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European Journal of Pharmacology 409 (2000) 331–335

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Short communication

A novel anti-rheumatic drug suppresses tumor necrosis factor-α and augments interleukin-10 in adjuvant arthritic rats

Masao Hisadome *, Tetsuko Fukuda, Hiroshi Sumichika, Tokushi Hanano, Kunitomo Adachi

Research Laboratories, Welfide Corporation, 955 Koiwai, Yoshitomi-cho, Chikujo-gun, Fukuoka 871-8550, Japan

Received 13 September 2000; received in revised form 3 November 2000; accepted 10 November 2000

Abstract

Tumor necrosis factor-α (TNF-α) plays an important role in the pathology of rheumatoid arthritis. When *N*-[1-(4-{[4-(pyrimidin-2-yl)piperazin-1-yl]methyl}phenyl)cyclopropyl]acetamide (Y-39041) (3–30 mg/kg) was orally administered to rats with established arthritis from day 15 to day 20, hindpaw volume was significantly reduced. This inhibitory activity of Y-39041 was kept up after administration was stopped. On day 17 Y-39041 suppressed lipopolysaccharide-induced TNF-α and interleukin-6 production in serum at doses of 3–30 mg/kg, and augmented interleukin-10 production at doses of 10 and 30 mg/kg. The finding that Y-39041 suppresses TNF-α and interleukin-6 production and augments interleukin-10 production could be beneficial in the therapy of chronic inflammatory diseases.

Keywords: Y-39041; Arthritis; TNF-α (Tumor necrosis factor-α); Interleukin-10; Lipopolysaccharide

1. Introduction

Chronic inflammation involves lymphocytes, macrophages and neutrophils in the inflamed joint. Macrophages play an essential role in the progression of inflammatory diseases. In fact, a large amount of tumor necrosis factor-α (TNF-α) is present in synovial fluid and synovial tissue in rheumatoid arthritis (Feldmann et al., 1996). Evidence has been provided that anti-TNFα antibodies and soluble TNF-α receptors are effective in rheumatoid arthritis (Newton and Decicco, 1999). Interleukin-10 has potent anti-inflammatory activity by suppressing TNF-α and interleukin-6 production by activated macrophages (Fiorentino et al., 1991). A recombinant human interleukin-10 has been clinically effective against steroid-resistant Crohn's disease (vanDeventer et al., 1997).

Down-regulation of TNF-α and up-regulation of interleukin-10 may be a new and rational approach for therapy of rheumatoid arthritis. In mice, combined treatment with anti-TNFα antibody and interleukin-10 had an additive effect against collagen-induced arthritis (Walmsley et al., 1996). This concept suggests that the combination of anti-TNFα antibody and interleukin-10 has a synergistic effect in the therapy of rheumatoid arthritis. However, there are potential problems in that immunogenicity and antibody production against biological drugs may be induced after the repeated administration of anti-TNFα antibody and interleukin-10. We looked for a novel disease-modifying anti-rheumatic drug that possessed anti-inflammatory activity on the basis of TNF-α suppression and interleukin-10 augmentation in vivo.

Studies based on this concept led to the identification of a novel synthetic dual regulator of TNF-α and interleukin-10, *N*-[1-(4-{[4-(pyrimidin-2-yl)piperazin-1-yl]methyl}phenyl)cyclopropyl]acetamide (Y-39041) (Hanano et al., 2000). In mice Y-39041 suppressed lipopolysaccharide-induced TNF-α production in serum (80% inhibition at 10–100 mg/kg, p.o.) and at the same time augmented interleukin-10 production (10-fold increase at 30 mg/kg, p.o.) (Fukuda et al., 2000). In addition, Y-39041 completely protected mice from lipopolysaccharide-induced death.

In adjuvant arthritic rats, the progression of arthritis is associated with TNF-α overproduction (DiMartino et al., 1997), suggesting that TNF-α can be a useful parameter for the evaluation of disease-modifying anti-rheumatic drugs. We investigated the effects of a novel disease-mod-

* Corresponding author. Tel.: +81-979-23-8970; fax: +81-979-23-8952.

E-mail address: hisadome@welfide.co.jp (M. Hisadome).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00866-9

ifying anti-rheumatic drug on paw edema and on lipopolysaccharide-induced TNF-α, interleukin-6 and interleukin-10 production in rats with established adjuvant-induced arthritis. Oral treatment with Y-39041 suppressed lipopolysaccharide-induced TNF-α and interleukin-6 production and augmented interleukin-10 production at doses that prevented paw inflammation.

2. Materials and methods

Male Lewis rats were purchased from Seac Yoshitomi (Fukuoka, Japan) and used at 7 weeks of age. Arthritis was induced by the intradermal injection (0.5 mg/rats) of heat killed Mycobacterium tuberculosis H35Rv-1 in liquid paraffin into the base of the tail. Y-39041 was synthesized in our laboratories. Y-39041 and prednisolone (Sigma, Missouri, USA) were suspended in 0.5% hydroxypropylmethylcellulose solution and were orally administered once a day from day 15. The volumes of both hindpaw were measured by aqueous plethysmography as a parameter of anti-arthritic activity.

Lipopolysaccharide (*Escherichia coli* 0111:B4, Difco Laboratories, Detroit, USA) was dissolved in 0.9% saline. Test compounds were administered 30 min prior to intraperitoneal injection of lipopolysaccharide (30 μg/kg) on day 17. Blood samples were obtained at 1.5 and 3 h after lipopolysaccharide injection. Serum was stored at −30°C until used for cytokine determination. The amounts of serum TNF-α and interleukin-10 were measured at 1.5 h after lipopolysaccharide injection and interleukin-6 was measured at 3 h, because serum TNF-α, interleukin-6 and interleukin-10 levels reached a maximum at this time in endotoxin-shock mice. The amounts of cytokines in serum were measured using enzyme-linked immunosorbent assay kits for murine TNF-α, interleukin-6 and interleukin-10 (Biosource International, California, USA). Assays were performed as indicated by the manufacturer's instructions. For cytokine productions, significant differences between arthritic rats and non-arthritic rats were determined by Student's *t*-test. Other significant differences were determined by the one-way analysis of Dunnett's method.

3. Results

Y-39041 (3–30 mg/kg, p.o.) significantly reduced the increase in hindpaw volume of rats with established adjuvant-induced arthritis at days 17, 19 and 21 in a dose-dependent manner when the compounds were administered therapeutically from day 15 to day 20 (Fig. 1: Experiment A). Y-39041 (10 mg/kg, p.o.) significantly reduced an increase in hindpaw volume on day 24 (day 4 after administration was stopped) and showed a mild, but not significant, inhibitory effect on day 27 (day 7 after administration was stopped) (Fig. 1: Experiment B). Prednisolone (3 mg/kg, p.o.) markedly reduced the increase in hindpaw volume on days 17, 19 and 21, and slightly reduced it on day 24. Moreover, prednisolone had no effect on day 27.

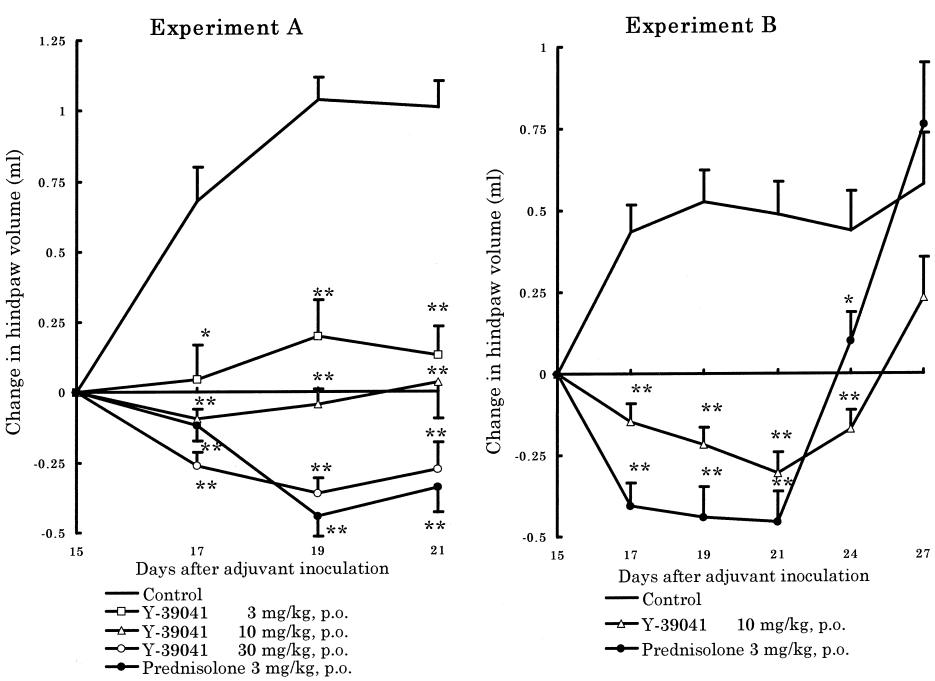

Fig. 1. Effect of Y-39041 on hindpaw swelling in rats with established adjuvant-induced arthritis. Data are shown as the means ± S.E.M. for eight animals/group. Test compounds were orally administered to rats from day 15 to day 20. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control (Dunnett method).

On day 17, Y-39041 (10 and 30 mg/kg, p.o.) and prednisolone (3 mg/kg, p.o.) significantly reduced the increased hindpaw volume. The production of lipopolysaccharide-induced TNF-α, interleukin-6 and interleukin-10 in adjuvant arthritic rats was significantly greater than that in non-arthritic rats (Fig. 2). Lipopolysaccharide-induced TNF-α production was significantly suppressed by Y-39041 (3–30 mg/kg) and prednisolone (3 mg/kg). The TNF-α levels of Y-39041 (10 and 30 mg/kg) treated rats were almost equal to those of non-arthritic rats. Lipopolysaccharide-induced interleukin-6 production was significantly suppressed by Y-39041 and prednisolone. In addition, an approximately fourfold increase in lipopolysaccharide-induced interleukin-10 production over that of the control group was observed after treatment with Y-39041 (10 and 30 mg/kg). Prednisolone showed no effect on interleukin-10 production.

4. Discussion

Adjuvant arthritis models in rats are often used for the evaluation of anti-rheumatic drugs. In synovial tissues of rats with adjuvant arthritis, the number of macrophages is increased during the established phase of arthritis and cell counts peak on day 25 (Halloran et al., 1996). This suggests that the activation of macrophages occurs when adjuvant-induced arthritis is established rather than when it is induced. Therefore, anti-inflammatory effects of TNF inhibitors or antagonists must be examined in the established phase of adjuvant-induced arthritis. In fact, metalloproteinase inhibitors (DiMartino et al., 1997) and p38 mitogen-activated protein kinase inhibitors (Badger et al., 1996), which suppress monocyte-derived cytokine production, are effective in rats with established adjuvant-induced arthritis. The oral treatment with Y-39041 reduced hind-

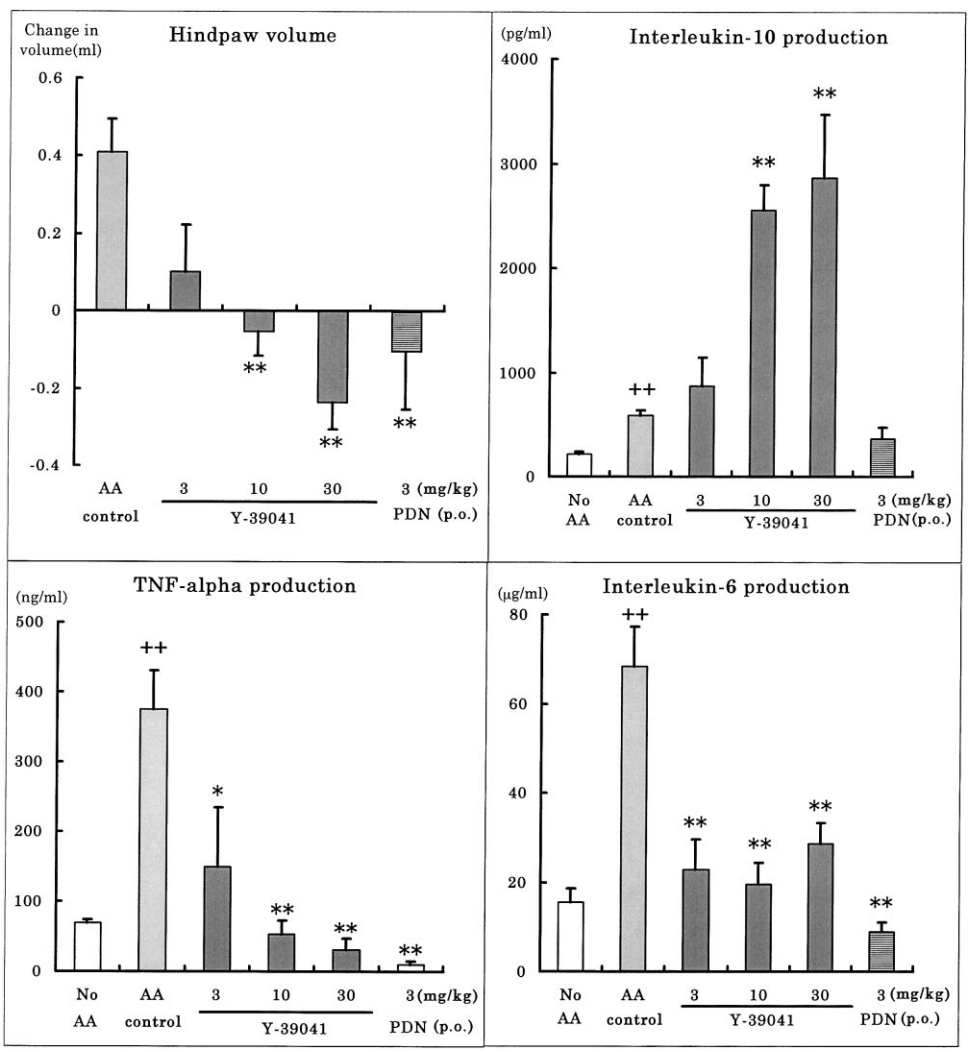

Fig. 2. Effect of Y-39041 on hindpaw swelling and on lipopolysaccharide-induced TNF-α, interleukin-10 and interleukin-6 production in rats with established adjuvant-induced arthritis. Y-39041 and prednisolone (PDN) were orally administered from day 15 to day 17. Rats were injected with lipopolysaccharide (30 μg/kg, i.p.) on day 17. Data are shown as the means ± S.E.M. for five animals/group. $^{++}P < 0.01$ vs. no adjuvant arthritis (AA) (*t*-test); $^{*}P < 0.05$; $^{**}P < 0.01$ vs. adjuvant arthritis control (Dunnett method).

paw swelling, and this reducing activity was sustained after termination of drug administration. In contrasts, the inhibitory effect of prednisolone on hindpaw swelling disappeared immediately after drug administration was stopped. There was a pronounced rebound in hindpaw swelling after prednisolone was stopped. Therefore, the inhibitory effect of Y-39041 on the development of arthritis was considered to be different from that of prednisolone.

In adjuvant arthritic rats, elevated TNF-α levels have been observed in the plasma and joint tissues (Smith-Oliver et al., 1993). In this report, lipopolysaccharide-induced TNF-α production in rats with established adjuvant-induced arthritis was significantly higher than that in non-arthritic rats. This means that there was an enhanced response to lipopolysaccharide and overproduction of TNF-α after secondary costimulation in the established phase of adjuvant-induced arthritis. When TNF-α was neutralized in the synovial tissue culture, other proinflammatory cytokines were not produced, suggesting that TNF-α is situated upstream in the cytokine network, namely at its apex (Feldmann et al., 1996). This leads to the hypothesis that chronic inflammation is sustained by TNF-α and that TNF-α could be a good therapeutic target in rheumatoid arthritis. Therefore, TNF-α and, subsequently, produced interleukin-6 are key cytokines in chronic inflammatory diseases.

Y-39041 suppressed lipopolysaccharide-induced TNF-α and interleukin-6 production in adjuvant arthritic rats at doses that prevented hindpaw swelling. The compound also augmented lipopolysaccharide-induced interleukin-10 production at doses that prevented inhibited swelling. This suggests that the compound has an anti-arthritic effect through not only TNF-α and interleukin-6 suppression but also interleukin-10 augmentation. The suppression of interleukin-6 production by Y-39041 may be expressed following TNF-α inhibition because the maximum level of serum interleukin-6 was detected after a maximum level of TNF-α. We suppose that the sustained effect of Y-39041 on arthritis may be due to the effects on TNF-α suppression and interleukin-10 augmentation. The mode of action of Y-39041 on TNF-α suppression and interleukin-10 augmentation is unclear. Pentoxifylline, one of phosphodiesterase inhibitors, suppresses TNF-α production but augments interleukin-6 production (Schandene et al., 1992). Y-39041 suppressed lipopolysaccharide-induced interleukin-6 production and inhibited TNF-α. Y-39041 showed no effect on phosphodiesterase activity at 10^{-5} mol/l in vitro. In addition, Y-39041 had no binding affinity for TNF-α, cyclooxygenase activity at 10^{-5} mol/l in vitro. Therefore, the pharmacological profile of Y-39041 is different from that of TNF-α antagonists, nonsteroidal anti-inflammatory drugs and phosphodiesterase inhibitors.

Among the anti-rheumatic drugs, methotrexate has become an immunosuppressive treatment for rheumatoid arthritis (Maetzel et al., 1998). Methotrexate shows an excellent inhibition on hindpaw swelling in rats with adjuvant arthritis in the induction phase but not in the established phase (Williams et al., 1995). This suggests that methotrexate has anti-arthritic activity via inhibition of T lymphocyte activation because methotrexate suppresses cytokine production by activated T lymphocytes but not by activated macrophages in mice (Neurath et al., 1999). Recently, the combination of anti-TNFα antibody and methotrexate (Maini et al., 1998) was found to have a synergistic effect on rheumatoid arthritis. Therefore, suppression of both T lymphocyte activation and macrophage activation should provide a good strategy for more effective treatment of rheumatoid arthritis. It is expected that the therapeutic effect of Y-39041 is similar to that of anti-TNFα antibody or soluble TNF receptor. The compound demonstrated a good bioavailability (76% at 30 mg/kg, p.o. in rats). Y-39041 possesses a very attractive pharmacological profile of anti-arthritic activity through TNF-α and interleukin-6 inhibition and interleukin-10 augmentation.

In conclusion, a novel synthetic compound, Y-39041, suppressed hindpaw swelling in rats with established adjuvant-induced arthritis and this suppressing activity was maintained after termination of administration. The compound suppressed lipopolysaccharide-induced TNF-α and interleukin-6 production, and augmented interleukin-10 production at doses that prevented hindpaw swelling. The findings suggest that Y-39041 would be beneficial in the therapy of TNF-α-associated diseases such as rheumatoid arthritis and Crohn's diseases.

Acknowledgements

We thank Dr. M. Terasawa for helpful discussion.

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