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Low-dose Pulse Methotrexate Inhibits Articular Destruction of Adjuvant Arthritis in Rats

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Abstract

The purpose of this study was to determine whether low-dose methotrexate pulse therapy, which had recently become important in the treatment of rheumatoid arthritis, was effective for controlling the progression of articular destruction in rats with adjuvant arthritis.

Intraperitoneal methotrexate at a dose of 0·05 or 0·1 mg kg^{-1} twice weekly inhibited inflammation in rats with adjuvant arthritis, as shown by reduction of the hind-paw volume. Methotrexate also inhibited articular destruction, as shown by X-ray findings.

Although the mechanisms by which low-dose pulse methotrexate acts on rat adjuvant arthritis are still unclear, our results imply that it might effectively slow the progression of articular destruction in rheumatoid arthritis in man. In addition, assessment of articular destruction in this animal model might be useful when evaluating new treatments for rheumatoid arthritis.

Low-dose methotrexate pulse therapy for rheumatoid arthritis has recently attracted attention because of its high efficacy (Furst & Kremer 1988). The major response to methotrexate therapy is an obvious improvement of the inflammatory process, but whether there is an inhibitory effect on the progression of articular destruction in rheumatoid arthritis is still controversial (Nordstrom et al 1987; Herborn et al 1991). In addition, pharmacological examination, especially for the pulse administration of methotrexate in animal arthritis models, has not been elucidated sufficiently.

Although rat adjuvant arthritis has been used in pre-clinical studies as one of the standard animal models of rheumatoid arthritis in man for evaluation of anti-inflammatory agents (Kerwar & Oronsky 1989), almost all studies using this animal model have only measured degree of articular inflammation. In this study, we thus investigated the effect of low-dose pulse methotrexate on the progression of articular destruction.

Materials and Methods

Induction of adjuvant arthritis

Inbred female Lewis rats, 180–200 g, were purchased from Nippon S. L. C. (Hamamatsu, Japan). Standard laboratory chow (Japan Kurea, Tokyo, Japan) and water were freely available. To produce an adjuvant mixture, dried heat-killed *Mycobacterium butyricum* (Sigma, St Louis, MO, USA; 10 mg) was mixed with heavy mineral oil (Sigma; 10 mL) and sonicated.

Each rat was immunized in the left hind foot-pad with 0·1 mL of the adjuvant mixture and all injected control rats developed polyarthritis. The rat hind-paw volume was measured just before injection and twice weekly from 1 day after adjuvant immunization by use of a MK-550 Volume Meter (Muromachi Machine, Tokyo, Japan).

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Administration of methotrexate

Methotrexate (Lederle (Japan)) doses of 0·025, 0·05, or 0·1 mg kg^{-1} were suspended in 0·5% carmellose (Wako Chemicals, Tokyo, Japan) and administered intraperitoneally twice weekly from 1 day after adjuvant immunization. In the control group, 0·5% carmellose alone was administered in the same schedule as methotrexate administration.

Hind-paw radiography

Rats were killed 22 days after adjuvant injection. The lower extremities were resected and were immediately fixed in 10% formalin. The limbs were then positioned over a cassette containing Kodak X-ray film and radiographs were obtained with a conventional microradiographic unit (SCMB-12, Softex, Tokyo, Japan) at 35 kV and 6 mA for 60 s.

Each hind-paw radiograph was evaluated by the method of Clark et al (1979) with some modifications. Briefly, four features, bone demineralization, bone erosion, periostitis and phalanges alignment, were evaluated individually and blindly by three researchers using grades of 0 to 4 (with 0 indicating normal and 4 indicating severe changes) for each feature. The sum of these four findings, defined as articular score, was then obtained for each hind-paw. The minimum and maximum articular scores were 0 and 16, respectively, and the mean score for each hind-paw was calculated from the evaluations of the 3 researchers.

Statistical analysis

Multiple Student's *t*-tests with Bonferroni's correction and a Dunnett's multiple range test were used for comparison among the different groups of rats for the data shown in Figs 1 and 2 and Table 1, respectively. A probability value of less than 0·05 was taken to indicate a significant difference.

Results

Hind-paw volume

The effects of various doses of methotrexate on adjuvant-injected left hind-paw inflammation are shown in Fig. 1. The mean left hind-paw volume increased equally in all groups of rats for 8 days after adjuvant injections (first-phase inflammation). Subsequently, the inhibitory effect of twice-weekly methotrexate on the increment of left hind-paw volume was significantly greater at 0·1 mg kg^{-1} than at 0·025 mg kg^{-1} ($P < 0·05$).

The effect of methotrexate administration on right hind-paw inflammation is shown in Fig. 2. There was no difference in the increment of right hind-paw volumes between control and 0·025 mg kg^{-1} groups. Twice-weekly methotrexate at both 0·05 and 0·1 mg kg^{-1}, however, significantly inhibited the increment of right hind-paw volume when compared with the control or 0·025 mg kg^{-1} groups.

Radiographic changes

Table 1 shows the effect of methotrexate on articular destruction in the hind-paws of the rats. At 0·025 mg kg^{-1} twice weekly methotrexate did not inhibit the progression of articular destruction. A twice-weekly dose of 0·1 mg kg^{-1}, on the other hand, significantly inhibited the progression of articular destruction in both hind-paws, as shown by the reduction of scores for most individual items and the articular score. At 0·05 mg kg^{-1} twice weekly, methotrexate had a non-significant inhibitory effect on the left hind-paw; it did, however, significantly inhibit the progression of articular destruction in the right hind-paw.

Representative radiographs of the hind-paws of rats from the control and 0·1 mg kg^{-1} twice-weekly methotrexate groups are shown in panels a and b, respectively, of Fig. 3.

Discussion

This study has demonstrated that low-dose pulse methotrexate administration at levels comparable with the usual therapeutic doses in patients with rheumatoid arthritis (Furst & Kremer 1988) inhibited the progression of articular destruction in rat adjuvant arthritis. Welles et al (1985) previously reported the efficacy of low-dose (0·15–0·6 mg kg^{-1} week^{-1}) methotrexate for rat adjuvant arthritis, but they performed only visual evaluation of hind-paw swelling. In this study, we demonstrated not only the anti-inflammatory effect of methotrexate (reduced

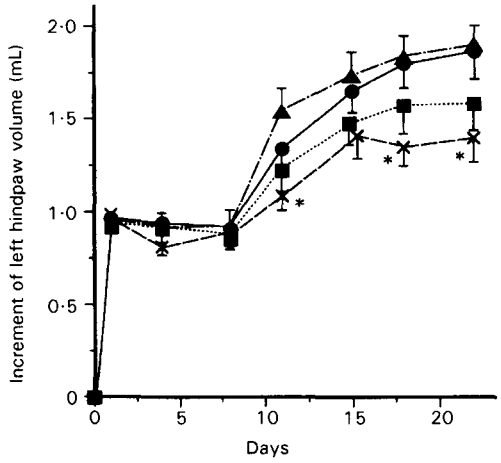

FIG. 1. Effect of various doses of methotrexate on the increment of left (adjuvant-treated) hind-paw volume. Methotrexate at a dose of 0 (control, ●), 0·025 (▲), 0·05 (■), or 0·1 (X) mg kg^{-1} was administered intraperitoneally twice weekly from 1 day after adjuvant immunization. Each point represents the mean ± s.e. (n = 7). *$P < 0·05$ compared with 0·025 mg kg^{-1} by multiple Student's *t*-tests with Bonferroni's correction on the same day of observation.

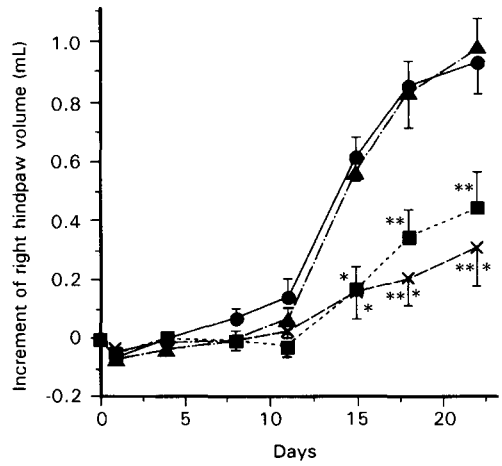

FIG. 2. Effect of various doses of methotrexate on the increment of right (untreated) hind-paw volume. Methotrexate at a dose of 0 (control, ●), 0·025 (▲), 0·05 (■), or 0·1 (×) mg kg^{-1} was administered intraperitoneally twice weekly from 1 day after adjuvant immunization. Each point represents the mean ± s.e. (n = 7). *$P < 0·05$ compared with control and **$P < 0·05$ compared with 0·025 mg kg^{-1} by multiple Student's *t*-tests with Bonferroni's correction on the same day of observation.

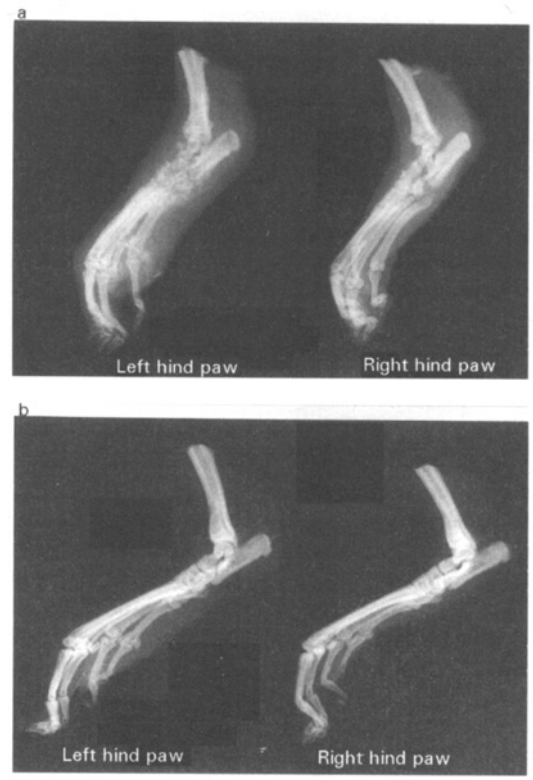

FIG. 3. Representative radiographs of rat hind-paws 22 days after adjuvant injection. a, Control left and right hind-paws showing severe soft tissue swelling and articular destruction. b, Left and right hind-paws of a rat given 0·1 mg kg^{-1} of methotrexate twice weekly showing marked inhibition of soft tissue swelling and articular destruction as compared with the control.

Table 1. Effect of low-dose methotrexate on articular destruction in the hind-paws of rats with adjuvant arthritis.

Methotrexate dose ($mg\,kg^{-1}$)	X-ray analysis (grade)				
	Bone demineralization	Erosion	Periostitis	Phalanges alignment	Articular score
Left hind-paw					
0	2·8 ± 0·3	3·1 ± 0·3	2·9 ± 0·4	2·7 ± 0·4	11·2 ± 1·2
0·025	3·0 ± 0·3	3·3 ± 0·2	3·1 ± 0·3	3·0 ± 0·2	12·4 ± 0·8
0·05	2·1 ± 0·3	2·4 ± 0·3	2·2 ± 0·4	1·8 ± 0·2	8·9 ± 1·2
0·1	1·8 ± 0·2*	2·0 ± 0·3*	1·7 ± 0·3*	1·9 ± 0·3	7·2 ± 1·1*
Right hind-paw					
0	2·1 ± 0·4	2·1 ± 0·5	1·6 ± 0·4	1·8 ± 0·5	7·6 ± 1·7
0·025	2·4 ± 0·3	2·2 ± 0·5	1·8 ± 0·4	1·4 ± 0·1	7·9 ± 1·1
0·05	1·2 ± 0·3	0·8 ± 0·2*	0·7 ± 0·1	0·2 ± 0·2**	2·9 ± 0·7*
0·1	1·0 ± 0·2*	0·8 ± 0·3*	0·8 ± 0·3	0·2 ± 0·2**	2·9 ± 0·8*

Data from the same batch of rats as used for Figs 1 and 2. At the end of 22 days the rats were killed and the hind-paws were subjected to radiographic analysis. Grade 0 indicates normal and grade 4 indicates maximum destruction for each item. Values represent the mean ± s.e. (n = 7). $*P < 0.05$, $**P < 0.01$ compared with control (Dunnett's multiple range test).

hind-paw volume) but also an inhibitory effect on articular destruction in a rat model of human rheumatoid arthritis.

Although various cytokines might influence articular destruction in rheumatoid arthritis in man, interleukin-1 and tumour necrosis factor (TNF) α seem to make a major contribution to tissue damage by induction of the release of proteolytic enzymes from synovial cells and chondrocytes (Arend & Dayer 1995). Connolly et al (1988) reported that methotrexate and non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and phenylbutazone, both significantly inhibited paw inflammation in adjuvant arthritis, but only methotrexate reduced interleukin-1 production by splenic leukocytes and reduced plasma acute phase reactants. Production of interleukin-1 by peritoneal macrophages from adjuvant-immunized rats is also reduced by methotrexate administration (Hu et al 1988). Interleukin-1 stimulates bone resorption and inhibits bone formation (Tatakis 1993), while also promoting cartilage damage in animal arthritis models (van de Loo et al 1995). Thus, inhibition of interleukin-1 by methotrexate might directly reduce bone destruction in rat adjuvant arthritis. Methotrexate is reported to reduce articular TNF in adjuvant arthritis, whereas ibuprofen, an NSAID, failed to reduce articular TNF levels significantly (Smith-Oliver et al 1993). Although the mechanisms of action of methotrexate on rat adjuvant arthritis still remain unclear, these reports suggest that reversal of the overproduction of cytokines, such as interleukin-1 and TNF might play a crucial role in the inhibition of inflammation and articular destruction observed in our study. Segal et al (1990) have also suggested that methotrexate has a major effect on the cascade of events initiated by cytokines such as interleukin-1, interleukin-6, and TNF α in patients with rheumatoid arthritis.

Recent clinical studies have shown that low-dose methotrexate pulse therapy results in clinical improvement of rheumatoid arthritis; it has not, however, been clearly demonstrated whether this therapy actually inhibits articular destruction (Furst & Kremer 1988). Our present results imply that low-dose pulse methotrexate therapy might prevent the progression of articular destruction in rheumatoid arthritis in man. It also appears that X-ray observation of articular damage in rat adjuvant arthritis might be a good method for pre-clinical evaluation of new treatment for rheumatoid arthritis.

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