Epoxyquinomicins A, B, C and D, New Antibiotics from *Amycolatopsis*

II. Effect on Type II Collagen-induced Arthritis in Mice

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The anti-arthritic effects of epoxyquinomicins on type II collagen-induced arthritis in DBA/1J mice were examined. Prophylactic treatment with epoxyquinomicins A, B, C and D (1~4 mg/kg) had potent inhibitory effects on type II collagen-induced arthritis. In contrast to nonsteroidal anti-inflammatory drugs (NSAIDs), epoxyquinomicin C (1~30 mg/kg) had neither an anti-inflammatory effect on carrageenan-induced paw edema in rats nor an analgesic effect on acetic acid-induced writhing in mice. These results suggest that the mode of action of epoxyquinomicins is different from that of NSAIDs and that epoxyquinomicins may become useful drugs for the treatment of rheumatoid arthritis.

In the preceding paper[1], we described the taxonomy of the producing strain, fermentation, isolation and antimicrobial activities of epoxyquinomicins A (**1**), B (**2**), C (**3**) and D (**4**) (Fig. 1).

In addition to epoxyquinomicins having chloro-substituted or non-substituted salicyloyl moiety, some compounds which possess 5,6-epoxy-4-hydroxy-2-cyclohexenone moiety in the structure have recently been reported to show anti-inflammatory activities[2,3]. Therefore we investigated whether epoxyquinomicins had similar activitis. In this paper, we describe anti-inflammatory activities of epoxyquinomicins.

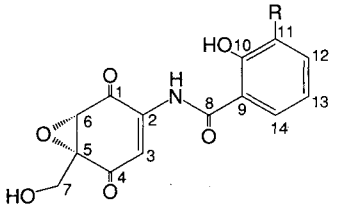
Physico-chemical properties and structure determination studies will be reported in the succeeding paper[4].

Materials and Methods

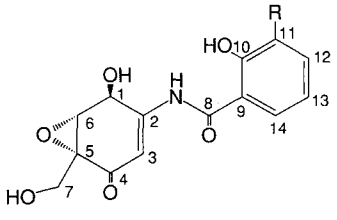
Animals

Male DBA/1J mice were purchased from Charles River Japan (Atsugi, Japan) and were used for the collagen-induced arthritis experiment. Male SD rats and ICR mice were purchased from Japan SLC (Hamamatsu, Japan), and were used for carrageenan-induced hind limb edema and for acetic acid-induced writhing, respectively. These animals were housed under standard laboratory conditions and were fed food and water *ad libitum*. Eight

Fig. 1. Structures of epoxyquinomicins A, B, C and D.



Epoxyquinomicin A (**1**): R = Cl
Epoxyquinomicin B (**2**): R = H



Epoxyquinomicin C (**3**): R = H
Epoxyquinomicin D (**4**): R = Cl

to 12 week-old mice were used for the arthritis experiment, and 4 week-old rats and mice were used for the hind limb edema and for acetic acid-induced writhing.

Drugs

In the experiment of arthritis, compounds **1**, **2**, **3** and **4** were used (see Fig. 1). In the experiments of carrageenan-induced edema and acetic acid-induced writhing, **3** was used, because of its stability, showed almost no toxicity and exhibited the same potency against arthritis as **4**.

Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were dissolved or suspended in physiological saline or 0.5% carboxymethylcellulose (CMC) (Wako pure Chemical Industries, Osaka, Japan).

Induction of Arthritis and Drug Administration

Bovine type II collagen was purchased from Cosmo-Bio (Tokyo, Japan) and was dissolved in 0.01 M acetic acid at a concentration of 2 mg/ml before use. DBA/1J mice were immunized by intradermal injection at the base of the tail with 100 μg of native collagen emulsified in an equal volume of Freund's complete adjuvant (Difco Labs., Detroit, MI, USA). Three weeks later, mice were boosted by i.p. injection with 100 μg of the same emulsified native collagen. Groups of 5 to 6 mice were treated i.p. with **1**, **3** and **4** (2 mg/kg, 4 mg/kg) 3 times per week (once a day on Monday, Wednesday and Friday) for 6 weeks, beginning at the time of antigen priming. Groups of 5 mice were also treated i.p. with **2** (1 mg/kg, 2 mg/kg) with the same schedule, because the drug at a dose of 4 mg/kg decreased the body weight of mice in a preliminary experiment. Groups of 3 and 7 to 9 mice were used for normal control (normal) and arthritis control (control), respectively. Normal and control mice received vehicle alone in the same manner.

Evaluation of Arthritis

The animals were observed clinically for their characteristic signs and symptoms. In the arthritis experiment, an arthritis score was employed. The mice were observed for clinical arthritis and were scored by grading each paw from 0 to 4 based on erythema and swelling of the joint (0=no erythema or swelling; 1=erythema or swelling of one toe; 2=erythema or swelling of two or more of the toe; 3=erythema and swelling of the entire paw; 4=complete erythema and swelling of the entire paw and incapacity to bend the ankle). As 4 legs were scored, the highest score was 16.

Carrageenan-induced Edema

Groups of 5 to 6 rats were injected s.c. with 0.1 ml of 1% carrageenan solution (Sigma Chemical Co., St. Louis, MO, USA) into the subplantar of region of the right hind paw 30 minutes after the i.p. treatment of **3** (1 mg/kg, 10 mg/kg) or vehicle, or after the i.p. or p.o. treatment of indomethacin (20 mg/kg). The volume of the left hind paw was measured by a volume meter (TK-105, Muromachi-kikai, Co., Tokyo) 2 hours before and at 1 hour intervals. Paw swelling percent was calculated as $[(Vt\text{-}V0)/V0 \times 100]$, where Vt was the paw volume after treatment of the drug and V0 was that before treatment of the drug.

Acetic Acid-induced Writhing

Groups of 10 mice were injected i.p. with 0.7% acetic acid solution in a volume of 0.1 ml/10 g b.w. Compound **3** (1 mg/kg, 10 mg/kg and 30 mg/kg) was administered i.p. 30 minutes before the injection of acetic acid solution. Indomethacin (3 mg/kg) was administered p.o. 1 hour before the injection of acetic acid solution. Control mice also received i.p. vehicle alone in the same manner. The writhings were counted for 10 minutes, starting 5 minutes after the injection of acetic acid solution.

Statistical Analysis

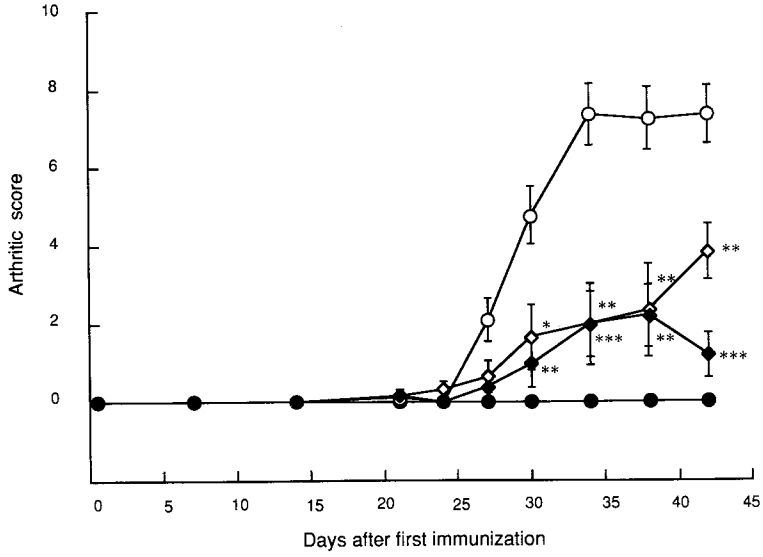
Results of the experiments were expressed as mean $\pm$ S.E. Mann-Whitney U-test (arthritis score) and Student's *t*-test (paw volume and count of writhing) were used to determine the significance of differences between experimental groups, and $P<0.05$ was defined as significant.

Results

Effects of Epoxyquinomicins on Collagen-induced Arthritis

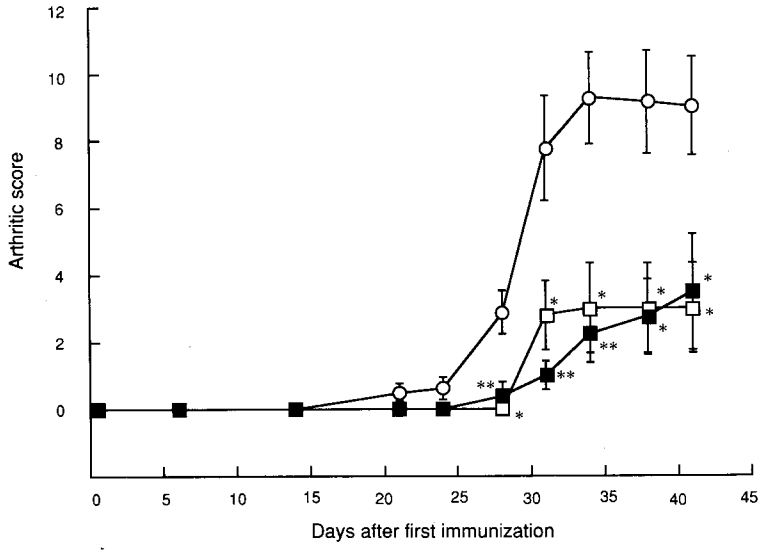
Effects of epoxyquinomicins on type II collagen-induced arthritis are shown in Fig. 2~5. The control groups started to have swelling and erythema in the paw joints at day 21 after antigen priming. The joint lesion in the control groups were evaluated in all the mice, and the arthritis score reached 6.3 to 9.3. Compounds **1** (2 mg/kg, 4 mg/kg), **2** (1 mg/kg, 2 mg/kg), **3** (4 mg/kg) and **4** (2 mg/kg, 4 mg/kg) had potent inhibitory effects on the articular lesions and decreased arthritis score ($P<0.05$, $P<0.01$ or $P<0.001$). Compound **3** (2 mg/kg) tended to reduce the arthritis score. The inhibitory effects of epoxyquinomicins were observed throughout the administration period.

Fig. 2. Effect of epoxyquinomicin A (EQM-A) on arthritic score in collagen-induced arthritic mice.
● Normal, ○ control, ◇ EQM-A 2 mg/kg, ◆ EQM-A 4 mg/kg.



Each value represents the mean ± S.E. significant difference from the control (vehicle): * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Fig. 3. Effect of epoxyquinomicin B (EQM-B) on arthritic score in collagen-induced arthritic mice.
○ Control, □ EQM-B 1 mg/kg, ■ EQM-B 2 mg/kg.



Each value represents the mean ± S.E. significant difference from the control (vehicle): * $P<0.05$, ** $P<0.01$.

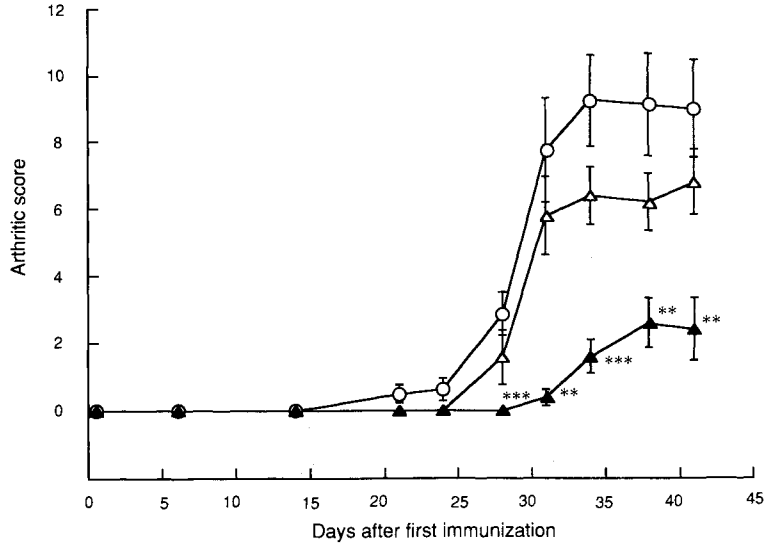
Effect of Epoxyquinomicin C on Carrageenan-induced Edema

The effects of **3** and indomethacin on carrageenan-induced hind edema were examined (Fig. 6). Compound **3** (1 mg/kg, 10 mg/kg) did not suppress progression of the carrageenan-induced hind paw edema. However, indomethacin (20 mg/kg) suppressed the edema.

Effect of Epoxyquinomicin C on Acetic Acid-induced Writhing

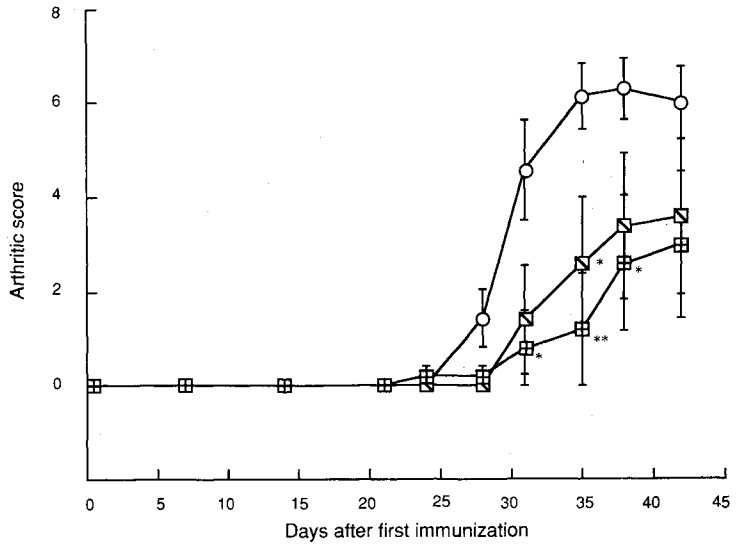
The effects of **3** and indomethacin on acetic acid-induced writhing were also examined (Fig. 7). Although indomethacin (3 mg/kg) decreased the counts of writhing, **3** (1 mg/kg, 10 mg/kg and 30 mg/kg) had no effect on it.

Fig. 4. Effect of epoxyquinomicin C (EQM-C) on arthritic score in collagen-induced arthritic mice.
○ Control, △ EQM-C 2 mg/kg, ▲ EQM-C 4 mg/kg.



Each value represents the mean±S.E. significant difference from the control (vehicle): ** $P<0.01$, *** $P<0.001$.

Fig. 5. Effect of epoxyquinomicin D (EQM-D) on arthritic score in collagen-induced arthritic mice.
○ Control, ⧅ EQM-D 2 mg/kg, ⊞ EQM-D 4 mg/kg.



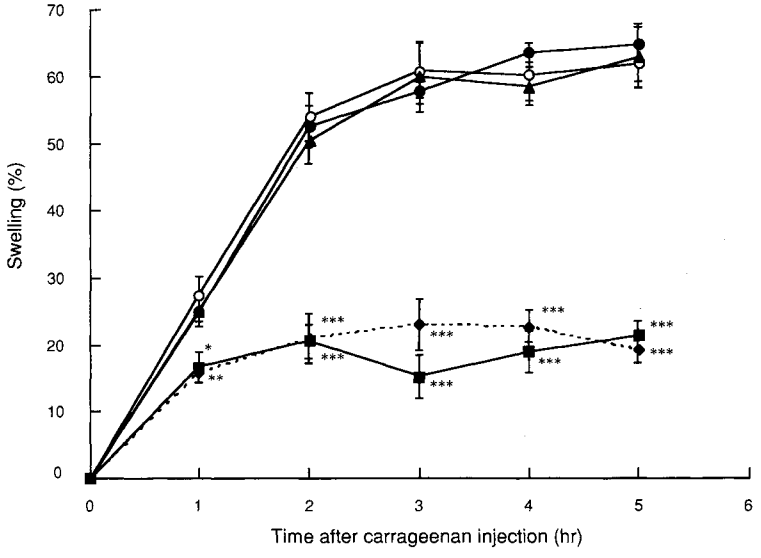
Each value represents the mean±S.E. significant difference from the control (vehicle): * $P<0.05$, ** $P<0.01$.

Discussion

Experimental animal models for arthritis are widely used for the evaluation of anti-rheumatic drugs. It is well known that type II collagen-induced arthritis in mice is a useful model in testing potential therapeutic agents for use in patients with RA, because this arthritic model shares similarities with RA in human patients[5]. In the present experiments, the effects of epoxyquinomicins were studied on type II collagen-induced arthritis in mice. Prophylactic treatment with epoxyquinomicins had potent inhibitory effects on type II collagen-induced arthritis in mice. These results provide evidence that epoxyquinomicins may become useful drugs for the treatment of rheumatoid arthritis (RA).

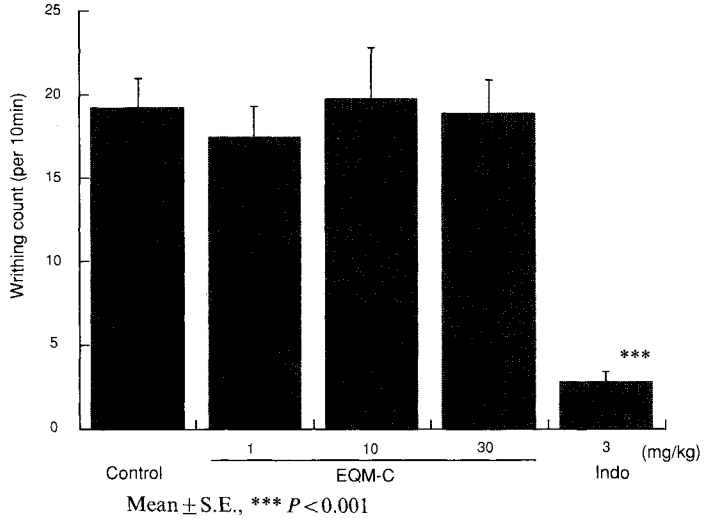
It is well known that not only disease-modifying anti-rheumatic drugs (DMARDs) such as bucillamine and actarit but also nonsteroidal anti-inflammatory drugs

Fig. 6. Effects of epoxyquinomicin C (EQM-C) and indomethacin (Indo) on rat hind edema induced by carrageenan.
○ Control, ● EQM-C 1 mg/kg, ▲ EQM-C 10 mg/kg, ■ Indo 20 mg/kg, ip, ◆ Indo 20 mg/kg, po.



* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Fig. 7. Effects of epoxyquinomicin C (EQM-C) and indomethacin (Indo) on acetic acid-induced writhing in mice.



Mean ± S.E., *** $P<0.001$

(NSAIDs) such as indomethacin exhibit an anti-arthritic effect on type II collagen-induced arthritis[6,7]. However, most DMARDs, unlike NSAIDs, have no acute anti-inflammatory effects on carrageenan-induced paw edema, no analgesic effects on acetic acid-induced writhing in mice, nor inhibitory activity in prostaglandin synthesis *in vitro*. In order to elucidate the mode of action of epoxyquinomicins, we examined the effects of **3** on carrageenan-induced edema in rats and on acetic acid-induced writhing in mice. The present experiment contributed evidence that **3** does not have anti-inflammatory action or analgesic action. In a preliminary experiment, results provided evidence that **3** at 300 μM did not inhibit the enzymatic activity of cyclooxygenase (unpublished data). These results suggest that the mode of the anti-arthritic action of **3** is different from those of NSAIDs.

In summary, epoxyquinomicins had a potent inhibitory effect on type II collagen-induced arthritis. In contrast to NSAIDs, **3** had neither an anti-inflammatory effect on carrageenan-induced edema in rats nor an analgesic effect on acetic acid-induced writhing in mice. These results suggest that the mode of action of epoxyquino-

micins are different from those of NSAIDs and that epoxyquinomicins may become excellent therapeutic agents for RA. The phamacokinetic studies and mode of action are now undergoing.

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