JPP
Journal of
Pharmacy and Pharmacology

JPP 2010, 62: 25–34

Received July 6, 2009
Accepted October 5, 2009
DOI 10.1211/jpp/62.01.0002
ISSN 0022-3573

Research Paper

Ketorolac trometamol topical formulations: release behaviour, physical characterization, skin permeation, efficacy and gastric safety

Doaa Ahmed El-Setouhy[a] and Sahar Mohy Ahmed El-Ashmony[b]

[a]Department of Pharmaceutics, Faculty of Pharmacy and [b]Department of Clinical Pharmacology, Faculty of Medicine, Cairo University, Cairo, Egypt

Abstract

Objectives The objective of this study was to improve systemic delivery of the highly analgesic ketorolac trometamol (ketorolac tromethamine) via the transdermal route, through cost-effective topical formulations, to avoid most of the problems associated with ketorolac trometamol therapy.

Methods In-vitro release behaviour of the drug from different microemulsion and emulgel formulations was evaluated. E2 emulgel (based on isopropyl myristate as penetration enhancer) and E7 emulgel (based on Brij 92 as penetration enhancer) were evaluated for their physical properties, rat skin permeation, in-vivo analgesic effect (hot-plate test and the paw pressure test), acute and chronic anti-inflammatory activity and gastric safety.

Key findings Isopropyl myristate and the synergistic effect of the two known penetration enhancers (propylene glycol and Brij 92) significantly modulated drug permeation and may be a promising approach for the transdermal delivery of ketorolac trometamol and other drugs. Selected in-vivo tested formulae (E2 and E7) caused significantly less ulcer score and less gastric erosion compared with oral ketorolac trometamol. E7 showed significantly higher analgesic and anti-inflammatory activity compared with E2 with no significant difference compared with oral ketorolac trometamol.

Conclusions The developed ketorolac trometamol E7 emulgel appeared promising for dermal and transdermal delivery of ketorolac trometamol, which would circumvent most of the problems associated with drug therapy.

Keywords analgesic; anti-inflammatory activity; ketorolac trometamol; transdermal delivery

Introduction

Ketorolac trometamol (ketorolac tromethamine) is one of the most potent non-steroidal anti-inflammatory drugs (NSAIDs) that is known to have potent analgesic and moderate anti-inflammatory effect. It is structurally and pharmacologically related to tolmetin and indometacin. Clinical studies have shown that a single dose of ketorolac is more effective than that of morphine, pethidine (meperidine) and pentazocine in severe to moderate postoperative pain.[1] It has been found effective in the treatment of trauma-related pain as well as pain associated with cancer.[2–4] Unlike narcotic analgesics, it has the advantage that it does not depress the respiratory and the central nervous system. It has no addiction potential associated with narcotic analgesics and hence it exhibits a more favourable safety profile.[5] The drug is currently administered intramuscularly and orally in a frequent dosing regimen (due to its short biological half-life; approximately 4–6 h) for the short-term management of postoperative pain. However, an injection is an invasive drug therapy, frequent dosing is inconvenient to the patient; and in addition ketorolac trometamol can cause gastrointestinal complaints associated with all NSAIDs such as gastrointestinal bleeding, perforation and peptic ulceration.[5,6]

Transdermal drug delivery appears to be an attractive noninvasive mode of drug delivery; it maintains drug blood levels for an extended period of time, eliminating a frequent dosing regimen, and minimizes gastrointestinal side effects.[7] However, the low permeability of the skin due to the barrier properties of the stratum corneum limits

Correspondence: Doaa Ahmed El-Setouhy, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt.
E-mail: doaaahmed@hotmail.com

the number of drugs that can be administered transdermally. Several approaches have been made to enhance percutaneous absorption of ketorolac trometamol, such as use of permeation enhancers, use of proniosomes, complexation with cyclodextrin, synthesis of a prodrug, iontophoresis and ultrasound.[7–12]

The aim of this study was to formulate ketorolac trometamol into different easily prepared transdermal formulations (utilizing relatively inexpensive readily available excipients and permeation enhancers) to facilitate its delivery to the blood circulation, depending primarily on their enhancing composition and without the aid of special instrumentation that is required for physical modalities (i.e. iontophoresis and phonophoresis) to enhance transdermal drug permeation. We tried to optimize ketorolac trometamol transdermal formulations with respect to macroscopic and microscopic behaviour, and in-vitro skin permeation. We assessed the efficacy and the gastric safety of selected formulations and compared them with those of a standard drug used systemically (orally).

Materials and Methods

Chemicals

Ketorolac trometamol was a gift from Amryia Co for Pharmaceutical Industries (Alexandria, Egypt). Sorbitan monooleate (Span 80), Pluronic F127, polyoxyethylene-2-oleyl ether (Brij 92) and isopropyl myristate were from Sigma Chemical Co (St Louis, MO, USA). carbomer 934p was from Goodrich Chemical Co (Cleveland, OH, USA). Hypromellose (hydroxypropyl methylcellulose 2910, 4000 cp) was from Tama (Tokyo, Japan). Polyoxyethylene sorbitan monoleate 80 (Tween 80), glycerin, propylene glycol, oleic acid, acetic acid and dextran 5% w/v BP 98 were from El-Nasr Co for Pharmaceutical Industries (Cairo, Egypt). Isopropyl palmitate, transcutol, acetonitrile HPLC grade were from Merk Co (Hohenbrunn, Germany). Spectra/Por dialysis membrane, molecular weight cut-off 12 000–14 000 was from Spectrum Labs. Inc. (Rancho Domingues, CA, USA). Formalin (38–40%) was from Abou Za'able Co for Insecticides and Industrial Detergents (Cairo, Egypt). Ketolac tablets were from Amriya Co for Pharmaceutical Industries (Alexandria, Egypt).

Construction of pseudo ternary phase diagram

Mixtures of oil, surfactant (Tween 80) and cosurfactant (propylene glycol, transcutol or glycerin) at the desired weight ratios (3 : 1, 2 : 1, 1 : 1) of surfactant to cosurfactant respectively were prepared. The ratio of oil to the mixture of surfactant and cosurfactant was varied from 9 : 1 to 1 : 9. The amount of water added was varied to give water concentrations in the range of 0–90% by weight at 10% intervals.[13] The systems were marked as being optically clear or turbid and as fluids or gels. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after tilting to an angle of 90°. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous-type microemulsions.

The microemulsion formulations were selected from 3 : 1 phase diagram at different component ratios as described in Table 1. Microemulsions containing glycerin as cosurfactant were excluded because they were gels with a gelatin-like appearance (these do not spread on the skin easily).

Microemulsions with oil-rich compositions were selected based on the assumption that they would be most compatible with sebum and would therefore be effective in facilitating transfollicular transport of hydrophilic drugs.[14] The effects of the content and type of oil and type of cosurfactant were evaluated.

Preparation of ketorolac trometamol emulgel and gel formulations

The composition of ketorolac trometamol emulgels and gels is shown in Table 1. The emulsifiers used were 2.5% hypromellose or a combination of surfactant emulsifier

Table 1 Composition of different ketorolac trometamol topical formulations, and viscosity of gel and emulgel formulations

Component	Composition wt% microemulsion (M) formulations							Component	Composition wt% gel and emulgel (E) formulations						
	M1	M2	M3	M4	M5	M6	M7		E1	E2	E3	E4	E5	E6	E7
Isopropyl myristate	24	24						Isopropyl myristate	10	5	10	5			
Isopropyl palmitate						24	24	Hypromellose	2.5	2.5			2.5		0.5
Oleic acid			24	12	24			Carbomer 934p			1	1		1	
Tween 80	42	42	42	36	42	42	42	Pluronic F127							20
Transcutol		14		12	14	14		Tween 80			2	2			
Propylene glycol	14		14				14	Span 80			0.5	0.5			
Glycerin								Brij 92							5
Water	20	20	20	40	20	20	20	Propylene glycol	5	5	5	5	5	5	30
								Methyl hydroxybenzoate	0.03	0.03	0.03	0.03	0.03	0.03	0.03
								Propyl hydroxybenzoate	0.01	0.01	0.01	0.01	0.01	0.01	0.01
								Purified water to	100	100	100	100	100	100	100
									Viscosity ($\times 10^3$ cps)						
									280	332	2250	2400	196	580	3980

The final concentration of ketorolac trometamol in all formulations was 3% w/w.

(blend of Tween 80/Span 80 at ratio 4 : 1) stabilized by polymeric thickener (1% carbomer 934). The aqueous phase (containing 3% w/w ketorolac trometamol) was prepared by dispersing hypromellose in purified water at 80°C or carbomer 934p in purified water into which a blend of Tween 80 / Span 80 was dissolved. The pH was neutralized to 6–6.5 using triethanolamine. The gels were stored at 4°C for 24 h before the addition of the oil phase. The oil phase was then added to the gels and the mixture was homogenized for 3 min at 25 000 rev/min using a Diax 900 homogenizer (Heidolph Instruments GMDH & Co., Kelheim, Germany).

The ketorolac trometamol gels were prepared by dispersing the hypromellose in purified water at 80°C or carbomer 934p into purified water followed by neutralization to pH 6–6.5 using triethanolamine. Ketorolac trometamol (3% w/w) was dissolved in water before dispersion of the polymer. Methyl and propyl hydroxybenzoate (parabens) were dissolved in propylene glycol which was mixed with the aqueous phase (for emulgels) or purified water (for gels).

The cold technique was used for preparation of Pluronic emulgel. Pluronic F127 was added into cold water containing 3 g ketorolac trometamol with gentle stirring, and the solution was left overnight in a refrigerator to complete polymer dissolution. Hypromellose was added as an emulsifying agent to stabilize the gel system. Brij 92, dissolved in propylene glycol, was added and the mixture was homogenized for 3 min at 25 000 rev/min using the Diax 900 homogenizer.

In-vitro release study

The dissolution of different formulations was performed using USP basket type dissolution apparatus (Pharmatest, PTW, Type I, Hainburg, Germany), modified by the addition of two open-sided glass tubes covered at one end with synthetic membrane.[15] The dissolution medium was 900 ml Sørensen's phosphate buffer pH 7.4 which was agitated at 100 rev/min and maintained at 37 ± 0.5°C. Samples of 5 ml were withdrawn at predetermined time intervals over 4.5 h. The drug content was analysed spectrophotometrically at 323 nm. Data were analysed using the following equation:

$$\frac{M_t}{M_\infty} = k\sqrt{t} \qquad (1)$$

where M_t / M_∞ is the drug fraction released at time t and k is a constant depending on the geometric and structural characteristics of the system.[16] The effective diffusion coefficient of each formulation was calculated from the slope of cumulative amount of drug released versus square root of time plot.

Ex-vivo skin permeation study of selected formulae

Newborn albino rats (2–3 days old) were killed by cervical dislocation and full thickness skin was excised.[17] The dermal surface was carefully cleaned to remove subcutaneous tissue without damaging the epidermal surface. The study was carried out using a double open-sided glass tube covered at one end with rat skin sealed with a rubber band, and the other end was attached to a dissolution apparatus shaft with the aid of sticking tape. The tube was held under the surface of the receptor medium (50 ml phosphate buffer, pH 7.4, agitated by a magnetic stirrer at 300 rev/min and maintained at 37°C) in a 100-ml beaker. Such assembly has been validated in a previous study.[18] The available skin permeation area was 3.14 cm^2. The glass tube was filled with the test formula (1 g) which was spread on the dorsal side of the rat skin. Samples (2 ml) were withdrawn at predetermined time intervals and compensated immediately with fresh receptor medium over 24 h. The drug content in the withdrawn samples was analysed by a slight modification of a previously reported HPLC method.[19] Dilution corrections were made in calculating cumulative amount permeated using the equation:

$$Q = V_r Cn + V_s \Sigma Cm \qquad (2)$$

where Q is the current cumulative mass of drug transported across skin at time t, C_n represents the drug concentration of the receptor medium at each sampling time and ΣC_m represents the sum total of previously measured concentrations ($m = 1 - n-1$). V_r is the receptor medium volume and V_s is the sample volume.[20]

The cumulative amounts of ketorolac trometamol permeated per unit area of rat skin were plotted as a function of time. The steady-state flux (J_{ss}) was calculated from the slope of the linear portion of the cumulative amount permeated through rat skin per unit area versus time plot.[21] Permeability coefficient K_p was calculated according to following equation:

$$K_p = J_{ss}/C_o \qquad (3)$$

where C_o is the drug concentration in the donor solution (3×10^4 μg). E2, E5 and E7 were selected for this study.

Physical characterization of ketorolac trometamol formulations

The viscosity and pH of the prepared gel and emulgel formulations were measured at room temperature. Formulae E2 and E7 were observed directly and three months after preparation for macroscopic (consistency, colour, homogeneity, and creaming or phase separation) and microscopic properties (particle size; Leica Microsystems Imaging Solutions Ltd, UK).

Assessment of the analgesic and anti-inflammatory effects

The experiments were conducted according to the Guidelines for Animal Care and Treatment of the European Community. The protocol of this study was reviewed by the Research Ethics Committee (REC) of the Pharmacology Department affiliated to the Faculty of Medicine, Cairo University.

Animals used and experimental design

This study included 96 adult male albino rats (180–200 g) obtained from the animal house of the Institute of Ophthalmological Research, Giza, Egypt. The rats were housed individually and light was maintained on a 12-h light/dark cycle. The animals were fed a standard laboratory diet and water was freely available. They were acclimatized for one week and randomly allocated in the following groups.

Group A (n = 48)

This group was used to assess the analgesic and acute anti-inflammatory effects of the E2 and E7 formulae and to compare them with commercial oral ketorolac tablets. Animals were divided into six groups (eight rats each): Group A_{cont}; this group received distilled water (by oral gavage) in the same amount given for oral drug therapy on the first and on the second day of the experiment. Group A_{oral}; this group received oral ketorolac trometamol 3.2 mg/kg (by oral gavage).[22] Group $A_{E2\ paw}$; in this group E2 was applied to the plantar region of the left hind paw in a dose equivalent to that used orally. Group $A_{E7\ paw}$; in this group E7 was applied to the plantar region of the left hind paw in a dose equivalent to that used orally. Group $A_{E2\ back}$; in this group E2 was applied to the shaved back (3 × 4 cm area was shaved one day before the experiment) in a dose equivalent to that used orally. Group $A_{E7\ back}$; in this group E7 was applied to the shaved back (as the previous group) in a dose equivalent to that used orally.

On the first day of the experiment all groups were treated once as previously mentioned. Thirty minutes after treatment, groups $A_{cont,}$ A_{oral}, $A_{E2\ back,}$ and $A_{E7\ back}$ were subjected to the hot plate test as previously described by Wiesenfeld-Hallin *et al*.[23] using a Ugo-Basile model hot plate, no: 32097 (Varese, Italy). The time for jumping latency was estimated at 15, 30, 45 and 60 min. The rats were left to rest for 15 min after which the paw pressure test was performed on the same four groups as described by Randall and Selitto.[24] The paw pressure test was based on the use of mechanical stimuli (analgesimeter: Ugo-Basile model no: 21025 COMERIO, Varese, Italy) where the weight (g) required to elicit nociceptive responses such as paw flexion (reflex withdrawal of the paw) was measured.

On the following day all six study groups were re-treated with a single dose of ketorolac trometamol (oral, E2 and E7) as previously mentioned. Thirty minutes following treatment, rats were injected with dextran (4% w/v) 0.1 ml into the subplantar region of the left hind paw for induction of acute oedema.[25] Paw volume was measured immediately and 1, 2, 3 and 6 h after dextran injection using a digital plethysmometer (model no.L.E 7500; Ugo-Basile, Varese, Italy).

Oral ketorolac trometamol and the topical formulae E2 and E7 were freshly prepared daily before use.

Group B (n = 48)

This group was used to assess the chronic anti-inflammatory effects of E2 and E7 formulae and to compare it with commercial oral ketorolac. Animals were divided into six groups (eight rats each). Group B_{cont}; this group received daily distilled water in the same amount given for oral drug therapy. The other five groups were B_{oral}, $B_{E2\ paw}$, $B_{E7\ paw}$, $B_{E2\ back}$ and $B_{E7\ back}$.

Oral ketorolac trometamol and the emulgel formulae (E2 & E7) were given to the B groups (in the same doses as group A) once per day for the duration of the experiment (11 days). On the first and the third days of therapy, animals in the six B groups were injected with 0.1 ml formaldehyde solution (2% w/v) into the plantar region of the left hind paw as a model of chronic arthritis.[26] Formaldehyde injection was performed 30 min after drug dosing. The mean increase in the paw volume of each treated group was measured at day 3, 5, 7, 9 and 11 using a digital plethysmometer:

% inhibition of paw oedema in rats at any given time (T time) = ((mean paw volume of control group − mean paw volume of treated group) / mean paw volume of control group) × 100.

All animals were killed at the end of a specified period using diethyl ether and their stomachs were excised for histopathological examinations. The effective skin areas on the back where the drug was applied in group $B_{E2\ back}$ and group $B_{E7\ back}$ were excised for skin drug content analysis.

Histopathological examination

Gross examination of stomach: scoring of gastric lesions

Stomachs were opened by an incision along the greater curvature; the lesions were examined by a (× 3) magnifying lens and accurately measured. Each haemorrhagic lesion was measured along its greatest length. The individual lengths were summed to obtain a total lesion length in each animal and expressed in millimetres. Five petechiae were taken as equivalent to a 1-mm ulcer.[27]

Microscopic examination of stomach

Sections of the stomach were stained with routine haematoxylin and eosin (H&E) stains and examined microscopically. The results were recorded and tabulated according to the following grading system.[28] Grade 0: normal gastric mucosal cells appeared intact with normal shape and location; grade 1 ulcer: erosion of the superficial epithelial layer; grade 2 ulcer: erosion of the superficial layer and less than one third the thickness of the mucosa; grade 3 ulcer: erosion or necrosis of more than one third of the mucosal surface.

Statistical analysis

The differences in the results of in-vitro release and ex-vivo skin permeation studies were evaluated using one-way analysis of variance (to test the significant effect of different formulations on the obtained data) followed by post hoc analysis (LSD) for significance at $P < 0.05$ (for pair-wise comparison of any two formulations) using the software SPSS (SPSS Inc., Chicago, USA). Independent sample *t*-test was used for comparative study concerning particle size of E2 and E7 at 0.05 level of significance.

For assessment of the analgesic, acute and chronic anti-inflammatory effects, the mean and the standard deviation were used as suitable statistical parameters to summarize the data. The differences among the mean values of: jumping latency at different time interval, weights in grams (paw pressure test), paw volume after dextran injection, paw volume after formaldehyde injection, and ulcer score were tested using one-way analysis of variance with multiple comparisons using post hoc Bonferroni test at $P \leq 0.05$. The f-statistical results of the above mentioned test rejected the null hypothesis (no chance difference between groups). The Kruskal-Wallis test was used to detect significant differences among percentage values related to grades of gastric erosion. A P value less than 0.05 was considered statistically significant.

Results

In-vitro release study

Spectra/Por dialysis membrane was used for the study; these membranes usually have a porous substructure made of a mixed hydrophobic/hydrophilic matrix, which makes it similar to skin with regard to affinity for certain molecules.[29] A linear relationship between cumulative amounts of drug released from different microemulsion, emulgel, and gel formulations and square root of time was obtained ($r \geq 0.97$). These results indicated that the release of ketorolac trometamol from different formulations followed the Higuchi diffusion model, and the rate controlling step in the release process was the diffusion of the dissolved drug through the vehicle.[30] Statistical analysis of data revealed that different formulations significantly affected the diffusion coefficient. The ranking of the diffusion coefficients (Table 2) of the microemulsion formulations was: M2 > M6 ≈ M7 > M1 > M3 ≈ M4 ≈ M5.

With the gel and emulgel formulations, the values of the diffusion coefficients calculated from the entire dissolution profiles did not correlate well with the amount of drug released. The obtained diffusion coefficient (from the entire dissolution profiles) for E1 was close to that obtained for E4 and smaller than those of E5 and E6. However, data calculated from the initial segment of the dissolution curves (after 60 min) were better indicators of release behaviour of the investigated preparations.

The ranking of diffusion coefficients (Table 2) from the gel and emulgel formulations was: E2 ≈ E1 > E5 > E6 > E4 > E3 > E7.

In general, it was observed that the release of the drug from its gels and emulgels was higher than its release from the microemulsion formulations (Table 2). E2 showed the highest diffusion coefficient among all the tested ketorolac trometamol topical formulations. Therefore, it was chosen along with E7 (which showed a lower diffusion rate value) and E5, which gave comparable drug release to E2 (E5 is based on hypromellose polymer as E2 but it was free from the penetration enhancer isopropyl myristate) for the skin permeation study to correlate between the in-vitro release and skin permeation study.

Emulgels were chosen since they resumed the favourable characteristics of both oil-in-water (O/W) emulsions (in term of viscosity, consistency and drug release) and those of hydrophilic gels (manageability).[31]

Characterization of E2 and E7 emulgel formulae

The organoleptic properties of the formulations varied depending on their composition. Taste was not determined as these formulae were intended for topical use. All freshly prepared formulations had a pleasant, smooth texture and appearance. They rubbed in quickly and gave a sensation of freshness and immediate hydration. E2 was a yellowish white soft homogeneous cream whilst E7 was a transparent homogeneous solid-like gel. Hypromellose gel (E5) was a transparent yellow soft gel.

All tested formulations did not show any changes in appearance, homogeneity or consistency after three months of storage at room temperature. Only E2 showed a slight creaming towards the end of the storage period. The pH of the selected formulations was between 6 and 7 and remained stable for the entire storage period.

The particle size of the freshly prepared selected emulgels was 2.72 ± 0.8 (E2) and 2.46 ± 0.91 μm (E7). The microscopic analysis was repeated three months after preparation. Droplet size remained almost constant with time, showing only a slight increase in size (3.19 ± 0.93 and 2.94 ± 0.46 μm for E2 and E7, respectively), which was not significant.

Ex-vivo skin permeation study of selected formulae

Ketorolac trometamol flux from hypromellose gel (E5) reached an apparently constant value after nearly six hours. For E2 and E7 the flux continuously increased throughout the experiment (Figure 1, Table 2). E7 showed a significantly higher flux value compared with E2 and E5.

Based on the above results E7 (highest flux value) and E2 (significantly lower but seemed to be with acceptable flux value) were chosen for the subsequent in-vivo study in a rat

Table 2 Diffusion properties of the different ketorolac trometamol topical formulations

Properties	Formula						
	E1	E2	E3	E4	E5	E6	E7
Diffusion coefficient ($\times 10^3$, cm^2/h)	17.290 ± 0.46	18.868 ± 0.79	12.778 ± 0.8	13.124 ± 0.59	16.463 ± 0.75	14.725 ± 0.032	6.324 ± 0.37
J_{ss} ($\mu g/cm^2$ h)		92.62 ± 0.87			6.23 ± 0.57		99.158 ± 2.6
K_p (cm/h)		3.1×10^{-3}			2.1×10^{-4}		3.3×10^{-3}
Skin content ($\mu g/g$)		5.99 ± 0.26					2.99 ± 0.41
	M1	M2	M3	M4	M5	M6	M7
Diffusion coefficient ($\times 10^3$, cm^2/h)	3.956 ± 0.091	7.071 ± 0.35	3.014 ± 0.47	2.76 ± 0.098	3.333 ± 0.24	5.946 ± 0.53	5.478 ± 0.035

Diffusion coefficient was through synthetic membrane for all prepared ketorolac trometamol topical formulations. Maximum flux (across excised rat skin; J_{ss}), permeability coefficient (K_p) and skin content were measured for selected formulae only.

paw oedema model, and to compare the effects of E2, E7 and oral ketorolac trometamol on gastric mucosa.

The hot plate test (Table 3)

The ketorolac trometamol E2 and E7 emulgel formulae applied on the back of rats significantly ($P < 0.05$) increased the mean jumping latency at all time intervals compared with the group A_{cont} without treatment. The peak effect was observed 30 min after drug use. There was no significant difference ($P > 0.05$) observed between oral ketorolac trometamol and E7 applied on the back. Meanwhile, the antinociceptive effect in group $A_{E2\ back}$ was significantly less than that in groups A_{oral} and $A_{E7\ back}$ ($P < 0.05$).

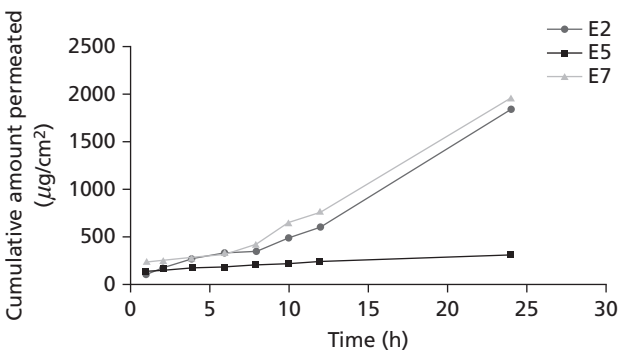

Figure 1 Skin permeation profiles of ketorolac trometamol through rat skin from selected formulae. Plotted results are mean ± SD of three determinations.

The mechanical noxious stimulus (paw pressure test)

E2 and E7 applied on the back of rats significantly ($P < 0.05$) increased the mean threshold for mechanical nociceptive stimuli versus the control group. The mean weights were 201.9 ± 6.9, 402 ± 7.8, 338 ± 7.1 and 400.6 ± 6.4 g for groups A_{cont}, A_{oral}, $A_{E2\ back}$ and $A_{E7\ back}$, respectively. There was no significant difference observed between E7 and the oral ketorolac trometamol ($P > 0.05$). Analgesic effect of E2 was significantly less than that of oral ketorolac trometamol and the E7 formula ($P < 0.05$).

Dextran-induced inflammation (Table 4)

Injection of dextran into the footpad of group A_{cont} resulted in an increase in the mean paw volume over the following six hours. Prior topical use of E2 and E7 on the rat paw (groups $A_{E2\ paw}$ and $A_{E7\ paw}$) and on the rat back (groups $A_{E2\ back}$ and $A_{E7\ back}$) significantly attenuated the increase in the paw volume observed in group A_{cont} ($P < 0.05$). Regarding the two emulgel formulae, the effect was significantly more in E7 vs E2 ($P < 0.05$). Furthermore, the attenuation showed by E7 used on the paw was higher than that of the oral ketorolac. However, the difference between the two groups was not significant ($P > 0.05$). Percentage inhibition was 57.7, 28.6, 58.9, 23.2 and 46.4% in the groups A_{oral}, $A_{E2\ paw}$, $A_{E7\ paw}$, $A_{E2\ back}$ and $A_{E7\ back}$ on the third hour, respectively. Formulations applied at site of inflammation (paw) showed significantly higher activity compared with those applied away from the inflammation site (back) ($P < 0.05$). The

Table 3 Time course of the analgesic activity of oral ketorolac trometamol and ketorolac trometamol emulgel formulae

Study group	Mean jumping latency (s) ± SD at different time intervals			
	15 min	30 min	45 min	60 min
Group A_{cont}	18.1 ± 2.4	17.6 ± 1.7	17.6 ± 1.3	18.2 ± 3.1
Group A_{oral}	55.2 ± 4.1*	55.8 ± 4.4*	53.1 ± 5.4*	47.2 ± 4.8*
Group $A_{E2\ back}$	40.7 ± 2.5*,†	41.5 ± 3.3*,†	40.3 ± 3.3*,†	38.8 ± 2.8*,†
Group $A_{E7\ back}$	56.3 ± 4.6*	55.3 ± 4.7*	50.8 ± 3.4*	49.6 ± 4.1*

Emugels E2 and E7 were applied on the rat back. The hot-plate test was used for the evaluation. $^{*}P < 0.05$ significantly different from control nontreated group. $^{\dagger}P < 0.05$ significantly different from group A_{oral} and group $A_{E7\ back}$. $n = 8$ in each group.

Table 4 The effects of oral ketorolac trometamol and ketorolac trometamol emulgel formulae on dextran-induced paw oedema in rats

Study group	Mean paw volume ± SD at different time intervals			
	1 h	2 h	3 h	6 h
Group A_{cont}	0.85 ± 0.02	1.0 ± 0.02	1.68 ± 0.03	1.09 ± 0.03
Group A_{oral}	0.57 ± 0.05*	0.63 ± 0.02*	0.71 ± 0.02*	0.85 ± 0.02*
Group $A_{E2\ paw}$	0.72 ± 0.02*,†	0.86 ± 0.05*,†	1.2 ± 0.02*,†	0.98 ± 0.02†,*
Group $A_{E7\ paw}$	0.55 ± 0.02*,‡	0.62 ± 0.02*,‡	0.69 ± 0.02*,‡	0.83 ± 0.05*,‡
Group $A_{E2\ back}$	0.77 ± 0.02*,†,‡,§	0.89 ± 0.05*,†,‡,§	1.29 ± 0.05*,†,‡,§	1.01 ± 0.02*,†,§
Group $A_{E7\ back}$	0.65 ± 0.02*,†,‡,§	0.75 ± 0.05*,†,‡,§	0.90 ± 0.05*,†,‡,§	0.90 ± 0.05*,†,‡,§

Emugels E2 and E7 were applied on the rat paw and the rat back. $^{*}P < 0.05$ significantly different from control nontreated group. $^{\dagger}P < 0.05$ significantly different from group A_{oral}. $^{\ddagger}P < 0.05$ significantly different from group $A_{E2\ paw}$. $^{\S}P < 0.05$ significantly different from group $A_{E7\ paw}$. $n = 8$ in each group.

difference was not significant between group $A_{E2\ paw}$ and group $A_{E2\ back}$ in the reading taken at six hours ($P > 0.05$).

Formaldehyde-induced arthritis in rats (Table 5)

The pretreatment with E2 or E7 on the rat paw and on the rat back produced a significantly inhibitory effect on developing paw oedema from day 3 to 11 of the experiment ($P < 0.05$). The percentage inhibition was 51.4, 28.6, 53.5, 17.2 and 38.4 in the groups B_{oral}, $B_{E2\ paw}$, $B_{E7\ paw}$, $B_{E2\ back}$ and $B_{E7\ back}$ at day 5, respectively. The inhibitory effect of E7 was significantly more compared with that of E2 ($P < 0.05$). The observed inhibitory effect (from day 3 to day 11 of the experiment) of the E7 formula was slightly more than that of oral ketorolac. This difference was significant ($P < 0.05$). Also, the inhibitory effects of both emulgel formulae were significantly higher when applied on the paw compared with the back of rats ($P < 0.05$).

Gross examination of stomach (Figure 2)

In some sporadic cases, rats from the control group and from all the emulgel-treated groups showed minimal petechiae in the mucosa with no significant difference in their mean ulcer scores ($P > 0.05$). The ulcer scores (mean in mm ± SD) in the groups B_{cont}, $B_{E2\ paw}$, $B_{E7\ paw}$, $B_{E2\ back}$, and $B_{E7\ back}$ were 0.5 ± 0.3, 0.7 ± 0.4, 0.6 ± 0.5, 0.9 ± 0.3 and 0.8 ± 0.4, respectively. All rats treated orally with ketorolac showed variable numbers of petechiae and/or haemorrhagic linear lesions. The mean ulcer score of group B_{oral} (18.7 ± 2.6) was significantly higher than those of the group B_{cont} and the emulgel-treated groups ($P < 0.05$).

Histopathological examination of stomach (Figure 3)

Microscopic examination of gastric specimens revealed that all rats of the control group showed intact gastric mucosa. Both ketorolac trometamol emulgel formulae caused only sporadic cases of grade 1 gastric erosion (12.5% for groups $B_{E2\ paw}$, $B_{E7\ paw}$ and $B_{E2\ back}$, and 25% for group $B_{E7\ back}$) with no significant difference from control group values ($P > 0.05$). The rats treated orally with ketorolac showed grade 1, 2 and 3 gastric erosions in variable percentages (100, 50 and 25%, respectively).

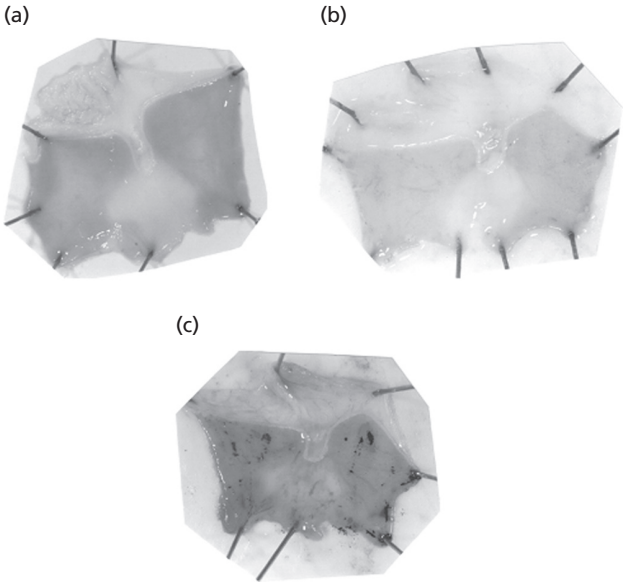

Figure 2 Naked eye pictures of rat stomach. (a) Normal gastric mucosa; (b) minimal petechial haemorrhagic lesions (obtained from the control and the ketorolac trometamol emulgel-treated groups); and (c) linear haemorrhagic lesions (obtained from ketorolac trometamol orally treated groups.

Discussion

The release rate determination is one of the most important studies to be conducted for all controlled release delivery systems. This is because drug concentration on the surface of the stratum corneum should be maintained consistently and be substantially greater than the drug concentration in the body, to achieve a constant rate of drug permeation.[32]

The microemulsion formulations M3, M4 and M5, containing oleic acid, showed the lowest diffusion coefficient. This was because oleic acid had the greater solubilizing capacity of ketorolac trometamol (0.77 mg/ml) compared with isopropyl myristate (0.17 mg/ml) and isopropyl palmitate (0.13 mg/ml). The solvent where the drug is least soluble should provide the highest drug release. Ho *et al.*[33] found a correlation between penetration rate and solubility of the drug in the respective vehicle, where the increase in drug solubility in the vehicle slows down the penetration of the

Table 5 The effects of oral ketorolac trometamol and ketorolac trometamol emulgel formulae on formaldehyde-induced paw oedema in rats

Study group	Mean paw volume ± SD at different time intervals					
	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Group B_{cont}	0.86 ± 0.05	2.08 ± 0.06	3.25 ± 0.08	3.28 ± 0.04	3.23 ± 0.04	3.13 ± 0.04
Group B_{oral}	0.86 ± 0.05	1.06 ± 0.07*	1.58 ± 0.08*	1.42 ± 0.05*	1.42 ± 0.06*	1.36 ± 0.06*
Group $B_{E2\ paw}$	0.86 ± 0.02	1.44 ± 0.06*,†	2.32 ± 0.06*,†	2.23 ± 0.06*,†	1.89 ± 0.11*,†	1.80 ± 0.06*,†
Group $B_{E7\ paw}$	0.86 ± 0.05	0.98 ± 0.05*,†,‡	1.51 ± 0.08*,†,‡	1.40 ± 0.06*,†,‡	1.31 ± 0.06*,†,‡	1.32 ± 0.05*,†,‡
Group $B_{E2\ back}$	0.88 ± 0.04	1.58 ± 0.07*,†,‡,§	2.69 ± 0.06*,†,‡,§	2.49 ± 0.07*,†,‡,§	2.41 ± 0.03*,†,‡,§	2.07 ± 0.06*,†,‡,§
Group $B_{E7\ back}$	0.86 ± 0.02	1.24 ± 0.05*,†,‡,§	2.0 ± 0.03*,†,‡,§	1.90 ± 0.03*,†,‡,§	1.81 ± 0.07*,†,‡,§	1.64 ± 0.06*,†,‡,§

Emugels E2 and E7 were applied on the rat paw and the rat back. $^{*}P < 0.05$ significantly different from control nontreated group; $^{\dagger}P < 0.05$ significantly different from group B_{oral}; $^{\ddagger}P < 0.05$ significantly different from group B_{E2paw}; $^{\S}P < 0.05$ significantly different from group B_{E7paw}. $n = 8$ in each group.

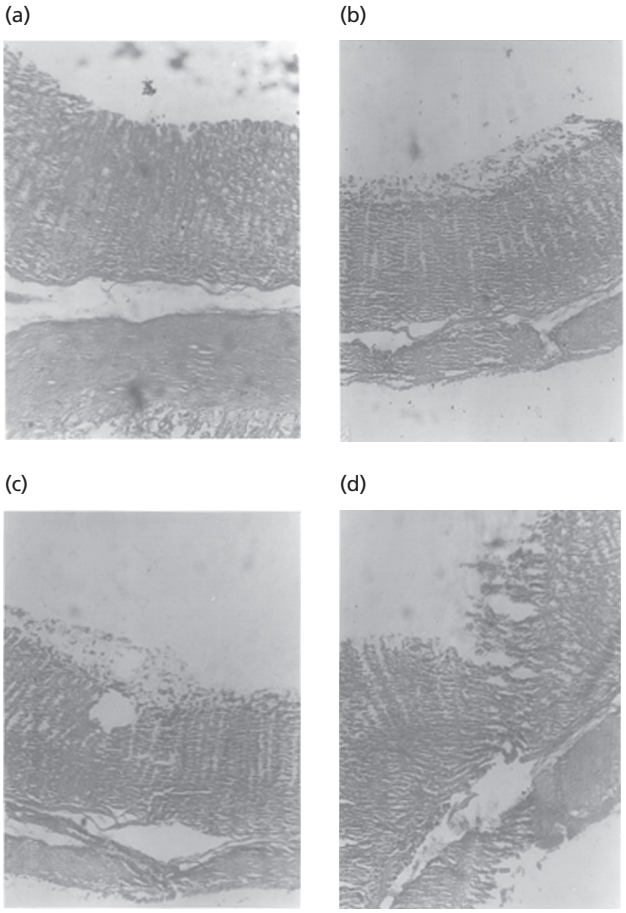

Figure 3 Histopathological stained sections of rat stomach showing gastric mucosa. (a) Normal gastric mucosa obtained from the control group; (b) grade 1 gastric mucosal erosion obtained from the ketorolac trometamol emulgel-treated groups; (c) grade 2 and (d) grade 3 gastric mucosal erosion, obtained from ketorolac trometamol orally treated groups.

drug through the synthetic membrane. M7 showed slightly higher release rate than M5 and M6. However there was no significant difference between diffusion rates of drug through M5, M4 or M3.

M6 and M7 exhibited a significantly higher drug release compared with M1; this may have been due to lower affinity between isopropyl palmitate and ketorolac trometamol compared with isopropyl myristate.

Formulation M2 showed the significantly highest diffusion coefficient among all the microemulsion formulations ($P < 0.05$). This may have been due to its composition, which combined the enhancing effect of both isopropyl myristate, reported as an excellent penetration enhancer, and transcutol. Similar synergies have been reported elsewhere.[7,34]

The difference in drug release rate from microemulsion formulations containing different amounts of oil (12 and 24%, w/w) was not significant. It was considered that the high content of surfactant mixture in microemulsion may have made the effect of oil on release rate less pronounced.[35] The effect of co-surfactant on release rate was only pronounced when transcutol was combined with isopropyl myristate (M2).

With the gel and emulgel formulations, the amount of oil (high and low concentration) had no significant effect on drug release rate from the different emulgel formulations ($P > 0.05$). The lower amount of drug released from E6 compared with that from E5 may have been due to the higher viscosity of E6, as shown in Table 1. It may have been due also to the entrapment of the drug in the network structure of carbomer 934p.[36] Similar results were observed with the emulgel formulations (E1 and E2) containing the polymeric emulsifier hypromellose, which showed significantly higher drug diffusion rates ($P < 0.05$) compared with the emulgel formulations (E3 and E4) prepared with traditional combination of surfactant emulsifier and polymeric thickener, and may be explained on the basis of viscosity differences. E7 showed the lowest drug release, most probably due to its high viscosity.

The results of the skin permeation study revealed that E7 and E2 showed a significantly higher flux value compared with E5 ($P < 0.05$). This may have been due to the composition of both E2 and E7 as they contained well known penetration enhancers, also the particle diameters of E2 and E7 were smaller than 3 μm; and it was previously reported that particles of < 3 μm are randomly distributed in the stratum corneum and penetrate the skin.[37] E7 showed a significantly higher flux value compared with E2. These results were not consistent with in-vitro release data, where E2 and E5 gave a significantly higher release rate than E7 (due to much higher viscosity of E7). This could have two possible explanations. Firstly, Ho *et al*.[33] proved that there was no significant correlation between viscosity of the gel matrix and drug penetration rate through the skin, and hence the viscosity of the gel matrix showed only a minor influence on the drug penetration. On the other hand the two factors that might have influenced penetration rate through the skin were partition of the drug between gel matrix and the skin, and modification of the skin structure by different penetration enhancers. Secondly, E7 was composed of two well known penetration enhancers, propylene glycol and Brij 92. It has been reported that the activity of some enhancers could be significantly increased when applied in combination with propylene glycol.[38]

The therapeutic performance of selected formulae was compared with oral ketorolac trometamol. Both formulae showed noticeable analgesic and anti-inflammatory activity with less adverse effects compared with oral ketorolac trometamol, which were obvious from histological examination. As with most NSAIDs, ketorolac trometamol is a weak acid and becomes concentrated in an acidic medium, with subsequent trapping in the stomach and a higher incidence for gastric complications.[39] E7 showed a significantly greater activity compared with E2; this result coincided with the skin permeation results. E7 was nearly equipotent to oral ketorolac trometamol. No immediate (acute effect) or delayed (chronic effect) skin damage or irritation was observed in any of the animals used.

For both formulations, the amount of drug remaining in the skin (Table 2) was markedly higher than the IC50 value of ketorolac required for cyclooxygenase-2-inhibition in the fibroblast cultures from human skin (0.097 μg/g), therefore a local anti-inflammatory effect could be expected.[40]

Formulations applied at the site of inflammation (paw) showed significantly higher activity compared with those applied away from the inflammation site (back); this may have been due to a combined local (as concluded from skin content analysis) and systemic effect of formulations applied on the paw.[40] Also, being a weak acid, ketorolac trometamol is concentrated in inflamed acidic tissues.[39] This might be another explanation for the higher activity of ketorolac trometamol when applied at the site of inflammation (paw).

Conclusions

The two selected ketorolac trometamol emulgel formulations (E2 and E7) modulated local and transdermal delivery of the drug. The developed E7 ketorolac trometamol emulgel appeared promising for dermal and transdermal delivery of ketorolac trometamol, which would circumvent problems and adverse effects associated with its oral therapy.

Declarations

Conflict of interest

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

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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