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Evaluation of Percutaneous Drug Permeation Using a Lateral Sectioning Approach

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1. Introduction

Local enhanced tissue delivery (LETD) of non-steroidal anti-inflammatory drugs (NSAIDs) provides a means to avoid adverse effects, such as gastrointestinal disorders (Hawkey & Truelove, 1983; Taha et al., 1994). Regarding the skin dispositions of drugs, it is considered to be more important to evaluate the drug LETD from the formulation than the drug uptake into the body from the cutaneous microvascular network (Morgan et al., 2003). Assessment of drug concentrations in the skin is crucial for the development of topical products, such as patches, and other transdermal formulations. Several studies have demonstrated the skin permeabilities and local tissue concentrations of NSAIDs after topical administration (Singh & Rogerts, 1994; Grupta et al., 1995). However, the drug concentrations in those *in vitro* studies were calculated as accumulated volumes across the skin, and the drug contents within the skin consequently remain unclear. It has also been suggested that the diffusion length of the viable epidermis and dermis is longer *in vitro* than *in vivo* (Scheuplein, 1976), thereby leading to possible discrepancies in the permeation data between *in vitro* and *in vivo* studies. Almost all previous *in vivo* studies have evaluated drug concentrations within the whole skin layer after topical application. In addition, only one or two points of the skin were sampled and measured for their drug concentrations, and it therefore remains unclear how drugs permeate the skin layer from the surface to the deep regions over time.

To solve such problems, we developed a new evaluation method involving transdermal pharmacokinetics (Goi et al., 2010). In this chapter, we first explain the details of the dermal lateral sectioning approach and clearly summarize the results. Next, we apply this evaluation method to a more realistic model. Finally, we analyze the correlation between the time course changes of the drug concentration at the deepest layer of skin and the expression rate of drug efficacy, and suggest that our lateral sectioning approach is an ideal method for predicting therapeutic expression.

2. Evaluation method of transdermal drug permeability

The aim of this study was to establish a new method for clearly analyzing the flow of drugs in the skin layers and evaluating the drug levels in the target area of the skin tissue.

2.1 Animals

All experiments were carried out in accordance with the Institutional Animal Care and Use Committee of Mikasa Seiyaku Co. Ltd. (Tokyo, Japan). Male hairless rats (8 weeks of age, weighing 190–250 g; Japan SLC Inc., Shizuoka, Japan) were used in all experiments. The rats were housed in an animal room with a room temperature of 23±2°C, relative humidity of 55±15% and 12-h/12-h light/dark cycle (lights on at 07:00). The animals had free access to a mouse/rat diet (#5002, pellet form; PMI Nutrition International Inc., Richmond, IN) and tap water throughout the experiments.

2.2 Drugs

Flurbiprofen and ketoprofen are racemic propionic acid-derived NSAIDs with proven efficacy and safety in the treatment of osteoarthritis, rheumatoid arthritis and acute musculoskeletal disorders (Brogden et., 1979; Buchanan & Kassam, 1986; Waikakul et al., 1997). As a matter of course, tape-type patches of flurbiprofen (FP-T) and ketoprofen (KP-T) have been used and investigated for their anti-inflammatory and analgesic effects on local tissues (Martens, 1997; Mazieres, 2005).

The FP-T tape-type patches (ZEPOLAS® TAPE; Mikasa Seiyaku Co. Ltd., Tokyo, Japan) contained 2.2% flurbiprofen (1.1 mg/4 cm²) in a hydrophobic adhesive-like styrene/isoprene/styrene copolymer without water. The KP-T tape-type patches (MOHRUS® TAPE; Hisamitsu Pharmaceutical Co. Ltd., Tosu, Japan) contained 2% ketoprofen (1.1 mg/4 cm²). Both patches are widely used clinically. Other chemical agents used were flurbiprofen and ketoprofen (purity, >99.7%; Daito Pharmaceutical Co. Ltd., Toyama, Japan).

2.3 *In vivo* evaluation of the transdermal permeability of drugs

Rats were anesthetized with ether and 2.0×2.0 cm flurbiprofen or ketoprofen patches were individually applied to the center of their abdomen. The region with the applied patch was covered with a bonded-fiber fabric bandage and the body was wrapped with an adhesive elasticized bandage (Elastopore® No. 50; Nichiban Co. Ltd., Tokyo, Japan) to prevent the patch from becoming dislodged. The rats were then housed individually in blanket cages (240 mm width × 380 mm length × 200 mm height) and supplied with food and water *ad libitum*. Both types of patches were removed at 2, 4, 8, 12, 14, 16 and 24 h after application, including an extra time point of 1 h after application for FP-T, and the rats were euthanized by collection of whole blood from the abdominal aorta under ether anesthesia. The area of skin tissue where each patch was applied was removed and the harvested skin tissues were embedded in OCT compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan) and frozen at –80°C.

The most important part of the technique was the procedure for freezing skin tissues in a flat manner. As shown in Fig. 1, the embedding agent was poured into a case to about half-full and frozen in advance. The case was then left for about 15 min at room temperature and 1) the surface of the embedding agent was lightly rubbed with the flat part of a finger to make the surface parallel. 2) Subsequently, the harvested skin tissues were placed on the flat surface and 3) pins were placed at the corners of the skin tissues to clarify the outline (because the place where the skin existed became unknown after the OCT compound was frozen). 4) Finally, the embedding agent was gently poured into the rest of the case and frozen at –80°C.
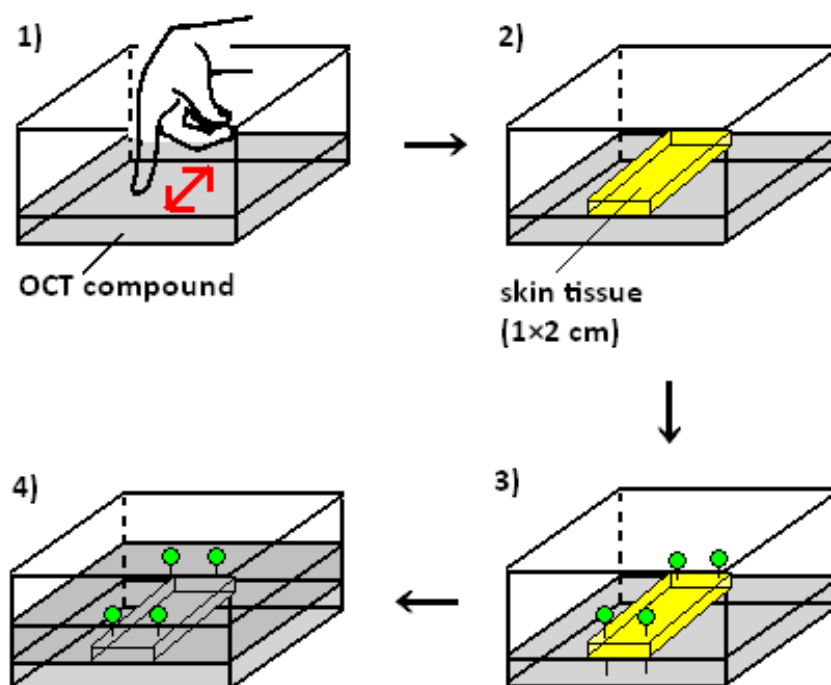
Fig. 1. Procedure for freezing skin tissues in a flat manner.

One-half of each frozen skin tissue sample (1.0×2.0 cm) was cut with a microtome (Cryotome CR-502; Nakagawa Co. Ltd., Tokyo, Japan) into 20 μm-thick slices parallel to the surface. The drug concentrations in the individual slices were measured after the slices were weighed. At this point in the slicing, the most crucial part of the handling was how the initial slice of frozen skin was cut parallel to the plane surface of the skin. When continued slicing became able to catch the embedded skin surface along the way, the angle of the cutting plane was adjusted and the slicing was operated several more times. This procedure was repeated until the cut plane became parallel to the surface of the skin tissue. Once the slicing of the skin was initiated, the operation was carried through without any further adjustments. The drug concentrations were obtained by dividing the measured amount of the drug in each skin slice by the weight of the slice. The microtome was not able to slice frozen samples thicker than 20 μm. The other half of each frozen skin tissue sample was sliced perpendicular to the skin surface and stained with hematoxylin and eosin (HE) for measurements of the skin thickness. Images were obtained using a fluorescence microscope (IX81-ZDC; Olympus, Tokyo, Japan). For measurement of the thickness of each layer, 10 points of the layer in a microscopic field were randomly picked up and the thickness was calculated using the software Lumina Vision LV-WIN TM-SOFT (Mitani Co. Ltd., Fukui, Japan). The average value from each image was taken as the individual thickness of each layer.

The thicknesses of the stratum corneum, epidermis and other tissues (dermal and subcutaneous tissues) were measured (Table 1) when the rats were 8 weeks of age (n=4). The whole skin thickness was approximately 700 μm and 35 pieces of skin tissue were theoretically obtained when the frozen tissues were sliced laterally every 20 μm. However, it was necessary to take account of individual differences and surface irregularities. Therefore, the skin tissue was sliced an extra five times (=100 μm thickness) and weighed, and the drug concentrations in the skin layers from 0 to 800 μm were measured. Images of the slicing and measuring protocol are shown in Fig. 2.

stratum corneum	epidermis	dermal and subcutaneous tissues
15.9 ± 0.9	38.4 ± 2.0	659.8 ± 32.6

(µm)

Table 1. Skin layer thicknesses of hairless rats

2.4 Analytical methods

The frozen skin tissue samples were dissolved in a solution at room temperature. Briefly, a piece of skin sample (about 3 mg) was added to 250 µL of 1 mol/L KOH and incubated at 60°C for 15 min. Next, 750 µL of 5% (v/v) H_3PO_4 was added, and 500 µL of the resulting solution was subjected to solid-phase extraction (Oasis® MAX 96-Well Plate 30 µm; Waters, Milford, MA) after the cartridge had been successively prewashed with 1 mL of methanol and 1 mL of water. The cartridge was consecutively rinsed with 1 mL of 50 mM sodium acetate (pH 7.0) and 1 mL of methanol, and eluted with a mixed solution (isopropanol/acetonitrile/formic acid = 58:40:2). A 5-µL aliquot of each eluate was analyzed by HPLC and LC/MS.
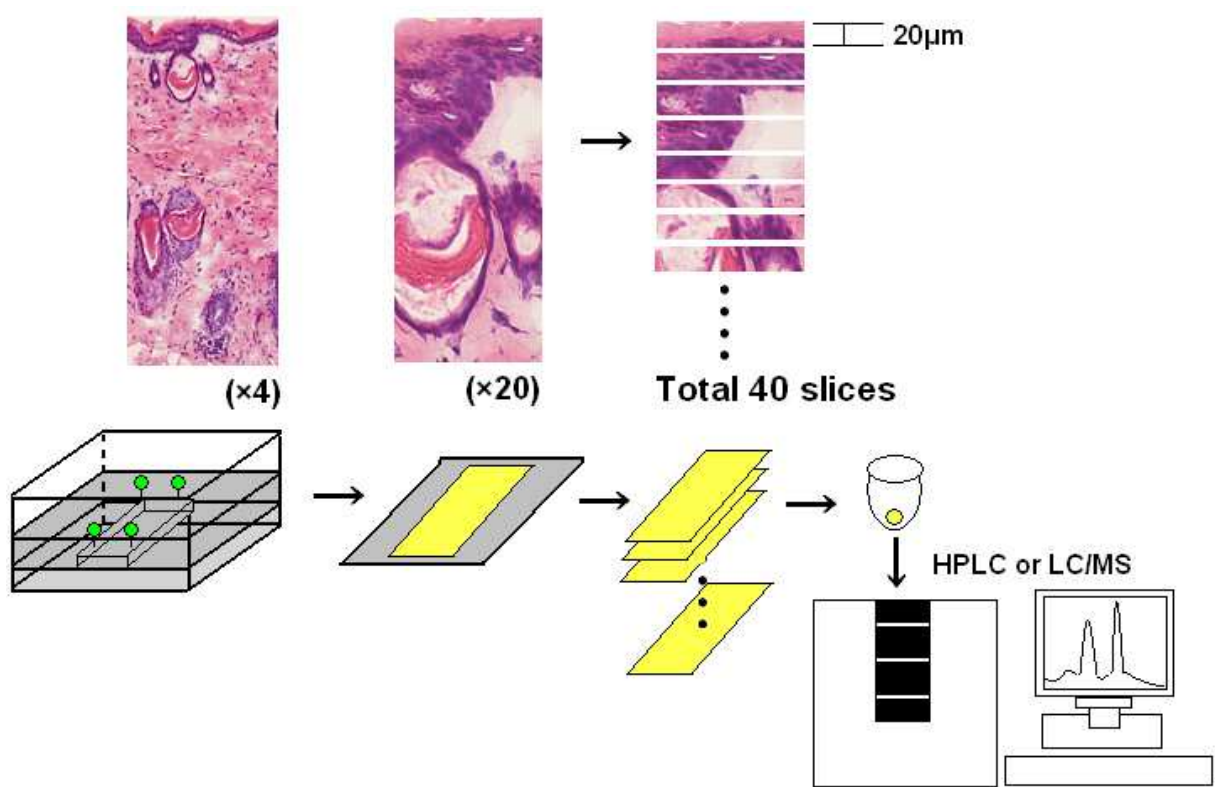

Fig. 2. Images of skin slicing and analysis of the drug contents of individual slices.

The concentrations of flurbiprofen in the dissolution media were determined by HPLC. The HPLC conditions were as follows: pump, LC-20AD; detector, RF-10A$_{XL}$; system controller, CBM-20A; auto-injector, SIL-20AC$_{HT}$; column oven, CTO-20AC (Shimadzu Co. Ltd., Kyoto, Japan); column, ZORBAX SB-C18 (150×4.6 mm, 1.8-µm particles; Agilent Technologies Inc.,

Wilmington, DE); column temperature, 60°C. The mobile phase consisted of acetonitrile and 0.1% (v/v) formic acid at a ratio of 60:40, delivered at a flow rate of 1 mL/min. The fluorescence was determined at 310 nm (emission) and 260 nm (excitation). The retention time of flurbiprofen was approximately 5.5 min.

The concentrations of ketoprofen were measured by LC/MS. Mass spectrometry was used to confirm the chromatographic profile obtained by a diode-array detector. The Shimadzu LCMS-2010A system used was equipped with a column (ZORBAX SB-C18, 150×2.1 mm, 3.5-μm particles; Agilent Technologies Inc.), a photodiode array detector (SPD-M20A) and a single quadrupole analyzer (LCMSF-2010EV). The other pieces of equipment were the same as those used for the HPLC analyses. Identification was achieved using the full-scan mode at a mass range of *m/z* 100-500. The mobile phase consisted of acetonitrile and 0.1% (v/v) formic acid at a ratio of 60:40, delivered at a flow rate of 0.2 mL/min.

The accuracy values of blank skin samples with added flurbiprofen and ketoprofen (2.5, 10 and 80 ng/mL) were in the ranges of 102.7–111.5% and 98.1–107.1%, respectively, within 24 h for the sample preparation and measurements by HPLC and LC/MS described above. The recovery rates of the individual drugs from the skin tissue samples were both >93.0%.

The flurbiprofen and ketoprofen contents of the FP-T and KP-T patches were also measured by HPLC. The residual ratios were calculated as follows:

$$\text{Residual ratio (\%)} = \frac{\text{Drug contents in patches after application}}{\text{Drug contents in patches before application}} \times 100 \tag{1}$$

The HPLC conditions were as follows: column, Mightysil RP-18 GP (150×4.6 mm, 5.0-μm particles; Kanto Chemical Co. Inc., Tokyo, Japan); column temperature, 40°C. The mobile phase consisted of acetonitrile and 0.1% (v/v) formic acid at a ratio of 60:40. The retention times of flurbiprofen and ketoprofen were both approximately 4.0 min. The other conditions were the same as those used for the skin tissues.

2.5 Statistical analysis

The results were expressed as means ± SD. The significance of differences between measurements for the FP-T-applied and KP-T-applied groups was evaluated using the Mann–Whitney *U* test. The criterion for statistical significance was a value of *P*<0.05 for all statistical evaluations.

3. Estimation of the percutaneous permeation of flurbiprofen and ketoprofen from the surface to the deep layer

To examine the drug permeation in the skin tissue, the skin areas where the tapes were applied were cut into 20 μm-thick horizontal slices from the surface, and the drug concentrations in the slices were measured individually. Later, we calculated each drug concentration in the skin tissue per 100 μm thickness, corresponding to five pieces of skin tissue, to clearly indicate the drug distributions, because we considered that it was not easy to evaluate the drug disposition in the finely-divided state (every 20 μm).

3.1 Single application of FP-T and KP-T

The drug levels in the skin tissues after application of FP-T and KP-T are shown in Fig. 3. Initially, the flurbiprofen concentrations in the skin layers from 0 to 400 µm were highest at 2 h after FP-T application and then decreased gradually, although the concentrations in the skin layers from 0 to 300 µm increased transiently after 12 h (Fig. 3A). The flurbiprofen concentrations at 2 h after application of FP-T were 2270±770, 1410±260, 1110±200 and 650±160 ng/mg of skin layer from 0 to 100 µm, 100 to 200 µm, 200 to 300 µm and 300 to 400 µm, respectively. Subsequently, the flurbiprofen concentrations in the skin layers from 400 to 800 µm, except for 500 to 600 µm, were highest at 4 h after FP-T application and then decreased until 24 h after FP-T application (Fig. 3C). On the other hand, the ketoprofen concentrations in the skin layers from 0 to 500 µm reached their peaks after 4 h of tape application (Fig. 3B and D).
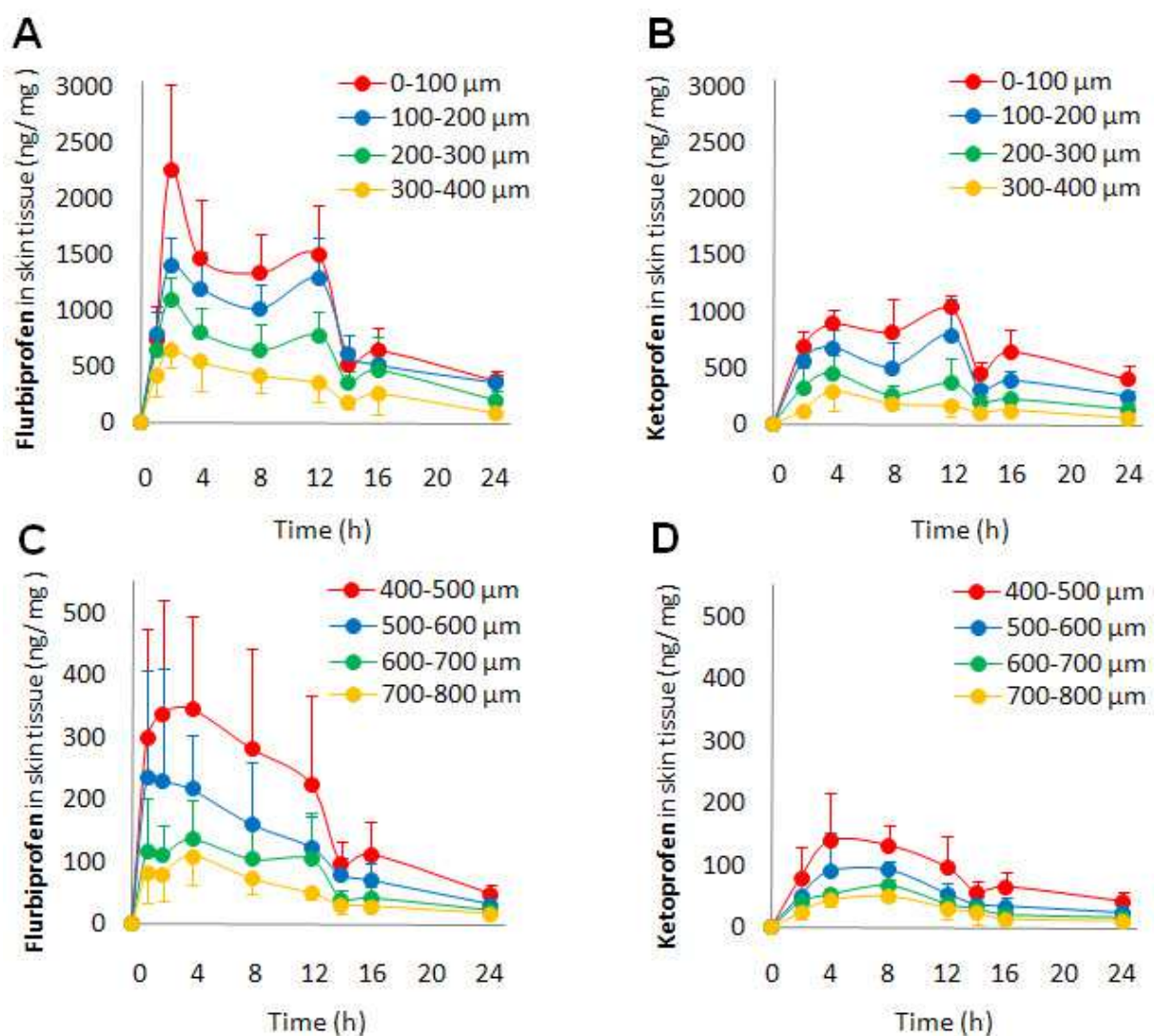

Fig. 3. Time-course profiles of the skin tissue concentrations of flurbiprofen and ketoprofen after FP-T and KP-T application. (A) Flurbiprofen concentrations in the layers from 0 to 400 µm. (B) Ketoprofen concentrations in the layers from 0 to 400 µm. (C) Flurbiprofen concentrations in the layers from 400 to 800 µm. (D) Ketoprofen concentrations in the layers from 400 to 800 µm. Each point represents the mean ± SD (n=5).

The ketoprofen concentrations at 4 h after application of KP-T were 900±130, 680±170, 460±160 and 300±160 ng/mg of skin layer from 0 to 100 µm, 100 to 200 µm, 200 to 300 µm and 300 to 400 µm, respectively. Similar to the case for flurbiprofen, the ketoprofen concentrations in the skin layers from 0 to 300 µm increased transiently after 12 h. The ketoprofen concentrations in the skin layers from 500 to 800 µm were highest at 8 h after KP-T application and decreased until 24 h (Fig. 3D).

Traditionally, information on percutaneous absorption has been obtained using *in vitro* skin diffusion chambers, mathematical models (Potts & Guy, 1992; Singh & Roberts, 1996) and indirect methods such as radiolabeled drug absorption techniques *in vivo* (Schaefer et al., 1978). However, percutaneous absorption depends on various multiple components, and consequently the series of equations is complicated. Autoradiography has the advantage that the drug levels are represented visually, but is not considered to be a quantitative method. Our technique of lateral sectioning of frozen skin tissues and measurement of the drug concentration in each slice may be primitive, but allowed us to clearly understand the diffusion of drugs in the skin layers.

Table 2 shows the time courses of the changes in the residual ratios of flurbiprofen and ketoprofen after topical application of FP-T and KP-T. No significant differences between flurbiprofen and ketoprofen were observed with respect to the residual ratios until 12 h after patch application, suggesting that the drug release rates of FP-T and KP-T were nearly equal and that the higher skin concentrations of flurbiprofen shown in Fig. 3 followed another mechanism that was separate from the drug release rate. From 12 to 16 h, a high volume of flurbiprofen was released compared with ketoprofen. Therefore, flurbiprofen seemed to be released from FP-T to the greatest possible extent until 16 h and no more flurbiprofen was subsequently released until 24 h, whereas ketoprofen was continuously released from KP-T until 24 h after application.

	Time after tape application (h)					
	2	4	8	12	16	24
Flurbiprofen	73.7 ± 2.6	65.1 ± 2.6	45.3 ± 6.2	37.3 ± 2.0	22.7 ± 1.2*	22.0 ± 2.6
Ketoprofen	76.8 ± 4.0	60.0 ± 2.9	48.7 ± 3.8	40.8 ± 6.2	34.7 ± 5.7	22.3 ± 2.8

(%)

Table 2. Residual ratios of flurbiprofen and ketoprofen after tape application. Each value represents the mean ± SD (n=4). *$P<0.05$ *vs.* ketoprofen.

3.2 Second application of FP-T

Since the FP-T patch is removed and replaced with a new patch every 12 h, we also investigated the flurbiprofen concentrations in the skin tissues after a second application of FP-T. The second FP-T was applied to the same skin site as the first patch under the assumption that patches are applied to the same affected area more than once. The time courses of the changes in the concentrations of flurbiprofen in the whole skin layer (0 to 800 µm) after tape application are shown in Fig. 4. It was clearly demonstrated that flurbiprofen rapidly reached the deepest layer of the whole skin within a short time, but

there was a low content of flurbiprofen in the area deeper than 600 µm from 16 to 24 h after administration with only a single application. This was because a very low amount of flurbiprofen was released from FP-T from 16 to 24 h, as shown in Table 2. On the other hand, the flurbiprofen concentration in all regions of the whole skin increased promptly after 14 h and a high concentration of flurbiprofen was maintained until 24 h after application, even in the deepest layer in cases where a second application was carried out.

The time courses of the changes in the concentrations of flurbiprofen and ketoprofen in the deepest skin layer (700 to 800 µm) after FP-T application are shown in Fig. 5. This layer is adjacent to the intramuscular tissue and plays a major role in the evaluation of drug permeability and pharmacodynamics. The concentration of flurbiprofen (109.4±44.8 ng/mg) was significantly higher than that of ketoprofen (44.4±9.8 ng/mg) at 4 h after tape application, but there were no significant differences in the concentrations of the two drugs at 12 h and thereafter, except for 16 h. However, the flurbiprofen concentration in the deepest skin tissue layer increased again at 2 h (total exposure, 14 h) after the second application of FP-T (115.3±44.6 ng/mg) and was maintained at a significantly higher level than ketoprofen for the next 10 h (total exposure, 24 h).
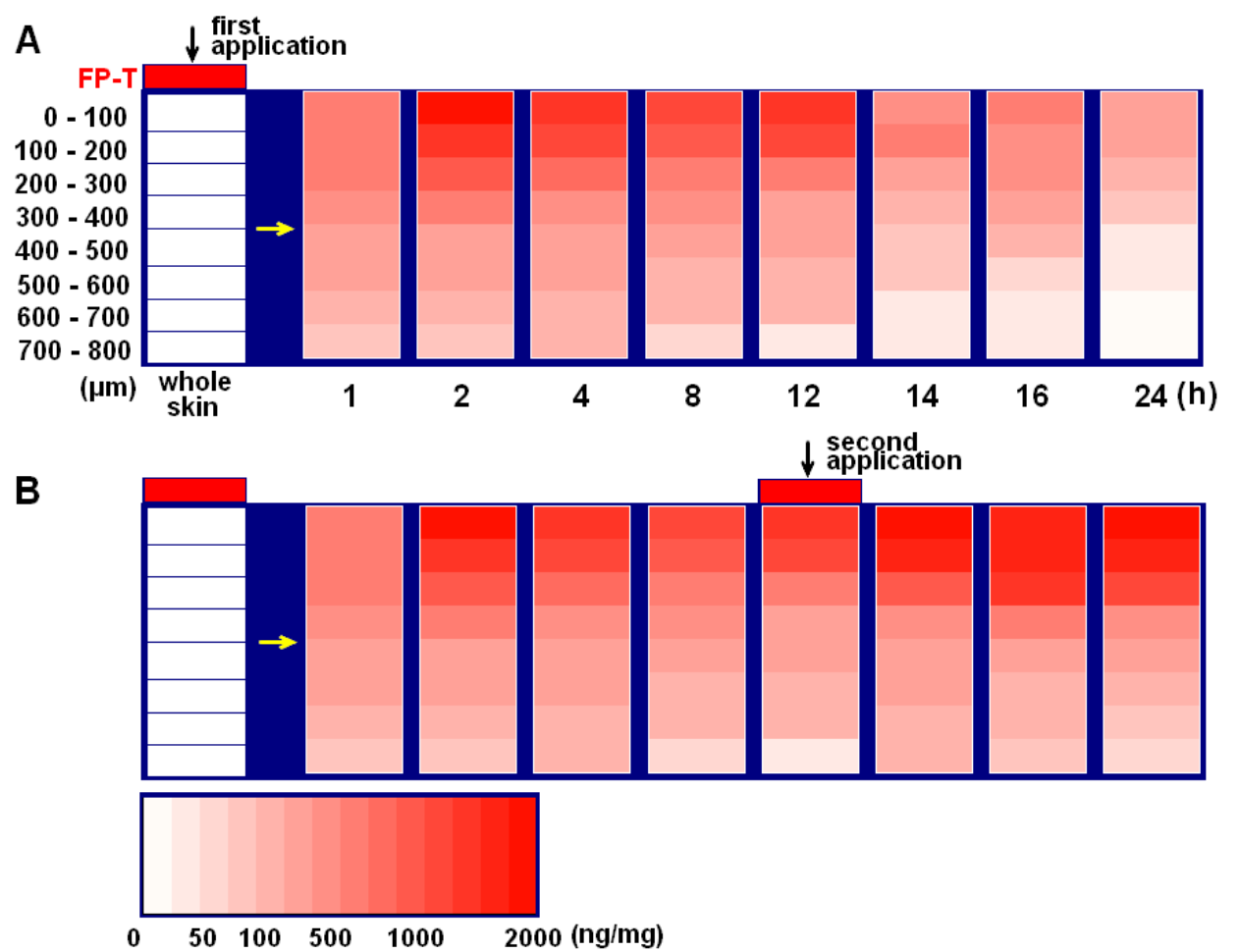

Fig. 4. Time-course profiles of the skin tissue concentrations of flurbiprofen after FP-T application. (A) Single application. (B) First and second applications. The color density is dependent on the drug concentration in the skin tissues.

A second application of FP-T reduced the time required to reach the maximal concentration in the deepest skin layer (700 to 800 µm) from 4 h to 2 h after administration (Fig. 5). The skin permeation of a drug is generally enhanced by occlusive application (Schaefer & Redelmeier, 1996). Hydration of corneocytes and weakening of the stratum corneum barrier function induced by 12 h of continuous FP-T application appeared to comprise the main mechanisms for how the permeability rate of flurbiprofen released from the new FP-T patch was increased. In addition, the flurbiprofen contents in the superficial layer (0 to 300 µm) increased slightly at 12 h after administration compared with the contents at 8 h (Fig. 3A). This observation means that enormous proportions of the flurbiprofen were stored in the superficial layer, and that this layer acted as a drug reservoir. Fundamentally, stored flurbiprofen is slowly released to the deeper tissues (Sugawara et al., 1987). However, it should be considered that the powerful driving force produced by the application of a second FP-T extruded the deposited flurbiprofen and a greater amount of flurbiprofen was able to permeate quickly into the deepest layer of the skin as a result. These findings are consistent with a previous study in which T_{max} was decreased by successive patch applications (Taburet et al., 1995).
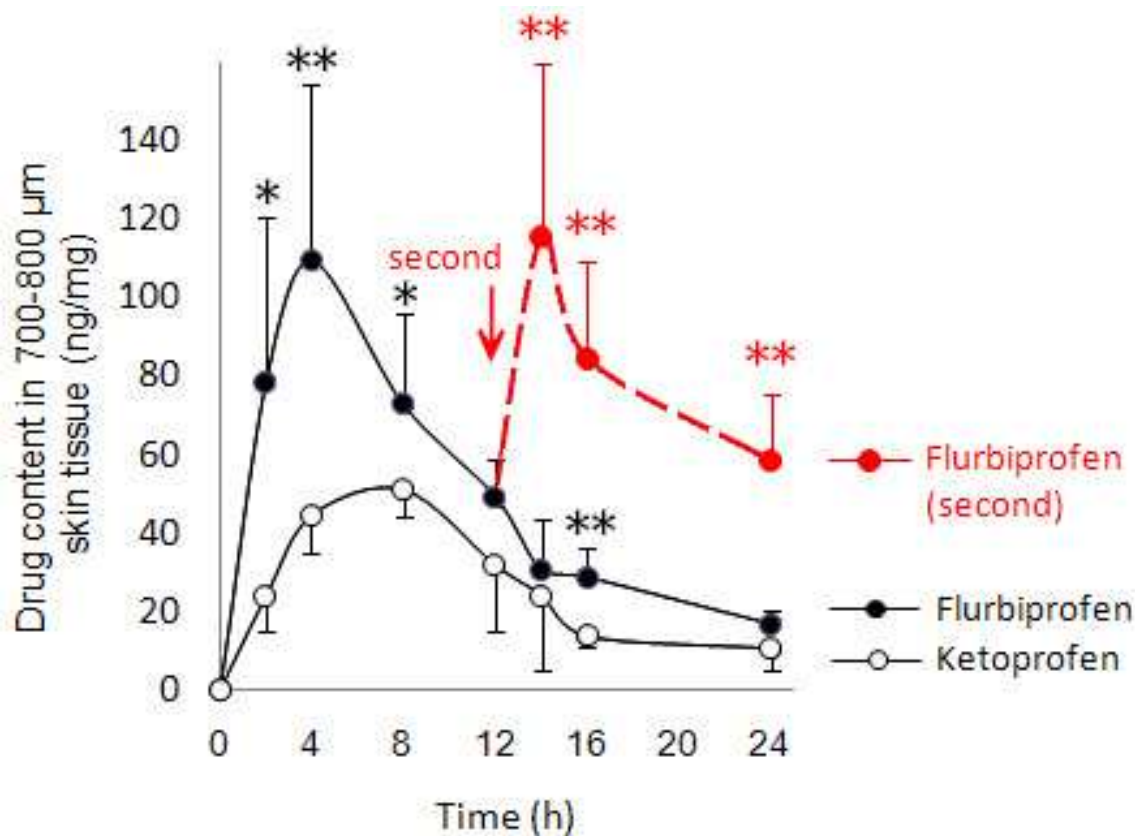

Fig. 5. Time-course profiles of the skin tissue concentrations of flurbiprofen and ketoprofen in the layer from 700 to 800 µm after FP-T and KP-T application. The unbroken lines for flurbiprofen and ketoprofen show data for continuous FP-T and KP-T administration for 24 h. The broken line for flurbiprofen shows data after a second administration of FP-T from 12 to 24 h (the previous FP-T was removed and the new patch was applied at 12 h). Each point represents the mean ± SD (n=5). *P<0.05 and **P<0.01 vs. ketoprofen.

3.3 Effect of stratum corneum stripping on the transcutaneous permeability of flurbiprofen

What factors would affect the LETD of NSAIDs in practical usage? The FP-T patch is removed and replaced with a new patch every 12 h as mentioned above. We observed that the stratum corneum was hardly stripped when FP-T was removed after 4 h of adhesion, but was slightly stripped to about 1.5-μm thickness after removal of the first FP-T patch at 12 h (Table 3). To investigate the effect of this decrease in the thickness of the stratum corneum on the transcutaneous penetration of flurbiprofen, we measured the concentrations of flurbiprofen in skin layers of 100-μm thickness with and without tape-stripping.

Normal	Time after FP-T administration (h)			
	2	4	8	12
15.9 ± 0.9	15.1 ± 1.0	15.2 ± 1.6	14.5 ± 0.6	14.5 ± 0.9
				(μm)

Table 3. Stratum corneum thicknesses after removal of adherent FP-T at a fixed time. Each value represents the mean ± SD (n=4).
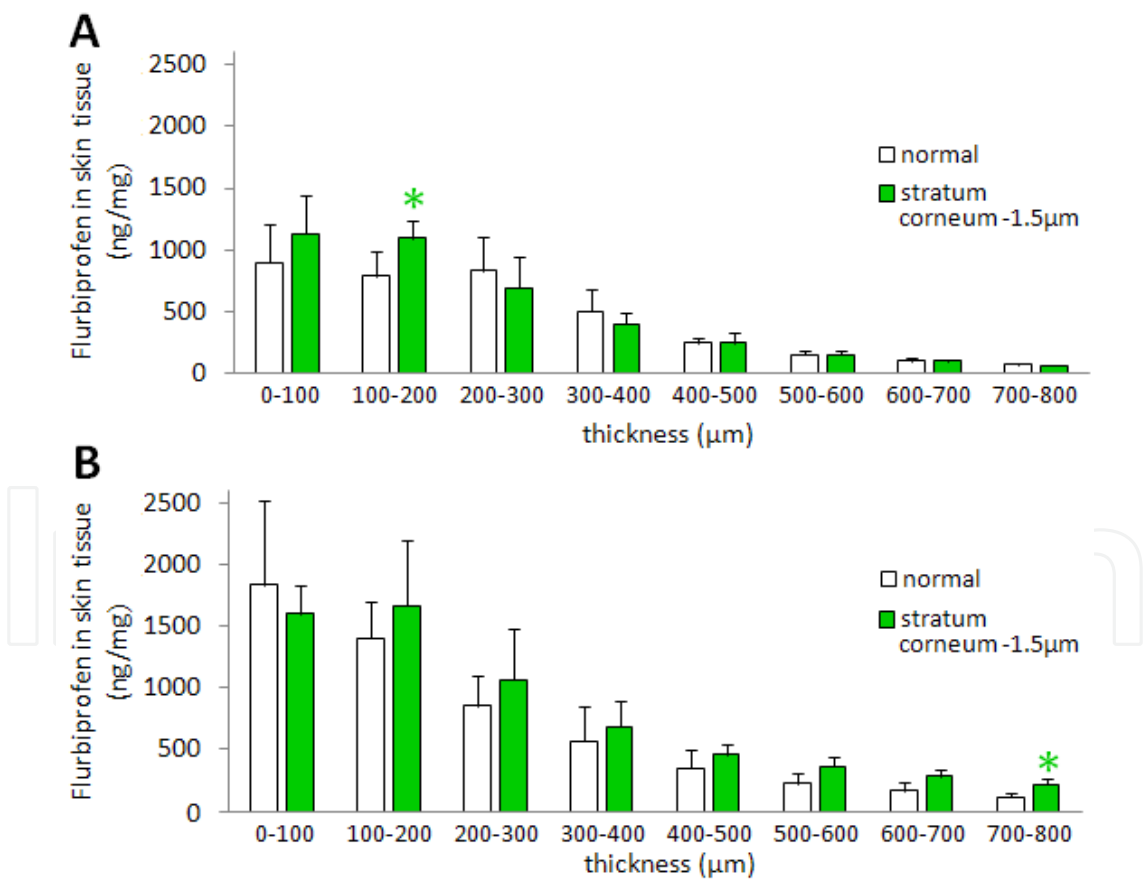

Fig. 6. Effect of stratum corneum stripping on the transcutaneous permeability of flurbiprofen. The skin tissue concentrations of flurbiprofen per 100-μm thickness are expressed after FP-T application for 2 h (A) and 4 h (B). The data represent means ± SD (n=4). *$P<0.05$ *vs.* the non-stripped group (=normal).

Tape-stripping is used to measure drug uptake into the skin (Touitou et al., 1998; Bashir et al., 2001). The tapes have different propensities to cause barrier disruption. Some individuals do not exhibit increased TEWL despite an equivalent mass of stratum corneum being removed compared with those who do show a response (Bashier et al., 2001). In our situation, the abdominal skin of some rats was tape-stripped using two strokes of adhesive tape (Transpore® Surgical Tape; Sumitomo 3M Co. Ltd., Tokyo, Japan) before placing FP-T patches. The score of TEWL was 9.3 ± 0.4 g/m²h before stripping and increased slightly to 11.6 ± 0.5 g/m²h after two strokes of tape-stripping. Therefore, it seemed that two strokes of tape-stripping replicated the decrease in thickness of the stratum corneum when FP-T was removed after 12 h of adhesion without disrupting the horny layer formation. We compared the drug concentrations in the skin tissues between the stripping and non-stripping models to evaluate the effect of stratum corneum stripping on the transcutaneous permeability of flurbiprofen.

At the time when the skin tissue was previously stripped and FP-T had been administered for 2 h, the skin tissue concentrations of flurbiprofen were higher than those without stripping in the layers from 0 to 200 µm, and a significant difference was observed in the layer from 100 to 200 µm (Fig. 6A). In the deeper layers beyond 200 µm, there were no significant differences in the flurbiprofen concentrations between the two groups. Likewise, at the time when the skin tissue was previously stripped and FP-T had been administered for 4 h, the skin tissue concentrations of flurbiprofen were higher than those without stripping in the layers from 100 to 800 µm, and a significant increase was observed in the deepest layer (Fig. 6B). Therefore, the transcutaneous permeability of flurbiprofen into the deepest layer of the skin tissue was not affected by stratum corneum stripping when FP-T had been applied for 2 h, but was affected by stripping after FP-T had been administered for 4 h. Our evaluation method revealed that only a small 10% decrease in the thickness of the stratum corneum could change the drug LETD to the deepest layer.

4. Pharmacokinetics

4.1 Pharmacokinetic parameters

Blood samples (0.2 mL) were collected from the jugular vein of the rats at 2, 4, 8, 12, 14, 16 and 24 h after both types of patches were applied. The analytical methods were slightly modified compared with those for the skin tissues. Briefly, 50 µL of plasma sample was applied to measure the drug contents. The mobile phase of HPLC consisted of acetonitrile and 0.1% (v/v) formic acid at a ratio of 55:45, delivered at a flow rate of 1 mL/min, and the mobile phase of LC/MS consisted of acetonitrile and 0.1% (v/v) formic acid at a ratio of 50:50, delivered at a flow rate of 0.2 mL/min.

The pharmacokinetic parameters following topical administration of flurbiprofen and ketoprofen are summarized in Table 4. These pharmacokinetic values are based on the plasma concentration/time profiles of the drugs after administration of one patch. FP-T exhibited higher and more rapid flurbiprofen permeation compared with ketoprofen permeation from KP-T. As shown in Table 4, FP-T exhibited a higher C_{max} value (4.6 ± 0.7 µg/mL) and shorter T_{max} value (6.3 ± 2.1 h) than KP-T. Moreover, FP-T administration produced a high AUC_{0-12h} value of 44.5 ± 7.3 µg·h/mL, which was five

times higher than that of KP-T (8.6±1.8 µg·h/mL). The plasma concentration of flurbiprofen peaked at 6.3±2.1 h after the first FP-T application, although this peak was shortened to 3.2±1.1 h after the second FP-T application. The C_{max} and AUC_{0-12h} values for the second FP-T application were slightly increased, and the $T_{1/2}$ value after the successive applications was longer than that after the first FP-T application. The drug delivery during the second patch application indicated relatively good permeability as mentioned previously, and this is probably the reason why the shorter T_{max} value and higher C_{max} and AUC values were observed.

	Flurbiprofen		Ketoprofen
	first	second	
Dose (mg/kg)	4.5	4.5	4.5
C_{max} (µg/ml)	4.6 ± 0.7	5.9 ± 0.4	1.5 ± 0.2
T_{max} (h)	6.3 ± 2.1	3.2 ± 1.1	19.2 ± 3.4
$T_{1/2}$ (h)	7.3 ± 1.1	10.3 ± 1.3	—
AUC_{0-12h} (µg·h/ml)	44.5 ± 7.3	55.2 ± 2.6	8.6 ± 1.8
A.B. (%)	43.0	53.4	14.8

Table 4. Pharmacokinetic parameters following topical administration of flurbiprofen and ketoprofen. Each value represents the mean ± SD (n=3–5). C_{max}: maximum plasma concentration; T_{max}: time to reach C_{max}; $T_{1/2}$: terminal half-life; AUC_{0-12h}: area under the plasma concentration-time curve from time 0 to 12 h; A.B.: absolute bioavailability in an intravenous group.

4.2 Differences in the time-course changes in the skin and plasma concentrations between flurbiprofen and ketoprofen

The time-course changes in the flurbiprofen concentrations in the skin layer from 700 to 800 µm were very similar to the plasma concentrations of flurbiprofen after both single and repeated applications (Fig. 7A). In contrast, the transcutaneous absorption of ketoprofen into the systemic circulation was relatively slow, and the plasma concentration of ketoprofen peaked about 8 h later than the peak in the skin layer from 700 to 800 µm (Fig. 7B).

The two formulations of NSAIDs examined have their own characteristic features. FP-T with a twice-daily application frequency induced a quick increase in the skin concentration of flurbiprofen and maintained a high level in the deepest layer via LETD (Fig. 7A). This shows the potential for prompt onset and prolonged duration of NSAID activity in intramuscular tissues. However, the systemic concentrations of the drug increased at the same rate as the concentrations in the deepest layer of the skin, which may increase the risk of adverse effects. On the other hand, the diffusion of ketoprofen from KP-T to the maximal depth of the skin by LETD was relatively slow (Fig. 7B), suggesting the possibility that several hours are required to realize its efficacy as an anti-inflammatory drug. However, the plasma concentrations of ketoprofen absorbed by the systemic circulation route were relatively low, which may lower the risk of adverse

effects. These observations are consistent with previous reports indicating that ketoprofen is an effective and safe therapeutic option for the treatment of local painful inflammation (Mazieres, 2005; Flouvat et al., 1989). In many studies, including the present study, pharmacokinetics parameters have been evaluated using the time courses of the total plasma or serum concentrations. However, the theory of practical pharmacokinetics emphasizes the importance of the unbound fraction of a drug in understanding concepts such as clearance, apparent volume of distribution and pharmacodynamic action (Wright et al., 1996). Borgå & Borgå (1997) reported that the lowest dissociation constant (highest affinity) was found with flurbiprofen, which exhibited an 80-fold difference compared with the highest affinity for ketoprofen. This high affinity of flurbiprofen for albumin may contribute to a reduction in the incidence of gastrointestinal disorders, although its plasma concentration was still much higher than that of ketoprofen.
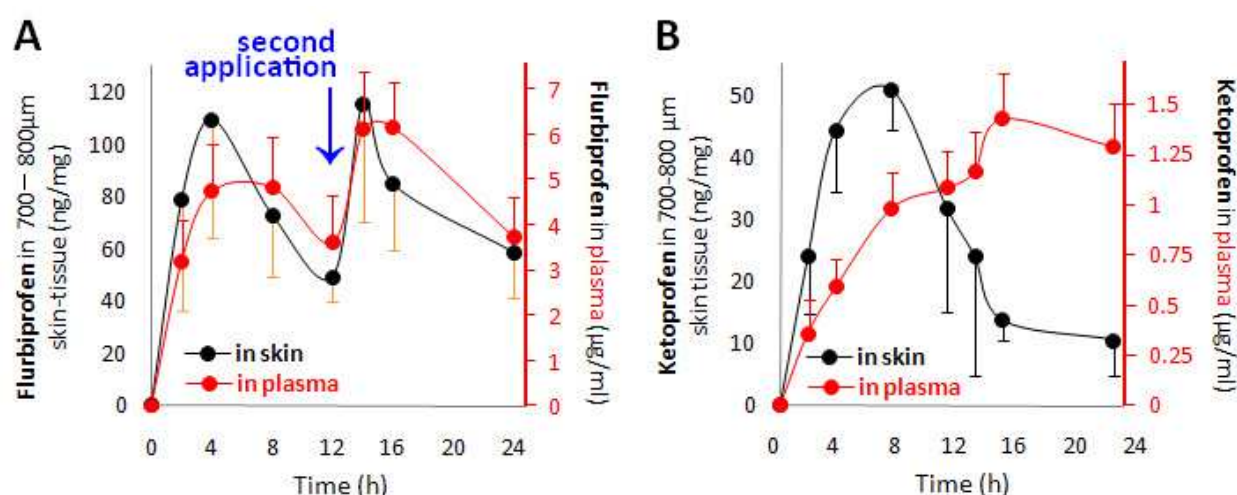

Fig. 7. Time-course profiles of the skin tissue and plasma concentrations of flurbiprofen and ketoprofen after FP-T and KP-T application. (A) The skin tissue concentrations of flurbiprofen in the layer from 700 to 800 μm are expressed on the left axis and the plasma concentrations of flurbiprofen are expressed on the right axis. (B) The skin tissue and plasma concentrations of ketoprofen are expressed in the same way as described for (A). Each point represents the mean ± SD (n=3–5).

Skin concentrations result from the balance of permeation into the tissue and clearance from the tissue, combined with partitioning into the various skin components (Kretsos et al., 2008). Percutaneous absorption depends on the physicochemical properties of the compound, and small moderately lipophilic molecules are generally the most readily absorbed. The log Ko/w values for flurbiprofen and ketoprofen are 3.75 and 2.76, respectively (Valko et al., 2003). The binding affinity of flurbiprofen for albumin is superior to that of ketoprofen, as mentioned above, and the binding ratios of the two drugs are 99.9% and 98.7%, respectively (Valko et al., 2003). It is notable that both the viable epidermis and dermis contain, on average, about 2% extravascular albumin, to

which both flurbiprofen and ketoprofen can bind (Bert et al., 1986). The high lipophilicity and an effect of the superior protein-binding activity on the clearance mechanism are likely to be the major reasons why the skin concentration of flurbiprofen was higher than that of ketoprofen. Besides, other important factors may affect the transdermal absorptions of the two drugs. In general, LETD-mediated drug permeation has been explained in terms of passive diffusion of non-ionized compounds (Potts, 1992). However, several studies have focused on the possible involvement of transporters expressed in the skin, similar to the case for other tissues (Schiffer et al., 2003; Bonen et al., 2006; Li, 2006). Organic anion transporter 2 (OAT2) is thought to be a candidate for an exchanger involved in the uptake and/or efflux of flurbiprofen in the skin, and the flurbiprofen permeability in the absorptive direction was higher than that in the secretory direction (Ito et al., 2007). The superior permeability of flurbiprofen in the skin may be attributable to a more predominant exchange capacity with several transporters compared with other NSAIDs.

5. Pharmacodynamics

From our lateral sectioning approach, it was predicted that FP-T had the potential for prompt onset and prolonged duration of NSAID activity, while KP-T needed several hours to demonstrate its efficacy as an anti-inflammatory drug in intramuscular tissues. Therefore, we investigated whether the results for the percutaneous drug permeation using lateral slicing were in accordance with the drug efficacies.

5.1 Topical anti-inflammatory efficacy in carrageenin-induced rat paw edema

The topical anti-inflammatory efficacy was evaluated for carrageenin-induced inflammation edema in Wistar rats weighing 124±11 g. The activities of the drugs were evaluated by measuring the changes in paw volume with a plethysmometer (TK-101CMP; Muromachi Co. Ltd., Tokyo, Japan). FP-T or KP-T (0.5×3.5 cm) was applied to the right paw for 3 h with the help of adhesive tape, at 0.5 h prior to the carrageenin injection. Acute inflammation was produced by injecting 0.1 mL of 1% (w/v) carrageenin solution into the subplantar region of the right hind paw. The paw volume was measured at 0, 3, 4 and 5 h after the injection.

The percentage of swelling in the paw was calculated by the following formula.

$$\text{Swelling (\%)} = \frac{b - a}{a} \times 100 \tag{2}$$

where a is the paw volume before producing the edema and b is the paw volume measured after producing the edema.

In this acute model, the percentage swelling was promptly suppressed by flurbiprofen released from FP-T, whereas it required some time to be suppressed by ketoprofen from KP-T (Fig. 8). In addition, the FP-T efficacy was much stronger than that of KP-T. These findings support the accuracy of the evaluation method of percutaneous drug permeation using a lateral sectioning approach.
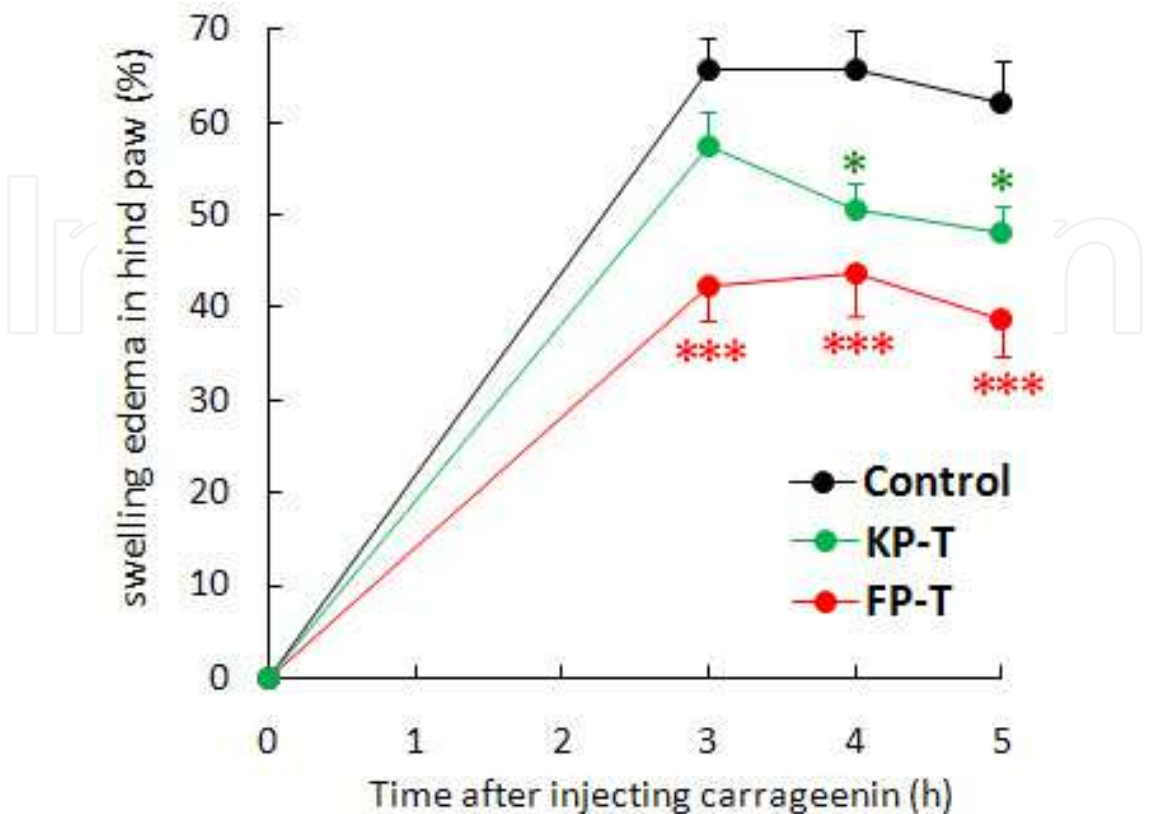
Fig. 8. Effects of FP-T and KP-T on edema produced by carrageenin in the hindpaw of rats. Each point represents the mean ± SE (n=7). *$P<0.05$ and ***$P<0.001$ *vs.* Control by Dunnett's test.

5.2 Antinociceptive efficacy in the inflamed paw pressure test

This experiment was performed according to the method of Randall & Selitto (1957) using Wistar rats weighing 114±19 g. FP-T or KP-T (0.5×3.5 or 1.0×3.5 cm) was applied to the right paw for 3 h with the help of adhesive tape, at 0.5 h prior to an injection of baker's yeast. Briefly, 0.1 mL of a 10% (w/v) solution of baker's yeast in saline was given by subplantar injection into the hind paw. Nociception was measured by applying an increased weight to the paw with a pressure meter (Ugo Basile, Varese, Italy) until the rats vocalized or drew their feet back. The algesic threshold was measured at 2, 3 and 4 h after the injection and the level of the algesic threshold was expressed as the sum of the algesic thresholds between 2 and 4 h.

As shown in Fig. 9, FP-T significantly increased the paw pressure threshold required for the nociceptive response, indicating a probable analgesic action. Besides, the drug efficacy depended on the applied area of FP-T. However, KP-T had no effect on the nociceptive response, even when its applied area was expanded.

From these pharmacodynamics studies, our predictions derived from the lateral sectioning approach were consistent with the facts that FP-T had the potential for prompt onset and prolonged duration of NSAID activity and that KP-T required a reasonable time to demonstrate its efficacy.
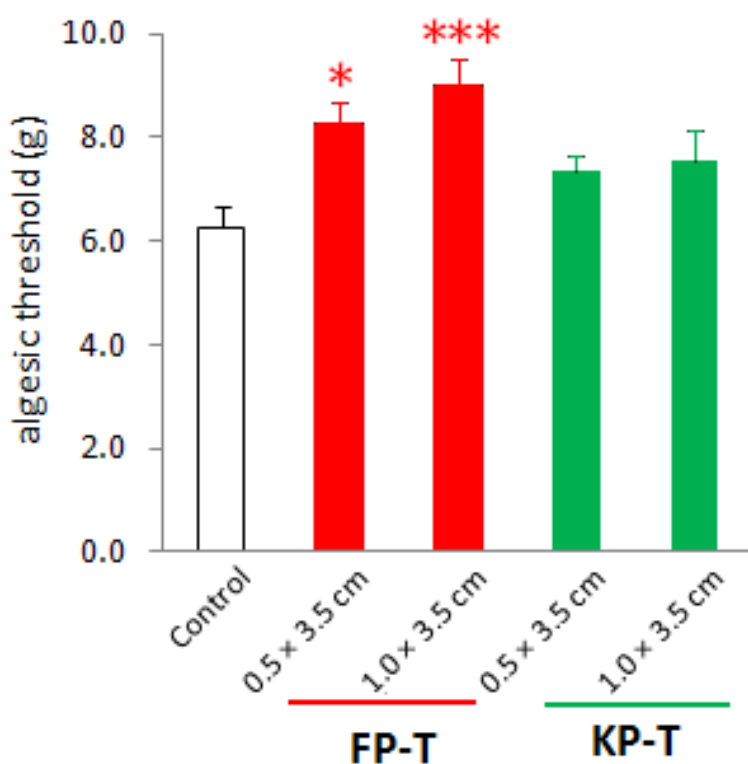
Fig. 9. Effects of FP-T and KP-T on nociception produced by baker's yeast in the hindpaw of rats. Each column represents the mean ± SE (n=10). *$P<0.05$ and ***$P<0.001$ *vs.* Control by Dunnett's test.

6. Evaluation of the technique

There are several limitations to our technique for evaluating transdermal permeability. First, information on the percutaneous absorption is obtained from invasive skin biopsies and this method cannot be applied to humans. Next, this technique is limited by the location. As previously mentioned, this technique depends on the procedure for freezing and slicing samples in a flat manner. Therefore, the abdominal skin is a suitable area for removal and preparation as samples. However, there are many sites in the body other than the abdominal area that are not flat, such as the circumferences of joints, and we ought to measure the drug concentrations at these sites. Our pharmacodynamic studies focused on the hindpaw and not on the abdominal area, although our predictions were derived using the abdominal region. Nevertheless, the therapeutic expressions were accordance with the efficacy predictions. This means that the lateral sectioning approach at the abdominal area could be applied to understand drug flow in other places of the body. Furthermore, it is well known that rodent skin is generally more permeable than human skin (Poet et al., 2000). Therefore, another method is required to precisely calculate the drug permeability in human skin. However, our lateral sectioning approach can simply and clearly indicate the time courses of the flow of drugs in skin tissues and the obtained data should facilitate the prediction and estimation of the pharmacodynamics of patches with other transdermal formulations. Recently, a hydrogel patch has been applied as a transcutaneous vaccination

system against viral and bacterial infections (Ishii et al., 2008; Matsuo et al., 2011). In this case, the drug levels in the epidermal layer where Langerhans cells reside are crucial, and there is no point in measuring the total amounts of drugs that permeate the whole skin. Furthermore, we can check the conformational changes of the protein in the process of passing through the stratum corneum to judge whether the activity of the protein is maintained at that site.

As a matter of course, the sites in the skin where different drugs have efficacy tend to differ. However, our slicing method makes it possible to evaluate the drug levels at the skin sites where individual drugs exert their beneficial effects. These studies focused on the skin region for the present data, and clarification of the musculocutaneous pharmacokinetics is an issue for future studies. In addition, we would like to concentrate on the surrounding environments of the drugs in the skin tissues.

7. Conclusions

The present experimental data suggest that our approach involving lateral sectioning of frozen skin tissues and measurement of the drug concentration in each slice makes it possible to easily and clearly determine the tissue concentrations of drugs in skin layers adjacent to the target area, albeit in a rodent model. This technique has the advantage of simplicity and therefore has the potential to be applied to evaluations of the pharmacokinetics of other transdermal formulations.

8. References

Bashir, S. J.; Chew, A. L.; Aniqboqu, A.; Dreher, F. & Maibach, H. I. (2001). Physical and physiological effects of stratum corneum tape stripping. Skin Research and Technology, Vol.7, No.1, (February 2001), pp. 40-48, ISSN 0909-752X

Bert, J. L. (1986). Pearce RH, Mathieson JM. Concentration of plasma albumin in its accessible space in postmortem human dermis. *Microvascular Research*, Vol.32, No.2, (September 1986), pp. 211-223, ISSN 0026-2862

Bert, J. L.; Mathieson, J. M. & Pearce, R. H. (1982). The exclusion of human serum albumin by human dermal collagenous fibres and within human dermis. Biochemical Journal, Vol.201, No.2, (February 1982), pp. 395-403, ISSN 0264-6021

Bonen, A.; Heynen, M. & Hatta, H. (2006). Distribution of monocarboxylate transporters MCT1-MCT8 in rat tissues and human skeletal muscle. *Applied Physiology and metabolism*, Vol.31, No.1, (February 2006), pp. 31-39, ISSN 1715-5312

Borgå, O. & Borgå, B. (1997). Serum protein binding of nonsteroidal antiinflammatory drugs: A comparative study. *Journal of Pharmacokinetics and Biopharmaceutics*, Vol.25, No.1, (February 1997), pp. 63-77, ISSN 0090-466X

Brogden, R.N.; Heel, R. C.; Speight, T. M. & Avery, G. S. (1979). Flurbiprofen: a review of its pharmacological properties and therapeutic use in rheumatic diseases. *Drugs*, Vol.18, (December 1979), pp. 417-438, ISSN 0012-6667

Buchanan, W. W. & Kassam, Y. B. (1986). European experience with flurbiprofen. A new analgesic/anti-inflammatory agent. *American Journal of Medicine*, Vol.80, No.3A, (March 1986), pp. 145-152, ISSN 0002-9343

Flouvat, B.; Roux, A. & Delhotal-Landes, B. (1989). Pharmacokinetics of ketoprofen in man after repeated percutaneous administration. *Arzneimittelforschung*, Vol.39, No.7, (July 1989), pp. 812-815, ISSN 0004-4172

Goi, N.; Morishita, K.; Taniguchi, A. & Ishii, T. (2010). Evaluation of percutaneous permeation of flurbiprofen and ketoprofen after application of transdermal patches using a lateral sectioning approach in hairless rats. *Pharmaceutical Development and Technology*, Vol.15, No.6, (December 2010), pp. 658-665, ISSN 1083-7450

Gupta, E.; Wientjes, M. G. & Au, J. L. (1995). Penetration kinetics of 2′, 3′-dideoxyinosine in dermis is described by the distributed model. *Pharmaceutical Research*, Vol.12, No.1, (January 1995), pp. 108-112, ISSN 0724-8741

Hawkey, C. J. & Truelove, S. C. (1983). Inhibition of prostaglandin synthetase in human rectal mucosa. *Gut*, Vol.24, (March 1983), pp. 213-217, ISSN 0017-5749

Ishii, Y.; Nakae, T.; Sakamoto, F.; Matsuo, K.; Quan, Y. S.; Kamiyama, F.; Fujita, T.; Yamamoto, A.; Nakagawa, S. & Okada, N. A transcutaneous vaccination system using a hydrogel patch for viral and bacterial infection. *Journal of Controlled Release*, Vol.131, No.2, (October 2008), pp. 113-120, ISSN 0168-3659

Ito, K.; Kato, Y.; Tsuji, H.; Nguyen, H. T.; Kubo, Y. & Tsuji, A. (2007). Involvement of organic anion transport system in transdermal absorption of flurbiprofen. *Journal of Controlled Release*, Vol.124, (August 2007), pp. 60-68, ISSN 0168-3659

Kretsos, K.; Miller, M. A.; Zamora-Estrada, G. & Kasting, B. (2008). Partitioning, diffusivity and clearance of skin permeants in mammalian dermis. *International Journal of Pharmaceutics*, Vol.346, (January 2008), pp. 64-79, ISSN 0378-5173

Li, Q.; Tsuji, H.; Kato, Y.; Sai, Y.; Kubo, Y. & Tsuji, A. (2006).Characterization of the transdermal transport of flurbiprofen and indomethacin. *Journal of Controlled Release*, Vol.110, No.3, (February 2006), pp. 542-556, ISSN 0168-3659

Martens, M. (1997). Efficacy and tolerability of a topical NSAID patch (local action transcutaneous flurbiprofen) and oral diclofenac in the treatment of soft-tissue rheumatism. *Clinical Rheumatology*, Vol.16, (January 1997), pp. 25-31, ISSN 0770-3198

Matsuo, K.; Ishii, Y.; Quan, Y. S.; Kamiyama, F.; Mukai, Y., Okada, N. & Nakagawa, S. (2011). Characterization of transcutaneous protein delivery by a hydrogel patch in animal, human, and tissue-engineered skin models. Biological and Pharmaceutical Bulletin, Vol.34, No.4, (2011), pp. 586-589, ISSN 0918-6158.

Mazieres B. (2005). Topical ketoprofen patch. *Drugs in Research and Development*, Vol.6, No.6, (2005), pp. 337-344, ISSN 1174-5886

Morgan, C.J.; Renwick, A. G. & Friedmann, P. S. (2003). The role of stratum corneum and dermal microvascular perfusion in penetration and tissue levels of water-soluble drugs investigated by microdialysis. *British Journal of Dermatology*, Vol. 148, (March 2003), pp. 434-443, ISSN 0007-6963

Poet, T. S.; Corley, R. A.; Thrall, K. D.; Edwards, J. A.; Tanojo, H.; Weitz, K. K.; Hui, X.; Maibach, H. I. & Wester, R. C. (2000). Assessment of the percutaneous absorption of trichloroethylene in rats and humans using MS/MS real-time breath analysis and physiologically based pharmacokinetic modeling. *Toxicological Sciences*, Vol.56, No.1, (July 2000), pp. 61-72, ISSN 1096-6080

Potts, R. O. & Guy, R. H. Predicting skin permeability. *Pharmaceutical Research*, Vol.9, No.5, (May 1992), pp. 663-669, ISSN 0724-8741

Randall, L. O. & Selitto, J. J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Archives internationals de pharmacodynamie et de therapie*, Vol.111, No.4, (September 1957), pp. 409-419, ISSN 0301-4533

Schaefer, H & Redelmeier, T. E. (1996). Skin Barrier, S Karger Pub, ISBN 978-3805563260, Basel, Switzerland

Schaefer, H.; Stüttgen, G.; Zesch, A.; Schalla, W. & Gazith, J. (1978). Quantitative determination of percutaneous absorption of radiolabeled drugs in vitro and in vivo by human skin. *Current Problems in Dermatology*, Vol.7, (1978), pp. 80-94, ISSN 1421-5721

Scheuplein, R. J. (1976). Percutaneous absorption after twenty-five years: or "old wine in new wineskins". *Journal of Ivestigative Dermatology*, Vol.67, (July 1976), pp. 31-38, ISSN 0022-202X

Schiffer, R.; Neis, M.; Höller, D.; Rodríguez, F.; Geier, A.; Gartung, C.; Lammert, F.; Dreuw, A.; Zwadlo-Klarwasser, G.; Merk, H.; Jugert, F. & Baron, J. M. (2003). Active influx transport is mediated by members of the organic anion transporting polypeptide family in human epidermal keratinocytes. *Journal of Investigative Dermatology*, Vol.120, No.2, (February 2003), pp. 285-291, ISSN 0022-202X

Singh, P. & Roberts, M. S. (1994). Skin permeability and local tissue concentrations of nonsteroidal anti-inflammatory drugs after topical application. *Journal of Pharmacology and Experimental Therapeutics*, Vol.268, No.1, (January, 1994), pp. 144-151, ISSN 0022-3565

Singh, P. & Roberts, M. S. (1996) Local deep tissue penetration of compounds after dermal application: structure-tissue penetration relationships. *American Society for Pharmacology and Experimental Therapeutics*, Vol.279, No.2, (November 1996), pp. 908-917, ISSN 0022-3565

Sugawara, S.; Ohno, H.; Ueda, R.; Ishigami, M.; Fujiwara, H.; Hashimoto, S.; Satoh, Y. & Yamazaki, K. (1987). A study of the concentration in the synovial fluid and tissues of patients treated with the transdermal topical application of flurbiprofen (FP-A). *Therapeutic Research*, Vol.6, No.1, (January 1987), pp. 289-294, ISSN 0289-8020

Taburet, A. M.; Singlas, E.; Glass, R. C.; Thomas, F. & Leutenegger, E. (1995). Pharmacokinetic comparison of oral and local action transcutaneous flurbiprofen in healthy volunteers. *Journal of Clinical Pharmacy and Therapeutics*, Vol.20, No.2, (April 1995), pp. 101-107, ISSN 0269-4727

Taha, A. S.; Dahill, S.; Sturrock, R. D.; Lee, F. D. & Russell, R. I. (1994). Predicting NSAID related ulcers – assessment of clinical and pathological risk factors and importance of differences in NSAID. *Gut*, Vol.35, (July 1994), pp. 891-895, ISSN 0017-5749

Touitou, E. Meidan, V. M. & Horwitz, E.(1998). Methods for quantitative determination of drug localized in the skin. *Journal of Controlled Release*, Vol.56, (December 1998), pp. 7-21, ISSN 0168-3659

Valko, K.; Nunhuck, S.; Bevan, C.; Abraham, M. H. & Reynolds, D. P. (2003). Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. Journal of Pharmaceutical Sciences, Vol.92, Vol.92, (November 2003), pp. 2236-2248, ISSN 0022-3549

Waikakul, S.; Penkitti, P.; Soparat, K. & Boonsanong, W. (1997). Topical analgesics for knee arthrosis: a parallel study of ketoprofen gel and diclofenac emulgel. *Journal of the*

Medical Association of Thailand, Vol.80, No.9, (September 1997), pp. 593-596, ISSN 0125-2208

Wright, J. D.; Boudinot, F. D. & Ujhelyi, M. R. (1996). Measurement and analysis of unbound drug concentrations. Clinical Pharmacokinetics, Vol.30, No.6, (June 1996), pp. 445-462, ISSN 0312-5963
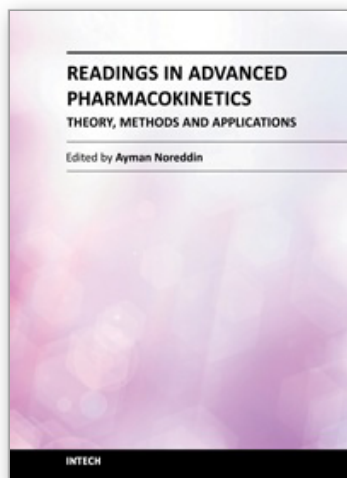
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