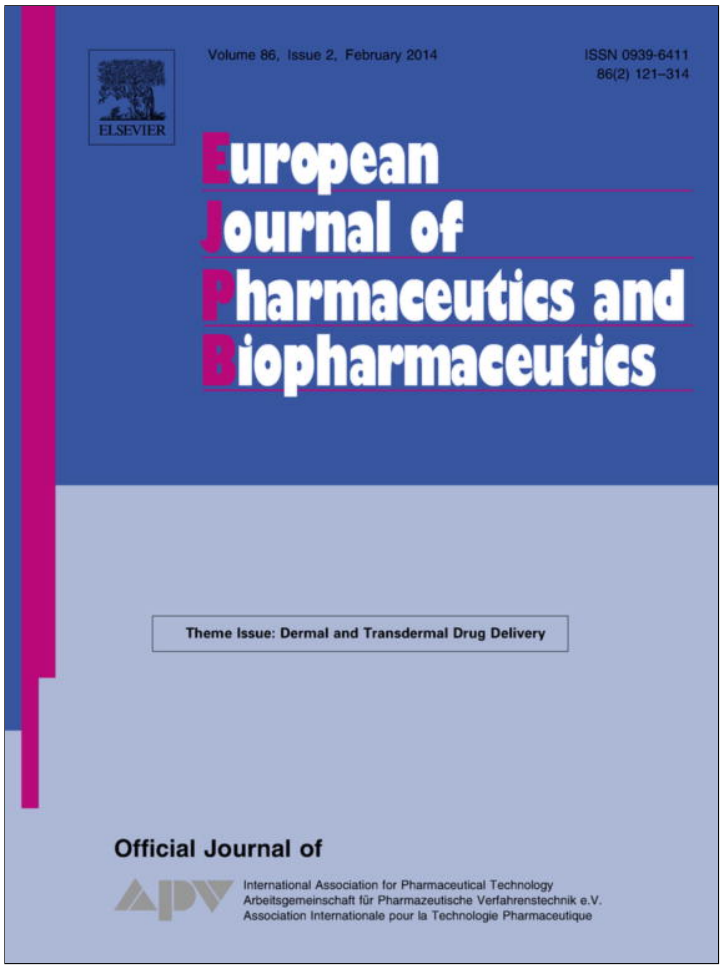

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Research paper

Iontophoretic transport kinetics of ketorolac *in vitro* and *in vivo*: Demonstrating local enhanced topical drug delivery to muscle

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ABSTRACT

The objective of the study was to investigate the iontophoretic delivery kinetics of ketorolac (KT), a highly potent NSAID and peripherally-acting analgesic that is currently indicated to treat moderate to severe acute pain. It was envisaged that, depending on the amounts delivered, transdermal iontophoretic administration might have two distinct therapeutic applications: (i) more effective and faster local therapy with shorter onset times (e.g. to treat trauma-related pain/inflammation in muscle) or (ii) a non-parenteral, gastrointestinal tract sparing approach for systemic pain relief. The first part of the study investigated the effect of experimental conditions on KT iontophoresis using porcine and human skin *in vitro*. These results demonstrated that KT electrotransport was linearly dependent on current density – from 0.1875 to 0.5 mA/cm^2 – ($r^2 > 0.99$) and drug concentration – from 5 to 20 mg/ml ($r^2 > 0.99$). Iontophoretic permeation of KT from a 2% hydroxymethyl cellulose gel was comparable to that from an aqueous solution with equivalent drug loading (584.59 ± 114.67 and 462.05 ± 66.56 μg/cm^2, respectively). Cumulative permeation (462.05 ± 66.56 and 416.28 ± 95.71 μg/cm^2) and steady state flux (106.72 ± 11.70 and 94.28 ± 15.47 μg/cm^2 h), across porcine and human skin, were statistically equivalent confirming the validity of the model. Based on the results *in vitro*, it was decided to focus on topical rather than systemic applications of KT iontophoresis *in vivo*. Subsequent experiments, in male Wistar rats, investigated the local enhancement of KT delivery to muscle by iontophoresis. Drug biodistribution was assessed in skin, in the *biceps femoris* muscle beneath the site of iontophoresis ('treated muscle'; TM), in the contralateral muscle ('non-treated muscle'; NTM) and in plasma (P). Passive topical delivery and oral administration served as negative and positive controls, respectively. Iontophoretic administration for 30 min was superior to passive topical delivery for 1 h and resulted in statistically significant increases in KT levels in the skin (91.04 ± 15.48 vs. 20.16 ± 8.58 μg/cm^2), in the *biceps femoris* at the treatment site (TM; 6.74 ± 3.80 vs. <LOQ), in the contralateral site (NTM; 1.26 ± 0.54 vs. <LOQ) and in plasma (P; 8.58 ± 2.37 μg/ml vs. <LOD). In addition to increasing bioavailability, iontophoretic administration of KT showed clear selectivity for local delivery to the *biceps femoris* at the treatment site – the TM:NTM ratio was 5.26 ± 1.45, and the TM:P and NTM:P ratios were 0.75 ± 0.32 and 0.14 ± 0.04, respectively. Furthermore, the post-iontophoretic concentration of KT in the 'treated' *biceps femoris* muscle and the muscle:plasma ratio were also superior to those following oral administration of a 4 mg/kg dose (6.74 ± 3.80 vs. 0.62 ± 0.14 μg/g and 0.75 ± 0.32 vs. 0.14 ± 0.03, respectively). In conclusion, the results demonstrate that iontophoresis of ketorolac enables local enhanced topical delivery to subjacent muscle; this may have clinical application in the treatment of localised inflammation and pain.

1. Introduction

Ketorolac (KT), which is marketed as the racemic mixture of the tromethamine salt (Fig. 1), is a potent nonsteroidal anti-inflammatory drug (NSAID) and analgesic indicated as a safe alternative to opioids [1]. It is a non-selective cyclooxygenase inhibitor,

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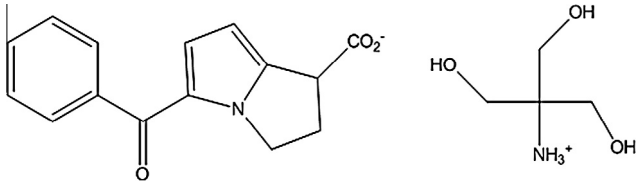

Fig. 1. Structure of ketorolac tromethamine (MW = 376.4, pK_a = 3.54).

blocking arachidonic acid metabolism, and hence decreasing prostaglandin and thromboxane biosynthesis [2]. Its oral administration is complicated due to many adverse effects, including gastrointestinal bleeding (especially in the elderly), perforation, and peptic ulceration [3,4]. Parenteral administration (i.m. and i.v.) is used to treat severe pain, e.g. after major surgery. However, its invasiveness and associated issues of patient non-compliance limit its general use.

As a consequence, there have been several attempts to develop transdermal systems to deliver ketorolac [5–8]. These would have two distinct therapeutic applications: (i) for systemic pain relief – e.g. as an alternative to parenteral administration to treat postoperative pain [9–11] or (ii) for more effective relief of musculoskeletal pain by enabling high local drug concentrations to be achieved quickly through enhanced targeted delivery to structures subjacent to the application site [12].

KT is a small hydrophilic molecule (376.4 D, log$P$ −0.58, pK_a 3.5) that does not readily partition into the lipidic stratum corneum. In order to overcome its poor cutaneous permeation, earlier studies have used chemical penetration enhancers (CPE), but only limited enhancing effects have been obtained with clinically accepted CPEs. In contrast, its physicochemical properties make KT an excellent candidate for iontophoresis [13]. Indeed, electrically-assisted delivery of ketorolac was reported to be superior to placebo in decreasing pain experienced by patients suffering from rheumatic pain in a double-blind clinical study measuring the degree of pain using a VAS scale [14]. However, there was no quantification of KT delivery, and based on the details reported, the iontophoretic conditions were not optimal since a silver cathode was used (instead of silver chloride, implying that the cathode was electrolysing water), and the application area was not clearly defined. More recently, the effect of formulation conditions on the iontophoretic transport of KT was investigated using rat skin *in vitro* [15]. However, a meaningful evaluation of the feasibility of using iontophoresis as a means to deliver therapeutic amounts of KT in humans requires a more realistic *in vitro* model.

In addition to increasing the amount of drug delivered, iontophoresis can also be used to achieve local therapeutic concentrations more quickly – thereby providing faster symptoms relief in the treatment for localised pain. There are reports that after topical application, drug accumulation can be enhanced at various subjacent structures such as underlying muscle and synovium [16,17]. The hypothesis is that local drug accumulation does not occur solely as a consequence of the topically applied drug being first absorbed into the systemic circulation and then distributed via the local vasculature, but that it is transported from the skin directly to the subjacent muscle [17]. The existence of such a 'local enhancement' effect would have direct implications on potential therapeutic applications.

The aim of this study was to determine whether iontophoretic transport of KT was sufficient – (i) to achieve input rates necessary for systemic effect and/or (ii) to enable locally enhanced drug delivery after short duration current application and so achieve higher local drug concentrations that might translate into faster onset of pain relief in patients. Initial studies investigated the effect of iontophoretic conditions on KT electrotransport *in vitro* using both porcine and human skin; the results from aqueous solutions were compared to those from a gel formulation. Based on the results of the *in vitro* experiments, subsequent *in vivo* studies addressed the questions whether it was possible to obtain a local enhancement of topical KT delivery after short duration iontophoresis. These studies were conducted in rats *in vivo,* and drug distribution was assessed in the skin, in the *biceps femoris* muscle beneath the site of iontophoresis, in the contralateral muscle distal from the application site and in the plasma. Results were compared to experiments following passive topical application and oral administration which served as negative and positive controls, respectively. The objective was to determine whether iontophoresis resulted in superior delivery of the anti-inflammatory drug directly to the muscle beneath the site of application demonstrating transport from the skin to the muscle without transit via the systemic circulation.

2. Materials and methods

2.1. Chemicals and reagents

Ketorolac tromethamine (KT), silver wire and silver chloride were purchased from Sigma–Aldrich (Buchs, Switzerland). Sodium chloride and methanesulfonic acid (>99% pure) were purchased from Fluka (Buchs, Switzerland). Ammonium acetate, citric acid and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) were purchased from Acros Organics (Chemie Brunschwig; Basel, Switzerland). PVC tubing (3 mm ID, 5 mm OD, 1 mm wall) used to prepare salt bridge assemblies was obtained from Fisher Bioblock Scientific S.A. (Illkirch, France). All solutions were prepared using deionised reverse osmosis filtered water (resistivity $\geqslant$18 MΩ cm). All other chemicals were at least of analytical grade.

2.2. Skin source

Porcine ears were obtained from a local abattoir (CARRE; Rolle, Switzerland), and the skin was excised (thickness 750 μm) with an air dermatome (Zimmer; Etupes, France), wrapped in aluminium foil and stored in polyethylene bags at −20 °C for a maximum period of 2 months. Human skin samples were collected immediately after surgery from (i) the Department of Plastic, Aesthetic and Reconstructive Surgery, Geneva University Hospital (Geneva, Switzerland), (ii) Clinique Vert-Pré (Geneva, Switzerland) and (iii) Hôpital de la Tour (Geneva, Switzerland). The fatty tissue was removed, and the skin was wrapped in Parafilm™ before storage at −20 °C for a maximum period of 7 days. The study was approved by (i) the Central Committee for Ethics in Research (CER: 08-150 (NAC08-051); Geneva University Hospital) and (ii) the Commission d'Ethique pour la Recherche Clinique en Ambulatoire (Protocol 10-25; Association des Médecins du Canton de Genève et Société Médicale).

2.3. Animals

In vivo experiments were conducted using a total of 18 male Wistar rats, weighing from 160 to 200 g ('Biotério Central', University of São Paulo, Brazil). The animals were housed at 24–26 °C, exposed to daily 12:12 h light/dark cycles (lights on at 6 a.m.) and had access to food and water *ad libitum*. The animal protocol was approved by the University Ethics Commission for the Use of Animals (CEUA) of the campus of Ribeirão Preto – University of São Paulo (Authorisation number: 10.1.1687.53.2).

2.4. Stability studies

2.4.1. KT stability in the presence of skin

Epidermal and dermal stabilities of the drug were determined by placing 1 ml of 10 mg/ml KT solution in contact with epidermis (1.6 cm^2) and dermis (1.6 cm^2), respectively. Samples were collected every 2 h for 6 h, diluted and analysed by the HPLC method described below ($n = 4$).

2.4.2. KT stability in the presence of current

Two millilitre of 10 mg/ml KT solution or 2 g of a hydroxyl methyl cellulose (2% w/w) gel containing 10 mg/g of KT was subjected to a current of 0.5 mA for 6 h, salt bridges ensured connectivity between the cathode and the formulation. Samples were collected every hour, diluted and analysed by the HPLC method described below ($n = 4$).

2.5. In vitro iontophoretic set-up and protocol

The experimental set-up used was similar to that described in earlier studies [18,19]. The skin was clamped in vertical diffusion cells (area 1.6 cm^2). The donor compartment was filled with 1 ml of drug solution and was connected to the cathode via a salt bridge assembly (3% agarose in 0.1 M NaCl). The receptor compartment was used as the anode and was filled with 10 ml of 25 mM HEPES and 133 mM NaCl (pH 7.4) solution. During the experiment, samples (1 ml) were withdrawn from the receptor compartment hourly for 6 h and replaced with fresh receptor fluid. Constant current was applied using Ag/AgCl electrodes connected to a power supply (Kepco® APH 1000 M, Flushing, NY). At the end of the permeation experiment (6 h), the diffusion cells were dismantled, and the skin surface washed in running water to remove residual formulation. The KT bound to the skin was extracted by cutting the skin samples into small pieces and soaking them in 5 ml of 25 mM HEPES and 133 mM NaCl (pH 7.4) solution. Samples were left overnight under constant stirring at ambient temperature. They were then filtered and analysed using the HPLC method described below (see Section 2.8). A passive control was performed following the exact same conditions, except that no current was applied.

2.6. In vitro transport studies

2.6.1. Effect of current density

The effect of the magnitude of the applied current on KT electrotransport kinetics was determined by investigating delivery at three different current densities (0.1875, 0.3125 and 0.5 mA/cm^2) applied for 6 h using 10 mg/ml KT aqueous solution (pH 5.9) as the cathodal donor solution ($n \geqslant 4$ in all experiments).

2.6.2. Effect of concentration

The effect of drug concentration on KT delivery was studied by comparing KT iontophoresis at 0.5 mA/cm^2 for 6 h from formulations containing 5, 10 and 20 mg/ml of KT in aqueous solution (pH 5.9) ($n \geqslant 4$ in all experiments).

2.6.3. Effect of formulation

KT delivery following iontophoresis for 6 h at 0.5 mA/cm^2 from a 2% hydroxyl methyl cellulose (w/w) gel containing 10 mg/g of the drug (pH 6.5) was compared to that from an aqueous formulation under the same conditions ($n \geqslant 4$ in all experiments).

2.6.4. Comparison of ketorolac iontophoresis across porcine and human skin

Iontophoretic delivery of KT across dermatomed porcine skin and full thickness human skin samples was compared under the same experimental conditions – 10 mg/ml KT iontophoresed for 6 h at 0.5 mA/cm^2 ($n \geqslant 4$ in both experiments).

2.7. In vivo set-up and protocol

2.7.1. Topical iontophoretic delivery

Rats used for the topical iontophoretic (and passive control) delivery studies had the thigh hair from the left hind limb trimmed 24 h before the experiments. They were anaesthetised a few minutes before formulation application with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). The drug solution (2 ml) containing 20 mg/ml of KT was applied to the skin surface via an open glass chamber (area 1.13 cm^2), and silicone grease was used to prevent leaks. The donor compartment was connected to the cathode via a salt bridge assembly (3% agarose in 0.1 M NaCl). An Ag counter electrode was introduced in a second glass chamber, containing only buffer solution and 133 mM of NaCl (silicone grease was again used to prevent leaks). A Phoresor II (model PM 850, Iomed, Inc., Salt Lake City, UT) delivered a total charge of 16.95 mA min in 30 min (0.5 mA/cm^2 min) (Fig. 2).

After current application for 30 min, the skin exposed to the drug was cleaned with cotton wool and then a blood sample taken by cardiac puncture either immediately (Group 1) or after an additional 30 min to allow the drug to distribute (Group 2). The drug was extracted from the plasma and analysed using the procedure described below (see Section 2.8). The animals were then sacrificed using carbon dioxide, and the skin and both the left (beneath the treated area) and right (non-treated area) *biceps femoris* muscles were excised from the animals for drug extraction and analysis. The KT that remained in the skin and muscles was extracted by cutting the samples into small pieces and soaking them in 4.5 ml of methanol:water (1:2) solution following 1 min of homogenisation using an Ultraturrax homogeniser (Turratec TE 102E, Brazil). The samples were then filtered and analysed by HPLC.

2.7.2. Topical passive delivery

This was performed using the same conditions as described above for the iontophoretic studies except that no current was applied. In this group, the formulation was applied for 60 min and followed immediately by cardiac puncture; drug levels in the plasma were determined as described below. This group was considered as the negative control.

2.7.3. Oral delivery

An oral delivery group was used as a positive control for the *in vivo* experiments. Animals were fasted for 6 h prior to experiments, but water was available *ad libitum* at all times. Each rat received a dose of 4 mg/kg KT. Drug concentrations in muscle and

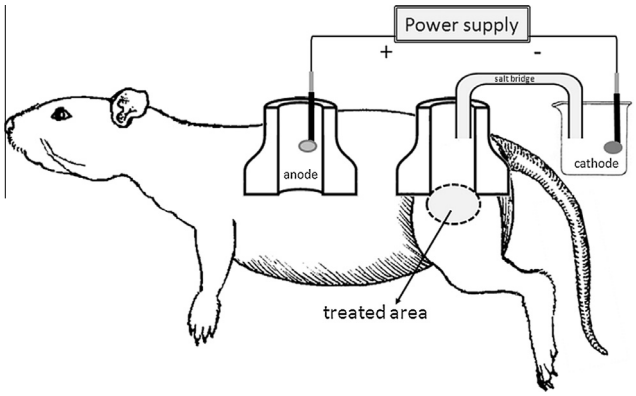

Fig. 2. Schematic representation of the experimental set-up for the *in vivo* studies.

plasma were determined after 60 min according to the protocol described below.

2.8. Analytical methods for quantification of KT in vitro and in vivo

KT was analysed using a P680A LPG-4 pump equipped with an ASI-100 autosampler and a UV/Vis detector (UVD 170U) (Dionex, Voisins LeBretonneux, France) and a Lichrospher® column (125 × 4 mm) packed with 5 μm C18 silica reversed-phase particles (BGB Analytik AG; Boeckten, Switzerland). The mobile phase comprised 75% ammonium acetate buffer (100 mM; pH 4.5) and 25% acetonitrile [15]. The flow rate was 1 ml/min, and the injection volume was 25 μl. KT was detected using its absorbance at 314 nm. The method was validated and showed good precision (RSD < 1%) and accuracy (>94%) both intra- and inter-day. Repeatability of the analytical method was calculated (according to ICH guidelines) with 5 replicates at 5, 10 and 25 μg/ml. The values obtained were, respectively, 0.70%, 0.56% and 0.20% for intra-day analysis and 0.77%, 0.95% and 0.75% for inter-day analysis. The specificity of the analytical method was verified with respect to endogenous compounds present in the skin. The limits of detection (LOD) and quantification (LOQ) were 0.05 and 0.15 μg/ml, respectively.

The methods to extract KT from skin and muscle were validated by spiking the tissue samples with two different known amounts of drug in ethanol solution. After solvent evaporation, tissue samples were subjected to the extraction procedure (see Sections 2.5 and 2.7.1), and the recovery of KT was determined by calculating the ratio of the amount extracted from the samples to the amount added, determined by direct injection of spiking solution in the absence of tissue. The recovery rates are shown in Table 1.

Plasma extraction was performed according to a previously described method [20]. Briefly, 300 μl of plasma was added to 900 μl of methanol. The mixture was vortex-mixed for 60 s and centrifuged at 10,000*g* for 5 min. Then, 800 μl of supernatant was added to 20 μl of hydrochloric acid (2 M), vortex-mixed for 60 s and centrifuged at 10,000*g* for 2 min. The supernatant was analysed directly by the HPLC method described above. The extraction method was validated, and the recovery rates are shown in Table 1.

2.9. Analytical method for quantification of Na^+ and K^+

Concentrations of Na^+ and K^+ in the donor solution were measured before and after iontophoresis of KT (10 mg/ml solution) at 0.5 mA/cm^2 for 6 h using High Performance Anion Exchange Chromatography with Suppressed Conductivity Detection; this enabled the contribution of cation transport from the receiver compartment to the overall current flow to be determined.

The system consisted of a GP50 gradient pump, ED50A electrochemical detector, AS50 autosampler, AS50TC thermal compartment, a cation Self-Regenerating CSRS®-ULTRA Suppressor connected in auto suppression recycle mode and Chromeleon® chromatography workstation (Dionex Corporation, Sunnyvale, CA, USA). A Dionex IonPac® CS14 analytical column (4 × 250 mm) and IonPac CG14 guard column (4 × 50 mm) were used for analysis. The mobile phase consisted of 25 mM methanesulfonic acid solution. It was prepared in deionised water and purged with helium to minimise carbonate. The flow rate was 1.0 ml/min, and the injection volume was 20 μl. The sodium peak eluted at 2.9 min and that of potassium at 3.8 min. The method was validated and showed good precision (RSD < 5%) and accuracy (>90%) both intra- and inter-day. The limits of detection (LOD) and quantification (LOQ) were 0.5 and 1.5 μg/ml, respectively, for sodium and 1.0 and 3.0 μg/ml, respectively, for potassium.

Table 1
Recovery of ketorolac from porcine skin, rat skin, muscle and plasma.

	Theoretical added concentration (μg/ml)	Recovery (%)
Porcine skin	10.0	82.16 ± 12.78[a]
	20.0	83.51 ± 16.37[a]
Rat skin	5.0	86.29 ± 7.48[a]
	10.0	84.33 ± 6.15[a]
Muscle	5.0	80.52 ± 11.16[b]
	10.0	74.05 ± 9.35[b]
Plasma	0.5	88.30 ± 16.79[c]
	1.0	87.52 ± 0.94[c]
	2.5	93.82 ± 1.98[c]

[a] Mean ± SD (n = 4).
[b] Mean ± SD (n = 2).
[c] Mean ± SD (n = 3).

2.10. Statistical analysis

Data were expressed as mean ± SD. Outliers determined using the *Grubbs test* were discarded. Results were evaluated statistically using either analysis of variance (ANOVA followed by *Student–Newman–Keuls test*) or *Student's t-test*. The level of significance was fixed at $\alpha = 0.05$.

3. Results and discussion

3.1. KT stability in presence of skin and electric current

The percentages of drug in solution after exposure for 6 h to porcine epidermis and dermis were 100.46 ± 0.55 and 71.13 ± 2.76%, respectively, of those observed initially. After 6 h of current application, the percentages of KT in solution or in the gel were 83.60 ± 5.88 and 97.04 ± 3.13%, respectively, of those at t = 0 h. Thus, it was concluded that KT was sufficiently stable for the purposes of the planned experiments. The superior stability observed using the gel in the presence of current may be due to a stabilising effect of the polymer network that protected the molecule from degradation.

3.2. KT transport through porcine skin in vitro

The iontophoretic delivery of KT has been previously investigated using rat skin *in vitro* [15]. However, the permeability barrier of rat skin is considered to be less efficient than that of human (or porcine) skin due, at least in part, to significant follicular transport [21,22]. Therefore, extrapolation of results obtained with rat skin may overestimate drug penetration into human skin. In order to have a more accurate and quantitative evaluation of the potential of iontophoresis for KT delivery and to determine the effect of experimental parameters on electrotransport, these *in vitro* permeation experiments were performed using porcine ear skin, which is generally considered to be a good model for transdermal delivery across human skin [23,24]. In control experiments, KT was not detected in either the receiver phase or after skin extraction (LOQ = 0.15 μg/ml) following passive diffusion for 6 h. In contrast, current application resulted in appreciable KT delivery. The effect of current density on KT electrotransport was investigated by determining cumulative iontophoretic permeation (Q) and steady state iontophoretic flux (J) at 0.1875, 0.3125 and 0.5 mA/cm^2 (Fig. 2). It was found that both cumulative KT permeation (Q; 189.72 ± 23.60, 315.42 ± 44.95 and 462.05 ± 66.56 μg/cm^2, respectively) and flux (J; 44.78 ± 6.10, 72.95 ± 10.67 and 106.72 ± 11.71 μg/cm^2 h, respectively) increased linearly as a function of current density (Q; r^2 = 0.995 and J; r^2 = 0.996). Statistical analysis (ANOVA ($\alpha = 0.05$) followed by *Student–Newman–Keuls test*) showed that there was a statistically significant difference between cumulative KT permeation at the

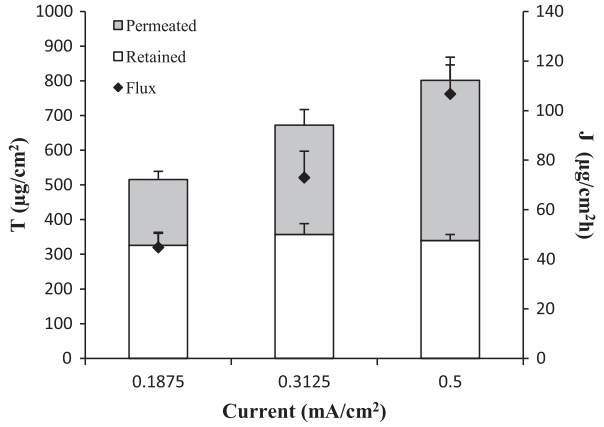

Fig. 3. Effect of current density (0.1875, 0.3125 and 0.5 mA/cm^2) on total delivery (T) of ketorolac – the sum of the amounts permeated across and retained within the membrane – and the steady state flux (J) after 6 h of transdermal iontophoresis using a 10 mg/ml solution. (Mean ± SD; $n \geqslant 4$).

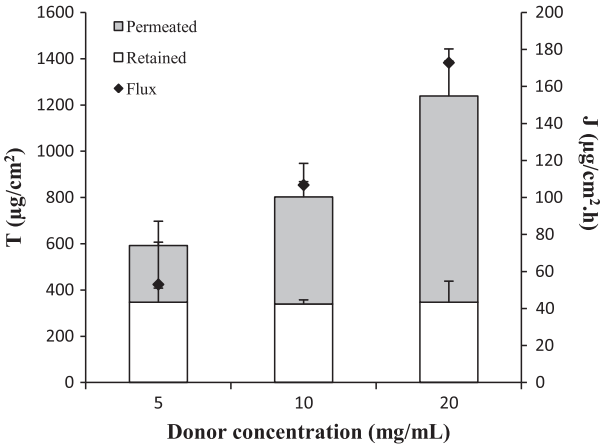

Fig. 4. Effect of donor concentration (5, 10 and 20 mg/ml) on the total delivery (T) of ketorolac – sum of the amounts permeated across and retained within the membrane – and the steady state flux (J) after 6 h of transdermal iontophoresis at 0.5 mA/cm^2. (Mean ± SD; $n \geqslant 4$).

different current densities used. The extraction experiments showed that significant quantities of KT were also retained within the membrane during iontophoresis. However, an increase in the applied current density did not produce a statistically significant increase in the amount of drug present in the skin (325.83 ± 34.58, 356.97 ± 31.42 and 339.77 ± 17.33 µg/cm^2, at 0.1875, 0.3125 and 0.5 mA/cm^2, respectively) (Fig. 3). Considering the amounts of drug permeated across and retained within the skin, the iontophoretic delivery efficiency increased upon increasing the applied current (8.25 ± 0.93, 10.76 ± 1.22, 12.83 ± 1.34%, at 0.1875, 0.3125 and 0.5 mA/cm^2, respectively).

Iontophoresis of KT solutions at 5, 10 and 20 mg/ml for 6 h at 0.5 mA/cm^2 also displayed a linear response with respect to both cumulative permeation (Q; 245.28 ± 104.53, 462.05 ± 66.56 and 891.08 ± 150.58 µg/cm^2, respectively) and iontophoretic flux (J; 53.00 ± 24.84, 106.72 ± 11.70 and 172.85 ± 7.42 µg/cm^2 h, respectively) as a function of increasing drug concentration (Q; $r^2 = 0.995$ and J; $r^2 = 0.983$) (Fig. 4). The differences were shown to be statistically significant (ANOVA ($\alpha = 0.05$) followed by *Student–Newman–Keuls* test). Although KT was the only anion present in the donor solution connected to the cathode, it was not the only charge carrier responsible for carrying the current. Small inorganic cations present in the skin, e.g. Na^+ and K^+, have high mobility and during cathodal iontophoresis migrate from the skin towards the negatively charged electrode. The iontophoretic current is the sum of the component currents due to these individual ion flows. Proof of the existence of these cation flows from the skin towards the cathode, and their magnitude was provided by measurement of Na^+ and K^+ concentrations in the donor solution after 6 h of iontophoresis; the concentration of Na^+ increased from 1.23 ± 0.05 to 117.85 ± 11.22 µmol/ml after 6 h of iontophoresis at 0.5 mA/cm^2 (~100-fold increase); K^+ was not detected in the donor solution before current application, but the post-iontophoretic concentration was 2.11 ± 1.15 µmol/ml. The transport number describes the fraction of the total charge carried by an ion and can be expressed by the following equation [13,25]:

$$t_i = \frac{J_i z_i F}{I_d}$$

where J_i is the flux, z_i refers to the charge, F is the Faraday constant and I_d is the applied current density. The transport numbers for Na^+, K^+ and KT were 0.652, 0.012 and 0.022, respectively; thus, only 2% of the total current was carried by the drug. The remaining current was most probably transported by Cl^- present in the epidermis [26]. The transport number for KT, t_{KT}, is determined by physicochemical (charge (z_{KT}) and mobility (u_{KT})) and formulation (c_{KT}) parameters and their values relative to those of the other charge carriers in the system [13,25,26]:

$$t_{KT} = \frac{z_{KT} u_{KT} c_{KT}}{\sum_{n=0}^{i} z_i u_i c_i}$$

The extraction experiments showed that, as in the case of increasing current, the increase in donor drug concentration did not produce a statistically significant change in the amount of drug retained in the skin (347.50 ± 60.77, 339.77 ± 17.33 and 347.51 ± 90.69 µg/cm^2, respectively) (Fig. 4).

The lack of dependence between the amount of drug retained in the skin and either the applied current density or drug concentration was also observed in our earlier studies with dexamethasone sodium phosphate [27]. It was proposed that as the skin is negatively charged under physiological conditions, there may be fewer potential binding sites for anions; moreover, those that exist may be readily saturated. This may be an advantage for the enhancement of local KT availability, since a smaller fraction of the drug entering the skin may be lost through binding to these sites enabling greater amounts to reach the underlying muscle. A correlation between the unbound fraction of a drug in viable skin and the drug clearance from viable skin to the muscle has been observed in rats *in vivo* [28].

The drug was also successfully iontophoresed from a hydroxyl methyl cellulose (2% w/w) gel formulation containing 10 mg/g KT. The cumulative permeation and steady state flux after iontophoresis for 6 h at 0.5 mA/cm^2 were 584.59 ± 114.67 µg/cm^2 and 128.39 ± 41.98 µg/cm^2 h, respectively. There was no statistically significant difference between the cumulative iontophoretic permeation and drug flux observed with the gel or the solution (Student's t-test; $\alpha = 0.05$) (Fig. 5). The ability to deliver KT from a gel formulation is obviously advantageous, since this could easily form the basis of a drug reservoir in an iontophoretic patch system [29,30].

3.3. Comparison of ketorolac iontophoresis across porcine and human skin

Cumulative permeation (462.05 ± 66.56 and 416.28 ± 95.71 µg/cm^2) and steady state flux (106.72 ± 11.70 and 94.28 ± 15.47 µg/

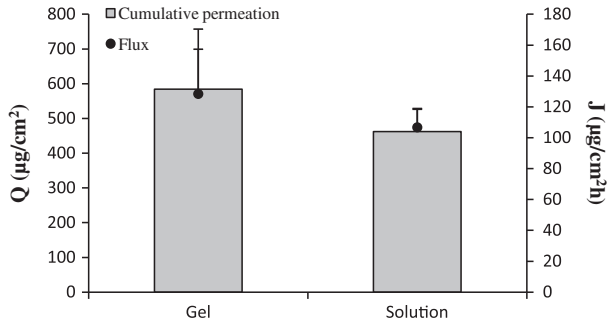

Fig. 5. Comparison of cumulative permeation (Q) and steady state flux (J) of ketorolac from either a 10 mg/ml solution or a 2% hydroxyl methyl cellulose gel after iontophoresis at 0.5 mA/cm^2 for 6 h. (Mean ± SD; $n \geqslant 4$).

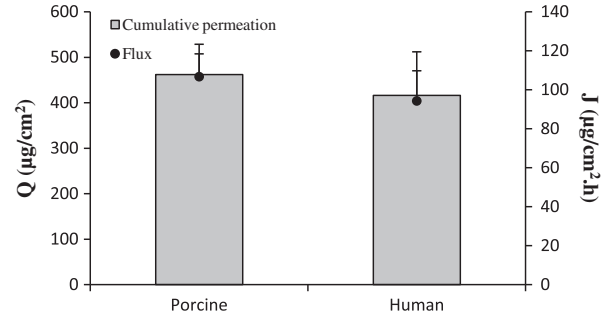

Fig. 6. Comparison of cumulative permeation (Q) and steady state flux (J) of ketorolac across porcine and human skin after iontophoresis at 0.5 mA/cm^2 for 6 h using a 10 mg/ml solution. (Mean ± SD; $n \geqslant 4$).

cm^2 h) across porcine and human skin were statistically equivalent (Student's t-test; $\alpha = 0.05$) (Fig. 6).

3.4. Comparison with earlier studies

Cumulative permeation and steady state flux of KT observed in these studies were superior to those achieved with CPEs using rat skin *in vitro* [5]. The highest flux achieved in those studies was 66.38 μg/cm^2 h (formulation containing 20 mg/ml of KT and 10% eucalyptus oil) [5], which is approximately 3-fold lower than the flux achieved with iontophoresis and the same drug concentration in the present study (Fig. 4). In terms of iontophoretic transport, it is worth noting that although KT flux obtained across human and porcine skin *in vitro* was equivalent (Fig. 6), the flux across porcine ear skin (53.00 ± 24.84 μg/cm^2 h) after 6 h iontophoresis at 0.5 mA/cm^2 of a 5 mg/ml drug solution was ~60% of that obtained using rat skin (91.35 ± 8.40 μg/cm^2 h) under the same conditions – highlighting the greater permeability of rat skin over porcine skin [15].

3.5. In vivo studies

Ketorolac has good oral bioavailability (>90%) but a relatively modest half-life (~5–6 h). It is available as 10 mg tablets or as a solution for injection; the intravenous single dose for adults is 15–60 mg. Based on the results obtained from the *in vitro* experiments in the first part of this study, systemic delivery of ~15 mg KT – at the lower limit of therapeutic dosing – would require application of an iontophoretic current of 0.5 mA/cm^2 for 8 h and a 10 cm^2 patch using a 2% drug load. Given these somewhat aggressive conditions, this was not considered practical for clinical use. Therefore, it was decided to focus the planned *in vivo* studies on the use of iontophoresis for locally enhanced topical delivery – in particular, with respect to the levels observed in subjacent muscle following short duration current application that might be of clinical interest in the treatment of musculoskeletal pain and inflammation. The key objective was to see whether topical iontophoresis was able to produce superior drug levels in the tissue directly beneath the current application site (as opposed to the contralateral site) and hence demonstrate that drug passed directly from the skin to the muscle without first entering the systemic circulation and establishing an equilibrium with the tissue. As mentioned above, it is generally accepted that rat skin is more permeable than human skin; nevertheless, for practical reasons, the rat was chosen as the first animal model to study KT distribution in tissues *in vivo*. Moreover, in all experiments, the contralateral muscle was used as an internal control/reference. Passive and oral delivery experiments were also performed as negative and positive controls, respectively.

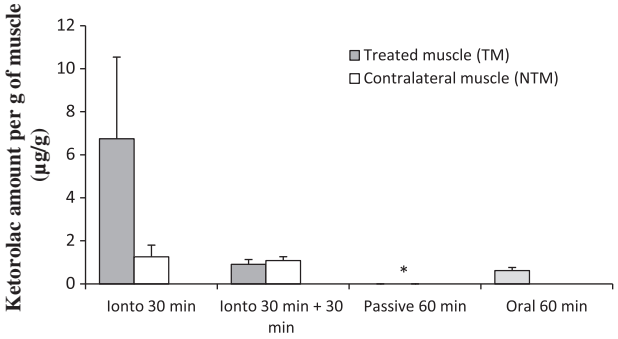

Fig. 7. Amount of ketorolac per gram of *biceps femoris* muscle assessed after: (i) 30 min iontophoresis, (ii) 60 min of delivery including 30 min of iontophoresis and an additional 30 min, (iii) 60 min of passive delivery and (iv) 60 min after oral treatment. (Mean ± SD; $n \geqslant 4$). * Values were below LOQ.

Passive permeation experiments for 60 min resulted in very poor permeation and skin retention of KT. Drug levels in the muscle beneath the treated site (TM) and distal from the treated site in the non-treated area (NTM) were below the limit of quantification (LOQ), and plasma levels were below the limit of detection (LOD). The amount retained in the skin at the treatment site was 20.16 ± 8.58 μg/cm^2 (Fig. 7). In contrast, cathodal KT iontophoresis for 30 min resulted in significant drug transport into and across the skin. The amounts recovered from the skin immediately after 30 min of iontophoresis and after an additional 30 min were 91.04 ± 15.48 and 31.32 ± 13.77 μg/cm^2, respectively – demonstrating that approximately two thirds of the drug loading in the skin was distributed to other tissues within 30 min. A local enhancement of topical KT delivery effect was observed in the *biceps femoris* muscle below the treated area (TM). Samples excised immediately after 30 min of iontophoresis contained approximately 5-fold more drug than samples excised after an additional 30 min (6.74 ± 3.80 vs. 0.91 ± 0.22 μg/g, respectively). This time-dependence was not observed in the contralateral muscle (NTM; 1.26 ± 0.54 vs. 1.08 ± 0.18 μg/g, respectively) (Student's t-test; $\alpha = 0.05$) (Fig. 7). These results provided clear evidence that KT was transported from the skin into the subjacent muscle following topical iontophoresis without first passing through the systemic circulation.

Quantification of KT levels in the plasma (Fig. 8) enabled the muscle-to-plasma ratios for the 'Treated Muscle' (TM/P) and contralateral or 'Non-Treated Muscle' (NTM/P) to be calculated. Immediately after current application for 30 min, TM/P ratio was ~0.78 as compared to the NTM/P ratio of 0.13.

Thus, drug accumulation was approximately 6-fold higher in the *biceps femoris* muscle under the site of iontophoretic application (Fig. 9). Muscle-to-plasma (M/P) ratios after oral delivery were

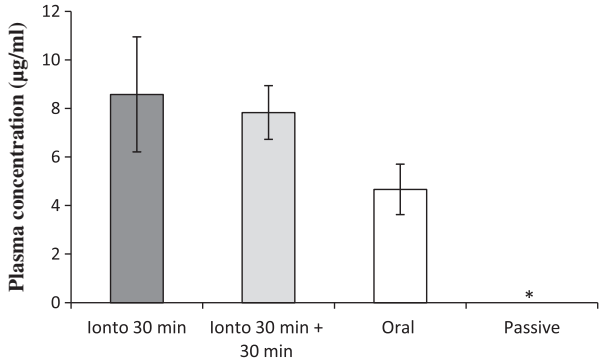

Fig. 8. Ketorolac concentrations in the plasma: (i) after 30 min iontophoresis (0.5 mA/cm^2) of a solution containing 20 mg/ml of the drug; 'Ionto 30 min' and 'Ionto 30 min + 30 min' represent samples collected immediately and 30 min after current cessation, (ii) 60 min after administration of an oral dose of 4 mg/kg and (iii) 60 min of passive application of a 20 mg/ml solution (Mean ± SD; $n \geqslant 4$). * Values were below LOD.

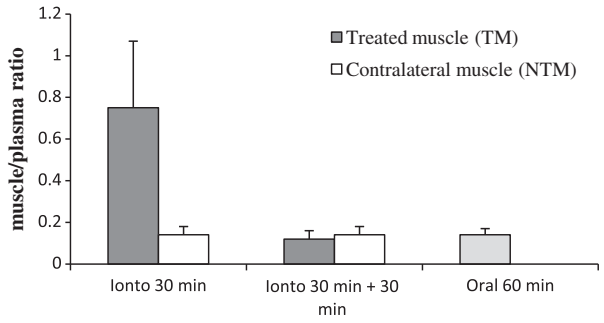

Fig. 9. Muscle-to-plasma (M/P) ratios of ketorolac after: (i) 30 min iontophoresis, (ii) 60 min of delivery including 30 min of iontophoresis and an additional 30 min, (iii) 60 min after oral treatment (Mean ± SD; $n \geqslant 4$). TM is the *biceps femoris* muscle beneath the treated area and NTM is the contralateral muscle.

approximately the same as the (NTM/P) ratio after 30 min of iontophoresis (~0.13). These values are in the same range as those found in mice 1 h following an oral dose of 2 mg/kg where the M/P ratio was ~0.09 [31].

These results clearly demonstrate that iontophoresis enables *local enhanced topical drug delivery* meaning that at least during the iontophoretic application, the drug concentration is significantly enhanced in subjacent structures to the application site, e.g. the underlying muscles. It has been recently reported that the transport of highly plasma protein bound drugs, e.g. ketorolac (>99%), from the skin to deeper tissues may be facilitated by convective blood, lymphatic and interstitial transport in addition to tissue diffusion itself [32]. It can be envisaged that iontophoresis when coupled with these physiologic transport processes may enable therapeutic levels to be achieved in a shorter time, enabling faster onset of relief from localised pain/inflammation. More studies are obviously required to demonstrate the therapeutic benefits of topical KT iontophoresis under clinical conditions and to determine the appropriate therapeutic regimen.

4. Conclusion

Topical iontophoresis for 30 min achieved local enhancement of ketorolac delivery resulting in superior drug levels in the skin and in the subjacent muscle at the treatment site as compared to the contralateral site. Given that passive application for 60 min resulted in drug levels that were below the LOQ (0.15 µg/ml), the results suggest that it may be possible to use this method as a means to provide faster onset of therapeutic effect in patients. The linear dependence of flux on both current density and drug concentration is an advantage for dose optimisation and the individualisation of treatment. These results should encourage investigation into the potential clinical benefits of iontophoresis for treating localised pain and inflammation.

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