Effect of FTY720, a novel immunosuppressant, on adjuvant-induced arthritis in rats

M. Matsuura, T. Imayoshi, K. Chiba and T. Okumoto
Pharmacology, Drug Development Laboratories, Welfide Corporation, 955 Koiwai, Yoshitomi-cho, Chikujo-gun, Fukuoka 871-8550, Japan,
Fax: +81 979238952, e-mail: matsuura_mamoru@welfide.co.jp

Received 21 June 1999; returned for revision 26 August 1999; returned for final revision 25 February 2000; accepted by M. Katori 9 April 2000

Abstract. Objective and Design. Anti-arthritic effect of FTY720, a novel immunosuppressant, was compared with those of immunosuppressants cyclosporin A and tacrolimus in adjuvant-induced arthritis in rats.

Material. Male LEW rats.

Treatment: FTY720 (0.03–0.3 mg/kg), cyclosporin A (1–10 mg/kg) or tacrolimus (0.3–3 mg/kg) were orally administered to rats for 21 days beginning on the day (day 0) of adjuvant inoculation. In addition, the anti-arthritic effect of FTY720 (0.3 mg/kg) and cyclosporin A (10 mg/kg) were evaluated by administration to animals for 5 consecutive days (days 2–6, 6–10, and 10–14).

Methods. Adjuvant-induced arthritis was produced by intradermal injection of 0.5 mg heat-killed Mycobacterium tuberculosis. Hindpaw edema was measured plethysmographically. The day of arthritis onset was determined macroscopically. Bone degradation was determined by radiography. Peripheral blood leukocytes were classified microscopically.

Results. All test compounds inhibited the incidence of arthritis, hindpaw edema, and bone destruction. In addition, FTY720 but not cyclosporin A or tacrolimus markedly decreased the number of peripheral blood lymphocytes. FTY720 treatment on days 6 to 10 inhibited the bone destruction and hindpaw edema.

Conclusion. These results suggest that the anti-arthritic effect of FTY720 in this adjuvant-induced arthritic model was more potent than those of cyclosporin A and tacrolimus. FTY720 administered on days 6 to 10 showed the inhibitory effect on the bone destruction and hindpaw edema. FTY720 may be effective in the treatment of rheumatoid arthritis.

Key words: Adjuvant-induced arthritis – Peripheral lymphocyte – FTY720 – Cyclosporin A – Tacrolimus

Introduction

Immunosuppressants such as cyclosporin A and tacrolimus have been reported to be effective against graft rejection and autoimmune diseases in animal models. Cyclosporin A and tacrolimus have similar mechanisms which inhibit the accumulation of IL-2 mRNA in activated human peripheral blood T cells [1]. These two compounds have also been extensively evaluated in clinical trials for the therapy of rheumatoid arthritis, in which some efficacy has been shown [2–7]. The use of cyclosporin A is limited, however, by its renal toxicity at therapeutic doses [8].

FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride) is a potent immunosuppressant with a mechanism different from that of cyclosporin A and tacrolimus [9]. This compound is more potent than cyclosporin A and tacrolimus in prolonging allograft survival in rat skin [10, 11], rat cardiac [12], and canine renal transplantation [13] models. In allograft models, FTY720 selectively decreased the number of CD3-positive mature T cells without affecting CD45RA-positive B cell counts [11, 12]. The immunosuppressive mechanism of FTY720 has been found to be related to acceleration of homing of peripheral blood lymphocytes to mesenteric and peripheral lymph nodes and Peyer’s patches [9, 11]. In addition, unlike cyclosporin A and tacrolimus, FTY720 does not affect cytokine production at therapeutic doses [9, 11]. The suppressive effect of FTY720 on T cell-mediated immune responses has been clarified in various animal models. For example, FTY720 has been reported to prolong host survival in lethal graft-vs-host reaction in rat [14]. In the present study, we compared the anti-inflammatory activity of FTY720 with those of cyclosporin A and tacrolimus in adjuvant-induced arthritic rats. In addition, we evaluated the influence of the administration schedule of FTY720 on its anti-arthritic effect.
Materials and methods

Animals

Male LEW rats were purchased from Seac Yoshitomi, Ltd. (Fukuoka, Japan), and used at 7 weeks of age (187–223 g). The animals were housed at a constant temperature of 23 ± 2°C and relative humidity of 55 ± 5% and provided free access to standard chow and water. Rats were handled under the supervision of the Animal Ethics Committee of Wel-fide Corporation.

Drugs

FTY720 was synthesized by Taito Co., Ltd. (Tokyo, Japan). Cyclosporin A and tacrolimus were purchased from Sandoz Co., Ltd. (Basel, Switzerland) and Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. FTY720 and tacrolimus were dissolved and diluted in distilled water. Cyclosporin A was dissolved and diluted in olive oil (Wako Pure Chemical Industries, Ltd. Tokyo, Japan). Compounds were given orally to rats at a constant volume of 5 mL/kg body weight. In preliminary examination, we confirmed that there was no difference in onset day, incidence, peripheral blood lymphocyte count and hindpaw edema of adjuvant-induced arthritic model between the distilled water control and olive oil control.

Adjuvant-induced arthritis

Adjuvant-induced arthritis was produced by intradermal injection of 0.5 mg of heat-killed Mycobacterium tuberculosis suspended in 0.1 mL liquid paraffin into the base of the tail of rats on day 0. Hindpaw edema was measured with an electronic water plethysmograph (TK-105, Muromachi Kikai Co., Tokyo, Japan) on days 0, 10, 15, 18, and 21. The day of onset of arthritis was determined as the day on which hindpaw edema or redness was detectable. The incidence of arthritis was inspect-ed daily until 21 days. On day 21, the animals were weighed and then sacrificed. Radiographs of the hind feet were taken with an X-ray unit (Muromachi Kikai Co., Tokyo, Japan) on days 0, 10, 15, 18, and 21. The day of onset of arthritis was determined as the day on which hindpaw edema or redness was detectable. The incidence of arthritis was inspected daily until 21 days. On day 21, the animals were weighed and then sacrificed. Radiographs of the hind feet were taken with an X-ray unit (CM8-80, SOFTEX, Tokyo, Japan). Bone destruction was evaluated according to a scoring method described elsewhere [15, 16]. The degree of bone destruction was scored from 0 to 3 (0, normal bone; 1, mild; 2, moderate; 3, severe changes). Maximum score for both legs was thus 6 per animal. Compounds were administered for 21 days (days 0–20) or 5 days (days 2–6, 6–10, or 10–14).

Measurement of leukocyte counts

Peripheral blood samples were obtained on day 21 after the 21-day treatment and on days 10, 15, and 20 after the 5-day treatment. Total peripheral blood cells were counted with a blood cell counter (Celltac MEK-4000 or MEK-5153, Nihon Kohden Co., Tokyo, Japan). Blood smears were then prepared to count lymphocytes, neutrophils, and monocytes by Wright-Giemsa staining.

Statistical analysis

Statistical significance for the incidence of arthritis was tested by the Fisher’s exact test with Hommel’s multiple comparison test. Bone destruction was tested by the nonparametric Dunnett’s test. Hindpaw volume and cell counts was tested by the Dunnett’s test.

Results

Effect of 21-day treatment with FTY720, cyclosporin A, and tacrolimus on adjuvant-induced arthritis

The incidence of arthritis in the arthritic controls reached 100% by day 10 after adjuvant inoculation (Table 1). Compounds were administered orally for 21 days from the day of inoculation. FTY720 gave 5 arthritis-free rats of 7 animals at 0.3 mg/kg. Cyclosporin A and tacrolimus also produced arthritis-free rats at their highest doses. Body weight decreased with disease progression in arthritic controls. All compo-unds tested dose-dependently prevented this weight loss at the doses at which they inhibited the incidence of arthritis (data not shown).

Because bone destruction scores were almost equal for right and left hindpaws in both the arthritic controls and compound-treated rats, mean values were calculated from the scores for both legs (Table 1). Mean bone destruction scores of the normal and arthritic controls on day 21 were 0 and 4.3, respectively. FTY720 significantly decreased the score at 0.1 and 0.3 mg/kg. Cyclosporin A also significantly inhibited bone destruction at 3 and 10 mg/kg. Tacrolimus showed significant inhibition at 1 and 3 mg/kg.

Mean hindpaw volume of the arthritic controls significantly increased from day 15 (Fig. 1). FTY720 completely inhibited this increase in hindpaw volume at 0.3 mg/kg. Cyclosporin A (3 mg/kg) and tacrolimus (1 mg/kg) also completely inhibited paw edema, but at dosages higher than that of FTY720.

The number of peripheral blood cells in arthritic controls significantly increased as compared with the normal controls on day 21, by 1.8-fold for lymphocytes, 7.4-fold for neutrophils, and 4.8-fold for monocytes (Fig. 2). FTY720 significantly decreased the number of peripheral blood lymphocytes to less than the normal control level without affecting neutrophil or monocyte counts. At the highest doses,

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Incidence of arthritis</th>
<th>Bone destruction score (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>–</td>
<td>0 / 7</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Arthritic control</td>
<td>–</td>
<td>7 / 7</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>FTY720</td>
<td>0.03</td>
<td>7 / 7</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7 / 7</td>
<td>0.6 ± 0.3 **</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>2 / 7</td>
<td>0.0 ± 0.0 **</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>1</td>
<td>7 / 7</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7 / 7</td>
<td>0.4 ± 0.3 **</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2 / 7</td>
<td>0.0 ± 0.0 **</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.3</td>
<td>7 / 7</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7 / 7</td>
<td>0.7 ± 0.4 **</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1 / 7#</td>
<td>0.0 ± 0.0 **</td>
</tr>
</tbody>
</table>

Compounds were administered orally for 21 days beginning the day of inoculation. The data represent the means ± S.E. of 7 rats. # P<0.05 (Fisher’s exact test with Hommel’s multiple comparison test) vs. ar-thritic control. ** P<0.01 (nonparametric Dunnett’s test) vs. arthritic control. 