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ORIGINAL ARTICLE

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Effects of FR167653, a dual inhibitor of interleukin-1 and tumor necrosis factor, on adjuvant arthritis in rats

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Abstract The effects of FR167653, a dual inhibitor of interleukin (IL)-1β and tumor necrosis factor (TNF)-α production, on the development of adjuvant arthritis were investigated in rats. Female Sprague–Dawleys rats weighing 130 g with adjuvant arthritis were administered FR167653 0.1, 1, 10, or 30 mg/kg, and their arthritis scores, soft tissue X-rays, and histological findings were then compared with those of a control group. Body weights were also recorded. FR167653 suppressed the severity of adjuvant arthritis in a dose-dependent manner. Administration of the drug had little effect on body weight. FR167653 might locally affect arthritic joints to prevent inflammation. However, further experiments are necessary to elucidate the underlying mechanisms.

Key words Adjuvant arthritis · Cytokine · Cytokine suppressive agent · FR167653

Introduction

Adjuvant arthritis in rats mimics many of the symptoms found in inflammatory polyarticular arthritis, and thus it is the principal animal model used to evaluate antiarthritic drugs.[1,2] Synoviocytes from adjuvant arthritis rats release elevated levels of interleukin (IL)-1 and tumor necrosis factor (TNF).[1,3] FR167653 is a potent antiinflammatory agent synthesized by the Fujisawa Pharmaceutical, Osaka, Japan. It is used to prevent the onset and development of septic shock and disseminated intravascular coagulation by inhibiting the production of IL-1α (IL-1α), IL-1β, and TNF-α in monocytes and lymphocytes.[4,5] It was therefore decided to investigate its effects on the development of adjuvant arthritis in rats.

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Materials and methods

Animals

Female Sprague–Dawleys rats, 4–6 weeks old, were purchased from Japan Charles River Breeding Laboratories (Kanagawa, Japan). They were allowed 1 or 2 weeks to acclimatize to their new environment, and were used when they weighed about 130 g. All the animals received standard laboratory chow and water ad libitum.

Materials

Mycobacterium butyricum was obtained from DIFCO Laboratories (Detroit, MI, USA). Liquid paraffin and methyl cellulose were purchased from Kanto Chemical (Tokyo, Japan). FR167653 (1-[7-(4-fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo[5,1-c][1,2,4]triazin-2-yl]-2-phenylethanedione sulfate monohydrate) was kindly provided by Fujisawa Pharmaceutical.

Adjuvant arthritis model

Adjuvant arthritis was induced in rats by a single intradermal injection of 0.2 ml of adjuvant at the base of the tail. The adjuvant was prepared by adding dried, heat-killed *M. butyricum*, which was finely powdered with a mortar and pestle, to liquid paraffin at a concentration of 10 mg/ml following sterilization by autoclaving.

Effects of FR167653 on adjuvant arthritis

Each control and experimental group contained five rats. FR167653 in 0.5% methylcellulose at concentrations

of 0.1, 1, 10, or 30 mg/kg/0.2 ml was administered by subcutaneous injection of 0.2-ml aliquots once daily from the day when the adjuvant was given to day 21 of the experimental. As a control, the same volume of methylcellulose alone was given. Rat body weights and serum cytokines in each group were measured at regular intervals throughout the experiment. The severity of the arthritis on day 21 in each paw of each rat was scored as: 0 = no arthritis, 1 = extreme redness and/or minimal swelling, 2 = medium swelling, 3 = severe swelling and 4 = severe swelling and nonweightbearing.[6] Histological and soft tissue X-ray examinations were also performed. For the histological analysis, a hind foot was amputated and immediately fixed with 15% formalin on day 21. Hematoxylin and eosin staining was performed on the sagittal section of the foot. Soft tissue X-ray was performed with Fuji film IX FR (Fuji Photo Film, Tokyo, Japan) under 100 kV and 120 s exposure (Softex ESM-2 type, Softex, Japan). Each hind-paw radiograph was evaluated by the method of Clark et al.[7] with some modifications. Briefly, the five features of foot pad swelling, bone erosion, bone demineralization, periostitis, and joint space narrowing were evaluated blindly using grades of 0–4 (with 0 indicating normal and 4 indicating severe damage). The radiological score was termed the radiological index.

Statistical analysis

The Bartlett test was used to test the normality and homogeneity of variances among the different groups. Fischer's protected least-significant difference or the Bonferroni–Dunn test was used to compare the different groups of rats, as shown in Figs. 1, 3, and 5. A probability value of less than 0.05 was taken to indicate a significant difference.

Results

Weight change in rats administered FR167653

A statistically significant weight loss was apparent on day 7 in rats given 10 or 30 mg/kg of FR167653 (10 mg/kg, $P = 0.024$; 30 mg/kg, $P = 0.012$). However, at the other times no significant differences were seen between the control and FR167653 rats. Little weight gain occurred in rats in any group after day 14 (Fig. 1).

Onset of adjuvant arthritis in the FR167653 groups and controls

Adjuvant arthritis developed from day 12 in the control groups and those treated with 0.1 and 1 mg/kg FR167653, while it was first apparent on day 16 in the 10 and 30 mg/kg groups. Adjuvant arthritis had developed in all rats to varying degrees by day 21 (Fig. 2).

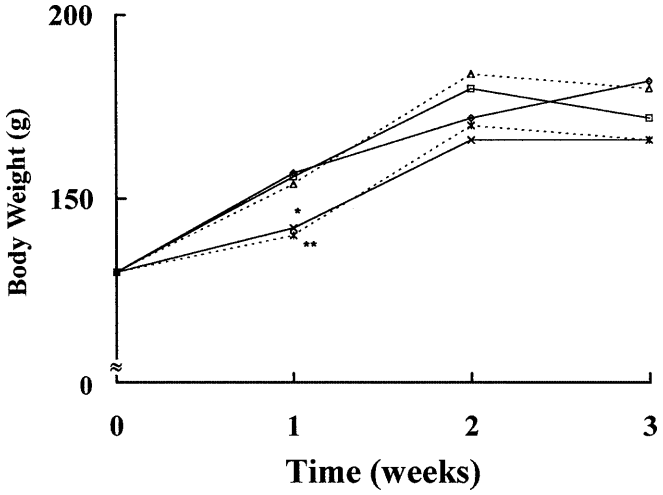

Fig. 1. Weight change in adjuvant arthritis rats administered FR167653. Change in mean body weight. Note the statistically significant differences between the control and 10 (*, $P = 0.024$) and 30 mg/kg (**, $P = 0.012$) FR167653 groups after 1 week. *Diamond*, control; *square*, 0.1 mg/kg; *triangle*, 1 mg/kg; *cross*, 10 mg/kg; *star*, 30 mg/kg

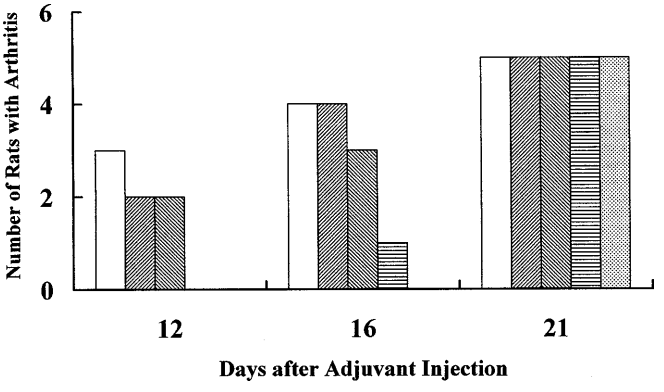

Fig. 2. Onset of adjuvant arthritis administered FR167653. Time dependence of disease development in control (*open box*), 0.1 mg/kg (*forward shading*), 1 mg/kg (*backward shading*), 10 mg/kg (*horizontal shading*), and 30 mg/kg (*stippled*) FR167653 groups

Arthritis evaluation

The mean arthritis scores on day 21 were 10.4 ± 3.6 in the control group and 12.4 ± 3.3 and 7.2 ± 4.6, respectively, in the 0.1 and 1 mg/kg FR167653 groups. A statistically significant decrease to 3.6 was observed for both the 10 (±2.6; SD) and 30 mg/kg (±1.7; SD) groups (10 mg/kg, $P = 0.0039$; 30 mg/kg, $P = 0.0039$) (Fig. 3).

Soft tissue X-ray findings

Soft tissue X-rays showed severe hind foot pad swelling, demineralization, and joint destruction of the rats in the control and 0.1 mg/kg FR167653 groups. However, the extent of those features decreased with increased concentra-

tions of FR167653, with an almost normal appearance in some rats given FR167653 10 or 30 mg/kg (Fig. 4). The mean radiological index on day 21 was 3.5 ± 0.58 in the control group, and 3.8 ± 0.5 and 3 ± 1.22, respectively, in the 0.1 and 1 mg/kg FR167653 groups. A statistically significant decrease was observed for both the 10 (0.8 ± 0.84) and 30 mg/kg (1 ± 0.89) groups (10 mg/kg, $P = 0.0003$; 30 mg/kg, $P = 0.0007$) (Fig. 5).

Histological findings

A histopathological assessment of the foot pads of rats in the control and 0.1 mg/kg FR167653 groups revealed a marked infiltration of neutrophils and lymphocytes, with disruption and loss of articular cartilage. However, inflammatory cell invasion and articular cartilage destruction were less prominent with increasing concentrations of FR167653 (Fig. 6). Little difference was observed in the relative proportion of neutrophils and lymphocytes in the FR167653-treated rats.

Discussion

Rat adjuvant arthritis has been used in preclinical studies as a standard animal model for the chronic inflammation occurring in human rheumatoid arthritis,[8] with applications in the evaluation of antiinflammatory,[1] and immunosuppressive agents,[9,10] as well as proteinase inhibitors.[11] In the present study, FR167653, a potent antiinflammatory drug that inhibits IL-1β and TNF-α production,[4,5] inhibited the onset of rat adjuvant arthritis (Fig. 2), with a significant reduction in arthritic changes at high concentrations (Fig. 3). Soft tissue X-ray and especially histological findings provided further evidence of improvement (Fig. 4). Weight loss is a marker for toxicity in animals,[8] but there was only a limited variation in body weights between FR167653-treated and control rats in this experiment, except for a transient decrease with the high dose (Fig. 1). Thus, FR167653 effectively suppressed adjuvant arthritis in rats with little toxicity.

TNF-α and IL-1β are known to be elevated in local joints in adjuvant arthritis rats.[1,3] FR167653 inhibits the produc-

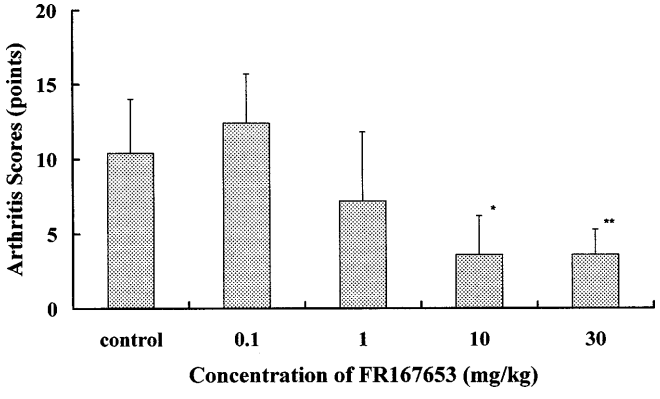

Fig. 3. Arthritis evaluation. Scores showing statistically significant differences between the control and 10 (*, $P = 0.0039$) and 30 mg/kg (**, $P = 0.0039$) FR167653 groups. Bars above symbols indicate mean ± SD

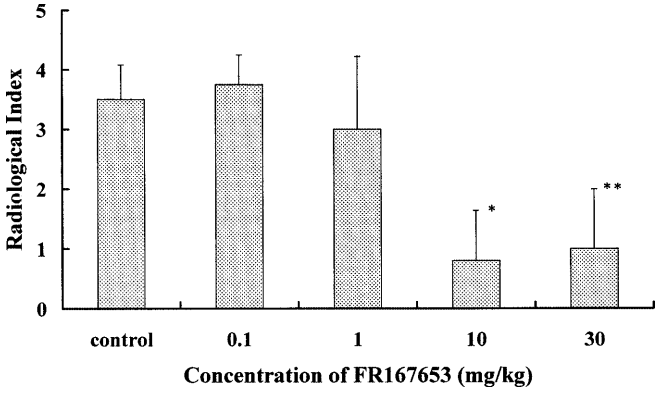

Fig. 5. Radiological index. Scores showing statistically significant differences between the control and 10 (*, $P = 0.0003$) and 30 mg/kg (**, $P = 0.0007$) FR167653 groups. Bars above symbols indicate mean ± SD

Fig. 4. Soft tissue X-ray findings. Lateral soft tissue X-ray views showing severe hind foot pad swelling, demineralization, and joint destruction of control (**A**) and 0.1 mg/kg FR167653 rats. However, the extent of these features is less prominent in the 1 mg/kg FR167653 group, and in the 10 (**B**) and 30 mg/kg (**C**) FR167653 groups the appearance is nearly normal

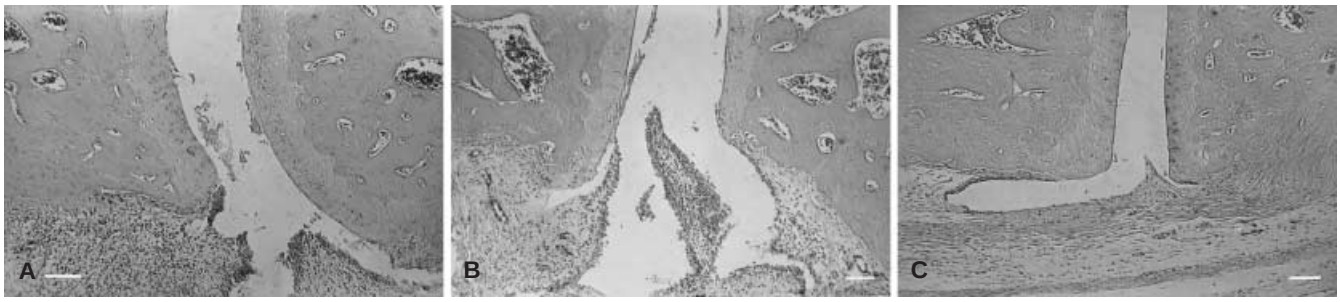

Fig. 6. Histological findings. Histology of control (**A**) and 0.1 mg/kg (**B**) foot pads showing infiltrates of neutrophils and lymphocytes with disruption and loss of articular cartilage. However, the alterations are less pronounced with higher concentrations of FR167653 (**C**, 30 mg/kg) (HE, ×79). *Bar* 100 μm

tion of TNF-α and IL-1β from lymphocytes and monocytes.[4] However, its inhibitory effect is thought to be different from that of antagonists. Moreover, FR167653 protects rats from several forms of organ dysfunction due to the inhibition of cytokine production.[5] Those results and the present experimental data suggest that FR167653 might inhibit the production of TNF-α and IL-1β from lymphocytes and monocytes in local joints with adjuvant arthritis. If this is the case, the measurement of local cytokines such as TNF-α and IL-1β might be appropriate. On the other hand, it is possible that this drug may have antiinflammatory effects unrelated to these functions, or which do not inhibit the synthesis of these cytokines under certain conditions. Further study should concentrate on the local environment in affected joints. In conclusion, the study showed that the administration of FR167653 effectively suppresses adjuvant arthritis in rats. This potent antiinflammatory drug might be a useful therapeutic tool for the control of chronic inflammatory diseases. Further studies are necessary to elucidate the underlying mechanisms of action.

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