Regular Article

JTE-952 Suppresses Bone Destruction in Collagen-Induced Arthritis in Mice by Inhibiting Colony Stimulating Factor 1 Receptor

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial inflammation and structural destruction of the joints. Bone damage occurs in an early stage after onset and osteoclast activation plays a substantial role in its progression. Colony stimulating factor 1 receptor (CSF1R) is a receptor protein tyrosine kinase specifically expressed in monocytic-lineage cells such as macrophages and osteoclasts. Here, we investigated the effect of JTE-952, a novel CSF1R tyrosine kinase inhibitor, on osteoclast formation in vitro and on bone destruction in a mouse model of collagen-induced arthritis. JTE-952 completely inhibited osteoclast differentiation from human monocytes, with an IC50 of 2.8 nmol/L, and reduced osteoclast formation from the synovial cells of RA patients. Detectable levels of colony stimulating factor 1 (CSF1), a ligand of CSF1R, were observed in the synovial tissues of the arthritis model, similar to those observed in the pathology of human RA. JTE-952 significantly suppressed increases in the bone destruction score, the number of tartrate-resistant-acid-phosphatase-positive cells, and the severity of arthritis in the model mice. We also examined the efficacy of JTE-952 combined with methotrexate. This combination therapy more effectively reduced the severity of bone destruction and arthritis than monotherapy with either agent alone. In summary, JTE-952 potently inhibited human osteoclast formation in vitro and suppressed bone destruction in an experimental arthritis model, especially when combined with methotrexate. These results indicate that JTE-952 should strongly inhibit bone destruction and joint inflammation in RA patients and effectively prevent the progression of the structural destruction of joints.

Key words JTE-952; colony stimulating factor 1 receptor; osteoclast; bone destruction; rheumatoid arthritis; collagen-induced arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by synovial inflammation and progressive structural destruction of the cartilage and bone in multiple affected joints.1 It is widely acknowledged that the progression of joint damage occurs in an early stage, soon after onset.2–5 The pathological processes of joint damage are influenced by direct or indirect interactions with synovial cells and a wide range of cell types associated with the adaptive and innate immune systems and are mediated by inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin 1 (IL1), IL6, IL17, receptor activator of nuclear factor-kappaB ligand (RANKL), and matrix metalloproteinase.5,6

In clinical practice, traditional disease-modifying antirheumatic drugs (DMARDs) such as methotrexate (MTX) are frequently used to suppress joint inflammation in the treatment of RA and attenuate disease progression.7 Biological DMARDs such as etanercept (a soluble TNF-α receptor) and tocilizumab (an anti-IL6 receptor antibody), which inhibit disease activity and lead to remarkable clinical improvement, have been approved.8,9 However, these agents are not always effective in all patients with RA, and even if joint inflammation is controlled, bone destruction gradually progresses in the long term, which results in the irreversible structural destruction of the joints,10–13 and these patients may ultimately require orthopedic treatments, such as artificial joints. Therefore, drugs that effectively inhibit bone destruction and prevent the structural destruction of the inflamed joints may satisfy unmet needs in terms of improving of QOL in RA patients.

Colony stimulating factor 1 receptor (CSF1R) is a receptor protein tyrosine kinase specifically expressed in monocytic-lineage cells, including monocytes, dendritic cells, and macrophages. Osteoclasts, which are large multinucleated cells responsible for the dissolution and absorption of bone, differentiate from monocytic-lineage cells, and the activation of CSF1R signals increases the number of the osteoclasts formed.14,15 The levels of colony stimulating factor 1 (CSF1), a ligand of CSF1R, are higher in the peripheral blood or synovial fluid of RA patients than in that of healthy subjects or patients with other joint diseases, such as osteoarthritis.16 Recently, IL34 was identified as a second ligand of CSF1R.17,18 IL34 is also expressed in the synovial tissues and sera of RA patients,19,20 although its role in arthritis is not fully understood. Therefore, osteoclast differentiation and activation, which are induced by signals from CSF1R, are thought to occur in the joint lesions of RA patients and may be respon-

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sible for the progression of bone destruction and consequent joint damage. Numerous small-molecule CSF1R inhibitors have been developed that inhibit both joint inflammation and inflammatory bone erosion in animal models of arthritis, but these are limited by poor kinase selectivity.

JTE-952 is a novel, orally available type II kinase inhibitor of CSF1R developed by Japan Tobacco Inc. JTE-952 clearly inhibits human CSF1R kinase activity (at an IC₅₀ of 11.1 nmol/L) in vitro, with no obvious inhibitory activity against other kinases, except tropomyosin-related kinase A. JTE-952 (≥3 mg/kg administered orally) has an anti-inflammatory effect in vivo in mice, evident as the suppression of CSF1-mediated enhancement of lipopolysaccharide (LPS)-induced TNF-α production. Based on these findings, the aim of this study was to clarify the effect of JTE-952 on osteoclast formation in vitro and on bone destruction in an animal model of arthritis, focusing on its effects on the pathology of RA.

MATERIALS AND METHODS

Compounds JTE-952, (2S)-3-[[2-(3-[4-(4-cyclopropylbenzyloxy)-3-methoxyphenyl]azetidine-1-yl) carbonyl]-pyridin-4-yl]methoxy]propane-1,2-diol, was chemically synthesized at the Central Pharmaceutical Research Institute, Japan Tobacco Inc. (Osaka, Japan). For the cell-based assays, JTE-952 (purity 97.5%) was dissolved in dimethyl sulfoxide and diluted in culture medium immediately before use. The final concentration of dimethyl sulfoxide was less than 0.1%. For the animal studies, JTE-952 and MTX (Sigma-Aldrich Co. LLC, St. Louis, MO, U.S.A.) were suspended in a 0.5% (w/v) aqueous solution of methylcellulose (MC).

In Vitro Human Osteoclast Formation Assay Human peripheral blood was collected from healthy in-house volunteers in tubes containing citric acid and was used for experiments on the same day. The monocytes were purified from the peripheral blood by negative selection with immunomagnetic beads (human Monocyte Isolation Kit II, Miltenyi Biotec K.K., Tokyo, Japan) and suspended in Dulbecco’s modified Eagle’s medium (DMEM; Wako Pure Chemical Industries, Ltd., Tokyo, Japan) and diluted in culture medium immediately before use. The cells were then stained for TRAP with a TRAP staining kit. TRAP-positive and multinucleated cells were counted as osteoclasts under a microscope by a blinded observer, and the average number for duplicate wells was considered the number of osteoclasts from each donor.

Measurement of Cytokine Levels in Synovial Tissues of Patients with RA The synovial tissues of six RA patients aged 61–77 years were obtained from HSRRB (five of the six patients were women, knee joints; one of the six patients was a man, a wrist joint). These patients had been taking DMARDs such as MTX, selective cyclooxygenase-2 inhibitor, and/or corticosteroid, but none were taking any biological agent. A part of each synovial tissue was placed in a separate tube and 15 µL of ice-cold lysis buffer containing 2 mmol/L ethylenediaminetetraacetic acid (EDTA) and protease inhibitors in phosphate-buffered saline was added per 1 mg of tissue. The tissues were homogenized with a mixer mill (Retsch MM400, Verder Scientific Co., Ltd., Tokyo, Japan) for 2 min at 30 Hz. After centrifugation (10 000 × g, 4 °C), the concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF) and CSF1 in the supernatant were measured with specific enzyme-linked immunosorbent assay (ELISA) kits (Quantikine ELISA, R&D Systems Inc., Minneapolis, MN, U.S.A.). Levels below the quantification limit were coded as 0 pg/mL.

Animals Male DBA/1J mice were supplied by Charles River Laboratories Japan, Inc. (Yokohama, Japan) when 6–7 weeks old. All animals were housed under specific-pathogen-free conditions at a room temperature of 23 ± 3 °C and air humidity of 55 ± 15% under a 12-h light/dark cycle and given access to standard laboratory chow diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. All procedures related to the use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Japan Tobacco Inc. and were performed in accordance with standards published by the National Research Council (Guide for the Care and Use of Laboratory Animals, NIH OACU) and the National Institutes of Health Policy on Human Care and Use of Laboratory Animals.
laren-induced arthritis (CIA) was induced in DBA/1J mice aged 10 weeks with a previously described method. Type II collagen (Collagen Research Center, Kiyose, Japan) derived from bovine articular cartilage was dissolved at a concentration of 4 mg/mL in a 0.01 mol/L aqueous acetic acid solution and emulsified with an equal volume of Freund's complete adjuvant containing 2 mg/mL heat-killed Mycobacterium tuberculosis H37Ra (Difco Laboratories, Detroit, MI, U.S.A.). Arthritis was induced by the intradermal injection of 100 µL of 2 mg/mL type II collagen emulsion at the base of the tail on days 1 and 22 of the experiment. All mice were immunized, except those in the normal group, in which arthritis was not induced. The severity of arthritis was scored for the digits of each limb using a three-point scale ranging from 0 to 2 (0, normal; 1, swelling of one finger; 2, swelling of two or more fingers) on days 22, 25, 27, 29, 32, and 35. The severity of arthritis was scored for the carpus and tarsus of each limb on a three-point scale ranging from 0 to 2 (0, normal; 1, erythema and mild-to-medium swelling; 2, erythema and severe swelling). The total arthritis score for each mouse was expressed as the sum of the scores for the four limbs (maximum possible score: 16). Scoring was blind for each group.

**Drug Treatment**  Arthritic mice were randomized and grouped for drug treatment based on their body weight on day 22 when arthritis had not developed. JTE-952 (1, 3, 10, and 30 mg/kg) and vehicle (0.5% MC) were orally administered when arthritis had not developed. JTE-952 (1, 3, 10, and 30 mg/kg) and vehicle (0.5% MC) were orally administered in a randomized manner and completely inhibited osteoclast formation at concentrations of ≥100 nmol/L. The IC50 value for JTE-952 on osteoclast formation was 2.8 ± 1.0 nmol/L. JTE-952 also reduced the TRAP-5b activity in the culture supernatant, with an IC50 value of 3.5 ± 1.1 nmol/L (Fig. 1C).

**IC50 Determination**  The IC50 was calculated from the concentration of JTE-952 and the residual (%) osteoclast formation activity or the residual (%) TRAP-5b activity, with a logistic function in the SAS software (SAS Institute Japan Ltd., Tokyo, Japan).

**RESULTS**

**Effects of JTE-952 on Human Osteoclast Formation in Vitro** To examine the effects of JTE-952 on human osteoclast formation, monocytes were stimulated with CSF1 and RANKL for 7 d in the presence of JTE-952. Human monocytes that had differentiated into osteoclasts (red staining), which were identified by TRAP-positive staining and multinucleation under a microscope (Fig. 1A, Control), were identified by TRAP-positive staining and multinucleation under a microscope (Fig. 1A, Control). However, negligible osteoclasts were observed in the CSF1-unstimulated group ([] CSF1; stimulated with RANKL alone). JTE-952 reduced the number of osteoclasts in a concentration-dependent manner and completely inhibited osteoclast formation at concentrations of ≥30 nmol/L (Figs. 1A, B). The IC50 value for JTE-952 on osteoclast formation was 2.8 ± 1.0 nmol/L. JTE-952 also reduced the TRAP-5b activity in the culture supernatant, with an IC50 value of 3.5 ± 1.1 nmol/L (Fig. 1C).

**Effects of JTE-952 on Osteoclast Formation among Synovial Cells of RA Patients** When synovial cells isolated from two RA patients were cultured for 15 d, osteoclasts, which were characterized by TRAP-positive staining and multinucleation, formed among the fibroblast-like cells, as observed with microscopy (data not shown). Osteoclasts formed even without CSF1 or RANKL ([] group; 8 and 24 cells/well in patient 1 and patient 2, respectively) and the addition of CSF1 and RANKL increased the numbers of osteoclasts (Control group; cultured with CSF1 and RANKL, 36 and 52 cells/well in patient 1 and patient 2, respectively; Fig. 2). JTE-952 concentration-dependently reduced the number of RA-synovial-cell-derived TRAP-positive osteoclasts and completely inhibited their formation at concentrations of ≥100 nmol/L. However, 300 nmol/L JTE-952 did not affect the number of fibroblast-like cells.

**GM-CSF and CSF1 Protein Levels in Synovial Tissues of RA Patients** In the synovial tissues from six RA patients who had undergone synovectomy, GM-CSF levels in the synovia were below the limit of quantification in all patients.
GM-CSF and CSF1 Protein Levels in Synovial Tissues of CIA Mice

The GM-CSF levels in the synovia were below the limit of quantification (≤0.5 ng/mg tissue) on days 29 and 35, whereas CSF1 was clearly detected at both time points (8.9 and 8.0 ng/mg tissue on days 29 and 35, respectively; Fig. 4). The average arthritis scores for the mouse synovia were 8.9 on day 29 and 13.2 on day 35 (maximum possible score: 16).

GM-CSF and CSF1 Protein Levels in Synovial Tissues of RA Patients

Synovial tissues from six RA patients were homogenized with a mixer mill. After centrifugation, the concentrations of GM-CSF and CSF1 in the supernatant were measured with specific ELISA kits. Levels below the limit of quantification were coded as 0 pg/mL. Results are expressed as the mean (ng/mg tissue) and individual values (○).

Fig. 1. Effects of JTE-952 on Osteoclast Formation from Human Monocytes

Human monocytes purified from peripheral blood were cultured in the presence of CSF1 and RANKL with the indicated concentrations of JTE-952 for 7 d. (A) Representative pictures of osteoclasts detected with TRAP staining. The cell shown with an arrow is a representative osteoclast. Cells of the (-) CSF1 group were cultured in the presence of RANKL alone. (B) Effect of JTE-952 on number of TRAP-positive multinucleated cells (with at least three nuclei). (C) Effect of JTE-952 on TRAP-5b activity in the culture supernatant. Results are expressed as mean percentages (±standard error of the mean (S.E.M.)) of the vehicle control value (n = 3).

Fig. 2. Effects of JTE-952 on Osteoclast Formation from Synovial Cells of RA Patients

Synovial cells from two RA patients, isolated with enzymatic dispersion, were cultured in the presence of CSF1 and RANKL with the indicated concentrations of JTE-952 for 15 d. Osteoclasts were detected with TRAP staining, and the number of TRAP-positive multinucleated cells (at least three nuclei) was counted in a blinded manner. The cells of the (-) group were cultured with MEM alone. Results are expressed as the mean number of TRAP-positive cells from the two RA patients and their individual values (○; patient 1, ○; patient 2, △).

Fig. 3. GM-CSF and CSF1 Protein Levels in the Synovial Tissues of RA Patients

Synovial tissues from six RA patients were homogenized with a mixer mill. After centrifugation, the concentrations of GM-CSF and CSF1 in the supernatant were measured with specific ELISA kits. Levels below the limit of quantification were coded as 0 pg/mL. Results are expressed as the mean (ng/mg tissue) and individual values (○).
Effects of JTE-952 on Bone Destruction and Joint Inflammation in CIA Mice

Prophylactic Effects

JTE-952 was orally administered at a dose of 1, 3, 10, or 30 mg/kg once daily for 14 d, from day 22 onwards. The bone destruction score of the vehicle-treated group on day 35 was significantly higher than that of the normal group (Fig. 5A). JTE-952 dose-dependently inhibited the increase in the bone destruction score. Statistically significant differences were observed at JTE-952 doses of ≥3 mg/kg and the percentage inhibition was 35.3, 68.4, 72.9, and 83.5% at doses of 1, 3, 10, and 30 mg/kg, respectively. Osteolytic bone lesions were observed in the metacarpophalangeal joints and carpal joints of the vehicle-treated group, and these radiographic findings were suppressed by JTE-952 (Fig. 5B). JTE-952 also dose-dependently inhibited the increase in the number of TRAP-positive cells in the knee joints (Fig. 5C). Statistically significant differences were observed at JTE-952 doses of ≥3 mg/kg, and the percentage inhibition was 20.3, 37.7, 71.0, and 88.0% at doses of 1, 3, 10, and 30 mg/kg, respectively. The vehicle-treated CIA mice showed an arthritis score of 2.4 ± 0.5 on day 27, which gradually increased to a maximum score of 12.0 ± 0.9 on day 35 (Fig. 5D). JTE-952 inhibited this increase in the arthritis score in an approximately dose-related manner, with signifi-
Bone destruction in RA leads to structural destruction of the joint. Therefore, it is important to control the progression of this bone destruction by suppressing synovial inflammation in the early stage of RA. Bone destruction is strictly regulated by the process of bone resorption by osteoclasts, and the CSF1/CSF1R axis is specifically involved in the differentiation of monocyte-lineage cells into osteoclasts and their activation. We have previously reported that JTE-952 potently inhibits the tyrosine kinase activity of CSF1R, which results in a potent anti-inflammatory effect in vivo. This effect is highly selective and JTE-952 displays no significant inhibitory activity against other kinases, except tropomyosin-related kinase A. Therefore, in the present study, we investigated the effects of JTE-952 on osteoclast formation in vitro and on bone destruction in an experimental model of arthritis, focusing on its effects on RA.

JTE-952 inhibited human osteoclast formation from monocytes obtained from the peripheral blood and the TRAP-5b activity in the culture supernatants, with IC50 values of 2.8 and 3.5 nmol/L, respectively. In this study, no apparent progression of osteoclast formation was observed in the absence of CSF1, even in the presence of RANKL, which indicates that CSF1 plays an important role in the process of differentiation of monocyte-lineage cells into osteoclasts. In RA, synovial cells, including tissue macrophages and synovial fibroblasts, contribute to osteoclast formation at sites of bone erosion, and synovial fibroblasts are sites of CSF1 production in the joints of patients with inflammatory arthritis. Therefore, we next examined the effects of JTE-952 on osteoclast formation in synovial cells obtained from RA patients. Interestingly, as well as an increase in number of fibroblast-like cells, the synovial cells derived from the synovial tissues of RA patients differentiated into osteoclasts in the absence of exogenous CSF1 and RANKL, and the addition of CSF1 and RANKL further increased the number of osteoclasts. Although JTE-952 had no apparent inhibitory effect on the proliferation of the fibroblast-like cells, it inhibited osteoclast formation from synovial cells in all the donors examined. These results suggest that JTE-952 inhibits osteoclastogenesis in the RA synovium.

Monocytes in the peripheral blood can differentiate into inflammatory M1 or anti-inflammatory M2 macrophage subtypes. In a GM-CSF environment, tissue macrophages are differentiated and activated toward M1 macrophages, which produce large amounts of proinflammatory cytokines, such as TNF-α and IL6. Conversely, polarization to M2 macrophages that produce low levels of proinflammatory cytokines was observed in a CSF1 environment. Notably, CSF1 levels in the peripheral blood and synovial fluid of RA patients are higher than in healthy subjects or patients with other joint diseases such as osteoarthritis, and these levels correlate with disease severity. GM-CSF was also detected in the synovial fluid and plasma of RA patients and a GM-CSF-blocking antibody was effective against joint inflammation and bone destruction in experimental arthritis. Regarding osteoclastogenic ability, there is a significantly positive correlation between the ratio of M1/M2 monocyte-lineage cells and the number of osteoclasts in RA patients. These findings imply that the local cytokine microenvironment in the synovial tissues of RA patients, including the quantitative balance of...
CSF1 and GM-CSF, is closely associated with osteoclastogenic activity. Therefore, before considering the effects of JTE-952, we evaluated the protein levels of GM-CSF and CSF1 in synovial tissues obtained from RA patients. We found higher levels of CSF1 compared with GM-CSF in all the RA synovia evaluated. The differentiation to osteoclasts in the absence of exogenous factors during osteoclast formation from RA synoviocytes (Fig. 2) can be explained, in part, by the influence of endogenous CSF1 produced by the synovial fibroblasts of RA patients. These results are also consistent with a previous report\(^{16}\) that suggested that osteoclasts in the synovia of RA patients are strongly affected by regional levels of CSF1.

To test the possibility that JTE-952 inhibits bone destruction \textit{in vivo}, we used a mouse model of CIA. Mouse CIA is a disease model of chronic arthritis, with synovial inflammation and bone erosion, which are similar to the symptoms of human RA.\(^{37}\) First, to estimate the cytokine environment at the joint locus, we evaluated the protein levels of GM-CSF and CSF1 in the synovial tissues in the early (day 29) and late (day 35) clinical stages after CIA onset. The levels of CSF1 in the synovial tissues were equally high in both stages, unlike those of GM-CSF. The marked increase in CSF1 from the onset of CIA demonstrated that CSF1 plays a pivotal role in the pathological process of CIA. These results are similar to those of the pathological analysis of human RA, shown in Fig. 3. Therefore, it was reasonable to use the CIA mouse model to predict the effect of JTE-952 on bone destruction and joint inflammation in human RA pathology.

We then evaluated the inhibitory effect of orally administered JTE-952 on bone destruction and joint inflammation in the CIA model. JTE-952 was administered before disease onset (days 22–35) and significantly and dose-dependently suppressed the bone destruction score and TRAP-positive cell number in the knee joints at doses of \(\geq 3\) mg/kg. The disease severity after treatment with 30 mg/kg JTE-952 was almost identical to that in the normal group, in which arthritis was not induced. These results indicate that JTE-952 reduces the osteoclast number in the synovium and inhibits bone destruction by inhibiting the CSF1R signal \textit{in vivo}. JTE-952 also attenuated the increase in the arthritis score, which seems to reflect the severity of joint inflammation. The anti-inflammation effect of JTE-952 was statistically significant at doses of \(\geq 3\) mg/kg, although at maximum efficacy, inhibition was only partial. The effect of a neutralizing anti-CSF1R antibody on mouse CIA has been demonstrated in a previous report by Tho \textit{et al.}\(^{20}\) In that study, although the anti-CSF1R antibody fully inhibited bone destruction, the arthritis score was only partially suppressed. The partial efficacy of the anti-CSF1R antibody on the arthritis score was thought to reflect the maximum possible contribution of CSF1 to joint inflammation in mouse CIA. The inhibitory effect of JTE-952 (30 mg/kg) on the arthritis score was also partial in our study and was similar to that of the anti-CSF1R antibody. Inflammatory factors produced by cells that rarely express CSF1R, such as T cells, may be partly responsible for the proportion of joint inflammation that failed to respond to JTE-952. Future studies should measure CSF1 levels in the synovial tissue after JTE-952 treatment and the inflammatory response involved in joint inflammation not controlled by JTE-952. Recent evidence indicates that osteoclasts accumulate and are activated in the synovial tissue and play a pivotal role in joint destruction in RA.\(^{39}\) Therefore, JTE-952 strongly suppresses bone destruction in RA patients and may also maximally inhibit the associated joint inflammation, depending on the contribution of CSF1. Further studies are required to evaluate whether JTE-952 has beneficial effects on global joint functions in arthritis, including cartilage destruction and joint movement.

RANKL is another molecule essential for osteoclastogenesis. The role of RANKL in the bone destruction associated with RA has been suggested by several observations.\(^{40–42}\) RANKL is upregulated in RA lesions, acts directly on osteoclast precursor cells to stimulate their differentiation into mature osteoclasts, and regulates the activation of mature osteoclasts. In Japan, denosumab, a neutralizing anti-RANKL antibody, has been used clinically to suppress the progression of the bone erosion associated with RA. However, although denosumab reduces the progression of bone erosion, it reportedly has no protective effect on the progression of joint-space narrowing or RA disease activity.\(^{43}\) Furthermore, although there was little evidence of bone destruction in a serum-transfer arthritis model with RANKL knockout, joint inflammation occurred to the same extent as in wild-type mice, when measured as the increase in the clinical score.\(^{44}\) These lines of evidence indicate that anti-RANKL therapy may have little effect on joint inflammation or disease activity. Conversely, as demonstrated in our present and previous studies,\(^{29}\) JTE-952 exerts an inhibitory effect on joint inflammation in the CIA mouse model. Indeed, LPS-stimulated TNF-\(\alpha\) production by whole blood obtained from CIA mice treated with JTE-952 was suppressed by up to 50\% compared with that of vehicle-treated CIA mice (data not shown). The percentage inhibition of TNF-\(\alpha\) production was similar to the maximum efficacy of inhibition mediated by JTE-952 against joint inflammation, which was measured as an increase in the arthritis score. These results demonstrate that JTE-952 suppressed proinflammatory cytokine production in the CIA mouse model. JTE-952 completely inhibited the production of proinflammatory cytokines that were induced by LPS-stimulated monocytes/macrophages \textit{in vitro}, such as TNF-\(\alpha\) and IL6. TNF-\(\alpha\) was reported to cooperate with IL6 to regulate the differentiation of osteoclasts and osteoclast-mediated bone erosion, independent of RANK.\(^{45}\) Therefore, these results suggest that unlike anti-RANKL antibodies, JTE-952 might provide benefit by inhibiting joint inflammation, in addition to its direct suppressive effect on osteoclastogenesis by inhibiting CSF1R. To test this hypothesis, further detailed research with appropriate reference agents is required.

MTX is frequently used as an anchor drug in the treatment of RA.\(^{46,47}\) However, although monotherapy with MTX has a strong anti-inflammatory effect, its effect on the progression of bone destruction is small.\(^{48}\) In this study, we examined the efficacy of 3 mg/kg JTE-952 combined with 0.3 mg/kg MTX (equivalent to the dose of JTE-952 [3 mg/kg] that suppressed the arthritis score) in the treatment of bone destruction and joint inflammation after their repeated oral administration. Monotherapy with JTE-952 inhibited bone destruction, but not significantly, in this experiment. When combined with a dose of MTX that had no overt inhibitory effect on bone destruction when used as a monotherapy, JTE-952 exerted a greater inhibitory effect than JTE-952 alone. The combined effect was not statistically significantly different from the effect of JTE-952 alone but differed significantly from that in the vehi-
cle-treated group. Furthermore, JTE-952 combined with MTX complementarily and synergistically inhibited the increase in the arthritis score. Thus, JTE-952 inhibited bone destruction and joint inflammation more effectively in the CIA mouse model when it was used in combination with MTX. MTX suppresses the expression of RANKL and increases the secretion of osteoprotegerin, a natural inhibitor of RANKL, by synovial fibroblasts.\(^9\) MTX also inhibits the production of cytokines, such as interferon-\(\gamma\), by pathogenic T cells and suppresses arthritis in the mouse CIA model.\(^9\) Therefore, the combined effects of JTE-952 and MTX are probably attributable to the synergistic effects of the different mechanisms of action of these agents. Hence, the combination may be more beneficial for the joint destruction and inflammation associated with RA than a monotherapy with either agent alone.

In conclusion, we have demonstrated that JTE-952, a novel CSF1R kinase inhibitor, suppresses osteoclast formation from monocytes and synovial cells in RA patients \textit{in vitro}. The oral administration of JTE-952 reduced the number of osteoclasts in the CIA mouse model and suppressed bone destruction. JTE-952 more effectively inhibited bone destruction and joint inflammation when administered in combination with MTX. These results indicate that JTE-952 inhibits both bone destruction and joint inflammation in RA patients and effectively prevents the progression of the structural destruction of joints.

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