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Pharmacokinetics and Pharmacodynamics of Ketoprofen Plasters

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ABSTRACT: Ketoprofen plasters of 70 cm^2 size using DuroTak® acrylic adhesive polymers were developed either containing 30 mg (Ketotop-L) or 60 mg drug (Ketotop-P). The *in vitro* skin permeation profile was obtained in hairless mouse skin and showed the permeation rate of Ketotop-P to be twice that of Ketotop-L. The plasma concentration profile of ketoprofen was determined in Sprague-Dawley rats after applying a $3 \times 3\,cm^2$ plaster. AUC_{0-24h} and C_{max} of Ketotop-P were 260.92 μg · h/ml and 25.09 μg/ml, respectively, which were about twice the values of Ketotop-L. The hind paw edema induced by carrageenan injection was measured for 6 h after applying a $2 \times 2\,cm^2$ plaster, and the area under the time-response curve (*AUR*) value was significantly lower in Ketotop-P attached rats (180.70% · h) than in those with the Ketotop-L (298.65% · h) and the control (407.04% · h) groups, indicating a stronger anti-inflammatory action of Ketotop-P. However, the analgesic effect of the two formulations did not show a statistically significant difference. In conclusion, Ketotop-P was able to achieve higher plasma concentration of ketoprofen, thereby exhibiting higher and more constant anti-inflammatory effect compared with Ketotop-L. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: ketoprofen; plaster; pharmacokinetics; pharmacodynamics; anti-inflammation; analgesic

Introduction

Ketoprofen [2-(3-benzoylphenyl) propionic acid] is a nonsteroidal anti-inflammatory drug (NSAID) used for the treatment of osteoarthritis and rheumatoid arthritis [1,2]. However, as in the case of most NSAIDs, oral ketoprofen formulations cause stomach irritation, hepatotoxicity and kidney failure [3,4]. Due to these adverse effects, the need for a non-oral delivery system of ketoprofen has been called for, and as a result, several topical forms of ketoprofen have been developed [5–7]. Topical application of ketoprofen on the joints or muscles can increase the drug concentration at the target site as well as lower the concentration in the systemic circulation, thereby reducing stomach irritations and liver toxicity [8,9]. High levels of ketoprofen in intra-articular adipose tissue and in capsular tissue were observed in gel formulations [10].

Compared with when ketoprofen was administered orally at a 50 mg dose, a 30 mg plaster significantly decreased the plasma drug concentration by 120 orders of magnitude while maintaining about a 4–7 times higher concentration at the target sites (i.e. meniscus and

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cartilage) [11]. It therefore seems possible to develop a safer and more effective ketoprofen plaster by doubling the drug concentration, since it is likely that the plasma concentration will remain lower than similar dosed oral formulations while the drug would be more concentrated in the intra-articular tissue.

The total amount of ketoprofen absorbed is proportional to the flux and surface area of the plaster. According to Fick's diffusion equation, the flux of drug through the skin is linearly dependent on the loading dose of the drug in the plaster. However, although the systemic level of the drug may increase as the surface area of the plaster increases, 70 cm^2 size is most popularly used. Therefore, while maintaining the size of the plaster at a constant 70 cm^2, the effect of the loading dose (30 mg and 60 mg) on the flux and pharmacodynamic behavior has been evaluated in order to develop a more effective topical plaster of ketoprofen for the treatment of arthritis.

Experimental

Materials

Ketoprofen was purchased from Aventis Pharma AS (Antony, France). DuroTak® acrylic adhesive polymers were kindly supplied by National Starch & Chemical Company (Bridgewater, NJ, USA) as a gift. Siliconized polyester release liner was obtained from Loparex (Apeldoorn, Netherland). Polyester woven backing was obtained as a gift from Sam Jin Ltd (Seoul, Korea). All other chemicals were reagent grade or better, and were used as received.

The animals used for the *in vitro* and *in vivo* studies were male hairless mice (6–7 weeks) and male Sprague Dawley rats, respectively, which were purchased from Orient Co., Ltd (Gyunggi-do, Korea). They had free access to food and water until they were used for experiments and maintained at a room temperature of $22 \pm 2\,^{\circ}C$ and relative humidity of $55 \pm 5\%$.

Fabrication of the plaster

Ketoprofen, enhancers and various additives were added with organic solvent-born acrylic adhesives (DuroTak®). The mixture was thoroughly mixed by using a mechanical stirrer at 800 rpm for 1 h under occluded condition until a clear solution was obtained. After degassing the mixture using an ultrasonicator, the mixture was cast on the release liner using a micrometer adjustable lab coaters (LC-100, Chemsultants Inc, OH, USA), which was set to contain 30 mg or 60 mg ketoprofen in $7 \times 10\,cm^2$ plasters. After drying the plasters in an oven for 20 min at 80 °C, they were transferred onto woven-fabric, the backing material, and then pressed using a roller. Final plaster products were cut into 70 cm^2 sizes and sealed in an aluminum pouch until used for future evaluation. Plasters were cut into $1.5 \times 1.5\,cm^2$ sizes for the *in vitro* permeation studies, $3 \times 3\,cm^2$ for the PK studies and $2 \times 2\,cm^2$ for the PD studies.

In vitro skin permeation study

In vitro skin permeation of ketoprofen across the hairless mouse skin was conducted using Keshary-Chien permeation cells (surface area of 2.14 cm^2) at 37 °C. Mice were humanely killed by cervical dislocation, and then full-thickness skin (about 4 cm^2) was surgically removed from the dorsal site of each mouse. After carefully removing the subcutaneous fat and washing with normal saline, the skin specimen was cut into appropriate sizes and kept at −70 °C until used. Ketoprofen plasters (1.5 cm × 1.5 cm) was applied to the stratum corneum side of the skin, and then mounted between the donor and receptor cells (stratum corneum side facing the donor cells). The receptor half-cells were filled with isotonic phosphate buffered saline (pH 7.4) solution (12.0 ml), which was magnetically stirred at 600 rpm. At predetermined time intervals, 1.0 ml of receptor solution was withdrawn, and refilled with the same volume of fresh receptor solution. Samples were kept in a freezer (−20 °C) until analysed by HPLC.

Pharmacokinetics study

Male Sprague-Dawley rats (230–250 g) were lightly anesthetized by intramuscular injection of ketamine (25 ml/g) and acepromazine (5 ml/g), and abdominal hair was removed with a hair clipper. After 1 day, rats were fixed at supine

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position under light ether anesthesia. Then, the femoral artery of the rats was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling, which was connected with a disposable syringe (1 ml) filled with heparin in normal saline (25 units/ml). After complete recovery (1 h) from anesthesia, each plaster (3 cm × 3 cm) was applied on the abdominal skin of rat and was covered with polyurethane tape (4 cm × 4 cm) to prevent the detachment of the plasters. At predetermined time intervals, blood samples (less than 250 μl each) were withdrawn from the femoral artery for 24 h, and were immediately centrifuged for 5 min at 5000 rpm. Plasma (100 μl) was kept at −20 °C until analysed.

Mean values of 3–4 rats in each group were obtained. From the plasma-concentration profiles of ketoprofen after administration in rats, the AUC_{24h} was calculated from the trapezoidal rule. Time (T_{max}) to reach the maximum plasma concentration (C_{max}) was directly read from the profiles.

HPLC analysis of ketoprofen

Analysis of ketoprofen from the formulation in the *in vitro* skin permeation experiment was done by HPLC (Hewlett Packard 1100 series, USA) equipped with a UV detector (254 nm), automatic sampler and isocratic pump. The column used was YMC-AM C18 (YMC, Japan), and the injection volume was 20 μl. The mobile phase was a mixture of acetonitrile, methanol and 0.1% acetic acid at the ratio of 40:40:20 (v/v/v) and the flow rate was 1.0 ml/min.

The concentration of ketopropen in plasma was analysed by HPLC by a literature method [12–14]. A Shiseido C18 MG 5 μm (size 4.6 mm i.d. × 250 mm) column and acetonitrile and acetate buffer (0.05 M, pH 5.0) (42:58, v/v) as mobile phase were used. The internal standard (60 μl, naproxen 10 μg/ml) and 1 N HCI (400 μl) were added to 100 μl of plasma and thoroughly vortexed after which 1 ml of ether was added and vortexed again. The upper ether layer was taken after the solution was centrifuged. After evaporating the ether layer, 200 μl of mobile phase was added to reconstitute the sample and analysed by HPLC (injection volume 20 μl). Ketoprofen was detected at 254 nm by UV at a flow rate of 1.0 ml/min. The ratio of the peak areas of ketoprofen and internal standard from the chromatograms obtained was used to calculate the concentration with reference to the calibration curve previously obtained.

Anti-inflammatory effect by hind paw edema study

The anti-inflammatory effect of ketoprofen was measured using the carrageenan-induced hind paw edema model reported in the literature [15,16]. Subjects were male Sprague-Dawley male rats (160–200 g). The left hind paw of each animal was carefully shaved without damaging the skin 1 day before the experiment. On the next day, the shaved area was again treated with hair removal gel containing thioglycolic acid for 10 min to remove residual hairs and washed off. Then 2 h after hair removal, each plaster (2 cm × 2 cm) was tightly adhered to the shaved area of the left hind paw, and was covered with polyurethane tape (3 cm × 3 cm). For the control group, only urethane tape without the plaster was applied. One hour after the plaster adhesion, 60 μl of carrageenan suspension (0.5% in normal saline) was injected into the sole of the left hind paw. Using a plethysmograph (type 7140, Ugo Basile, Comerio, Italy), the volume of the left paw was measured immediately (0 h) and 1, 2, 3, 4, and 5 h after the carrageenan injection to observe the changes in the paw volume due to edema. The amount of swelling was quantified as follows:

$$\% \text{ swelling} = \frac{V_1 - V_0}{V_0} \times 100$$

where, V_1 is the volume of paw at 1, 2, 3, 4 or 5 h after the carrageenan injection, and V_0 is the volume of paw immediately after the carrageenan injection.

The percent inhibition of swelling at 1, 2, 3, 4 and 5 h after the carrageenan injection compared with the control was calculated using the following equation:

$$\% \text{ inhibition} = \left[1 - \frac{\% \text{ swelling of treatment group}}{\% \text{ swelling of control group}}\right] \times 100$$

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Analgesic effect by radiant heat study

Thermal hyperalgesia test was conducted in Sprague-Dawley rats (male, 160–200 g) by using the radiant heat method to evaluate the analgesic effect of the ketoprofen plasters. One day before the experiment, the rats were divided into corresponding groups by weight (10 rats each group), and the left hind paw of each animal was carefully shaved without damaging the skin using an electrical clipper. On the day of the experiment, residual hairs were completely removed using thioglycolic acid-containing hair removal gel that again was completely washed off after 10 min. Then 2 h after wash-off of hair removal gel, radiant heat (Ugo Basile Type 7360, Tail Flick Tester, Intensity 35) was applied to the sole of the left hind paw in order to measure the base level of the paw withdrawal latency (PWL, sec) in response to the radiant heat. After measurements of the base PWL level, each plaster (2 cm × 2 cm) was tightly adhered to the shaved area of the animals of the corresponding groups (Ketotop-P or Ketotop-L). Also, a piece of urethane tape (3 cm × 3 cm) was used to secure the adhesion of the plaster. For the control group, only a piece of urethane tape without a plaster was applied. One hour after plaster adhesion, 60 µl of 0.5% carrageenan suspension in normal saline was injected into the sole of the left hind paw, and then PWLs in response to the radiant heat were measured at 1, 2, 3 and 4 h after the carrageenan injection (i.e. after 2, 3, 4, 5 h after plaster application).

Data analysis

For comparison of mean values between the plasters, the Student's *t*-test at the $p < 0.05$ level was used.

Results and Discussion

Ketotop-L, which has been on the market in Korea since 1994, contains 30 mg of ketoprofen in a 70 cm^2 dissolution-matrix type plaster. However, an increase in drug efficacy for a more effective therapy had been called for by patients, which could be achieved by either increasing the plaster size or increasing the loading dose. Since it is not practical to increase the plaster size, the latter method seemed more feasible. Therefore, the pharmacokinetic (PK) and pharmacodynamic (PD) aspects of the plaster when the loading dose was doubled have been evaluated and gave the following results.

In vitro skin permeation study

Figure 1 and Table 1 show results of the *in vitro* skin permeation of the 70 cm^2 ketoprofen plaster either containing 30 mg (Ketotop-L) or 60 mg (Ketotop-P) drug. The amount of drug permeated over a 24 h period was 337.94 µg/cm^2 and 703.40 µg/cm^2, respectively, while the average permeation rate was 13.92 µg/cm^2/h and 29.95 µg/cm^2/h, respectively, indicating that the degree of skin permeation of Ketotop-P was about twice that of Ketotop-L. However, the lag times were not significantly different from one another. It can be predicted that when Ketotop-P is applied to *in vivo* conditions, it would distribute a higher concentration of drug in local tissue as well as in plasma compared with Ketotop-L.

Validation of HPLC analysis of ketoprofen

Analysing ketoprofen in plasma by HPLC resulted in good separation with no interfering peaks. The linear range was from 0.5–20 µg/ml ($r^2 = 0.9999$), with a detection limit of 0.5 µg/ml.

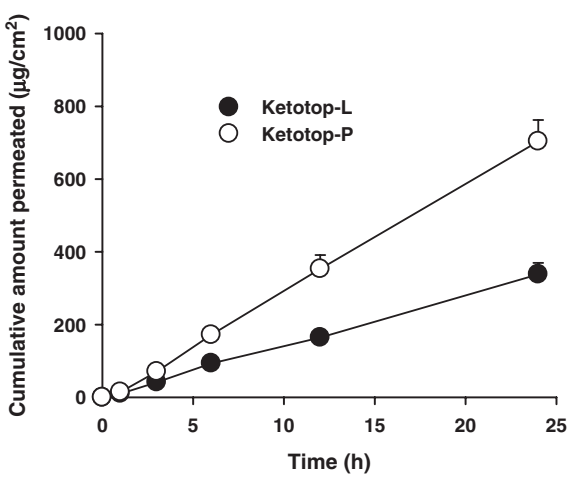

Figure 1. *In vitro* hairless mouse skin permeation profiles of ketoprofen from plasters containing 30 mg (Ketotop-L) and 60 mg (Ketotop-L) of ketoprofen in 70 cm^2 plasters. Each value is the mean ± SD ($n = 6$)

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Table 1. *In vitro* permeation rate of ketoprofen from plasters using hairless mouse skin

Plaster type	Cumulative amount permeated after 24 h (μg/cm^2)	Permeation rate (μg/cm^2/h)	Lag time (h)
Ketotop-L (30 mg ketoprofen/70 cm^2)	337.94 (± 31.56)	13.92 (± 1.25)	−0.18 (± 0.90)
Ketotop-P (60 mg ketoprofen/70 cm^2)	703.40 (± 58.98)	29.95 (± 1.49)	0.42 (± 0.29)

Each value is the mean ± SD of six determinations.

Table 2. Validation of HPLC analysis of ketoprofen in rat plasma

Plasma concentration (μg/ml)	Precision (%CV)		Accuracy (%)	
	Intra-day ($n = 4$)	Inter-day ($n = 4$)	Intra-day ($n = 4$)	Inter-day ($n = 4$)
0.5 (LOQ)	0.31	0.28	87.91	94.70
1	0.59	0.60	93.23	101.08
3	1.80	1.81	100.78	101.35
5	2.93	2.94	99.80	99.09
10	5.94	5.96	102.20	100.29
20	11.51	11.88	99.46	99.95

The precision and accuracy of ketoprofen were determined at plasma concentrations of 0.5, 1, 3, 5, 10 and 20 μg/ml. Intra-day precision was determined by analysing four sets of data obtained on the same day, while inter-day validation was done for 4 days. Accuracy was determined by dividing the average value quantitated from the calibration curve by the known concentration and was expressed in percent (%). As shown in Table 2, intra- and inter-day precision for the assay over the concentration range was below 11.51% and 11.88%, and the accuracy ranged between 87.91–102.20% and 94.70–101.35%, respectively. The method developed for the determination of ketoprofen in rat plasma was found to have adequate sensitivity and reproducibility, and thus was applicable to pharmacokinetic studies.

Pharmacokinetics of ketoprofen plasters

Plasma concentration profiles from patches applied on the abdomens of four rats are shown in Figure 2. The plasma concentration of Ketotop-P was about twice that of Ketotop-L over the 24 h period, while maximum level was reached after 2–3 h of plaster application. Analysis of the pharmacokinetic parameters is shown in Table 3.

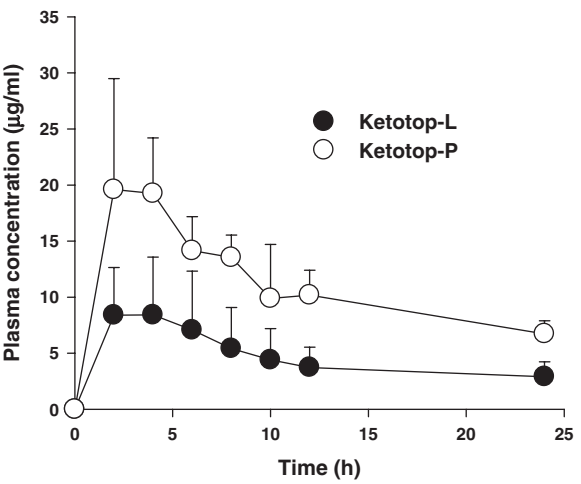

Figure 2. Plasma concentration profiles of ketoprofen after applying Ketotop-L or Ketotop-P (3 cm × 3 cm each) on the abdominal skin of Sprague-Dawley rats (mean ± standard deviation, $n = 3-4$)

The AUC_{24h} values calculated using the trapezoidal equation was 110.34 μg · h/ml for Ketotop-L while 260.92 μg · h/ml for Ketotop-P. The C_{max} of Ketotop-L was 9.01 (± 4.89) μg/ml, while that of Ketotop-P was 25.09 (± 5.12) μg/ml indicating that the blood concentration was significantly higher with the use of Ketotop-P. These data are

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Table 3. Pharmacokinetic parameters of ketoprofen plasters (3 cm × 3 cm) in rats after applying onto the abdominal skin

Parameter	Ketotop-P (60 mg ketoprofen/70 cm^2)	Ketotop-L (30 mg ketoprofen/70 cm^2)
AUC_{24h} (μg·h/ml)	260.92 (± 31.10)	110.34 (± 60.94)
C_{max} (μg/ml)	25.09 (± 5.12)	9.01 (± 4.89)
T_{max} (h)	3.00 (± 1.15)	2.50 (± 1.00)

Each value is the mean ± standard deviation ($n = 3-4$).

consistent with the *in vitro* skin permeation data shown in Figure 1 and Table 1. The T_{max} value was 2.5 h and 3.0 h for Ketotop-L and Ketotop-P, respectively, and was not statistically different.

The permeability in rat and mouse skin is generally known to be higher than in human skin [17]. When the animal study results are applied to human subjects, the plasma concentration of ketoprofen is expected to be lower. Moreover, Rolf *et al*. reported that C_{max} values for an oral (50 mg tablet) formulation was 2600 ng/ml while that for transdermal delivery (30 mg plaster) was only 21 ng/ml [11]. At the target site, however, concentration at the meniscus was determined to be 86 ng/g tissue for the oral formulation while being 350 ng/g tissue for the transdermal delivery system. The transdermal delivery resulted in about a 120 times less plasma concentration compared with the oral type while maintaining a higher concentration at the target site. Increasing the dose two-fold in the transdermal formulation would thus be able to increase the concentration at the target site while maintaining a low plasma concentration guaranteeing safety compared with the oral administration.

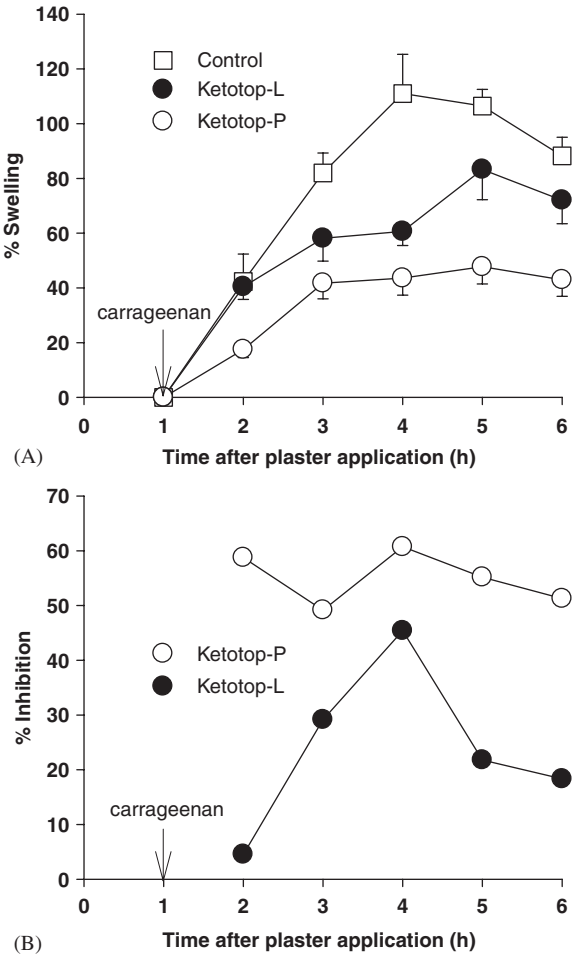

Figure 3. The effect of ketoprofen plasters on (A) the percent swelling of hind paw and (B) the percent inhibition of the edema induced by carrageenan injection in rats (mean-standard error, $n = 7$)

Anti-inflammatory effect by hind paw edema study

Results of the anti-inflammatory effect of the Ketotop plasters using the hind paw edema measurement of carrageenan induced edema is shown in Figure 3. The control group which was not treated with the plasters showed maximum swelling after 3 h of carrageenan injection. However, when Ketotop-P or Ketotop-L was applied on the paw, a significant reduction in edema was observed for 6 h (Figure 3A). In order to compare the change in the % swelling quantitatively over a 6 h period, area under the time-response curve (*AUR*) values were calculated [18]. The AUR_{6h} of the control was 407.04% · h while that of Ketotop-L was 298.65% · h and that of Ketotop-P was 180.70% · h (Table 4). The significant decrease in AUR_{6h} values shows that considerable anti-inflammatory activity was observed in rats with ketoprofen plasters. Moreover, the anti-inflammatory effect of Ketotop-P was more significant than that of Ketotop-L.

Figure 3B shows the % inhibition of swelling to the control group. In the case of Ketotop-L, the inhibition effect increased over a 4 h period after application of plaster while a decrease was observed thereafter. However, in the case of

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Table 4. *AUR* values representing % swelling over a 6 h period and analgesic effect over a 5 h period

Area under the time-response curve (*AUR*)	Control	Ketotop-L	Ketotop-P
AUR_{6h} (% swelling · h)[a]	407.04 (± 31.20)	298.65[c] (± 33.25)	180.70[d,e] (± 23.99)
AUR_{5h} (s · h)[b]	38.39 (± 1.71)	51.60[d] (± 1.70)	58.23[d] (± 3.04)

[a] Each value is the mean ± standard error ($n = 7$).
[b] Each value is the mean ± standard error ($n = 10$).
[c] $p < 0.05$.
[d] $p < 0.01$ compared with control.
[e] $p < 0.05$ compared with Ketotop-L.

Ketotop-P, the % inhibition effect was maintained up to 6 h after application. This shows that Ketotop-P was able to deliver enough ketoprofen constantly for a longer period, thereby maintaining a higher anti-inflammatory effect compared with Ketotop-L.

Analgesic effect by radiant heat study

Experimental plasters were attached to the hind paw of rats and carrageenan was administered after 1 h. On the foot where carrageenan was applied, radiant heat (beam intensity 35) was illuminated and paw withdrawal latency (PWL, s) was measured (Figure 4). The *AUR* values over a 5 h period were calculated to compare the changes in analgesic effect quantitatively. The control exhibited PWL of 38.39 s · h while that of Ketotop-L was 51.60 s · h and that of Ketotop-P, 58.23 s · h (Table 4). A significant analgesic effect was observed for both Ketotop-P and Ketotop-L groups for 5 h compared with the control group. However, unlike the anti-inflammatory effect, a statistically significant difference in analgesic activity was not observed between the two formulations. Studies are under way to further investigate the factors influencing the analgesic effect of ketoprofen plasters.

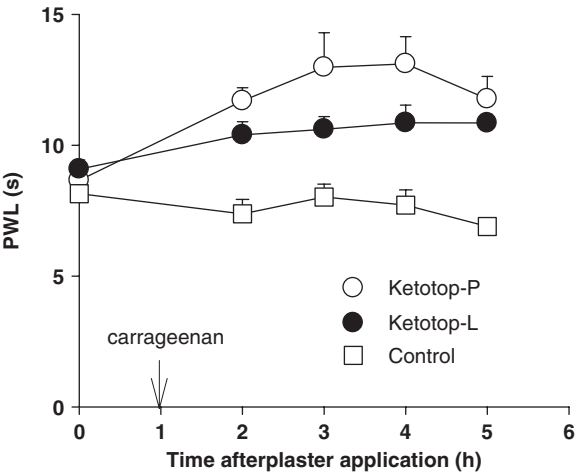

Figure 4. The effect of ketoprofen plasters on the paw withdrawal latency (PWL) when the radiant heat was applied to the sole of the left hind paw in rats. PWL in response to the radiant heat were measured after 1, 2, 3 and 4 h after the carrageenan injection in the left hind paw (mean ± standard error, $n = 10$)

Conclusions

Ketotop-P showed two times higher blood concentration and *AUC* value compared with Ketotop-L as well as a significant increase in anti-inflammatory activity. Although the analgesic effect obtained by measuring the PWL did not reveal a significant difference between the two plasters ($p = 0.068$), the ability of Ketotop-P to maintain a stronger and constant anti-inflammatory effect up to a 6 h period makes it more preferable to Ketotop-L in treating arthritis related diseases.

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