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PHARMACEUTICAL NANOTECHNOLOGY

Development, Characterization, and *In Vitro* and *In Vivo* Evaluation of Benzocaine- and Lidocaine-Loaded Nanostructrured Lipid Carriers

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ABSTRACT: The present study concerns the *in vitro* and *in vivo* evaluation of benzocaine (BENZO) and lidocaine (LIDO) topical delivery from nanostructured lipid carriers (NLCs). Morphology and dimensional distribution of NLCs have been, respectively, characterized by differential scanning calorimetry (DSC) and photon correlation spectroscopy. The release pattern of BENZO and LIDO from NLCs was evaluated *in vitro* determining drug percutaneous absorption through excised human skin. Radiant heat tail-flick test was carried out in mice to determine the antinociceptive effect of BENZO and LIDO from NLC. DSC studies revealed that the inner oil phase of NLC plays a significant role in stabilizing the particle architecture and increasing the drug solubility. *In vitro* evidences show that BENZO and LIDO, when incorporated in viscosized NLC dispersions, exhibited a lower flux with respect to formulations containing the free drugs in the aqueous phase. *In* vivo study enabled to demonstrate that BENZO and LIDO can be released in a prolonged fashion when incorporated into lipid carriers. The results obtained pointed out NLC capability to act as an effective drug reservoir, thus prolonging the anesthetic effect of BENZO and LIDO. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:1892–1899, 2011

Keywords: Calorimetry (DSC); Particle sizing; Percutaneous; Ultrasound; Lipids; Nanostructured lipid carriers; Radiant heat tail-flick test; Dermal targeting.

INTRODUCTION

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are interesting colloidal carriers presenting several attractive features for topical drug delivery.[1–3] First, these carriers are composed of no toxic biodegradable lipids, which are excipients generally used in commercially available topical preparations. Furthermore, because of their solid lipid matrix, a controlled release from these carriers is possible. This becomes an important tool when it is necessary to supply the drug over prolonged period of time, to reduce systemic absorption, and when drug produces irritation in high concentration.[4–6]

Although both SLNs and NLCs are submicron size particles (40–1000 nm) and are based on solid lipids, they can be distinguished by their inner structure. NLCs show a more complex architecture that guarantees higher stability and drug loading compared with "old" SLNs.[7] This "second generation" of lipid particles is composed of a solid lipid and an oil phase that seems to be organized in nanocompartments inside solid lipid matrix. Some evidences suggest that, after the formulation, the drug remains in the liquid lipid surrounded by the solid lipid.[8] This provides some degree of mobility to the drug that contributes to stability even when the solid lipid undergoes polymorphic changes.

Another important feature of NLCs is their ability to control the release of active compounds from the matrix.[7] Similar evidences were demonstrated in previous works of our research group regarding the evaluation of the percutaneous absorption of different nonsteroidal anti-inflammatory drugs from NLCs. The results showed a decreased permeation of the active compounds and the formation of a drug reservoir

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in the stratum corneum (SC), probably due to the interaction between lipid carrier and SC lipids.[4,5,8,9] This evidence, regarding lipid carrier inability to enhance the delivery of therapeutic agents into the skin, is still object of scientific debate as widely reported in literature.[10–12]

Benzocaine (BENZO) and lidocaine (LIDO) are local anesthetics often used to alleviate pain after surgery, trauma, or medical procedures.[13,14] They induce pain relief by binding to the sodium channel of excitable membranes, thus blocking the influx of sodium ions and the propagation of the nervous impulse.[15] Their action is characterized by a rapid but short effect, compared with the potential duration of pain, so the development of a topical drug delivery system which is not only able to release the anesthetic in a prolonged fashion at the site of action but also to reduce the risk of systemic toxicity, could be particularly useful.

Hence, the aim of the present study is to design and characterize BENZO- and LIDO-loaded NLCs, to investigate the percutaneous absorption of these local anesthetics from NLCs employing *in vitro* and *in vivo* techniques, and to assess the possibility of achieving a drug-sustained release from NLCs into the skin. Percutaneous absorption has been studied *in vitro*, using excised human skin membranes (i.e., Stratum Corneum Epidermis or SCE), and *in vivo*, determining the antinociceptive effect in mice by the radiant heat tail-flick test.

MATERIALS AND METHODS

Materials

Compritol© 888 ATO (glyceryl behenate, tribehenin), a mixture of mono-, di-, and triglycerides of behenic acid (C22), was a gift of Gattefossè (Milan, Italy). Miglyol© 812 (caprylic/capric triglycerides) was provided by Eingemann & Veronelli S.p.A (Milan, Italy). Lutrol© F68 was a gift of BASF ChemTrade GmbH (Burgbernheim, Germany). Xanthan gum, BENZO, and LIDO were purchased from Sigma–Aldrich corporation (St. Louis, Missouri). High-performance liquid chromatography (HPLC)-grade solvents and water were purchased from CarloErba Reagents (Milan, Italy). When not specified, chemicals and reagents were of the highest purity grade commercially available.

NLCs Preparation

Blank and drug-loaded NLCs were prepared by ultrasonication (US) method following the procedure reported elsewhere.[5] Briefly, Compritol© 888 ATO (4 g) was melted at 80°C, and Miglyol© 812 (1.52 g) and BENZO or LIDO (80 mg) were added. The melted lipid phase was dispersed in the hot (80°C) surfactant solution (Lutrol© F68, 1.35 %, w/v) by using a high-speed stirrer (UltraTurrax T25; IKA-Werke GmbH & Co. KG, Staufen, Germany) at 8000 rpm. The obtained pre-emulsion was ultrasonified by using a UP 400 S (Ultraschallprozessor, Dr. Hielscher GmbH, Germany) maintaining the temperature at least 5°C above the lipid melting point. After US method, the obtained dispersion was cooled in an ice bath in order to solidify the lipid matrix and to form NLCs. Blank and drug-loaded SLNs were prepared and characterized as well.

Preparation of the Formulations

NLCs were formulated into hydrogels using glycerol and xanthan gum as excipients.[5] Briefly, hydrogel formulations were produced adding to BENZO- or LIDO-loaded NLC suspensions (89 %, w/w), 10 % (w/w) of glycerol, and 1 % (w/w) of xanthan gum (Table 1).

Control formulations were prepared in the same way by using BENZO or LIDO aqueous suspensions (89 %, w/w) instead of the NLC suspensions (Table 1). Hydrogels were stirred at 1000 rpm for 5 min and then stored at 4°C before use.

Determination of Drug Loading

The percentage of BENZO or LIDO entrapped in the lipid matrix was determined as follows: NLC dispersion was filtered by using a Pellicon XL tangential ultrafiltration system (Millipore, Milan, Italy) equipped with a polyethersulfone Biomax 10 membrane. The amount of retained material was freeze-dried, dissolved in chloroform, and analyzed by ultraviolet (UV) spectrophotometry at 285 and 263 nm for BENZO and LIDO, respectively (Lambda 52, PerkinElmer, Waltham, Massachusetts). Calibration curves for the validated UV assays of BENZO and LIDO were performed on five solutions in the concentration ranges of 5–40 and 50–500 μg/mL, respectively.

Correlation coefficient was above 0.990. Each point represents the average of three measurements and the error was calculated as standard deviation (±SD). BENZO or LIDO incorporation efficiency was expressed as drug recovery, calculated from Eq. 1:

$$\text{Drug recovery}\,(\%) = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug fed to the system}} \times 100 \quad (1)$$

Possible lipid interferences during UV determination of BENZO or LIDO were also investigated by comparing the two standard curves of each drug alone and in presence of lipids. The differences observed between the standard curves were within the experimental error, thus inferring that no lipid interference occurred (data not shown).

Table 1. Composition (%, w/w) of LIDO (A–C) and BENZO (D–F) Formulationspresentation.: OK

Constituents	Formulation Code					
	A[a]	B	C[a]	D[b]	E	F[b]
LIDO suspension in distilled water	89	–	–	–	–	–
NLCs loaded LIDO suspension	–	89	–	–	–	–
NLCs not loaded suspension + free LIDO	–	–	89	–	–	–
BENZO suspension in distilled water	–	–	–	89	–	–
NLCs loaded BENZO suspension	–	–	–	–	89	–
NLCs not loaded suspension + free BENZO	–	–	–	–	–	89
Glycerol	10	10	10	10	10	10
Xanthan gum	1	1	1	1	1	1

Hydrogels were stirred at 1000 rpm for 5 min and then stored at 4°C before use.
[a]LIDO amount was correspondent to LIDO content in formulation B.
[b]BENZO amount was correspondent to BENZO content in formulation E.

Particle Size Distribution

Mean particle size of the lipid dispersions was measured by photon correlation spectroscopy (PCS). A Zetamaster (Malvern Instrument Ltd., Sparing Lane South, Worcs, England), equipped with a solid-state laser having a nominal power of 4.5 mW with a maximum output of 5 mW at 670 nm, was employed. Analyses were performed using a 90° scattering angle at 20 ± 0.2°C. Samples were prepared by diluting 10 μL of NLC suspension with 2 mL of deionized water previously filtered through a 0.2 μm Acrodisc LC 13 PVDF filter (Pall-Gelman Laboratory, Ann Harbor, Michigan). During the experiment, refractive index of the samples always matched the liquid (toluene) to avoid stray light.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) studies were performed using a Mettler TA STAR^e system equipped with a DSC 822^e cell and a Mettler STAR^e V8.10 software (Mettler Toledo International Inc., Greifensee, Zurich, Switzerland). The reference pan was filled with 120 μL of distilled water. The calorimetric system was calibrated, in transition temperature and enthalpy changes, by using indium and palmitic acid (purity ≥ 99.95% and ≥99.5%, respectively; Fluka, Buchs, Switzerland) following the procedure of the Mettler STAR^e software. The DSC measurements were carried out on the following samples: unloaded NLCs and SLNs, BENZO- or LIDO-loaded NLCs, and BENZO- or LIDO-loaded SLNs.

Each sample (120 μL) was transferred into a 160 μL calorimetric pan, hermetically sealed, and submitted to DSC analysis as follows: (i) a heating scan from 25°C to 95°C, at the rate of 2°C/min and (ii) a cooling scan from 95°C to 25°C, at the rate of 4°C/min; for at least three times. Each experiment was carried out in triplicate.

In Vitro Studies

Skin Membrane Preparation

Samples of adult human skin (mean age 36 ± 8 years) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at 60 ± 1°C for 2 min,[16] after which SCEs were removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in desiccators at ~25% relative humidity. The dried samples were wrapped in aluminum foil and stored at 4 ± 1°C until use. Previous research works demonstrated the maintenance of SC barrier characteristics after storage in the reported conditions.[17] Besides, preliminary experiments were carried out in order to assess the barrier integrity of SCE samples by measuring the *in vitro* permeability of [^{3}H]water through the membranes using the Franz cell method described below. The value of calculated permeability coefficient (P_m) for [^{3}H]water agreed well with those previously reported.[18]

In Vitro Skin Permeation Experiments

Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in Franz-type diffusion cells supplied by LGA (Berkeley, California).

The exposed skin surface area was 0.75 cm^2 and the receiver compartment volume was of 4.5 mL.

The receptor compartment was filled with a water–ethanol solution (50:50, to allow the establishment of the sink conditions and to sustain permeant solubilization),[19] stirred at 500 rpm, and thermostated at 35 ± 1°C during all the experiments.

Approximately 100 mg of each formulation (A–F) were placed on the skin surface in the donor compartment and the latter was covered with Parafilm© (Pechiney Plastic Packaging Company, Chicago, IL, USA). Each experiment was run in duplicate for 24 h using three different donors. At predetermined intervals, samples (200 μL) of receiving solution were

withdrawn and replaced with fresh solution. The samples were analyzed for drug content by HPLC as described below. BENZO and LIDO fluxes through the skin were calculated by plotting the cumulative amounts of drug penetrating the skin against time and determining the slope of the linear portion of the curve and the χ-intercept values (lag time) by linear regression analysis. Drug fluxes ($\mu g \cdot h/cm^2$), at steady state, were calculated by dividing the slope of the linear portion of the curve by the area of the skin surface through which diffusion took place.

In Vivo Study

Animals

Male CD1 mice (30–35 g) were used for these experiments. Mice were maintained on 12-h light/dark cycle with food and water available *ad libitum*. Mice were housed in groups of five until tested. All procedures were approved by the Institutional Animal Care and Use Committee.

Protocol

Animals were randomly divided into different experimental groups, and 50 mg of A–F formulations was applied on the midline of the tail to cover approximately 2–2.5 cm.

Radiant Heat Tail-Flick Test

Antinociception was assessed by using a tail-flick apparatus (Ugo Basile Srl. Varese, Italy). A noxious heat stimulus was applied via a focused, radiant heat light source to the dorsal surface of the tail. Tail-flick test started 5 min after the local application of different preparations, and the test was conducted every 5 min for the first 30 min and then every 15 min for a total of 75 min.

Antinociception was expressed as percentage of the maximum possible effect (% MPE). For each animal, the % MPE was calculated using the following formula (Eq. 2):

$$\%\text{MPE} = \frac{(\text{postdrug latency} - \text{predrug latency})}{(\text{cutoff time} - \text{predrug latency})} \times 100 \quad (2)$$

The baseline tail-flick latency (predrug) was calculated as the mean of three different measurements taken at 10-min intervals. Baseline latencies were typically ranged from 2.5 to 3.0 s. A maximum cutoff latency of 10 s was set to avoid tissue damage in analgesic animals. Results are expressed as mean ± standard error mean of 8–10 animals per group.

High-Performance Liquid Chromatography

The HPLC apparatus consisted of a Shimadzu LC10 AT Vp (Milan, Italy) equipped with a 20 μL loop injector and a SPD-M 10 A Vp Shimadzu photodiode array UV detector.

Chromatography was performed using a Jupiter Phenomenex C_{18} RP column (particle size 5 μm; 250 × 4.6 mm ID; Phenomenex, Torrance, California). The mobile phase was composed of 40% water and 60% acetonitrile, and the detection was effected at 285 and 263 nm for BENZO and LIDO, respectively. The flow rate was set at 1 mL/min.

Table 2. Particle Size and Drug Loading (Expressed as Percentage of Drug Recovery) of SLNs and NLCs Containing BENZO and LIDO

Sample	Particle Size	Drug Recovery (%)
BENZO-loaded SLNs	415.6 ± 54.8	75.1
BENZO-loaded NLCs	386.1 ± 65.6	84.3
LIDO-loaded SLNs	398.8 ± 31.9	72.3
LIDO-loaded NLCs	342.0 ± 23.9	80.6

Statistical Analysis

Statistical analysis of *in vitro* data was performed using the Student's *t*-test. Group comparisons, in *in vivo* study, were performed by a two-way analysis of variance followed by the Bonferroni–Dunn *post hoc* pairwise comparison procedure. A probability, P, of less than 0.05 was considered significant in this study.

RESULTS

Preparation and Characterization of BENZO- and LIDO-Loaded NLCs

The US method used in this work produced lipid particles with a mean diameter ranging from 310 to 415 nm (Table 2). From the analysis of the data, the presence of Miglyol© was useful to increase NLCs drug recovery in comparison with SLNs, although particle size evaluation pointed out no significant differences between BENZO-loaded SLNs and NLCs ($P > 0.05$) and a significant difference between LIDO-loaded SLNs and NLCs ($P < 0.05$) (Table 2).

In order to acquire insights about the mechanism involving BENZO and LIDO release from NLCs, we have submitted to DSC analysis both unloaded and loaded NLCs, and unloaded and loaded SLNs. Unloaded and loaded NLCs will be taken into account first and their calorimetric curves are shown in Figure 1. Unloaded NLCs are characterized by a peak at 67.0°C and two shoulders at about 70.2°C and 73.0°C, respectively. With respect to the unloaded NLCs, in BENZO as well as in LIDO-loaded NLCs, the calorimetric curve maintains the shape; the temperature of the peak remains almost unchanged. These results indicate that the drugs distribute in the NLC structures without disturbing it. Unloaded and loaded SLNs were prepared and submitted to DSC

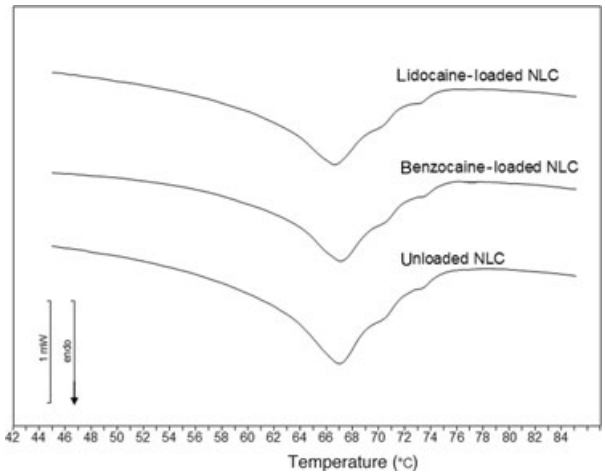

Figure 1. Calorimetric curves, in heating mode, of unloaded and loaded NLCs.

analyses to get information about drug localization inside the lipid matrix of NLCs. The calorimetric curves are shown in Figure 2. Unloaded SLNs exhibit a narrow peak at 71.9°C and a shoulder at about 74.3°C. In BENZO-loaded SLNs, the narrow peak shifts toward lower temperature. In LIDO-loaded SLNs, the peak shifts toward lower temperature and broadens. Comparing the results of SLNs and NLCs, it emerges that the two drugs do not produce appreciable effect on the NLCs, whereas they affect the SLNs behavior; in particular, BENZO produces a destabilization of the system and LIDO causes the stabilization , in addition to the decrease of the cooperativity of the system.[20]

In Vitro Skin Permeation Experiments

In Figure 3, the plots of the cumulative amounts of BENZO and LIDO permeated through human SCE membranes as a function of time are shown. Drug flux values for formulations A–F are reported in Figure 4. Statistical analysis revealed significant differences between the steady-state flux value obtained with LIDO- or BENZO-loaded NLC formulations (forms B and E) and the value registered with formulations C and F (free LIDO or BENZO and not loaded NLCs, respectively; $P < 0.05$). Moreover, both A and D formulations (NLC free) showed flux values of the two anesthetics considerably higher than B and E formulations ($P < 0.01$).

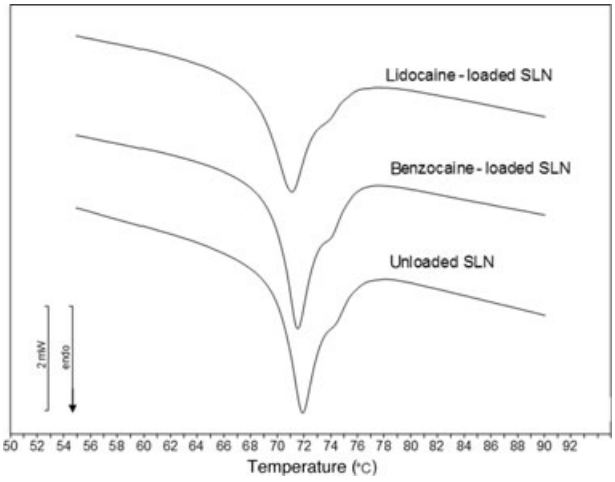

Figure 2. Calorimetric curves, in heating mode, of unloaded and loaded solid lipid nanoparticles.

In Vivo Study

Radiant heat tail-flick test has been used to assess antinociceptive effect of LIDO- and BENZO-loaded NLCs, particularly, this *in vivo* test gave us further evidences about drug release profile from lipid particles compared with control forms (A and D).

As reported in Figure 5, all the LIDO-based formulations increased the tail-flick test latency significantly. Particularly, formulations A and C showed a rapid but short-lasting antinociceptive effect, with a peak effect seen after 20 min from the application. Interestingly, the topical application of formulation B produced a more prolonged antinociceptive effect when compared with A or C forms.

As regards BENZO-containing forms, the topical application of formulation D produced a statistically significant analgesic effect only 20 min after the application (Fig. 6). Moreover, formulation E was able to induce a pronounced antinociceptive effect that persisted for 1 h after the application. On the contrary, formulation F was devoid of any analgesic activity.

Finally, as regards blank forms (vehicle drug free), no change of basal thermal sensitivity in the tail-flick test has been observed.

DISCUSSION

The US method used in this work confirmed its suitability for NLCs production. In fact, we have successfully used this method in our previous works, producing lipid nanoparticles characterized by a mean diameter ranging from 90 to 400 nm and a high drug loading capacity ranging from 80% to 88%.[4,5,8,9,21] This procedure shows the advantages to be safe, avoiding the use of organic solvents, and simple to control and setting up instrumental parameters.

It is a fact that high-pressure homogenization is generally the most suitable procedure because of its easy scalability, but according to the experimental evidences obtained in our studies, US method appeared likewise efficacious.

The results of the present study pointed out the higher drug loading capability of NLCs compared with the "old" SLNs; in fact, the addition to the solid lipid matrix of Miglyol 812© increased drug solubility, minimizing the physical exclusion phenomenon

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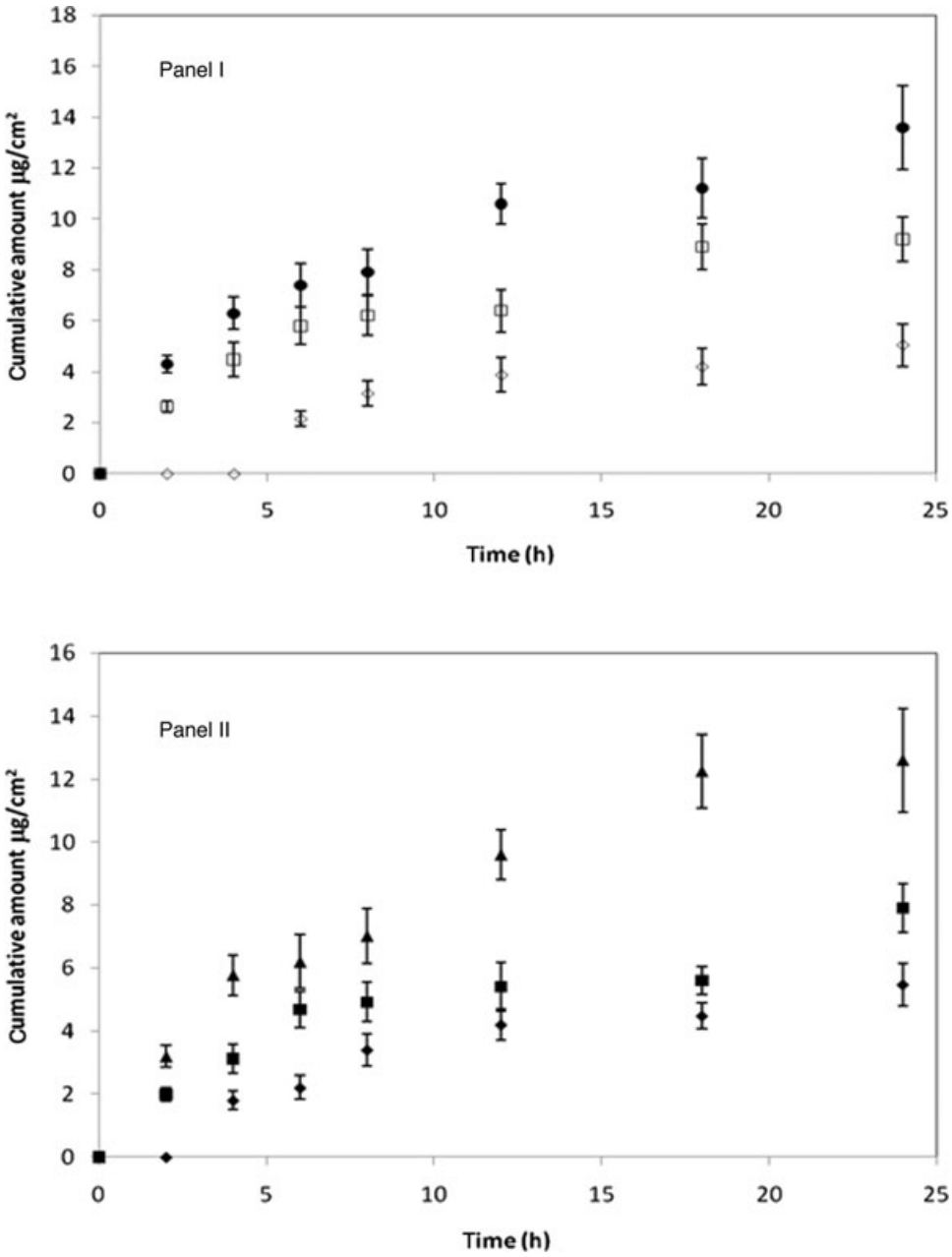

Figure 3. Permeation profiles of LIDO (panel I) and BENZO (panel II) from formulations (A–F) through SCE membranes using Franz-type diffusion cells. Formulation A containing free LIDO (●); formulation B containing LIDO-loaded NLCs (◊); formulation C containing free LIDO and not loaded NLCs suspension (□); formulation D containing free BENZO (▲); formulation E containing BENZO-loaded NLCs (◆); and formulation F containing free BENZO and not loaded NLCs suspension (■).

of drugs typical of SLNs. The last phenomenon, particularly, is due to phase transition phenomena occurring in triglycerides (α, β', and β) such as Compritrol© (particularly the conversion of the α and β' states of Compritol© to stable β ones) and is well documented in literature.[22,23]

PCS studies gave us evidences about the nanodimensional characteristics of the lipid carriers. This feature acquires an essential value because both SLNs and NLCs are maybe the only vehicles escaped from the witch-hunt, for everything is nano. The "nano problem" is strictly related to the potential toxicity of nanosized materials and to the limited experience about the mechanism of interaction of these materials with the body. But not all the nanomaterials are equal. Particularly, the lipid nanoparticles are made of compounds, such as the glycerides, which are easily degraded by inner natural processes. This complete biodegradation is the outstanding advantage of lipid nanoparticles.

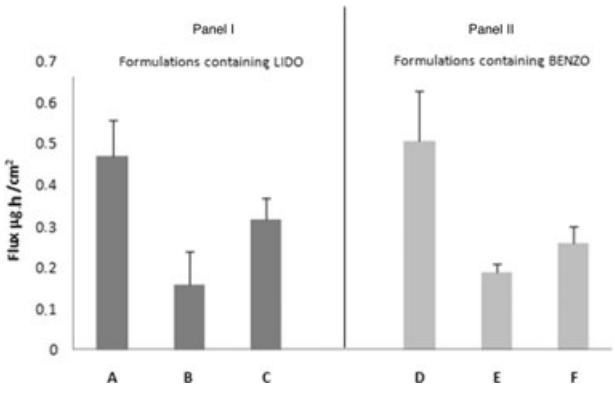

Figure 4. Steady-state fluxes of LIDO (Panel I) and BENZO (Panel II) through excised human skin from A–F formulations.

DSC studies revealed a different behavior of SLNs and NLCs toward the two anesthetics, LIDO and BENZO. From the analysis of calorimetric profiles of the two kinds of lipid particles, we consider plausible to suppose that the inner oil phase of NLCs plays a significant role in stabilizing the particle architecture and increasing the drug solubility. It can be hypothesized that NLCs solid lipid matrix is characterized by oil "clusters" in which drugs are entrapped in such a way that do not modify NLCs architecture. Vice versa, from the analysis of SLN calorimetric curves, drugs seem to affect significantly lipid matrix thermal behavior.

In vitro and *in vivo* evidences obtained in this work showed a drug release profile from NLCs very similar to that observed in our previous works.[4,5,9] The interaction between drug and NLC lipid matrix seems to play a significant role in modulating the release

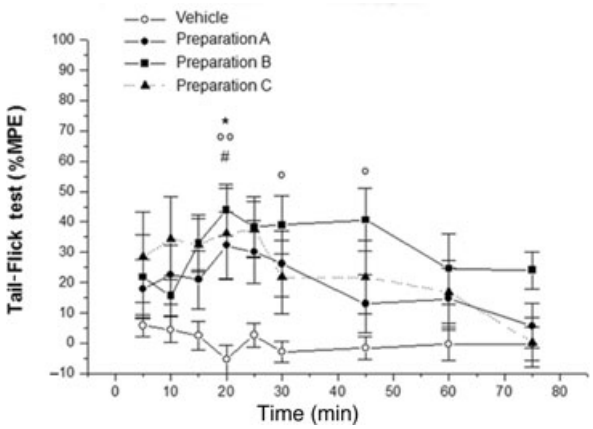

Figure 5. Time course of the antinociceptive effect of A–C forms containing LIDO in tail-flick test. Legend: $^{*}P < 0.05$, preparation A versus vehicle; $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$, preparation B versus vehicle; $^{\#}P < 0.05$, preparation C versus vehicle. Two-way ANOVA followed by Bonferroni's *post hoc* test. TFL, tail-flick latency; %MPE, percentage of the maximum possible effect.

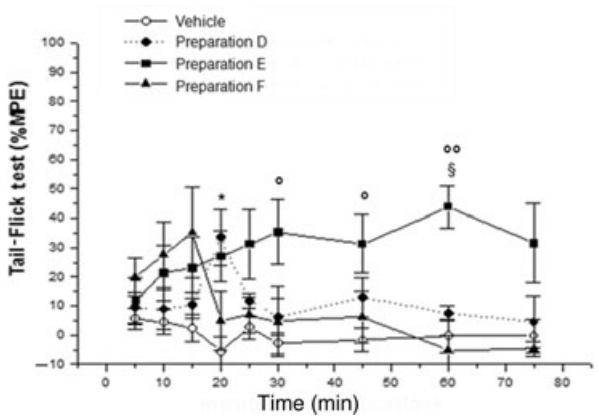

Figure 6. Time course of the antinociceptive effect of D–F forms containing BENZO in tail-flick test. Legend: $^{*}P < 0.05$, preparation D versus vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, preparation E versus vehicle; $^{\#}P < 0.05$, preparation F versus vehicle; $^{\S}P < 0.05$, preparation F versus preparation D. Two-way ANOVA followed by Bonferroni's *post hoc* test. TFL, tail-flick latency; %MPE, percentage of the maximum possible effect.

of drug enclosed. This release, particularly, could be influenced by a certain chemical affinity between the drug and the oil phase characterizing NLCs architecture. The role of NLC lipid phases in drug release has been studied by different researchers. Castelli et al.[8] studied the interaction between lipids and drugs in an NLC system using indomethacin as model compound and by means of calorimetric experiments, demonstrated that NLC lipid matrix interacted with the drug modulating its release.

Results of *in vitro* study showed that BENZO and LIDO formulated in NLCs (formulations B and E) were characterized by a decreased permeation through the skin that could be related to a high reservoir capacity of the SC. In previous works, we have just hypothesized that drug reservoir formation was probably due to the interaction between the lipid components of NLCs and the SC lipid matrix.[4,5,8,9] In these studies, we have observed that reservoir formation is strongly speeded by chemical analogies by lipid structure of vehicle and SC lipids. This assumption was later confirmed in a more recent work[24] regarding the evaluation of percutaneous absorption of the model drug naproxen from SC lipid liposomes (SCLLs), that is, liposomes formulated reproducing the physiological lipid composition of human skin SC. The results reported that the lipid bilayers of SCLLs mix easily with SC physiological lipids forming a drug reservoir characterized by a high capacity. NLCs formulated in this study are made of lipids [Compritol® 888 ATO is a mixture of mono-, di-, and triglycerides of behenic acid (C22)] showing chemical analogies with SC lipids (C24–C26 in ceramides and C22–C24 in free fatty acids).

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In vivo experiments corroborated *in vitro* evidences. The anesthetic activity of BENZO- and LIDO-loaded NLCs revealed a more interesting delayed and sustained activity compared with the other formulations. As reported in Figures 5 and 6, LIDO and BENZO encapsulation in NLCs (forms B and E, respectively) guaranteed a prolonged antinociceptive effect that could be explained confirming the previous assumption that NLCs provoke the accumulation of the two anesthetics into the upper skin layers, thus reducing the drug flux and creating a reservoir able to prolong the skin residence time. The other formulations containing LIDO and BENZO were characterized by a rapid onset of action with a peak effect seen after 20 min from the application. The antinociceptive effect was rapid but short lasting because the two anesthetics were rapidly absorbed and cleared off by systemic circulation.

CONCLUSION

The NLCs employed in our study were endowed with a high encapsulation efficacy and with a well-determined size distribution. NLCs formulation containing BENZO and LIDO allowed a significant improvement of their therapeutic effectiveness in terms of duration of action. Particularly, *in vitro* and *in vivo* results pointed out NLCs capability to act as an effective drug reservoir, thus prolonging the anesthetic effect of BENZO and LIDO. This study provides further evidences about NLCs targeting and prolonged release effects in dermal delivery.

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