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In Vivo Pharmacokinetics of Ketoprofen after Patch Application in the Mexican Hairless Pig

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ABSTRACT: To evaluate the pharmacokinetics of topical drugs, *in vitro* permeation studies are performed using sacrificed pig skin or human tissues resected at surgery; however, these methods have their limitations in *in vivo* pharmacokinetics. This study examined the usefulness of Mexican hairless pigs for *in vivo* pharmacokinetic study, especially the drug concentration in the tissues. A ketoprofen patch was applied on the back of Mexican hairless pigs for 24 h, followed by sequential collection of blood specimens from 0 to 36 h ($n = 3$). Also, the skin, subcutaneous fat, fascia and muscle from the center of the site of application were excised at 12 h after the application ($n = 4$). Ketoprofen was first detected in the plasma at 8 h, the concentration increasing up to 24 h; the plasma concentration began to decrease after the removal of the ketoprofen patch. Ketoprofen concentrations in the tissues decreased with increasing depth of the tissues, but the values in the deep muscles, being the lowest among the tissues examined, were still higher than those in the plasma. While the data of drug concentration in human tissue are difficult to test, the Mexican hairless pig model appears to be attractive for *in vivo* pharmacokinetic studies of topically applied ketoprofen. Copyright © 2009 John Wiley & Sons, Ltd.

Key words: ketoprofen; Mexican hairless pig; patch; pharmacokinetics; topical application

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used widely for pain relief in musculoskeletal disorders [1]. Although oral formulations of NSAIDs are currently popular, they are associated with a high incidence of adverse effects, including stomach irritation, hepatotoxicity and kidney failure [2,3]. In order to minimize the incidence of systemic events related to oral formulations of NSAIDs, topical forms of the drugs have been developed and the ketoprofen patch is one such product [4].

To evaluate the pharmacokinetics of topically applied drugs, *in vitro* permeation studies [5,6] and *in vivo* pharmacokinetic studies have been performed using small animals [7]; however, the results obtained from such studies may not be directly applicable to humans. There have been several reports describing the NSAID concentrations in human tissues after transdermal

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application [8–11], but the information from these studies remains limited. Larger animals will be preferable for *in vivo* pharmacokinetic studies for obtaining results applicable to humans.

Miniature pigs are used widely for medical research because of their easy handling and raising [12]. Among miniature pigs, it was considered that hairless pigs might be highly suitable for the evaluation of topical drugs, because the structure of the hairless pig skin is similar to that of human [13]. This study evaluated the *in vivo* pharmacokinetics of ketoprofen applied topically on the back of Mexican hairless pigs. The results demonstrated the usefulness of the Mexican hairless pig for the study of NSAID patches.

Materials and Methods

Animals

To collect sequential blood specimens, three Mexican hairless pigs [14] (National Livestock Breeding Center, Ibaraki, Japan) aged 12 months old and weighing 23.1–38.0 kg were used. To obtain en bloc tissue specimens from the back, four Mexican hairless pigs aged 5–22 months old and weighing 9.8–30.8 kg were used. All Mexican hairless pigs used were bred under specific pathogen-free (SPF) conditions. All the pigs had free access during the study period to food and water in a postoperative care cage which was 40 cm in width, 121 cm in depth, and 109 cm in height. The schemes of the animal experiments had been investigated and permitted by The Judging Committee of Experimental Animal Ethics of Jichi Medical University.

Topical application of ketoprofen

The sparse hairs on the back of the animals were shaved with an electrical clipper and the application area was swept with dry cotton. The ketoprofen patch (10 cm × 7 cm; Hisamitsu Pharmaceutical Co., Inc., Tokyo, Japan) containing 20 mg of ketoprofen was applied on the back of the Mexican hairless pigs. The medial margin of the patch was located at 3 cm to the left of the spinous processes of the thoracic vertebrae.

Plasma preparation

One day before the experiment, a central venous catheter was placed in the right medial cervical vein in all the animals under general anesthesia for blood sampling. Five milliliter blood samples were collected in heparinized syringes at 0, 1, 2, 4, 6, 8, 12 and 24 h, after which the ketoprofen patch was removed; thereafter, blood samples were collected again at 28, 32 and 36 h. The collected blood samples were immediately centrifuged at 3000 rpm for 15 min. Plasma was separated and the plasma samples were cryopreserved at −20°C until analysis.

Tissue sampling

The ketoprofen patches were applied on the backs of the animals for 12 h. Immediately after removal of the patches and wiping off of the drug remaining on the skin surface with wet cotton, the skin, subcutaneous fat, fascia and muscle at the center of the patch application site were excised en bloc (2 cm × 2 cm × 3 cm) under anesthesia induced by intramuscular injection of 10 mg/kg of ketamine, 2 mg/kg of xylazine and 0.02 mg/kg of atropine. The specimens were then divided into five sections; skin, subcutaneous fat, fascia, superficial muscle up to 5 mm thickness, and the remaining deeper muscle. All specimens were cryopreserved at −20°C until the analysis.

Ketoprofen concentration analysis

Fifty milligrams of tissues were homogenized in methanol. Ketoprofen in the homogenates and plasma (0.25 ml) was acidified and extracted by liquid–liquid extraction with diethyl ether. After evaporation of the organic phase, the residue was dissolved in a methanol/water mixture and transferred into vials. Ketoprofen extracted from the plasma and tissues was assayed by high-performance liquid chromatography with positive ion spray ionization tandem mass spectrometry detection (LC-MS-MS; 2695 separation module (Waters) and API-4000 (Applied Biosystems/MDS SCIEX).

Results and Discussion

Mexican hairless pigs are descendants of Iberian pigs. The name 'hairless' derives from its main

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characteristic, namely, the absence of hair (or sparse hair) on the skin (Figure 1A).

A topical patch containing 20 mg of ketoprofen was applied to the back of Mexican hairless pigs for 24 h (Figure 1B). Blood was collected at 0, 1, 2, 4, 6, 8, 12, 24 (prior to patch removal) 28, 32 and 36 h after the patch application. Ketoprofen was first detected in the plasma at 8 h, and the concentration increased steadily up to 24 h (Figure 2). After removal of the ketoprofen patch, the plasma concentration decreased, but the drug could still be detected until the 36 h time-point.

Next, the ketoprofen concentrations in the tissue specimens were measured. Ketoprofen patches were applied on the back of Mexican hairless pigs for 12 h. Immediately after removal of the patch, the skin, subcutaneous fat, fascia and muscle at the center of the patch application site (Figure 3A) were excised en bloc (2 cm × 2 cm × 3 cm). The muscle was divided into superficial muscle up to 5 mm thickness and the remaining deep muscle. The highest ketoprofen concentration was obtained in the skin, followed by that in the subcutaneous fat, fascia, superficial muscle and deep muscle, in that order (Figure 3B). Interestingly, ketoprofen concentrations in the tissues decreased with increasing depth of the tissues, even though the composition of the tissues, tissue permeability to ketoprofen and the vascularity in the layers are totally different. The ketoprofen concentration in the deep muscle was the lowest among the tissues examined, but it was still higher than that in the plasma. There was no marked difference in ketoprofen concentrations in the

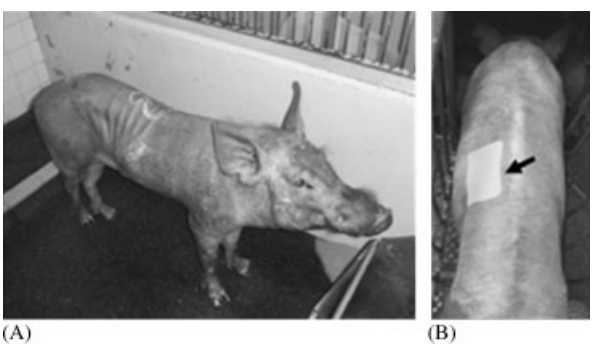

Figure 1. Mexican hairless pig. (A) Typical appearance of a 12-month-old Mexican hairless pig. (B) Ketoprofen patch (arrow) applied on the back of the Mexican hairless pig

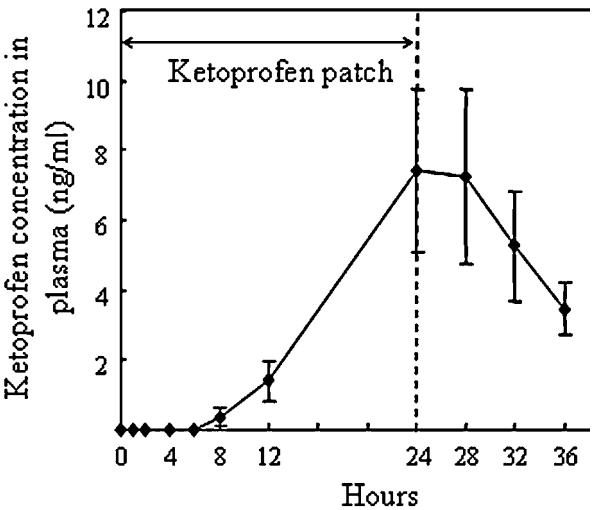

Figure 2. Ketoprofen concentrations in the plasma. Average values with SEM are shown ($n = 3$)

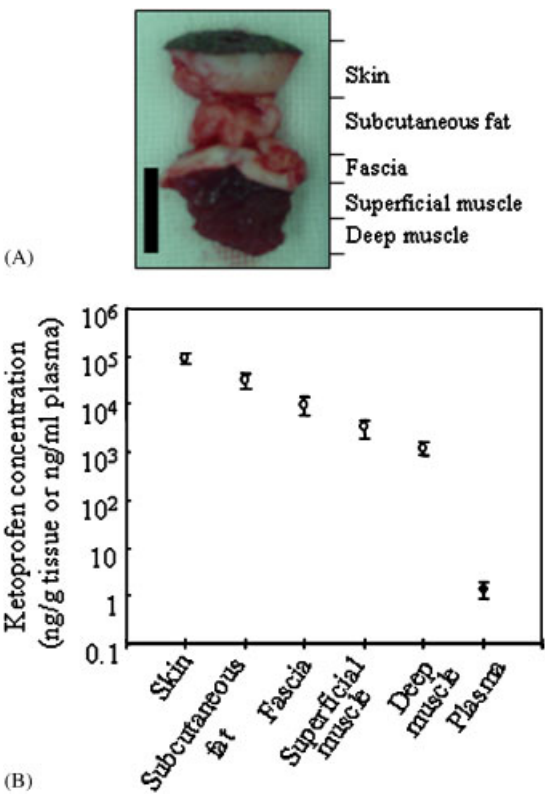

Figure 3. Ketoprofen concentration in the tissues. (A) Tissues from the center of the patch application site were excised en bloc and examined. Scale bar: 10 mm. (B) Ketoprofen concentration in each tissue at 12 h after the ketoprofen patch application. Average values with SEM are shown ($n = 4$)

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tissues and plasma between small and big animals in this study.

In our previous research conducted in humans, the ketoprofen concentrations in the plasma reached their maximum at 13 h and decreased thereafter when a 20 mg ketoprofen patch was applied on the back of humans for 24 h (data not shown). The pharmacokinetics of ketoprofen in the plasma was thus different in between humans and Mexican hairless pigs. One of the possible explanations is that the difference in the thickness of the stratum corneum, which represents a rate-limiting factor for percutaneous absorption. Since the stratum corneum in Mexican hairless pigs is thicker than that in humans, the percutaneous absorption rate in the Mexican hairless pig could be expected to be slower.

At 12 h after the application, even though the ketoprofen concentrations in the plasma did not reach their peak levels, the ketoprofen concentrations in the tissues under the patch application site decreased with increasing depth of the tissues. Furthermore, the concentrations in the deep muscles were still higher than the plasma concentrations at this time-point. These findings indicate that ketoprofen may be directly delivered to the deep tissues without passing through the blood stream.

At present, in order to evaluate the pharmacokinetics of topically applied drugs, *in vitro* permeation studies are often performed using the skin from the sacrificed mouse, rat [7] or pig [5,15]. Our study has the advantage of being able to obtain the living animal data that include the effect of blood flow.

The percutaneous absorption of ketoprofen from topical application is known to be influenced by differences in skin structure at various regions of the body [16]. In a clinical situation, ketoprofen patches are applied not only to the back, but also to varied positions of the entire body in humans. In this study, the ketoprofen patches were applied only to the back of the pigs. When investigating the abdominal site or leg of large four-footed animals previously, some failures were experienced. For example, the patches peeled off or slipped from the abdominal site from kicks of the hind legs, and the abdominal sites became dirty from the feces or urine. In order to avoid these technical failures, only the back skin of large four-footed animals was investigated.

It is difficult to obtain data of drug concentrations in tissues from non-human primates. The FDA recommends having a pre-clinical model in pigs (personal communications). Based on the results of our study, the Mexican hairless pig may serve as a suitable model for *in vivo* pharmacokinetic analysis of topically applied drugs, especially ketoprofen, in solid tissues, which is difficult to obtain in humans.

Implications

This study evaluated the *in vivo* pharmacokinetics of ketoprofen applied topically in Mexican hairless pigs. Peak plasma levels were observed 24 h after the ketoprofen patch application on the back and the ketoprofen concentrations in the tissues decreased with increasing depth of the tissues. Mexican hairless pigs are attractive models for the pharmacokinetic study of topically applied ketoprofen.

Acknowledgements

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