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Research Paper

Enhanced oral bioavailability of etodolac by self-emulsifying systems: in-vitro and in-vivo evaluation

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Abstract

Objectives The objective of this study was to prepare a self-emulsifying drug delivery system (SEDDS) for oral bioavailability enhancement of a poorly water-soluble drug, etodolac. The SEDDS formulations were optimized by evaluating their ability to self-emulsify when introduced to an aqueous medium under gentle agitation, and by determination of the particle size of the resulting emulsion.

Methods An optimized formulation of SEDDS (composed of 20% etodolac, 30% oil Labrafac WL1349, 10% Lauroglycol 90 and 40% Labrasol) was selected for bioavailability assessment in rabbits. The anti-inflammatory effect was also determined in rats, and compared with powder drug and etodolac suspension in water (50 mg/kg).

Key findings The peak plasma concentration of 16.4 ± 1.1 μg/ml appeared after 1.3 ± 0.2 h, whereas with powder drug and etodolac suspension the values were 7.5 ± 0.5 and 10.6 ± 0.7 μg/ml at 4.2 ± 0.4 and 2.4 ± 0.2 h, respectively. The AUC_{0-8} of the etodolac SEDDS formulation was 2.3 times that of the pure drug and 1.4 times that of the suspension form. SEDDS formulation exhibits a 21% increase in paw thickness compared with a 39% increase on oral administration of etodolac suspension after 4 h at the same dose of the drug (20 mg/kg).

Conclusions The result indicates the utility of SEDDS for the oral delivery of etodolac and potentially other lipophilic drugs.

Keywords bioavailability; dissolution; etodolac; paw oedema; self-emulsifying drug delivery system

Introduction

The oral route is preferred for drug therapy of chronic conditions. However, numerous potent lipophilic drugs exhibit low oral bioavailability due to their poor aqueous solubility. For this class of compounds, defined by Amidon *et al*.[1] as 'low solubility/high permeability class', dissolution in the environmental lumen is the rate-controlling step in the absorption process. Efforts are ongoing to enhance the oral bioavailability of lipophilic drugs in order to increase their clinical efficacy. Various formulation strategies are reported in the literature, including the use of surfactants, cyclodextrins, nanoparticles, solid dispersions, micronization, liposomes and lipids, with each formulation approach having its specific advantages and limitations.[2–4]

In recent years much attention has been focused on lipid-based formulations,[5,6] with particular emphasis on self-emulsifying drug delivery systems (SEDDS).[7] SEDDS are isotropic mixtures of oils and surfactants, sometimes including cosurfactants, that emulsify under conditions of gentle agitation. These mixtures are similar to those that are encountered in the gastrointestinal (GI) tract, where the digestive mobility of the stomach and intestine provide the agitation required for self-emulsification *in vivo*.[8] The spontaneous formation of fine oil-in-water (o/w) emulsions or microemulsions on drug release in the GI tract advantageously presents the drug in a dissolved form and the small droplet size provides a large surface area for drug absorption.[9]

Factors controlling the in-vivo performance of SEDDS include their ability to form small droplets of oil (5 μm) and the polarity of the oil droplets, which promotes faster drug release into the aqueous phase.[10] The smaller oil droplets provide a large interfacial area for pancreatic lipase to hydrolyse triglycerides and thereby promote the rapid release of the drug and/or formation of mixed micelles of bile salts containing the drug.[11] The surfactants used are known to improve the bioavailability by various mechanisms, including (1) improved drug dissolution, (2) increased intestinal epithelial permeability and (3) increased tight junction permeability.[12]

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Recently, a few studies have reported enhancement in the bioavailability of poorly soluble drugs when formulated as SEDDS.[13] A marketed formulation of ciclosporin (Sandimmune Neoral), a microemulsion preconcentrate with self-emulsifying properties, is reported to improve oral bioavailability and reduce inter-and intra-subject variability in ciclosporin pharmacokinetics.[14]

Etodolac is a pyranocarboxylic acid-derived non-steroidal anti-inflammatory drug with analgesic activity. Etodolac is effective in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis, and in the alleviation of postoperative pain. The aim of the current investigation was to develop and characterize the optimal formulation of SEDDS containing the model compound and to assess its bioavailability and anti-inflammatory effect compared with pure powdered etodolac.

Materials and Methods

Materials

Etodolac was obtained from Pharco Co., Cairo, Egypt. The oils used include Labrafac WL1349 (medium-chain triglycerides, hydrophilic lipophilic balance (HLB) = 2) and Labrafil M 1944 CS (composed largely of triglycerides based on oleic and linoleic acid (C18) and pegylated derivatives, HLB = 4), Labrafil M 2125 (linoleoyl macrogolglycerides, HLB = 4) and Labrasol (as a surfactant, caprylocaproyl macrogolglycerides, polyoxylglycerides, HLB = 14). The co-surfactants used, Transcutol P (diethylene glycol monoethyl ether, HLB = 14), Lauroglycol 90 (propylene glycol monolaurate, HLB = 5) and Capryol 90 (propylene glycol monocaprylate, HLB = 6), were kindly supplied by Gattefosse (Saint-Priest-Cedex, France). Peanut oil, soya bean oil and oleic acid were obtained from Fluka, UK. Polyvinyl pyrrolidone (PLASDONE K90, PVP K90) was obtained from ISP Tecnologies Inc., Wayne, NJ, USA. All organic solvents were high-performance liquid chromatography (HPLC) grade. All other chemicals and solvents were of analytical grade.

Methods

Solubility of etodolac

The solubility of etodolac in various oils, surfactants and cosurfactants was determined. An excess of etodolac (approximately 500 mg) was placed in 2 ml of the vehicle in screw-capped glass vials and the mixture was heated at 60°C in a water bath using a vortex mixer to facilitate the solubilization. Mixtures were equilibrated at 30°C for 48 h in a water bath and then centrifuged at 30 000 rev/min for 10 min (Hitachi, Tokyo, Japan) to separate the undissolved drug. Aliquots of supernatant were diluted with methanol and quantified by an HPLC system (Shimadzu, Japan) consisting of Class VP computer software, an LC 10AD VP pump, and an SPD 10A VP variable wavelength ultraviolet absorbance detector. The chromatographic column was a 5 μm, 150 $\times$ 3.9 mm Nova-Pak C18 (Waters Corp., Milford, MA, USA). The mobile phase, a mixture of a 0.04 M solution of potassium dihydrogen phosphate and acetonitrile (70 : 30, v/v, pH 6.0), was filtered through a 0.45 μm membrane filter and eluted at a flow rate of 1 ml/min. Effluents were monitored at 276 nm. The inter- and intra-day variance of this HPLC method was within the acceptable range.

Preparation of self-emulsifying formulations

Approximately 5–7 g of each formulation was prepared. After optimization of the best stable formulations as determined by preliminary experiments (Table 2), a series of SEDDS formulations was prepared, containing varying concentrations of surfactant (20–40%), cosurfactant (10–30%) and oil (30%). Etodolac was dispersed into the mixture of oil and surfactant–cosurfactant with constant stirring and kept at 50–60°C for 10 min to obtain a good blend of the oil–surfactant mixture in a liquid state. The mixture was cooled to ambient temperature. In all formulations, the level of etodolac was constant (i.e. 20% w/w of the vehicle). At this level, the filling volume of a size 0 capsule represents 100 mg of etodolac. A quantity of 500 mg of each formulation was accurately placed into air-filled soft gelatin capsules using a syringe with an 18G needle, after which the resulting hole was sealed with molten gelatin. The chemical stability of the formulations was assessed prior to, and during, the course of the study by a validated HPLC method, in order to monitor the concentration of etodolac. The composition of the capsules was gelatin, glycerin, methylparaben, propylparaben and purified water.

Assessment of the efficiency of self-emulsification

A visual test to assess the self-emulsification properties reported by Khoo *et al.* was modified and adapted in the present study.[15] The efficiency of self-emulsification was assessed using a standard USP dissolution apparatus II (Erweka, DT-6, Germany). One millilitre of each formulation was added dropwise to 200 ml of either 0.1 M HCl or purified water at 37°C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rev/min. The tendency to emulsify spontaneously and also the progress of emulsion droplets were observed. The tendency to form an emulsion was visually assessed using the following grading system: A, denoting a rapidly forming clear microemulsion; B, denoting a rapidly forming, slightly-less-clear emulsion which had a bluish-white appearance; C, denoting a bright white emulsion; D, denoting a dull, grayish-white emulsion with a slightly oily appearance that was slow to emulsify; E, denoting a formulation which exhibited poor or minimal emulsification with large oil droplets present on the surface. The dispersibility of the formulation on addition to the aqueous medium was also noted. All studies were repeated twice, with similar observations being made between repeats.

Emulsion droplet size analysis

Twenty-five microlitres of each formulation was diluted with water to 25 ml in a volumetric flask and gently mixed by inverting the flask. The droplet size distribution of the resultant emulsions was determined by photon correlation spectroscopy, which analyses the fluctuations in light scattering due to Brownian motion of the particles,[16] using a Malvern particle size analyzer (Model no. 3000, 63 mm lens, Malvern Instruments, Malvern, UK). Light scattering was monitored at 25°C at a 90° angle. Purified water filtered through a 0.22 μm

cellulose filter was used as the dissolution medium. The values of mean emulsion droplet diameters were compared.

Drug release studies

Dissolution studies of the SEDDS containing 20% etodolac in soft gelatin capsules were conducted (comparing with 100 mg pure powder filled in hard gelatin capsules using lactose as the diluent) using USP 23,[17] Apparatus II (Erweka DT-6, Germany) with three replicates. The dissolution media were 900 ml of simulated gastric fluid without pepsin (SGF; pH 1.2) and water. The paddle rotation speed was kept at 50 rev/min. In all experiments, 5 ml of dissolution samples was withdrawn at 5, 10, 20, 30, 45, 60, 90 and 120 min and replaced with an equal volume of the fresh medium to maintain a constant total volume. Samples were filtered through 0.2 μm Millipore filters and analysed by UV spectrophotometry at 276 nm. Cumulative percentages of the drug dissolved from the preparation were calculated.

The best formulation (small droplet and maximum in-vitro dissolution) was selected for further in-vivo study in animals.

In-vivo absorption study

The bioavailability of three formulations of etodolac was assessed in rabbits. The study was performed in accordance with the guidelines of the local institutional animal ethics committee, King Saud University, Riyadh, Saudi Arabia. Eighteen New Zealand male rabbits, weighing 2.5–3.0 kg, were obtained from the Laboratory Animal Center. All animals were housed individually in standard cages on a 12 h light–dark cycle with a temperature-controlled environment at 20 ± 2ºC and 50 ± 5% relative humidity. Animals were fed with standard animal chow daily and had free access to drinking water.[18] The rabbits were fasted for 12 h before drug administration but were allowed free access to water. The animals were divided at random into three groups (six animals each), and each animal received one of the following dosage forms: etodolac powder-filled capsule with lactose as a diluent, an optimized self-emulsifying formulation and etodolac suspension in water with 1% povidone as suspending agent (one formulation each group) corresponding to a dose of 100 mg (50 mg/kg). The formulations were administered by the oral route with a gastric catheter which was subsequently flushed with 10 ml of water. A 1-week washout period was allowed between two successive dosings. About 2 ml of blood sample was collected in polypropylene tubes containing 57 U heparin (15 μl). Blood samples were collected through the peripheral ear vein prior to dosing and at designated time intervals after dosing (0.25, 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 h). Plasma was separated by centrifugation (4°C, 2500 rev/min, 15 min) and kept frozen (–70°C) until analysis. The concentration of etodolac in rabbit plasma was determined by HPLC.

Sample processing and analytical method

Plasma samples were analysed for etodolac using a published HPLC method with slight modification.[19] The method may be described as follows: 100 μl of plasma was placed in a plastic centrifugation tube and then 300 μl of acetonitrile containing naproxen as internal standard (10 μg/ml) was added. After vigorous shaking for 30 s, the mixture was centrifuged at 18 000 rev/min for 15 min at room temperature. The organic layer was transferred to a new tube and evaporated by nitrogen pursing and the residue was reconstituted in 100 μl of mobile phase. After vortex-mixing for 10 min, 50 μl of the sample was used for HPLC as described below.

HPLC analysis

Chromatography was carried out on a Shimadzu HPLC column (SCL-10A VP, Japan) equipped with Class VP software for data processing. Samples were analysed on a reverse-phase 15 cm × 3.9-mm i.d., VP-ODS C_{18} column (Shimadzu, 5 μm, Japan), attached to a C_{18} precolumn (Shimadzu SPD-10A). A VP-UV detector was set at 276 nm and the mobile phase was pumped at a flow rate of 1.5 ml/min. The mobile phase consisted of a 0.04 M solution of potassium dihydrogen phosphate and acetonitrile in a proportion of 70 : 30, with pH 6.0 (adjusted with 0.1 M KOH). The mobile phase passed through a 0.45 μm nylon filter (Millipore, USA) and was degassed by ultrasonication under vacuum before use. The volume of the injected sample was 20 μl. Under these conditions the retention times were 3.14 min for naproxen and 4.5 min for etodolac.

Calibration curves of etodolac were prepared with blank plasma that was spiked with known amounts of the drug, utilizing its HPLC peak area ratios to the internal standard. The mean best-fit linear regression equation was used to estimate the concentrations of etodolac at different time intervals.

Assay validation

The detailed analytical method validation was based on the recommendations published as a conference report of the Washington Conference on Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies.[19] The Washington Conference provides guiding principles for the validation of analytical methods for the assay of drugs and their metabolites, i.e. xenobiotics, in biological fluids. Before any assay, the specificity, linearity and repeatability of the method were demonstrated and the limit of quantification and detection were determined. The specificity of the method is documented by the absence of interference from endogenous substances from drug-free rabbit plasma. No endogenous rabbit plasma components corresponding to either the drug or the internal standard were observed at the retention times.

Pharmacokinetics and statistical analysis

Pharmacokinetic analysis was performed by means of a model-independent method using the PCSTAT\STRIPE computer program, Version 1A (issued by Department of Pharmacodynamics, University of Illinois at Chicago, 1984). The area under the plasma concentration versus time curve from zero to 8 h ($AUC_{0–8\ h}$) was calculated using the trapezoidal rule.[20] The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were directly obtained from plasma data. For each formulation, mean percentage absorbed–time plots were obtained by deconvolution of the corresponding mean concentration versus time plots using the Wagner–Nelson method.[21] These plots allowed the construction of percentage

unabsorbed–time plots, which were used for the evaluation of the absorption rate constants.[22]

$$A_t = (C_t + k_e \times AUC_{0-t})/k_e \times AUC_{0-\alpha}$$

A_t is the fraction of drug absorbed at time t, C_t is the concentration of drug in the plasma at time t, k_e is the elimination rate constant, AUC_{0-t} is the area under the curve from time = zero to time = t and $AUC_{0-\alpha}$ is the area under the plasma curve from time = zero to time = infinity.

The significance differences observed for the mean pharmacokinetic parameters after oral administration of etodolac formulations to rabbits was evaluated using analysis of variance (ANOVA) at a significance level of $P \leq 0.05$. Subsequent analysis was performed using Tukey multiple comparisons employing Graphpad INSTAT, Graphpad Software V 2.04. Results are presented as mean values ± SD.

Carrageenan-induced paw oedema

Male Wister rats weighing 200 ± 10 g with free access to water but that had fasted overnight (18 h) were divided into four groups with three rats in each group. A volume of 0.1 ml of 1% carrageenan solution (Sigma, Germany) was injected into the right-hand paw. One hour after carrageenan injection, the first group was given a blank lipid dispersion (1 ml/kg). The second group was given etodolac powder suspended in aqueous 1% povidone solution at 20 mg/kg, while the third group was given etodolac SEDDS formulation at the same dose. A fourth group (control group) received only tap water. Paw thickness was measured from ventral to dorsal surfaces, using dial calipers, immediately prior to carrageenan injection, and then at hourly intervals for 7 h. The mean swelling for three rats was calculated. The inflammation responses are expressed as the percentage increase in paw thickness compared with pre-injection values, plotted against time.

Results and Discussion

Solubility of etodolac in various oils and surfactants

Etodolac is an interesting possibility for developing oil- and surfactant-based dosing vehicles suitable for oral administration of compounds, thus increasing their oral bioavailability. The aqueous solubility of etodolac, at about 0.20 mg/ml, is comparatively low; but its solubility in oily formulations is usually high. The solubilities of etodolac in various surfactants and oils are presented in Table 1. These components are soluble in each other and form homogenous liquids. Capryol 90, Labrasol, Labrafac WL 1349 and Lauroglycol 90 exhibited higher solubility than other vehicles. The oil Labrafac WL 1349, a medium-chain fatty acid glyceride, was selected for formulation development because it forms a distinct core in the interior of the surfactant aggregate and subsequently enhances intestinal absorption.[23] On the other hand, Labrasol (HLB = 14), which was chosen as the surfactant, exhibited the maximum solubility of etodolac at 147 ± 8.3 mg/ml (Table 1). Lauroglycol 90 (HLB = 4) and Capryol 90 (HLB = 6) were chosen as cosurfactants for the optimal SEDDS formulation.

Table 1 Solubility of etodolac in various vehicles

Vehicles	Solubility of etodolac (mg/ml), mean ± SD
Soyabean oil	45.8 ± 3.7
Peanut oil	67.3 ± 5.5
Oleic acid	102.3 ± 3.7
Labrafil M 2125	68.6 ± 3.2
Labrafac WL1349	97.3 ± 4.2
Transcutol P	121.6 ± 7.6
Labrasol	147.4 ± 8.3
Labrafil M 1944	78.5 ± 3.2
Lauroglycol 90	87.3 ± 3.8
Capryol 90	98.5 ± 4.3

Formulation and in-vitro assessment of the SEDDS

Drug loading per formulation was a critical design factor, dependent on etodolac solubility in the various formulations and the maximum volume reasonably encapsulated in a soft gelatin capsule. Twenty per cent w/w etodolac was readily soluble in each of the prepared formulations (Table 2).

Lipophilic surfactants with HLB < 10 (Capryol 90, Lauroglycol 90) are capable of promoting some emulsification of the oil, but the resulting emulsions are normally crude in terms of size; 500 nm show turbidity. Hydrophilic surfactants with HLB > 10 (Labrasol, Transcutol P), on the other hand, provide fine, uniform emulsion droplets.[24] Based on these considerations, a surfactant with high HLB value (Labrasol) and two low-HLB-value cosurfactants (Lauroglycol 90, Capryol 90) were selected. Accordingly, six formulations (Table 2) were developed for further characterization.

Emulsification assessment and droplet size analysis

The efficiency of self-emulsification was used to evaluate the formulations, with studies being conducted in an acidic medium (0.1 M HCl) and water to simulate the range of conditions encountered in the GI tract. Table 2 presents the result of the visual assessment and particle size determination of the different SEDDS formulations studied. The emulsification characteristics differed slightly between the different dissolution media, with better visual grading and smaller particle size values resulting when the formulations were dispersed in the acidic medium.

The droplet size analysis showed the quality of emulsion formed. It was observed that increasing the surfactant concentration (from 20 to 40% w/w) in SEDDS formulations decreased the mean droplet size of the emulsion formed (Figure 1). The smallest droplet size was observed when the surfactant concentration was at 40%, whether the cosurfactant used was Lauroglycol 90 or Capryol 90. Such a decrease in droplet size may be the result of more surfactant being available to stabilize the oil–water interface. Furthermore, the decrease in the droplet size reflects the formation of a better, close-packed film of the surfactant at the oil–water interface, thereby stabilizing the oil droplets.[25] A decrease in droplet size was observed with an increase in the surfactant/cosurfactant ratio (Table 2).

Table 2 Composition of SEDDS formulations

Vehicle	Composition (% w/w)					
	I	II	III	IV	V	VI
Etodolac	20	20	20	20	20	20
Labrafac WL 1349	30	30	30	30	30	30
Lauroglycol 90	25	20	10			
Capryol 90				25	20	10
Labrasol	25	30	40	25	30	40
S/CoS	1/1	1.5/1	4/1	1/1	1.5/1	4/1
Visual grading						
0.1 M HCl	C	B	A	B	B	A
Water	D	C	A	C	B	B
Dispersity index						
0.1 M HCl	0.51	0.34	0.13	0.47	0.29	0.15
Water	0.70	0.47	0.11	0.48	0.26	0.19
Mean particle size (nm)						
0.1 M HCl	330 ± 7.7	131 ± 2.1	52 ± 0.9	366 ± 6.7	232 ± 3.6	102 ± 3.2
Water	710 ± 4.3	206 ± 3.4	68 ± 1.3	556 ± 4.6	126 ± 4.8	107 ± 2.5

A, rapidly forming clear microemulsion; B, rapidly forming less clear emulsion; C, bright white milky emulsion; D, dull grayish white emulsion, with oily appearance of oily droplets. S/CoS, surfactant/cosurfactant. Mean ± SD, $n = 3$.

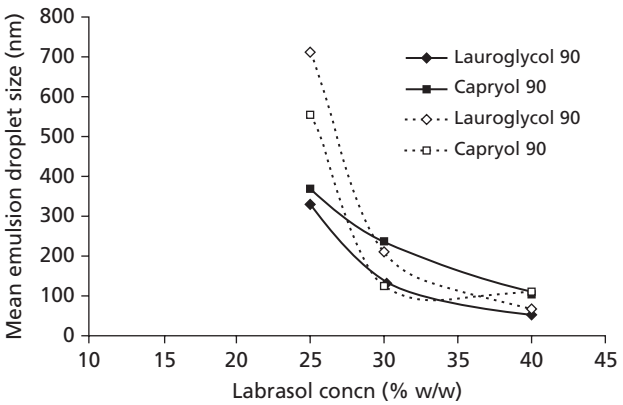

Figure 1 Effect of surfactant (Labrasol) concentration on mean emulsion droplet diameter. Water (- - - -), 0.1 M HCl (–).

In-vitro dissolution testing

Dissolution studies of etodolac SEDDS containing 20% etodolac and pure etodolac were conducted in media with different pH values. As seen in Figure 2a and b, pure etodolac shows pH-dependent and incomplete dissolution behaviour. Etodolac is a weakly acidic drug, having a pK_a of 4.65, and its solubility is known to increase rapidly with pH values above the pK_a of the drug.[26]

Etodolac dissolution at pH 1.2 was lower than the dissolution in water. After 60 min, cumulative etodolac released was 3% and 13% in simulated gastric fluid and water, respectively. On the other hand, the dissolution of etodolac from SEDDS formulations was not influenced by pH. Etodolac from SEDDS was completely and rapidly dissolved, regardless of the fluid condition; more than 90% of etodolac was released within 30 min in both of the dissolution media tested. The SEDDS formulations provided complete dissolution of the drug within 30 min in both of the dissolution media (Figure 2a,b).

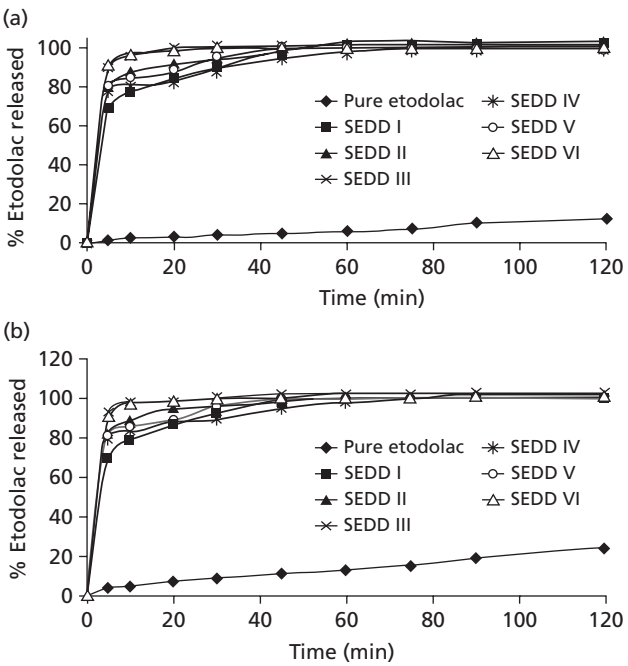

Figure 2 Dissolution profiles of pure etodolac and SEDDS formulations in different dissolution media. (a) 0.1 M HCl; (b) water. Each data point is the mean ± SD from three experiments ($n = 3$).

Assay validation

The HPLC chromatograms of plasma samples collected before and after the administration of etodolac showed no interference from endogenous compounds. The retention time of etodolac was 4.45 min. A weighted linear regression of the peak area ratio against concentration was performed for etodolac. Validation of the HPLC method for etodolac detection in plasma is summarized in Table 3. The observed

Table 3 Validation of HPLC technique for the analysis of etodolac in rabbit plasma

Sensitivity (μg/ml)	0.125
Linearity (μg/ml)	
Range (μg/ml)	0.5–25 μg/ml
Regression equation (n = 8)	Y = 3814.267X + 6124.912
Correlation coefficient (R^2)	0.9987
Recovery range (%)	93.7–100.2
Accuracy	
Recovery at low concentration	96.4
Recovery at high concentration	98.5
Precision	
Intra-day RSD (%), n = 4	0.9–4.6
Inter-day RSD (%), n = 8	1.9–5.0

RSD, relative standard deviation.

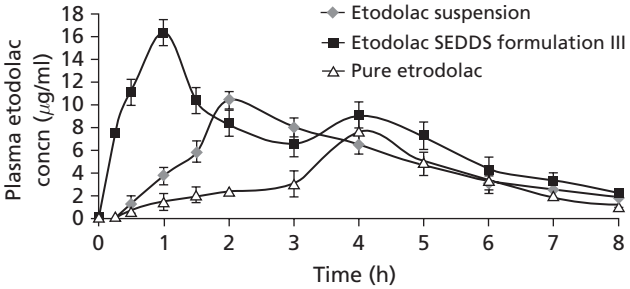

Figure 3 Mean plasma concentration–time profiles for different etodolac dosage formulations. From a single-dose of 50 mg/kg, in a three-way, crossover bioavailability study in rabbits. Each data point is the mean ± SD from six experiments (n = 6).

peak area ratios were linear over the concentration range of 0.5–25 μg/ml in rabbit plasma. The standard curves were fitted to a linear regression, $y = ax + b$, where y is the peak area ratio of etodolac to internal standard, a is the slope of calibration curve and b is the intercept (± SD, n = 6). Slope, intercept and R^2 were 3814.267 ± 132.45, 6124.912 ± 176.37 and 0.9987 ± 0.0032, respectively, for the six calibration curve plots. The recovery rate of etodolac in plasma was 96.0% ± 5.34. The inter-assay relative standard deviation (RSD) for the standards of the calibration curve (n = 8) ranged from 1.9 to 5.0%. The intra-assay RSD (n = 4) was between 0.9 and 4.6%. Using a signal/noise ratio of 4 : 1, the limit of quantification (LOQ) was measured as the lowest amount of analyte that can be reproducibly quantified above the baseline noise. A practical limit of quantification giving a good precision and acceptable accuracy was 0.125 μg/ml. The inter- and intraday accuracy (expressed as the percentage error of the determined concentration compared with the added concentration) did not exceed 5%.

In-vivo absorption study

In the current investigation, a SEDDS formulation of etodolac administered orally to rabbits was compared to the same dose of etodolac (50 mg/kg) in a suspension formulation containing 1% povidone as a suspending agent and also against pure etodolac powder. The mean plasma etodolac concentration versus time profiles of the three formulations are shown in Figure 3. Pure etodolac powder showed the lowest average plasma concentration. The peak plasma concentration (C_{max}) for etodolac powder was found to be 7.5 μg/ml and the time to attain peak concentration (t_{max}) was 4.2 h. Etodolac powder showed significantly lower plasma levels up to the first 2 h when compared with the etodolac SEDDS formulation ($P < 0.001$), at 2–4 h ($P < 0.05$). On the other hand, etodolac suspension showed significantly lower plasma levels at the first hour mark when compared with the etodolac SEDDS formulation ($P < 0.001$) after 1 h ($P < 0.01$) and 1.5 h ($P < 0.05$). Mean plasma concentrations were not significantly different among all three dosage forms from 4 h up to 8 h ($P > 0.05$).

Double peaks exist in the plasma concentration–time curves of etodolac from SEDDS formulation, one at 1 h and the other at 4 h. Several hypotheses based on region-dependent variation in absorption, significant enterohepatic recirculation, glucuronoid conjugates of etodolac and intestinal reconversion of metabolite have been proposed to account for this observation.[27] Yüksel *et al*. also observed double peaks and enhanced bioavailability from lipid-based formulations of piroxicam.[28] The authors suggested that a higher amount of drug becomes available for absorption due to rapid dissolution of the drug.

The mean etodolac pharmacokinetic parameters determined from the individual profiles after oral administration of the three formulations are summarized in Table 4. Peak concentration was reached in 1.3 h for the etodolac SEDDS formulation, while the equivalent figures were 4.0 and 2.4 h for the pure etodolac and the etodolac suspension, respectively, indicating a faster absorption of etodolac from the liquid dispersion. The mean t_{lag} of the SEDDS formulation

Table 4 Pharmacokinetic parameters after oral administration of etodolac formulation

Formulations	C_{max} (μg/ml)	t_{max} (h)	AUC_{0-8} (μg min/ml)	K_{el} (h)	$t_{1/2}$ (min)	MRT (h)	t_{lag} (min)	Absorbed in 1 h (%)
Pure etodolac	7.5 ± 0.5	4.2 ± 0.4	25.3 ± 0.9	0.4 ± 0.1	110.6 ± 2.4	5.3 ± 0.3	40.0 ± 6.6	7.6 ± 4.3
Etodolac suspension	10.6 ± 0.7[a]	2.4 ± 0.2[a]	40.3 ± 0.9[b]	0.4 ± 0.2	118..8 ± 1.4[f]	4.1 ± 0.4	32.0 ± 8.4	34.3 ± 2.8
Etodolac SEDDS	16.4 ± 1.1[b,d]	1.3 ± 0.2[c]	58.0 ± 1.5[b,e]	0.4 ± 0.1[f]	112.4 ± 1.2[f]	3.8 ± 0.5	4.0 ± 1.4	69.9 ± 5.5

Etodolac equivalent to 50 mg/kg. Mean ± SD, n = 6. C_{max}, maximum concentration; t_{max}, time to reach maximum concentration; AUC, area under curve; K_{el}, elimination rate constant; $t_{1/2}$, time for half concentration; t_{lag}, lag time; MRT, mean residence time. [a]$P < 0.05$, when compared with the parameter of pure powder by the ANOVA test, [b]$P < 0.001$ compared with the parameter of pure powder by the ANOVA test, [c]$P < 0.01$ compared with the parameter of pure powder by the ANOVA test, [d]$P < 0.05$ compared with the parameter of etodolac suspension by the ANOVA test, [e]$P < 0.01$ compared with the parameter of etodolac suspension by the ANOVA test, [f]$P > 0.05$ compared with the parameter of pure powder by the ANOVA test.

was 4.0 ± 1.4 min, which is significantly shorter than the value observed with pure etodolac and the etodolac suspension of 40.0 ± 6.6 and 32.0 ± 8.4 min, respectively ($P < 0.01$).

According to a statistical comparison of the mean pharmacokinetic parameters in Table 4, there are significant differences between the three products. The etodolac SEDDS formulation exhibited significantly higher plasma concentration (C_{max} = 16.4 μg/ml) and shorter time to reach peak concentration (t_{max} = 1.3 h) when compared to the other dosage forms. This result indicates that the lipophilic drug is present in solution or in small droplets of oil, leading to elimination of the dissolution step and the maintenance of the drug in a dissolved state during transport to the unstirred water layer of the intestinal membrane.[29] The presence of the surfactant (Labrasol, HLB = 14) may affect the bioavailability of the drug in the SEDDS.[30] Furthermore, the liquid SEDDS is likely to be type III in the lipid formulation classification system.[31] In fact, the main advantage of lipid formulation is the maintenance of the drug in solution throughout its period in the GI tract.[32] In this study, the SEDDS formulation can form a fine oil-in-water microemulsion in the GI tract where the drug is presented in small droplets of oil.

In order to demonstrate a faster absorption of the etodolac SEDDS formulation, particularly during the first hour after administration, the percentage of etodolac absorbed after 1 h was estimated (Table 4, Figure 4). A statistically significant difference was revealed for the percentage of etodolac absorbed. The percentages absorbed from the etodolac SEDDS formulation were two and eight times higher than those observed with the etodolac suspension and pure etodolac, respectively. Figure 4 presents a plot of the percentage of etodolac unabsorbed versus time after oral administration of the three formulations. A linear relationship can be observed ($R^2 > 0.987$) between logarithm unabsorbed values and time on rectilinear coordinates, which suggests apparent first-order absorption. The apparent first-order absorption rate constants (k_1) were estimated from the slope of the curves multiplied by 2.303, and were found to be 0.311 ± 0.045, 1.381 ± 0.132 and 1.699 ± 1.10 per hour for pure etodolac, etodolac suspension and the etodolac SEDDS formulation, respectively. These results suggest that the absorption rate of the drug from the SEDDS formulation is faster than that from the other formulations.

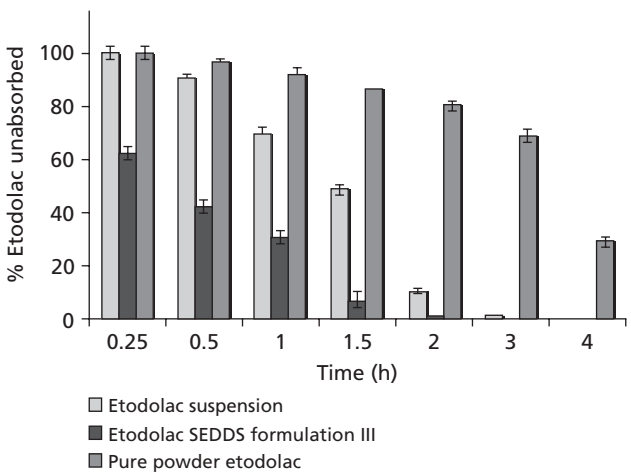

Figure 4 Percentage etodolac unabsorbed versus time for different etodolac dosage forms. Time after oral administration of 50 mg/kg. Each data point is the mean ± SD from six experiments (n = 6).

Anti-inflammatory effect of etodolac formulations

Induction of acute inflammation in control rats resulted in a prominent increase in paw thickness, beginning 1 h after intraplantar injection of carrageenan and reaching a peak of inflammation after 4 h. Administration of the etodolac SEDDS formulation containing 20 mg/kg of etodolac significantly ($P < 0.05$) suppressed the maximal oedema responses attained over 7 h, from 80 to 27% (Figure 5). A dose of 20 mg/kg of etodolac suspension in water produced little anti-inflammatory effect. The pronounced anti-inflammatory effect of the etodolac dispersion was attributed to an increased availability due to enhanced gastrointestinal (GI) absorption of the drug as a result of its improved dissolution rate, and accumulation of this colloidal dispersion at the oedematous tissue because of increased vascular permeability. The control group of rats showed almost the same results as that of the group administered drug-free lipid dispersion. These results were in agreement with the results of Srinath and Diwan,[33] who compared anti-inflammatory activity of lipid-based indometacin formulations with free indometacin.

Conclusions

The results of this study demonstrate the importance of enhancing dissolution of class II drugs (with high permeability and low solubility), thus increasing their in-vivo absorption. SEDDS may be a promising approach for the rapid onset and the effective absorption by oral administration of etodolac and could increase bioavailability for other poorly water-soluble drugs. The spontaneous formation of an emulsion on drug release in the GI tract advantageously presents the drug in a

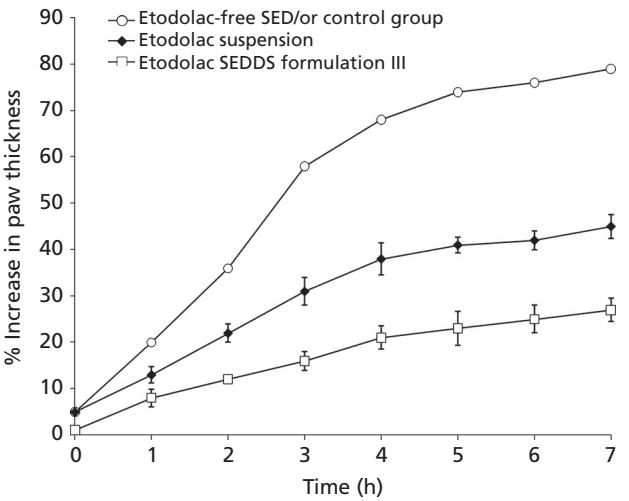

Figure 5 Effect of etodolac formulation on carrageenan-induced paw oedema. Dose of 20 mg/kg administered to rats. Each data point is the mean ± SD from three experiments.

solubilized form, and the small droplet size provides a large interfacial surface area for drug absorption. This new formulation, SEDDS, could be advantageous with regard to a rapid onset of action, especially in various painful conditions where an acute analgesic effect is desired. Liquid SEDDS formulations of etodolac in soft gelatin capsules also have the advantage of pH-independent rapid dissolution of the drug, ease of preparation and use of edible and non-toxic excipients.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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