Iontophoretic Transdermal Delivery of Ketoprofen: Novel Method for the Evaluation of Plasma Drug Concentration in Cutaneous Vein

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The objective of our study is to establish a novel method for the in vivo evaluation of transdermal delivery. In this study, cathodal iontophoresis of ketoprofen, a nonsteroidal anti-inflammatory drug, was performed in the thoracic area of rats at a constant direct current, and blood samples were collected from cutaneous vein passing through the thoracic part of the body. After the iontophoresis, the plasma ketoprofen concentration in cutaneous vein ipsilateral to the application site was significantly higher than that in systemic vein. On the other hand, the plasma concentration in cutaneous vein contralateral to the application site was not significantly different from that in systemic vein. A comparison of the time-course curves demonstrated that, for the duration of iontophoresis, the plasma ketoprofen concentration in cutaneous vein ipsilateral to the application site increased with the amount of ketoprofen absorbed in the skin. These results suggest that the plasma concentration in the cutaneous vein ipsilateral to the application site is related with the transfer of drug from skin to cutaneous blood circulation. Therefore, the measurement of plasma concentration in cutaneous vein close to the application site would allow us to directly quantify the local behavior of iontophoretic transdermal absorption.

Key words: transdermal delivery; iontophoresis; cutaneous vein; plasma concentration; ketoprofen

During the last 2 decades, transdermal drug delivery by iontophoretic application has been developed for fundamental studies and clinical use.1–4 Iontophoresis is a physical method for enhancing the permeability of drugs through skin. The principal of iontophoresis is the migration of charged drugs to an electrode of an opposite charge under an electrical potential gradient.

Early studies of in vivo transdermal delivery were based on the hypothesis that drug molecules absorbed into the skin were transferred to the blood flow in cutaneous microcirculation and were transported to the rest of the body through systemic circulation.4–7 Therefore, from a number of studies, it is suggested that cutaneous blood flow plays an important role in the in vivo transdermal absorption of drugs.8–15 Singh and Roberts demonstrated that the decrease in cutaneous blood flow caused by phenylephrine, a local vasoconstrictive agent, led to a decrease in the transfer of drug from rat dermis.13 Gao et al. proposed a pharmacokinetic model in which the drug was removed to cutaneous blood flow.15 Thus, the measurement of drug concentration in cutaneous vein would allow us to evaluate the transfer of drug from skin to cutaneous blood flow. Recently, the contribution of cutaneous blood flow to transdermal absorption of drugs has been studied using a skin flap.16–20 However, as skin flap is isolated from the body of the animal with a pair of artery and vein, the cutaneous circulatory system of the skin flap is not considered to be intact. Therefore, it is desired to establish an experimental method using intact animals. In the present study, the objective is to demonstrate a method for the measurement of drug concentrations in the cutaneous vein of intact rat. In the experiment, cathodal iontophoresis of ketoprofen (M.W. 254; pKa 4.60), a nonsteroidal anti-inflammatory drug, was performed on the thoracic part of rats, and the plasma concentration in cutaneous vein close to the application site was measured. The lateral thoracic vein was used as cutaneous vein.19 Moreover, the plasma concentration in cutaneous vein was compared with that in systemic vein.

Ketoprofen has been proven effective in treating rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute musculoskeletal diseases.20–22 Ketoprofen is available in a wide range of dosage forms, including topical gel and tape for local treatment.20,22 The local treatment of ketoprofen requires transporting a sufficient amount to the internal tissues of joints, which are deep below the application site.20 Thus, it is valuable to clarify the role of cutaneous blood flow in drug distribution to deeper tissues.

MATERIALS AND METHODS

Materials and Animals Ketoprofen was purchased from Wako Pure Chemical Industries, Ltd. (analytical grade, product number 115-00381). All other chemical reagents were of analytical grade. Male Sprague-Dawley rats (8–10 weeks old) were purchased from Charles River Japan.

Iontophoresis of Ketoprofen Ketoprofen solution of 1 mg/ml was prepared with 50 mM phosphate buffer solution (pH 7.4). Rats were lightly anesthetized with ether. Their body temperature was maintained at 37°C throughout the experiment by placing them on a heating pad. The hair of the thoracic and abdominal area was removed with electrical clippers. The reservoirs (made of silicone, 9.5 mm i.d., 20 mm height) were fixed using adhesive to the thoracic skin of the rat, as shown in Fig. 1. Ketoprofen solution (1.2 ml) and 50 mM phosphate buffer solution (1.2 ml) were placed in the drug and buffer reservoirs, respectively. A platinum electrode (8 mm diameter) was immersed in each reservoir. Cathodal iontophoresis was performed with an electrical stimulating apparatus (Nippion Koden, SEN7203). A constant direct current of 0.5 mA/0.7 cm2 was applied for 30 min. After the current was turned off, the electrodes and the reservoirs were removed from the skin, and the surface of the applied area was washed with 1 ml of saline 3 times.

Blood Sampling Blood samples were collected from the lateral thoracic vein and inferior vena cava as follows, with

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the animals under ether anesthesia. After the termination of iontophoresis, an incision was made in the thoracic and epigastric skin of each rat. The subcutaneous surface of the thoracic skin at the application site was exposed by carefully reversing the thoracic skin. Blood samples (200—250 μl) were collected by bleeding the lateral thoracic vein using a syringe needle. Subsequently, the abdomen of each rat was opened through a midline incision. Blood samples from the inferior vena cava were collected by venipuncture. The plasma samples were separated by centrifugation (3000 rpm×10 min, 4°C) and stored in a sampling tube at −20°C prior to analysis.

**Sampling of Whole Skin** The whole skin corresponding to the applied area was excised and weighed. The skin samples were added to 2 ml of saline and acetonitrile (1:1, v/v) and homogenized under cooling at 4°C. After centrifugation (3000 rpm×10 min, 4°C), the clear supernatant was stored in a polypropylene tube at −20°C prior to analysis.

**Assay of Plasma Concentration** Plasma concentrations of ketoprofen were determined by HPLC analysis. The plasma (0.05—0.2 ml) was acidified by adding 1 ml hydrochloric acid (0.1 ml), then extracted with ether (5 ml). The ether layer was separated by centrifugation (3000 rpm×10 min, 4°C) and evaporated to dryness under a nitrogen stream. The mobile phase (0.2 ml) was added to dissolve the residue. A 100 μl aliquot of the final preparation was injected into an octadecyl reversed-phase column (YMC-Pack ODS-AM312, 150×6.0 mm i.d., S-5 μm, 120 Å) using a mobile phase composed of 50 μM phosphoric acid—acetonitrile—methanol (40:30:30, v/v/v) at a flow rate of 0.8 ml/min. The column temperature was maintained at 40°C. Ketoprofen in effluent from the column was detected at 256 nm by UV spectrophotometer. Ketoprofen was quantitated by means of a calibration curve of the peak area versus standard concentration. The limit of quantitation was 0.01 μg/ml of ketoprofen and the calibration curves were linear through at least 1 μg/ml (linear least squares regression, 0.999—0.9999).

**Assay of the Amount of Ketoprofen Absorbed in Whole Skin** Ketoprofen concentrations in excised whole skin were determined by HPLC analysis. A 20 μl aliquot of the supernatant obtained from homogenization of the excised whole skin was injected into an octadecyl reversed-phase column (YMC-Pack ODS-AM312, 150×6.0 mm i.d., S-5 μm, 120 Å) using a mobile phase composed of 0.05% phosphoric acid—acetonitrile (55:45, v/v) at a flow rate of 1 ml/min. The column temperature was ambient (room temperature, 23—25°C). Ketoprofen in effluent from the column was detected at 256 nm by UV spectrophotometer. Ketoprofen was quantitated by means of a calibration curve of the peak area versus standard concentration. The limit of quantitation was 0.01 μg/ml of ketoprofen and the calibration curves were linear through at least 1 μg/ml (linear least squares regression, 0.999—0.9999). The amount of absorbed ketoprofen in skin was calculated as the amount per unit area.

**RESULTS**

Cathodal iontophoresis of ketoprofen onto the right thoracic skin of rats was performed in vivo for a period of 30 min. After the termination of iontophoresis, the plasma ketoprofen concentration in cutaneous vein on the same side (ipsilateral) as the application site was compared with that in systemic vein (Fig. 2). The plasma concentration in cutaneous vein ipsilateral to the application site, 1.08±0.45 μg/ml (mean±S.D.), was significantly higher than that in systemic vein, 0.14±0.03 μg/ml (p<0.01). On the other hand, as shown in Fig. 2, the plasma concentration in cutaneous vein on the contralateral side, 0.22±0.04 μg/ml, was similar to that in systemic vein (p>0.05). These results suggest that ketoprofen molecules absorbed at the application site are transferred to the cutaneous vein on the ipsilateral side and are transported to the contralateral side by systemic circulation.

Passive diffusion of ketoprofen was performed as a control for cathodal iontophoresis. The plasma concentrations after a 30-min application are shown in Fig. 3. The ketoprofen con-
Fig. 3. Comparison of Plasma Ketoprofen Concentrations after (A) Passive Diffusion and (B) Anodal Iontophoresis

Passive diffusion (without current) and anodal iontophoresis (0.5 mA/0.7 cm²) was performed for 30 min, and immediately after the application, blood samples were collected from the following veins: cutaneous vein ipsilateral to the application site (solid), systemic vein (open). Data represent the mean ± S.D. of 4 experiments.

Fig. 4. Time-course Curves of (A) the Amount of Ketoprofen Absorbed in Skin, (B) Plasma Ketoprofen Concentration in Cutaneous Vein Ipsilateral to the Application Site and (C) Plasma Ketoprofen Concentration in Systemic Vein during and after Cathodal Iontophoresis

Cathodal iontophoresis (0.5 mA/0.7 cm²) was performed for the period of 0 to 30 min. Data represent the mean ± S.D. of at least 4 experiments.

Fig. 5. Relationship between the Amount of Ketoprofen Absorbed in Skin and Plasma Ketoprofen Concentration in Cutaneous Vein Ipsilateral to the Application Site

Data in Fig. 4(B) was plotted against that in Fig. 4(A). Data represent the mean ± S.D. of at least 4 experiments.

The time-course curves obtained from cathodal iontophoresis are shown in Fig. 4. The amount of ketoprofen absorbed in the skin increased immediately after starting iontophoresis and decreased gradually after the termination of iontophoresis (Fig. 4A). The plasma ketoprofen concentration in cutaneous vein ipsilateral to the application site increased immediately after starting iontophoresis and seemed to reach a plateau value at 15 min. After the termination of iontophoresis, the plasma concentration in cutaneous vein decreased and neared to that in systemic vein (Fig. 4B). There was no significant difference between the plasma concentrations in cutaneous and systemic veins at 30 min after the termination of iontophoresis. The time-course curves in Fig. 4A and B were similar to the current pattern of cathodal iontophoresis. These results demonstrate that the absorption into the skin and the transfer from the skin to cutaneous blood circulation were responsive to the current application of iontophoresis. On the other hand, the time-course curve of the plasma concentration in systemic vein was not consistent with the current pattern (Fig. 4C).

Using the data in Fig. 4A and B, the relationship between the amount of ketoprofen absorbed in whole skin and the plasma concentration in cutaneous vein was evaluated. As shown in Fig. 5, the plasma concentrations in cutaneous vein were proportional to the amounts of ketoprofen absorbed. This result suggests that the transfer of ketoprofen from the application site to cutaneous blood flow is dependent on the amount of ketoprofen present at the application site.

DISCUSSION

The measurement of drug concentrations in systemic vein has been a basic and useful method for the evaluation of in vivo transdermal delivery. However, systemic drug concentrations do not reveal the events that occur below the application site. In our study, transdermal iontophoretic absorption of ketoprofen in the thoracic area of intact rats was examined, and the plasma concentrations in lateral thoracic vein
were measured. As shown in Fig. 1, the lateral thoracic vein is a cutaneous vein passing through the subcutaneous layer beneath the application site for iontophoresis. Therefore, this experimental system is able to demonstrate the role of cutaneous blood circulation at the application site.

It is hypothesized that drug molecules absorbed into skin are transported to cutaneous blood circulation and are transported to the rest of the body through systemic circulation. Comparing Fig. 4A with B, the profile of the amount of ketoprofen absorbed into the skin was almost identical to the plasma concentration profile in cutaneous vein ipsilateral to the application site. These results support the hypothesis that ketoprofen absorbed in the skin is transferred to the cutaneous blood circulation. On the other hand, the plasma concentration in cutaneous vein contralateral to the application site was equivalent to that in systemic vein (Fig. 2). This result suggests that ketoprofen is transported to the contralateral side via systemic circulation. A similar conclusion was drawn by Cross et al. regarding the contribution of systemic circulation to anodal iontophoresis of ethanolamine. They reported that the distribution of ethanolamine to the skin on the contralateral side resulted from systemic circulation, although the plasma concentration in cutaneous vein was not measured.

The mechanisms by which the drug is transferred from skin to cutaneous blood are not fully understood. Siddiqui et al. and Singh et al. investigated the effect of the lipophilicity of drugs on the clearance from rat dermis. The results obtained with steroids by Siddiqui et al. showed a dependence on lipophilicity. In contrast, Singh et al. found no apparent dependence on lipophilicity with steroids, non-steroidal anti-inflammatory drugs and basic drugs. These results were obtained from the measurement of drug concentrations in the dermis below the application site. In our study, by measuring plasma concentrations in cutaneous vein below the application site, the relationship between the plasma concentration in cutaneous vein and the amount of ketoprofen absorbed in the skin was evaluated. As shown in Fig. 5, the ratio of the plasma concentration in cutaneous vein to the amount absorbed seemed to be constant. This finding suggests that the transfer of ketoprofen from skin to cutaneous blood is governed by partitioning between the tissue in the skin and cutaneous blood. Therefore, it is possible that the lipophilicity of a drug is a significant factor in its transfer from skin to cutaneous blood flow.

As mentioned in the Introduction, the local treatment of ketoprofen is required to transport a sufficient amount to deep tissues below the application site. There is an assumption that the transport of drug to deep tissues involves direct penetration or systemic blood supply. Ballerini et al. supposed that the distribution of ketoprofen to the synovial fluid of the knee-joint was the result of direct penetration, when ketoprofen gel was applied to the circumference of the knee in patients. Radermacher et al. conducted a similar study with diclofenac gel and concluded that the distribution to the synovial fluid occurred mainly through systemic blood supply. In our study, it was demonstrated that the plasma ketoprofen concentration in cutaneous vein was significantly increased during the iontophoresis, as compared with that in systemic vein (Fig. 4B and C). From this result, it can be speculated that a sufficient amount of ketoprofen is transported through local blood flow to deeper tissues, such as the synovial membrane of the knee-joint. Therefore, the iontophoresis of ketoprofen might be applicable to the local treatment of rheumatoid arthritis, although conventional products, such as topical ketoprofen gel and tape, have been used for treating minor rheumatologic disease.

The measurement of cutaneous plasma concentration is available not only for the evaluation of iontophoresis, but also passive transdermal delivery. As shown in Fig. 3, systemic ketoprofen concentration after passive application for 30 min was below the limit of detection. On the other hand, the plasma concentration in cutaneous vein was detectable after application for 30 min (Fig. 3), but not for 15 min (data not shown). This finding suggests a time-lag of approximately 30 min in passive diffusion, which represents the time taken to diffuse through the superficial layers of the skin (stratum corneum and viable epidermis) and to transfer to the cutaneous blood circulation. In the case of cathodal iontophoresis (Fig. 4B), the time-lag obtained from the profile of cutaneous ketoprofen concentration was less than 5 min, which was apparently shorter than that in the case of passive diffusion. The difference in time-lag may reflect the enhancing effect of cathodal iontophoresis on the diffusion of ketoprofen through the superficial layers of skin to the cutaneous vasculature. The time-lag in the plasma concentration profile for cutaneous vein might provide the direct evaluation of the parameters of diffusion, such as a diffusion coefficient, under the in vivo condition.

In conclusion, this study has contributed to the establishment of a novel method for the in vivo evaluation of transdermal drug delivery. The results showed that the measurement of the plasma concentration of a drug in cutaneous vein ipsilateral to the application site would allow one to clarify the role of cutaneous blood circulation in local therapy. Cutaneous blood circulation might be a more effective for the delivery of drugs to deep tissues below the skin than direct penetration.

REFERENCES AND NOTES

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