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NOTE

Suppression of type II collagen-induced arthritis by ICM0301B, a new angiogenesis inhibitor

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The anti-arthritic effect of ICM0301B, a new angiogenesis inhibitor, was examined using a type II collagen-induced arthritis model for human arthritis in DBA/1J mice. ICM0301B by s.c. injection (100 mg kg^{-1} per day) suppressed not only body weight reduction and swelling of limbs but also radiographic changes such as bone lesions. The efficacy of ICM0301B was approximately equal to that of a non-steroidal antiinflammatory drug, indomethacin (1 mg kg^{-1} per day). As ICM0301B inhibits angiogenesis in a rat aorta organ culture model and growth of human umbilical vain endothelial cells induced by basic fibroblast growth factor, the observed anti-arthritic effect of ICM0301B might be partly attributed to the anti-angiogenic activity.

ICM0301A and B(Figure 1)were found in the culture of a fungus in the course of screening of anti-angiogenic compounds. ICM0301A and B inhibited specifically not only the growth of human umbilical vain endothelial cells induced by basic fibroblast growth factor but also angiogenesis in a rat aorta organ culture model.[1,2]

Human rheumatoid arthritis is a chronic inflammatory joint disease, which is characterized by proliferation of synoviocytes and massive infiltration of leukocytes.[3,4] For the treatment of rheumatoid arthritis, non-steroidal anti-inflammatory drugs and steroids have been used, though their therapeutic effects are not satisfactory. The disease modifying anti-rheumatoid agents has also reported to show therapeutic effects on rheumatoid arthritis by improving the immunological abnormalities of this disease.[5–7] However, disease modifying anti-rheumatoid agents are more or less ineffective in the late phase of the disease and adverse effects often limit their use. Recently, biological drugs such as antibodies against TNF-α and IL-6 have been used for the therapies of rheumatoid arthritis.[8–10] Angiogenesis might be one of targets for the development of antirheumatoid drugs, as angiogenesis is related to the development of rheumatoid arthritis.[11,12] Therefore, we examined the antirheumatoid activity of IMC0301B, an angiogenesis inhibitor using a mouse arthritic model.

Induction of arthritis by type II collagen was performed as follows: Bovine type II collagen (Cosmo-Bio, Tokyo, Japan) was dissolved in 0.01M acetic acid at a concentration of 2 mg ml^{-1} before use. DBA/1J mice (10-weeks old, female, Charles Liver Japan) were immunized by intradermal injection at the base of the tail vain with 100 μg of native collagen emulsified in an equal volume of Freund's complete adjuvant (Difco Labs., Detroit, MI, USA) on day 0. On day 20, antigen production was boosted by an i.p. injection with 100 μg of the same emulsified native collagen. Groups of 10 mice were treated subcutaneously (100 mg kg^{-1}) with ICM0301B once daily for 2 weeks (day 21–34) and intraperitonealy with indomethacin (1 mg kg^{-1}) once daily for 4 weeks (day 21–48) with the exception of Sunday. Control mice immunized with collagen were similarly treated with vehicle only. Normal mice, which were not immunized with collagen, were treated with vehicle alone. As ICM0301B had high hydrophobicity, ICM0301B was resolved in saline with a non-ionic surfactant, Cremophor EL (BASF Co., Germany) for s.c. injection.

Macroscopic evaluation of arthritis was performed as follows: Animals were observed clinically for their characteristic signs and symptoms, which were scored by grading each paw from 0−4 according to the following criteria: 0= no erythema or swelling of the joint; 1= erythema and swelling of one toe; 2= erythema or swelling of two toes or more; 3= erythema and swelling of the entire paw; 4= complete erythema and swelling of the entire paw and incapacity to bend the ankle. The volume of swelling was also measured on both hind paws with a foot volume meter (7140, LMS Co. Ltd, Japan) and the mean volume of right and left paws was calculated.

Radiographic evaluation of arthritis was performed as follows: Skeletal changes were radiographically examined with a soft X-ray unit (μFX-1000, Fujifilm Co. Ltd, Japan) at the end of the experiment (day 59). Bone changes in the calcaneus, talus, metatarsus, tarsal bones, and proximal phalanges of both hind limbs were grades on a

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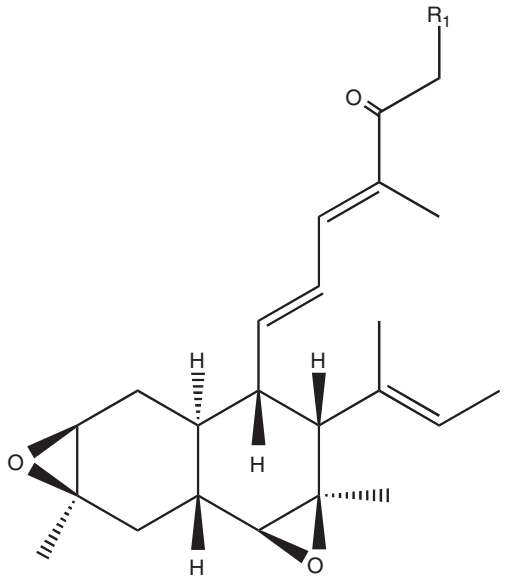

ICM0301A $R_1=CH_3$

ICM0301B $R_1=H$

Figure 1 Structure of ICM0301A and B.

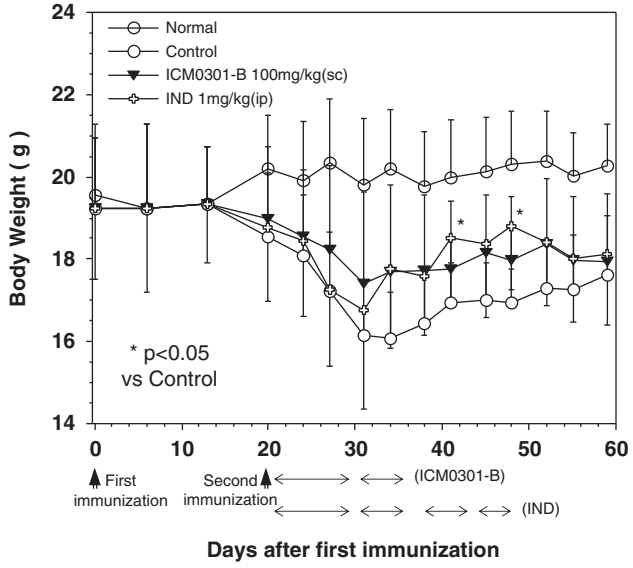

Figure 2 Effect of ICM0301B on body weight in collagen- induced arthritic mice.

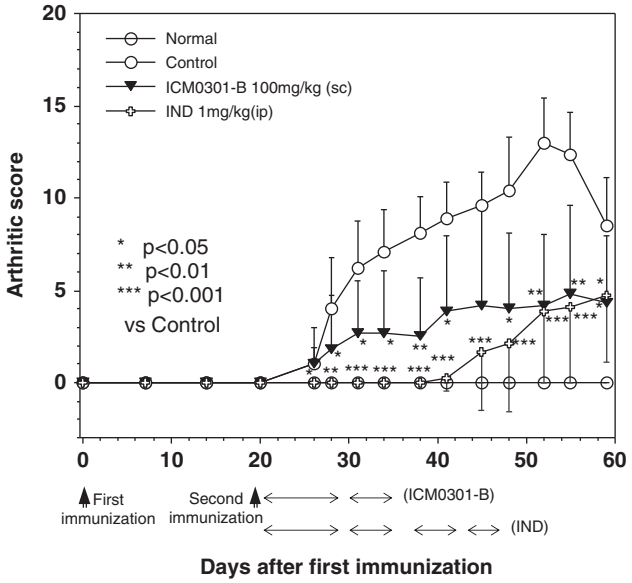

Figure 3 Effect of ICM0301B and indomethacin on arthritic score in collagen-induced arthritic mice.

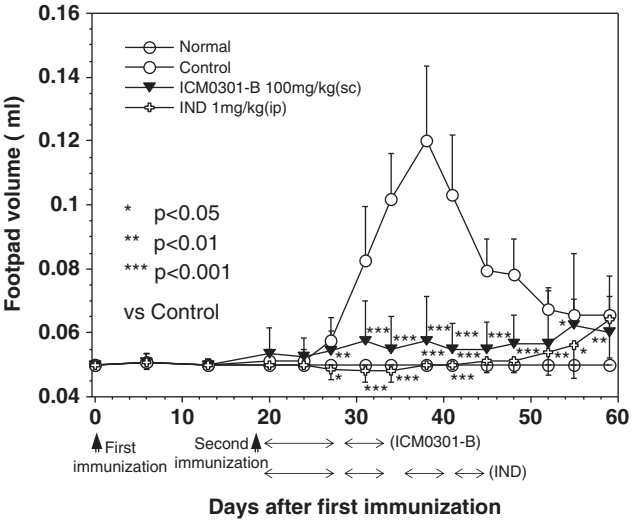

Figure 4 Effect of ICM0301B and indomethacin on paw swelling in collagen-induced arthritic mice.

scale of 0–3 according to the following criteria: 0 = no change; 1 = slight destruction and decreased density of bone; 2 = moderate destruction and decreased density of bone; 3 = severe destruction and decreased density of bone. For each animal, the total score was calculated by summing up all the bone score.

The body weight of mice was monitored in each experimental group. In all the groups treated with collagen, body weight gains were suppressed as compared with normal group, and the body weight decreased immediately after the second immunization on day 20 (Figure 2). Significant differences were observed in the body weight between control and treated group with indomethacin at day 41 and 48, though similar differences in the body weight between control and treated group with ICM0301B was found.

Arthritic score and food pad volume were monitored in each experimental group. In control group, erythema and swelling of the paw joints started to appear after antigen priming (day 20) and the arthritic score (Figure 3) and paw swelling (Figure 4) reached the maximum on day 52 and 38, respectively. IMC0301B and indomethacin suppressed significantly both arthritic score and paw swelling. Suppression of arthritic score and paw swelling by indomethacin was stronger than that of IMC0301B. The period of suppression by ICM0301B had continued after stopping of administration (day35). On the other hand, the suppression in arthritic

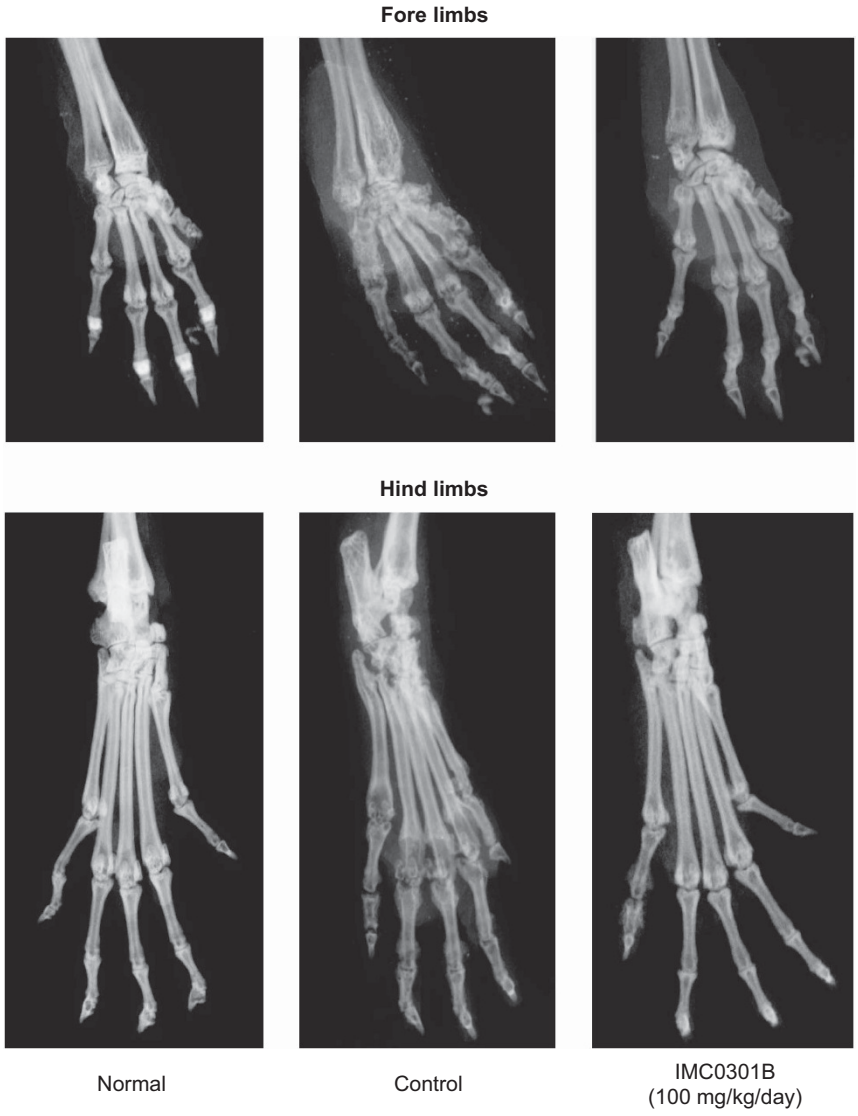

Figure 5 Representative radiographs of limbs from normal, control and ICM0301B treated mice.

score and paw swelling by indomethacin was reduced after day 45 and day 52, respectively in spite of continuing administration.

Radiographic analysis was examined in each experimental group. IMC0301B markedly suppressed bone lesions (Figures 5 and 6) as well as arthritic score and paw swelling. Indomethasin also suppressed bone lesions.

In other same experiment using this arthritic model, ICM0301B exerted same anti-arthritic effect as mentioned above. I.v. injection of IMC0301B (25 mg kg^{-1} per day) failed to suppress arthritis. Its high instability in the body by metabolism (data not shown) partially causes the inefficacy of IMC0301B by i.v. injection. Gradual absorption of IMC0301B by s.c. injection may cause its revelation of anti-arthritic effect. Acute toxic symptoms by i.p. and i.v. injection (100 mg kg^{-1}) of IMC0301B were not founded.

It is interesting that the anti-arthritic effect of IMC0301B had continued after the end of its administration. Although the precise mechanism responsible for the anti-arthritic action of IMC0301B remains unclear, the beneficial effect of ICM0301B might be attributed to its antiangiogenic activities.

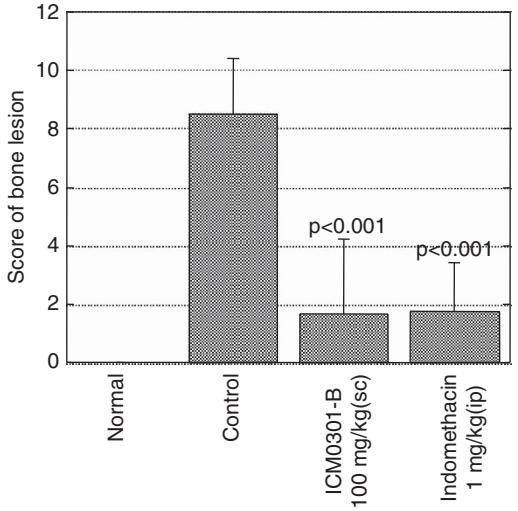

Figure 6 Effect of ICM0301B and indomethacin on total bone score in collagen-induced arthritic mice.

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