

Anti-inflammatory Effect of Flurbiprofen Tape Applied Percutaneously to Rats with Adjuvant-Induced Arthritis

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ABSTRACT—The anti-inflammatory effect of flurbiprofen tape (FP-T) by topical application was investigated, and the findings were compared with the results of oral administration of flurbiprofen to adjuvant arthritic rats. The topical application of FP-T significantly suppressed both applied and non-applied hind paw edema, with a potency similar to that seen with the oral administration of flurbiprofen. Body weight also increased with these treatments. Plasma levels of flurbiprofen differed little between topical application of FP-T and oral administration of flurbiprofen. Gastric damage induced by topical application of FP-T was significantly less than that seen in case of oral administration of flurbiprofen. These results suggest that the anti-inflammatory effects of FP-T cannot be entirely explained by flurbiprofen permeating inflamed tissue below the application site; rather, flurbiprofen penetrating into the systemic circulation may explain these actions.

Keywords: Anti-inflammatory, Tape, Flurbiprofen, Transdermal penetration

A poultice or ointment containing non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, ketoprofen and flurbiprofen, is widely used to treat patients with various local inflammatory diseases and arthropathies. These external drugs are characteristic in that their topical application has anti-inflammatory and analgesic effects on local inflamed tissue and also reduces adverse effects, such as gastric damage, because of the low blood concentration of the drugs (1, 2). The flurbiprofen tape (FP-T) is an adhesive tape containing 0.15 mg of flurbiprofen per cm², and it has a higher transdermal permeation capacity than other external materials. The anti-inflammatory effects and plasma flurbiprofen concentrations after topical application of FP-T were compared with those after oral administration of flurbiprofen to rats with adjuvant arthritis. We also examined the effect of FP-T and flurbiprofen on rat gastric mucosae.

MATERIALS AND METHODS

Materials

Flurbiprofen and *Mycobacterium butyricum* were purchased from Kaken Pharma (Tokyo) and Difco Laboratories (Detroit, MI, USA), respectively.

Preparation of FP-T

Preparation of FP-T was as follows: Fifty-eight grams of natural rubber was dissolved in toluene, shaken at room temperature for 4 hr and then stored overnight. Ten grams of polybutene, 14.7 g of liquid paraffin, 15 g of petroleum resin, 1.5 g of flurbiprofen and 0.8 g of peppermint oil were added to the solution, and the preparation was shaken for 3 hr at room temperature. The mixture was coated onto polyethylene terephthalate film and dried for 3 hr at room temperature. Nonwoven fabric was used for the backing film of FP-T. The content of flurbiprofen was 0.15 mg per cm², and the thickness was 100 μ m. Preliminarily, we examined the effects of FP-T containing several amounts of flurbiprofen on carrageenin-induced paw edema in rats. The FP-T applied to the hind paw suppressed paw edema in a dose-dependent manner. From the findings, we decided that the content of flurbiprofen should be 0.15 mg per cm².

Rat adjuvant arthritis

Male Lewis rats, aged 6 weeks and purchased from Seiwa Experimental Animals (Fukuoka) were kept in an air-conditioned room (22 \pm 1 $^{\circ}$ C, 55 \pm 10%), with lights on 12 hr per day (8:00–20:00). Standard chow and water were provided ad libitum for one week before the start of the experiments. Adjuvant arthritis was induced by the

method of Winder et al. (3). In brief, rats were injected s.c. with 0.1 ml of 0.6% *Mycobacterium butyricum*-liquid paraffin into the root of the tail. On day 20, the rats were selected according to the degree of swelling in the right and left hind paws and randomly assigned to three groups: Group 1 (n=10), non-treated control; Group 2 (n=10), topical application of FP-T (3.5 cm × 4 cm); Group 3 (n=10), oral administration of flurbiprofen (0.75 mg/kg). The FP-T was applied to the right hind paw for 6 hr per day (10:00–16:00), and the flurbiprofen suspended in 5% gum arabic was orally administered once a day (10:00) on day 20 until day 28. After the FP-T was applied for 6 hr, the tapes were removed. The flurbiprofen content of the tapes removed was measured by using an HPLC system. The amount of flurbiprofen released was calculated from the flurbiprofen content of the tapes removed. The dose of FP-T calculated based on the release amount of flurbiprofen from FP-T was about 1 mg/kg/day. The volumes of the right and left hind paw were measured using a plethysmometer (TK-102; Unicom, Chiba) on days 0, 20, 23 and 27. The percentage of swelling was expressed as the ratio of swelling hind paw volume to the initial volume.

Plasma concentration of flurbiprofen

On day 27, after the measurement of hind paw volume, drugs were again administered to the rats. Under ether anesthesia, 0.5 ml of blood was withdrawn from an or-

bital vein into a heparinized tube 1, 3, 6, 8, 12 and 24 hr after drug administration. The blood was centrifuged at 3,000 rpm for 5 min to obtain plasma samples. Each sample was analyzed as follows: A 100- μ l aliquot of a plasma sample was added to 400 μ l of 10% acetic acid and 100 μ l of internal standard solution (0.1 μ g of 4-biphenylacetic acid / 100 μ l of acetonitrile – 0.1% phosphoric acid (1:1)). The solution was centrifuged at 10,000 rpm for 2 min. The supernatant obtained was applied to a column of Bond Elut C₁₈ (Varian, Harbor City, CA, USA), which was activated prior to use by passing through it 1 ml of methanol and 2 ml of water. The column was washed with 1 ml of distilled water, and flurbiprofen and internal standard were eluted with 400 μ l of methanol. The eluate was dried with N₂ gas at 40°C. The residue was dissolved in 200 μ l of mobile phase and 20 μ l of this solution was injected on to the HPLC system equipped with a fluorescence detector, using excitation and emission wavelengths of 246 and 314 nm, respectively. HPLC analysis was performed by using an Inertsil ODS-2 column (GL Science, Tokyo) with a mobile phase consisting of acetonitrile – 0.1% phosphoric acid (1:1) and a flow rate of 1.0 ml/min.

Gastric mucosal irritation

On day 28, FP-T and flurbiprofen were administered to the rats which had been fasted for 18 hr. The animals were killed 6 hr after the drug administrations; and the

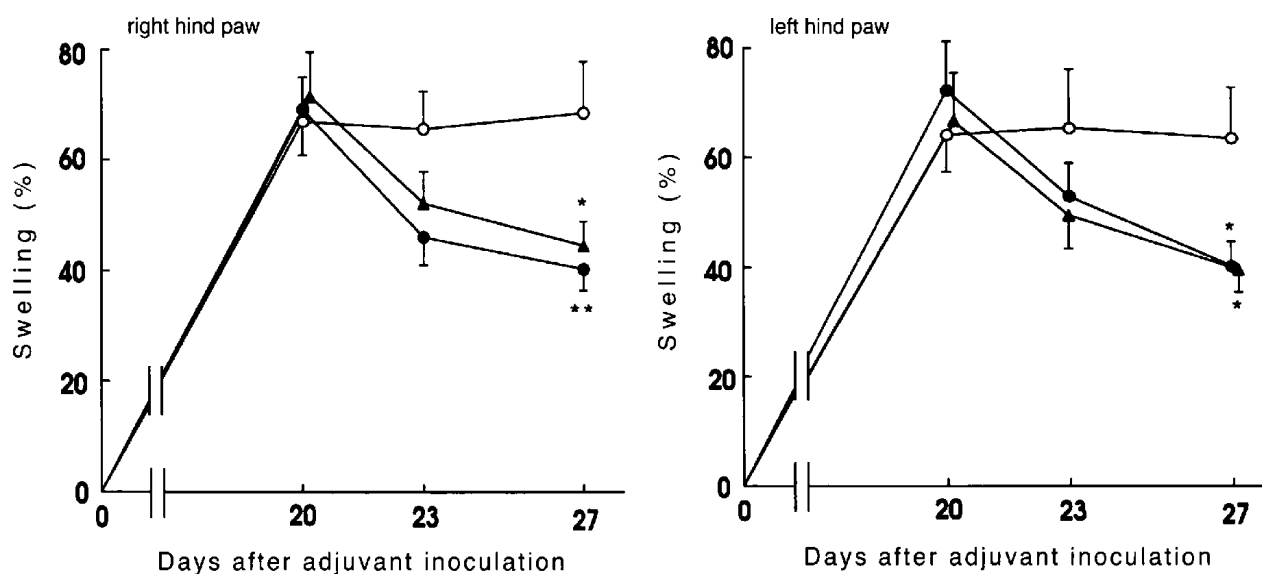


Fig. 1. Effects of FP-T and flurbiprofen (p.o.) on adjuvant-induced paw edema in rats. Treatment was started 20 days after adjuvant inoculation. FP-T was applied topically to the right hind paw for 6 hr per day, and flurbiprofen was orally administered once a day for 8 days. The volumes of the right (left panel) and left (right panel) hind paw were measured on days 0, 20, 23 and 27. Values each represent a mean \pm S.E.M. (n=10). ○: non-treated control; ●: FP-T; ▲: flurbiprofen, 0.75 mg/kg, p.o. *P<0.05, **P<0.01, significantly different from the non-treated control group by one-way analysis of variance and Dunnett's Multiple Range test.

stomach of each was excised, inflated by 10 ml of 10% formalin solution and immersed in the same solution for 2 hr. The length of gastric irritation in the stomach was measured according to Okabe et al. (4).

Statistical analyses

Results are expressed as means \pm S.E.M. Values were statistically examined by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Range test, Student's *t*-test or the Mann Whitney *U*-test. A *P* value of less than 0.05 was regarded as significant.

RESULTS

Effects of FP-T on adjuvant arthritis in rats

The anti-inflammatory effect of FP-T was evaluated by inhibition of hind paw edema in rats with adjuvant arthritis. The effect of FP-T on adjuvant arthritis was a significant inhibition in both the FP-T-applied right paw and the non-applied left paw. The degree of inhibition by FP-T on swelling in the FP-T-applied and non-applied paws were the same. The anti-inflammatory effect by FP-T was the same as that seen with oral administration of flurbiprofen (Fig. 1). Body weight gain by treatment with topical application of FP-T and oral administration of flurbiprofen is given in Table 1. Body weight significantly increased with these treatments.

Plasma concentrations of flurbiprofen

The profiles of plasma concentration of flurbiprofen after topical application of FP-T and oral administration of flurbiprofen are shown in Fig. 2. Although the maximum plasma concentration of flurbiprofen after oral administration of flurbiprofen was higher compared to the case of topical application of FP-T, the flurbiprofen levels in the plasma were much the same as those observed with topical application of FP-T and oral administration of flurbiprofen.

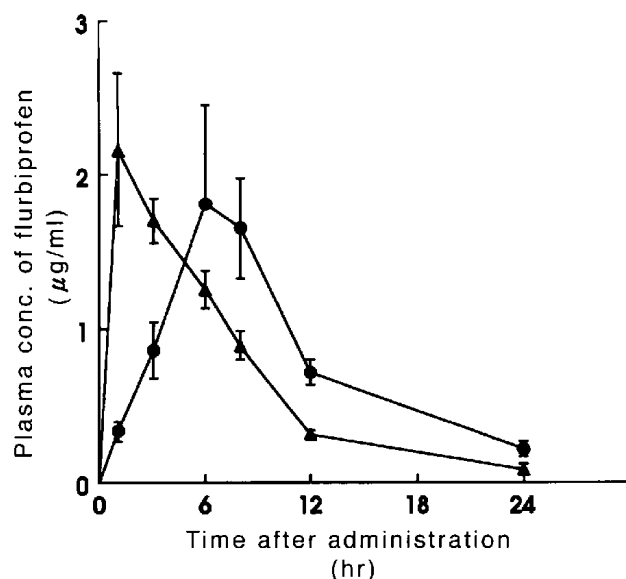


Fig. 2. Plasma concentration of flurbiprofen after topical application of FP-T and oral administration of flurbiprofen to rats. FP-T (3.5 cm \times 4 cm) was applied topically to the right hind paw for 6 hr, and the flurbiprofen (0.75 mg/kg, p.o.) was orally administered. Values each represent a mean \pm S.E.M. (*n* = 5). \bullet : FP-T, \blacktriangle : flurbiprofen (p.o.).

Effect of FP-T on gastric mucosae

The incidence of gastric damage 6 hr after topical application of FP-T and after oral administration of flurbiprofen was 50% and 80%, respectively. The ulcer index for FP-T was significantly lower compared to that for the oral administration of flurbiprofen (Table 2).

DISCUSSION

FP-T was developed as an adhesive tape with a high percutaneous absorption capacity and low gastric ulcerogenesis. We found that topical application of FP-T suppressed both FP-T applied paw edema and non-

Table 1. Body weight gain after topical application of FP-T and oral administration of flurbiprofen to adjuvant arthritic rats

Groups	n	Body weight (g)	
		days after adjuvant inoculation	
		day 20	day 27
Non-treated control	10	190.1 \pm 6.7	211.3 \pm 8.5
FP-T (3.5 cm \times 4 cm) 6 hr/day	10	190.7 \pm 3.4	222.4 \pm 3.1***
Flurbiprofen, 0.75 mg/kg, p.o./day	10	184.6 \pm 7.1	211.3 \pm 6.3*

The treatment was started 20 days after adjuvant inoculation. FP-T was applied topically to the right hind paw for 6 hr per day, and flurbiprofen was orally administered once a day for 8 days. Values each represent the mean \pm S.E.M. **P* < 0.05, ****P* < 0.001, significantly different from day 20 by Student's *t*-test.

Table 2. Gastric lesion induced by topical application of FP-T and oral administration of flurbiprofen in adjuvant arthritic rats

Groups	Incidence of lesions	Ulcer index (mm)
FP-T (3.5 cm × 4 cm) 6 hr/day	5/10 (50%)	0.95 ± 0.34*
Flurbiprofen, 0.75 mg/kg, p.o./day	8/10 (80%)	5.14 ± 2.08

Ulcer index values each represent the mean ± S.E.M. of 10 animals. * $P < 0.05$, significantly different from the group given flurbiprofen at 0.75 mg/kg, p.o./day by the Mann-Whitney *U*-test.

applied paw edema of rats with adjuvant arthritis. Anti-inflammatory effects of topical application of poultices or ointments containing NSAIDs are attributed to actions on inflamed tissue below the applied site, without any systemic influence, and these externally applied compounds are free from adverse effects that develop after oral administration of such drugs (2). However, we found that the anti-inflammatory effects of FP-T were exerted on not only FP-T-applied paw edema but also non-applied paw edema, with the same potency. Oral administration of flurbiprofen (0.75 mg/kg/day) also suppressed both the right and left paw edema. The activities of orally administered flurbiprofen were the same as those of topically applied FP-T. It seems that the anti-inflammatory effect of FP-T cannot be explained only by the action of flurbiprofen permeating the inflamed tissue below the applied site. The distribution of flurbiprofen absorbed transdermally may be mainly via the blood stream. The increase of body weight by FP-T or oral administration of flurbiprofen suggests that the systemic condition of rats was improved by these treatments. In addition, although the time-lag effect was observed in the case of topical application of FP-T, the plasma concentrations of flurbiprofen after topical application of FP-T were the same as those after oral administration of flurbiprofen (5). Furthermore, we preliminarily examined the effect of FP-T applied to the dorsal skin on hind paw edema induced by carrageenin in rats. The FP-T applied to the dorsal skin significantly suppressed the paw edema (data not shown). These results indicate that the anti-inflammatory effects of FP-T may be exerted via the systemic circulation of flurbiprofen absorbed transdermally. Further studies on FP-T, such as the dose-response relationship of the anti-inflammatory effect or analgesic effect, may be necessary.

Radermacher et al. (6) described drug concentrations in synovial fluid after cutaneous application of diclofenac gel in inflammatory and degenerative disease, at least 85% of the dose was considered to penetrate via the systemic circulation. Singh and Roberts (7) reported that local, direct penetration of NSAIDs was evident up to a depth of about 3 to 4 mm below the applied site, with

distribution to deeper tissue being predominant through the systemic blood supply. This evidence supports our postulation.

On the other hand, gastric damages following topical application of FP-T was lower compared to findings in the case of oral administration of flurbiprofen, yet the anti-inflammatory activities were almost equivalent. Gastric damage caused by some acidic NSAIDs is due to direct actions on gastric mucosa (8–10).

The transdermal delivery system (TDS) has been reviewed by Cleary (11). Ongoing studies of FP-T are being done to explore possible applications for local inflammatory diseases, arthropathies and rheumatoid arthritis.

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