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Research Paper

In-vitro and in-vivo transdermal iontophoretic delivery of tramadol, a centrally acting analgesic

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Abstract

Objectives The feasibility of transdermal delivery of tramadol, a centrally acting analgesic, by anodal iontophoresis using Ag/AgCl electrodes was investigated *in vitro* and *in vivo*.
Methods To examine the effect of species variation and current strength on skin permeability of tramadol, in-vitro skin permeation studies were performed using porcine ear skin, guinea-pig abdominal skin and hairless mouse abdominal skin as the membrane. In an in-vivo pharmacokinetic study, an iontophoretic patch system was applied to the abdominal skin of conscious guinea pigs with a constant current supply (250 μA/cm^2) for 6 h. An intravenous injection group to determine the pharmacokinetic parameters for estimation of the transdermal absorption rate in guinea pigs was also included.
Key findings The in-vitro steady-state skin permeation flux of tramadol current-dependently increased without significant differences among the three different skin types. In the in-vivo pharmacokinetic study, plasma concentrations of tramadol steadily increased and reached steady state (336 ng/ml) 3 h after initiation of current supply, and the in-vivo steady-state transdermal absorption rate was 499 μg/cm^2 per h as calculated by a constrained numeric deconvolution method.
Conclusions The present study reveals that anodal iontophoresis provides current-controlled transdermal delivery of tramadol without significant interspecies differences, and enables the delivery of therapeutic amounts of tramadol.
Keywords analgesic; anodal iontophoresis; tramadol hydrochloride; transdermal delivery

Introduction

Tramadol hydrochloride is a centrally acting synthetic opioid analgesic that has a dual mechanism of action, binding to μ-opioid receptors and weakly inhibiting the neuronal reuptake of norepinephrine and serotonin.[1,2] Tramadol hydrochloride has been used to treat moderate to moderately severe chronic pain for over 30 years.[3,4] Tramadol hydrochloride is available in many dosage forms, such as injection (intravenous and intramuscular) and oral formulations (immediate release and extended release).[4] These routes of administration are subject to problems with patient discomfort associated with multiple injections and swallowing that can lead to low patient compliance. Other issues are high labour costs due to the need for frequent injections, risks of toxicity due to sudden peaks in plasma drug concentrations after intravenous injection, and variable interpatient bioavailability after oral administration.[5] Transdermal drug delivery, a non-invasive drug administration route, offers the potential benefits of simplicity, efficacy and patient acceptance by maintaining a constant blood drug concentration for an extended period of time with acceptable interpatient variations.[6–8] In addition, the transdermal delivery system can decrease the possible abuse and addiction potential of tramadol[9,10] by avoiding peak and trough plasma concentrations and by reducing the total amount of medication input.[11,12] Hence, a transdermal delivery system is a desirable alternative administration route for tramadol hydrochloride for patients with chronic pain.

Transdermal drug delivery technologies are divided into passive and active methods. For a drug to be delivered passively via the skin, it needs to have low molecular mass (up to a few hundred Daltons), high lipophilicity and low to modest daily dose up, typically less than

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50 mg.[8,13–15] Due to the large amounts of daily dose (50–400 mg) and low lipophilicity (*n*-octanol/water log partition coefficient (log P) at pH 7: 1.35) of tramadol hydrochloride, it is unlikely that passive diffusion across the skin could deliver therapeutic amounts of tramadol from reasonably sized patches. Iontophoresis, an active transdermal drug delivery technology, delivers charged or neutral (but polar) drugs across the skin via application of a small electric current on the skin, resulting in higher flux of molecules that otherwise have negligible permeation through the skin.[16–19] Anodal iontophoresis is the method whereby a cationic drug is delivered across an epithelial barrier when placed under a positively charged delivery electrode (anode) from which it is repelled. A counter electrode completes the circuit by drawing physiological anions (i.e. Cl^-) from the body. Anodal iontophoresis has been widely used for transdermal delivery of cationic compounds and positively charged macromolecules.[20,21] The ionization constant (pKa) of tramadol hydrochloride is 9.41, and anodal iontophoretic technology is thus applicable to transdermal delivery of tramadol. However, to date there have been no reports regarding transdermal iontophoretic delivery of tramadol.

The aim of the present study was to evaluate the feasibility of transdermal iontophoretic delivery of tramadol using anodal iontophoretic patches *in vitro* and *in vivo*. This is the first evaluation of the feasibility of delivering therapeutic amount of tramadol by iontophoresis.

Materials and Methods

Chemicals

Tramadol hydrochloride (DongBang Future Technology & Life Co., Ltd, Seoul, Korea), was dissolved in an aqueous solution with 1% (w/v) Tween 80 (MP Biomedicals, LLC, Morgan Irvine, CA, USA) and 3% (w/v) hydroxypropyl cellulose-H (Nippon Soda Co., Ltd, Tokyo, Japan) for iontophoretic experiments. Buspirone hydrochloride was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA) for use as an internal standard in liquid chromatography tandem mass spectrometry (LC/MS/MS). High-performance liquid chromatography (HPLC) grade acetonitrile and ammonium acetate were purchased from Merck KGaA (Darmstadt, Germany) and Wako Pure Chemical Industries, Co., Ltd (Osaka, Japan), respectively.

Animals

Fresh porcine ears from 6–7-month old male pigs were obtained from a local abattoir. Frozen 7-week-old hairless mouse abdominal skin was purchased from Japan SLC, Inc. (Hamamatsu, Japan). Male Hartley guinea pigs, 9 weeks old, 495–550 g, were purchased from Japan SLC, Inc. The guinea pigs were housed individually at room temperature (23 ± 1°C), humidity 55 ± 7%, with a 12-h night/day cycle, and supplied with a standard pellet diet and water *ad libitum*. The experiments were designed according to the Guidelines for the Care and Use of Laboratory Animals adopted by the Committee of Animal Care and Use (CACU) at TTI ellebeau Inc., and the protocols were reviewed and approved by the CACU.

Iontophoretic patch system

The iontophoresis patch used in the present study was composed of an electrode sheet and a reservoir retaining anodal or cathodal solution. Polyester nonwoven cloth coated with silver/silver chloride composition paste (DuPont, Research Triangle Park, NC, USA) was used as both anodal and cathodal electrodes. Each electrode was housed in a reservoir and connected to an electric current controller (SDPS-518; Syrinx Inc. Tokyo, Japan). The reservoir was comprised of polyester nonwoven material and housed in polyethylene foam to form an adhesive pad with flexibility to allow for animal motions. Volumes and active surface areas of the reservoir for in-vitro and in-vivo iontophoretic patches were 180 µl with 0.79 cm^2 (diameter: 1 cm) and 260 µl with 1.13 cm^2 (diameter: 1.2 cm), respectively.

In-vitro protocol

Skin preparation

Porcine ear skin was prepared for the skin permeation study according to reported methods.[22–24] Ears were cleaned under running cold water and the hairs were shaved. The whole skin was carefully removed from the outer region of the ear and separated from the underlying cartilage with a scalpel. The skin was then dermatomed with an average thickness of 750 µm using a skin graft knife (PM-14701; Padgett Instruments, Inc., Kansas, MO, USA). The pieces of skin obtained (3.5 cm × 3.5 cm) were individually wrapped in plastic films and stored for no more than 1 month at −80°C until use. Fresh dermatomed porcine ear skin (without frozen storage) was also used for the skin permeation experiment to compare transdermal permeability of tramadol between fresh and frozen skin.

Guinea pigs were asphyxiated using carbon dioxide and the abdominal skin was shaved and excised. The excised skin was immediately dermatomed to a 750-µm thickness using the skin graft knife, and used immediately for the skin permeation study.

Frozen full-thickness hairless mouse skin was stored for no more than 1 month at −80°C until use. In the case of frozen skin, the required pieces of skin were thawed at room temperature for 30 min.

Skin permeation study

The in-vitro skin permeation study for tramadol hydrochloride by anodal iontophoresis was carried out using PermeGear side-by-side horizontal diffusion cells (orifice diameter: 10 mm; PermeGear Inc., Hellertown, PA, USA) with fresh or frozen porcine ear skin, fresh guinea-pig abdominal skin or frozen hairless mouse abdominal skin as the membrane.[18,24–26] The anodal iontophoresis patch containing 10 mg of tramadol hydrochloride was clamped between the skin and donor chamber, and tightly attached on the stratum corneum side of the skin. An AgCl electrode was placed in the sampling port of the receptor chamber as the cathode. The receptor chamber was filled with 3.4 ml of saline and the temperature of the solution in donor and receiver chambers was maintained at 32°C. To examine current-dependent transdermal delivery of tramadol, both anodal and cathodal electrodes were connected to the electric current controller, and constant current (three levels: 125, 250 and 380 µA/cm^2) was

applied for 4 h. Samples (200 µl) from the receiver chamber were collected, with replacement of saline, at 0, 60, 120, 180 and 240 min, and stored at −20°C until analysed by HPLC.

HPLC for in-vitro samples

The samples were assayed using reverse phase ultra fast liquid chromatography (UFLC). The UFLC system comprised a system controller CBM-20A, an auto-sampler SIL-20A HT, an on-line degasser DGU-20A3, a UV-VIS detector SPD-20A, a column oven CTO-20A and two solvent delivery unit LC-20AD (Shimadzu Co., Kyoto, Japan), and was equipped with a CAPCELL PAK C18 UG120 column (4.6 × 150 mm; particle size, 5 µm) purchased from Shiseido Co., Ltd (Tokyo, Japan). The mobile phase comprising 50% (v/v) acetonitrile with 0.1% (v/v) acetic acid (AcOH) and 50% (v/v) 10 mM sodium dodecyl sulfate solution with 0.1% (v/v) AcOH was delivered at a flow rate of 1 ml/min. The column oven was maintained at 50°C. The injection volume was 10 µl. Tramadol was detected at 272 nm by UV detection. Standard curves were linear over the range 1–300 µg/ml ($r^2 > 0.999$), and the limit of quantification (LOQ) was 1.0 µg/ml.

Data analysis

The cumulative amount of tramadol permeated was plotted against time, and then the steady-state skin permeation fluxes were calculated from the straight line portion of the curve and the intercept of the straight line on the *x*-axis gave the lag time.[26] The experiment was performed in triplicate or quadruplicate and the values were expressed as means ± SD. Statistical analysis of the effects of species differences and increasing current on the steady-state skin permeation flux and the lag time was performed using a one-way analysis of variance followed by Bonferroni's method.[21,26] Linear regression analysis was performed to examine correlation between the steady-state skin permeation flux and current intensity. All statistic analyses were performed using SPSS Statistics 17.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

In-vivo protocol (pharmacokinetic study)

Transdermal iontophoretic administration

Before application of iontophoretic patches, the hairs of the abdominal area were shaved. The iontophoretic anodal patch containing 20 mg of tramadol hydrochloride and cathodal patch containing saline were placed on the abdominal area of the guinea pig, separated by about 2 cm, and fixed into position on the skin using a medical elastic bandage (Multipore; 3 M, Tokyo, Japan). The electrodes in both patches were connected to the electric current controller and a constant current (250 µA/cm^2) was applied. Animals with the tramadol iontophoretic patches but no current applied served as the control group (passive condition group). During the current application, the animals were housed individually in plastic cages (14 × 21 × 12 cm) in a conscious and freely moving condition, and were supplied with a standard pellet diet and water *ad libitum*. Blood samples (150 µl) were collected via a cannula (pre-inserted into the jugular vein 2 days before iontophoretic administration) at 1, 1.5, 2, 2.5, 3 and 6 h and stored at −20°C until analysed by LC/MS/MS. At 10 min and 24 h after the termination of current supply, macroscopic observation on the skin to which the patches were applied was performed in a single-blind manner. The animals with iontophoretic patches containing saline instead of tramadol served as the reference group for macroscopic observation.

Pharmacokinetic parameters of tramadol after intravenous administration were required to calculate the transdermal absorption rate by the numeric deconvolution method. Therefore, 2.5 mg of tramadol hydrochloride in 0.5 ml of saline was intravenously injected via a hind limb vein in conscious guinea pigs, and blood samples (150 µl) were collected via a cannula (pre-inserted into the jugular vein) at 5, 10, 20, 30, 60, 90, 120, 180 and 240 min; the plasma fractions were stored at −20°C until used in the LC/MS/MS analysis.

LC/MS/MS for in-vivo samples

A plasma sample (20 µl) was transferred to a 1.5-ml polypropylene tube and then 250 µl of internal standard working solution (30 ng/ml buspirone acetonitrile) and 200 µl of water were added. After vortex mixing for 10 s, the mixture was centrifuged at 4°C, 16 200 g for 10 min. The supernatant was transferred to injection vials and a 5-µl aliquot was injected into the LC/MS/MS. The LC/MS/MS assay was performed according to reported methods with modifications.[27,28] The system included the Prominence UFLC system (Shimadzu Co.) and API 4000 mass spectrometer with Turbo Ion spray (Applied Biosystems/MDS Sciex, Toronto, ON, Canada) operating in the positive ion mode. The chromatographic separations were performed on a CAPCELL PAK C18 column (2 × 50 mm; particle size, 3 µm) purchased from Shiseido Co., Ltd. Mobile phase A consisted of water with 10 mmol/l ammonium acetate and mobile phase B was 100% acetonitrile. The gradient was as follows: 0–0.50 min, solvent B maintained at 10% B; 0.50–4.00 min, linear gradient from 10 to 80% B; 4.00–5.00 min, maintained at 80% B. The flow rate was 0.25 ml/min and 5 µl was injected for each analysis. The column and autosampler were maintained at 40°C and 4°C, respectively. Quantitation was done using multiple reaction monitoring mode to monitor protonated precursor→product ion transition of m/z 264.1→58 for tramadol, and 387→122 for internal standard. Standard curves were linear over the range 1.5–1500 ng/ml ($r^2 > 0.999$) and the LOQ was 1.5 ng/ml.

Data analysis

All pharmacokinetic data analysis was performed using WinNonlin 5.2.1 (Pharsight Inc., St. Louis, MO, USA). The plasma concentration versus time profile after intravenous injection was analysed using a one-compartment model, and pharmacokinetic parameters such as elimination rate constant (kel) and volume of distribution (V) were calculated and used in the constrained numeric deconvolution. The transdermal absorption rate (µg/cm^2 per h) during the iontophoretic delivery was determined based on the constrained numeric deconvolution method, a model-independent analytic deconvolution technique: intravenous data were analysed as a first step of the deconvolution to estimate the tramadol disposition function, and then iontophoretic data were deconvolved in a second step to estimate the unknown transdermal input profile given the

disposition function estimated in the first step.[29–32] The experiment was performed in triplicate and the values were expressed as means ± SD.

Results

In-vitro iontophoresis

As shown in Figure 1, the cumulative amounts of tramadol permeated across frozen porcine ear skin, fresh guinea-pig abdominal skin and frozen hairless mouse abdominal skin increased in a time- and current-dependent manner when constant current (175, 250 and 380 μA/cm^2) was applied for 4 h. The skin permeation profiles of tramadol across frozen and fresh porcine ear skin at 250 μA/cm^2 were found to be nearly identical over 4 h. The amounts of tramadol permeated across the different skin types when no current was applied (passive condition) were below the LOQ of the HPLC analysis over 4 h.

Tables 1 and 2 show the effects of species variation and increasing current on the steady-state skin permeation flux of tramadol, and the lag-time of percutaneous delivery of tramadol, respectively. The steady-state skin permeation fluxes at each of three currents (175, 250 and 380 μA/cm^2) showed no significant differences among the three skin types (pig, guinea pig, hairless mouse). In all the skin types, the steady-state percutaneous flux increased current-dependently, and excellent linear relationships were observed between the steady-state skin permeation flux of tramadol and current intensity ($r^2 = 0.999$ in porcine skin; $r^2 = 0.992$ in guinea-pig skin; $r^2 = 0.998$ in hairless mouse skin). Unlike the steady-state skin permeation fluxes, the lag times showed a significant difference between pig and the other two species (guinea pig and hairless mouse) at each of the three current applications: the lag time of percutaneous delivery of tramadol across pig skin was significantly longer than that across guinea pig skin and hairless mouse skin at all current intensities used. Increasing current intensity had no effect on the lag time in any of the skin types evaluated in the study.

Taken together, anodal iontophoresis provided a current-dependent increase in the steady-state skin permeation flux of tramadol without significant interspecies differences among pig, guinea pig and hairless mouse skin, regardless of whether fresh or frozen skin was used. On the other hand, the lag time of percutaneous delivery of tramadol induced by iontophoresis exerted a significant interspecies difference but not current dependency.

In-vivo iontophoresis

Plasma levels of tramadol rapidly decreased after intravenous injection and were fitted to first-order elimination kinetics

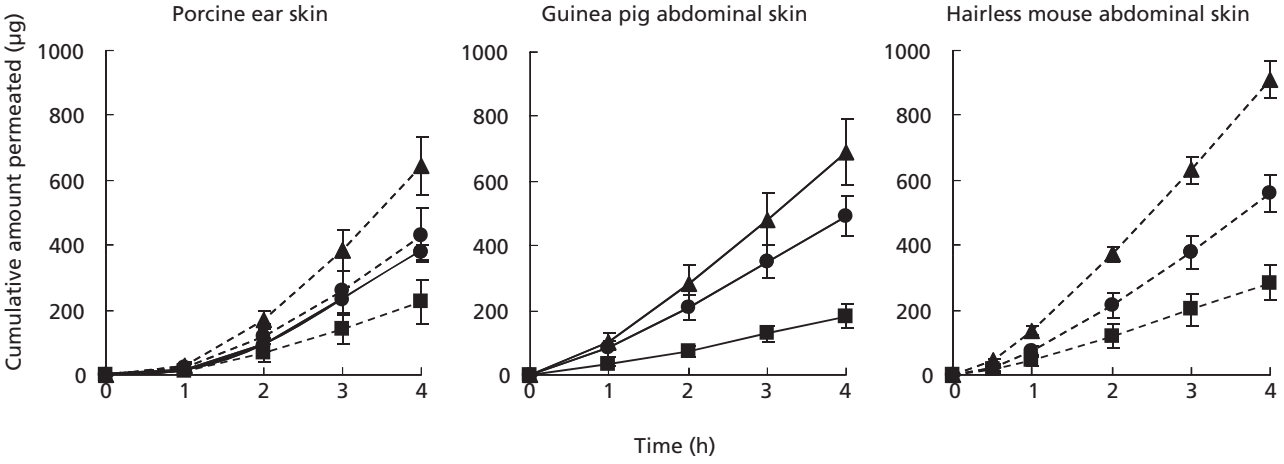

Figure 1 Effect of current strength on in-vitro skin permeation profiles of tramadol across porcine ear skin, guinea-pig abdominal skin and hairless mouse abdominal skin. Current intensity: 125 μA/cm^2 (■); 250 μA/cm^2 (●); 380 μA/cm^2 (▲). Solid lines represent fresh skin preparations. Dashed lines represent previously frozen skin. Data represent the mean ± SD ($n = 3$–4).

Table 1 Effect of species variation and increasing current on the steady-state skin permeation flux of tramadol

Current intensity (μA/cm^2)	Steady-state skin permeation flux (μg/cm^2 per h)		
	Porcine ear skin	Guinea pig abdominal skin	Hairless mouse abdominal skin
125	104 ± 25.9	68.2 ± 13.3	101 ± 16.3
250	214 ± 33.4**	180 ± 16.9**	205 ± 16.3**
380	302 ± 40.4**,##	262 ± 282**,##	324 ± 19.5**,##

Data represent mean ± SD ($n = 3$–4). **$P < 0.01$, significantly different compared with 125 μA/cm^2 in the same skin type. ##$P < 0.01$, significantly different compared with 250 μA/cm^2 in the same skin type.

Table 2 Effect of species variation and increasing current on the lag time of percutaneous delivery of tramadol

Current intensity (μA/cm^2)	Lag time (h)		
	Porcine ear skin	Guinea pig abdominal skin	Hairless mouse abdominal skin
125	1.25 ± 0.183	0.613 ± 0.026**	0.543 ± 0.247**
250	1.31 ± 0.192	0.530 ± 0.160**	0.767 ± 0.165*
380	1.32 ± 0.026	0.655 ± 0.154**	0.610 ± 0.020**

Data represent mean ± SD ($n = 3$–4). *$P < 0.05$ and **$P < 0.01$, significantly different compared with porcine ear skin at the same current, respectively.

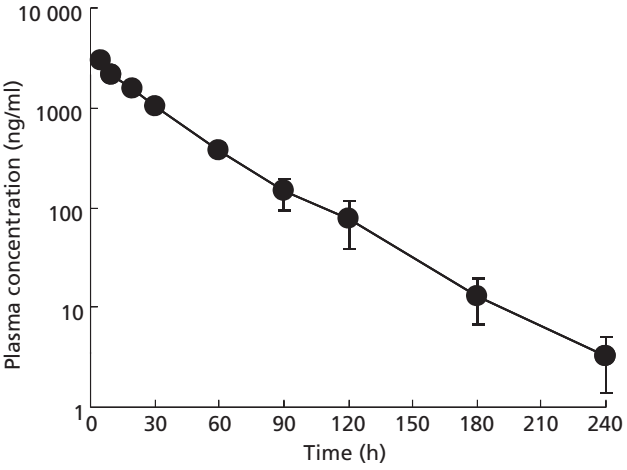

Figure 2 Plasma concentrations of tramadol (2.5 mg) after intravenous administration in guinea pigs. Data represent the mean ± SD ($n = 3$).

(Figure 2); the $t^1/_2$, kel and V were 0.291 ± 0.019 h, 2.39 ± 0.153 h^{-1} and 1349 ± 104 ml/kg, respectively.

When the anodal iontophoresis patch system was applied to the abdominal skin of conscious guinea pigs, plasma concentrations of tramadol increased steadily and reached steady state 3 h after the initiation of current supply (250 μA/cm^2), and the average plasma concentration of tramadol at steady state was 336 ± 83.3 ng/ml (Figure 3). On the other hand, plasma levels of tramadol in guinea pigs treated with the tramadol patch without current supply were below the LOQ of the LC/MS/MS analysis. Very slight erythema was observed in the skin when 250 μA/cm^2 current was applied for 6 h in combination with the tramadol patch or saline patch (incidence rate: 3/3 in each group). This macroscopic change disappeared completely within 24 h after removal of the patch. No erythema was observed at the site with the tramadol patch alone without applied current. The in-vivo transdermal absorption rate, determined by the numeric deconvolution method, increased steadily and reached steady state in 3 h of the iontophoresis, and the average absorption rate at steady state was 499 ± 161 μg/cm^2 per h (Figure 3).

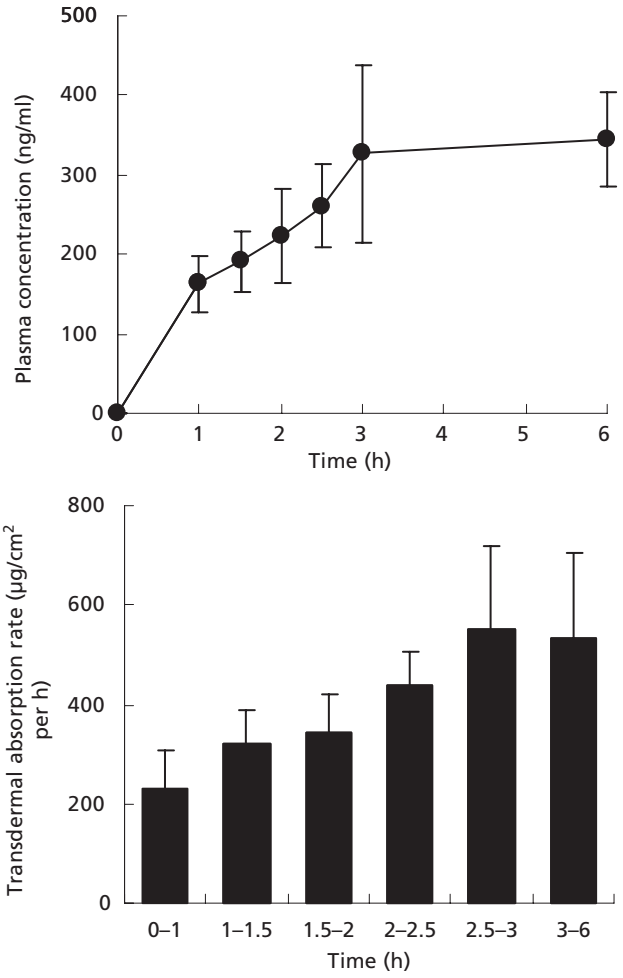

Figure 3 In-vivo pharmacokinetics of tramadol during the transdermal iontophoretic administration (applied current: 250 μA/cm^2 for 6 h) in guinea pigs. Data represent the mean ± SD ($n = 3$).

Discussion

The composition and architecture of the stratum corneum render it a protective barrier against transdermal administration of therapeutic agents. In spite of the many advantages offered by transdermal drug delivery by passive permeation, the route is limited to delivery of small, relatively lipophilic molecules into the systemic circulation due to the barrier function of stratum corneum.[13,18] It is difficult to exploit the transdermal route to deliver therapeutic amounts of tramadol by conventional passive transdermal administration, since tramadol hydrochloride is hydrophilic (log P at pH 7.0 is 1.35) and its oral daily dose is quite large (50–400 mg) with high bioavailability (~70%).[3,4] Iontophoresis is a technique used to enhance the transdermal delivery of compounds through the skin via application of a small electric current, and enables transdermal delivery of relatively large amounts of hydrophilic charged molecules compared with the conventional passive transdermal approach.[16–18,33] The pKa of tramadol hydrochloride is 9.41, and so anodal iontophoresis of tramadol hydrochloride is probably the most promising transdermal drug delivery system to attain therapeutic blood levels of tramadol.

To evaluate transdermal absorption of a molecule, the most relevant membrane is human skin, however, human skin specimens of sufficient size and quality are not readily accessible and are only available in limited amounts. Porcine skin, the histological and biochemical properties of which have been repeatedly shown to be similar to human skin,[34–36] is readily obtainable from abattoirs for in-vitro skin permeation studies. Rodents (mice, rats and guinea pigs) are conventionally used for both in-vitro skin permeation studies and in-vivo pharmacokinetic studies due to their availability, although these types of skin often exert higher transdermal permeation rates than human skin, especially for passive type formulations.[36–38] In addition to interspecies differences based on anatomical and biochemical properties of skin, the storage conditions of skin prior to the experiment (i.e. fresh or frozen) can sometimes influence the skin permeability of

drugs.[34] To examine the effect of species variation and skin storage conditions prior to the experiment on the in-vitro transdermal permeability of tramadol induced by anodal iontophoresis, fresh and frozen porcine ear skin, fresh guinea pig abdominal skin and frozen hairless mouse abdominal skin were used in the present in-vitro skin permeation studies. The in-vitro steady-state skin permeation flux of tramadol showed no significant differences among the three skin types (pig, guinea pig and hairless mouse) regardless of whether fresh or frozen skin was used, when the same current intensity was applied. Additionally, the skin permeation profiles for tramadol across fresh and frozen porcine ear skin were nearly identical. Accordingly, the present findings indicate that the in-vitro steady state of transdermal delivery of tramadol induced by anodal iontophoresis is hardly affected by skin anatomical differences and storage conditions. There are several reports demonstrating that in-vitro skin permeability induced by anodal iontophoresis is not influenced by skin type and storage conditions. Iontophoretic transport of hydromorphone was found to be similar in porcine and human skin,[39] and that of lithium was also almost identical through human, pig and rabbit skin.[40] There were also no significant differences in the steady-state fluxes of timolol across fresh rat, rabbit, mouse and guinea-pig skin and frozen human skin.[25] In addition to these previous findings, the present results provide further evidence that anodal iontophoresis substantially reduces the interspecies differences in the transdermal permeation of compounds normally observed in passive diffusion of drugs. Contrary to the steady-state skin permeation flux, the lag time of in-vitro percutaneous delivery of tramadol showed significant differences between porcine skin and rodent skin (guinea pig and hairless mouse). Differences in chemical composition and biophysical properties between porcine skin and the two rodent skin types may affect the affinity of tramadol with the skin tissues, causing the delay in reaching a steady-state flux.

Controlling plasma levels of tramadol is crucial in the treatment of chronic pain to maintain therapeutic plasma concentration with high accuracy, to avoid the risk of toxicity due to sudden peaks in plasma levels and abuse/addiction potential due to overexposure. Although one of benefits of transdermal iontophoresis is controlled drug delivery based on the percutaneous permeation of drug in proportion to current, some molecules have been reported to exert a non-current-dependent relationship between flux and current.[41] In the present skin permeation study, the in-vitro steady-state skin permeation flux of tramadol increased in a current-dependent manner in all three skin types (porcine ear, guinea pig abdomen and hairless mouse abdomen). These findings indicate that the percutaneous delivery of tramadol can be controlled directly by varying the current strength. Thus, individual dose requirements of patients for chronic pain management can be defined without the risk of side-effects by adjusting current strength.

Anesthetized rats have been used for the in-vivo assessment of transdermal iontophoretic drug delivery in various studies.[22,42,43] The pharmacokinetic behaviour of tramadol has been reported to be affected by anaesthesia,[44] and so in-vivo pharmacokinetic evaluation using conscious animals appears to be preferable for tramadol. In a preliminary study, guinea pigs exerted stable and calm behaviour without any interruption of the experimental procedure during current supply under the conscious and free-moving condition compared with rats. Therefore the in-vivo pharmacokinetic properties of tramadol were evaluated in conscious free-moving guinea pigs. In the pharmacokinetic study for intravenous injection, tramadol was eliminated according to the one-compartment model in guinea pigs as well as in rats[45] and humans.[46,47] The obtained pharmacokinetic data such as kel and V were used in the numeric deconvolution method to determine the in-vivo transdermal absorption rate of tramadol in guinea pigs. When the anodal iontophoretic patch system was applied in combination with a constant current supply (250 $\mu A/cm^2$) for 6 h on the abdominal skin of conscious guinea pig, both plasma concentrations and in-vivo transdermal absorption rate of tramadol reached a peak 3 h after the initiation of current supply and were sustained for the subsequent 3 h. This result indicates that the transdermal input rate of tramadol was in equilibrium with its elimination rate and the steady-state plasma concentration of tramadol was maintained under constant current supply. In contrast to the present result, a passive-type transdermal delivery system of tramadol using a polymeric matrix was reported not to sustain stable efficacy after reaching the peak effect, but nevertheless to provide prolonged efficacy compared with oral administration in rats.[12] One of advantages of iontophoretic transdermal drug delivery over passive transdermal delivery is the maintenance of stable blood concentrations of drugs and therefore efficacy as a result of controlling the transdermal transport of permeates based on the amount of applied current.[8,12,16,18] The present result is in agreement with this technical characterisation of transdermal iontophoretic drug delivery. Iontophoresis by itself is known to have a potential for skin irritation, especially at high current intensity.[16,18] Although the application of the tramadol patch alone for 6 h without current supply showed no macroscopic alternations at the skin, the iontophoresis (250 $\mu A/cm^2$ for 6 h) in combination with either the tramadol patch or saline patch caused a very slight but reversible erythema. This observation suggests that the slight macroscopic alteration caused by the anodal iontophoresis of tramadol is probably due to the continuous current supply and not tramadol permeating the skin.

The in-vivo steady-state transdermal absorption rate of tramadol (499 $\mu g/cm^2$ per h) was almost 2.8-times higher than the in-vitro steady-state skin permeation flux (180 $\mu g/cm^2$ per h) in guinea pigs when a constant current (250 $\mu A/cm^2$) was applied. Higher in-vivo transdermal delivery flux compared with in-vitro percutaneous permeation flux has been reported for several molecules.[42,43] Dermal blood supply has been found to play a significant role in the systemic and underlying tissue solute absorption during iontophoretic delivery.[48] Conscious guinea pigs were used in the present in-vivo pharmacokinetic study, and therefore intact cutaneous microcirculation may provide efficient clearance of tramadol from the skin.

Pig skin has been suggested as the most relevant surrogate for human skin to predict percutaneous penetration of compound in humans, since its histological and physiological properties are similar to those of human skin.[35,36] Rodent skin has been conventionally used for evaluation of skin

permeability for various compounds, however, it is often more permeable, especially for passive-type transdermal formulations, than pig and human skin.[36] In the present study, the in-vitro steady-state skin permeation of tramadol did not show any significant interspecies differences among pig, guinea pig and hairless mouse. A quantitative comparison of permeability between human and animal skin (pig and guinea pig), statistically analysing previously published reports, demonstrated that in-vitro permeability of both pig and guinea pig skin is equally correlated with that of human skin.[34] Accordingly, plasma concentrations of tramadol in humans was extrapolated from the in-vitro skin permeation flux of tramadol across pig skin (214 μg/cm^2 per h at 250 μA/cm^2) and the in-vivo transdermal absorption rate determined in guinea pig (499 μg/cm^2 per h at 250 μA/cm^2). The iontophoretic delivery of tramadol has been reported to be based on the one-compartment continuous infusion model with zero-order absorption.[29,42,49,50] Tramadol is known to be eliminated according to the one-compartment model in humans.[46,47] Therefore, pharmacokinetic parameters of tramadol in humans cited from a previous report[46] were applied to Equation 1, based on the one-compartment continuous infusion model with zero-order absorption and first-order elimination:

$$Cp = KA/(kel_h \times V_h) \times (1 - e^{-kel_h \times t}) \quad (1)$$

where Cp is the plasma concentration of tramadol in humans; K is the in-vitro steady-state skin permeation flux across porcine skin (214 μg/cm^2 per h) and in-vivo steady-state transdermal absorption rate in guinea pigs (499 μg/cm^2 per h) determined in the present study at 250 μA/cm^2; A is the area of the patch for human use: 10~20 cm^2; kel_h is the elimination rate constant in humans, calculated from the $t^1/_2$ (4.38 h) after oral administration of tramadol hydrochloride (50 mg) using an equation of $kel = \ln 2/t^1/_2$; and V_h is the volume of distribution in humans, calculated from the pharmacokinetic parameters, such as AUC (1138.52 μg h/l) and F (bioavailability, 68.4%), after oral administration of tramadol hydrochloride (50 mg) using an equation of $AUC = F \times dose/(kel_h \times V_h)$. As a result of this calculation, plasma concentrations of tramadol at steady-state in humans during iontophoretic administration (250 μA/cm^2) were estimated to range from 70 to 320 ng/ml using a 10~20-cm^2 patch. The therapeutic serum level of tramadol in humans was reported to be 100–300 ng/ml.[51] Taken together, anodal iontophoresis can attain therapeutic blood concentrations of tramadol with reasonable patch sizes with acceptable current intensity.

Tramadol hydrochloride is classified as a 'step II' opioid in the World Health Organization pain treatment ladder, and it has been used to treat moderate to moderately severe pain for 30 years.[3] In the management of chronic pain, both 'as needed' and 'around the clock' administration of analgesics has been required.[52] Therefore, there are many formulations for tramadol hydrochloride with differing pharmacokinetic profiles: for example rapid increase of plasma concentrations induced by intravenous injection and immediate release type oral tablets, and sustainable plasma levels induced by extended release oral tablets.[4] Intravenous injection and immediate release oral formulations must be taken several times a day due to the short half-life, and may lead an increased risk of adverse events associated with high peak plasma concentrations.[3] Extended release type oral formulations take approximately 4 days to achieve steady-state plasma concentrations during multiple dosing, possibly due to saturation of the hepatic 'first-pass' effect.[4,53] In addition, oral formulations are not suitable for patients experiencing nausea and vomiting, both common adverse reactions caused by tramadol. Opioid abuse and addiction are serious medical and social problems. Oral controlled-release formulations of opioids including tramadol have been reported to possess a potential risk for abuse and overdosing due to dose dumping caused by uncontrolled release of drugs when co-ingested with alcohol.[54] Transdermal delivery decreases the possible abuse and overdosing potential of tramadol by not only avoiding peak and trough plasma concentrations but also reducing the risk of dose dumping observed in oral controlled release formulations.[11,12] The present study demonstrates that anodal iontophoresis can control skin permeation flux of tramadol in a current-dependent manner and deliver therapeutic amounts of tramadol. Thus, transdermal iontophoresis is a potential alternative administration route for tramadol in the treatment of chronic pain, with improved compliance and reduced risk of adverse events and abuse/addiction as observed with existing formulations.

Conclusions

In an in-vitro skin permeation study, anodal iontophoresis with constant current supply provided current-dependent transdermal delivery of tramadol with no significant interspecies differences among pig, guinea pig and mouse skin, regardless of skin storage condition (fresh or frozen). In an in-vivo pharmacokinetic study using conscious guinea pigs, the steady-state transdermal absorption rate was found to be sufficient to maintain therapeutically relevant blood concentrations of tramadol for the management of chronic pain.

Declarations

Conflict of interest

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

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References

1. Dayer PL *et al*. The pharmacology of tramadol. *Drugs* 1994; 47(Suppl. 1): 3–7.
2. Lewis KS, Han NH. Tramadol: a new centrally acting analgesic. *Am J Health Syst Pharm* 1997; 54: 643–652.
3. Mongin G. Tramadol extended-release formulations in the management of pain due to osteoarthritis. *Expert Rev Neurother* 2007; 7: 1775–1784.
4. Leppert W, Luczak J. The role of tramadol in cancer pain treatment – a review. *Support Care Cancer* 2005; 13: 5–17.
5. Grond S *et al*. Clinical pharmacokinetics of transdermal opioids: focus on transdermal fentanyl. *Clin Pharmacokinet* 2000; 38: 59–89.
6. Berner B, John VA. Pharmacokinetic characterisation of transdermal delivery systems. *Clin Pharmacokinet* 1994; 26: 121–134.
7. El-Setouhy DA, El-Ashmony SM. Ketorolac trometamol topical formulations: release behaviour, physical characterization, skin permeation, efficacy and gastric safety. *J Pharm Pharmacol* 2010; 62: 25–34.
8. Prausnitz MR, Langer R. Transdermal drug delivery. *Nat Biotechnol* 2008; 26: 1261–1268.
9. Raffa RB. Basic pharmacology relevant to drug abuse assessment: tramadol as example. *J Clin Pharm Ther* 2008; 33: 101–108.
10. Klotz U. Tramadol – the impact of its pharmacokinetic and pharmacodynamic properties on the clinical management of pain. *Arzneimittelforschung* 2003; 53: 681–687.
11. Ammar HO *et al*. Polymeric matrix system for prolonged delivery of tramadol hydrochloride, part I: physicochemical evaluation. *AAPS PharmSciTech* 2009; 10: 7–20.
12. Ammar HO *et al*. Polymeric matrix system for prolonged delivery of tramadol hydrochloride, part II: biological evaluation. *AAPS PharmSciTech* 2009; 10: 1065–1070.
13. Brown MB *et al*. Dermal and transdermal drug delivery systems: current and future prospects. *Drug Deliv* 2006; 13: 175–187.
14. Naik A *et al*. Transdermal drug delivery: overcoming the skin's barrier function. *Pharm Sci Technol Today* 2000; 3: 318–326.
15. Prausnitz MR *et al*. Current status and future potential of transdermal drug delivery. *Nat Rev Drug Discov* 2004; 3: 115–124.
16. Batheja P *et al*. Transdermal iontophoresis. *Expert Opin Drug Deliv* 2006; 3: 127–138.
17. Dixit N *et al*. Iontophoresis – an approach for controlled drug delivery: a review. *Curr Drug Deliv* 2007; 4: 1–10.
18. Nair V *et al*. Transdermal iontophoresis. Part I: basic principles and considerations. *Methods Find Exp Clin Pharmacol* 1999; 21: 39–51.
19. Vemulapalli V *et al*. Synergistic effect of iontophoresis and soluble microneedles for transdermal delivery of methotrexate. *J Pharm Pharmacol* 2008; 60: 27–33.
20. Ackaert OW *et al*. Transdermal iontophoretic delivery of a novel series of dopamine agonists in vitro: physicochemical considerations. *J Pharm Pharmacol* 2010; 62: 709–720.
21. Nicoli S *et al*. Effect of lipopeptides and iontophoresis on aciclovir skin delivery. *J Pharm Pharmacol* 2010; 62: 702–708.
22. Cazares-Delgadillo J *et al*. Using transdermal iontophoresis to increase granisetron delivery across skin in vitro and in vivo: effect of experimental conditions and a comparison with other enhancement strategies. *Eur J Pharm Sci* 2010; 39: 387–393.
23. Patel SR *et al*. In vitro and in vivo evaluation of the transdermal iontophoretic delivery of sumatriptan succinate. *Eur J Pharm Biopharm* 2007; 66: 296–301.
24. Sylvestre JP *et al*. In vitro optimization of dexamethasone phosphate delivery by iontophoresis. *Phys Ther* 2008; 88: 1177–1185.
25. Kanikkannan N *et al*. In vitro transdermal iontophoretic transport of timolol maleate: effect of age and species. *J Control Release* 2001; 71: 99–105.
26. Kumar MG, Lin S. Transdermal iontophoresis of hydromorphone across hairless rat skin in vitro. *Pharm Dev Technol* 2009; 14: 106–115.
27. Patel BN *et al*. An accurate, rapid and sensitive determination of tramadol and its active metabolite O-desmethyltramadol in human plasma by LC-MS/MS. *J Pharm Biomed Anal* 2009; 49: 354–366.
28. Shilo Y *et al*. Pharmacokinetics of tramadol in horses after intravenous, intramuscular and oral administration. *J Vet Pharmacol Ther* 2008; 31: 60–65.
29. Fiset P *et al*. Biopharmaceutics of a new transdermal fentanyl device. *Anesthesiology* 1995; 83: 459–469.
30. Hung OR *et al*. Pharmacokinetics of inhaled liposome-encapsulated fentanyl. *Anesthesiology* 1995; 83: 277–284.
31. Park K *et al*. Passive versus electrotransport-facilitated transdermal absorption of ketorolac. *Clin Pharmacol Ther* 1998; 63: 303–315.
32. Verotta D. An inequality-constrained least-squares deconvolution method. *J Pharmacokinet Biopharm* 1989; 17: 269–289.
33. Balaguer-Fernandez C *et al*. Combined strategies for enhancing the transdermal absorption of midazolam through human skin. *J Pharm Pharmacol* 2010; 62: 1096–1102.
34. Barbero AM, Frasch HF. Pig and guinea pig skin as surrogates for human in vitro penetration studies: a quantitative review. *Toxicol In Vitro* 2009; 23: 1–13.
35. Dick IP, Scott RC. Pig ear skin as an in-vitro model for human skin permeability. *J Pharm Pharmacol* 1992; 44: 640–645.
36. Godin B, Touitou E. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. *Adv Drug Deliv Rev* 2007; 59: 1152–1161.
37. Fang JY *et al*. Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations: in vitro and in vivo studies. *Int J Pharm* 1999; 178: 83–92.
38. Sato K *et al*. Species differences in percutaneous absorption of nicorandil. *J Pharm Sci* 1991; 80: 104–107.
39. Padmanabhan RV *et al*. In vitro and in vivo evaluation of transdermal iontophoretic delivery of hydromorphone. *J Control Release* 1990; 11: 23–135.
40. Phipps JB *et al*. Iontophoretic delivery of model inorganic and drug ions. *J Pharm Sci* 1989; 78: 365–369.
41. Medi BM, Singh J. Electronically facilitated transdermal delivery of human parathyroid hormone (1-34). *Int J Pharm* 2003; 263: 25–33.
42. Chaturvedula A *et al*. Dermal, subdermal, and systemic concentrations of granisetron by iontophoretic delivery. *Pharm Res* 2005; 22: 1313–1319.
43. Luzardo-Alvarez A *et al*. In vivo iontophoretic administration of ropinirole hydrochloride. *J Pharm Sci* 2003; 92: 2441–2448.
44. Zhao Y *et al*. Pharmacokinetics of tramadol in rat plasma and cerebrospinal fluid after intranasal administration. *J Pharm Pharmacol* 2008; 60: 1149–1154.
45. Parasrampuria R *et al*. Route-dependent stereoselective pharmacokinetics of tramadol and its active O-demethylated metabolite in rats. *Chirality* 2007; 19: 190–196.
46. Kotb HI *et al*. Pharmacokinetics of oral tramadol in patients with liver cancer. *J Opioid Manag* 2008; 4: 99–104.
47. Murthy BV *et al*. Pharmacokinetics of tramadol in children after i.v. or caudal epidural administration. *Br J Anaesth* 2000; 84: 346–349.
48. Cross SE, Roberts MS. Importance of dermal blood supply and epidermis on the transdermal iontophoretic delivery of monovalent cations. *J Pharm Sci* 1995; 84: 584–592.

49. Nugroho AK *et al*. Compartmental modeling of transdermal iontophoretic transport II: in vivo model derivation and application. *Pharm Res* 2005; 22: 335–346.
50. Nugroho AK *et al*. Pharmacokinetics and pharmacodynamics analysis of transdermal iontophoresis of 5-OH-DPAT in rats: in vitro-in vivo correlation. *J Pharm Sci* 2006; 95: 1570–1585.
51. Barkin RL. Extended-release tramadol (ULTRAM ER): a pharmacotherapeutic, pharmacokinetic, and pharmacodynamic focus on effectiveness and safety in patients with chronic/persistent pain. *Am J Ther* 2008; 15: 157–166.
52. Evans HC, Easthope SE. Transdermal buprenorphine. *Drugs* 2003; 63: 1999–2012.
53. Hair PI *et al*. Tramadol extended-release tablets. *Drugs* 2006; 66: 2017–2030.
54. Traynor MJ *et al*. Influence of alcohol on the release of tramadol from 24-h controlled-release formulations during in vitro dissolution experiments. *Drug Dev Ind Pharm* 2008; 34: 885–889.