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Research paper

Microdialysis assessment of percutaneous penetration of ketoprofen after transdermal administration to hairless rats and domestic pigs

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ABSTRACT

The study was performed to evaluate the percutaneous penetration of ketoprofen after transdermal administration using a microdialysis technique in pigs, in comparison with rats. Ketoprofen release from patches was determined by analysis of the remaining drug content after application to hairless rats and pigs. Skin and knee joint penetration of ketoprofen was tested by microdialysis, and recovery was determined by retrodialysis. Residual rates in hairless rats and pigs were 68.1 ± 1.6% and 81.7 ± 4.4%, respectively, at 10 h. The average recoveries of ketoprofen over 480 min in the skin and knee joint cases were 72.0 ± 3.4% and 9.8 ± 6.2% in rats and 72.3 ± 2.5% and 57.6 ± 3.1% in pigs, respectively. In rats, ketoprofen was rapidly absorbed with transdermal administration, with C_{max} values of 191.7 ± 76.2 and 35.5 ± 21.7 ng/mL and AUC_{0-8h} values of 918.2 ± 577.5 and 195.9 ± 137.1 ng h/mL, respectively, for the skin and knee joint. The C_{max} values for the pig were 20.9 ± 18.5 and 3.7 ± 3.0 ng/mL, with AUC_{0-8h} values of 73.1 ± 69.2 and 16.1 ± 16.1 ng h/mL. Ketoprofen concentrations within skin and knee joint of non-application sites in rats and pigs were less than 0.8 ng/mL. Transdermal administration of ketoprofen significantly reduced prostaglandin E2 levels in the skin of the application site and showed a tendency for inhibition in the knee joint. We thus demonstrated that topical patches containing ketoprofen can deliver the drug through the skin and knee joint of pigs and rats via direct diffusion, and microdialysis data with the pig may be useful for the prediction of human tissue penetration of drugs with transdermal administration.

1. Introduction

Transdermal drug delivery offers many important advantages, including protection of active compounds from gastric enzymes, avoidance of the hepatic first-pass effect, and reduction in the risk of the systemic adverse effects [1,2]. Since ketoprofen has many appropriate characteristics, with a low molecular weight, a low melting point, and high lipophilicity, it can more readily permeate the skin than other NSAIDs like indomethacin and diclofenac [3,4]. Several topical formulations of ketoprofen are therefore now commonly used to reduce swelling and inflammation and to relieve pain in the clinical field. In particular, topical patch ketoprofen formulations offer one of the most widely used tools for topical NSAID treatment in Japan, allowing steady supply of the drug into tissues over more than 12 h. A typical ketoprofen patch contains 0.3% ketoprofen (30 mg per sheet) and approximately 56% water with some water-soluble polymers. It has been reported that the residual drug in the patch at 24 h after application in rats is approximately 40% of the administered dose [5]. Since the patch contains large quantities of water, this facilitates maintaining moist skin with only a small amount of stripped stratum corneum and a low risk of skin irritation [6].

Since the pharmacological potency of NSAIDs usually depends on the drug concentration, it is important to examine penetration into target tissues for pharmacodynamic evaluation. Recently, we have demonstrated that the ketoprofen patch can provide the drug in sufficient amounts to inhibit prostaglandin E2 production in the skin and knee joint in rats [7]. However, drug absorption through the skin exhibits species differences, and it is well known that drug absorption in rodent skin, such as that of rats or mice, is generally greater than in human skin [8,9]. In contrast, histological and biochemical properties of pig skin have been shown to be similar to the human case, and there is abundant evidence substantiating the value of the minipig and domestic pig in animal models for human skin permeability [8,9]. Domestic pigs display especially close permeation characteristics to human skin and are more readily available and economical than minipigs [10].

Determination of drug concentrations is generally carried out by the traditional method with homogenized tissues, but this needs many animals and continuous sampling from individuals is difficult. In contrast, microdialysis is currently attracting attention

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as a technique that allows continuous sampling of unbound drug in skin in vivo [11,12]. Since the protein-bound fraction of a drug cannot traverse cell membranes, it is the unbound fraction that usually exhibits pharmacologic effects. While microdialysis for animal models with rats has been established as an estimation tool for cutaneous penetration of topical drugs [13,14], data for pigs are limited.

In the present study, we therefore examined drug amounts released from ketoprofen patches on application to hairless rats and pigs. Furthermore, we investigated the change in concentrations in skin and knee joints in hairless rats and domestic pigs following exposure to topical ketoprofen patches by microdialysis.

2. Materials and methods

2.1. Animals

Studies were carried out in accordance with the Institutional Animal Care and Use Committee of Nipro Patch Co., Ltd. Male HWY hairless rats (240–420 g; Japan SLC, Inc., Shizuoka, Japan) and female mixed strain domestic pigs (19–26 kg, Narc Co., Chiba, Japan) were used. They were housed at 23 ± 2 °C, with a relative humidity of 55 ± 15% and a 12-h/12-h light/dark cycle (light on at 8:00 a.m.). All animals were shaved in application sites for patches before starting the test.

2.2. Drugs

Patches were obtained from Nipro Patch Co., Ltd. (Miltax®, Saitama, Japan). Each contained 0.3% ketoprofen and approximately 56% water with some water-soluble polymers like sodium polyacrylate, sodium carboxymethylcellulose, and gelatin and additives like *N*-methyl-2-pyrrolidone and L-menthol. Other chemical agents used were ethyl carbamate (Wako Pure Chemical Industries, Ltd., Osaka, Japan), isoflurane (Mylan Inc., PA, USA), thiopental sodium (Mitsubishi Tanabe Pharma Co., Osaka, Japan), medetomidine (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), midazolam (Astellas Pharma Inc., Tokyo, Japan), and heparin sodium (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan).

2.3. Evaluation of residual ketoprofen in patches

The residual rate was calculated from the remaining drug content in the patch after finishing the application as an index of the drug amount release, and drug content in the pre-application patch was defined as 100%. The skin of the abdominal region in rats and the hip region in pigs was shaved, and two patches were applied to each containing ketoprofen at 1.6 mg per 7.5 cm^2 (2.5 × 3 cm) for rats and 2.1 mg per 10 cm^2 (3.2 × 3.2 cm) for pigs. The patches were removed and collected, one each at 10 and 24 h after application, and stored at room temperature until assayed for ketoprofen by HPLC.

2.4. In vivo evaluation of probe recovery

In vivo recovery of ketoprofen in hairless rats and domestic pigs was determined by retrodialysis methods [15].

Rats were anesthetized with ethyl carbamate (1.25 g/kg, i.p.) and placed on a temperature-controlled (37 °C) heating pad. The skin of the abdominal region and knee joint was shaved and a linear microdialysis fiber (0.22 mm OD; 0.20 mm ID, Eicom Co., Kyoto, Japan) of length 30 mm for skin and 5 mm for the knee joint and a molecular mass cutoff of 50 kDa was inserted using a 23-gauge guide cannula, resurfacing through an exit puncture as reported previously [7]. The guide was then withdrawn leaving membranes placed within the skin and knee joint. Dialysis probes were inserted into dermis or subcutaneous tissues and the knee joint cavity (Fig. 1C–E). After implantation, the inlet tube of the probe was connected to a microinjection pump (Harvard Apparatus, MA, USA). PBS solution containing 1 μg/mL of ketoprofen was passed through the probe using an injection pump at a 1.2 μL/min and dialysate samples were collected every 60 min for 480 min.

Pigs were anesthetized with medetomidine (40 μg/kg, i.m.), midazolam (0.2 mg/kg, i.m.), and thiopental sodium (5 mg/kg, i.v.) and then maintained on isoflurane (1–3%) in oxygen and placed on a temperature-controlled (37 °C) heating pad. The skin of the hip and bilateral knee joints was shaved and two linear microdialysis fibers of length 30 mm for skin and 15 mm for knee joints were inserted using an 18-gauge guide cannula. After implantation, the inlet tube of the probe was connected to a microinjection pump. PBS solution containing 1 μg/mL of ketoprofen was passed through the probe using an injection pump at a 1.5 μL/min, and dialysate samples were collected every 60 min for 480 min. Rising perfusate flow can be induced to increase the dialysate volume with decrease in the recovery. Since we needed more dialysate volume for the analysis of drug and prostaglandin E2 concentrations in pig microdialysis study, perfusate flow in pig microdialysis made it faster than in rats.

The recovery was determined from the ratio of the concentration loss to the initial concentration in the perfusate:

$$\text{Recovery } (\%) = [(\text{influent dialysate amount-effluent dialysate amount})/\text{influent dialysate amount}] \times 100$$

2.5. In vivo microdialysis study

For the determination of drug concentrations in rats, microdialysis probes were inserted within two sites in the skin and bilateral knee joints as described above and illustrated in Fig. 1A. Probe perfusion was started with a flow rate of a 1.2 μL/min with PBS, and a recovery period of 60 min was allowed for insertion trauma to subside. After 60-min baseline sampling of dialysate, patches containing ketoprofen 0.51 mg per 2.4 cm^2 (0.8 × 3 cm) were applied at the one side point of the skin and knee joint where the microdialysis probe was implanted. Each other point was a non-application site. Dialysate was then sampled at 60-min intervals for up to 480 min after administration. Plasma was collected via the carotid artery catheter.

For the determination of drug and prostaglandin E2 concentrations in pigs, microdialysis probes were inserted within two sites in the skin and bilateral knee joints as indicated above and Fig. 1B. Probe perfusion of skin and knee joint was started with a flow rate of a 1.5 μL/min with PBS, and a recovery period of 60 min was allowed for insertion trauma to subside. After 60-min baseline sampling of dialysate, patches containing ketoprofen 7.5 mg per 35 cm^2 (7 × 5 cm) were applied at one side point of the skin and knee joint where the microdialysis probes were implanted. Each other point was a non-application site of drug. Dialysate was then sampled at 60-min intervals for up to 480 min after administration. Plasma was collected via the central venous catheter. Ketoprofen concentrations in dialysate were measured as unbound fractions, and ketoprofen concentrations in plasma were measured as total bound and unbound fractions. All samples were stored at −40 °C until assayed for ketoprofen and prostaglandin E2.

2.6. Sample analysis

The initial and residual ketoprofen contents of the patches were determined by HPLC. Analytes were separated by L-column (5 μm,

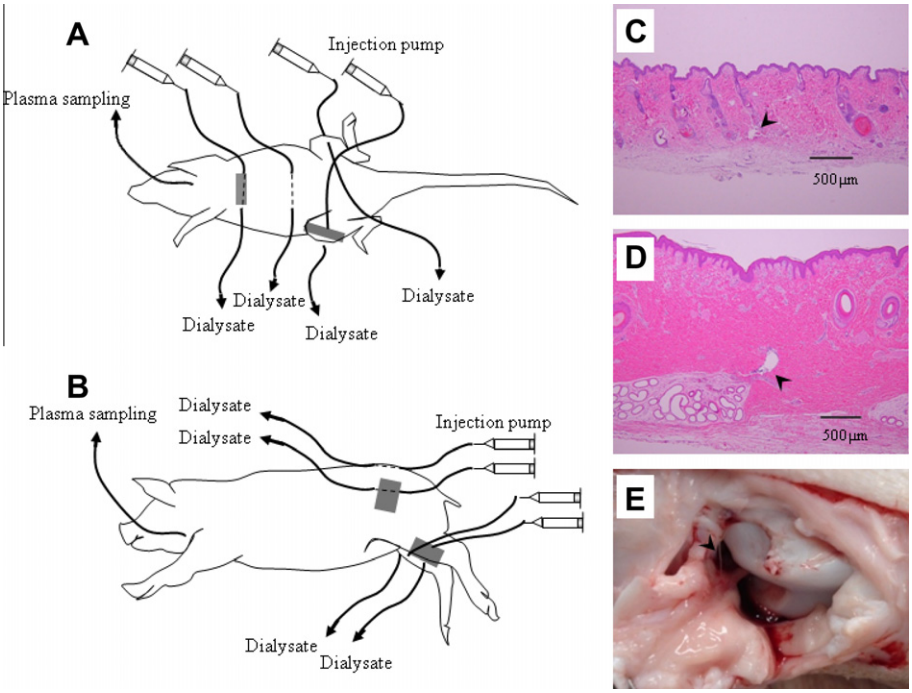

Fig. 1. Schematic diagrams and photomicrographs of skin and knee joint in the rat (A and C) and pig (B, D, and E) showing location of probes indicated by arrow heads. Hematoxylin and eosin staining; original magnification 40× (C and D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

150 × 4.6 mm) (Chemicals Evaluation and Research Institute, Tokyo, Japan) and maintained at 25 °C. The mobile phase consisted of methanol/0.2% acetic acid (75/25, v/v). The peak area correlated linearly with ketoprofen concentration in the range from 3 to 91 μg/mL for rats (r^2 = 0.999) and from 32 to 240 μg/mL for pigs (r^2 = 0.999). Limit of detection for ketoprofen was 1.1 μg/mL, and the coefficient of variation (CV) was 0.02% at 3 μg/mL, 0.20% at 60 μg/mL, and 0.16% at 240 μg/mL. The solvent was analyzed for ketoprofen by dissolving patches in methanol, using methyl 4-hydroxybenzoate as the internal standard. Residual rates in patches after application were expressed as the ratio to the initial content in the patch.

Analysis of ketoprofen in dialysate and plasma was performed with LC-MS/MS (API 2000, Applied Biosystems Ltd., CA, USA). Analytes were separated by UG-120 (5 μm, 150 × 3 mm) (Shiseido Co. Ltd., Tokyo, Japan) and maintained at 40 °C. The mobile phase consisted of acetonitrile/0.1% ammonium formate (60/40, v/v). The peak area correlated linearly with ketoprofen concentration in the range from 1 to 500 ng/mL (r^2 = 0.998). The limit of detection for ketoprofen was 1 ng/mL, and coefficient of variation (CV) was 15% at 5 ng/mL, 12% at 20 ng/mL, and 5% at 50 ng/mL. A 50-μL aliquot of dialysate was added to diclofenac sodium solution (50 μL) as an internal standard and then mixed for 30 s. A 150-μL aliquot of plasma was added to the mobile phase (150 μL) and diclofenac sodium solution (150 μL) and then mixed for 30 s. After solid-phase extraction using Waters Oasis MAX cartridges, the extract was evaporated to dryness under a gentle stream of nitrogen at 50 °C, and the preparation was dissolved in 200 μL of the mobile phase. Solvents from both dialysate and plasma were then routinely analyzed for ketoprofen.

Prostaglandin E2 was assessed by enzyme immunoassay (EIA) according to the manufacturer's protocol (Cayman Chem. Co., Ann Arbor, MI), with a stated detection limit of 30 pg/mL. The specificity of the EIA was 100%, and cross-reactivity with other eicosanoids was <2%.

2.7. Statistical analysis

The results are expressed as mean ± SD values. Differences were statistically examined using one-way analysis of variance. Individual differences between means were examined with the *t*-test. The criterion for statistical significance was $P < 0.05$ with all statistical evaluations.

3. Results

3.1. Evaluation of residual rates of ketoprofen in the patches

Residual rates of the drug in hairless rats and pigs were 68.1 ± 1.6% and 81.7 ± 4.4% at the 10-h time point and 61.9 ± 1.5%

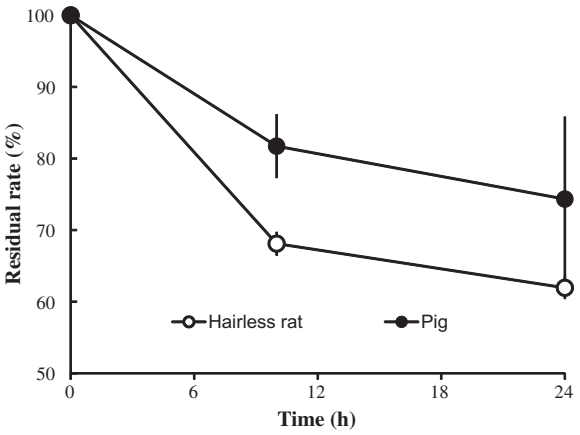

Fig. 2. Profiles of residual content of ketoprofen in patches with single-dose administration in the hairless rat and pig. Mean ± SD values from four to five experiments.

and 74.3 ± 11.5% at the 24-h time point, respectively (Fig. 2). Total amounts of ketoprofen released from the patches after application in hairless rats and pigs were 0.062 ± 0.035 and 0.038 ± 0.009 mg/cm^2 at the 10-h time point and 0.074 ± 0.040 and 0.053 ± 0.024 mg/cm^2 at the 24-h time point, respectively.

3.2. In vivo evaluation of probe recovery

All dialysis membranes showed steady loss of ketoprofen for 480 min through the microdialysis probes.

The average recoveries of ketoprofen over 480 min in the skin and knee joint of the rat were 72.0 ± 3.4% and 9.8 ± 6.2%, respectively (Fig. 3A). Recovery with dialysis membranes of 5 mm length was much lower than with those of 30 mm length.

The average recoveries of ketoprofen over 480 min in the skin and knee joint of the pig were 72.3 ± 2.5 and 57.6 ± 3.1%, respectively (Fig. 3B). Recovery with dialysis membranes of 15 mm was lower than with those of 30 mm length and much higher than with those of 5 mm length inserted into the rat knee joint.

3.3. In vivo microdialysis study

Ketoprofen absorption within skin and knee joints after transdermal administration in rats was rapid and showed similar values at 120 through 480 min (Fig. 4). Ketoprofen was absorbed with C_{max} values of 191.7 ± 76.2 and 35.5 ± 21.7 ng/mL and AUC_{0-8h} values of 918.2 ± 577.5 and 195.9 ± 137.1 ng h/mL, respectively, for the skin and knee joint. The C_{max} value within the skin was approximately 5.4 times higher than within the knee joint. Ketoprofen concentrations within the rat skin and knee joint of non-application site were less than 0.8 ng/mL.

After transdermal administration in the pig, C_{max} values were 20.9 ± 18.5 and 3.7 ± 3.0 ng/mL and AUC_{0-8h} values were 73.1 ± 69.2 and 16.1 ± 16.1 ng h/mL, respectively, for the skin and knee joint (Fig. 5). The increment of ketoprofen absorption was comparatively slow and continued at least for a period of 480 min. Ketoprofen concentrations within the pig skin and knee joint of non-application site were again less than 0.8 ng/mL. The C_{max} values within the rat skin and knee joint were approximately 9.1 and 9.6 times higher than in the pig for the two tissues.

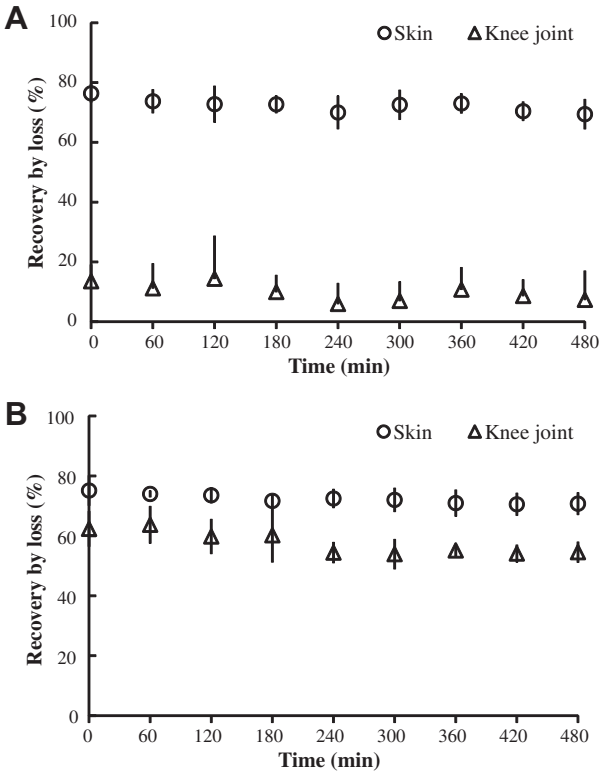

Fig. 3. Loss of ketoprofen assessed by retrodialysis with skin and knee joint microdialysis probes in rats (A) and pigs (B). Data are mean ± SD values from five to six experiments.

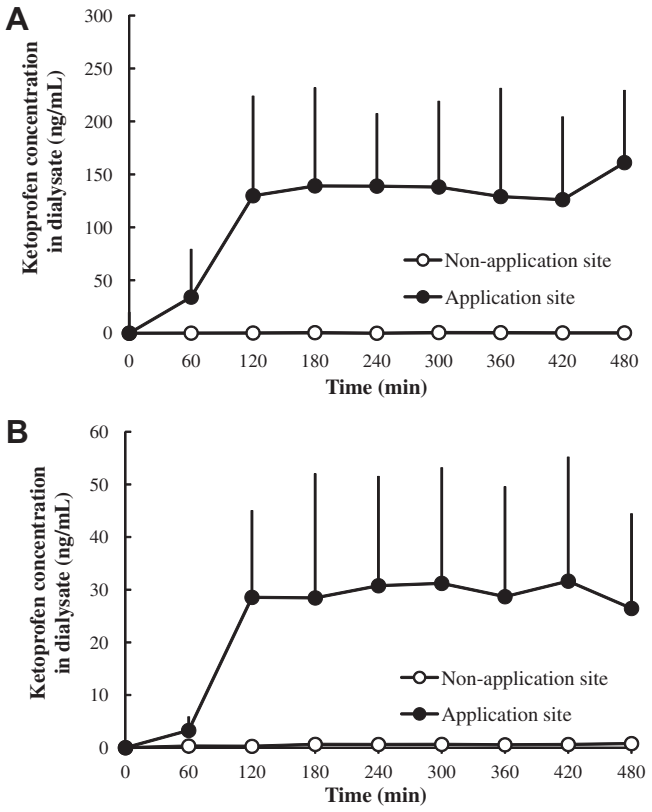

Fig. 4. Concentration–time profiles for ketoprofen in the skin (A) and knee joint (B) after transdermal administration in rats. Data are mean ± SD values from five experiments.

Time course change in prostaglandin E2 levels in the skin and knee joint dialysates after transdermal administration in pigs is shown in Fig. 6. The baseline levels of prostaglandin E2 in the skin and knee joint were 2537.3 ± 1748.6 and 2222.7 ± 1727.9 pg/mL in application sites and 1990.7 ± 1466.2 and 1752.1 ± 1728.8 pg/mL in non-application sites, respectively. Prostaglandin E2 concentrations in the skin and knee joint markedly decreased until 180 min after start of dialysate sampling, with the comparative steady levels reached at 240 min through 480 min. Transdermal administration of ketoprofen significantly reduced prostaglandin E2 levels in the skin of application sites at 240 min through 420 min. Prostaglandin E2 levels in the knee joint of application sites were transiently elevated at the 60-min time point. No significant changes were observed in steady levels at 240 min through 480 min.

Ketoprofen was found in the rat and pig plasma after transdermal administration, with C_{max} values of 460.0 ± 152.6 and 4.2 ± 3.2 ng/mL and AUC_{0-8h} values of 2046.0 ± 826.4 and 14.2 ± 11.8 ng h/mL, respectively (Fig. 7A). The C_{max} value in rat plasma after transdermal administration was approximately 110 times higher than that in the pig plasma. The predicted ketoprofen concentration profile in rats compensated for administration dose and body weight in pigs is shown in Fig. 7B. Predicted C_{max} and AUC_{0-8h} values in rat plasma after transdermal administration were 55.2 ± 18.3 ng/mL and 245.5 ± 99.2 ng h/mL, respectively. The C_{max} value in rat plasma was approximately 13 times that in pig plasma.

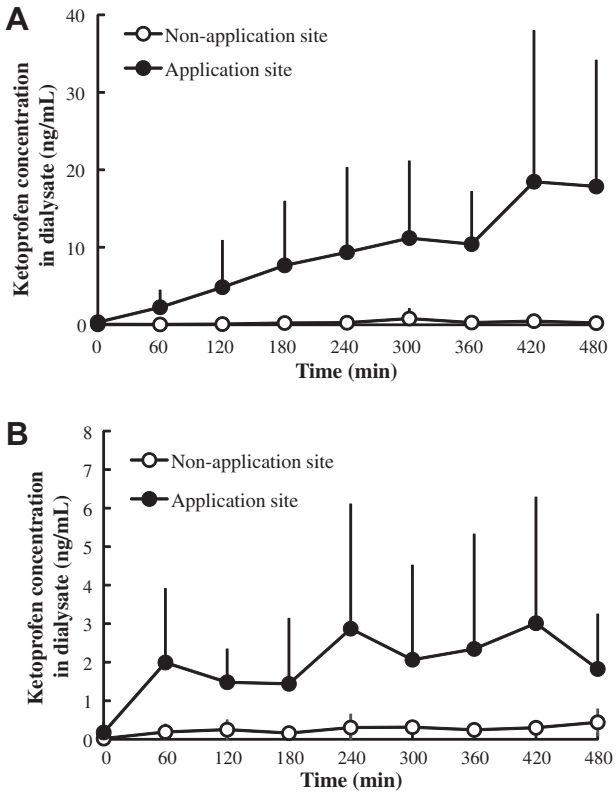

Fig. 5. Concentration–time profiles for ketoprofen in the skin (A) and knee joint (B) after transdermal administration in pigs. Data are mean ± SD values from five experiments.

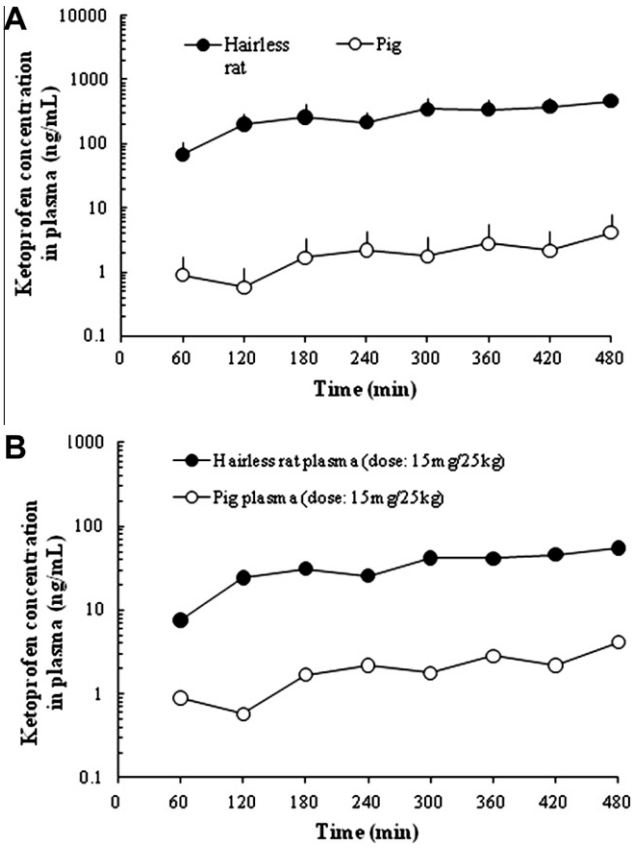

Fig. 7. Comparison of concentration–time profiles for ketoprofen in plasma after transdermal administration in rats and pigs. The observed ketoprofen concentration (A) and predicted ketoprofen concentration profiles (B) are shown. The predicted ketoprofen concentration profile in rats was calculated by compensating observed values with administration dose and average body weight in pigs. Data are mean ± SD values from five experiments.

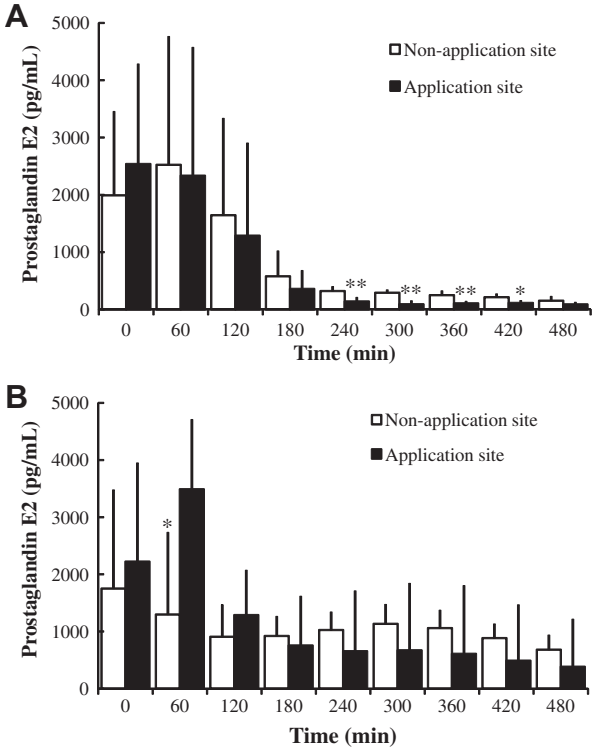

Fig. 6. Concentration–time profiles for prostaglandin E2 in the skin (A) and knee joint (B) after transdermal administration in pigs. Data are mean ± SD values from five experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. non-application sites.

4. Discussion

Microdialysis in the skin is an appropriate technique for the evaluation of drug absorption after topical administration. In fact, many researchers have reported drug penetration from topical formulations containing NSAIDs, such as ketoprofen, diclofenac, and felbinac, using dermal microdialysis studies in rats, pig, and man [16]. Bioavailability is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action [as defined in 21 CFR 320.1 (e)] [17]. It is hence important that the drug absorption from topical patches is determined in active sites for demonstrating pharmacodynamic potency. Since prostanoids are produced by chondrocytes, synoviocytes, and subchondral osteoblasts within sites of osteoarthritis or rheumatoid joints and are involved in the pathogenesis of arthritis [18,19], one of the activity sites of NSAIDs is intra-articular. In a previous study, it was established that transdermal ketoprofen patches can deliver the drug in sufficient amounts to inhibit prostaglandin E2 production in the skin and knee joints in rats [7]. However, since species differences in the skin permeability of drugs have been extensively reported [8,9], it was not clear whether those results reflect the human case. Indeed, the present results indicated the existence of an appreciable species difference in the residual rate with ketoprofen patches after transdermal administration. In clinical studies, it was observed that residual rates of the ketoprofen patch 12 h after topical administration in human are approximately 86.7% (unpublished data). On the other hand, residual rates of the patch in the rat

and pigs were 68.1 ± 1.6 and 81.7 ± 4.4%% at 10 h, respectively. Thus, the ketoprofen amount released from the patch after application in the rat is higher than that in the human case, whereas that in pig is comparatively similar.

Calibration methods in microdialysis studies are important when quantitative information on extracellular fluid concentrations of a drug is desired. The most common calibration method is in vivo retrodialysis, which predicts the relative recovery of drug from the extracellular fluid to dialysate [12]. In the present retrodialysis study, the average recoveries of probe inserted in rat skin and knee joint were 72.0 ± 3.4% and 9.8 ± 6.2%, respectively. Since drug recovery from tissue to perfusate is the same as drug loss from perfusate to tissue across the probe membrane [15], this system is in line with approximately 72% and 10% drug levels in the skin and knee joints. The probe recovery in the knee joint was much lower than in the skin, while relative recoveries of 20% or more in retrodialysis are usually recommendable, facilitating the evaluation of drug concentration [15]. The recovery is influenced by the probe design, physico-chemical properties of drug, and flow rate of perfusate [11]. Especially, probe design and length is an important factor for obtaining higher recovery. Longer probes are therefore to be recommended but the articular cavity of the rat is small and we used the largest microdialysis probe (5 mm) that can be inserted into a rat knee joint. Thus, high probe recovery cannot always be obtained. On the other hand, since we could use a longer microdialysis fiber in the pig retrodialysis study (15 mm), relative recoveries in pig skin and knee joint 55% or more could be obtained. Hence, microdialysis in the pig knee joint should provide more reliable values than with the rat.

Ketoprofen was readily found within patch-applied skin and knee joints of rats and pigs, while it was hardly detectable in non-application sites. This result confirmed that penetration of the drug was by direct diffusion, rather than via the systemic circulation. The topical patch could thus provide the drug to the target tissues without raising the risk of systemic adverse effects. The C_{max} values within the patch-applied skin and knee joint of rats were much higher than in pigs. Predicted ketoprofen concentrations in rat plasma also were approximately 13 times higher than in pig plasma. Since the striatum corneum is known to be the primary and main barrier to percutaneous absorption of drugs [20], differences in skin penetration between the pig and rat might be due to morphological and physiological variation in its thickness and lipid content. Similarities between pig and human skin are well established from skin permeation studies and by physiological and morphological examination [21,22]. Therefore, skin and knee joint penetration of ketoprofen in man might be closer to the concentration–time profile of pigs than that of rats.

Analgesic activity of ketoprofen in arthritis patients is related to drug concentration within target tissues, and our results showed that ketoprofen concentrations within the knee joint after topical application in pigs are much lower than within the skin. In a clinical study, the ketoprofen concentration in the synovial fluid of osteoarthritis patients after repeated application of patches for three days was 3.8 ng/mL and this was similar to our result that indicated a C_{max} value of 3.7 ± 1.4 ng/mL in the knee joint of pigs [23].

Microdialysis can give valuable information regarding change in endogenous substances in target tissues. In a previous study, we indicated that ketoprofen could inhibit prostaglandin production in the skin and knee joint of rats when transdermal administration maintained drug concentrations in target tissues at more than 1 ng/mL [7]. The present study also demonstrated that transdermal administration of ketoprofen significantly reduced prostaglandin E2 levels in the skin of application sites at 240 min through 420 min. However, although transdermal administration of ketoprofen in the knee joint maintained drug concentrations in target tissues at more than 1 ng/mL, significant effects were not observed. In the present study, transient increment of prostaglandin E2 within both tissues was observed immediately after insertion of microdialysis probe, with subsequent decrease over more than 3 h thereafter. Probe implantation causes skin trauma with increase in blood flow and erythema, whealing of the skin, and histamine release [24,25]. Hence, the damage inflicted by probe or guide cannula injection should be allowed to decline before onset of experimentation. A previous study observed a return to blood flow baseline after 45 min following insertion of a probe with a 23-gauge guide cannula in man [26]. The increment of histamine levels also recovered by 40 min after probe injection [27]. We therefore allowed a recovery period of 60 min, although it was clear that influence persisted for more than 3 h. In general, there is less damage from insertion of probes or guide cannulas with smaller diameters. The increment of blood flow may also subside faster in rats than in humans. In fact, we earlier observed a normalization of the increased prostaglandin E2 production 60 min after insertion of a probe with a 23-gauge guide cannula in rats [7]. Thus, effects of ketoprofen on prostaglandin E2 production in the knee joint might not be sufficiently reflected by tissue damage. Since assessment of change in prostanoid levels within target tissues can provide valuable information on therapeutic activity, it is necessary to examine the study procedures in detail in the future.

5. Conclusion

To our knowledge, this is first report of ketoprofen transdermal diffusion into knee joints using a microdialysis technique in pigs. Since pig and human skin has many common characteristics, this approach using the pig model should facilitate the prediction of the pharmacokinetics and pharmacodynamics of drug transfer from topical patches in human beings. Moreover, we have demonstrated that topical patches containing ketoprofen can deliver the drug within the skin and knee joints of pigs and rats via direct diffusion.

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