ELSEVIER

European Journal of Pharmacology 344 (1998) 261–267

ejp

Effects of JTE-522, a specific inhibitor of cyclooxygenase-2, on the recurrence of allergic inflammation in rats

Hisae Niki [a], Masateru Yamada [a], Kouya Yamaki [a], Suetsugu Mue [b], Kazuo Ohuchi [a,*]

[a] *Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba Aramaki, Aoba-ku, Sendai, Migagi 980-8578, Japan*

[b] *Department of Health and Welfare Science, Faculty of Physical Education, Sendai College, Funaoka, Miyagi 989-1693, Japan*

Received 18 November 1997; revised 8 December 1997; accepted 12 December 1997

Abstract

JTE-522, 4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide, is a selective inhibitor of cyclooxygenase-2 at the enzyme level (IC_{50} is 6.4×10^{-7} M for sheep cyclooxygenase-2, but it does not inhibit sheep cyclooxygenase-1 at concentrations up to 10^{-4} M). In rat peritoneal macrophages in culture, it markedly inhibited cyclooxygenase-2-dependent prostaglandin E_2 production and weakly inhibited cyclooxygenase-1-dependent prostaglandin E_2 production, as did the selective cyclooxygenase-2 inhibitor NS-398 ([*N*-2(cyclohexyloxy-4-nitrophenyl)]-methanesulfonamide). In addition, the anti-inflammatory activity of JTE-522 was evaluated, using a model of recurrent air pouch-type allergic inflammation in rats. JTE-522, injected into the pouch just after a second antigen challenge, suppressed the accumulation of pouch fluid, the infiltration of leukocytes and the prostaglandin E_2 content in the pouch fluid, as did NS-398 and indomethacin. These findings indicated that JTE-522 is a selective cyclooxygenase-2 inhibitor in cell culture systems and that the suppression by JTE-522 of the recurrence of allergic inflammation is due to the inhibition of cyclooxygenase-2. © 1998 Elsevier Science B.V.

Keywords: Allergic inflammation, recurrence of; JTE-522; NS-398; Indomethacin; Cyclooxygenase-1; Cyclooxygenase-2; Prostaglandin E_2

1. Introduction

It is reported that increases in arachidonic acid metabolism at inflammatory sites are due to the induction of cyclooxygenase-2 protein expression in inflammatory cells by several kinds of extracellular stimuli (Lee et al., 1992; O'Sullivan et al., 1992; Sano et al., 1992; Evett et al., 1993; Dubois et al., 1994; Mitchell et al., 1994; Seibert et al., 1994; Appleton et al., 1995; Niki et al., 1997). In contrast, cyclooxygenase-1 is constitutively expressed in almost all types of cell (Funk et al., 1991; Simmons et al., 1991; O'Neil et al., 1994; Smith et al., 1994) and is probably involved in cellular housekeeping such as aggregation of platelets and gastric mucosal cytoprotection (DeWitt and Smith, 1988, 1990; Merlie et al., 1988). It is accepted that the side effects observed in organs such as the stomach (Carson et al., 1987; Brooks and Day, 1991) and kidneys (Clive and Stoff, 1984; Black, 1986) induced by classical cyclooxygenase inhibitors (non-selective inhibitors of cyclooxygenase-1 and -2) are due to the inhibition of cyclooxygenase-1, a housekeeping gene product. Therefore, much effort has been made to develop selective inhibitors of cyclooxygenase-2, such as NS-398 (Futaki et al., 1994), nimesulide (Barnett et al., 1994; Taniguchi et al., 1995), DuP 697 (Copeland et al., 1994), SC-58125 (Seibert et al., 1994) and meloxicam (Engelhardt et al., 1996).

Recently, JTE-522 (4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide), the chemical structure of which is shown in Fig. 1, has been synthesized as a novel selective inhibitor of cyclooxygenase-2. It inhibits sheep cyclooxygenase-2 with an IC_{50} of 6.4×10^{-7} M and human recombinant cyclooxygenase-2 with an IC_{50} of 8.5×10^{-8} M, but does not inhibit sheep cyclooxygenase-1 or human platelet cyclooxygenase-1 at concentrations up to 10^{-4} M (Matsushita et al., 1997a,b). The first aim of the present study is to clarify whether JTE-522 causes selective inhibition of cyclooxygenase-2 in cell

* Corresponding author. Tel.: +81-22-2176860; fax: +81-22-2176859; e-mail: ohuchi-k@mail.pharm.tohoku.ac.jp

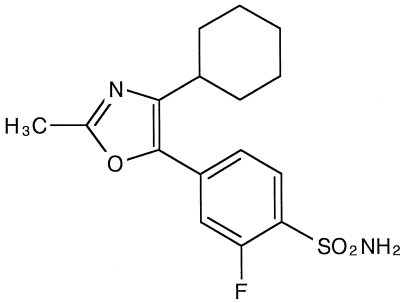

Fig. 1. Chemical structure of JTE-522 (4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide).

culture systems. We recently have developed two cell culture systems in which prostaglandin E_2 production is dependent upon cyclooxygenase-1 and cyclooxygenase-2, respectively (Yamada et al., 1997). The second aim is to determine its anti-inflammatory activity using a model of recurrent allergic inflammation in rats in which cyclooxygenase-2 plays an important role in the pathogenesis of the recurrence (Niki et al., 1997).

2. Materials and methods

2.1. Preparation of rat peritoneal macrophages

A solution of soluble starch (Wako Pure Chemical Ind., Osaka, Japan) and bacto peptone (Difco Laboratories, Detroit, MI), 5% (w/v) each, that had been autoclaved at 120°C for 15 min was injected intraperitoneally into specific pathogen-free male Sprague–Dawley rats (300–350 g) (Charles River Japan, Kanagawa, Japan), at a dose of 5 ml per 100 g body weight. 4 days later, the rats were killed by cutting the carotid artery under diethylether anesthesia and the peritoneal cells were harvested (Ohuchi et al., 1985b). The rats were treated in accordance with procedures approved by the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Tohoku University, Japan.

2.2. Macrophage cultures for measurement of cyclooxygenase-1-dependent prostaglandin E_2 production

Macrophages were cultured to measure cyclooxygenase-1-dependent prostaglandin E_2 production as described recently (Yamada et al., 1997). The peritoneal cells were suspended in Eagle's minimal essential medium (Nissui, Tokyo, Japan) containing 10% (v/v) calf serum (Flow Laboratories, North Rydge, N.S.W., Australia), penicillin G potassium (Meiji Seika Co., Tokyo, Japan) (18 μg/ml), streptomycin sulfate (Meiji Seika Co.) (50 μg/ml) and cycloheximide (Sigma Chemical Co., St. Louis, MO) (1 μM) at a density of 1.5×10^6 cells per ml of the medium. 1 ml of the cell suspension was poured into each well of a 12-well plastic tissue culture plate (Coster Co., Cambridge, MA) and the plates were incubated for 2 h at 37°C. The cells were then washed three times with medium to remove non-adherent cells (Ohuchi et al., 1985b). The adherent cells were incubated for 4 h at 37°C in 1 ml of medium containing cycloheximide (1 μM). After three washes, the cells were further incubated for 4 h at 37°C in 1 ml of medium containing arachidonic acid (Sigma Chemical Co.) (10 μM), cycloheximide (1 μM) and the indicated concentrations of drugs. After incubation, the conditioned medium was collected to determine the prostaglandin E_2 concentration.

2.3. Macrophage cultures for measurement of cyclooxygenase-2-dependent prostaglandin E_2 production

Macrophages were cultured to measure cyclooxygenase-2-dependent prostaglandin E_2 production as described recently (Yamada et al., 1997). The peritoneal cells were suspended in Eagle's minimal essential medium containing 10% (v/v) calf serum, penicillin G potassium (18 μg/ml) and streptomycin sulfate (50 μg/ml), at a density of 1.5×10^6 cells per ml. 1 ml of the cell suspension was poured into each well of a 12-well plastic tissue culture plate (Coster Co.) and the plates were incubated for 2 h at 37°C. The wells were then washed three times with medium to remove non-adherent cells (Ohuchi et al., 1985b). The adherent cells were incubated for 4 h at 37°C in 1 ml of medium containing aspirin (Sigma Chemical Co.) (100 μM). After three washes to remove free aspirin, the cells were further incubated for 4 h at 37°C in 1 ml of medium containing 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (Sigma Chemical Co.) (16.2 nM) and the indicated concentrations of drugs in the absence of arachidonic acid. After incubation, the conditioned medium was collected to determine the prostaglandin E_2 concentration.

2.4. Drug treatment of macrophages in culture

The drugs used for preincubation of the cells were cycloheximide and aspirin (Sigma Chemical Co.). They were dissolved in ethanol and added to the medium. To examine the effects on prostaglandin E_2 production, JTE-522 (Japan Tobacco, Osaka, Japan), NS-398 ([*N*-2(cyclohexyloxy-4-nitrophenyl)]-methanesulfonamide, Funakoshi Co., Tokyo, Japan) and indomethacin (Sigma Chemical Co.) were used. They were dissolved in ethanol and added to the medium. The final concentration of ethanol was adjusted to 0.1% (v/v). The control medium contained the same amount of the vehicle.

2.5. Measurement of prostaglandin E_2 concentrations

The conditioned medium was centrifuged at 1500 *g* and 4°C for 5 min and the prostaglandin E_2 concentration in

the supernatant fraction was radioimmunoassayed (Ohuchi et al., 1985b). Prostaglandin E_2 antiserum was purchased from PerSeptive Diagnostics, Cambridge, MA, USA.

2.6. Immunization and induction of allergic inflammation

Male rats of the Sprague–Dawley strain, 39 to 41 days old and weighing 150 to 160 g (Charles River Japan), were immunized with the antigen, azobenzenearsonate-conjugated acetyl bovine serum albumin with the aid of Freund's complete adjuvant (Tsurufuji et al., 1982). Briefly, 5 mg of the antigen dissolved in 250 μl of saline was emulsified with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, MI) and 100-μl aliquots of the emulsion were injected intradermally at 2 nuchal and 3 lumbar sites of each rat. 9 days after immunization, 10 ml of air was injected subcutaneously in the dorsum of rats under light diethylether anesthesia, to make an ellipsoid shaped air pouch. 24 h after the injection of air, 2 mg of the antigen dissolved in 4 ml of a sterile 2% (w/v) solution of sodium carboxymethylcellulose (Cellogen F3H, Dai-ichi Kogyo Seiyaku, Niigata, Japan) in 0.9% (w/v) NaCl solution supplemented with antibiotics (0.1 mg penicillin G potassium and 0.1 mg dihydrostreptomycin sulfate per 1 ml of the solution) was injected into the air pouch as the first antigen challenge (Tsurufuji et al., 1982). The animal experiments were done in accordance with procedures approved by the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Tohoku University, Japan.

2.7. Induction of recurrence of allergic inflammation

As a second antigen challenge to induce recurrence of allergic inflammation, 3 mg of the antigen dissolved in 0.5 ml of a sterile 0.9% NaCl solution was injected into a granulation capsule holding about 8 ml of exudate that had formed over a period of 5 days after the first antigen challenge (Watanabe et al., 1987). 24 h after the second antigen challenge, rats were killed by cutting the carotid artery under diethylether anesthesia and the pouch fluid was collected. Pouch fluid volume, the number of leukocytes in the pouch fluid and the amount of prostaglandin E_2 in the pouch fluid were determined as described below.

2.8. Drug treatment in *vivo*

Drugs used were JTE-522 (Japan Tobacco), NS-398 (Funakoshi Co.) and indomethacin (Sigma Chemical Co.). Each was dissolved in ethanol and appropriately diluted with sterile saline solution and 0.5 ml of the drug solution was injected into the pouch just after the second antigen challenge. The final concentration of ethanol in the antigen solution was adjusted to 2%. The control rats received the same amount of sterile saline solution containing 2% ethanol.

2.9. Determination of total number of leukocytes in the pouch fluid

The pouch fluid was centrifuged at $1000 \times g$ and 4°C for 5 min to precipitate the cells. The cells were suspended in phosphate-buffered saline (PBS, pH 7.4) and the total number of cells was determined by using a hemocytometer. In some cases, differential counting of the infiltrating leukocytes was performed after May-Giemsa staining.

2.10. Determination of prostaglandin E_2 concentrations in the pouch fluid

1 ml of the supernatant fraction of the pouch fluid, to which 5×10^{-4} μCi of [^{3}H]prostaglandin E_2 (160 Ci/mmol, [5,6,8,11,12,14,15-^{3}H(N)] prostaglandin E_2, NEN/DuPont, Wilmington, DE) had been added, was applied to a SEP-PAK C_{18} cartridge (Waters Associates, Milford, MA). The lipophilic fraction containing prostaglandin E_2 was eluted with methanol, evaporated and dissolved in an aliquot of 0.01 M Tris–HCl buffer (pH 7.4) containing 0.1% (w/v) gelatin, 0.15 M NaCl and 10 mg/ml sodium azide. A portion of the solution was used for the radioimmunoassay of prostaglandin E_2 (Watanabe et al., 1987; Niki et al., 1997). Another portion was used for the measurement of radioactivity for the calculation of recovery over the entire procedure (Watanabe et al., 1987; Niki et al., 1997). Prostaglandin E_2 antiserum was purchased from PerSeptive Diagnostics, Cambridge, MA, USA.

2.11. Statistical analysis

The results were analyzed for statistical significance by Dunnett's test for multiple comparisons and Student's *t*-test for unpaired observations.

3. Results

3.1. Effects of JTE-552 on cyclooxygenase-1-dependent prostaglandin E_2 production

In the presence of the protein synthesis inhibitor cycloheximide, so that cyclooxygenase-2 protein was not induced during the incubation period, the addition of arachidonic acid significantly increased prostaglandin E_2 production (Fig. 2). Under this condition, prostaglandin E_2 production stimulated by the addition of arachidonic acid is regarded to be mainly due to cyclooxygenase-1 (Yamada et al., 1997). In fact, the inhibition of prostaglandin E_2 production by NS-398, a selective cyclooxygenase-2 inhibitor, was more than 10-fold weaker than that elicited by

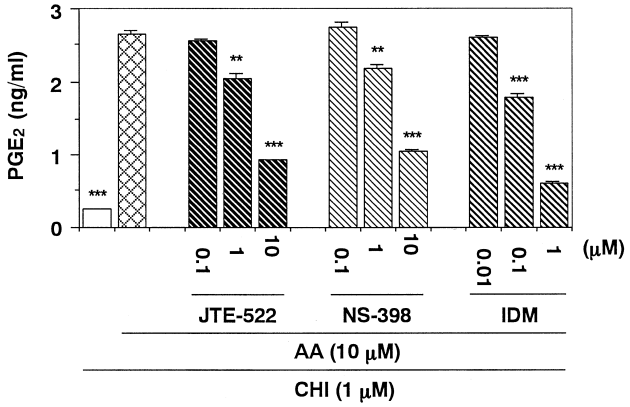

Fig. 2. Effects of JTE-522 on cyclooxygenase-1-dependent prostaglandin E_2 production. Rat peritoneal macrophages (1.5×10^6 cells) were preincubated at 37°C for 2 h in 1 ml of medium. After three washes, the adherent cells were further incubated at 37°C for 4 h in 1 ml of medium containing cycloheximide (CHI, 1 μM). The cells were then washed three times and further incubated at 37°C for 4 h in 1 ml of medium containing arachidonic acid (AA, 10 μM), CHI (1 μM) and the indicated concentrations of JTE-522, NS-398 or indomethacin (IDM). Prostaglandin E_2 (PGE_2) concentrations in the conditioned medium are shown. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: $^{**}P < 0.01$, $^{***}P < 0.001$ versus AA control.

indomethacin, a non-selective cyclooxygenase-1/cyclooxygenase-2 inhibitor (Fig. 2). JTE-522 also weakly inhibited prostaglandin E_2 production (Fig. 2). IC_{50} values of JTE-522, NS-398 and indomethacin for cyclooxygenase-1-dependent prostaglandin E_2 production were 3.3, 4.5 and 0.19 μM, respectively.

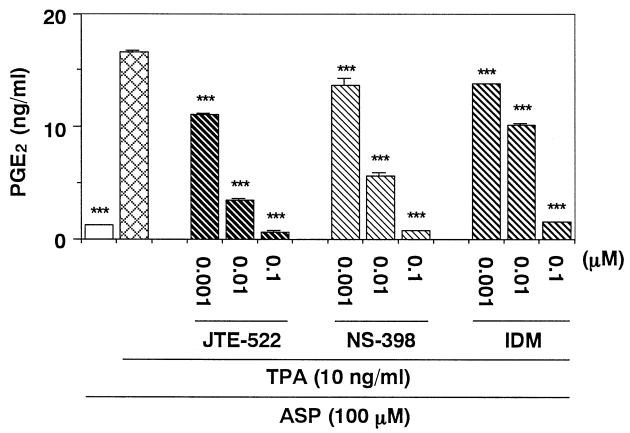

Fig. 3. Effects of JTE-522 on cyclooxygenase-2-dependent prostaglandin E_2 production. Rat peritoneal macrophages (1.5×10^6 cells) were preincubated at 37°C for 2 h in 1 ml of medium. After three washes, the adherent cells were further incubated at 37°C for 4 h in 1 ml of medium containing aspirin (ASP, 100 μM). The cells were then washed three times and further incubated at 37°C for 4 h in 1 ml of medium containing TPA (16.2 nM, 10 ng/ml) and the indicated concentrations of JTE-522, NS-398 or indomethacin (IDM). Prostaglandin E_2 (PGE_2) concentrations in the conditioned medium are shown. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: $^{***}P < 0.001$ versus TPA control.

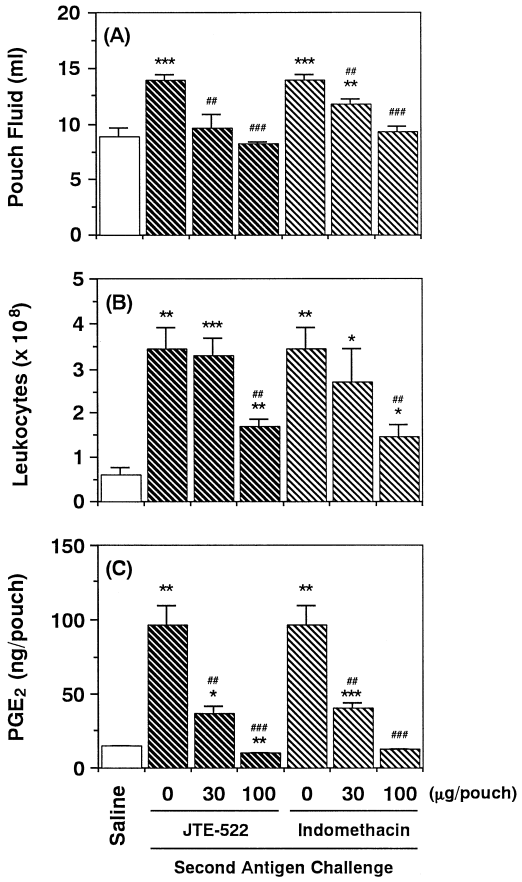

Fig. 4. Effects of JTE-522 on the recurrence of allergic inflammation. Five days after the first antigen challenge, 3 mg of the antigen dissolved in 0.5 ml of sterile saline solution was injected into the pouch. Immediately after the second antigen challenge, JTE-522 or indomethacin dissolved in 0.5 ml of sterile saline solution was injected into the pouch. 24 h later, the pouch fluid was collected and pouch fluid volume (A), total number of leukocytes (B) and prostaglandin E_2 (PGE_2) content in the pouch fluid (C) were determined. Values are the means from 6 rats with S.E.M. shown by vertical bars. Statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the group without second antigen challenge. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus corresponding control.

3.2. Effects of JTE-552 on cyclooxygenase-2-dependent prostaglandin E_2 production

Prostaglandin E_2 production stimulated by 12-*O*-tetradecanoylphorbol 13-acetate in macrophages in which preexisting cyclooxygenase-1 and -2 has been inactivated by treatment with aspirin is regarded to be due to newly synthesized cyclooxygenase-2 (Yamada et al., 1997). Under this condition, all three drugs over the same concentration range inhibited prostaglandin E_2 production in a concentration-dependent manner (Fig. 3). IC_{50} values of JTE-522, NS-398 and indomethacin for cyclooxygenase-2-dependent prostaglandin E_2 production were 0.0022, 0.0048 and 0.020 μM, respectively. The ratio of the IC_{50} for cyclooxygenase-1 to the IC_{50} for cyclooxygenase-2 was about 1500 (JTE-522), 940 (NS-398) and 10 (indo-

methacin), respectively. These findings indicated that JTE-522 is a more selective inhibitor of cyclooxygenase-2 than NS-398 in cell culture systems.

3.3. Effects of JTE-552 on the recurrence of allergic inflammation

The second antigen challenge of the immunized rats induced the recurrence of allergic inflammation. Pouch fluid volume, number of leukocytes in the pouch fluid and prostaglandin E_2 content in the pouch fluid at 24 h were significantly increased by the second antigen challenge (Figs. 4 and 5). In this model of recurrent allergic inflammation, the effect of intrapouch injection of JTE-522 (Fig. 4) or NS-398 (Fig. 5) on the recurrence of allergic inflammation was compared with that of indomethacin. Accumulation of pouch fluid by the second antigen challenge was suppressed dose dependently by JTE-522, NS-398 and indomethacin. At a dose of 100 μg per pouch, pouch fluid accumulation was almost completely inhibited by JTE-522, NS-398 or indomethacin. Significant inhibition was also observed at a dose of 30 μg per pouch (Fig. 4A and Fig. 5A). In contrast, significant inhibition of leukocyte infiltration was observed only at a dose of 100 μg per pouch (Fig. 4B and Fig. 5B). Histological investigation revealed that more than 93% of the infiltrating leukocytes were neutrophils, 6% were monocytes and the rest were eosinophils. Treatment with JTE-522, NS-398 or indomethacin did not change the population of leukocytes (data not shown). The prostaglandin E_2 content in the pouch fluid was also decreased dose dependently by JT-522, NS-398 and indomethacin. At a dose of 100 μg per pouch, the prostaglandin E_2 content in the pouch fluid was decreased to the level of the group receiving no antigen (Fig. 4C and Fig. 5C). Significant suppression was also observed at a dose of 30 μg per pouch (Fig. 4C and Fig. 5C). Comparison of the anti-inflammatory effects of JTE-522 with those of NS-398 showed that JTE-522 is as effective as NS-398 (Figs. 4 and 5).

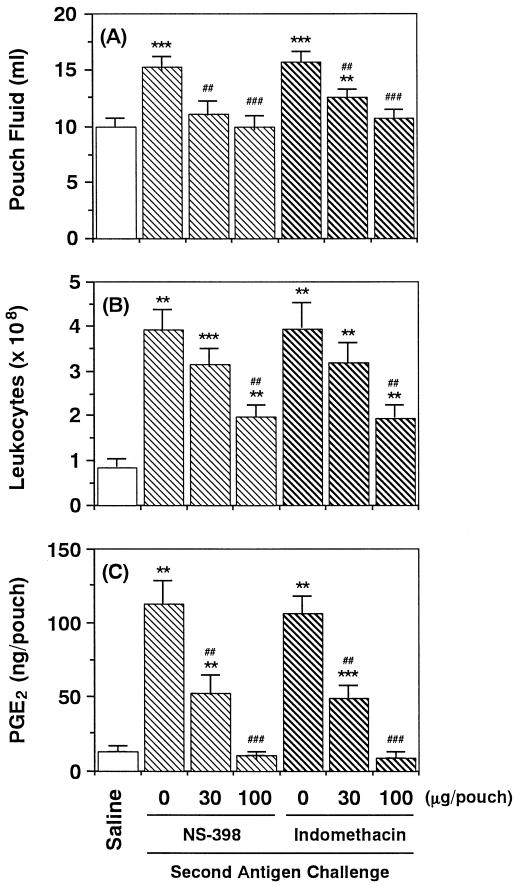

Fig. 5. Effects of NS-398 on the recurrence of allergic inflammation. Five days after the first antigen challenge, 3 mg of the antigen dissolved in 0.5 ml of sterile saline solution was injected into the pouch. Immediately after the second antigen challenge, NS-398 or indomethacin dissolved in 0.5 ml of sterile saline solution was injected into the pouch. 24 h later, the pouch fluid was collected and pouch fluid volume (A), total number of leukocytes (B) and prostaglandin E_2 (PGE_2) content in the pouch fluid (C) were determined. Values are the means from 6 rats with S.E.M. shown by vertical bars. Statistical significance: $^{**}P < 0.01$, $^{***}P < 0.001$ versus the group without second antigen challenge. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus corresponding control.

4. Discussion

It has been reported that JTE-522 is a selective inhibitor of cyclooxygenase-2 at the enzyme level. It inhibits sheep cyclooxygenase-2 with an IC_{50} of 6.4×10^{-7} M and human recombinant cyclooxygenase-2 with an IC_{50} of 8.5×10^{-8}, but does not inhibit sheep cyclooxygenase-1 and human platelet cyclooxygenase-1 at concentrations up to 10^{-4} M (Matsushita et al., 1997a). In addition, the ratio of the IC_{50} value for sheep cyclooxygenase-1 to that for cyclooxygenase-2 of JTE-522 is more than 156, while that of NS-398, ibuprofen and indomethacin is more than 55, 0.63 and 0.023, respectively (Matsushita et al., 1997b). The first aim of the present study was to clarify whether JTE-522 selectively inhibits cyclooxygenase-2 in cell culture systems, because penetration of the cell membrane is an important event in the expression of such activity in vivo. Recently, we developed two different cell culture systems in which prostaglandin E_2 is produced dependent upon cyclooxygenase-1 or upon cyclooxygenase-2, respectively (Yamada et al., 1997). Cyclooxygenase-1-dependent prostaglandin E_2 production is induced by the addition of arachidonic acid to the medium of rat peritoneal macrophages that have been pretreated with cycloheximide, a protein synthesis inhibitor, to suppress the induction of cyclooxygenase-2 (Yamada et al., 1997). Under such culture conditions, prostaglandin E_2 production was only slightly inhibited by NS-398, a selective inhibitor of cyclooxygenase-2, but was strongly inhibited by indomethacin, a non-selective inhibitor of cyclooxygenase-1/cyclooxygenase-2 (Yamada et al., 1997; Fig. 2). West-

ern blot analysis demonstrated that the level of cyclooxygenase-2 protein in the cells is low and is not changed by the addition of arachidonic acid; cyclooxygenase-1 protein is more clearly detectable than cyclooxygenase-2 protein (Yamada et al., 1997). Therefore, prostaglandin E_2 production under these culture conditions is suggested to be dependent upon cyclooxygenase-1 (Yamada et al., 1997). Under these conditions, JTE-522, as well as the selective cyclooxygenase-2 inhibitor NS-398, showed less than one tenth the inhibitory activity of the non-selective cyclooxygenase-1/cyclooxygenase-2 inhibitor indomethacin (Fig. 2).

Cyclooxygenase-2-dependent prostaglandin E_2 production is induced by stimulation with 12-*O*-tetradecanoylphorbol 13-acetate of rat peritoneal macrophages that have been pretreated with aspirin to inactivate pre-existing cyclooxygenase (Rome et al., 1976; Roth et al., 1983). Under these conditions, the protein level of cyclooxygenase-2 in the cells is increased and prostaglandin E_2 production is inhibited by the selective inhibitors of cyclooxygenase-2, NS-398 and nimesulide (Yamada et al., 1997). With this cell culture system, the IC_{50} value of JTE-522 for cyclooxygenase-2 was calculated to be 0.0022 μM, while that of NS-398 was 0.0048 μM (Fig. 3). Therefore, JTE-522 is a more selective inhibitor of cyclooxygenase-2 than NS-398.

The second aim of the present study was to establish whether JTE-522 exerts anti-inflammatory activity in a model of recurrent allergic inflammation in rats (Watanabe et al., 1987). In the air pouch-type allergic inflammation model, the first challenge with antigen injected into the air pouch made on the dorsum of immunized rats induces a prominent local anaphylactic increase in vascular permeability, followed by delayed-type hypersensitivity reactions (Ohuchi et al., 1985a; Hirasawa et al., 1986). 5 days after the first antigen challenge, vascular permeability, the number of leukocytes infiltrated into the pouch fluid and the prostaglandin E_2 content in the pouch fluid decreased; the granulation tissue pouch holds about 8 ml of fluid (Watanabe et al., 1987). However, the second antigen challenge at this stage induces recurrence of allergic inflammation; the pouch fluid volume, the number of leukocytes in the pouch fluid and the content of prostaglandin E_2 in the pouch fluid increased time dependently (Watanabe et al., 1987). Recently, we reported that the recurrence of allergic inflammation in rats is suppressed by the selective cyclooxygenase-2 inhibitor, NS-398, as well as by the non-selective cyclooxygenase-1/cyclooxygenase-2 inhibitor, indomethacin (Niki et al., 1997). Western blot analysis demonstrated that cyclooxygenase-2 protein is not detected in the granulation tissue before the second antigen challenge, but after the second antigen challenge, the amount of cyclooxygenase-2 protein in the granulation tissue increases with time up to 6 h and was constant up to 48 h (Niki et al., 1997). In contrast, no prominent changes in the amount of cyclooxygenase-1 protein are observed after the second antigen challenge (Niki et al., 1997). Therefore, this model might be suitable for the evaluation of selective cyclooxygenase-2 inhibitors in vivo. The present study demonstrated that JTE-522 shows potent anti-inflammatory activity similar to that of indomethacin (Fig. 4), as does NS-398 (Fig. 5). Therefore, it is strongly suggested that JTE-522 exerts its anti-inflammatory activity by inhibiting cyclooxygenase-2 induced in the inflammatory locus by the second antigen challenge. Recently, the anti-inflammatory activity of orally administered JTE-522 was also observed in carrageenin-induced rat paw edema and adjuvant-induced arthritis in rats (Matsushita et al., 1997a,b).

As to side effects, it is reported that JTE-522 has little ulcerogenic effect at doses up to 300 mg/kg, while indomethacin has a strong and dose-dependent ulcerogenic effect at 1 to 10 mg/kg in fasted rats (Matsushita et al., 1997a,b). Taken together, JTE-522 might prove a useful non-steroidal anti-inflammatory drug with fewer side effects than conventional non-selective inhibitors of cyclooxygenase-1 and cyclooxygenase-2.

In conclusion, JTE-522 proved to be a specific inhibitor of cyclooxygenase-2 in rat peritoneal macrophages in culture. In addition, JTE-522 suppressed the recurrence of allergic inflammation in rats as potently as the selective cyclooxygenase-2 inhibitor NS-398 did, suggesting that the anti-allergic inflammatory effect of JTE-522 is due to the inhibition of cyclooxygenase-2 at the inflammatory site.

References

Appleton, I., Tomilson, A., Mitchel, J.A., Willoughby, D.A., 1995. Distribution of cyclooxygenase isoforms in murine chronic granulomatous inflammation. Implication for future anti-inflammatory therapy. J. Pathol. 176, 413–420.

Barnett, J., Chow, J., Ives, D., Chiou, M., Mackenzie, R., Osen, E., Nguyen, B., Tsing, S., Bach, C., Freire, J., Chan, H., Sigal, E., Ramesha, C., 1994. Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. Biochim. Biophys. Acta 1209, 130–139.

Black, H.E., 1986. Renal toxicity of non-steroidal anti-inflammatory drugs. Toxicol. Pathol. 14, 83–90.

Brooks, P.M., Day, R.O., 1991. Nonsteroidal anti-inflammatory drugs: Differences and similarities. N. Engl. J. Med. 324, 1716–1725.

Carson, J.L., Strom, B.L., Soper, K.A., West, S.L., Morse, M.L., 1987. The association of nonsteroidal anti-inflammatory drugs with upper gastrointestinal tract bleeding. Arch. Int. Med. 147, 85–88.

Clive, D.M., Stoff, J.S., 1984. Renal syndromes associated with nonsteroidal antiinflammatory drugs. N. Engl. J. Med. 310, 563–572.

Copeland, R.A., Williams, J.M., Giannaras, J., Nurnberg, S., Covington, M., Pinto, D., Pick, S., Trzaskos, J.M., 1994. Mechanism of selective inhibition of the inducible isoform of prostaglandin G/H synthase. Proc. Natl. Acad. Sci. USA 91, 11202–11206.

DeWitt, D.L., Smith, W.L., 1988. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc. Natl. Acad. Sci. USA 85, 1412–1416.

DeWitt, D.L., Smith, W.L., 1990. Cloning of sheep and mouse prostaglandin endoperoxide synthase. Methods Enzymol. 187, 469–479.

Dubois, R.N., Award, J., Morrow, J., Roberts II, J.L., Bishop, P.R., 1994. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. J. Clin. Invest. 93, 493–498.

Engelhardt, G., Bogel, R., Schnitzer, C., Utzmann, R., 1996. Meloxicam: Influence on arachidonic acid metabolism. Part 1. In vitro findings. Biochem. Pharmacol. 51, 21–28.

Evett, G.E., Xie, W., Chipman, J.G., Robertson, D.L., Simmons, D.L., 1993. Prostaglandin G/H synthase isoenzyme 2 expression in fibroblasts: Regulation by dexamethasone, mitogens and oncogenes. Arch. Biochem. Biophys. 306, 169–177.

Funk, C.D., Funk, L.B., Kennedy, M.E., Pong, A.S., Fitzgerald, G.A., 1991. Human platelet/erythroleukemia cell prostaglandin G/H synthase: cDNA cloning, expression and gene chromosomal assignment. FASEB J. 5, 2304–2312.

Futaki, N., Takahashi, S., Yokoyama, M., Arai, I., Higuchi, S., Otomo, S., 1994. NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro. Prostaglandins 47, 55–59.

Hirasawa, N., Ohuchi, K., Sugio, K., Tsurufuji, S., Watanabe, M., Yoshino, S., 1986. Vascular permeability responses and the role of prostaglandin E_2 in an experimental allergic inflammation of air pouch type in rats. Br. J. Pharmacol. 87, 751–756.

Lee, S.H., Soyoola, E., Chanmugam, P., Hart, S., Sun, W., Zhong, H., Liou, S., Simmons, D., Hwang, D., 1992. Selective expression of a mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. J. Biol. Chem. 267, 25934–25938.

Matsushita, M., Masaki, M., Wakitani, K., Tanaka, T., 1997a. Anti-inflammatory, anti-pyretic and analgesic effects of JTE-522, a novel selective inhibitor of COX-2 in rats. Jpn. J. Pharmacol. 73 (Suppl. I), 145P.

Matsushita, M., Masaki, Y., Yagi, Y., Tanaka, T., Wakitani, K., 1997b. Pharmacological profile of JTE-522, a novel prostaglandin H synthase-2 inhibitor, in rats. Inflamm. Res. In press.

Merlie, J.P., Fagan, D., Mudd, J., Needleman, P., 1988. Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J. Biol. Chem. 263, 3550–3553.

Mitchell, J.A., Belvisi, M.G., Akarasereenont, P., Robbins, R.A., Kwon, O.J., Croxtall, J., Barnes, P.J., Vane, J.R., 1994. Induction of cyclooxygenase-2 by cytokines in human pulmonary epithelial cells: Regulation by dexamethasone. Br. J. Pharmacol. 113, 1008–1014.

Niki, H., Tominaga, Y., Watanabe-Kobayashi, M., Mue, S., Ohuchi, K., 1997. Possible participation of cyclooxygenase-2 in the recurrence of allergic inflammation in rats. Eur. J. Pharmacol. 320, 193–200.

Ohuchi, K., Hirasawa, N., Watanabe, M., Tsurufuji, S., 1985a. Pharmacological analysis of the vascular permeability response in the anaphylactic phase of allergic inflammation in rats. Eur. J. Pharmacol. 117, 337–345.

Ohuchi, K., Watanabe, M., Yoshizawa, K., Tsurufuji, S., Fujiki, H., Suganuma, M., Sugimura, M., Levine, L., 1985b. Stimulation of prostaglandin E_2 production by 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-type and non-TPA-type tumor promoters in macrophages and its inhibition by cycloheximide. Biochim. Biophys. Acta 834, 42–47.

O'Neil, G.P., Mancini, J.A., Kargman, S., Yergey, J., Kwan, M.Y., Falqueyret, J.P., Abromovitz, M., Kennedy, B.P., Ouellet, M., Cromlish, W., Culp, S., Evans, J.F., Ford-Hutchinson, A.W., Vickers, P.J., 1994. Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vaccinia virus: Inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxyeicosatetraenoic acid. Mol. Pharmacol. 45, 245–254.

O'Sullivan, M.G., Chilton, F.H., Huggins, E.M. Jr., McCall, C.E., 1992. Lipopolysaccharide priming of alveolar macrophages for enhanced synthesis of prostanoids involves induction of a novel prostaglandin H synthase. J. Biol. Chem. 267, 14547–14550.

Rome, L.H., Lands, W.E., Roth, G.J., Majerus, P.W., 1976. Aspirin as a quantitative acetylating reagent for the fatty acid oxygenase that forms prostaglandins. Prostaglandins 11, 23–30.

Roth, G.J., Machuga, E.T., Ozols, J., 1983. Isolation and covalent structure of the aspirin-modified, active-site region of prostaglandin synthase. Biochemistry 22, 4672–4675.

Sano, H., Hla, T., Maier, J.A., Crofford, L.J., Case, J.P., Maciag, T., Wilder, R.L., 1992. In vivo cyclooxygenase expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis. J. Clin. Invest. 89, 97–108.

Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Maeferrer, J., Perkins, W., Lee, L., Isakson, P., 1994. Pharmacological and biochemical demonstration of the role Bof cyclooxygenase 2 in inflammation and pain. Proc. Natl. Acad. Sci. USA 91, 12013–12017.

Simmons, D.L., Xie, W., Chipman, J., Evett, G.E., 1991. Multiple cyclooxygenase: Cloning of a mitogen inducible form. In: Bailey, J.M. (Ed.), Prostaglandins, Leukotrienes, Lipoxins and PAF. Plenum Press, New York, pp. 67–78.

Smith, W.L., Meade, E.A., DeWitt, D.L., 1994. Interactions of PGH synthase isozymes-1 and -2 with NDAIDs. Ann. N. Y. Acad. Sci. 744, 50–57.

Taniguchi, Y., Ikesue, A., Yokoyama, K., Noda, K., Debuchi, H., Nakamura, T., Toda, A., Shimeno, H., 1995. Selective inhibition by nimesulide, a novel non-steroidal anti-inflammatory drug, with prostaglandin endoperoxide synthase-2 activity in-vitro. Pharm. Sci. 1, 173–175.

Tsurufuji, S., Yoshino, S., Ohuchi, K., 1982. Induction of an allergic air-pouch inflammation in rats. Int. Arch. Allergy Appl. Immunol. 69, 189–198.

Watanabe, M., Ohuchi, K., Tsurufuji, S., 1987. Recurrence of allergic inflammation or air-pouch type in rats and possible participation of prostaglandin E_2. Int. Arch. Allergy Appl. Immunol. 83, 390–397.

Yamada, M., Niki, H., Yamashitas, M., Mue, S., Ohuchi, K., 1997. Prostaglandin E_2 production dependent upon cyclooxygenase-1 and cyclooxygenase-2 and its contradictory modulation by auranofin in rat peritoneal macrophages. J. Pharmacol. Exp. Ther. 281, 1005–1012.