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Inhibition of Histone Deacetylase Suppresses Osteoclastogenesis and Bone Destruction by Inducing IFN-β Production

Takahiro Nakamura,* Toshiro Kukita,† Takeo Shobuie, Kengo Nagata,† Zhou Wu,† Kenji Ogawa,† Takao Hotokebuchi,‡ Osamu Kohashi,* and Akiko Kukita‡*

Osteoclasts are bone-resorptive multinucleated cells that are differentiated from hemopoietic cell lineages of monocyte/macrophages in the presence of receptor activator of NF-κB ligand (RANKL) and M-CSF. Downstream signaling molecules of the receptor of RANKL, RANK, modulate the differentiation and the activation of osteoclasts. We recently found that histone deacetylase inhibitors (HDIs), known as anticancer agents, selectively suppressed osteoclastogenesis in vitro. However, the molecular mechanism underlying inhibitory action of HDIs in osteoclastogenesis and the effect of HDIs on pathological bone destruction are still not remained to be elucidated. In this study, we show that a depsipeptide, FR901228, inhibited osteoclast differentiation by not only suppressing RANKL-induced nuclear translocation of NFATc1 but also increasing the mRNA level of IFN-β, an inhibitor of osteoclastogenesis. The inhibition of osteoclast formation by FR901228 was abrogated by the addition of IFN-β-neutralizing Ab. In addition, treatment of adjuvant-induced arthritis in rats revealed that FR901228 inhibited not only disease development in a prophylactic model but also bone destruction in a therapeutic model. Furthermore, immunostaining of the joints of therapeutically treated rats revealed significant production of IFN-β in synovial cells. Taken together, these data suggest that a HDI inhibits osteoclastogenesis and bone destruction by a novel action to induce the expression of osteoclast inhibitory protein, IFN-β. The Journal of Immunology, 2005, 175: 5809–5816.
promoter (23). In contrast, HDIs also repress the expression of several genes, although the molecular mechanism is still not known. Among those genes, inflammatory cytokines such as TNF-α and IFN-γ are suppressed by suberylanilide hydroxamic acid (24). In addition, NaB and TSA have also been found to suppress NF-κB activation in colon cells (25), suggesting that HDIs may exhibit anti-inflammatory properties. However, whether HDIs have such an effect in vitro and their target genes are still not well understood.

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease characterized by chronic inflammation of synovial joints and concomitant destruction of cartilage and bone (26). Studies of RA indicate that osteoclasts play a central role in bone destruction (27, 28). Several studies also have shown that RANKL and RANK are expressed in synovial cells involved in bone destruction in RA (29–31). Adjuvant-induced arthritis (AA) is a rat RA model that mimics many of the clinical and pathological features of human RA.

In the present study, we investigated the effect of a HDI, FR901228, on osteoclastogenesis and bone resorption and its molecular mechanism using in vitro culture and examined whether FR901228 has an effect on inflammatory bone destruction using a human RA.

Animals and chemicals
FR901228 was kindly provided by Fujisawa Pharmaceutical. α-MEM was purchased from Invitrogen Life Technologies. FCS was purchased from BioWhittaker. Recombinant human TNF-α was purchased from Boehringer Mannheim. Recombinant human soluble RANKL (sRANKL) and human M-CSF were obtained from PeproTech. 1α,25-dihydroxy vitamin D3 (1α,25-(OH)2D3) was purchased from BIOMOL. Anti-NF-κB p65, NFATc1, actin, and anti-rat IFN-β Abs were purchased from Santa Cruz Biotechnology. Anti-p38/MAPK and phosphorylated p38/MAPK (pT180/ T182) Abs were obtained from BD Biosciences. Anti-mouse IFN-β-neutralizing Ab was purchased from Yamasa Shoyu. Mycobacterium tuberculosis H37 Ra and IFA were purchased from Difco. Male Sprague-Dawley rats (aged 5–7 wk) were obtained from Seac. Yoshitomi. Female Lewis rats were purchased from Charles River Laboratories. All experiments were conducted under clean room facilities in accordance with the Saga University Guidelines for Animal experiments.

Osteoclast formation in the cell cultures
Cultures of rat bone marrow cells were conducted as described by Kukita et al. (32). Briefly, whole bone marrow cells were cultured for 5 days in α-MEM containing 15% FCS for 3 days in the presence of 10−8 M 1α,25-(OH)2D3, with or without FR901228 (0.8 ng/ml). After culture, the slices were subjected to ultrasonication to remove attached cells. Resorption pits on the slices were examined using a Jeol JSM-5200LV scanning electron microscope.

RT-PCR analysis
RAW-D cells were cultured in the presence or absence of sRANKL and/or FR901228 for 3 days. Total RNA was extracted by using a commercial kit (Isogen; Nippon Gene) and subjected to PCR using a RT-PCR kit (Takara). The following primers were used for RT-PCR analysis: mouse calcitonin receptor (CTR) (sense, 5’-TTTCAAGAACCTTAGGCTGAG-3’; and antisense, 5’-CAAGGCAGACCTTGTGGAGGA-3’); mouse cathepsin K (sense, 5’-ATGTTGGGCTCTCAAGTT-3’; and antisense, 5’-CCA CAAGATTCGAGGACT-3’); mouse p21WAF1 (sense, 5’-TCCAGAC ATTCAGACCAAGG-3’; and antisense, 5’-GTTTTGCCGCTTGAG ATGTTCC-3’); mouse M KK-M (sense, 5’-TCCAAACCTACCATTCCTG-3’; and antisense, 5’-TGCAAGATACCTACCTAAGG-3’); and mouse SOCS-3 (sense, 5’-GAGATGTGGCC GAAAACAAGA-3’; and antisense, 5’-CCACAGCTTTAGATACAC ACAGTGC-3’). PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide. As an internal control for RNA quantity, the same cDNA was amplified using primers specific for mouse GAPDH mRNA (sense, 5’-CATGGGAGAGGTTGGGCTC-3’; and antisense, 5’-AAGCAGTACATTGGGAT-3’).

Protein isolation and Western analysis
RAW 264 cells were grown on 10-cm dishes in the presence or absence of sRANKL and FR901228. For isolation of cell extracts, the cells were washed with ice-cold PBS and suspended in SDS buffer (0.125 M Tris-HCl (pH 6.8), 2% SDS, and 10% glycerol). Nuclear extracts were prepared according to methods as described previously (35). Briefly, the cells were washed with ice-cold PBS and incubated at 4°C for 15 min in buffer containing 10 mM HEPES-KOH (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 2 mM MgCl2, and 5 μM amido-PMSF. The cell were then added with Nonidet P-40 at the concentration of 0.5% and gently mixed. The homogenate was centrifuged at 15,000 rpm for 1 min at 4°C, and supernatant was used as cell extract. The nuclear pellet was resuspended in buffer containing 20 mM HEPES-KOH (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 5 μM amido-PMSF and mixed at 4°C for 30 min. Protein concentrations of nuclear extracts were determined using BCA protein assay kits (Pierce). Equal amounts of proteins were loaded on 12% PAGE and then transferred to a PVDF membrane and incubated with rat anti-NF-κB p65 (1/500 dilution) and/or rat anti-κB p50 (1/250 dilution) Abs. The filters were then incubated with HRP-conjugated goat anti-mouse or anti-rabbit Abs. Detection of bands was performed with a ECL Western blotting system (Amersham Biosciences).

Induction of AA
All animal procedures were conducted with the approval of the Saga University Animal Research Ethics Committee. AA was induced as described by Zhang et al. (36). Briefly, CFA, emulsion of H37Ra in incomplete adjuvant (10 mg/ml), was prepared. LEW rats were intradermally injected at the base of the tail with 0.1 ml of CFA. FR901228 was dissolved and diluted with 10% low-fat milk at room temperature for 1 h, and then injected with anti-NF-κB p65 (1/500 dilution), anti-NFATc1 (1/500 dilution), anti-p38/MAPK (1/2500 dilution), anti-phosphorylated p38/MAPK (1/2500 dilution), and anti-actin (1/500 dilution) for 1 h. The filters were then incubated with HRP-conjugated goat anti-mouse or anti-rabbit Abs. Detection of bands was performed with a ECL Western blotting system (Amersham Biosciences).
Radiological and histological studies

At the end of the experiment (day 21), the hind paws of rats were imaged on ultra-speed radiographic film (Kodak), and the level of bone destruction was analyzed by a soft x-ray analysis system (SOFRON: SRO-M-I; Sofron). Tissue sections were prepared as described previously (38). The sections were scored at low and high power for bone erosion according to the method described by Bendele et al. (39) with minor modifications. Briefly, bone erosion was scored 0 to 5 according to the following criteria: 0, none; 1, minimal (not readily apparent on low magnification); 2, mild (more numerous areas of resorption, not readily apparent on low magnification); 3, moderate (obvious foci of resorption, numerous osteoclasts); 4, marked (large erosions extending into bone cortices, more numerous osteoclasts); and 5, extensive erosions (markedly disrupted joint architecture). Four to eight H&E sections were scored for each animal, and the mean score for each histopathological parameter was calculated for each animal. The sections were also stained with TRAP.

Immunocytochemistry

Immunostaining was performed as described previously (40). After decalcification, the tissue blocks were washed overnight in PBS containing 30% sucrose, embedded in OCT compounds, and frozen in dry ice/isopentane. Sections (10-μm thick) of joints including tarsal areas were prepared by using Frigocut 2800N (Leica). Sections were incubated with goat polyclonal anti-rat IFN-γ Ab (1/200 dilution) in a humidified chamber overnight at 4°C after blocking nonspecific binding with 10% donkey serum for 30 min at room temperature. Sections were incubated with biotinylated anti-goat IgG (1/200 dilutions) for 1 h at room temperature followed by a DAB substrate kit (Vector Laboratories). Color development was performed with peroxidase-conjugated streptavidin-biotin complex (1/300 dilution) for 1 h at room temperature. Sections were incubated with goat anti-rat IFN-γ Ab (1/200 dilution) in a humidified chamber overnight at 4°C after blocking nonspecific binding with 10% donkey serum for 30 min at room temperature. Color development was performed with peroxidase-conjugated streptavidin-biotin complex (1/300 dilution) for 1 h at room temperature. Color development was performed with peroxidase-conjugated streptavidin-biotin complex (1/300 dilution) for 1 h at room temperature.

Results

FR901228 inhibits osteoclast differentiation in vitro

In this study, we used a despsipeptide, FR901228, which is a structurally unrelated compound of HDIs, TSA or NaB. Therefore, we first examined the effect of FR901228 on osteoclastogenesis in rat bone marrow cultures. In these cultures, the formation of TRAP-positive MNCs is inhibited by the addition of osteoprotegrin (OPG), thereby dependent on RANKL (Fig. 1A). As shown in Fig. 1, A and B, FR901228 strongly inhibited the formation of TRAP-positive MNCs in a dose-dependent manner. An inhibitory effect was observed as low as 0.2 ng/ml FR901228. We then examined whether FR901228 inhibited the bone resorption activity of osteoclasts formed in rat bone marrow culture. Typical resorption pits were formed in the absence of FR901228, whereas formation was markedly reduced in the presence of FR901228 (Fig. 1, F and G). We further examined the effect of FR901228 on osteoclastogenesis using a clone of macrophage cell RAW264, RAW-D, that efficiently differentiates into osteoclasts in the presence of sRANKL. FR901228 inhibited TRAP-positive MNC formation from this cell line (Fig. 1, C and E). We then determined, using semiquantitative RT-PCR, whether the inhibitory effect of FR901228 correlates with expression of osteoclast-specific phenotypes such as CTR and cathepsin K. The level of CTR and cathepsin K mRNA was decreased in the tissue treated with FR901228 (Fig. 1H). In the previous study, we found that HDIs suppressed differentiation into...
osteoclasts but not into macrophages (12). Therefore, we also analyzed the effect of FR901228 on the expression of macrophage-associated phenotypes such as Mac-1 (CD11b) and F4/80 in bone marrow macrophages induced by M-CSF. FR901228 had no effect on the expression of either Mac-1 or F4/80 (data not shown). These results indicate that like TSA and NaB, FR901228 selectively inhibits osteoclastogenesis in vitro and that HDAC plays a key role in differentiation of osteoclasts.

**Effect of FR901228 on the activation of signaling proteins of sRANKL and the expression of possible target genes**

To understand the molecular mechanism by which FR901228 inhibits osteoclastogenesis, we analyzed the effect of FR901228 on the activation of the downstream signals stimulated with sRANKL using RAW-D cells. RANKL stimulates the nuclear translocation of transcription factors such as NF-κB and NFATc1. Nuclear extracts of RAW-D cells stimulated with sRANKL for 30 min or 24 h were analyzed using Abs to NF-κB p65 subunit or NFATc1, respectively. Pretreatment of RAW-D cells with FR901228 did not affect the nuclear translocation of NF-κB p65 but strongly inhibited that of NFATc1 as shown in Fig. 2A. Activation of p38/MAPK is another important pathway activated by RANKL (5). However, FR901228 did not affect phosphorylation levels of p38/MAPK protein (Fig. 2B).

Treatment with HDIs may affect the mRNA levels of target genes, which may be involved in osteoclast signaling. It is reported that TSA affects the promoter of genes for p21<sub>WAF1</sub>, MKP-M, and IFN-β. Activation with RANKL affects the expression of cell cycle inhibitors such as p21<sub>WAF1</sub> or p27<sub>KIP1</sub> (41). In addition, MKP-M is a member of the MKP family, which regulates the activity of MAPKs (42). Furthermore, IFN-β is known to be an inhibitor of osteoclastogenesis (10, 11). Therefore, we analyzed the expression levels of these mRNAs in RAW-D cells stimulated with or without sRANKL in the presence or in the absence of FR901228 by RT-PCR. As shown in Fig. 2C, FR901228 (0.8 ng/ml) did not affect the expression of p21<sub>WAF1</sub> or MKP-M in the presence and in the absence of sRANKL. In contrast, FR901228 strongly stimulated gene expression of IFN-β mRNA in RAW-D cells, both in the presence and in the absence of sRANKL. The expression of IFN-β was also increased by RANKL alone. It is reported that IFN-β inhibits osteoclastogenesis by affecting c-Fos expression (10). In the presence of FR901228, the level of c-Fos mRNA decreased both in the presence and in the absence of sRANKL (Fig. 2C). SOCS-3 is known to block signaling of IFNs and is also induced by sRANKL (11). FR901228 decreased the expression of SOCS-3 mRNA. These data together indicate that FR901228 not only suppresses the activation of RANKL-mediated signals, it also stimulates RANKL-counteracted signals.

We further analyzed whether the inhibition of osteoclastogenesis by FR901228 is mediated by the production of IFN-β. As shown in Fig. 3, the addition of neutralizing Ab against IFN-β did not increase the formation of TRAP-positive MNCs in the culture, whereas it dose-dependently decreased the inhibitory effect of FR901228. The addition of control rat IgG to the culture had no effect on the inhibitory effect of FR901228. These data indicate that induction of IFN-β production is a central action of FR901228-mediated inhibition of osteoclastogenesis.

**FR901228 prevents the development of AA in rats**

To investigate the effect of FR901228 in vivo, we analyzed whether FR901228 affects bone destruction or inflammation of AA. We first studied the effect of FR901228 on development and severity of arthritis by injecting FR901228 into rats simultaneously with CFA. In rats immunized with CFA, onset of arthritis appeared around day 11. FR901228 dose-dependently suppressed the development and severity of AA as measured by arthritis score (Fig. 4A) and hind paw swelling (data not shown). Complete suppression was seen at a dose of FR901228 (0.5 mg/kg). The average body weight of rats decreased after onset of arthritis in CFA immunized rats (Fig. 4B). The treatment with rats with 0.2 or 0.5 mg/kg FR901228 markedly improved the reduction of their body weight. At the end of the experiment (day 21), we examined gross appearance and radiographs of hind paws. As shown in Fig. 4C, joint bone destruction of CFA-immunized rats was severe, whereas in CFA-immunized rats treated with FR901228, bone destruction was completely prevented. We further analyzed H&E and TRAP-stained section of joint tissues of rats. CFA-challenged rats developed severe arthritis characterized by inflammatory cell infiltration and bone destruction (Fig. 5B). In contrast, inflammation and bone destruction were markedly suppressed in rats treated with
To investigate whether decreased bone loss on FR901228 treated rats is based on decreased osteoclast formation, TRAP staining of joint sections was compared. A number of TRAP-positive osteoclasts were seen in the edge of bone erosion in CFA-immunized rats (Fig. 5, E and F), but in the section of FR901228-treated, CFA-immunized rats, no osteoclasts were seen (data not shown). These results indicate that FR901228 is a strong inhibitor of disease development and bone destruction in AA.

Therapeutic treatment with FR901228 suppresses bone destruction in AA

To investigate whether FR901228 has therapeutic effect on inflammation and bone destruction in AA, we started to inject FR901228 into rats after onset of arthritis (day 11). Therapeutic treatment with FR901228 (0.5 and 1.0 mg/kg) did not significantly decrease the arthritis score of AA (Table I and Fig. 4C). At the end of the experiment, we compared radiographs of hind paws of CFA-immunized rats treated with and without FR901228 (Fig. 4C). Surprisingly, the bone destruction of rats treated with FR901228 1.0 mg/kg was less severe than in nontreated rats. We further performed histological studies to know the effect of FR901228 on bone destruction and osteoclast formation. As shown in Fig. 5, G and H, bone destruction and the number of TRAP-positive osteoclasts significantly decreased. Pathology of the sections of both joints (ankle, tarsal bone, and calcaneus) was evaluated according to the method as described previously. The average bone erosion score was significantly reduced by therapeutic treatment with 1.0 mg/kg FR901228 (Fig. 5H). We performed this experiment three times and obtained same results (data not shown).

Detection of IFN-β production in the synovial cells of AA in rats

To understand the mechanism of the inhibitory effect of bone destruction by FR901228, we further analyzed whether FR901228 treatment induces the production of IFN-β, using immunohistochemistry. Fig. 6 indicates the comparison of the staining of synovial cells with anti-IFN-β Ab in both nontreated and FR901228-treated AA rats. In AA control rats, there were few positive staining cells, whereas in FR901228 treated AA rats, there are a number of positively stained cells in the synovial membrane, and some mononuclear cells were strongly stained with IFN-β.

Discussion

HDIs results in hyperacetylation and modify gene expression either positively or negatively in a cell-specific manner (13). In the
Previous study, we found that HDIs such as TSA and NaB inhibited differentiation into osteoclasts specifically (12). These results suggest that the expression of genes regulated by HDAC may be involved in osteoclastogenesis. In the present study, we demonstrate that IFN-β is a target gene for HDI FR901228, and IFN-β induction is a central feature in the suppressive effect of osteoclastogenesis by FR901228.

The IFNs are a family of glycoproteins that consist of type I, IFN-αβ, and type II, IFN-γ. The gene for type I IFN, IFN-β, is maintained silence without specific extracellular signals. The virus-responsive element in promoter region is required for the virus-induced transcriotional activation of the IFN-β gene. The region consists of a complex enhancer comprising DNA binding sites for several transcription factors such as NF-κB, IFN regulatory factors, and activating transcription factor 2 (23). IFN-β is induced by LPS and TNF-α, and the induction is mediated by proteins such as IFN-β regulatory factor 3 in in vitro cultures (43, 44). Recently, Takayanagi et al. (10) and Hayashi et al. (11) showed that IFN-β is also induced by RANKL in bone marrow macrophage cells. We also found that IFN-β expression is induced by RANKL in RAW-D cells. On the other hand, there are some negative regulatory domains in IFN-β promoter, which contributes constitutive repressed state of the promoter (45). However, the mechanism of negative regulation of the IFN-β gene remains less well understood. Shestakov et al. (23) showed that the silent state of the promoter correlates with the state of hypoacetylation of histone H4 using TSA. The data suggest that FR901228 treatment

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** H&E and TRAP staining of joints. Tarsal area of normal rats (A), CFA-immunized rats (B, E, and F), and CFA-immunized rats prophylactically (C) or therapeutically (D, G, and H) treated with FR901228 were histologically analyzed. Representative sections stained with H&E (A–E) or TRAP (F and H) are shown. E–H show high magnification. (Bars indicate 500 μm in A–D and 5 μm in E–H). CFA-immunized rats developed severe arthritis characterized by inflammatory cell infiltration (asterisk) (B), bone destruction (arrowheads) (B), and the induction of a number of TRAP-positive osteoclasts (arrows) (F). Therapeutic treatment of rats with FR901228 1.0 mg/kg did not prevent the infiltration of inflammatory cells (asterisk) (D) but prevented bone destruction and the formation of TRAP-positive osteoclasts. 1. Histological bone erosion score was reduced in CFA-immunized rats prophylactically treated with FR901228 (0.5 mg/kg) and also in rats therapeutically treated with FR901228 (1.0 mg/kg). Each value represents the mean ± SEM of bone erosion score of four rats. Data were analyzed by Student’s t test. *, p < 0.05 and **, p < 0.01 compared with a group of CFA-immunized rats.

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** Detection of IFN-β-positive synovial cells in AA rats therapeutically treated with FR901228. Representative immunostaining of synovial cells with anti-IFN-β Ab is shown. In the group with CFA-immunized rats, few slightly positive cells were observed in synovial cells (A and C), whereas in the group with CFA-immunized rats therapeutically treated with 1.0 mg/kg FR901228, a number of strongly IFN-β-positive cells are seen (arrows) (B and D). Bars indicate 100 μm in A and B and 50 μm in C and D, respectively.

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<th>Days after Injection (day)</th>
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*FR901228 (0.5 or 1.0 mg/kg) or vehicle (10% HCO60 saline) was injected into CFA-immunized rats i.v. on day 11. Injections were given twice a week until the end of the experiment. Data are shown as mean ± SEM of four rats.
derepresses the negative regulation of IFN-β by affecting acetylation state of IFN-β promoter.

In this study, the treatment with both of FR901228 and RANKL stimulated IFN-β expression more strongly than RANKL alone. It is interesting how RANKL counteracts the inhibitory effect of IFN-β. Hayashi et al. (11) have shown that RANKL also induced the expression of SOCSs, a suppressor of the IFN signaling. In our study, the mRNA expression level of SOCS-3 was increased by RANKL but decreased by FR901228 in RAW-D cells. Down-regulation of SOCS-3 by FR901228 seems to strengthen IFN-β expression in the presence of RANKL. In contrast, Takayangi et al. (10) have reported that IFN-β decreases the expression of c-Fos induced by RANK. In our study, FR901228 decreased mRNA expression levels of c-Fos, which was thought to be down-regulated by IFN-β.

TSA and NaB inhibited the activation of p38/MAPK and NF-κB in our previous study (12), and several HDIs are shown to inhibit NF-κB activation (24, 25). Unlike TSA and NaB, FR901228 did not inhibit the nuclear translocation of NF-κB and activation of p38/MAPK. However, FR901228 strongly inhibited the nuclear translocation of Nfatc1, which is activated by RANKL. Nfatc1 was recently reported as a regulator of osteoclastogenesis, which is involved in terminal differentiation of osteoclasts (6). Nfatc1 binding sites are present within the promoter region of the osteoclast-specific genes such as TRAP and cathepsin K (6, 46). Translocation of Nfatc1 to nuclei is regulated by Ca2+/calcinurin-dependent phosphatase pathway (47). Recently, it is shown that HDI NaB inhibited IL-2 production by affecting cellular localization of Nfatc1 protein (48). Interestingly, it is reported that in addition to calcium-mediated signals, the TRAF6 and c-fos pathways were required in RANKL-induced Nfatc1 expression (6). The data suggest that the down-regulation of c-fos gene by FR901228-induced IFN-β may reduce Nfatc1 expression. To elucidate the mechanism of inhibition of Nfatc1 activation by FR901228, further study is necessary.

Thus, we found some differences in the inhibitory signals by TSA and NaB and FR901228. In addition, some genes whose expression is affected by FR901228 were different from those of TSA and NaB. HDACs comprise a family and are grouped into two classes, class I and class II (49). Several structural classes of compounds in HDIs have shown different specificity to HDAC subtypes. TSA inhibits all HDACs of both class I and II, whereas trapoxin cannot inhibit HDAC6, class II HDAC (50). The reduced form of FR901228 inhibited class I enzymes, HDAC1 and HDAC2, more strongly than class II enzymes, HDAC4 and HDAC6 (18). The difference in the response genes between TSA and FR901228 may be caused by the specificity of HDI.

In vitro studies have suggested that HDIs have anti-inflammatory actions (24). In addition, several reports suggest that IFN-β has anti-inflammatory effect because IFN-β enhances the production of anti-inflammatory cytokines such as IL-10 antagonist in chondrocytes and synovial cells (51). Treatment of collagen-induced arthritis rats with IFN-β reduced inflammation and bone destruction (52). However, we couldn’t observe the inhibition of inflammation of AA by therapeutic treatment with FR901228. Recent reports show that earlier treatment of AA with TSA and phenylbutyrate before the onset of AA inhibited the inflammation, but late therapeutic treatment had no significant effect (53). HDI treatment may not enough for the suppression of active inflammation like AA.

In contrast, FR901228 strongly suppressed bone destruction in AA. Because osteoclast formation was markedly decreased, the inhibition of bone destruction is thought to be caused by inhibition of osteoclastogenesis. Immunostaining studies provide the evidence that IFN-β is produced by some mononuclear cells in the synovial membrane. Because synovials infiltrate includes RANK-positive cells (30), IFN-β-positive cells might be macrophage osteoclast precursor cells. Alternatively, IFN-β might be produced by T lymphocytes, which affect osteoclastogenesis. Although further study is required to determine the details of IFN-β-producing cells, IFN-β produced in synovial cells by FR901228 is thought to affect osteoclastogenesis from RANK-positive cells. However, we can’t exclude the possibility of parallel effect of FR901228 in AA model. Several inflammatory cytokines are also involved in bone destruction of RA (30). FR901228 may reduce the production of inflammatory cytokines directly or indirectly via IFN-β, which affects osteoclastogenesis. FR901228 also strongly suppressed disease development and severity of AA. AA is a T cell-mediated autoimmune disease, whose progression is dependent on the expression of Th1 type cytokines. Nfatc1 is required for activation of T cell. Recently, Mishra et al. (54) showed that HDIs down-regulated not only Th1 cytokine but also Th2 cytokine using MRL/lpr/lpr mouse, which is a model for human systemic lupus erythematosus. Inhibition of AA development by FR901228 may be mediated by the inhibition of Nfatc1-dependent signal.

In conclusion, we show that inhibition of HDAC inhibits osteoclastogenesis in vitro and in vivo by not only suppressing osteoclast-specific signal but also inducing production of IFN-β, an inhibitor of osteoclastogenesis. This study demonstrates that HDI has a novel action to induce IFN-β production selectively and has therapeutic benefit in the treatment of bone destruction of inflammatory diseases such as RA. Type I IFNs are known to inhibit tumor cell growth and stimulate the immune system (55), and systemic IFN-β therapy results in long-term survival in mice with colorectal liver metastases (56). Induction of IFN-β might be a part of the efficacy of cancer therapy by FR901228. Our data provide insight for treatment of not only rheumatoid arthritis but also cancer.

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Disclosures
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