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Evaluation of Alternative Strategies to Optimize Ketorolac Transdermal Delivery

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ABSTRACT

In the present study, 2 alternative strategies to optimize ketorolac transdermal delivery, namely, prodrugs (polyoxyethylene glycol ester derivatives, I-IV) and nanostructured lipid carriers (NLC) were investigated. The synthesized prodrugs were chemically stable and easily degraded to the parent drug in human plasma. Ketorolac-loaded NLC with high drug content could be successfully prepared. The obtained products formulated into gels showed a different trend of drug permeation through human stratum corneum and epidermis. Particularly, skin permeation of ester prodrugs was significantly enhanced, apart from ester IV, compared with ketorolac, while the results of drug release from NLC outlined that these carriers were ineffective in increasing ketorolac percutaneous absorption owing to a high degree of mutual interaction between the drug and carrier lipid matrix. Polyoxyethylene glycol esterification confirmed to be a suitable approach to enhance ketorolac transdermal delivery, while NLC seemed more appropriate for sustained release owing to the possible formation of a drug reservoir into the skin.

KEYWORDS: ketorolac, lipid carrier, NLC, prodrug, transdermal delivery.

INTRODUCTION

Ketorolac is a nonsteroidal agent with powerful analgesic and low antiinflammatory activity, widely used in the management of both moderate and severe pain.[1] Although oral bioavailability of ketorolac was reported to be 90% with very low hepatic first-pass elimination, the biological half-life of 4 to 6 hours requires frequent administration to maintain the therapeutic effect.[2] The long-term use of currently available dosage forms of ketorolac may result in gastrointestinal ulceration and acute renal failure.[3]

In recent years, the development of transdermal dosage forms has been attracting increasing attention, owing to the several advantages that this administration route offers. Transdermal delivery systems, when compared with conventional formulations, generally show a better control of blood levels, a reduced incidence of systemic toxicity, no hepatic first-pass metabolism, and a higher compliance.[4] Nevertheless, drug delivery via the skin is not an easy task because of the formidable barrier properties of the stratum corneum (SC). The majority of drugs do not appear to penetrate the skin at a sufficiently high rate to have therapeutic effectiveness. The feasibility of transdermal route is thus limited to powerful actives presenting the appropriate features such as appropriate low molecular weight and high lipophilicity. Ketorolac shows high analgesic activity and a small molecular weight, but it appears to be not lipophilic enough (with a reported log P of 1.04)[5] to penetrate the skin. Different approaches have been studied to overcome these limitations in order to develop efficient transdermal delivery systems.[6,7] Some of these strategies have been also proposed to enhance the penetration through the skin of ketorolac[5,8-11] but not always with excellent results.[11] Nevertheless, prodrug approach and, most recently, the use of colloidal carriers represent very promising strategies to improve ketorolac transdermal delivery.[5,8,12]

The objective of this research was to evaluate novel ketorolac polyoxyethylene glycol esters prodrugs and ketorolac-loaded nanostructured lipid carriers (NLC) as tools to enhance ketorolac percutaneous absorption. Ketorolac polyoxyethylene glycol esters were synthesized and characterized for partition coefficients, and both chemical and enzymatic stabilities. NLC were prepared and characterized for particle size distribution and drug loading. To investigate and compare the effectiveness of the 2 approaches in enhancing ketorolac in vitro skin permeation, hydrogels containing ketorolac

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prodrugs and ketorolac-loaded NLC were prepared, and the penetration through human SC and epidermis in vitro was evaluated.

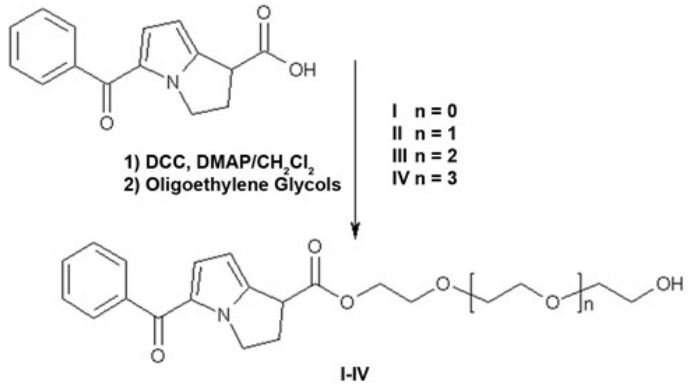
Materials and Methods

Materials

Compritol 888 ATO (glyceryl behenate, tribehenin), a mixture of monoglycerides, diglycerides, and triglycerides of behenic acid (C_{22}), was a gift of Gattefossè Italy srl (Milan, Italy). Miglyol 812 (caprylic/capric triglycerides) was provided by Eingemann and Veronelli SpA (Milan, Italy). Lutrol F68 was a gift of BASF ChemTrade GmbH (Burgbernheim, Germany). Ketorolac, N,N–dicyclohexylcarbodiimide (DCC) and 4-dimethylamminopyridine (DMAP) were purchased from Sigma Chemical (Milan, Italy). Diethylene glycol, triethylene glycol, tetraethylene glycol, pentaethylene glycol were purchased from Lancaster (Milan, Italy). High-performance liquid chromatography (HPLC)-grade acetonitrile and water were purchased from Carlo Erba reagents (Carlo Erba, Milan, Italy). Column chromatography was performed on Carlo Erba silica gel (70-230 mesh; Carlo Erba) and Carlo Erba silica gel 60 (230-400 mesh; Carlo Erba). Thin layer chromatography (TLC) was performed using plates coated with silica gel 60F 254 nm purchased from Merck (Darmstadt, Germany). All other chemicals were of reagent grade and used without further purification.

General Procedure for the Synthesis of Ketorolac Oligoethylene Esters

Ketorolac oligoethylene esters were prepared in 2-step synthesis, starting from the acid precursor by the activation with DCC, in presence of DMAP. The intermediate was treated in situ with diethylene, triethylene, tetraethylene, and pentaethylene glycols to give the corresponding esters (I-IV). As reported in Scheme 1, to a stirred solution of ketorolac (1.18 mmol) in dry CH_2Cl_2 (2 mL) was added N,N-dicyclohexylcarbodiimide (1.0 M in CH_2Cl_2, 1.4 mmol), 4-dimethylamminopyridine (3 mg), and oligoethylene glycol (2.95 mmol). The mixture was stirred for 2 hours at room temperature, and the resulting suspension was filtered and the organic layer was washed with brine, dried over sodium sulfate anhydrous, and evaporated to dryness in vacuo. The products were purified by flash chromatography.



Scheme 1. Scheme of ketorolac derivatives (I-IV) synthesis.

Ketorolac Oligoethylene Esters Characterization

The products obtained were all oils and they failed to crystallize. ^1H and ^{13}C spectra were recorded on a Brüker AC-300 (Rheinstetten, Germany), using $CDCl_3$ as solvent. ^1H and ^{13}C nuclear magnetic resonance (NMR) chemical shifts of esters I to IV are reported in parts per million (ppm) relative to tetramethylsilane (TMS) and ^1H-NMR signals were quoted as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad).

Determination of Chemical and Enzymatic Stability

To evaluate the chemical and enzymatic stability of esters I to IV, initially, 100 μL of an acetonitrilic ester solution (10^{-4} M) was diluted with 6 mL of phosphate buffered saline (PBS) (pH 7.4; μ = 0.5).

Chemical stability was determined following the disappearance of ester on 3 mL of PBS at 32°C by HPLC. Enzymatic hydrolysis of esters was determined as previously reported.[13] Plasma fractions (4 mL) were diluted with 1 mL of PBS (80% plasma). The reaction was started by adding 100 μL of a stock solution of esters I to IV in methanol (1.0 mg/mL) to 5 mL of plasma prethermostated at 37°C ± 0.2°C during the experiments. Aliquots (300 μL) were withdrawn at intervals and deproteinized by adding 600 μL of 0.01 M HCl in methanol. After centrifugation at 5000*g* for 5 minutes, 25 μL of the clear supernatant was analyzed for ketorolac or esters I to IV concentration by HPLC. Pseudo-first order rate constant for chemical and enzymatic hydrolysis were determined from the slopes of linear plots of the logarithm of residual esters against time.

Partition Coefficient of Ketorolac Esters

The partition coefficients n-octanol-PBS (pH 7.4, μ = 0.5) of ketorolac and their esters (I-IV) were determined at room temperature, according to the method described by Inagi and colleagues.[14] The solute concentrations in the aqueous phase, before and after partition, were determined by HPLC.

Nanostructured Lipid Carrier Preparation

NLC were prepared using the high speed stirring/ultra sonication method. In brief, Compritol 888 ATO (4 g) was melted at 80°C and Miglyol 812 (1.52 g) and ketorolac

Table 1. Composition (% wt/wt) of Gel Formulation A to G

Constituents	Gel Code*						
	A	B	C	D	E	F	G
Ketorolac†	15	—	—	—	—	—	—
Ketorolac-loaded NLC suspension‡	—	—	—	—	—	98	—
Blank NLC suspension + free ketorolac	—	—	—	—	—	—	98
Ester I†	—	15	—	—	—	—	—
Ester II†	—	—	15	—	—	—	—
Ester III†	—	—	—	15	—	—	—
Ester IV†	—	—	—	—	15	—	—
Carbomer	1	1	1	1	1	1	1
Triethanolamine	1	1	1	1	1	1	1
Distilled water	83	83	83	83	83	—	—

*Gel pHs ranged from 6.4 to 6.8.

†The value represents the percentage of ethanol added to the formulation in which ketorolac or its derivatives were solubilized. In all the formulations, the drug molar fraction was the same as in Formulation A.

‡NLC indicates nanostructured lipid carriers.

(110 mg) were added. The hot lipid phase was dispersed in a surfactant solution (Lutrol F68, 1.35% wt/vol), at 8000 rpm, 80°C for 1 minute, by using a high-speed stirrer (Ultra Turrax T25, IKA-Werke GmbH and Co KG, Staufen, Germany). The obtained pre-emulsion was ultrasonified by using a UP 400 S (Ultraschallprozessor, Dr. Hielscher GmbH, Teltow, Germany). In order to prevent recrystallization during homogenization, production temperature was kept at least 5°C above the lipid melting point. After the homogenization, the obtained nanoemulsion (O/W) was cooled down in an ice bath; the lipid recrystallized forming NLC dispersion.

Loading Determination

The percentage of ketorolac 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 Biomax10 membrane. An amount of retained material was freeze dried, dissolved in chloroform, and analyzed by UV spectrophotometry at 319 nm (Jasco V-520, Rome, Italy). Calibration curve for the validated UV assays of ketorolac was performed on 5 solutions in the concentration range 0.9 to 40 µg/mL. Correlation coefficient was >0.990. Each point represents the average of 3 measurements, and the error was calculated as ±SD.

Ketorolac incorporation efficiency was expressed both as drug recovery and drug content, calculated using the following equations[15]:

$$Drug\ Recovery(\%) = \frac{Mass\ of\ Ketorolac\ in\ Nanoparticles}{Mass\ of\ Ketorolac\ Fed\ to\ the\ System} \times 100 \quad (1)$$

$$Drug\ Content(\%) = \frac{Mass\ of\ Ketorolac\ in\ Nanoparticles}{Mass\ of\ Nanoparticles\ Recovered} \times 100 \quad (2)$$

Size Distribution

Mean particle size and population distribution of the bulk particle dispersion were measured by photon correlation spectroscopy (PCS) by using a Zetamaster (Malvern Instruments Ltd, Worcestershire, UK) equipped with a solid state laser having a nominal power of 4.5 mW with a maximum output of 5 mW 670 nm. Analyses were performed using a 90° scattering angle and at 20°C ± 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 polyvinylidene fluoride (PVDF) filter (Pall-Gelman Laboratory, Ann Harbor, MI). During the experiment, refractive index of the samples always matched liquid (toluene) to avoid stray light.

Preparation of Gel Formulations

To investigate in vitro skin permeation performances of ketorolac polyoxyethylene glycol esters prodrugs and ketorolac-loaded NLC, 7 gel formulations were prepared (Table 1). Formulations A to E were prepared by dispersing Carbopol 934P (Carbomer) in water and then adding an alcoholic solution in which ketorolac (4 mmol) or its esters I to IV (4 mmol) were solubilized with constant stirring. The dispersion was then neutralized and made viscous by the addition of triethanolamine. Formulations F and G were obtained by adding, respectively, a dispersion of NLC-encapsulating ketorolac or a dispersion made of blank NLC and free ketorolac to a carbomer dispersion obtained as previously reported. Finally, triethanolamine was added in order to neutralize and make the suspensions viscous. All the formulations were stored at 4°C before use.

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°C ± 1°C for 2 minutes,[16] after which SC and epidermis (SCE) were removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in a desiccator at approximately 25% relative humidity (RH). The dried samples were wrapped in aluminum foil and stored at 4°C ± 1°C until use.[17] Preliminary experiments were performed in order to asses SCE samples for barrier integrity by measuring the in vitro permeability of [^{3}H]water through the membranes using the Franz cells described below in the In Vitro Skin Permeation Experiments section. The value of the permeability coefficient (P_m) for tritiated water was found to be $1.6 \pm 0.2 \times 10^{-3}$ cm/h, which agreed well with those for tritiated water reported by others using human SCE samples.[18]

In Vitro Skin Permeation Experiments

Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 hour before being mounted in Franz-type diffusion cells supplied by Laboratory Glass Apparatus Inc (Berkeley, CA). The exposed skin surface area was 0.75 cm^2, and the receiver compartment volume was of 4.5 mL. The receptor compartment contained a water-ethanol solution (50:50) that, to allow the establishment of the "sink condition" and to maintain permeant solubilization,[19] was stirred and thermostated at 32°C ± 1°C during all the experiments.[20]

Approximately 100 mg of each gel formulation (A-G) was placed on the skin surface in the donor compartment and the latter was covered with Parafilm M barrier film. Each experiment was run in duplicate for 36 hours using 3 different donors (n = 3). At intervals, samples (200 μL) of receiving solution were withdrawn and replaced with fresh solution. The samples were analyzed for ketorolac content by HPLC as described in the High-Performance Liquid Chromatography section. Drug fluxes (nmol/cm^2/h), 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. Statistical analysis of data was performed using Student *t* test.

High-Performance Liquid Chromatography

The HPLC apparatus consisted of Hewlett-Packard chromatograph HP 1100 (Milan, Italy) equipped with a 20 μL loop injector and a Hewlett-Packard photodiode array UV detector.

Chromatography analysis was performed using a variation of the method reported by Alsarra et al.[8] Particularly, the added modifications concerned the use of a convex gradient and a different UV detector setting to optimize the chromatographic determination of ketorolac and its esters. As regards the convex gradient, the analysis starts from 95/5 (vol/vol) to 2/98 (vol/vol) water/acetonitrile over 31 minutes, using a Jupiter Phenomenex RP C_{18} column (particle size, 5μm; 25 × 4.6 mm inner diameter (id); Phenomenex, Torrance, CA). Detection was effected at 260 nm, and the flow rate was set at 1 mL/min. The retention times for ketorolac and its derivatives are reported in Table 2.

Results and Discussion

Ketorolac Oligoethylene Esters Characterization

5-Benzoyl-2,3-dihydro-1H- pyrrolizine-1-carboxylic acid diethylene glycol ester (I)

Ester I was purified by flash silica gel column chromatography eluting with $CHCl_3$/MeOH (98:2) and obtained in 85% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.90 (m, 2H, CH-C$\underline{H}_2$-CH_2), 3.63 (t, 2H, COO-CH_2-C$\underline{H}_2$-O, *J* = 3.95 Hz); 3.75 (m, 4H); 4.15 (dd C$\underline{H}$-CH_2-CH_2, *J* = 5.70; 5.92 Hz); 4.35 (t, 2H, COO-C$\underline{H}_2$-CH_2-O, *J* = 3.95 Hz); 4.55 (m, 2H NC$\underline{H}_2$); 6.16 (d, 1H, CH = CH, *J* = 3.95 Hz); 6.85 (d, 1H, CH = CH, *J* = 3.95 Hz); 7.47 (dd, Ar-H 2H, *J* = 7.45; 7.02 Hz); 7.56 (t, 1H, Ar-H, *J* = 7.02); 7.84 (d, 2H, Ar-H, *J* = 7.45). ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 31.27; 42.83, 47.63, 64.77, 69.15,

Table 2. Molecular Mass, Retention Time, Chemical and Enzymatic Hydrolysis, Partition Coefficient, and Calculated Water Solubility of Ketorolac and Its Esters (I-IV)*

Compound	M_r	$t_{½}$ (h)		Log P†	S_w (μmol/mL)	t_r
		Buffer pH 7.4†	Human Plasma†			
Ketorolac	255.27	—	—	1.14 ± 0.01	19.1	17.4
I	343.37	518.6 ± 92.3	4.2 ± 0.4	2.13 ± 0.02	28.3	25.0
II	387.43	487.3 ± 71.4	3.9 ± 0.3	1.98 ± 0.03	47.6	25.7
III	431.48	526.9 ± 87.3	3.2 ± 0.6	1.54 ± 0.01	113.0	26.3
IV	475.53	518.4 ± 82.1	3.7 ± 0.2	1.42 ± 0.01	152.0	27.9

*M_r indicates molecular mass; t_r, retention time; $t_{½}$, chemical and enzymatic hydrolysis; Log P, partition coefficient; and S_W, calculated water solubility.

†Data represent the mean ± SD of 3 determinations.

70.99, 71.10, 103.37, 125.24, 127.60, 128.38, 129.27, 131.76, 139.62, 142.68, 171.58, 185.22.

5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid triethylene glycol ester (II)

Ester II was purified by flash silica gel column chromatography eluting with $CHCl_3$/MeOH (98:2) and obtained in 80% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.90 (m, 2H, CH-C$\underline{H_2}$-CH_2), 3.63 (t, 2H, COO-CH_2-C$\underline{H_2}$-O, *J* = 3.95 Hz); 3.69 (m, 4H); 3.75 (m, 4H); 4.15 (dd C$\underline{H}$-CH_2-CH_2, *J* = 5.70; 5.92 Hz); 4.35 (t, 2H, COO-C$\underline{H_2}$-CH_2-O, *J* = 3.95 Hz); 4.55 (m, 2H NC$\underline{H_2}$); 6.16 (d, 1H, CH = CH, *J* = 3.95 Hz); 6.85 (d, 1H, CH = CH, *J* = 3.95 Hz); 7.47 (dd, Ar-H 2H, *J* = 7.45; 7.02 Hz); 7.56 (t, 1H, Ar-H, *J* = 7.02); 7.84 (d, 2H, Ar-H, *J* = 7.45). ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 31.27; 42.3; 46.0; 63.7; 67.7; 69.8; 70.9; 73.1; 111.8; 127.60; 128.6; 129.5; 132.4; 133.2; 135.7; 142.9; 172.0; 177.0.

5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid tetraethylene glycol ester (III)

Ester III was purified by flash silica gel column chromatography eluting with $CHCl_3$/MeOH (98:2) and obtained in 83% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.90 (m, 2H, CH-C$\underline{H_2}$-CH_2), 3.63 (t, 2H, COO-CH_2-C$\underline{H_2}$-O, *J* = 3.95 Hz); 3.69 (m, 8H); 3.75 (m, 4H); 4.15 (dd C$\underline{H}$-CH_2-CH_2, *J* = 5.70; 5.92 Hz); 4.35 (t, 2H, COO-C$\underline{H_2}$-CH_2-O, *J* = 3.95 Hz); 4.55 (m, 2H NC$\underline{H_2}$); 6.16 (d, 1H, CH = CH, *J* = 3.95 Hz); 6.85 (d, 1H, CH = CH, *J* = 3.95 Hz); 7.47 (dd, Ar-H 2H, *J* = 7.45; 7.02 Hz); 7.56 (t, 1H, Ar-H, *J* = 7.02); 7.84 (d, 2H, Ar-H, *J* = 7.45). ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 25.7; 42.3; 46.0; 63,7; 67.7; 69.8; 70.9; 73.1; 111.8; 124.9; 127.6; 128.6; 129.5; 132.4; 133.2; 135.7; 142.9; 172.0; 177.0.

5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid pentaethylene glycol ester (IV)

Ester IV was purified by flash silica gel column chromatography eluting with $CHCl_3$/MeOH (98:2) and obtained in 84% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.90 (m, 2H, CH-C$\underline{H_2}$-CH_2), 3.63 (t, 2H, COO-CH_2-C$\underline{H_2}$-O, *J* = 3.95 Hz); 3.68 (m, 12H); 3.76 (m, 4H); 4.15 (dd C$\underline{H}$-CH_2-CH_2, *J* = 5.70; 5.92 Hz); 4.36 (t, 2H, COO-C$\underline{H_2}$-CH_2-O, *J* = 3.95 Hz); 4.55 (m, 2H NC$\underline{H_2}$); 6.16 (d, 1H, CH = CH, *J* = 3.95 Hz); 6.84 (d, 1H, CH = CH, *J* = 3.95 Hz); 7.48 (dd, Ar-H 2H, *J* = 7.45; 7.02 Hz); 7.56 (t, 1H, Ar-H, *J* = 7.02); 7.84 (d, 2H, Ar-H, *J* = 7.45). ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 25.7; 43.5; 46.0; 61.4; 65.0; 69.4; 70,5; 72.7; 142.9; 110.2; 124.0; 127.6, 128.9; 129.7; 131.3; 132,7; 135.7; 172.7; 177.0.

Chemical and Enzymatic Stability of Ketorolac Esters

The conversion of ester prodrugs to the parent drug ketorolac was confirmed by the stability studies in PBS at pH 7.4 and human plasma at 37°C. The results (Table 2) showed that esters I to IV had a high stability in PBS but were readily hydrolyzed in the human plasma. No significant difference in their hydrolysis rates was observed as the length of the polyoxyethylene chain increased ($P > .05$). The same behavior has been observed with other prodrugs obtained conjugating polyoxyethylene groups to some interesting nonsteroidal antiinflammatory drugs (NSAIDs).[13,21]

Physicochemical Characteristics of Esters I to IV

The physicochemical properties of ketorolac and its various ester prodrugs are summarized in Table 2. The molecular weights of ketorolac and its prodrugs, ranging from 256 to 475.53 g/mol, fell in the appropriate range for transdermal delivery.[22]

Ketorolac esters I to IV showed a similar or an increased lipophilicity compared with the parent drug (Table 2). As the polyoxyethylene chain grew longer, this parameter decreased as a result of the increasing hydrophilicity introduced in these molecules by adding new polyoxyethylene units.

Owing to the oily consistency of the synthesized derivatives, water solubility was estimated using a theoretical method previously reported.[23-25] This method has been used successfully to calculate water solubility of both solid and oily compounds using Equations 3 and 4, respectively[13,21,26]:

$$Log\ S_W = -\ log\ P - 0.01\ MP + 1.05 \quad (3)$$

$$Log\ S_W = -\ 1.072\ log\ P + 0.672 \quad (4)$$

where S_W represents the calculated water solubility of a compound, MP is its melting point (165°C-167°C), and log P is the n-octanol-PBS partition coefficient. As may be noted in Table 2, for all the synthesized esters, an increase of this parameter was observed lengthening the polyoxyethylenic chain.

Characterization of Ketorolac-loaded NLC

The ultrasonic method used appeared suitable for the production of the lipid nanoparticles according to previous work.[27] PCS analyses showed mainly the presence of 2 distinct

particle populations with mean diameters of 90 nm and 220 nm.

NLC exhibited very high encapsulation efficiency as shown by drug recovery (92.3%) and drug content (1.26%) results. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be housed almost completely within the liquid oil (Miglyol 812) owing to the higher ketorolac solubility in this medium compared with the drug solubility observed in the lipid melt used for NLC preparation (Compritol 888 ATO) (data not shown). Similar behavior was observed for indomethacin-loaded NLC.[27] Possible lipid interferences during UV determination of ketorolac were also investigated by comparing the 2 standard curves of ketorolac alone and ketorolac plus lipids in chloroform solution in the range of 0.9 to 40 μg/mL. The differences observed between the standard curves were within the experimental error, thus implying that no lipid interference occurred (data not shown).

In Vitro Permeation

Figures 1 and 2 show the plots of the cumulative amounts of ketorolac and its derivatives permeated through human SCE membranes as a function of time. In particular, Figure 1 shows esters I to IV permeation profiles from B to E gel formulations, respectively; while Figure 2 reports the ketorolac permeation profiles from NLC containing forms (F and G), both compared with a control formulation (A) containing free ketorolac. Drug flux values (J_{SS}) from gels A to G calculated from the linear segments at the steady-state are reported in Figure 3. As shown in Figure 3, skin permeation of ester prodrugs was significantly enhanced, apart from ester IV, compared with ketorolac. Formulation B, containing ester derivative I, gave the maximum permeation rate of this compound (6.71 ± 0.43 nmol/cm^2/h), while there was no significant difference between ester II and III permeation profiles ($P > .05$). Polyoxyethylene glycols were used as promoieties, to design new ketorolac prodrugs, to combine within the same molecule 2 strategies widely employed to modify drug permeation profile and pharmacokinetic features such as the use of prodrug approach and of a penetration enhancer. Polyoxyethylene glycols possess skin penetration enhancing ability and are also common constituents of topical drug formulations.[21,28]

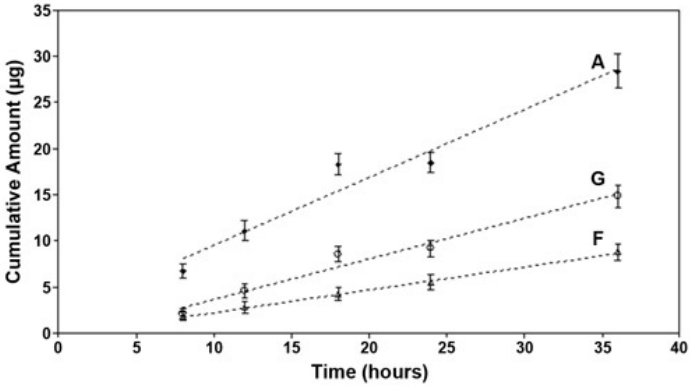


Figure 2. Permeation profiles of ketorolac through SCE membranes from a control gel (A) and NLC-containing forms (A, ketorolac; G, ketorolac-loaded NLC; F, blank NLC + free ketorolac).

The increase of prodrug skin permeation rates was found to be directly related to the molecule lipophilicity. Ester I, in fact, showed a higher permeation rate and log P value (2.1) compared with the other ester derivatives (Figure 1, Table 3). It showed remarkable water solubility (28.3 μmol/mL) higher than that of ketorolac but at the lowest level if compared with the other esters (Table 2). Considering skin structure, water solubility appears to be a parameter that drastically influences drug permeation profile. The skin is composed of a comparatively lipophilic SC and hydrophilic viable skin (epidermis and dermis). Although highly lipophilic drugs

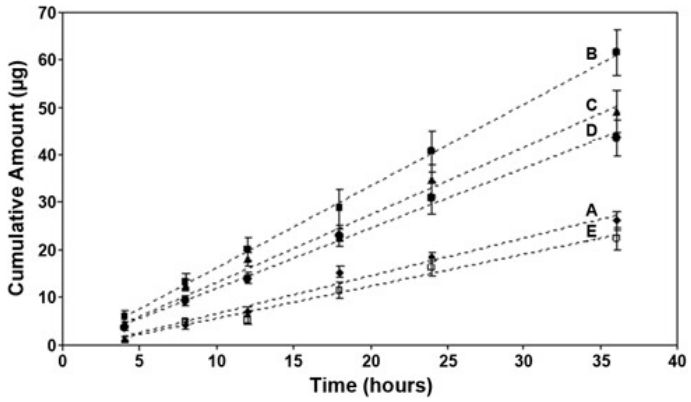


Figure 1. Permeation profiles of ketorolac and its esters through SCE membranes from different gel formulations (A, ketorolac; B, ester I; C, ester II; D, ester III; E, ester IV).

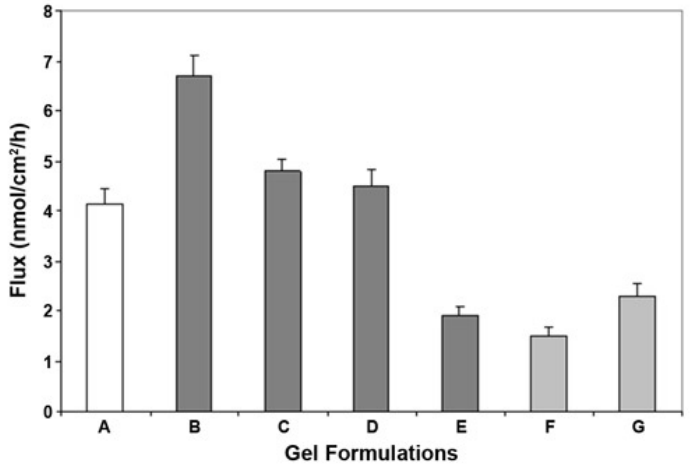


Figure 3. Drug steady-state fluxes through excised human skin from A to G gel formulations.

Table 3. Permeation Parameters for Ketorolac and Its Esters From Different Formulations

	Parameter			
Gel Code	*Lag Time (h)*	*Permeability Coefficient (P, 10^{-5} cm × h^{-1})*	*Partition Coefficient (K)*	*Diffusion Coefficient (D, 10^{-8} cm^{-2} × h^{-1})*
A	*1.85*	*10.6*	*0.70*	*25.4*
B	*0.58*	*23.0*	*0.58*	*80.9*
C	*1.44*	*19.2*	*0.98*	*32.6*
D	*1.50*	*17.2*	*0.92*	*31.4*
E	*1.77*	*8.9*	*0.56*	*26.6*
F	*2.42*	*3.8*	*0.32*	*19.4*
G	*2.27*	*5.8*	*0.47*	*20.7*

can easily get into the SC, they may not be able to escape from the SC into the viable epidermis. On the basis of the permeation results, ester I showed an optimal balance between lipophilicity and hydrophilicity. Notwithstanding, ester IV showed an increased lipophilic character compared with ketorolac; both its elevated molecular weight and its ability to form H-bond (owing to more oxyethylene groups) influenced its diffusional properties, reducing the permeation rate through the skin.[29,30]

NLC-containing formulations (F and G) gave the lowest ketorolac permeation rate among the formulations tested, and the flux values registered were considerably lower than control formulation (A; $P < .01$). Statistical analysis also revealed significant differences between the steady-state flux value obtained with ketorolac-loaded NLC formulation (F) and the value registered with gel G (free ketorolac and NLC; $P < .05$).

This behavior could be explained by taking into account potential interactions between ketorolac and the NLC lipids. These interactions became more evident when the drug was included in the nanoparticles. This finding could depend on a chemical affinity between the drug and the oil phase employed to formulate these lipidic carriers. In this respect the vehicle could produce a partial "sequestration" of the drug, thus reducing the amount available for diffusion. Castelli et al[31] studied the interaction between lipids and drug in an NLC system using indomethacin as model compound. The authors, by means of calorimetric experiments, demonstrated that NLC interacted with indomethacin, modulating its release, either when the drug was included in the lipid nanoparticles or when it was suspended together with blank NLC. Notwithstanding, indomethacin resulted more lipophilic than ketorolac; in our opinion, this evidence could be used to justify the slight differences observed comparing G formulation (blank NLC + free ketorolac) and F formulation (ketorolac-loaded NLC) fluxes through SCE membranes.

Table 3 reports other important drug permeation parameters such as the lag time (T_L), the diffusion coefficient (D), the permeability (P), and the partition coefficient (K) calculated for different formulations tested in the present study (A-G). In particular, the lag time represents the time period required to establish steady-state diffusion, and it corresponds to the nonlinear portion of a permeation profile, while the other parameters were obtained using the equations reported below[32]:

$$D = \frac{h^2}{6T_L} \tag{5}$$

$$P = \frac{J_{SS}}{C} \tag{6}$$

$$K = \frac{P \cdot h}{D} \tag{7}$$

where h is the barrier thickness for human skin, and C is the donor phase concentration. The h value is 16.8 μm assuming that the SC represents the main rate-limiting barrier.

Coefficient D, particularly, reflects the facility for the molecules to move through the membrane strata and is a function of the molecular structure of the diffusant.[33] As shown in Table 3, ester I showed the highest D value, and this result could be justified by its optimal log P value and lipophilicity. Furthermore, it showed a lower molecular weight compared with the other ester derivatives, and this could influence its permeation profile and diffusivity through SCE membranes.

The relative affinity of the drug for skin and vehicle is presented by the partition coefficient K. Generally a high K value indicates that the vehicle has a poor affinity for the drug. A low K value, indicating a high degree of mutual interaction, reflects the tendency of the drug to remain in the vehicle. As expected, K values obtained for the NLC-made formulations were lower than the value registered for control formulation, confirming the previous hypothesis and the permeation results.

Another interesting aspect should be considered to elucidate the mechanism controlling ketorolac release from NLC.

In a previous study, regarding the evaluation of indomethacin percutaneous absorption from NLC,[27] the formation of a drug reservoir in the SC, owing to the interaction between the lipid components of the nanoparticles and the SC lipid matrix, was demonstrated. In an in vivo experimental procedure, this reservoir was able to guarantee an indomethacin sustained release toward deeper skin layers.[27] Since the in vitro permeation profile obtained for indomethacin was similar to that obtained with ketorolac, it is speculated that this "skin-NLC" interaction could be, in part, responsible for drug release from the nanoparticles. However, in vivo study on human volunteers is in progress to confirm the results of in vitro permeation studies.

CONCLUSIONS

The reported results gave evidence for a different trend of SCE permeation of ketorolac, formulated as a prodrug or into lipid carriers, according to the employed strategy. All ester prodrugs except ester IV showed an enhancement in skin permeation compared with ketorolac. The ester I, characterized by an optimal balance between lipophilicity and hydrophilicity, showed the highest percutaneous flux. Concerning drug release from NLC, the results demonstrated that these carriers were ineffective in increasing ketorolac percutaneous absorption, probably because of a certain degree of interaction between the drug and the carrier-lipid matrix. However, NLC may potentially work well as a controlled release system, owing to their depot attitudes.[31]

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