomer, which was swollen in deionized water and left at room temperature to obtain a homogeneous

dispersion. After an overnight incubation, triethanolamine (1% w/w) was added to neutralize the solution up to pH 7.

Finally, Carbomer gels were diluted 1:1 (w/w) with the liposome suspensions characterized by a PC concentration of 100, 150 or 200 mg mL^{-1} (0.1, 0.15 or 0.2%, w/v) and a MN concentration of 1% (w/v), respectively. In the final liposomal formulations the resulting PC concentrations were 50, 75 or 100 mg mL^{-1} (0.05, 0.075 or 0.1%, w/v) while MN was 0.5% (w/v). Formulations were named as lip, lip(+) or lip(−) followed by 50, 75 or 100 according to the lipid charge and concentration.

Control formulations with empty liposomes (PC 0.10% w/v) were prepared and signed as blank, blank(+) and blank(−). Moreover control formulations with empty liposomes (PC 0.10% w/v) plus free MN were prepared and signed as $blank^{MN}$, $blank(+)^{MN}$ and $blank(-)^{MN}$. All the formulations were stored at 4°C.

For the storage stability study, samples of liposome gels (lip100, lip100(+) or lip100(−)) were stored at 20 and 40°C for four weeks and analysed by PCS after appropriate dilution.

In-vivo evaluation of MN-induced erythema

Instrument

MN-induced skin erythema was monitored by using a reflectance visible spectrophotometer X-Rite model 968, having 0° illumination and 45° viewing angle, as previously reported (Saija et al 2000). The instrument was calibrated with a supplied standard traceable to the National Bureau of Standards perfect white diffuser. The spectrophotometer was controlled by a conventional personal computer, which performed all colour calculations from the spectral data by means of a menu driven suite of programs (Spectrostart) supplied with the instrument. Reflectance filter colorimetry has been used extensively (Westerhof et al 1986) for designating the extent of erythema by measuring the skin colour surface in terms of CIE (Commission International d'Eclairage) L*a*b* colour space parameters, since some authors (Braue et al 1990; Muizzuddin et al 1990) found significant correlation between a* values and visual grading of skin erythema. Reflectance spectrometry provides skin reflectance spectra over the wavelength range 400–700 nm using illuminant C and 2° standard observer. From the spectral data obtained, the erythema index (EI) was calculated using equation 1 (Dawson et al 1980):

$$EI = 100\left[\log\frac{1}{R_{560}} + 1.5\left(\log\frac{1}{R_{540}} + \log\frac{1}{R_{580}}\right) - 2\left(\log\frac{1}{R_{510}} + \log\frac{1}{R_{610}}\right)\right] \quad (1)$$

Where 1/R is the inverse reflectance at a specific wavelength (560, 540, 580, 510 and 610 nm).

Protocol

In-vivo experiments were performed on ten volunteers of both sexes in the age range 25–35 years. They were recruited after medical screening including filling in a health questionnaire followed by physical examination of the application sites. The volunteers gave their written consent after they were fully informed of the nature of the study and of the procedures involved. The participants did not suffer from any ailment and were not on any medication at the time of the study. They were rested for 15 min before the experiments and room conditions were set at 22 ± 2°C and 40–50% relative humidity.

For each subject, eight sites on the ventral surface of each forearm were defined using a circular template (1 cm^2) and demarcated with permanent ink.

Formulations were applied, in duplicate, for 30 min using Hill Top chambers (1 cm^2) (Hill Top, Cincinnati, OH) containing 100 mg MN-containing formulations or blank formulations. After MN occlusive treatment, the chambers were removed and the skin surface was washed, to remove the formulations tested, and allowed to dry for 15 min. The induced erythema was monitored for 10 h. EI baseline values were taken before application of the formulations tested and they were subtracted from the EI va1ues obtained after MN application at each time point, to obtain ΔEI values. Plotting ΔEI values vs time, AUC values were determined for each subject by calculating the areas between the response curve and the *x*-axis.

Statistical analysis

Statistical differences of in-vivo data were determined using repeated measure analysis of variance followed by the Bonferroni–Dunn post-hoc pair-wise comparison procedure. $P < 0.05$ was considered significant.

Results and Discussion

Production and characterization of liposomes

The preparation of liposomes was performed by the reverse-phase evaporation method (REV). The choice of using this method could be criticized since it is time consuming with respect to the hydration method. However, it should be noted that one of the major drawbacks of the hydration method is the relatively poor encapsulation efficiency of amphiphilic and water soluble drugs (Betageri et al 1993). Since MN is an amphiphilic molecule (log P = 0.36) the reverse-phase evaporation method should be better indicated to improve MN encapsulation efficiency (Nastruzzi et al 1993).

After production and extrusion, liposome morphology was characterized by freeze-fracture electron microscopy analyses. Liposome dimensional distribution and electrophoretic mobility were respectively determined by PCS and by Zetasizer 3000. Table 1 reports mean diameters of neutral, cationic and anionic liposomal vesicles produced by the use of PC 100 mg mL^{-1} (0.1% w/v), as determined by PCS and expressed as Z average and as intensity. It was observed that neutral and charged liposomes were characterized by similar mean diameters and that, before the extrusion, they possessed a larger mean

Table 1 MN containing liposomes, mean diameter, polydispersity index, and charge as a function of the charge and of the extrusion. Phosphatidyl choline concentration was always 100 mg mL^{-1}

Liposome formulation	Z average (nm) ± s.d.	Polydispersity index	Intensity (nm) ± s.d.	Zeta potential (mV) ± s.d.
Lip	865.8 ± 1.2	1.00	845.2 ± 1.0	+5.1 ± 0.2
Lip ex	185.25 ± 1.0	0.14	184.8 ± 0.9	+5.2 ± 0.1
Lip(+)	880.1 ± 1.4	1.00	880.2 ± 1.2	+52.1 ± 0.1
Lip(+) ex	195.2 ± 0.7	0.28	180.4 ± 0.1	+52.2 ± 0.2
Lip(−)	854.5 ± 1.1	1.00	860.1 ± 1.3	−54.3 ± 0.3
Lip(−) ex	178.2 ± 0.5	0.31	180.3 ± 0.4	−54.1 ± 0.4

Lip, neutral liposomes; lip(+), positively charged liposomes; lip(−), negatively charged liposomes; ex, vesicles extruded through 400-nm pore size polycarbonate membranes and through 200-nm pore size polycarbonate membranes. Data were the mean of four determinations on different dispersions.

diameter (865.8, 880.1 and 854.4 nm for neutral, cationic and anionic liposome, respectively) and a high polydispersity index (PI = 1) which reflected a broad dimensional distribution. As expected, after the extrusion process the mean diameter and PI dramatically decreased, maintaining the same dimensional trend of vesicles before the extrusion.

Mean diameters of vesicles produced by the use of higher PC concentrations (150 and 200 mg mL^{-1}) slightly differed from those produced by PC 100 mg mL^{-1} (data not shown).

Moreover, as expected, liposomes produced by the use of PC alone possessed a zeta potential value around neutrality. Liposomes produced by PC and $DDAB_{18}$ displayed a net positive zeta potential, while liposomes produced by PC and DCP were characterized by a net negative zeta potential.

Figure 1 shows micrographs of neutral, cationic and anionic liposomes, obtained by the freeze-fracture electron microscopic technique. The presence of unilamellar vesicles for neutral and charged liposomal dispersions could be observed. Vesicles were characterized by regular spheroidal morphology and by the same dimensional range.

Liposomes were used for the in-vivo experiments without any further purification step. The free drug was not eliminated from the liposome preparations, since MN as an amphiphilic molecule possesses a high solubility in the aqueous phase and a high permeability through the liposome bilayer structure. These characteristics caused the rapid redistribution of MN between the internal and external space of liposomes. During the separation processes (gel permeation chromatography on Sepharose 4B and Sephacryl 1000, and centrifugation were performed, data not shown) the concentration gradient, generated by non-entrapped drug removal, led as a final consequence to a leakage of MN from the liposomes, thus preventing a correct evaluation of the encapsulation efficiency. This resulted in a progressive decrease of the total MN concentration within liposomes.

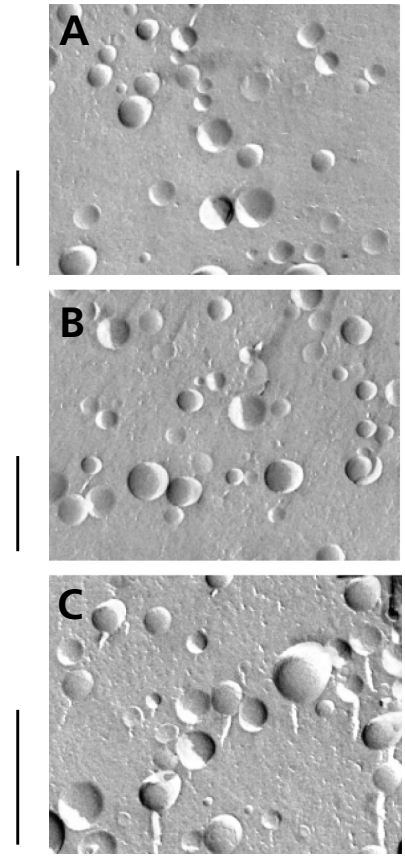

Figure 1 Freeze-fracture electron micrographs of neutral (A), positively-charged (B) and negatively-charged (C) liposomes. Liposomes were produced using phosphatidyl choline 100 mg mL^{-1} and methyl nicotinate 0.5% w/w. Liposome dispersions were subjected to extrusion through 200-nm pore size membranes. The bars equal 500 nm.

With respect to the production of viscous liposome formulations it is well known that the selection of lipid composition and additives must be done carefully to guarantee liposome integrity in the final product. The presence of surfactants in emulsion formulations can be a considerable problem for the stability of liposomes with respect to their molecular assembly structure. This was particularly important in this study, where surfactants had been included in the liposome composition. For these reasons surfactant free formulations were used based on Carbomer hydrophilic gel (Bonina et al 1995; Pavelic et al 2004).

A storage stability study was performed to investigate the suitability of liposome gel as a stable vehicle for dermal application in which liposomes would be distributed uniformly with their structure preserved. Liposomal gels were kept for four weeks at 20 and 40°C (stress testing). During these experiments, the size distribution and the mean diameter of the incorporated liposomes were determined. Results demonstrated the ability of the Carbomer gel to preserve the original size distribution of MN liposomes. The mean diameters of liposomes immediately

after incorporation in the gel vehicle were 185.2, 195.2 and 178.2 nm for lip100, lip100(+) and lip100(−), respectively. After four weeks of storage, the mean diameters changed from 185.2 to 195.5 nm (at 20°C) and to 205.7 nm (at 40°C) for lip, from 195.2 to 206.5 nm (at 20°C) and to 212.8 nm (at 40°C) for lip(+), and from 178.2 to 190.3 nm (at 20°C) and to 198.7 nm (at 40°C) for lip(−). The dimensional distribution remained almost unchanged for lip, lip(+) and lip(−), with PI values always between 0.1 and 0.35 (Pavelic et al 2004).

In-vivo evaluation of MN-induced erythema

The biological response of an active compound is directly related to the amount penetrated into the skin, therefore the percutaneous absorption of a drug is proportional to its pharmacological effect. Therefore, in this study the effect of the liposome formulation was investigated in-vivo, evaluating the MN percutaneous absorption following the erythema induced by the topical application of the drug. It is known that the intensity and the duration of vasodilatation generated by MN depend on its concentration in the dermal vasculature (Roskos et al 1990; Boelsma et al 2000). The choice of a 30-min contact time for all the tested formulations was made to observe the influence of the vehicle on the vasodilatory response in accordance with Ryatt et al (1986).

Figure 2 shows a typical ΔEI value vs time curve, obtained after the application of a control formulation containing 0.5% (w/v) MN. The in-vivo effect observed after the administration of the control formulation was in general less intense than that obtained after the application of the different liposomal suspensions. This evidence indicated an in-vivo delayed-release effect mediated by MN liposomal formulations.

The typical individual curves of the liposomal formulations are shown in Figure 3. In particular, panels A, B and C show the curves relative to liposomal formulations containing MN and different amounts of PC (50, 75 and 100 mg mL^{-1}, respectively). Figure 3D shows the curves relative to blank^{MN} formulations containing blank (not loaded) liposomes with a PC concentration of 100 mg mL^{-1} and free MN.

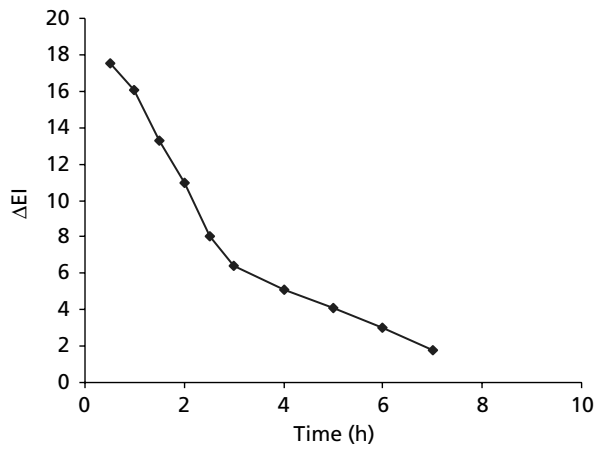

Figure 2 Typical individual ΔEI–time curves obtained after application of methyl nicotinate (0.05% w/v) carbomer based gel.

Data regarding blank formulations have not been reported because we did not observe any appreciable (and detectable) cutaneous erythema after the application of these control formulations.

As regards lip75 and lip100 formulations, it was evident that the erythema induced by positively-charged liposomes was substantially lower ($P < 0.05$) compared with that caused by neutral or negatively-charged liposomes. In the case of lip50, the curves of neutral, positively- and negatively-charged liposomes were almost superimposable.

Formulations lip75 and lip100 caused erythemas that were instrumentally detectable 10 h after their application.

We tried to elucidate the pharmacodynamic profile of MN response by determining the area under the curve (AUC) values within different time intervals, namely between 0 and 10 ($AUC_{0–10}$), 0 and 2 ($AUC_{0–2}$), and 2 and 10 h ($AUC_{2–10}$). The interval $AUC_{0–10}$ was used to evaluate the total response to MN, whereas more detailed information could be obtained by dividing the monitored period into two parts. $AUC_{0–2}$ could be related to the initial extent of erythema while $AUC_{2–10}$ allowed the evaluation of prolonged effects.

All the liposomal formulations, apart from blank^{MN}, showed similar $AUC_{0–2}$ values ($P > 0.05$), but only the formulations containing the highest amount of PC (lip75 and lip100) were characterized by $AUC_{0–2}$ values significantly lower ($P < 0.05$) than the corresponding $AUC_{2–10}$ values. The similar trend registered for $AUC_{0–2}$ values could depend on the amount of MN not included in liposomal vesicles, which was able to produce a rapid penetration through the skin eliciting an initial cutaneous erythema. The control formulation (free MN dispersed in a gel matrix) was evidence for this effect showing the highest $AUC_{0–2}$ values. Furthermore, during this first period of monitoring, PC concentration and charge of liposomes did not appear to influence the skin response to MN.

Regarding the $AUC_{2–10}$ values, the influence of liposomal charge on the MN pharmacodynamic response was observed only with lip75 and lip100. In particular, neutral and negatively-charged liposomes displayed higher $AUC_{2–10}$ values with respect to positively-charged ones (Figure 4). In the case of lip50, the values obtained with different charged liposomes were not significantly different ($P > 0.05$) and, as expected, the control formulation showed the lowest $AUC_{2–10}$ values.

Concerning the effect of PC concentration on in-vivo MN penetration, it could be noted that this parameter was able to modify the lasting of the vasodilatory effect. $AUC_{2–10}$ values registered for lip75 and lip100 formulations (particularly neutral and negative ones) showed that an increase of PC concentration produced an increase of the AUC values ($P < 0.05$).

This activity could be explained by hypothesizing a mechanism already observed in a recent study regarding

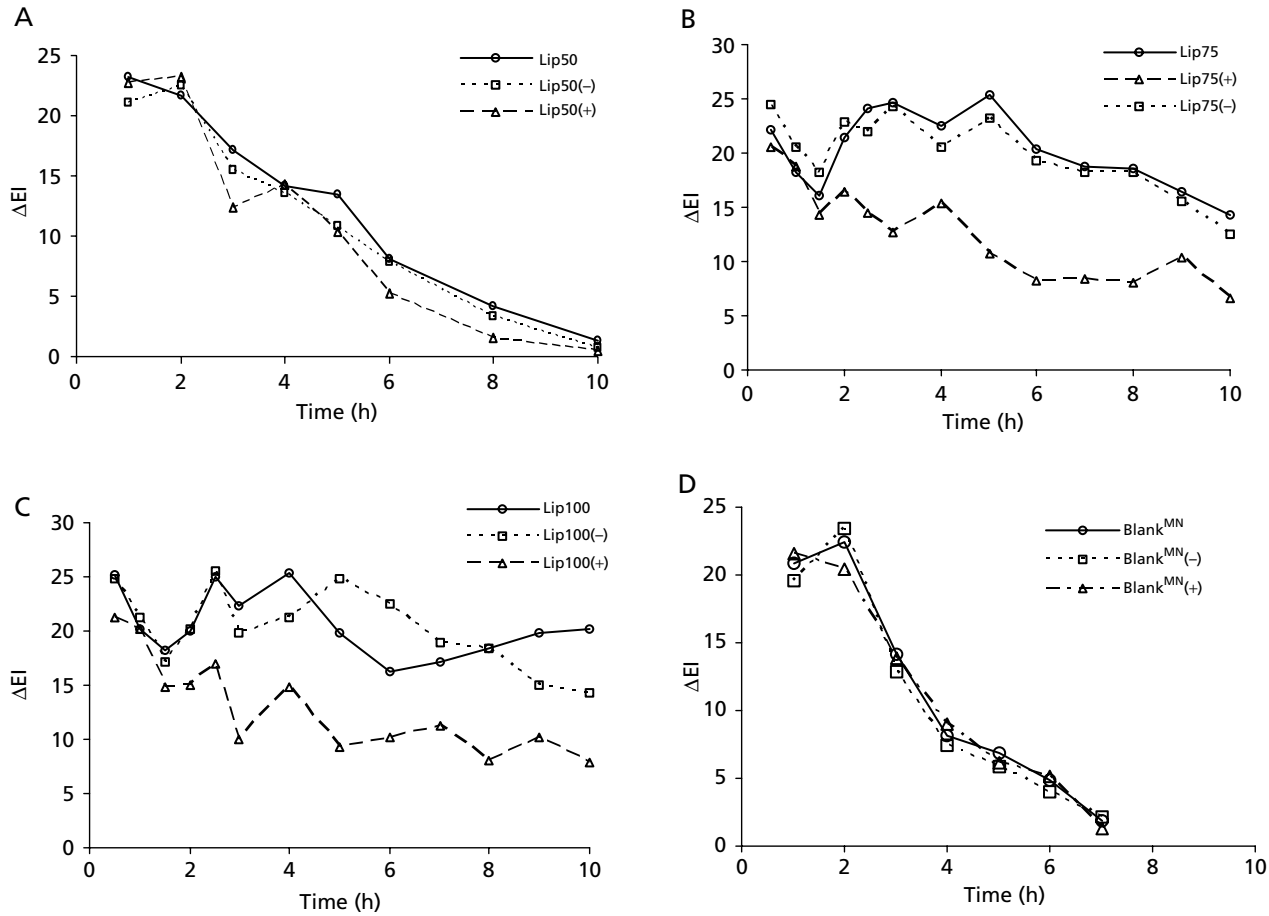

Figure 3 Typical individual ΔEI–time curves. A. Lip50 formulations (PC concn 50 mg mL^{-1}). B. Lip75 formulations (PC concn 75 mg mL^{-1}). C. Lip100 formulations (PC concn 100 mg mL^{-1}). D. $Blank^{MN}$ formulations (PC concn 100 mg mL^{-1}).

liposome drug sustained release (Puglia et al 2004). In particular, the sustained effect shown by lip75 and lip100 formulations might have been dependent on the formation of a MN reservoir in the stratum corneum which was able to guarantee MN sustained release towards deeper skin layers. This drug reservoir would have been produced by an interaction of a certain amount of liposome phospholipids with stratum corneum lipid structures. A low amount of lipids, as in lip50 formulations, produced, inevitably, a minor extent of interaction with the stratum corneum lipid components and consequently a scarce MN reservoir and delayed effect of the drug.

The effect of drug encapsulation was studied evaluating MN release from $blank^{MN}$ formulations. This time, values of AUC_{2-10}, strictly related to MN prolonged effect, appeared almost similar ($P > 0.05$) to control values (Figure 4). This demonstrated that drug inclusion in liposomal vesicles drastically influenced how long the vasodilatory effect lasted and consequently MN prolonged release.

Different considerations are necessary to elucidate the role of the liposomal charge in the formation of a MN depot into the stratum corneum.

Among lip75 and lip100 formulations, only the ones characterized by neutral or negative charge showed an interesting MN sustained release. These results seemed to agree with Katahira et al (1999), in which it was found that negatively-charged liposomes provided better drug retention in the skin compared with the positive ones. The authors observed that negatively charged and neutral liposomes mixed with the lipids of the stratum corneum were able to induce ultrastructural changes due to an impaired barrier function in the deeper layers of this stratum.

Different theories have been reported as regards the mechanism of interaction between positively-charged liposomes and skin. Katahira et al (1999), for instance, observed that the positive charges on the surface of liposomal formulations could bind to the negative charges of the stratum corneum enhancing the drug penetration through the skin. On the contrary, Ogiso et al (2001) observed a higher in-vivo percutaneous absorption of ethosuximide when the drug was encapsulated in negatively-charged liposomes compared with the drug permeation profile obtained with positively-charged liposomes.

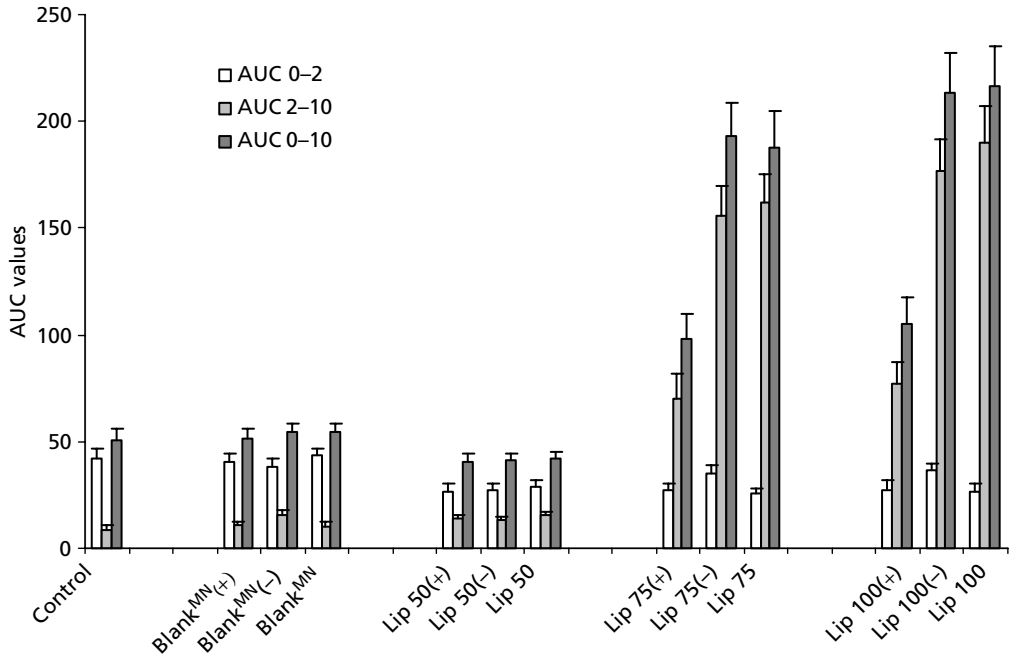

Figure 4 Comparative effects of different formulations on the in-vivo activity of MN. Histograms represent the mean values ($n = 10$) ± s.d. of area under the curve (AUC) corresponding to the indicated length of time after product application. PC concentrations were 50, 75 and 100 mg mL^{-1}.

In spite of the contrasting evidence, our results showed that the interaction between positively-charged liposomes and the skin produced only a reduced drug delayed effect. This could be noted comparing the AUC_{2-10} values obtained with lip75(+) and lip100(+) to the ones obtained with negatively-charged and neutral liposome containing forms.

In our opinion this data could be justified by the hypothesis of formation of both a mix of liposomal phosphatidyl choline with stratum corneum lipids and a fusion of liposomes onto the outer surface of the stratum corneum (Hofland et al 1995). The mix of liposomal PC with stratum corneum lipids might have led to the formation of a MN reservoir and therefore to the sustained release of MN. Moreover, the electrostatic interaction between the negatively-charged skin surface and the positively-charged liposomes could promote MN penetration and its consequent rapid depletion by the bloodstream in the vascularized section of the skin.

Conclusions

The liposomes exerted a 'delayed effect' on in-vivo MN percutaneous absorption. This effect was related to the presence of a charge on the liposomal structure: neutral and negatively-charged liposomes enabled a higher MN sustained release with respect to positively-charged liposomes. The charge effect was not found in the case of formulation lip50. The MN sustained-release could be attributed to an interaction between the PC present in the formulation and the lipid structure of the stratum corneum. This effect was influenced by the amount of PC in each formulation.

References

Betageri, G. V., Jenkins, S. A., Parsons, D. L. (1993) Preparation of liposomes. In: Betageri, G.V., Jenkins, S. A., Parsons, D. L. (eds) *Liposome drug delivery systems*. Technomic Publisher Co. Inc., Lancaster, USA, pp 1–26

Boelsma, E., Anderson, C., Karlsson, A. M. J., Ponec, M. (2000) Microdialysis technique as a method to study the percutaneous penetration of methyl nicotinate through excised human skin, reconstructed epidermis, and human skin in-vivo. *Pharm. Res.* **17**: 141–147

Bonina, F. P., Montenegro, L., Scorfani, N., Esposito, E., Cortesi, R., Menegatti, E., Nastruzzi, C. (1995) Effects of phospholipid based formulations on in vitro and in vivo percutaneous absorption of methyl nicotinate. *J. Control. Release* **34**: 53–63

Braue, E. H., Jr., Mershon, M. M., Wade, J. V., Litchfield, M. R. (1990) In vivo assessment of vesicant skin injury using Minolta Chroma Meter. *J. Soc. Cosm. Chem.* **41**: 259–265

Dawson, J. B., Barker, D. J., Ellis, D. J., Grassam, E., Catterill, J. A., Fischer, G. W., Feather, J. W. (1980) A theoretical and experimental study of light absorption and scattering by in vivo skin. *Phys. Med. Biol.* **25**: 696–709

Du Plessis, J., Weiner, N., Muller, D. (1994) The influence of in vivo treatment of skin with liposomes on the topical absorption of a hydrophilic and hydrophobic drug in vitro. *Int. J. Pharm.* **103**: R1–R5

El Maghraby, G. M. M., Williams, A. C., Barry, B. W. (2001) Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro. *J. Pharm. Pharmacol.* **53**: 1069–1077

Fang, J. Y., Hong, C. T., Chiu, W. T., Wang, Y. Y. (2001) Effect of liposomes and niosomes on skin permeation of enoxacin. *Int. J. Pharm.* **219**: 61–72

Glavas-Dodov, M., Goracinova, K., Mladenovska, K., Fredro-Kumbaradzi, E. (2002) Release profile of lidocaine HCl from topical liposomal gel formulation. *Int. J. Pharm.* **242**: 381–384

Hofland, H. E., Bouwstra, J. A., Bodde, H. E., Spies, F., Junginger, H. E. (1995) Interactions between liposomes and human stratum corneum in vitro: freeze fracture electron microscopical visualization and small angle X-ray scattering studies. *Br. J. Dermatol.* **132**: 853–866

Katahira, N., Murakami, T., Kugai, S., Yata, N., Takano, M. (1999) Enhancement of topical delivery of a lipophilic drug from charged multilamellar liposomes. *J. Drug Target.* **6**: 405–414

Kirjavainen, M., Urtti, A., Valjakka-Koskela, R., Kiesvaara, J., Monkkonen, J. (1999) Liposome-skin interactions and their effects on the skin permeation of drugs. *Eur. J. Pharm. Sci.* **7**:279–286

Koppel, D. E. (1972) Analysis of macromolecular polydispersity in intensity correlation spectroscopy: the method of cumulants. *J. Chem. Phys.* **57**: 4814–4820

Manosroi, A., Podjanasoonthon, K., Manosroi, J. (2002) Development of novel topical tranexamic acid liposome formulations. *Int. J. Pharm.* **235**: 61–70

Manosroi, A., Kongkaneramit, L., Manosroi, J. (2004) Stability and transdermal absorption of topical amphotericin B liposome formulations. *Int. J. Pharm.* **270**: 279–286

Masoliver, J., Llosa, J. (1990) Brownian motion of multidimensional systems in nonpotential velocity-dependent fields of force. *Phys. Rev. A.* **41**: 734–738

Muizzuddin, N., Marenus, K., Maes, D., Smith, W. P. (1990) Use of chromameter in assessing the efficacy of anti-irritants and tanning accelerators. *J. Soc. Cosm. Chem.* **41**: 369–378

Nastruzzi, C., Esposito, E., Pastesini, C., Gambari, R., Menegatti, E. (1993) Comparative study on the release kinetics of methyl nicotinate from topic formulations. *Int. J. Pharm.* **90**: 43–50

Ogiso, T., Yamaguchi, T., Iwaki, M., Tanino, T., Miyake, Y. (2001) Effect of positively and negatively charged liposomes on skin permeation of drugs. *J. Drug Target.* **9**: 49–59

Pavelic, Z., Skalko-Basnet, N., Jalsenjak, I. (2004) Liposomal gel with chloramphenicol: characterisation and in vitro release. *Acta Pharm.* **54**: 319–330

Puglia, C., Trombetta, D., Venuti, V., Saija, A., Bonina, F. (2004) Evaluation of in-vivo topical anti-inflammatory activity of indomethacin from liposomal vesicles (LUV). *J. Pharm. Pharmacol.* **56**: 1225–1232

Roskos, K. V., Bircher, A. J., Maibach, H. I., Guy, R. H. (1990) Pharmacodynamic measurements of methyl nicotinate percutaneous absorption: the effect of aging on microcirculation. *Br. J. Dermatol.* **122**: 165–171

Ryatt, K. S., Stevenson, J. M., Maibach, H. I., Guy, R. H. (1986) Pharmacodynamic measurement of percutaneous penetration enhancement in vivo. *J. Pharm. Sci.* **75**: 374–377

Saija, A., Tomaino, A., Trombetta, D., De Pasquale, A., Uccella, N., Barbuzzi, T., Paolino, D., Bonina, F. (2000) In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. *Int. J. Pharm.* **199**: 39–47

Schreier, H., Bouwstra, J. (1994). Liposomes and niosomes as drug carriers: dermal and transdermal drug delivery. *J. Control. Release.* **30**: 1–15

Sentjurc, M., Vrhovnik, K., Kristl, J. (1999) Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method. *J. Control. Release* **59**: 87–97

Szoka, F., Papahadjoupoolos, D. (1978) Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse phase evaporation. *Proc Natl Acad. Sci. USA* **75**: 4194–4198

Verma, D. D., Verma, S., Blume, G., Fahr, A. (2003) Particle size of liposomes influences dermal delivery of substances into skin. *Int. J. Pharm.* **258**: 141–151

Westerhof, W., Van Hasselt, B. A. A. M., Kammeijer, A. (1986) Quantification of UV-induced erythema with a portable computer chromameter. *Photodermatology* **3**: 310–314