Abstract. Objective and Design: To investigate the effect of JTE-522, a novel selective prostaglandin H synthase (PGHS)-2 inhibitor, on adjuvant-induced arthritis and bone changes.

Subjects: Male Lewis rats at 8 weeks old were immunized with heat-killed mycobacteria.

Treatment: JTE-522 (0.1–30 mg/kg) and indomethacin (0.1–3 mg/kg) were administered orally once-daily after immunization.

Methods: Paw swelling, bone changes in arthritic paws and vertebrae, urinary levels of deoxypyridinoline and pyridinium crosslinks, and the incidence of gastric lesions were determined in arthritic rats.

Results: JTE-522 (from 0.3 mg/kg) suppressed the development of paw swelling, and also reduced bone damage (score and bone mineral density) in arthritic paws and the urinary excretion of deoxypyridinoline and pyridinium crosslinks. However, JTE-522 did not cause gastric lesions even at 30 mg/kg in arthritic rats.

Conclusions: These results suggest that JTE-522 possesses potent anti-arthritis activities and suppressive activity on inflammatory bone resorption without gastric side effects.

Key words: JTE-522 – Adjuvant arthritis – Prostaglandin H synthase-2 (PGHS-2) – Bone change – Non-steroidal anti-inflammatory drugs (NSAIDs)

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of inflammatory diseases. It is well known that their effects are mainly due to the inhibition of prostaglandin H synthase (PGHS). Two isoforms of PGHS have been identified [1]. The constitutive enzyme, PGHS-1, is believed to be involved in the maintenance of essential physiological functions such as platelet aggregation [2] and gastric mucosal cytoprotection [3]. Another isozyme, PGHS-2, has been shown to be induced in a number of cells by pro-inflammatory cytokines [4] and stimuli [5, 6]. Recent studies have indicated that rheumatoid synovial tissues express PGHS-2 [7, 8] and that the activation of PGHS-2 in rheumatoid and osteoarthritic synovial cells is enhanced by pro-inflammatory cytokines [9, 10]. Current NSAIDs inhibit both PGHS-1 and PGHS-2, and long-term NSAIDs treatment is often limited by gastrointestinal ulcerogenicity that may result from the inhibition of PGHS-1. Therefore, selective PGHS-2 inhibitors would be expected to possess a very attractive drug profile for the treatment of rheumatoid arthritis and osteoarthritis.

We have recently shown that a newly synthesized agent, JTE-522 (4-[4-cyclohexyl-2-methyloxazol-5-yl]-2-fluorobenzensulfonamide), possesses the selective inhibitory activity of PGHS-2, with potent anti-inflammatory, antinociceptive and antipyretic activities, and very little gastrointestinal ulcerogenicity [11]. Adjuvant-induced arthritis in rats has been widely used as an experimental model for human rheumatoid arthritis and the evaluation of the effects of anti-rheumatic drugs. In addition, it has been reported that PGHS-2 is expressed in the joints of rats with adjuvant-induced arthritis as well as in rheumatoid synovial tissues [8]. In the present study, we therefore investigated the effects of JTE-522 on arthritis, bone changes and the incidence of gastric lesions in adjuvant-treated rats.

Materials and methods

All animal experiments were performed according to the guidelines of the International Association for the Study of Pain [12]. In addition, the experimental work was reviewed by the Japan Tobacco Ethical Committee for Animal Experimentation.

Animals

Male Lewis rats aged 7 weeks were obtained from Charles River Japan Laboratories (Hino, Shiga, Japan) and acclimatized for 1 week before the experiment. During the acclimatization and experiment, the animals were housed in a 12 h light/dark cycle (lights on from 7 a.m. to 7 p.m.) at...
23 ± 3°C and 55 ± 15% relative humidity with free access to commercial laboratory food and water.

**Drugs**

JTE-522 was synthesized at the Central Pharmaceutical Research Institute of Japan Tobacco Inc. (Takatsuki, Osaka, Japan). Indomethacin was obtained from Sigma (St. Louis, MO, USA). JTE-522 and indomethacin were suspended in 0.5% carboxymethylcellulose sodium (CMC-Na) solution and administered orally at a volume of 5 ml/kg body weight.

**Induction of adjuvant arthritis and drug administration**

The method as described by Newbould [13] was employed with slight modification. Briefly, arthritis was induced by subplantar injection of 0.1 ml of a 0.5% suspension of heat-killed Mycobacterium tuberculosis H37 RA (Difco Laboratories, Detroit, MI, USA) in paraffin oil into the right hindpaw. Two experiments were conducted in the study. In the first experiment, JTE-522 (0.1–3 mg/kg), indomethacin (0.1 and 0.3 mg/kg) or vehicle (0.5% CMC-Na) for the control were given p.o. once daily from the day of adjuvant injection (day 0) to day 27. In the second experiment, JTE-522 (3–30 mg/kg), indomethacin (0.3–3 mg/kg) or 0.5% CMC-Na were administered from day 0 to day 23.

**Measurement of hindpaw swelling**

Paw volume was measured with a plethysmometer (TK-101, Unicom, Yachiyo, Chiba, Japan).

**Determination of bone mineral density (BMD)**

At the end of the experiment, animals were sacrificed. Their hindlimbs and fourth and fifth lumbar vertebrae were excised for determination of BMD and radiographic assessment of bone changes. Bone minerals were measured using a dual-energy X-ray absorptiometer (DCS-600, Aloka, Tokyo, Japan). The mineralization profile of isolated hindlimb by lateral projection was recorded with the monitoring image. Values for total bone mineral content (BMC: mg), bone area (cm²), and BMD (mg/cm²) were obtained for each specimen. On the monitoring image of hindlimbs, each paw image was divided into 5 parts and data from the first and the second parts from the posterior heel side were represented as data for paw BMD. BMD data from the fourth and fifth lumbar vertebrae were selected to represent as data for lumbar vertebral BMD.

**Radiographic assessment of bone changes**

Radiographs of both hindlimbs were taken using an X-ray unit (CSMW, Softex). The severity of bone damage was assessed blindly from the radiographs by grading the osteoporosis, erosions and joint space narrowing in the distal tibia, tarsus, metatarsus and calcaneus. A grade of 0 to 3 was assigned to each bone area on the basis of the following scoring system: 0: no change; 1: dimming of bone surface and minimal exostosis in a few areas by lysis of the compact substance and periosteal new bone formation and/or mild osteoporosis in some areas with little change of bone area size; 2: dimming of bone surface in all areas, exostosis by new bone formation, joint space narrowing and moderate osteoporosis; 3: physiologic structures no longer detectable due to the irregular proliferation of spongiosa, entire loss of joint spaces, severe osteoporosis.

**Measurement of urinary deoxypyridinoline and pyridinium crosslinks**

On day 23 in the first experiment, urinary samples were collected from 9 a.m. to 4 p.m. Urinary deoxypyridinoline and pyridinium crosslinks (including both deoxypyridinoline and pyridinoline) were measured by ELISA system (Metra Biosystems, Inc. Mountain View, CA, USA). Creatinine level was determined by a modified Jaffe method whereby alkaline picrate solution forms a colored solution in the presence of creatinine [14]. Concentrations of deoxypyridinoline and pyridinium crosslinks were expressed relative to creatinine.

**Assessment of gastric lesions**

After sacrifice of rats in the second experiment, the stomachs were excised and then fixed with 10% formalin. Each stomach was opened by cutting along the greater curvature and all mucosal lesions were determined by visual inspection.

**Statistical analysis**

The statistical significance of parametric data between normal or adjuvant control and drug-treated groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett’s test. The significance of differences in parametric data between normal and adjuvant control groups was determined by Student’s t-test. The statistical evaluation for multiple group comparison of score was performed by the Kruskal-Wallis test followed by Mann-Whitney’s U-test. The significance of differences in score between normal and adjuvant control groups was determined by Mann-Whitney’s U-test. Differences giving a value of p < 0.05 were regarded as statistically significant.

**Results**

**Paw swelling**

In the adjuvant control group, primary swelling of adjuvant-injected hindpaw (right hindpaw) was observed 3 days after adjuvant injection, and secondary swelling reached a plateau 15 days after adjuvant injection (Fig. 1). The swelling of the uninjected hindpaw also reached a plateau 15 days after adjuvant injection. The ipsilateral foot volume increased from 1.6 ± 0.01 ml (n = 9) in the normal group to 4.6 ± 0.17 ml (n = 9) in the control group. JTE-522 and indomethacin...