

CONCISE REPORT

Inhibition of arthritis by systemic administration of endostatin in passive murine collagen induced arthritis

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Objective: To investigate the arthritis inhibiting effect of endostatin, known to have potent antiangiogenic activity, systemically given to animal models of rheumatoid arthritis (RA).

Methods: Four kinds of monoclonal anti-type II collagen antibody followed by lipopolysaccharide (LPS) three days later were given to 6 week old, female Balb/c mice to induce arthritis. Three groups of mice received 0.2 mg/kg/day, 2 mg/kg/day, and 10 mg/kg/day of endostatin, respectively, whereas a control group received phosphate buffered saline (PBS). Endostatin or PBS was given for 13 days, starting before the development of arthritis. Arthritis was evaluated by arthritis scores and hind paw thicknesses. Mice were killed for histological examination on the 22nd day after the administration of monoclonal anti-type II collagen antibody.

Results: Arthritis developed within three days after LPS administration in both the control and endostatin treatment groups. No difference in the development rate of arthritis was noted between the control and endostatin treatment groups. Arthritis scores remained significantly lower in the endostatin 10 mg/kg/day group than in the control group. Hind paw thicknesses also remained significantly smaller in the endostatin 10 mg/kg/day group than in the control group. Histopathological examination showed that synovial thickening and subchondral bone erosion improved more in the endostatin treatment groups than in the control group.

Conclusion: The systemic administration of endostatin had an arthritis inhibiting effect in RA animal models. Endostatin inhibited, in particular, pannus formation and bone destruction.

A few studies have reported the arthritis inhibiting effects of neovascularisation inhibitors given to rheumatoid arthritis (RA) animal models.^{1–3} Endostatin was isolated from the supernatant of mouse endothelioma cell cultures during screening for endogenous neovascularisation inhibitors produced by tumour cells using endothelial growth inhibitory activity as an indicator.⁴ Subsequent studies have shown that endostatin is part of the C terminal non-collagen region of type XVIII collagen.⁴ Matsuno *et al* reported that endostatin induced regression of human RA derived synovial tissue that had been implanted in SCID mice.⁵ Yin *et al* reported that arthritis was improved by the direct injection into the joint of arthritic mice of lentiviral vectors that had been genetically engineered to express the endostatin gene.⁶ Also, a report indicated that patients with RA actually had an imbalance in endostatin.⁷ These reports show the potential of endostatin for use as a therapeutic agent for arthritis. However, as far as we know, no studies have reported the therapeutic effect of the systemic administration of endostatin. Thus, in this study, we systemi-

cally administered endostatin to arthritic mice to see whether the arthritis improved or not.

MATERIALS AND METHODS

Purification of mouse recombinant endostatin

Human kidney cells (293-EBNA) expressing mouse endostatin were cultured.⁸ When they grew to confluence, they were cultured in serum-free medium, and mouse endostatin was purified from conditioned medium as previously described.⁸

Production of experimental arthritis and its evaluation

Four kinds of 2 mg monoclonal anti-type II collagen antibody (IBL Co, Gunma, Japan) were given intravenously (day 1) to 6 week old female Balb/c mice (Charles River Japan, Inc, Kanagawa, Japan). Three days later (day 4), 50 µg lipopolysaccharide (LPS) was given intraperitoneally to produce arthritis.⁹ Arthritis was graded as 0 (no swelling), 1 (mild swelling and redness), 2 (marked swelling or oedema), or 3 (ankylosis), and was evaluated based on the sum of the scores for the forepaws and hind paws.

Dosing of endostatin

Endostatin was given for 13 days (days 1–13) starting on the day of administration of monoclonal anti-type II collagen antibodies. Three groups of mice received 0.2 mg/kg/day (n=10), 2 mg/kg/day (n=10), and 10 mg/kg/day (n=4), respectively. These doses of endostatin were given subcutaneously into the back daily in two divided doses (in the morning and evening). A control group (n=10) received the same volume of phosphate buffered saline (PBS).

Histological examination of the foot joint

On day 22 after the administration of monoclonal anti-type II collagen antibody, mice were anaesthetised with diethylether; the right and left paws were removed, fixed in 10% formalin and embedded in paraffin. Sections were cut from the central portion of the talotibial joint and stained with haematoxylin and eosin. Histological features of periarticular inflammation (extent of inflammatory infiltration), synovial thickening (pannus formation with mesenchymal cell proliferation), and subchondral bone erosion were graded as 0 (normal), 1 (mild), 2 (moderate), or 3 (severe) in a double blind manner by pathologists. The sum of the grades for these features was referred to as the histological score.

Statistical analysis

Intergroup differences in time dependent changes in arthritis scores and hind paw thicknesses were assessed by repeated measure analysis of variance (ANOVA). Considering multiplicity, Scheffe's test was used to detect differences between time

Abbreviations: ANOVA, analysis of variance; LPS, lipopolysaccharide; PBS, phosphate buffered saline; RA, rheumatoid arthritis

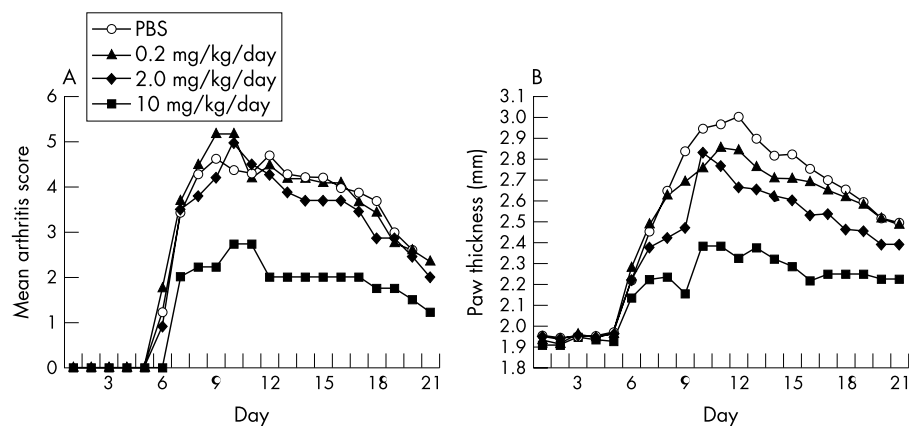


Figure 1 Time dependent changes in mean arthritis scores and hind paw thicknesses.

points. The Kruskal-Wallis test was used to compare differences in histological scores between the four groups. For post hoc tests to compare the control PBS group and each treatment group, Bonferroni's method was used, where p values $\leq 0.05/3$ ($=0.017$) were considered significant.

RESULTS

Arthritis was seen in the control mice from around day 3 after LPS administration. Arthritis developed in 9/10, 9/10, and 4/4 mice in the endostatin 0.2 mg/kg/day, 2 mg/kg/day, and 10 mg/kg/day groups, respectively, whereas development of arthritis in the control group was 100%. In this study, endostatin administration was started before the onset of arthritis, but did not decrease its onset rate.

Similar to the control group, the endostatin treatment groups developed arthritis around day 3 after LPS administration. The severity of arthritis was evaluated using two parameters. Figures 1A and 1B show changes in the mean arthritis score and in the mean paw thickness in each group, respectively. At the same time, the hind paw thickness and arthritis score in each mouse were analysed by the repeated measure ANOVA method, which showed significant differences in the time dependent change patterns of the arthritis score and hind paw thickness between the four groups. Furthermore, for detailed analysis of change patterns in each group, differences between time points were analysed by Scheffe's method. As a result, the 10 mg/kg/day group alone showed no difference between time points; specifically, in contrast with the other three groups, the 10 mg/kg/day group showed little time dependent change in the arthritis score as well as the hind paw thickness.

Next, the talotibial joint was examined histopathologically on the last day of the experiment. Histopathological data were analysed using histological scores. The Kruskal-Wallis test was used to compare differences between the four groups, followed by Bonferroni's method to compare the control group with each treatment group. As a result, the p values for the control group *v* the 0.2 mg/kg/day, 2 mg/kg/day, and 10 mg/kg/day groups were 0.072, 0.060, and 0.008, respectively, indicating a significant difference between the control and 10 mg/kg/day groups (fig 2A).

Synovial thickening, a parameter in the histological score, tended to be inhibited in a dose dependent manner in the endostatin treatment groups (fig 2B), with a significant difference between the control and 10 mg/kg/day groups. The same was true of subchondral bone erosion (fig 2C). Although periarticular inflammation tended to be somewhat inhibited in the 10 mg/kg/day group, the difference was not significant (fig 2D).

DISCUSSION

In this study, endostatin known to have potent antiangiogenic activity, was given to mice with experimental arthritis, and its

arthritis inhibiting effect was investigated. Except for endothelial cells, the actions of endostatin, particularly on immune system cells, have hardly been investigated. Thus, to evaluate the arthritis inhibiting effect of endostatin, we selected a model of passive experimental arthritis which would probably not involve immune system cells—that is, an experimental system to induce arthritis with four kinds of monoclonal antibody specific for type II collagen. Because 10–20 mg/kg/day of endostatin has been reported to exert an antitumour effect, we used doses up to 10 mg/kg/day in the experiment. An experimental study injecting recombinant human endostatin into mice subcutaneously reported its half life in blood to be 10 hours.¹⁰ Thus, endostatin was given subcutaneously twice daily to maintain its levels in blood. The administration was started before the onset of arthritis, and its onset rate and severity were observed.

We speculate that 10 mg/kg/day of endostatin does not have the effect of reducing the onset rate of arthritis. However, this dose reduced the severity of arthritis, which was confirmed statistically. Endostatin at a dose of 0.2 mg/kg/day did not have an arthritis inhibiting effect. The dosage of 2 mg/kg/day produced no difference in arthritis scores, but slightly reduced paw thicknesses. Although 2 mg/kg/day may inhibit arthritis to some degree, it could not be confirmed statistically. Histopathologically, synovial thickening and subchondral bone erosion tended to be inhibited in the 10 mg/kg/day group in comparison with the control group. To ascertain this, a histological grading system was used. As a result, the 10 mg/kg/day group differed significantly from the control group. Among the three parameters in the histological score, synovial thickening and subchondral bone erosion were inhibited in a dose dependent manner in the endostatin treatment groups; in particular, synovial thickening in the 10 mg/kg/day group significantly differed from that of the control group. On the other hand, periarticular inflammation was somewhat decreased in the 10 mg/kg/day group compared with the control group, but there was no significant difference among the 0.2 mg/kg/day, 2 mg/kg/day, and the control groups. It should be further investigated whether endostatin inhibits periarticular inflammation. These results suggest that endostatin inhibits pannus formation and bone destruction.

Mice were observed for other side effects produced by the systemic administration of endostatin, in addition to arthritis, but no mice died or developed disease symptoms other than arthritis, which was considered to be an advantage of using endostatin as a drug.

Although the maximum dose in this experiment was 10 mg/kg/day, it needs to be further investigated whether this dose has the maximum arthritis inhibiting effect. In an experiment expecting to achieve an antitumour effect, doses higher than 10 mg/kg/day were given. An administration method using an osmotic pump to maintain blood levels has recently been tried, enabling a reduction of the dose to one

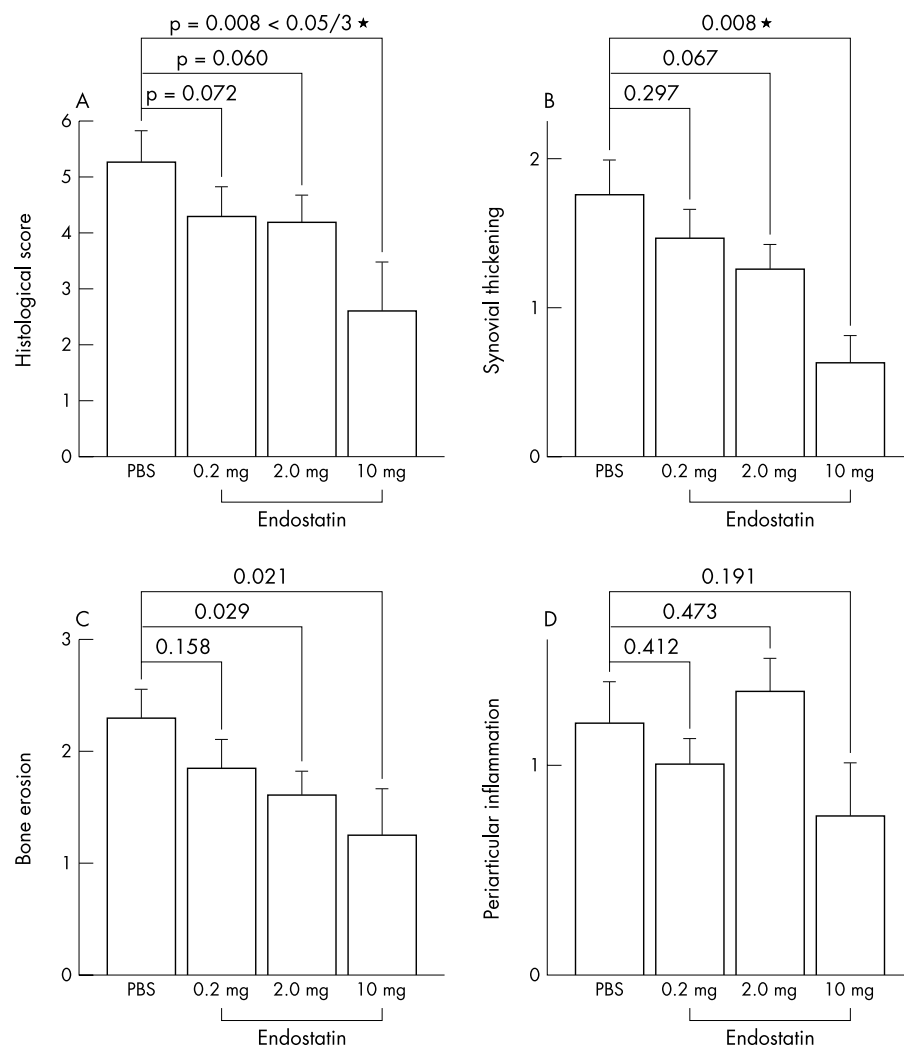


Figure 2 Comparison of the histological scores in the endostatin treatment groups and the control group.

fifth¹¹ Such an administration method should be tested in the future. This experiment used only a preventive protocol, but a therapeutic protocol needs to be studied in the future. Much study of whether endostatin administration can be a new treatment for inhibiting arthritis will be needed in the future. However, we consider that the systemic administration of endostatin is a strong candidate for a new therapeutic strategy for arthritis because of its low toxicity.

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