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Enhanced efficacy by percutaneous absorption of piroxicam from the poloxamer gel in rats

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Abstract

The pharmacokinetics and anti-inflammatory activity of piroxicam from the poloxamer 407 gel were determined to investigate percutaneous absorption of piroxicam from poloxamer gels in rats. The poloxamer 407 gel containing 1% piroxicam showed significant inhibition of carragenin-induced rat foot swelling when compared to the control group. The extent of inhibition of swelling (%) showed a linear relationship with the logarithm of piroxicam dose within ~0.4–3.2 mg/kg. The enhancing effect of polyoxyethylene-2-oleyl ether, non-ionic surfactant on the percutaneous absorption of piroxicam from poloxamer 407 gel was evaluated in rats. The piroxicam gel containing polyoxyethylene-2-oleyl ether increaesd the relative bioavailability ~1.8-fold compared with the gel without enhancer. Percutaneous administration of piroxicam gel containing polyoxyethylene-2-oleyl ether to rats showed a relatively constant, sustained blood concentration with minimal fluctuation.

Keywords: Piroxicam; Poloxamer 407 gel; Percutaneous absorption

1. Introduction

Transdermal therapy has become a useful drug delivery system in recent years. The skin is not only an administration route for local therapy, but also a route for drugs to achieve systemic effects. There are many reports (Huang et al., 1995; Yokomozo and Sagitani, 1996) on studies of topical delivery of anti-inflammatory drugs because it is possible to avoid the first-pass effects and the gastrointestinal disturbances which might occur when orally administered. Since the NSAIDs are administered for long term, it is desirable to reduce the adverse reactions. We, therefore, attempted to develop the piroxicam gel for percutaneous administration to overcome the side effects and to obtain a therapeutic plasma concentration (i.e. a sustained plasma concentration during dosing without a high initial peak concentration). In previous work (Shin et al., 1999) on piroxicam gels using various enhancers, polyoxyethylene-2-oleyl ether showed the best enhancing effects (enhancing factor, 2.84) through excised rat skin. The topical anti-inflammatory effects of piroxicam ointment have been studied by some researchers (Larson et al., 1980; Schiantarelli et al., 1982) but the pharmacokinetics

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that influence the percutaneous absorption of piroxicam from the poloxamer 407 gels have not yet been studied. The purpose of this study was to develop and confirm the enhancing efficacy of piroxicam gels using polyoxyethylene-2-oleyl ether as an enhancer in rats.

2. Materials and methods

2.1. Materials

Piroxicam from Chodang Pharm. Co. (Korea) and Poloxamer 407 from BASF (Germany) were used. Carrageenan and indomethacin were purchased from Sigma (USA). Acetonitrile and acetic acid were HPLC grade. All other chemicals were reagent grade and used as received.

2.2. Methods

2.2.1. Preparation of piroxicam test preparation

The poloxamer gel containing piroxicam described in the paper by Shin et al. (1999) was used for the percutaneous absorption tests. Briefly, poloxamer was added to water at ~ 5°C with gentle stirring, and the solution was left overnight in a refrigerator to complete polymer desolvation. One gram of piroxicam and 5 g of polyoxyethylene-2-oleyl ether dissolved in 40 ml of propylene glycol was added slowly with stirring to cold poloxamer solution prepared previously. The preparation was then brought to 100 ml and stored in a shaking water bath for 2 days.

The piroxicam solution for i.v. or oral administration was prepared by dissolving 200 mg of piroxicam in 100 ml of a sodium bicarbonate-buffered solution (pH 9.2).

2.2.2. Relationship between the dose of piroxicam and anti-inflammatory activity

After dissolving an appropriate dose (4, 8, 16 and 32 mg) of piroxicam in the 1% carrageenan solution, an appropriate volume of this carrageenan solution was injected in the sole of the foot. The dose of piroxicam injected was 0.4, 0.8, 1.6 and 3.2 mg/kg. The swelling (%) and inhibition of swelling (%) of each rat were obtained by methods mentioned previously.

2.2.3. Anti-inflammatory effects of the poloxamer 407 gel containing piroxicam on carrageenan-induced paw edema

Paw edema can be induced by murine carrageenan by injecting 1% carrageenan as described by Levy (1969) using male S.D. rats (Schrier et al., 1987). The anti-inflammatory effects of piroxicam gel were evaluated by applying the gel containing 1% piroxicam on the right hind paw of the rats. After 3 h, the rats were injected intradermally in the right hind paw with 0.1 ml of 1% carrageenan in physiological saline or 0.1 ml of normal physiological saline as control. Edema volume was measured by the plethysmometer (Ugo Bacile, Model 7150) 3 h after carrageenan injection. The extent of swelling (%) was calculated from the volume differences between immediately and 3 h after carrageenan injection (at least six rats per group).

$$\text{Swelling (\%)} = \frac{V - V_i}{V_i} \times 100$$

where, V is the volume 3 h after the injection in the sole of the foot and V_i is the volume immediately after the injection in the sole of the foot.

$$\text{Inhibition of swelling (\%)} = \left[1 - \frac{\text{swelling \% of group treated}}{\text{swelling \% of control}}\right] \times 100$$

2.2.4. Skin irritation study

Various drugs, when applied topically, might elicit primary skin irritation. This irritation might vary with the ability of the agent to cross the stratum corneum barrier and subsequently interact with the viable cells of the epidermis and dermis (Nangia et al., 1993). Two grams of poloxamer gel containing piroxicam were applied onto the dorsal skin of rats weighing 220–250 g and occluded with gauze and bandage. After 24 h, the gel was removed and the score of erythema was determined according to the method of Drazie (1959) as follows: 1, mild erythema; 2, moderate erythema; 3, severe erythema (Aioi et al., 1995). The score of eryrthema was evaluated by the method of Bratty et al. (1995).

Table 1
HPLC condition for the determination of piroxicam

Parameters	Conditions
Column	Novapak C_{18} column (4 μm, 3.9×150 mm^2)
Mobile phase	Acetonitrile:water:acetic acid (50:46:4), pH 3
Flow rate	0.8 ml/min
UV detector	320 nm
Temperature	Ambient
Injection volume	20 μl

2.2.5. Determination of piroxicam in rat plasma

A 0.1 ml aliquot of plasma was pipetted into a 15 ml centrifuge tube, along with 0.8 ml of Soerensens citrate buffer (pH 3) and 100 μl of internal standard (1 mg/ml of indomethacin) and mixed for 15 s by a vortex mixer. The mixture was shaken for 10 s and extracted with 5 ml of ethyl ether by mechanical shaking for 20 min. After centrifugation for 3 min at 3000 rpm, 3 ml of the ether phase was transferred to another tube and evaporated to dryness on a centrifugal evaporator. The residue was dissolved in 1 ml of HPLC mobile phase (Table 1); then 20 μl of this solution was injected into the HPLC (Tsai et al., 1985). HPLC conditions for the determination of piroxicam are as follows (Table 1).

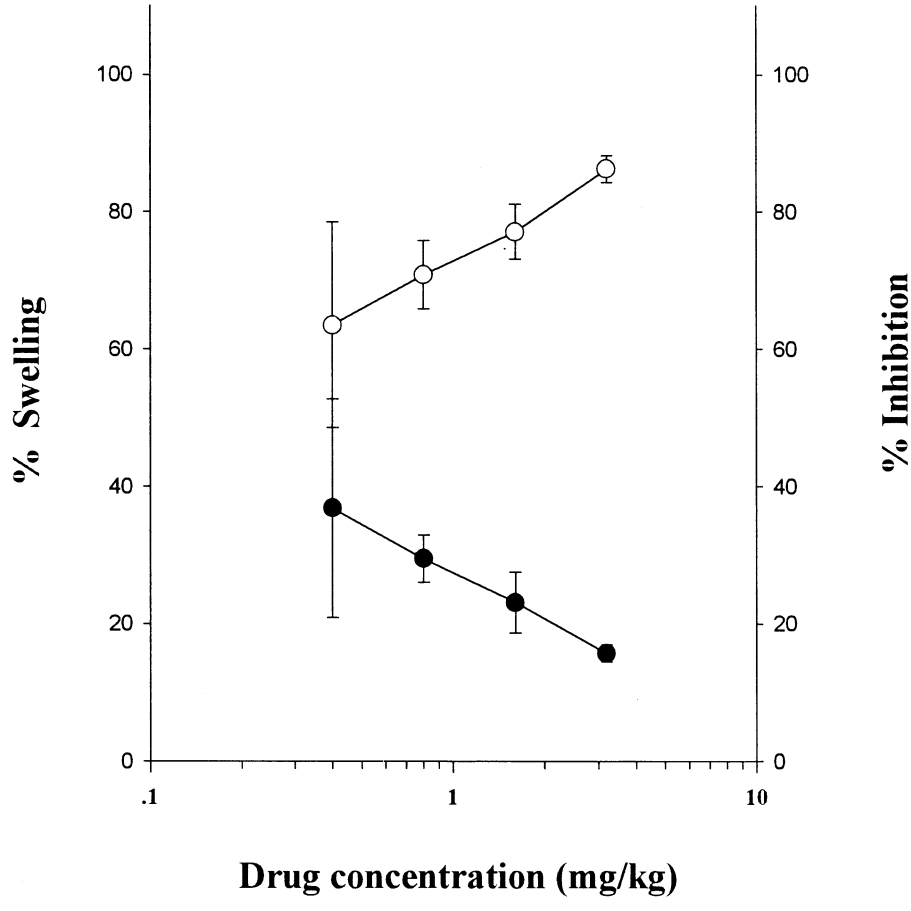

Fig. 1. Dose response of piroxicam after the sole of the paw injections on carrageenan-induced edema model. ●, % swelling; ○, % inhibition.

3. Results and discussion

3.1. Relationship between the dose of piroxicam and anti-inflammatory activity by carrageenan-induced edema model

Fig. 1 shows swelling (%) and inhibition (%) of swelling measured 3 h after injecting 1% carrageenan solution containing piroxicam in the sole of the foot against logarithmic dose of piroxicam. The relationship between log dose of piroxicam injected in the sole of the foot and % inhibition of swelling was shown in the following equation. The correlation coefficient was 0.9740.

$$\log D = 0.1249 \times I - 7.7979$$

where D is the dose and I is the % inhibition of swelling.

The extent of inhibition of swelling (%) showed a linear relationship with the logarithm of piroxicam dose within ~ 0.4–3.2 mg/kg. The dose of 0.4 mg/kg piroxicam showed $\sim 63\%$ inhibition of swelling which was known to be ~ 60–70% (Marsh and Schuna, 1986).

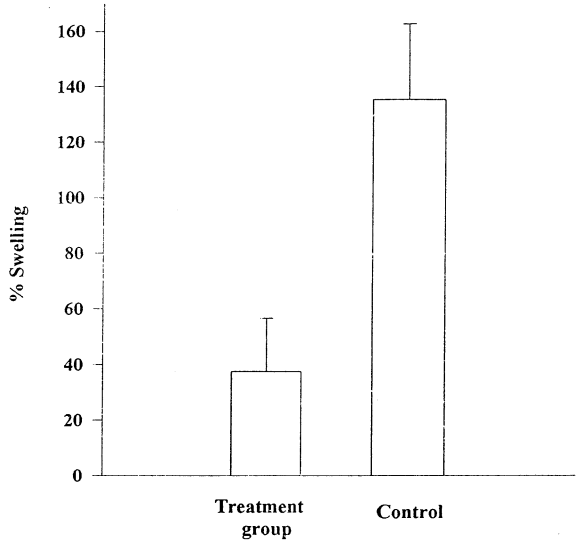

Fig. 2. Anti-inflammatory effects of poloxamer 407 gels containing 1% piroxicam by rat paw swelling methods.

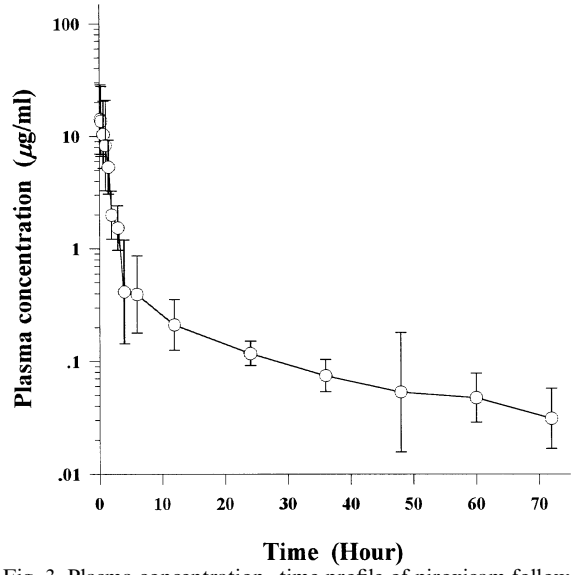

Fig. 3. Plasma concentration–time profile of piroxicam following i.v. administration (10 mg/kg) to rats ($n = 5$). The error bar represents the S.D. of the mean.

3.2. Anti-inflammatory effects of poloxamer gels containing piroxicam by rat paw swelling models

The poloxamer 407 gel containing 1% piroxicam showed great inhibition of swelling when compared to the control group. The group treated with gel showed $\sim 38\%$ swelling while the control group showed $\sim 135\%$ swelling (Fig. 2).

3.3. Skin irritation study of poloxamer gels containing piroxicam

The skin irritation study conducted on the rat by single application of the gel did not show any sign of irritation after 24 h (eight samples showed zero of the erythema score).

3.4. Pharmacokinetics

For the purpose of studying the biopharmaceutical aspects of percutaneous absorption of piroxicam, one of the prerequisites is the pharmacokinetic parameters after the i.v. administration. The plasma concentration-time curve for piroxicam after the i.v. administration of 10 mg/kg of piroxicam is shown in Fig. 3. Following oral administration of a single 10 mg/kg of piroxicam

Table 2
The comparison of $AUC_{0\rightarrow 72\,h}$ and Cl_t for i.v. and oral administration of piroxicam[a]

	i.v.	Oral
$AUC_{0\rightarrow 72\,h}$ (μg h per ml)	1764.11 ± 85.2	3609.29 ± 287.62
Cl_t (ml/h)	25.60 ± 1.18	0.33 ± 0.02

[a] Each value represents the mean ± S.D. of three determinations.

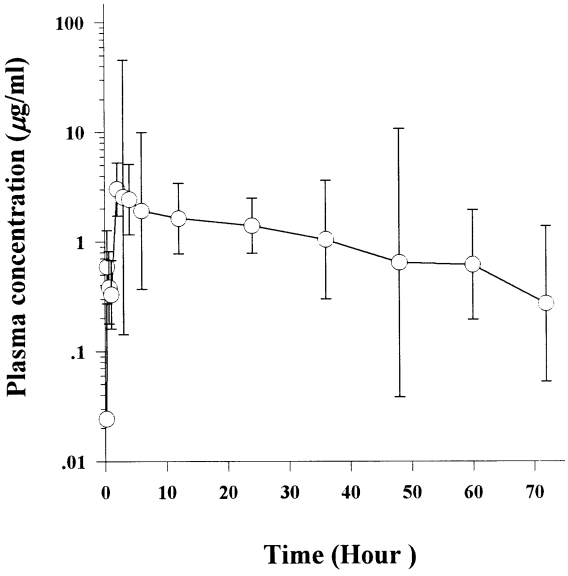

Fig. 4. Plasma concentration–time profile of piroxicam following oral administration. (10 mg/kg) to rats ($n = 5$). The error bar represents the S.D. of the mean.

to rats, the drug started to appear in the plasma within ~10–20 min and the peak plasma concentration of ~3.01 μg h per ml was reached within 2 h (Fig. 4). Following i.v. and oral administration to rats of a single 10 mg/kg of piroxicam, the value of $AUC_{0\rightarrow 72\,h}$ was ~1764.11 and 3609.29 μg/ml,

Table 3
The comparison of $AUC_{0\rightarrow 72\,h}$ for the percutaneous absorption of piroxicam gels with enhancer or not[a]

	Without enhancer	With enhancer
$AUC_{0\rightarrow 72\,h}$ (μg h per ml)	2986.45 ± 245.81	5298.87 ± 475.72

[a] Each value represents the mean ± S.D. of three determinations.

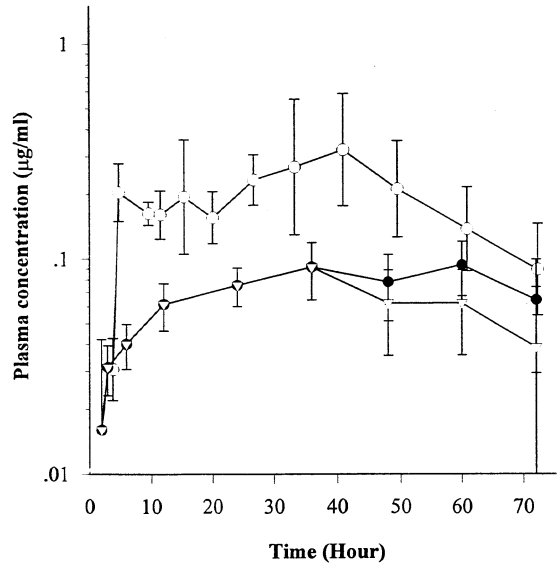

Fig. 5. Plasma concentration–time profile of piroxicam following percutaneous administration (240 mg/kg) of the piroxicam gel with enhancer to rats ($n = 5$). The error bar represents the S.D. of the mean. ○, with enhancer (polyoxyethylene-2-oleyl ether); ●, without enhancer; ▽, after removing the gels without enhancer.

respectively, and the total clearance was ~25.60 and 0.33 ml/h, respectively (Table 2). Fig. 5 shows the plasma concentration profile of piroxicam by percutaneous absorption from gel formulations. The percutaneous absorption of piroxicam from the gel containing polyoxyethylene-2-oleyl ether as an enhancer was higher than that from the gel without enhancer. After removing the gel, the plasma concentration of piroxicam decreased. Following percutaneous administration of a single 240 mg/kg of piroxicam to rats, the value of $AUC_{0\rightarrow 72\,h}$ of percutaneous absorption with enhancer was 5298.87 and that without enhancer was 2986.45 μg h per ml (Table 3).

From the results of the above conditions, as the piroxicam gel containing polyoxyethylene-2-oleyl ether as an enhancer was administered percutaneously to rats, the relative bioavailability showed ~1.8-fold increase compared with the group without enhancer. The value of $AUC_{0\rightarrow 72\,h}$ of percutaneous absorption without enhancer showed 0.83-fold compared with oral administration. However, the value of $AUC_{0\rightarrow 72\,h}$ of percutaneous absorption of piroxicam gel containing an en-

hancer, polyoxyethylene-2-oleyl ether, increased to 1.5-fold compared to oral administration.

Percutaneous administration of piroxicam gel containing polyoxyethylene-2-oleyl ether to rats showed a relatively constant, sustained blood concentration with minimal fluctuation.

Acknowledgements

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