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Evaluation of in-vivo topical anti-inflammatory activity of indometacin from liposomal vesicles

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Abstract

The aim of this study was to evaluate the in-vivo drug release profile of indometacin-loaded liposomes into the skin. Large unilamellar vesicles (LUVs), composed of dipalmitoyl-L-α-phosphatidylcholine and cholesterol (9:1), were obtained using the extrusion method and then incorporated in hydrogels (LUV-A and LUV-B). The delivery of indometacin from the liposomal system was evaluated by determining its in-vivo local anti-inflammatory activity after cutaneous application of liposomal gel formulations; the anti-inflammatory activity is directly proportional to the amount of drug that actually crosses the skin. UVB-induced erythema on healthy human volunteers was chosen as the inflammatory model and the extent of erythema was monitored by the non-invasive technique of reflectance spectrophotometry. The results showed that LUV dispersions containing indometacin provided a high percentage of entrapped drug (~84%). Furthermore, in-vivo findings revealed that the anti-inflammatory effect was more prolonged when indometacin was delivered from a liposomal gel formulation rather than from a gel formulation without liposomes. In particular, the indometacin-loaded gel formulation LUV-A showed a sustained effect, probably related to an interaction between LUV lipids and stratum corneum lipid structure. This interaction produces a depot in the stratum corneum that ensures sustained release of the drug to deeper skin layers.

Introduction

Liposomes have been studied extensively as carriers for dermal and transdermal administration of drugs and substances (Schmid & Korting 1996; Honzak et al 2000; Glavas-Dodov et al 2002; Manosroi et al 2002). It has been demonstrated that bioavailability of percutaneously administered drugs is improved when entrapped in liposomes (Katahira et al 1999). This arises from at least two contradictory properties of liposomes (Barry 2001). First, phospholipids and liposomes can act as permeability enhancers, increasing the percutaneous absorption of drugs administered to obtain systemic effects (Fang et al 2001; Kim et al 2002). On the other hand, in the case of drugs that should act topically (i.e. at the epidermal or dermal level), liposome-based formulations are known to localize the active substance at the skin level, acting as a drug reservoir (Singh & Vyas 1996; Kim et al 1997; Perugini et al 2000; Soltan Monem et al 2000; Glavas-Dodov et al 2002). This last mechanism is probably owing to the similar lipidic composition of vesicle bilayers and skin, leading to the fusion of vesicles in skin intercellular spaces (Egbaria & Weiner 1990; Bouwstra & Honeywell-Nguyen 2002).

Glavas-Dodov et al (2002) showed that soya lecithin/cholesterol liposomes entrapping lidocaine HCl in the inner water compartment act as reservoir systems for continuous drug delivery. Furthermore, Singh & Vyas (1996) demonstrated that a topical liposomal suspension containing the local anaesthetic, benzocaine, delivers the drug at a controlled rate over 24 h, and Perugini et al (2000) showed the controlled release of glycolic acid into the skin from phosphatidylcholine/cholesterol liposomes after topical application. We investigated the in-vivo percutaneous absorption of methyl nicotinate encapsulated in large unilamellar vesicles (LUVs) (Bonina et al 1995). After in-vivo topical application, this nicotinic acid ester rapidly crossed the skin and elicited a typical erythema, the intensity and duration of which depended on

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the drug concentration in the dermal vasculature. Sustained drug release was observed following topical application of methyl nicotinate-containing liposomal formulations. Liposomal vesicles have been used successfully for the dermal delivery of dipotassium glycyrrhizinate, an anti-inflammatory agent used to treat acute and chronic dermatitis (Trotta et al 2002). Agarwal et al (2001) used liposomes to deliver dithranol, one of the mainstays in the topical treatment of psoriasis.

In the above-mentioned examples, it has been demonstrated that for topical anti-inflammatory therapy, the entrapment of anti-inflammatory drugs in liposomes can help with the localized delivery of the drug. Furthermore, it has been observed that improved availability of the drug at the site reduced the dose and in turn the dose-dependent side-effects such as irritation and staining.

Indometacin, one of the most potent non-steroidal anti-inflammatory drugs, is widely used in the management of patients affected by dermatitis and rheumatic diseases (Heyneman et al 2000). Oral therapy with indometacin is very effective, but its clinical use is often limited because of its potential to cause adverse effects such as irritation and ulceration of the gastrointestinal mucosa. Also, it has a short elimination half-life and requires frequent dosing (Helleberg 1981; Srinath et al 2000). Several studies have been aimed at the development of an efficient means of indometacin topical administration to increase local soft-tissue and joint concentrations while reducing its systemic distribution and so avoiding its side-effects (Mikulak et al 1998; Stozkowska 2002; Miyazaki et al 2003).

The aim of the present study was to optimize the topical anti-inflammatory activity of indometacin by using a liposomal formulation as a topical controlled delivery system. Indometacin was encapsulated in LUVs composed of dipalmitoyl-L-α-phosphatidylcholine (DPPC) and cholesterol (CHOL) (9:1) and the physical characteristics were evaluated. We then studied the delivery of indometacin into the skin from the liposomal system by determining its in-vivo topical anti-inflammatory activity after cutaneous application of the liposomal formulation incorporated in a gel base. The anti-inflammatory activity is directly proportional to the amount of drug that actually crosses the skin. UVB-induced erythema in healthy human volunteers was chosen as an inflammatory model and the extent of erythema was monitored by reflectance spectrophotometry.

Materials and Methods

Materials

DPPC and CHOL were obtained from Avanti Polar Lipids (Birmingham, AL , USA). Sodium chloride (NaCl), sodium phosphate dibasic anhydrous (Na_2HPO_4), sodium phosphate monobasic anhydrous (NaH_2PO_4), ethanol absolute (EtOH) and indometacin were purchased from Sigma Chemical Co. (Milan, Italy), Triton X-100 was from Merck (Darmstadt, Germany), and 5(6)-carboxyfluorescein (CF) and Sephadex G-50 fine were from Fluka (Milan, Italy). Xanthan gum was a gift from Gattefossè (Milan, Italy). All other chemicals were of reagent grade and were used without further purification.

Preparation of liposomes

Multilamellar large vesicles were prepared by hydration of a dried lipid film containing a fixed molar ratio of DPPC and CHOL (9:1) in accordance with the method of Kirby & Gregoriadis (1986). Briefly, DPPC and CHOL were dissolved in $CHCl_3$ and EtOH, respectively. Then, 10 mL of this lipid solution (200 mg mL^{-1}) was mixed with 1 mL of indometacin solution (114.94 mg mL^{-1}) in EtOH. The mixture was dried under vacuum in a round-bottomed flask using a rotary evaporator; traces of residual solvents were removed over P_2O_5 in a vacuum desiccator for 24 h. The resulting dried lipid film was hydrated with 20 mL of phosphate-buffered saline (PBS; pH 7.4), vortexed and heated for 20 min at 50°C; the lipid dispersion was frozen and thawed five times using liquid N_2 and sonicated in a bath sonicator for 30 min at 50°C.

LUVs were obtained using the extrusion method (Hope et al 1985). LUVs were prepared by repeatedly passing (19 times for each membrane) the multilamellar large vesicle dispersion under pressure through pore-sized polycarbonate membranes (Nucleopore, 400- and 200-nm pore sizes; Milsch Equipment, Laudenbacg, Germany) in an extrusion device (LiposoFast-Basic; Avestin Inc., Ottawa, ON, Canada).

Vesicle size analysis

The size and size distribution of LUVs were determined by photon correlation spectroscopy. A PCS41 size analyser (Malvern Autosizer IIc; Malvern Instruments, Malvern, Worcestershire, UK) and a 5 mW He-Ne laser (Spectra Physics, Darmstadt, Hessen, Germany), set at $\lambda_{ex} = 633$ nm, were used. Data were collected with a Malvern 7032N 72 data channel correlator and the mean hydrodynamic diameter was calculated from a cumulative analysis of the intensity autocorrelation function. Before analysis, vesicle dispersions were appropriately diluted with PBS (pH 7.4) to avoid multiple scattering.

Determination of entrapment efficiency

Liposomal entrapment efficiency was determined using a small amount of liposomal suspension. Residual unencapsulated drug was removed by size exclusion chromatography on Sephadex G-50 spin columns at room temperature, using PBS (pH 7.4) as eluent (New 1990). The amount of drug entrapped in LUVs was then determined by liquid chromatography (Hess et al 2001) after dissolving an aliquot of the liposome suspension in EtOH.

Separation of unencapsulated indometacin

In-vivo studies were carried out after removing the unencapsulated indometacin from the liposomal suspension. The removal of non-encapsulated drug is an essential step because the entire purpose of liposomal incorporation of

indometacin could be defeated if the unencapsulated drug is present in the final product (Gulati et al 1998). In brief, the liposome dispersion was centrifuged at 585 144 *g* at 4°C for 20 min in order to separate the incorporated indometacin from the free indometacin. Liposomes and supernatant were then separated and the liposome pellet was redispersed in PBS (pH 7.4) to obtain a 1% indometacin concentration.

Evaluation of liposome membrane integrity

Liposome membrane integrity was determined by the CF fluorescence technique (Weinstein et al 1984; Fatouros et al 2001). Briefly, the DPPC/CHOL film, prepared as described above but without indometacin, was hydrated by using 10 mM PBS (pH 7.4) containing 0.82% NaCl and 50 mM CF, and then processed using the same procedure to obtain a dispersion of CF-encapsulated LUVs. Non-encapsulated dye was removed using a Sephadex G-50 column (1 × 30 cm). CF release was monitored on a Shimadzu RF-5301 PC spectrofluorophotometer (Shimadzu Corporation, Kyoto, Japan) equipped with a circulating water bath, using $\lambda_{ex} = 480$ and $\lambda_{em} = 521$ nm. Aliquots of the eluted liposomes (80 μL, 200 μg lipid mL^{-1}) were added to each cuvette (total volume 2 mL) and the fluorescence was recorded for 90 min. The permeability experiments were carried out at two different temperatures (25°C and 32°C). After the release measurement, 150 μL of a 10% (v/v) Triton X-100 solution were added to the liposome suspension in order to completely lyse the liposomes, and total CF was measured after Triton disruption. The rates of CF leakage are expressed as the percentage of total trapped CF released during the experiment:

$$\%\text{CF released} = ((F_t - F_0)/(F_T - F_0)) \times 100 \quad (1)$$

where F_t is the fluorescence intensity at a specified time, F_0 the fluorescence at zero time, and F_T the total fluorescence obtained by the addition of Triton. F_T was corrected for the dilution introduced by the addition of Triton. Incubation of liposomes with higher concentration of Triton did not affect the value of F_T, indicating that the release of dye from the liposomes was complete.

Preparation of hydrogels

LUV dispersions were further processed to hydrogel to obtain a topical formulation having the desired semisolid consistency. In order to gain insight about the mechanism involving indometacin release from LUVs, three different gel formulations were prepared. The gels were prepared with a weighted amount of xanthan gum (a gel-forming polymer), which was swollen in deonized water and stirred at approximately 1000 rev min^{-1} for 5 min. The final concentration of polymer (1% w/w) and of indometacin (1% w/w) was then adjusted by the addition of an appropriate amount of indometacin water solution (INDO), LUV encapsulating indometacin dispersion (LUV-A), or blank LUV and free indometacin (LUV-B). All the formulations were stored at 4°C.

In-vivo anti-inflammatory activity of LUV-A, LUV-B and INDO gel formulations on UVB-induced erythema

UVB-induced skin erythema was monitored by using a reflectance visible spectrophotomer X-Rite model 968, having 0° illumination and 45° viewing angle, as previously reported (Bonina et al 2001). The instrument was calibrated with a supplied white standard traceable to the National Bureau of Standards perfect white diffuser. The spectrophotomer was controlled by a IBM Pentium III 600 computer, which performed all colour calculations from the spectral data by means of a menu-driven suite of programs (Spectrostart; X-Rite Inc. Grandville, MI, USA) supplied with the instrument. Reflectance spectra were obtained 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 the equation reported by Dawson et al (1980):

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

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

In-vivo experiments were performed on twelve volunteers of both sexes, aged 25–35 years. The volunteers were recruited after medical screening including filling in a health questionnaire followed by physical examination of the application sites. After they were fully informed of the nature of the study and of the procedures involved, they gave their written consent. 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. Skin erythema was induced by UVB irradiation using an ultraviolet lamp model UVM-57 (UVP, San Gabriel, CA, USA). This source emits over the range of 290–320 nm with an output peak at 302 nm. The fluence rate measured at the skin surface was 0.80 mW cm^{-2}. The minimal erythemal dose was preliminarily determined and an irradiation dose corresponding to the double of the minimal erythemal dose was used throughout the study.

For each subject, ten sites on the ventral surface of each forearm were defined using a circular template (1 cm^2) and demarcated with permanent ink: one of the ten sites of each forearm was used as a control, three sites were treated with 200 mg of INDO gel formulation, another three sites were treated with 200 mg of LUV-A gel formulation, and the remaining three sites were treated with 200 mg of LUV-B gel formulation. The preparations were spread uniformly on the site using a solid glass rod. The sites were then occluded for 6 h using Hill Top Chambers (Hill Top Research, Cincinnati, OH, USA). After the occlusion period, the chambers were removed and the skin surface was washed to remove the gel and allowed to dry for 15 min. Each pretreated site, was exposed to UVB irradiation at different times after gel removal: one, three and 6 h later (t = 1, t = 3 and t = 6, respectively), and the induced erythema was monitored for 52 h. EI baseline values were taken at each

designated site before application of gel formulation and they were subtracted from the EI values obtained after UVB irradiation at each time point to obtain ΔEI values. For each site, the area under the response (ΔEI)–time curve (AUC) was computed using the trapezoidal rule.

Data analysis

Statistical differences were determined using repeated measure analysis of variance followed by the Bonferroni–Dunn post-hoc pair-wise comparison procedure. A P value of less than 0.05 was considered significant.

Results and Discussion

The aim of our study was to investigate if the topical anti-inflammatory activity of indometacin would be enhanced by using a liposomal formulation as a controlled delivery system. It is well known that many factors such as lamellarity, lipid composition, charge on the liposomal surface, mode of application and total lipid concentration may influence deposition of liposome-encapsulated drug into the skin (Kirjavainen et al 1996; Vrhovnik et al 1998; Verma et al 2003).

In the present study, we used DPPC/CHOL (9:1) LUVs; the lipid composition was chosen because neutral liposomes are suitable to enhance drug delivery of lipophilic drugs (Katahira et al 1999). The liposomal system was characterized by determining LUV physical size and entrapment efficiency. The mean hydrodynamic diameter of the LUVs, determined by photon correlation spectroscopy, was 200 ± 12.4 nm and the polydispersity index was 0.03. The extremely low value of the polydispersity index indicates a liposomal colloidal suspension of homogeneous size. Drug penetration in the skin is inversely related to the size of the liposome; in particular, only liposomes with size $\leq$300 nm are able to deliver their contents to some extent into the deeper layers of the skin (Verma et al 2003).

According to the physicochemical characteristics of indometacin (Beetge et al 2000), the LUV entrapment efficiency for indometacin was $84 \pm 1.8\%$.

Liposome membrane integrity was studied by measuring the rate of CF leakage from DPPC/CHOL (9:1) LUVs in PBS at 25°C and 32°C (physiological skin temperature). The gel with liposomes was prepared immediately after liposome purification. We determined the stability of the gel containing liposomes with encapsulated CF by analysing the dispersion obtained after gently mixing gel and water. The dispersion was centrifuged and the CF concentration was measured. We found that the percentage concentration was less than 0.5% (Figure 1). This indicates that the lipid composition used allows the preparation of liposomes that are stable at 25°C and 32°C.

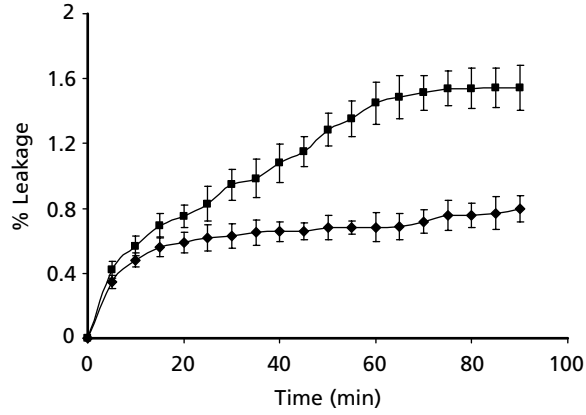

Figure 1 Effect of temperature on 5(6)-carboxyfluorescein leakage from DPPC/CHOL (9:1) liposomes. Leakage was measured during a 90-min period at 25°C (♦) and 32°C (■).

In-vivo anti-inflammatory activity

We investigated the delivery of indometacin in-vivo into the skin after topical application of LUV-A, LUV-B and INDO hydrogels. The anti-inflammatory effect of indometacin against UVB-induced erythema was monitored after topical application of the gel formulations. The intensity of erythema, determined by skin reflectance spectrophotometry, allowed us to indirectly and non-invasively evaluate the amount of indometacin released from the formulations used.

Different models for evaluating topical anti-inflammatory activity of non-steroidal anti-inflammatory drugs have been reported (Bouclier et al 1989). UVB- and methyl nicotinate-induced erythema are among the most used models in humans (Chan & Li Wan Po 1992; Bonina et al 2001). Reflectance filter colorimetry has been extensively used (Westerhof et al 1986) for designating the extent of erythema by measuring the skin surface colour in term of CIE (Commission International d'Eclairage) L*a*b* colour space parameters, since some authors found significant correlation between a* values and visual grading of skin erythema (Braue et al 1990; Muizzuidin et al 1990). Reflectance spectrometry provides skin reflectance spectra, generally over the range of 400–700 nm, from which it is possible to obtain EI values for more accurate and reliable evaluation of skin erythema (Anderson & Parrish 1981). From the ΔEI values, calculated at each site and at different times, it was possible to monitor the extent of UVB-induced skin erythema and the ability of liposomal gel formulations (LUV-A and LUV-B) to inhibit this process after their application on the skin.

Figure 2 shows the typical time courses of UVB-induced erythema on skin sites pre-treated with LUV-A, LUV-B or INDO gel formulations. By plotting ΔEI versus time, the AUC values were determined for each subject; the mean AUC values are given in Table 1. AUC values were inversely related to the ability of the tested substance to inhibit UVB-induced erythema. To better compare the results obtained, the percentage inhibition of UVB-induced erythema (PIE) was calculated as follows:

$$\mathrm{PIE} = (\mathrm{AUC}_{\mathrm{control}} - \mathrm{AUC}_{\mathrm{treated}}/\mathrm{AUC}_{\mathrm{control}}) \times 100 \qquad (3)$$

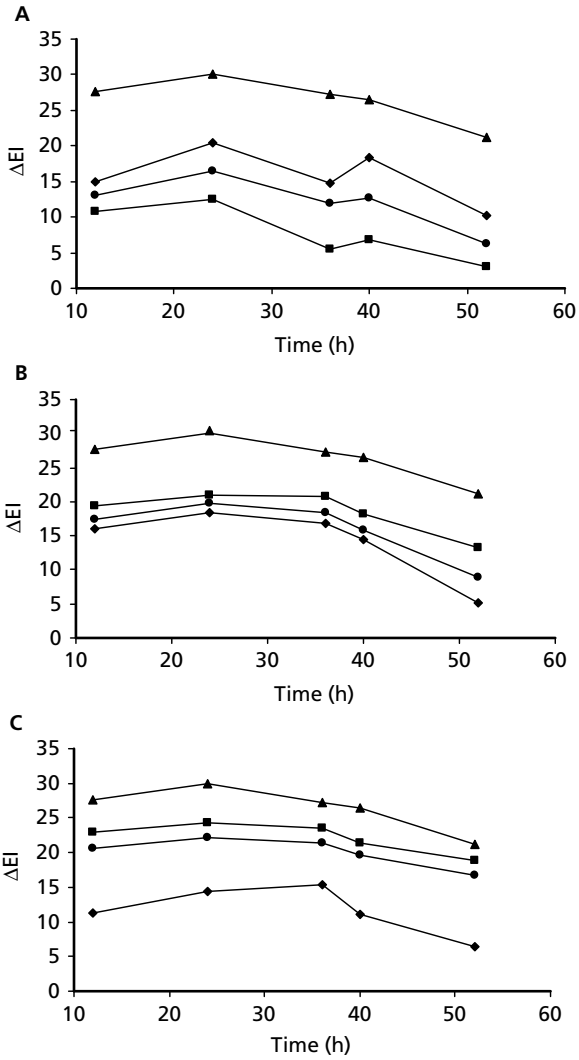

Figure 2 Change in erythema index (ΔEI) values versus time for one subject. INDO, LUV-A and LUV-B gel formulations were applied for 6 h. Skin sites were exposed to UVB radiation at 1 h (A), 3 h (B) and 6 h (C) after removal of the gels. Control (▲); LUV-A (◆); LUV-B (●); INDO (■). LUV-A, indometacin-loaded large unilamellar vesicle (LUV) gel formulation; gel formulation containing a suspension of LUVs and free indometacin (LUV-B); INDO, gel formulation without liposomes.

where $AUC_{control}$ is the area under the response–time curve on the vehicle-treated site (control), $AUC_{treated}$ is the area under the response–time curve on the drug-treated site.

Skin sites were exposed to UVB irradiation at different times (t = 1, 3 and 6 h) after removal of the gel formulation. As shown in Table 1, at t = 1 h the INDO formulation was more effective than the LUV-A formulation at inhibiting UVB-induced erythema ($P < 0.05$) and its AUC values were only slightly greater than those obtained with the LUV-B formulation ($P < 0.05$). The PIE value of the INDO formulation at t = 1 h (48.4%) was greater than that observed for the LUV-A formulation (31.7%), as demonstrated in Figure 3. The PIE value of the INDO formulation at t = 1 h was similar to that obtained with the LUV-B gel formulation (41.5%). Furthermore, the PIE value of the INDO formulation was markedly decreased at 3 h (27.4%) and it was about 12% after 6 h (Figure 3). Since it is reasonable to suppose that the anti-inflammatory activity of the drug is related to its tissue concentration, these results suggest rapid depletion of indometacin in the viable epidermis.

At 3 and 6 h after gel removal (Figure 2), the anti-inflammatory profile of the LUV-A formulation appeared different compared with that observed with the INDO formulation ($P < 0.05$), showing a similar trend as found at t = 1 h. Regarding LUV-A, the PIE value (Figure 3) was relatively lower (31.7 %) than that of the INDO formulation at t = 1 h, then it increased at t = 3 h (about 44%) and again at t = 6 h (about 50%). The LUV-B formulation, containing a suspension of LUVs and free indometacin (1%), at 3 and 6 h after gel removal, showed a similar anti-inflammatory profile to that observed with the INDO formulation. In particular, at t = 6 h, the LUV-B formulation had significantly different ($P < 0.05$) AUC values compared with those obtained with the INDO formulation, whereas at t = 3 h, it showed only slight or non-significant differences ($P = 0.05$). This anti-inflammatory profile is more clearly demonstrated by the PIE values shown in Figure 3.

Different considerations are necessary to elucidate the anti-inflammatory profiles observed for LUV-A and LUV-B gel formulations. In particular, the LUV-A formulation showed a sustained effect that could be accounted for by considering the formation of an indometacin reservoir in the stratum corneum. This reservoir is formed by the presence of liposomes in the stratum corneum layer as confirmed by experimental evidence reported elsewhere (Bonina et al 1995). The ability of the stratum corneum to act as a reservoir for drug transport through the skin has been demonstrated by Roberts et al (2004). Different hypotheses regarding the mechanism used by liposomes to cause drug accumulation within the stratum corneum have been proposed (Gabrijelcic et al 1994; Singh et al 1996). For example, Du Plessis et al (1994), studying the influences of liposomes on the in-vivo topical absorption of hydrophilic and hydrophobic substances, concluded that when active compounds are formulated in liposomal vehicles, a depot in the stratum corneum is formed guaranteeing sustained release of the drug towards deeper skin layers. To explain this, the authors suggested that the phospholipids may mix with the intercellular lipids and thereby cause a swelling effect giving the possibility to form an intracutaneous depot. In our opinion, this is the most probable mechanism that would explain our in-vivo results. Furthermore we believe, as others do (Barry 2001), that liposomes are not able to penetrate into viable skin, although occasional transport processes are reported (Mezei 1992).

The LUV-B gel was formulated in order to gain more information about the mechanism that involves indometacin release from liposomal vehicles. This formulation, in which indometacin was not included in LUVs, showed an opposite trend compared with the LUV-A formulation,

Table 1 $AUC_{0–52\,h}$ values obtained after pre-treating skin sites with LUV-A, LUV-B and INDO gel formulations containing indometacin (1%) and applying UVB radiation at 1, 3 and 6 h after removal of the gels

Subject	t = 1 h			t = 3 h			t = 6 h			Control
	LUV-A	LUV-B	INDO	LUV-A	LUV-B	INDO	LUV-A	LUV-B	INDO	
A	660.6	510.8	329.6	597.8	669.8	758.8	489.0	819.2	900.8	1083.2
B	714.2	596.6	504.3	607.3	709.1	704.2	492.4	793.2	915.3	1031.2
C	809.1	711.3	614.6	809.9	698.3	915.9	781.2	910.6	1036.2	1214.6
D	915.2	700.1	808.7	615.9	735.6	810.6	623.4	849.5	890.4	1203.4
E	713.4	629.6	630.6	555.3	867.4	724.8	472.3	911.7	832.9	1013.2
F	529.7	594.4	498.9	488.4	718.4	896.8	421.1	796.2	1061.1	922.6
G	723.7	644.4	564.5	612.4	735.1	795.7	546.6	813.5	939.5	1078.1
H	629.1	614.8	530.4	720.2	690.5	890.8	486.8	800.4	1012.4	1034.2
I	814.9	731.6	682.4	618.3	908.1	718.2	518.2	888.2	998.2	1115.9
L	827.1	742.7	529.2	648.3	828.3	857.4	664.1	827.4	848.5	1215.6
M	783.4	634.9	528.1	527.4	751.2	722.4	681.0	794.3	1118.4	1326.2
N	901.2	621.4	595.7	606.1	713.9	798.6	418.4	827.2	1001.1	984.4
Mean	751.8	644.4	568.1	617.3	752.1	799.5	549.5	835.9	962.9	1101.9

UVB-induced erythema was monitored for 52 h. $AUC_{0–52}$, area under the response–time curve. LUV-A, indometacin-loaded large unilamellar vesicle (LUV) gel formulation; gel formulation containing a suspension of LUVs and free indometacin (LUV-B); INDO, gel formulation without liposomes.

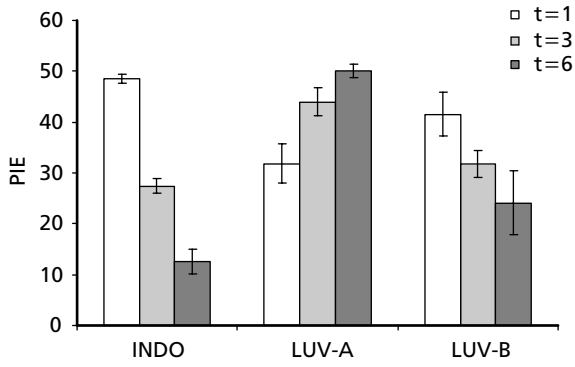

Figure 3 Percentage inhibition of UVB-induced erythema (PIE) by LUV-A, LUV-B and INDO gel formulations at 1, 3 and 6 h (t = 1, t = 3 and t = 6, respectively) after removal of the gels. LUV-A, indometacin-loaded large unilamellar vesicle (LUV) gel formulation; gel formulation containing a suspension of LUVs and free indometacin (LUV-B); INDO, gel formulation without liposomes.

that is to say we observed the lack of a sustained effect and a time-lasting anti-inflammatory activity.

The LUV-B trend appeared similar to that obtained with the INDO formulation, although some differences were observed. At t = 1 h, the LUV-B anti-inflammatory activity was less than that for the INDO formulation. At t = 3 and 6 h, greater anti-inflammatory activity was found for the LUV-B formulation compared with the INDO formulation. The results obtained with the LUV-B formulation could be owing to: (i) the release of free indometacin from the gel formulation into the skin, giving greater anti-inflammatory activity at t = 1 h compared with that observed with the LUV-A formulation; and (ii) an interaction between indometacin, intercellular lipids and LUV phospholipids, which could account for the residual anti-inflammatory activity of the LUV-B formulation at 3 and 6 h after its removal. So the anti-inflammatory profile of LUV-B appears similar to the INDO formulation, although compared with the latter, at t = 3 and 6 h, indometacin was still present in the skin in amounts that ensured appreciable PIE values (see Figure 3).

Conclusions

The LUVs (DPPC/CHOL 9:1) used in the present study to deliver indometacin into the skin have a high encapsulation efficiency (~84%) and are stable at 25°C and 32°C. Furthermore, in-vivo findings showed that the anti-inflammatory effect was more prolonged when the drug was delivered via the indometacin-loaded LUV formulation (LUV-A), compared with a gel formulation without liposomes (INDO) and a gel formulation containing a suspension of LUVs and free indometacin (LUV-B). This was probably owing to the formation of an indometacin reservoir at the stratum corneum layer. The results suggest a new opportunity for indometacin to be used in topical controlled release formulations. The use of liposomal formulations for topical application of active agents could promote drug accumulation into the skin, reducing systemic side-effects by modulating drug release.

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