CJ-023,423, a Novel, Potent and Selective Prostaglandin EP4 Receptor Antagonist with Antihyperalgesic Properties

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ABSTRACT

The prostaglandin (PG) EP4 receptor subtype is expressed by peripheral sensory neurons. Although a potential role of EP4 receptor in pain has been suggested, a limited number of selective ligands have made it difficult to explore the physiological functions of EP4 or its potential as a new analgesic target. Here, we describe the in vitro and in vivo pharmacology of a novel EP4 receptor antagonist, N-[[2-[4-(2-ethyl-4,6-dimethyl-1H-imidazo [4,5-c]pyridin-1-yl)phenyl][ethyl]amino]carbonyl]-4-methylbenzenesulfonamide (CJ-023,423). In vitro, CJ-023,423 inhibits [(3H)]PGE2 binding to both human and rat EP4 receptors with K of 13 ± 4 and 20 ± 1 nM, respectively. CJ-023,423 is highly selective for the human EP4 receptor over other human prostanoid receptor subtypes. It also inhibits PGE2-evoked elevation in intracellular cAMP at the human and rat EP4 receptors with pA2 of 8.3 ± 0.03 and 8.2 ± 0.2 nM, respectively. In vivo, oral administration of CJ-023,423 significantly reduces thermal hyperalgesia induced by intraplantar injection of PGE2 (ED50 = 12.8 mg/kg). CJ-023,423 is also effective in models of acute and chronic inflammatory pain. CJ-023,423 significantly reduces mechanical hyperalgesia in the carrageenan model. Furthermore, CJ-023,423 significantly reverses complete Freund’s adjuvant-induced chronic inflammatory pain response. Taken together, the present data indicate that CJ-023,423, a highly potent and selective antagonist of both human and rat EP4 receptors, produces antihyperalgesic effects in animal models of inflammatory pain. Thus, specific blockade of the EP4 receptor signaling may represent a novel therapeutic approach for the treatment of inflammatory pain.

Nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase (COX)-2 inhibitors are mainstays of the pharmacopoeia for the treatment of signs and symptoms of osteoarthritis and inflammatory pain of various etiologies. Their mechanism of action is to decrease prostaglandin (PG) synthesis by inhibiting COX activities. Two isoforms of COX, COX-1 and COX-2, have been identified. COX-1 is constitutively expressed throughout the body, and it is thought to play an essential role in normal gastrointestinal and renal function, whereas COX-2 is induced in the presence of inflammation. NSAIDs inhibit both isoforms and inhibition of COX-1 is thought to cause the adverse gastrointestinal effects such as gastric erosion, ulceration, and hemorrhage, whereas inhibition of COX-2 is associated with the therapeutic effects of NSAIDs. Thus, inhibition of PG synthesis by NSAIDs has demonstrated clear efficacy in the reduction of
PGE2-evoked elevation in intracellular cAMP at both human and rat provides the basis for novel therapeutic approaches for better pain management. It has downstream effects of COX, and particularly, the identification of EP1–4. These receptor subtypes are distinguished by their distinct pattern of tissue distribution, signaling pathways, and physiological functions (Coleman et al., 1994b). EP1 is coupled to intracellular Ca2+ mobilization, EP2 and EP4 are coupled to stimulation of adenylate cyclase via Gs protein, and EP3 is coupled to inhibition of adenylate cyclase via Gi protein. Studies performed either in mutant mice lacking the individual PG receptors (Murata et al., 1997; Minami et al., 2001; Stock et al., 2001) or with synthetic EP receptor agonist/antagonist (Minami et al., 1994; Nakayama et al., 2002) have not yet provided a coherent picture of which EP receptors are responsible for inflammatory pain. Recently it has been reported that EP2 knockdown with intrathecally delivered short hairpin RNA attenuates inflammation-induced thermal and mechanical behavioral hypersensitivity (Lin et al., 2006), suggesting that EP2 is a potential target for the pharmacological treatment of inflammatory pain. However, developing subtype-selective EP receptor antagonists has been difficult because of the existence of multiple PG receptor subtypes and the lack of the selectivity of synthetic PG analogs. Thus, defining the contribution of EP receptor subtype to pain sensitization on the basis of available antagonists remains elusive.

Here, we report the in vitro and in vivo characterization of a novel, potent, and selective EP4 antagonist, CJ-023,423 (Fig. 1). CJ-023,423 competitively inhibits PGE2 binding and PGE2-evoked elevation in intracellular Ca2+ AMP at both human and rat EP4 receptors. Oral administration of CJ-023,423 dose-dependently reduced hyperalgesia in animal models of inflammatory pain, and it produced comparable efficacy to NSAIDs, which block PG synthesis of all of the COX products. These data suggest that the EP4 receptor plays a dominant role in mediating the pronociceptive action of PGE2. It is downstream of COX, and particularly, the identification of it as a single PG receptor subtype responsible for pain provides the basis for novel therapeutic approaches for better tolerated analgesics.

### Materials and Methods

#### Compounds

The sodium salt of CJ-023,423 and 4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide (picroxamic), a prototypical NSAID used as a comparator, were synthesized in Pfizer Research Laboratories.

#### Stable Expression of Prostanoid Receptors in the Human Embryonic Kidney 293 Cell Line

Prostanoid receptor cDNA clones were obtained by reverse transcription and polymerase chain reaction. The amplified DNA fragment was isolated by electrophoresis on a 1.2% agarose gel, digested with restricted enzymes, and cloned into the appropriate site of the mammalian expression vector pcDNA3 (Invitrogen, Carlsbad, CA). To generate stable transfectants, plasmid DNA was transfected into HEK293 cells (American Type Culture Collection, Manassas, VA) using Lipofectamine (Invitrogen) according to the manufacturer’s instructions. HEK cells expressing the cDNA together with the G-418 (Geneticin; Invitrogen) resistance gene were selected in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Individual colonies were isolated after 2 weeks of growth under selection using the cloning ring method, and they were subsequently expanded into clonal cell lines. Expression of the receptors was assessed by receptor binding assays.

#### Prostanoid Receptor Radioligand Binding Assays

A competitive binding assay was performed in a final incubation volume of 1 ml in 10 mM MES/KOH buffer, pH 6, containing 10 mM MgCl2 and 1 mM EDTA for EP1, EP2, EP3, and FP or in 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM MgCl2 and 1 mM EDTA for DP and IP or in DMEM buffer containing 0.1% NaNO3, pH 7.4, for EP4. Saturation studies were conducted with [3H]PGE2 (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for EP1, [3H]PGD2 (GE Healthcare) for DP, [3H]PGF2α (GE Healthcare) for FP, and [3H]iloprost (GE Healthcare) for IP in concentrations ranging from 0.015 to 32 nM. Membranes were prepared from stably transfected HEK293 cells expressing EP subtypes as well as those for PGD2, PGE2, and prostacyclin. Assays were initiated by the addition of membrane proteins (EP1, 40 μg; EP2, 40 μg; EP3, 5 μg; EP4, 100 μg; DP, 30 μg; FP, 60 μg; and IP, 100 μg) into the incubation mixture. After 60-min incubation at room temperature, the reaction was terminated by rapid vacuum filtration over glass fiber filter papers presoaked in 0.2% polyethyleneimine (Branded Inc., Gaithersburg, MD) using a Brandel cell harvester followed by two washes with assay buffer. Receptor-bound radioactivity was quantified by liquid scintillation counter, and nonspecific binding was determined in the presence of unlabeled 10 μM of the corresponding nonradioactive prostanoid. IC50 values of CJ-023,423 from competitive binding assay were determined using 1 nM ligand concentration, [L]. To obtain Kd values of CJ-023,423, these parameters were applied to the Cheng and Prusoff (1973) equation

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K_d = IC_{50}/(1 + [L]/K_a)
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Displacement binding at the TP receptor prepared from human platelet membranes was performed at Cerep (Celle L’Evescault, France).

#### Isolation and Culture of Rat Dorsal Root Ganglion Cells

Primary cultures of dissociated neonatal DRG neurons were prepared according to the method described previously (Garland et al., 1995). In brief, Sprague-Dawley (SD) rats (1–4 days) were anesthetized, and the spinal cords were surgically removed and placed into ice-cold sterile Hanks’ medium (Invitrogen). DRG were digested by the serial addition of 0.1% collagenase (Sigma-Aldrich, St. Louis, MO) and then centrifuged. The cell pellet was resuspended in minimal essential medium (Invitrogen) supplemented with 10% FBS, 5 g/l glucose (Sigma-Aldrich), 40 mg/l gentamicin (Invitrogen), 100 U/ml penicillin (Invitrogen), 100 μg/ml streptomycin (Invitrogen), 100 ng/ml nerve growth factor (Sigma-Aldrich), and 10 μM cytokine arabinoside (Sigma-Aldrich). Cells were plated onto poly-d-lysine and laminin- precoated 96-well culture plates (BD Biosciences, San
A regression lines were calculated to ascertain the competitive antagonism of the antagonist against PGE2 plotted against log[antagonist concentration]. The slopes of linear regression lines were calculated from each experiment.

SD rats (115–250 g) were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). Animals were housed in pairs and in the absence of antagonist. Estimates of log[CR 1] were plotted against log[antagonist concentration]. The slopes of linear regression lines were calculated to ascertain the competitive antagonist behavior. The antagonist potency was expressed in terms of pA2 calculated from the equation pA2 = log[CR 1] − log[antagonist concentration] for each experiment.

Animals. All procedures were carried out with the approval of the Animal Ethics Committee at Pfizer’s Nagoya Laboratories (Aichi, Japan) according to the Laboratory Animal Welfare guidelines. Male SD rats (115–250 g) were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). Animals were housed in pairs with free access to food and water. The animals were kept under conditions of constant temperature (23 ± 2°C) and humidity (55 ± 15%) with a 12-h light/dark cycle (lights on 7:00 AM). Before the start of the experiment, the animals were housed under these conditions for 4 to 5 days. The rats were fasted overnight before experimental use, and each drug was orally administered suspended with 0.1% methylcellulose (MC; Wako Pure Chemicals, Osaka, Japan) in a volume of 10 ml/kg.

PGE2-Induced Thermal Hyperalgesia. Hyperalgesia was induced by intraplantar injection of 100 ng of PGE2 (Sigma-Aldrich) in 5% dimethyl sulfoxide/saline (100 µl). CJ-023,423 suspended in 0.1% MC was administered orally 30 min before PGE2 injection. Rats were placed in plastic cages of plantar test apparatus (Ugo Basile, Comerio, Italy), and the mobile radiant heat source was focused on the right hind paws of the rats. Latency (seconds) to the thermal stimulation was measured both before and after PGE2 injection. The change in thermal nociceptive threshold was calculated as follows: percentage of change in pain threshold = 100 × ([latency after PGE2 injection] − [latency before PGE2 injection])/(latency before PGE2 injection).

Carrageenan-Induced Mechanical Hyperalgesia. Hyperalgesia was induced by intraplantar injection of 0.1 ml of 1% (w/v) λ-carrageenan (Picnic A; Zushikagaku Laboratory, Tokyo, Japan). The test compounds suspended in 0.1% MC were administered orally at 5.5 h after the carrageenan injection. The pain threshold was measured using an algosy meter (Ugo Basile) at 4, 5, 6.5, 7.5, and 8 h after the carrageenan injection, and the change of pain threshold was calculated.

Complete Freund’s Adjuvant-Induced Weight-Bearing Deficit. In male 7-week-old SD rats (Charles River Laboratories Japan Inc.), 300 µg of Mycobacterium tuberculosis H37 RA (Difco, Detroit, MI) in 100 µl of liquid paraffin (Wako Pure Chemicals) (CFA) was injected into the right hind footpad. Two days after CFA injection, changes in hind paw weight distribution between the right (inflamed) and the left (contralateral) limbs were measured as an index of pain using a Linton Incapacitance tester (Linton Instrumentation, Norfolk, UK). Each animal was placed in the apparatus, and the weight load exerted by the hind paws was measured. The duration of the measurement was adjusted to 5 s. Rats with a weight load difference of 40 to 100 g on each paw were selected. Test compounds suspended in 0.1% MC were administered orally, and their analgesic effects were examined (0.5, 1, 2, 4, and 6 h after drug administration). Rats were kept fed during the experiment.

Statistics. Statistical analyses of the data were performed using the StatView (Abacus Concepts, Berkeley, CA) statistical package.

Fig. 1. Chemical structure of the EP4 antagonist CJ-023,423.

Fig. 2. A, competition binding profile of CJ-023,423 and PGE2 at the human and rat EP4 receptors. Membranes prepared from HEK293 cells stably transfected with cDNA encoding the human and rat EP4 receptors were incubated with 1 nM [3H]PGE2 and various concentrations of CJ-023,423. Sigmoidal competition curves were constructed by expressing the maximum specific binding of 1 nM [3H]PGE2 as 100%. The Ki value was calculated using the Cheng and Prusoff equation (1973). Ki = IC50/1 + [L]/Kd. Data shown are mean ± S.E.M. of three independent experiments with each point performed in duplicate. B, CJ-023,423-binding activity for human EP4, DP, FP, IP, and TP. Equilibrium competition assays were performed as described under Materials and Methods. Data are represented as the mean ± S.E.M. of the three independent experiments for human EP4 assay except for human EP4 binding to DP, FP, IP, and TP assay (n = 2) with each point performed in duplicate.
Differences between treatment groups were tested by one-way ANOVA and unpaired two-tailed Student’s *t* test. *P* values less than 0.05 at 95% confidence level were considered significant. All values in the text and figures are mean ± S.E.M.

### Results

**CJ-023,423 Inhibits PGE₂ Binding to Human and Rat EP₄ Receptors.** CJ-023,423 (Fig. 1) is a high-affinity EP₄ receptor-selective ligand. The affinity of CJ-023,423 against human and rat EP₄ receptors was determined by radioligand binding studies using membranes prepared from HEK293 cells stably expressing human EP₄ or rat EP₄ receptors. CJ-023,423 displaced [³H]PGE₂ binding to human recombinant EP₄ in a concentration-dependent manner with an IC₅₀ value of 14 ± 3.9 nM (n = 3), and *Kᵢ* values of CJ-023,423 and PGE₂ for the human EP₄ receptor were 13 ± 4 and 4 ± 1 nM, respectively (n = 3) (Fig. 2A). CJ-023,423 showed almost equal binding affinity for rat recombinant EP₄ with an IC₅₀ value of 14 ± 3.9 nM (n = 3) (Fig. 2A).
value of $27 \pm 1.3 \text{ nM} (n = 3)$ and a resultant $K_i$ value of $20 \pm 1 \text{ nM} (n = 3)$ (Fig. 2A). These data suggest that CJ-023,423 is a potent ligand at both human and rat EP₄ receptors. The compound was 200 times more selective for the human EP₄ receptor subtype than other prostanoid receptors [IC₅₀ > 20 μM for EP₁, EP₂, EP₃, FP, IP, and TP; IC₅₀ = 4.3 μM for DP (n = 2)] (Fig. 2B). Furthermore, receptor selectivity profile for CJ-023,423 was determined by evaluating the effects of the EP₄ receptor ligand on specific binding of radioligands to more than 50 receptors, which was performed by Cerep. CJ-023,423 (1 μM) did not exhibit any significant activity at all other receptors tested (Table 1).

**CJ-023,423 Inhibits PGE₂-Evoked Intracellular cAMP Elevation in HEK293 Cells Expressing EP₄ Receptors.** PGE₂ dose-dependently increased intracellular cAMP accumulation at human EP₄ receptors expressed in HEK293 cells. Increasing concentrations of CJ-023,423 produced a rightward shift in the concentration-response curve for PGE₂ without modulating the maximal cAMP production (Fig. 3A). The $pA_2$ value was calculated as $8.3 \pm 0.3$ with a slope of $1.3 \pm 0.1$ (n = 3) by Schild plot analysis (Arunlakshana and Schild, 1959). These experiments were repeated using HEK293 cells transfected with cDNA encoding the rat EP₄ receptor, resulting in a $pA_2$ value of $8.2 \pm 0.2$ with a slope of $1.2 \pm 0.1$ (n = 4) (Fig. 3B). CJ-023,423 did not show any agonist activity at the human and rat recombinant EP₄ receptors (data not shown). These data suggest that CJ-023,423 is a competitive antagonist at the human and rat EP₄ receptors.

**CJ-023,423 Inhibits PGE₂-Evoked Intracellular cAMP Elevation in Rat DRG Cells.** PGE₂ is known to mediate its hyperalgesic action through G protein-coupled receptors linked to Gₐ proteins and by activation of adenyl cyclase in primary sensory neurons (Ferreira and Nakamura, 1979). We have attempted to determine the contribution of the EP₄ receptor subtype in modulating adenyl cyclase activity in isolated DRG neurons. CJ-023,423 inhibited PGE₂-evoked intracellular cAMP elevation in rat DRG cells with an IC₅₀ of $41 \pm 15 \text{ nM} (n = 3)$ (Fig. 4). These data suggest that PGE₂-mediated signal transduction is mediated by EP₄ receptors in the rat DRG cells.

**Pharmacokinetic Profile of CJ-023,423.** The pharmacokinetic profile of CJ-023,423 in rats (Fig. 5) was characterized by moderate-to-high plasma clearance (3.1 l/h/kg), low oral bioavailability (F = 4.6%), a low-to-moderate volume of distribution (Vdss = 1.2 l/kg), and a moderate plasma half-life ($t_{1/2} = 2.0 \text{ h i.v.}$). $C_{\text{max}}$ and $T_{\text{max}}$ following oral administration at 3 mg/kg were 12 ng/ml and 0.083 h, respectively. CJ-023,423 plasma protein binding was high in rats (98.8%).

**CJ-023,423 Inhibits PGE₂-Induced Thermal Hyperalgesia in Rats.** Initial experiments with CJ-023,423 tested whether EP₄ has a predominant role in mediating PGE₂-evoked nociceptive responses. Intraplantar injection of 100 ng of PGE₂ into the rat footpad induced hyperalgesia to peripheral thermal stimuli, peaking 15 to 20 min after injection (Fig. 6A). Paw withdrawal latencies (PWL) in response to bright light irradiation of the paw were recorded both before and 15 min after PGE₂ injection. CJ-023,423 was administered orally 30 min before PGE₂ treatment. Untreated rats displayed a PWL of approximately $10.1 \pm 0.4$ s. PGE₂ injection reduced PWL by approximately 6 s, indicating thermal hyperalgesia of the injected paw. CJ-023,423 (1, 3, 10, and 29 mg/kg p.o.) significantly increased the PWL of the PGE₂-injected paw with an ED₅₀ of 12.8 mg/kg. These...
results suggest that CJ-023,423 reduces PGE2-dependent thermal hyperalgesia (Fig. 6B).

**CJ-023,423 Reverses Carrageenan-Induced Mechanical Hyperalgesia in Rats.** Carrageenan injection into the rat footpad resulted in the induction of hyperalgesia to peripheral mechanical stimuli, peaking 4 to 8 h after treatment. Paw withdrawal thresholds (PWT) were recorded before and after intraplantar injection of carrageenan. Untreated rats displayed a PWT of approximately 120 g. Carrageenan-treated rats reached maximum sensitivity 5 h after injection with a PWT of approximately 50 to 60 g. Test compounds were administered 5.5 h after carrageenan injection, and PWT was recorded in the carrageenan-treated paw 1, 2, and 2.5 h after dosing. CJ-023,423 (3, 10, 30, and 100 mg/kg p.o.) dose-dependently increased PWT (reduced hyperalgesia) on the carrageenan-treated paw with the maximal effect of 64% reversal at 100 mg/kg. (Fig. 7). These results indicate that CJ-023,423, when administered therapeutically, reduces carrageenan-induced mechanical hyperalgesia. Its therapeutic effect (100 mg/kg p.o.) was comparable with that observed with the dual COX-1/COX-2 inhibitor piroxicam (10 mg/kg p.o.) in terms of efficacy.

**CJ-023,423 Inhibits CFA-Induced Weight-Bearing Deficit in Rats.** CJ-023,423 was tested in the CFA model of chronic inflammatory pain. Intraplantar injection of 300 μg of CFA resulted in the development of pain in the paw associated with weight-bearing differences (WBD) between the inflamed and noninflamed hind paws. Two days postinjection, substantial WBD were observed between inflamed and noninflamed hind paws (72.5 ± 9.0 g). Following oral administration, CJ-023,423 (10, 19, 29, and 57 mg/kg p.o.) significantly decreased WBD at a dose of 19 mg/kg, and it was effective for up to 4 h. The maximal efficacy of CJ-023,423 (57 mg/kg p.o.) was approximately 70% reversal, and it was comparable with that of piroxicam (3 mg/kg p.o.), reaching a peak pharmacodynamic effect 1 to 2 h after dosing and returning to the baseline by 6 h after drug administration (Fig. 8).

**Discussion**

We have discovered that CJ-023,423 is a potent, competitive antagonist of human and rat prostanoid EP4 receptors with similar potency (pA2 = 8.3 ± 0.03 and 8.2 ± 0.2 nM, respectively), and it is at least 200 times more selective over other human prostanoid receptors, that is, EP1, EP2, EP3, DP, FP, IP, and TP receptors. Moreover, in vivo pharmacological antagonism of EP4 receptor with CJ-023,423 produces antihyperalgesic effects in rat models of inflammatory pain, suggesting that inflammatory pain can be treated by targeting a single PG receptor subtype EP4.

PGE2 has long been recognized as a key mediator of peripheral inflammatory pain. Treatment with a monoclonal anti-PGE2 antibody reverses carrageenan-induced hyperalgesia to the same extent as NSAIDs, indicating that PGE2 specifically plays a key role in inflammatory pain (Zhang et al., 1997). However, it has been challenging to get a clear understanding on the specific contribution each of the different EP receptor subtypes has on inflammatory pain. A genetic approach relying primarily on studying knockout mice has not yet provided conclusive evidence, and in some cases, contradictory findings regarding which EP subtype mediates the pronociceptive effects of PGE2. For example, abdominal writhing in response to an intraperitoneal proinflammatory
irritant is significantly attenuated in knockout mice for IP and EP3, but not for the EP1, EP2, or EP4 receptors (Ueno et al., 2001); however, another study found the importance of EP1 in nociception using a similar model (Stock et al., 2001).

The relevance of this acute visceral irritant model to somatic inflammatory pain is uncertain. More controversial is the contribution of the different EP receptor subtypes to spinal PGE2-evoked pain response. Whereas intrathecal PGE2 induces allodynia in EP3-knockout mice, but not in EP4-knockout mice, low doses of PGE2-induced hyperalgesia are mediated by the EP2 subtype (Minami et al., 2001). EP2-knockout mice also show a lack of intrathecal PGE2-evoked hyperalgesia (Reinold et al., 2005).

Few antagonists for the EP receptors are available; however, some selective EP1 receptor antagonists exist and the EP1 receptor subtype has been considered as a potential target for analgesia. Peripheral administration of the EP1-selective antagonist ONO-8711 effectively inhibits the mechanical hyperalgesia induced by incision (Omote et al., 2001). Moreover, intrathecal ONO-8711 inhibits the hyperalgesia induced by carrageenan (Nakao et al., 2002). To date, at least one EP1 receptor antagonist, ZD-6416, has entered clinical evaluation for visceral pain (Sarkar et al., 2001). However, its clinical development has been discontinued.

The antagonists for EP4 receptors used for pharmacological inactivation of EP4 subtype are not selective, and many of these compounds act at multiple prostanoid receptor subtypes. For example, the commonly used EP4 antagonist AH23848 (Coleman et al., 1994a; Boie et al., 1997) has a very weak affinity to EP4 receptors with a Ki value of 8010 nM, and it actually has the highest affinity for TP receptors. EP4α (Machwate et al., 2001) and the recently discovered GW6273678X (Wilson et al., 2006) are claimed to be potent and selective competitive antagonists of human EP4 receptors. However, EP4α possesses significant TP and EP3 receptor binding affinity, and GW6273678X, one of the most potent and selective human EP4 receptor antagonists reported so far, also has equal binding affinities for human EP3 and TP receptors.

CJ-023,423 is a highly potent human and rat EP4 subtype antagonist, although it has very weak affinity to the human DP receptor subtype (Ki = 2926 nM). Affinities for all other human prostanoid receptors are not substantial (Ki > 5000 nM). To our knowledge, CJ-023,423 is the most selective human EP4 receptor antagonist.

In an in vivo model of acute pain, PGE2-induced thermal hyperalgesia, CJ-023,423 demonstrates that the EP4 receptor has a predominant role in mediating PGE2-evoked nociceptive responses. This peripheral PGE2-induced thermal hyperalgesia is reduced in EP1-deficient mice, although its effect is not very robust (Moriyama et al., 2005). In the absence of receptor-specific antagonists, the cellular mechanisms that mediate the PGE2-evoked hyperalgesia are still unclear. In mouse DRG neurons, PGE2 increases transient receptor potential vanilloid 1 activity via EP1 and EP4 receptors through protein kinase C- and protein kinase A-dependent pathways, respectively (Moriyama et al., 2005). Rat DRG culture studies with antisense oligonucleotides for prostanoid EP receptor subtypes demonstrate that PGE2-induced sensitization of sensory neurons is dependent on the EP3 and EP2 receptors (Southall and Vasko, 2001). However, the recent study by use of potent and selective EP agonists (Suzawa et al., 2000; Wise, 2006) suggests that PGE2 acts via EP2 receptors to increase cAMP production. Our present results are consistent with these reports, and they confirm that PGE2 acts via EP2 receptors to increase adenyl cyclase activity in rat DRG cells. Although it may be

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**Fig. 7.** Analgesic effect of CJ-023,423 on carrageenan-induced mechanical hyperalgesia in rats. All drugs were administered orally 5.5 h after carrageenan injection. PWT was recorded 1, 2, and 2.5 h after dosing. **p < 0.01 versus vehicle control by one-way ANOVA followed by Bonferroni test; ##, p < 0.001 versus vehicle control by Student’s unpaired t-test. Data represent the mean ± S.E.M. (n = 6–7/group).

**Fig. 8.** Analgesic effect of CJ-023,423 on CFA-induced chronic inflammatory pain in rats. On day 2 after CFA injection, hind paw weight distribution was measured before and after drug administration using a Linton Incapacitance tester. All drugs were administered orally. **p < 0.05,** **p < 0.01 versus vehicle control by one-way ANOVA followed by Dunnett’s test. ##, p < 0.01 versus vehicle control by Student’s unpaired t-test. Data represents the mean ± S.E.M. (n = 9–10/group).
possible that other EP subtypes contribute to PGE₂-evoked pain signaling, our present in vitro and in vivo results strongly suggest that the EP₄ receptor subtype mediates PGE₂-induced hypersensitivity in rats.

Here, CJ-023,423 has been further characterized in both acute and chronic models of inflammatory pain. Carrageenan-induced hyperalgesia, a well characterized acute inflammatory pain in rodents, is attenuated by CJ-023,423, an effect that is similar to that seen with a high dose level of the nonselective COX inhibitor piroxicam. In an in vivo model of chronic pain, CFA-induced weight-bearing deficit, CJ-023,423 after CFA injection suppresses the pain response. This compound shows a comparable efficacy to an NSAID (piroxicam) in the hyperalgesic response at the highest dose level tested. However, it should be noted that CJ-023,423 is inferior to piroxicam in terms of in vivo potency and duration of action. In contrast to CJ-023,423, which has a moderate plasma half-life (2.0 h) and low oral bioavailability, piroxicam has superior pharmacokinetic profile with a longer plasma half-life (7.0 h) and much higher oral bioavailability (100%) (Kimura et al., 1997). EP₄ receptors are expressed by primary sensory neurons, and EP₄ levels increase in the DRG after peripheral inflammation (Oida et al., 1995; Kopp et al., 2004; Lin et al., 2006). Reverse transcription-polymerase chain reaction analysis of the inflamed L5 DRGs by CFA injection shows an increase in only EP₄ mRNA, but not in other EP receptors (EP₁, EP₂, and EP₃) (Lin et al., 2006). Thus, EP₄ may play a more substantial role in peripheral chronic inflammatory pain because of its increased levels after peripheral inflammation. The genetic inactivation of EP₄ using intrathecally delivered short hairpin RNA has demonstrated that the EP₄ receptor mediates inflammatory pain hypersensitivity in rats (Lin et al., 2006). EP₄ knockdown attenuates CFA-induced thermal and mechanical hypersensitivity. Our present results, using a highly selective EP₄ antagonist, are consistent with this recent finding.

In conclusion, EP₄ antagonists can effectively attenuate inflammatory pain hypersensitivity in preclinical models of hyperalgesia. EP₄ receptor-specific antagonists may, therefore, be useful drugs for the treatment of the signs and symptoms of osteoarthritis and inflammatory pain of various etiologies. EP₄ antagonists as new analgesics may have an advantage over other approaches that target prostaglandins such as NSAIDs. COX-2 inhibitors, or prostaglandin synthase inhibitors. This will be determined in part by the specific role of EP₄ in other PGE₂-mediated functions, such as renal homeostasis, endothelial and platelet function, control of blood pressure, and gastrointestinal mucosal function, which remain to be systematically investigated. The overall safety profile of selective EP₄ antagonists should, nevertheless, be different from COX-2 inhibitors and NSAIDs that block PG synthesis of all of the COX products. Targets downstream of COX inhibition, such as selective EP₄ antagonism, may therefore provide an opportunity for the development of more specific and better tolerated analgesics beyond COX inhibition.

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