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Percutaneous penetration of felbinac after application of transdermal patches: relationship with pharmacological effects in rats

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Abstract

We have evaluated the percutaneous penetration of felbinac following application of topical patches using a microdialysis technique, and have examined correlations with pharmacological effects. A linear microdialysis probe with a 20-mm dialysis fibre was inserted into the skin of anaesthetized rats. Probe perfusion was started at 2.0 μL min^{-1} with physiological saline and after a 60-min baseline sampling of dialysate, 0.1 mL croton oil was applied to the skin surface at a concentration of 8%, v/v. A felbinac patch was then applied to the same point 60 min thereafter and dialysate was sampled at 60-min intervals up to 300 min after patch application, for determination of concentrations of felbinac and prostaglandin (PG) E_2. Analgesic effects of felbinac patches in an iodoacetate-induced osteoarthritis model and an incisional pain model were evaluated using the weight bearing method. After application of patches, felbinac penetration into the skin was rapid, maximum concentrations in the dialysates with 0.07, 0.5 and 3.5% w/w felbinac patches being 0.046 ± 0.02, 0.104 ± 0.06 and 0.244 ± 0.2 μg mL^{-1}, respectively. Dermal administration of croton oil caused an increment in PGE_2 levels, which was significantly decreased by 0.5 and 3.5% felbinac patches 2–5 h after application. In pharmacological studies, 3.5% felbinac patches suppressed pain-associated behaviour induced by iodoacetate injection and plantar incision. These results suggested that the transdermal patch containing 3.5% felbinac may become a useful formulation.

Introduction

Osteoarthritis featuring chronically inflamed joints has a high morbidity rate. Age is the most important factor, so that patients with osteoarthritis increase as ageing of the population advances. In the clinical field, oral administration of non-steroidal anti-inflammatory drugs (NSAIDs) is widely used for the treatment of osteoarthritis pain, but in the long-term this raises the risk of adverse effects such as gastrointestinal disorders or renal insufficiency (Singh et al 1996; Solomon & Gurwitz 1997). Local administration of NSAIDs offers an alternative effective and safe route which can deliver drugs to inflammatory tissues without causing high plasma concentrations.

Felbinac is a major metabolite of fenbufen and various pharmacologic tests have indicated that it is the agent responsible for the anti-inflammatory effects resulting from the administration of fenbufen (Tolman et al 1976). Indeed it has shown anti-inflammatory and analgesic activity when administered topically (Hosie & Bird 1994). Felbinac has several advantageous characteristics for development of topical formulations, with a low molecular weight, high stability, oil solubility and no skin irritant properties. We have therefore developed a transdermal patch containing felbinac as a new approach to drug administration, with the aim of delivering high concentrations to inflammatory tissues. The patch features an oil-soluble base to facilitate skin permeability.

Assessment of drug concentrations in the skin is important in the development of topical products such as transdermal patches and microdialysis offers a technique for continuous sampling of unbound drug in the extracellular fluid of tissues in-vivo (Elmquist & Sawchuk 1997). Furthermore, microdialysis can be used to evaluate endogenous substances. Prostanoids are known to be involved in many physiological and pathological processes including

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generation of pain, inflammation and bone resorption (Laufer 2003), and prostaglandin (PG) E_2 in particular has been reported to have strong inflammatory activity. Hence the determination of low molecular weight PGE_2 concentrations in inflammatory tissues is very important to predict pharmacological activity of topical NSAID formulations.

We have investigated changes in felbinac and PGE_2 levels in skin following application of croton oil followed by 0.07, 0.5 and 3.5% w/w felbinac patches by microdialysis. The analgesic efficacy in the iodoacetate-induced osteoarthritis model was examined. In addition, because transdermal patches can deliver high concentrations of drug into the skin or muscle, it is expected to have good efficacy for soft tissue injury. Therefore, we investigated effects of the patches on incisional pain.

Materials and Methods

Animals

Studies were carried out in accordance with the Institutional Animal Care and Use Committee of Saitama Daiichi Pharmaceutical Co., Ltd. Male Wistar rats (180–250 g; Nihon SLC, Shizuoka, Japan) were used in all experiments. Rats were housed in an animal room with a room temperature of 23 ± 2°C, a relative humidity of 55 ± 15%, and a 12/12-h light–dark cycle (light on at 08:00 h). Animals had free access to a mouse/rat diet (F-2, pelleted form, Funabashi Farm, Chiba, Japan) and tap water throughout the experiment.

Drugs

Patches containing 0 (blank), 0.07, 0.5 and 3.5% w/w felbinac in a hydrophobic adhesive styrene/isoprene/styrene copolymer were obtained from Mikasa Seiyaku Co. Ltd (Tokyo, Japan), with a felbinac content of 0.04, 0.29 and 2 mg/4 cm^2, respectively. Sodium iodoacetate (iodoacetate, Sigma Chemical Co., St Louis, MO) was dissolved in 0.9% physiological saline (Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan). Other chemical agents used were croton oil (Sigma Chemical Co., St Louis, MO), pentobarbital sodium (Dainippon Pharmaceutical Co. Ltd, Osaka, Japan) and povidone–iodine solution (Meiji Seika Kaisha Ltd, Tokyo, Japan).

In-vivo evaluation of the recovery of the probe

In-vivo recovery of felbinac was determined by retrodialysis methods (Elmquist & Sawchuck 1997). Rats were anaesthetized with an intraperitoneal injection of urethane (1.25 g kg^{-1}) and placed on a temperature-controlled (36°C) heating pad. The skin of the abdominal region was shaved and a linear microdialysis probe with a 20-mm dialysis fibre (0.22 mm o.d.; 0.20 mm i.d.; Eicom Co., Kyoto, Japan) was inserted using a 23-gauge guide cannula resurfacing through an exit puncture. The guide was withdrawn leaving the membrane placed horizontally within the skin. After implantation in the rat, the inlet tube of the probe was connected to a microinjection pump (Harvard Apparatus, MA). Saline solution containing 0.5 mg mL^{-1} felbinac was passed through the probe using an infusion pump at 2.0 μL min^{-1} and dialysate samples were collected every 60 min for 360 min. The recovery was determined from the ratio of the concentration loss to the initial concentration in the perfusate:

$$\text{Recovery } (\%) = [(\text{influent dialysate amount} - \text{effluent dialysate amount})/\text{influent dialysate amount}] \times 100 \quad (1)$$

In-vivo microdialysis study

A probe was inserted within the skin as indicated above. Probe perfusion at 2.0 μL min^{-1} with physiological saline was started and a recovery period of 60 min was allowed for the insertion trauma to subside. After 60-min baseline sampling of dialysate, 0.1 mL croton oil was applied to the skin surface at a concentration of 8%, v/v, at the point where the microdialysis probe was cutaneously implanted. A felbinac patch was then applied to the same point 60 min thereafter and dialysate was sampled at 60-min intervals for up to 300 min after patch application. All samples were stored at –40°C until assayed for PGE_2 with an ELISA kit (Cayman Chemical, Ann Arbor, MI) and for felbinac by gas chromatograph mass spectrometry.

Analysis of felbinac in dialysate

Analysis of felbinac was performed with a gas chromatograph mass spectrometer (Trace DSQ; Thermo Electron Co., TX). Dialysate (20 μL) was mixed with ethyl acetate solution containing ketoprofen (50 μL), as an internal standard, HCl (0.1 M; 10 μL) and ethyl acetate (120 μL), and the ethyl acetate layer (120 μL) was then mixed with trimethylsilyldiazomethane (50 μL) and left to stand for 60 min at ambient temperature and analysed for felbinac.

Osteoarthritis pain model

Osteoarthritis was induced with an intra-articular injection of iodoacetate, as described by Guingamp et al (1997). Briefly, rats were anaesthetized with pentobarbital sodium (50 mg, i.p.) and received a single injection of iodoacetate (3 mg/site) or saline in a 25-μL volume into the left knee joint using a 27-gauge needle inserted through the patellar tendon. Pain behavioural tests were carried out before injection of iodoacetate, and at 1, 3, 5, 7, 9 and 11 days thereafter. Patches sized 4 cm^2 were applied to the left knee joint 4 h before the behavioural test and then removed 10 min before the test.

Incisional pain model

Surgery was performed by a modification of a described procedure (Brennan et al 1996). Briefly, rats were anaesthetized with pentobarbital sodium (50 mg kg^{-1}, i.p.) and the left plantar was prepared in a sterile manner with a 10% povidone–iodine solution. A 1-cm longitudinal incision was made with a number 11 blade, through the skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toe. The skin was then opposed with two single interrupted sutures using 5-0 nylon. The wound site was covered with povidone–iodine solution and the animals were allowed to recover in their

home cages. Pain behavioural tests were carried out before surgery and at 1, 2, 3, 4 and 7 days after surgery. Patches sized 4 cm^2 were applied to the left plantar 4 h before the behavioural test and removed 10 min before the test.

Pain behavioural tests

Hind limb weight bearing was measured using a Dual Channel Weight Averager (Linton Instrumentation, Diss, Norfolk, UK). The rats were placed in a Perspex chamber designed so that each hind paw rested on a separate transducer pad. The averager was set to record the load on the transducer over 5 s and the two numbers displayed represented the distribution of the rat's body weight on each paw. For each rat two readings from each paw were taken and then averaged. Data were presented as weight bearing differences.

Statistical analysis

The results were expressed as mean ± s.d. values. Differences in various treatments were statistically examined using one-way analysis of variance. Individual differences between means were examined with the Dunnett's parametric multiple comparison test. The criterion for statistical significance was $P < 0.05$ with all statistical evaluations.

Results

In-vivo evaluation of the recovery of the probe

The in-vivo recovery of the probe was determined by the retrodialysis method. The average recovery of felbinac over 360 min was 44.7 ± 2% (Figure 1). Dialysis membranes of 20-mm length showed steady loss of felbinac for 360 min through the microdialysis probe.

In-vivo microdialysis study

Time courses of change in felbinac concentrations in the skin for 300 min after application of patches are shown in Figure 2A. Felbinac penetration of the skin was rapid. Maximum concentrations in dialysates after application of 0.07, 0.5 and 3.5% felbinac patches were shown to be 0.046 ± 0.02, 0.104 ± 0.06 and 0.244 ± 0.2 $\mu g\,mL^{-1}$, respectively.

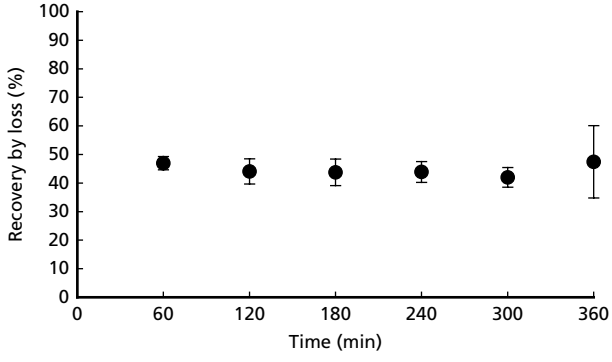

Figure 1 Loss of felbinac by retrodialysis with the microdialysis probe. Data are mean ± s.d. values for six experiments.

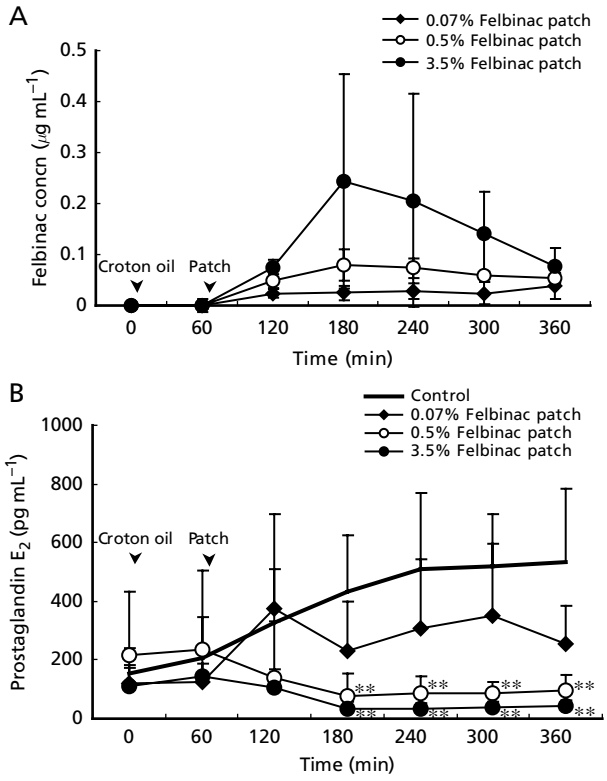

Figure 2 Concentration–time profiles of felbinac (A) and prostaglandin E_2 (B) in the skin after transdermal application of patches. Croton oil solution and felbinac patches were applied at 60 and 120 min after beginning of perfusion, respectively (indicated by arrow heads). Data are mean ± s.d. values for five to six experiments. $^{**}P < 0.01$ vs control.

Time courses of change in PGE_2 levels in the skin after application of croton oil and felbinac patches are shown in Figure 2B. Transdermal administration of 8% croton oil solution caused increment of PGE_2 levels, in a time dependent manner, with a plateau reached at 240 min through 360 min. The peak level of PGE_2 was 531.7 ± 250 pg mL^{-1} at 360 min, an approximately 3.5-fold elevation. Transdermal application of felbinac patches inhibited the increment of PGE_2 induced by croton oil in a concentration dependent manner, and 0.5 and 3.5% felbinac patches significantly decreased values in the skin 2–5 h after application. No significant changes were observed after application of 0.07% felbinac patches.

Effects on osteoarthritis pain

Injection of iodoacetate into the left knee resulted in a clear reduction in the amount of weight placed on the injected hind limb (Figure 3). Peak level of reduction in the amount of weight placed on the injected hind limb was 22.6 ± 11 g at 5 days after injection, and the reduction recovered to 3.6 ± 11 g on day 11. Felbinac patches inhibited the alteration of hind limb weight bearing, in a concentration-dependent manner, with 0.5 and 3.5% felbinac patches exerting

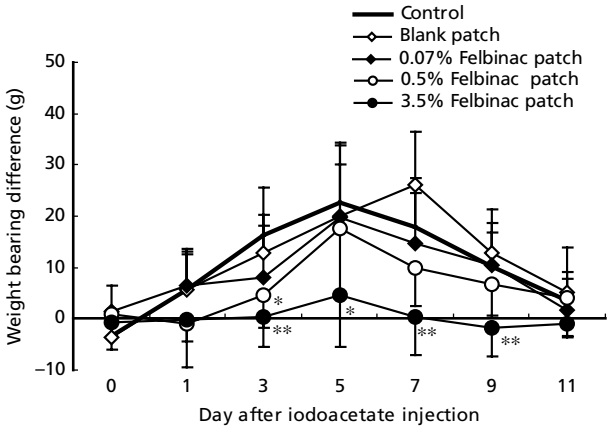

Figure 3 Effects of felbinac patches on iodoacetate-induced alteration of weight bearing in rats. Each patch was applied the left knee joint 4 h before behavioural tests, and removed 10 min before the tests. Data are mean ± s.d. values for eight rats. $*P < 0.05$, $**P < 0.01$ vs control.

significant effects on day 3 and days 3–9, respectively. Blanks and 0.07% felbinac patches did not influence the alteration of weight bearing.

Effects on incisional pain

Incision of the plantar surface of the left-hind paw caused reduction in the amount of weight placed on the incised paw (Figure 4). Peak reduction in the amount of weight placed on the injected hind paw reached 12.0±7 g at day 1 after incision. Blank, 0.07 and 0.5% felbinac patches did not influence the alteration of weight bearing, but 3.5% felbinac patches reversed the alteration, and exerted significant inhibition on day 2.

Discussion

Felbinac patches are designed as effective and safe formulations by which high concentrations of the drug can be achieved in inflammatory tissue through diffusion into the skin, in the absence of high plasma levels. Since the patch does not contain water, felbinac contained in the patch should essentially exist as a non-ionized drug. It is well documented that the general absorption of non-ionized drugs through skin is higher than with ionized drugs, which have high aqueous solubility (Inoue et al 2000). Thus, this formulation without water may contribute to high percutaneous permeability of the drug.

In this retrodialysis study, the average recovery of felbinac over 360 min was 44.7 ± 2%, with steady loss into the tissue. Since drug recovery from the tissue to perfusate is the same as drug loss from perfusate to tissue across the probe membrane (Elmquist & Sawchuck 1997), this microdialysis system may reflect approximately 45% of the drug level in the skin. The recovery of felbinac was relatively low, facilitating evaluation of drug concentration into the skin (Mathy et al 2005). A higher recovery could be obtained by increasing surface area of the microdialysis fibre or declining perfusate flow (Kreilgaard 2002). However, because declining perfusate flow induced decrease in dialysate volume, there was the risk that lack of dialysate volume would adversely affect precision in determination of the drug and PGE_2 concentrations in the dialysate. After administration of the patches, felbinac rapidly penetrated the skin, and felbinac levels in the dialysate increased in a concentration-dependent manner. The concentration of felbinac contained in each patch was associated with a 7-fold increment, but the differences in maximum felbinac levels in the dialysate after application of each patch were approximately 2- to 3-fold. Takizawa et al (2007) reported that differences in felbinac levels in the stratum corneum layer after application of the patches were approximately 7-fold, the results reflecting the difficulty in measuring in deep tissues of the skin, such as the dermis or subcutaneous tissue. We confirmed that our dialysis probes were inserted into subcutaneous tissue by pathological examination.

Felbinac suppresses prostanoid production, involved in pain and inflammation, mediated by inhibition of cyclooxygenase. In this study, 0.5 and 3.5%, but not 0.07%, patches completely inhibited PGE_2 production in the skin induced by croton oil. This indicated a threshold level of felbinac action higher than 0.046 $\mu g\,mL^{-1}$, the maximum level in dialysate after application of 0.07% felbinac patches. With another protocol, we preliminarily determined the IC50 of felbinac (the concentration required for 50% inhibition) for lipopolysaccharide-induced PGE_2 production in human skin fibroblasts to be approximately 0.049 $\mu g\,mL^{-1}$.

Prostanoids are produced by chondrocytes, synoviocytes, and subchondral osteoblasts within the osteoarthritis joint and are involved in the pathogenesis of osteoarthritis (Molloy & McCarthy 2005). Although complex animal models of osteoarthritis, such as surgical instability models in rabbits or dogs, have been studied for many years, these do not always reflect symptoms of osteoarthritis disease (Bendele 2001). The iodoacetate model of osteoarthritis is very simple, and shares characteristics with human osteoarthritis, such as cartilage lesions, suppression of mobility and inhibition of proteoglycan production (Janusz et al 2004). Injection of the metabolic inhibitor, iodoacetate, causes joint pathology via

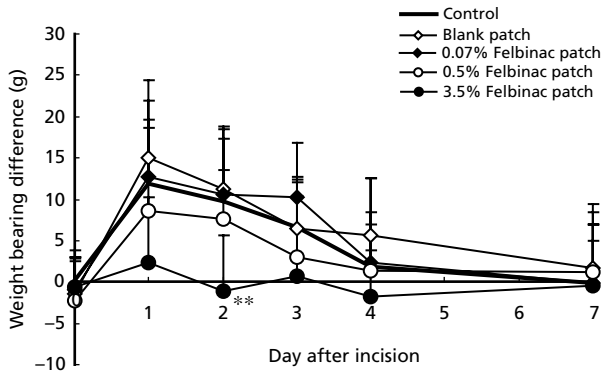

Figure 4 Effects of felbinac patches on plantar incision-induced alteration of weight bearing in rats. Each patch was applied the left knee joint 4 h before behavioural tests, and removed 10 min before tests. Data are mean ± s.d. values for eleven rats. $**P < 0.01$ vs control.

inhibition of glycolysis, thereby targeting vascular cartilage and causing chondrocyte death (Kalbhen 1987; van der Kraan et al 1989). Also, we have shown that rats injected with iodoacetate feature necrosis and fibrillation of articular cartilage, increase in osteoclasts, and proliferation of synovial membranes (data not shown). Fernihough et al (2004) reported that the NSAID diclofenac inhibited hyperalgesia induced by iodoacetate injection. In this study, we demonstrated that application of 0.5 and 3.5%, but not 0.07%, felbinac patches suppressed iodoacetate-induced pain measured by the weight bearing method. Effects of 0.5% felbinac patches against iodoacetate-induced pain were no more remarkable than against PGE_2 production in the skin. This result suggested that drug penetration of muscle following topical application was low compared with skin (Higaki et al 2002).

Topical application of felbinac is both highly effective and well tolerated in the treatment of acute soft tissue injuries (Leeb 1994). There is also a report that oral administration of indometacin or naproxen inhibited mechanical hyperalgesia and tactile allodynia in a rat model of incisional pain (Whiteside et al 2004). In this study, we have demonstrated that 3.5% felbinac patches showed analgesic effects against incision-associated pain in rats. To our knowledge this is the first report of the effects of topical administration of NSAIDs in an incisional pain model. It has been suggested that the incisional pain model has some similarities to the human post-operative pain state (Brennan et al 1996).

It is well known that rodent skin is generally more permeable than human skin (Catz & Friend 1990; Kakubari et al 2006), while drug permeability in pig skin is similar to that in man (Ngawhirunpat et al 2004). Human skin permeability of felbinac after application of the patch may be poor compared with rat skin. However, the aim of this study was to evaluate the relationship between the amount of drug penetration through the skin and the pharmacological effects. Evaluation methods for analgesic effects using pigs have yet to be established, whereas skin permeability and pharmacological activity can be readily determined in the rat. Drug permeability in human skin requires examination with another protocol, but then the results obtained in rats should facilitate prediction of the pharmacodynamics with felbinac patches.

Conclusions

We have demonstrated that transdermal administration of felbinac patches could result in high concentrations of the drug that could inhibit PGE_2 production in the skin, and inhibit osteoarthritis and incisional pain in rats. The results indicated that the felbinac transdermal patch may become a useful formulation.

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