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Formulation strategies to modulate the topical delivery of anti-inflammatory compounds

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Synopsis

The aim of this study was to assess the ability of some vehicles (emulsion and emulgel), containing hydrogenated lecithin as penetration enhancer, in increasing the percutaneous absorption of the two model compounds dipotassium glycyrrhizinate (DG) and stearyl glycyrrhetinate (SG). Furthermore SG-loaded solid lipid nanoparticles (SLNs) were prepared and the effect of this vehicle on SG permeation profile was evaluated as well. Percutaneous absorption has been studied *in vitro*, using excised human skin membranes (i.e., stratum corneum epidermis or [SCE]), and *in vivo*, determining their anti-inflammatory activity. From the results obtained, the use of both penetration enhancers and SLNs resulted in being valid tools to optimize the topical delivery of DG and SG. Soy lecithin guaranteed an increase in the percutaneous absorption of the two actives and a rapid anti-inflammatory effect in *in vivo* experiments, whereas SLNs produced an interesting delayed and sustained release of SG.

INTRODUCTION

During the recent decades, many cosmetic products with biologically active ingredients have been developed and marketed. This category of products showing medicinal or drug-like benefits is recognized with the term "cosmeceutical."

Cosmeceuticals are intended to carry out their function as protection, whitening, tanning, and antiaging. Like cosmetics, cosmeceuticals are applied topically but differ in that they contain potent ingredients that can influence the biological function of the skin and deliver nutrients to promote healthy skin.

The extracts of *Glycyrrhiza glabra* roots contain interesting compounds that are among the most important ingredients used to formulate innovative cosmeceuticals (1). The biological properties of this plant are associated with the presence of glycyrrhizin, a 3-diglucuronide derivative and with its aglycone, glycyrrhetic acid. Some interesting derivatives of these active compounds, such as dipotassium glycyrrhizinate (DG) and

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stearyl glycyrrhetinate (SG), are used for external use in cosmetic field as lenitive and antireddening agents (2) and in the field of dermatology, because they seem to improve the symptoms of acute and chronic dermatitis (3,4).

DG and SG show high chemical stability and emulsifying properties but show different physicochemical features and solubility; in fact, while DG has a good water solubility, SG is characterized by a pronounced lipophilic character. These physicochemical properties appear to be unsuitable because actives characterized by extremes of hydrophilic or lipophilic natures are not well absorbed (5).

Different physical and chemical approaches have been developed to overcome the skin barrier and to have better control of active transport across the skin (6–8). Among them, penetration enhancers are one of the most convenient materials and show relatively high effects, interacting with skin constituents to increase the flux of active substances (6,9).

Another new and very promising strategy is represented by the use of innovative carriers such as solid lipid nanoparticles (SLNs) (10). In recent years, an unmeasured number of research papers have been published describing the use of lipid based carriers for cosmetic application. SLNs with a solid particle matrix are developed from o/w emulsions by simply replacing the liquid lipid (oil) by a solid lipid, i.e., being solid at body temperature (11). Scientific literature reports, with different examples, the benefits associated to the application of lipid nanoparticle strategy to the formulation of products aimed to dermal administration (12–14). The main advantages recognized for these nanocarriers are the capability to enhance the drug penetration into the skin increasing treatment efficiency, to target the epidermis, and to reduce the systemic absorption and consequently the side effects of many drugs and cosmetic actives that should limit their activity to the skin layers. However, considering the chemical features of SLNs, these nanoparticles seem to be idoneous to vehiculate lipophilic compounds rather than hydrophilic ones.

The objective of this study was to assess the ability of some vehicles (emulsion and emulgel), containing hydrogenated lecithin as penetration enhancer, in increasing DG and SG percutaneous absorption. Furthermore, SG-loaded SLNs were prepared and the effect of this vehicle on SG permeation profile was evaluated as well. Percutaneous absorption has been studied *in vitro*, using excised human skin membranes (i.e., SCE), and *in vivo*, determining their anti-inflammatory activity.

MATERIALS AND METHODS

MATERIALS

DG and SG were purchased from Maruzen Pharmaceuticals Co., LTD. (Hiroshima, Japan). Brij® 721P (steareth-21), Brij® 72 (steareth-2), Arlamol® E (PPG-15 Stearyl Ether, butylated hydroxy toluene) were purchased from Croda Italiana S.P.A. (Mortara, Italy). Cetearyl alcohol, Myritol® 318 (caprylic/capric triglyceride), Cetiol® SB45 (*Butyrospermum parkii*), Pluronic® F68 (poloxamer 188), were purchased from BASF Corporation (Florham Park, NJ). Colonial monolaurin (glyceryl laurate) was purchased by Colonial Chemical, Inc. (South Pittsburg, TN). Dermosoft® OMP (methylpropandiol, caprylyl glycol, phenylpropanol) was purchased from Dr. Straetmans GmbH (Hamburg, Germany). Arginine was obtained by ACEF S.p.A. (Fiorenzuola d'Arda, , Italy). Lecinol® S-10 (hydrogenated lecithin) was obtained by Nikko

Chemical Co., LTD. (Tokyo, Japan). Silicol® 200 (dimethicone) was obtained by Esperis (Milan, Italy). Compritol® 888 ATO (glyceryl behenate, tribehenin), a mixture of mono-, di- and triglycerides of behenic acid (C_{22}), was a gift of Gattefossè (Milan, Italy). Carbopol® Ultrez 20 (acrylates/C10-30 alkyl acrylate crosspolymer) was obtained by The Lubrizol Corporation (Wickliffe, OH). All other materials were of analytical grade.

PREPARATION OF DG AND SG FORMULATIONS

The composition of emulsions A,C and B,D containing 0.5% of DG and SG, respectively, is reported in Table I. Briefly, the ingredients of the emulsion oily phase (cetearyl alcohol, Myritol® 318, Silicol® 200, colonial monolaurin, Brij® 721P, Brij® 72, Arlamol® E, Cetiol® SB45) were mixed at 60°C and then slowly added to the water phase (Dermosoft® OMP and water) using a turbomixer and maintaining the temperature of 60°C during the preparation. The water phases of C and D formulations contained 1% of Lecinol® S-10. Final formulations were made viscous by Carbopol® Ultrez 20 and arginine.

The composition of gel formulations E,G and F,H containing 0.5% of DG and SG, respectively, is reported in Table II. The gel formulations were prepared by dispersing Carbopol® Ultrez 20 in water at 60°C and then adding Dermosoft® OMP, DG and arginine with constant stirring. The water phases of G and H formulations contained 1% of Lecinol® S-10. All the formulations were stored at 4°C before use.

SLN PREPARATION

Blank and drug-loaded SLNs were prepared by ultrasonication (US) method following the procedure reported elsewhere (15). Briefly, Compritol® 888 ATO (5 g) was melted at

Table I
Composition of A–D emulsions (% w/w)

Trade name	INCI name	A	B	C	D
Cetearyl alcohol	Cetearyl alcohol	2.5	2.5	2.5	2.5
Myritol® 318	Caprylic/capric triglyceride	4	4	4	4
Silicol® 200	Dimethicone	1.5	1.5	1.5	1.5
Colonial monolaurin	Glyceryl laurate	1.5	1.5	1.5	1.5
Dermosoft® OMP	Methylpropandiol, caprylyl glycol, phenylpropanol	2.5	2.5	2.5	2.5
Brij® 721P	Steareth-21	2	2	2	2
Brij® 72	Steareth-2	3	3	3	3
Arlamol® E	PPG-15 stearyl ether, BHT	4	4	4	4
Arginine	Arginine	1	1	1	1
Cetiol® SB45	*Butyrospermum parkii*	5	5	5	5
Lecinol® S-10	Hydrogenated lecithin	—	—	1	1
Potassium glycyrrhizinate	Potassium glycyrrhizinate	0.5	—	0.5	—
Stearyl glycyrrhetinate	Stearyl glycyrrhetinate	—	0.5	—	0.5
Carbopol® Ultrez 20	Acrylates/C10-30, alkyl acrylate crosspolymer	0.3	0.3	0.3	0.3
Water	Aqua	72.2	72.2	71.2	71.2

Table II
Composition of E–H Gel Formulations (% w/w)

Trade name	INCI name	E	F	G	H
Dermosoft® OMP	Methylpropandiol, caprylyl glycol, phenylpropanol	2.5	2.5	2.5	2.5
Arginine	Arginine	1	1	1	1
Lecinol® S-10	Hydrogenated lecithin	—	—	1	1
Potassium glycyrrhizinate	Potassium glycyrrhizinate	0.5	—	0.5	—
Stearyl glycyrrhetinate	Stearyl glycyrrhetinate	—	0.5	—	0.5
Carbopol® Ultrez 20	Acrylates/C10-30 Alkyl acrylate crosspolymer	0.6	0.6	0.6	0.6
Water	Aqua	95.4	95.4	94.4	94.4

85°C and SG (1 g) was added. The melted lipid phase was dispersed in the hot (85°C) surfactant solution (Pluronic® F68, 1.5 g) by using a high-speed stirrer (UltraTurrax T25; IKA-Werke GmbH & Co. KG, Staufen, Germany) at 8000 rpm. The obtained preemulsion was ultrasonified by using a UP 400 S (Ultraschallprozessor, Dr. Hielscher GmbH, Teltow, 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 to solidify the lipid matrix and to form SLNs.

To acquire insights about the mechanism involving SG release from SLN, we prepared two different hydrogels (SLN-IN and SLN-OUT) using glycerol and xanthan gum as excipients (12).

Briefly, SLN-IN formulation was produced adding to SG-loaded SLN suspensions (89%, w/w), 10% (w/w) of glycerol, and 1% (w/w) of xanthan gum, whereas SLN-OUT was produced adding to a suspension of not loaded SLN and free SG (89%, w/w), 10 (w/w) of glycerol, and 1% (w/w) of xanthan gum. Hydrogels were stirred at 1000 rpm for 5 min and then stored at 4°C before use.

CHARACTERIZATION OF SG-LOADED SLN

Particle size distribution. Mean particle size of the lipid dispersions was measured by photon correlation spectroscopy (PCS). A Zetamaster (Malvern Instrument Ltd., 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 used. Analyses were performed using a 90° scattering angle at 20 ± 0.2°C. Samples were prepared by diluting 10 μl of SLN suspension with 2 ml of deionized water previously filtered through a 0.2-μm Acrodisc LC 13 PVDF filter (Pall-Gelman Laboratory, Ann Harbor, MI). During the experiment, refractive index of the samples always matched the liquid (toluene) to avoid stray light.

Determination of drug loading. The percentage of SG entrapped in the lipid matrix was determined as follows: a fixed amount of SLN dispersion was filtered using a Pellicon XL tangential ultrafiltration system (Millipore, Milan, Italy) equipped with a polyethersulfone Biomax 1000 membrane (Millipore) with a 1,000,000 daltons molecular weight cutoff. An amount of retained material was freeze dried, dissolved in dichloromethane, and analyzed by ultraviolet (UV) spectrophotometry at 240 nm (Spectrophotometer

UV-1700 Pharma Spec; Shimadzu, Milan, Italy). Calibration curves for validated UV assays of SG were performed on five solutions in the concentration range 8—80 mg/ml. The correlation coefficient was greater than 0.990.

Each point represented the average of three measurements, and the error was calculated as standard deviation (±SD). SG incorporation efficiency was expressed as active recovery and calculated using equation (1):

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

Possible lipid interferences during UV determination of SG were also investigated by comparing the standard curve of the substance alone and in the 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).

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 SCE was removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in a desiccator at approximately 25% relative humidity.

The dried samples were wrapped in aluminum foil and stored at 4 ± 1°C until use. Previous research work showed the maintenance of SC barrier characteristics after storage in the reported conditions (17). Besides, preliminary experiments were carried out 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 reported earlier (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, CA). The exposed skin surface area was 0.75 cm^2 and the receiver compartment volume was 4.5 ml.

The receptor compartment was filled with a water–ethanol solution (50:50) (to allow the establishment of sink conditions and to sustain permeant solubilization), stirred at 500 rpm, and thermostated at 32 ± 1°C during all experiments (19,20).

Approximately 100 mg of each formulation (A–H, SLN-IN, and SLN-OUT) was placed on the skin surface in the donor compartment and the latter was covered with Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL). Each experiment was run in duplicate for 24 h using three different donors (n = 6). At predetermined intervals, samples (200 μL) of receiving solution were withdrawn and replaced with fresh solution. The samples were analyzed for SG and DG content by HPLC as described below. The results were expressed as cumulative amount of SG and DG permeating the SCE membranes after 24 h. Statistical analysis of data was performed using student's *t*-test.

IN VIVO STUDIES

To investigate the relationship between *in vitro* skin permeation data and *in vivo* topical anti-inflammatory activity, we evaluated the ability of the formulations that showed the best *in vitro* profile, to inhibit the UV-induced skin erythema on healthy human volunteers.

Volunteers recruitment. *In vivo* experiments were performed on a group of ten volunteers of both sexes in the age range 25–35 years. They were recruited after medical screening, including the filling of a health questionnaire, followed by physical examination of the application sites. After they were fully informed on the nature of the study and on 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 prior to the experiments and room conditions were set at 22±2°C and 40–50% relative humidity.

In vivo anti-inflammatory activity. UVB-induced skin erythema was monitored by using a reflectance visible spectrophotometer X-Rite model 968 (X Rite Inc., Grandville, MI), calibrated and controlled as reported earlier (12,21).

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 equation (2) (22):

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

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

The skin erythema was induced by UVB irradiation using a UVM-57 ultraviolet lamp (UVP, San Gabriel, CA) whose specific parameters are reported elsewhere (12).

The minimal erythemal dose (MED) was preliminarily determined, and an irradiation dose corresponding to twice the value of MED was used throughout the study. For each subject, seven 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 seven sites of each forearm was used as control, three sites were treated with 300 mg of formulation D, and the remaining three with 300 mg of formulation G. The preparations were spread uniformly by means of a solid glass rod and then the sites were occluded for 6 h using Hill Top Chambers (Hill Top Research, Cincinnati, OH). After the occlusion period, the chambers were removed and the skin surfaces were gently washed to remove the gel and allowed to dry for 15 min. Each pretreated site was exposed to UVB irradiation 1, 3, and 6 h ($t = 1$, $t = 3$ and $t = 6$, respectively) after gel removal 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 a curve (AUC) was computed using the trapezoidal rule. The volunteers were again recruited to complete the experimentation after a washout period of 2 weeks and the same experimental procedure was repeated for the formulations SLN-IN and SLN-OUT.

To better outline the results obtained, the PIE was calculated from the AUC values using equation (3):

$$\text{Inhibition}\ (\%) = \frac{AUC_{(C)} - AUC_{(T)}}{AUC_{(C)}} \times 100 \tag{3}$$

where $AUC_{(C)}$ is the area under the response/time curve of the vehicle-treated site (control) and $AUC_{(T)}$ is the area under the response/time curve of the drug-treated site. Statistical differences of *in vivo* data were determined using repeated measure analysis of variance (ANOVA) followed by the Bonferroni–Dunn post hoc pairwise comparison procedure. A probability, p, of less than 0.05 was considered significant in this study.

HPLC ANALYSES

DG quantification was effected by HPLC. The HPLC apparatus consisted of a Shimadzu LC-10 AT VP (equipped with a 20-µl loop injector and a SPD-M10A VP Shimadzu photodiode array UV detector. Chromatography was performed using a Symmetry Shield C18 RP column (particle size, 5 µm, 250 × 4.6 mm i.d..; Phenomenex, Torrance, CA). The mobile phase was composed of 30% water (pH 3 adjusted with phosphoric acid) and 70% acetonitrile and the detection was effected at 250 nm. The flow rate was set at 1 ml/min. The retention time was 7.5 min.

UV ANALYSES

SG quantification was effected by UV analyses. The UV apparatus consisted of a spectrophotometer UV-1700 PharmaSpec, Shimadzu. Calibration curves for validated UV assays of SG were performed on five solutions in the concentration range 8—80 mg/ml using a wavelength of 240 nm. The correlation coefficient was greater than 0.990. Each point represented the average of three measurements, and the error was calculated as standard deviation (±SD).

RESULTS AND DISCUSSION

PREPARATION AND CHARACTERIZATION OF SG-LOADED SLN

SG-loaded SLN were prepared with Compritol® 888 ATO (glyceryl behenate, tribehenin), a mixture of mono-, di-, and triglycerides of behenic acid (C_{22}) as the solid lipid and Pluronic® F68 (poloxamer 188) as the surfactant. We decided to use these ingredients after a lipid screening for the identification of matrices for SG incorporation, which pointed out a high affinity of the active compounds toward Compritol® 888 ATO (data not shown). The use of SLN strategy to optimize DG permeation profile resulted to be difficult, because the active ingredient, even if showed a chemical structure similar to SG, was very hydrophilic and consequently unsuitable to be formulated in a SLN system.

Notwithstanding that the hot high pressure homogenization method is recognized as the most suitable procedure to produce lipid nanoparticles because of its easy scalability, the US method, in our experience, is a "cheap and fast" method suitable for the production of lipid nanoparticles.

Mean particle size data of SG-loaded SLN were obtained by PCS analyses. The results confirmed the efficiency of the US method: not loaded SLN showed a mean diameter of 280.9 ± 25.4 nm (polydispersity index: 0.237 ± 0.03) while for SG-loaded SLN a population with a mean diameter of 185.4 ± 12.8 nm was obtained (polydispersity index: 0.395 ± 0.01). As regards the drug loading, the SLN produced by the US method had a high incorporation efficiency showing drug recovery of 88%.

IN VITRO PERCUTANEOUS ABSORPTION STUDY

In vitro skin permeation experiments were performed using human SCE membranes instead of full-thickness skin because, as reported by others, the dermis *in vitro* can act as a significant artificial barrier to the absorption of lipophilic compounds (23).

In Figs 1 and 2, the cumulative amounts (Q_{24}) of SG and DG permeated through human SCE membrane are shown, respectively. From the results obtained, all the formulation containing Lecinol® S-10 (H, D, G, and C) produced an increase of percutaneous absorption of SG and DG compared to control formulations (F, B, E, and A), respectively.

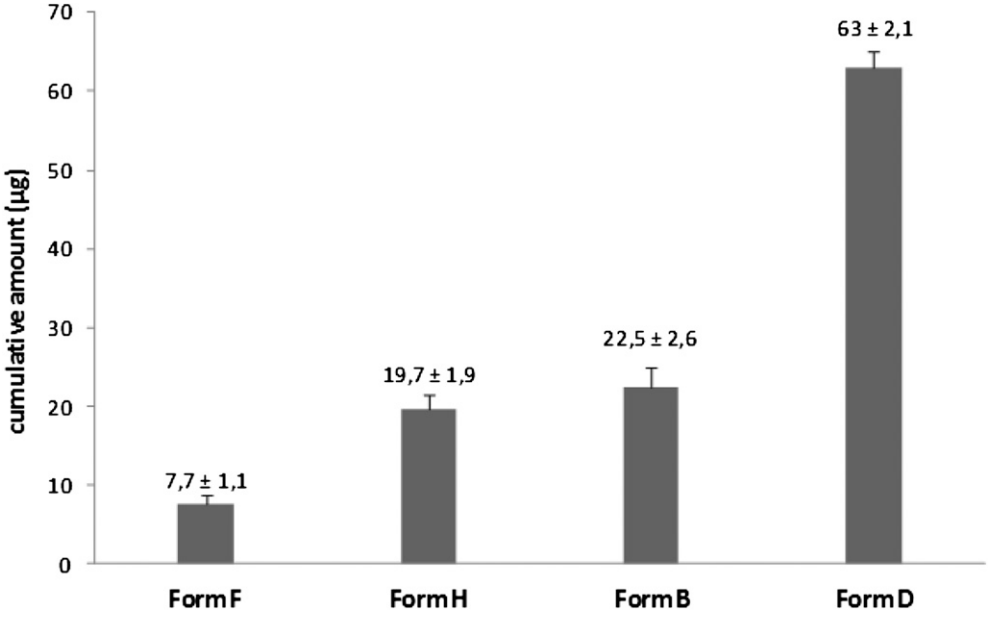

Figure 1. Cumulative amount (Q_{24}) of SG permeated during 24 h from F (gel containing 0.5% of SG), H (gel containing 0.5% of SG and 1% of soy lecithins), B (O/W emulsion containing 0.5% of SG), and D (O/W emulsion containing 0.5% of SG and 1% of soy lecithins).

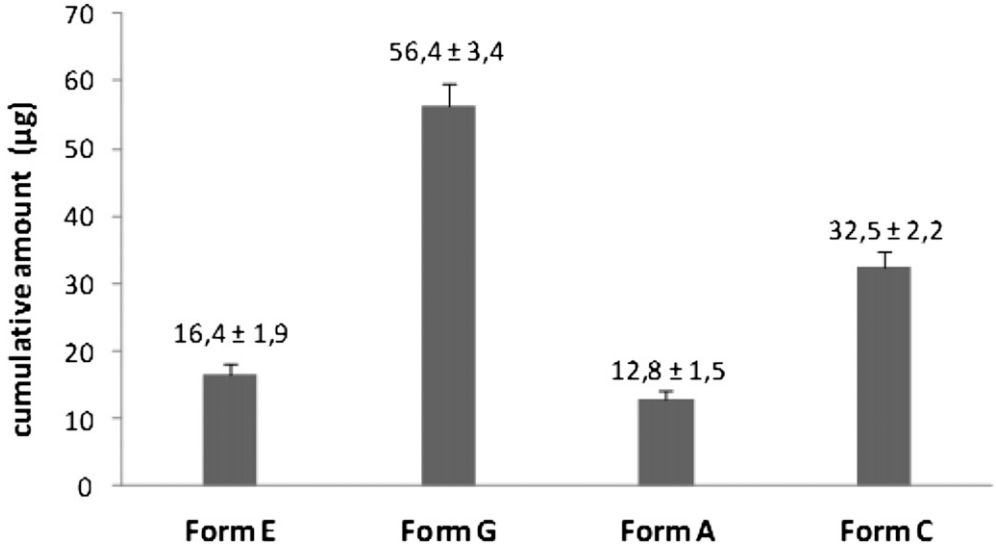

Figure 2. Cumulative amount (Q_{24}) of DG permeated during 24 h from F (gel containing 0.5% of SG), H (gel containing 0.5% of SG and 1% of soy lecithins), B (O/W emulsion containing 0.5% of SG), and D (O/W emulsion containing 0.5% of SG and 1% of soy lecithins).

This result is in line with the evidences reported in literature that point out a penetration enhancer effect of soy lecithin toward topically applied active substances (6). Lecithin interacts directly with stratum corneum packing and occludes the skin surface thus increasing tissue hydration, and consequently, the permeation of actives.

As regards SLN based formulations (Fig. 3), SG inclusion in lipid matrix of solid lipid nanoparticles (SLN-IN) produced an enhancement of active percutaneous absorption

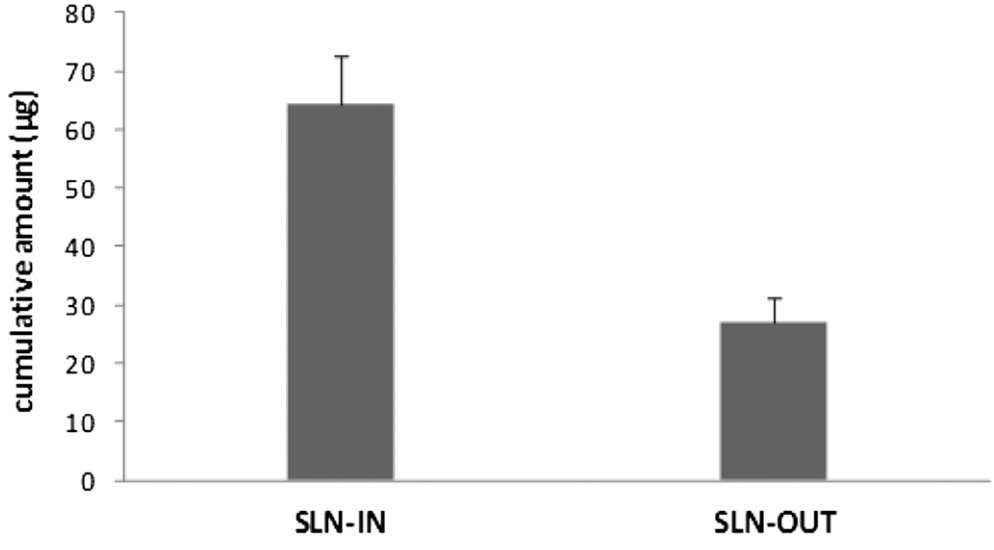

Figure 3. Cumulative amount (Q_{24}) of SG permeated during 24 h from SLN-IN (viscosized suspension of SG-loaded SLN) and SLN-OUT (viscosized suspension of not loaded SLN and "free" SG).

through the skin with respect to the formulation characterized by not loaded SLN and free SG suspension (SLN-OUT).

A different mechanism could explain the permeation enhancer effect of SLN observed in this study. Once applied to the stratum corneum the SLN, made of high biocompatible lipids, can fuse with stratum corneum lipids. This collapse of structure liberates permeant into a medium in which the active is poorly soluble. In these conditions an increase of SG thermodynamic activity is observed so facilitating the delivery.

IN VIVO ANTI-INFLAMMATORY ACTIVITY

Formulation D, G, SLN-IN, and SLN-OUT, showing the best *in vitro* profile, were further studied *in vivo* to determine their ability to inhibit the UVB-induced skin erythema on healthy human volunteers.

Skin reflectance spectrophotometry was used to determine the extent of the erythema and to assess the inhibition capacity of the formulations after their preventive application onto the skin. The AUC was determined for each subject plotting ΔEI values versus time. An inverse relationship was found between the AUC and the inhibition of UVB-induced erythema (Table III). Fig. 4 reports the PIE values.

Formulations D and G, containing SG and DG respectively, showed to be more effective than SLN-IN and SLN-OUT formulations, containing SG, in inhibiting the induced erythema 1 h after gel removal ($p < 0.05$), while at 3 and 6 h, the formulation SLN-IN showed the best inhibitory ability ($p < 0.05$; Fig. 4, Table III).

The mechanism described to justify the *in vitro* evidences appears to be useful also to find an explanation of *in vivo* results. In fact, soy lecithin, increasing skin hydration, produced

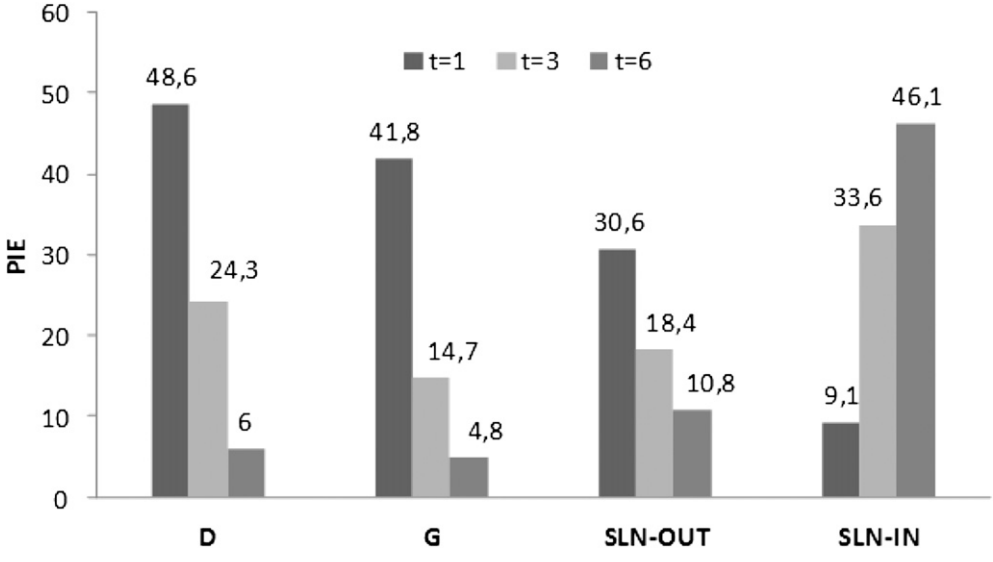

Figure 4. Percentage of inhibition of the UVB-induced erythema (PIE) by formulation D, SLN-IN, and SLN-OUT containing SG (0.5% w/w) or with formulation G containing DG (0.5% w/w). Data represent the mean for 10 subjects.

Table III
AUC_{0-52} Values Obtained Pretreating Skin Sites With Formulation D, SLN-IN, and SLN-OUT Containing SG (0.5% w/w) or With Formulation G Containing DG (0.5% w/w) and Applying UVB Radiations After 1 h ($t = 1$), 3 h ($t = 3$), or 6 h ($t = 6$) From Their Removal

	AUC_{0-52}												
	$t = 1$				$t = 3$				$t = 6$				
Subjects	D	G	SLN-IN	SNL-OUT	D	G	SLN-IN	SNL-OUT	D	G	SLN-IN	SNL-OUT	Control
A	690.1	747.3	1218.6	928.6	1019.2	1128.3	918.2	1116.3	1278.2	1266.5	796.2	1201.2	1418.2
B	715.3	791.2	1200.4	900.3	1115.6	1215.6	924.3	1022.4	1266.3	1288.4	774.3	1210.2	1575.6
C	628.6	801.3	1229.3	1017.1	998.2	1117.3	896.2	1128.3	1215.9	1321.3	726.5	1204.3	1449.8
D	657.8	816.1	1244.8	949.4	1011.2	1198.3	865.4	1129.4	1298.8	1304.5	748.6	1198.6	1320.6
E	731.4	788.4	1197.3	1000.3	1103.9	1088.3	928.3	1138.2	1299.5	1316.6	702.1	1192.1	1146.8
F	726.9	809.3	1208.1	921.3	1088.2	1156.2	919.3	1100.1	1264.6	1324.9	721.2	1194.3	1294.3
G	697.1	826.4	1224.3	916.2	963.4	1228.4	926.2	1099.6	1228.1	1288.6	716.4	1218.4	1421.2
H	701.3	747.3	1281.6	928.4	943.1	1103.6	888.2	1098.4	1300.4	1216.5	698.6	1221.6	1318.3
I	721.4	768.1	1291.5	896.5	995.9	1163.2	826.3	1122.2	1318.2	1284.4	688.5	1244.3	1221.1
L	654.1	724.4	1196.3	886.4	912.2	1212.8	849.5	1211	1294.6	1296.3	788.4	1198.6	1393.0
Mean	696.66	788.38	1232.88	939.79	1026.52	1155.47	899.16	1106.1	1274.44	1290.19	730.27	1209.44	1355.9

a rapid increasing of SG and DG percutaneous absorption and consequently an anti-inflammatory effect that occurs within the first 3 h. Instead the above mentioned fusion of SLN lipid matrix with stratum corneum lipids, could lead to the formation of a reservoir able to realize a SG sustained release toward deeper skin layers and consequently a prolonged anti-inflammatory activity.

In conclusion, the use of both penetration enhancers and SLN resulted to be valid tools to optimize the topical delivery of DG and SG. In particular, the use of soy lecithin guaranteed an increase in the percutaneous absorption of the two actives and a rapid anti-inflammatory effect in *in vivo* experiments.

Otherwise the results of the present study revealed an interesting delayed and sustained activity of SG-loaded SLN. Further *in vitro* and *in vivo* studies are in progress with the aim to maximize the results obtained with these two different strategies.

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