Inhibition of NF-κB signaling by fenofibrate, a peroxisome proliferator-activated receptor-α ligand, presents a therapeutic strategy for rheumatoid arthritis

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Abstract

Objectives

Inflammatory mediators such as interleukin-6 and tumor necrosis factor-α play an important role in the pathogenesis of rheumatoid arthritis (RA) by promoting chronic inflammation and joint damage. NF-κB is a transcriptional activator of genes for these cytokines. It also plays an important role in the regulation of osteoclast differentiation which plays a key role in joint destruction in RA. Ligands for peroxisome proliferator-activated receptor (PPAR) -γ have recently been reported to inhibit the development of RA. In this study, we investigated the role of PPARα in RA.

Methods

We analyzed the protein expression of PPAR-α and -γ in rheumatoid synovial fibroblasts (RSF) from RA patients and analyzed the effects of ligands for PPAR-α and -γ on cytokine production from RSF, NF-κB activations in RSF and osteoclast differentiation from osteocalst progenitor in the peripheral blood. Moreover, we analyzed the effects of oral administration of PPAR-α and -γ ligands on the development of adjuvant-induced arthritis (AIA) in female Lewis rats.

Results

We confirmed the expression of PPAR-α in RSF and also demonstrated that fenofibrate, a ligand for PPAR-α, inhibited cytokine production from RSF, NF-κB activation in RSF, and osteoclast differentiation from osteoclast progenitor cells. Furthermore, we demonstrated that fenofibrate inhibits the development of arthritis in a rat model of human RA.

Conclusions

These results indicate that fenofibrate suppresses the development of arthritis by inhibition of NF-κB signaling; therefore, this compound offers possible anti-rheumatic drug.

Key words

Peroxisome proliferator-activated receptor α, rheumatoid arthritis, NF-κB.

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Introduction

Rheumatoid arthritis (RA) is a chronic, destructive, inflammatory, polyarticular joint disease, characterized by massive synovial proliferation and subintimal infiltration of inflammatory cells, followed by the destruction of cartilage and bone (1). Inflammatory mediators such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) play important roles in the pathogenesis of RA. These cytokines are abundant in synovial tissues and fluid from RA patients and overexpression of these cytokines promotes chronic inflammation and joint destruction (2). TNF-α receptor antagonists, neutralizing antibodies against TNF-α and IL-1 receptor antagonists can control joint inflammation in most RA patients (3-5). Bone-resorbing osteoclasts are critical for erosive disease and for irreversible destruction of the bone in affected joints. Osteoclasts are of hematopoietic cell origin, probably from the CFU-M-derived monocyte-macrophage family, and their differentiation is regulated by inflammatory cytokines (6).

The NF-κB family of transcriptional activators regulates the expression of a variety of cytokines involved in osteoclast differentiation, including IL-1, TNF-α, IL-6, and granulocyte/ macrophage-colony stimulating factor (GM-CSF) (7). It has been reported that the nuclear shift of NF-κB localization causes cartilage destruction in the early stage of arthritis in a mouse model of RA, DBA/1 mice immunized with type II collagen (8). Expression of TNF-α and induction of NF-κB were reported to play important roles in the pathogenesis of reactive arthritis (9). Consistent with these findings, mice with a deletion of the gene encoding the p50/p52 heterodimer of the NF-κB/Rel family exhibit osteopetrosis due to a deficiency in osteoclast differentiation (10, 11). Therefore, NF-κB, which is responsible for the production of inflammatory molecules as well as for the differentiation of osteoclasts, is an important target of therapy for RA.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor family, the largest family of transcription factors (12). Three distinct members of the PPAR subfamily have been reported:α, δ (also called β, NUC-1) and γ, all of them being activated by naturally occurring fatty acids or fatty acid derivatives. PPARs heterodimerize with the retinoid X receptor and regulate the transcription of target genes through binding to specific response elements, PPREs, which consist of a direct repeat of the nuclear receptor hexameric DNA core recognition motif spaced by one nucleotide. In addition to the regulation of gene transcription via PPREs, PPARs modulate gene transcription in a DNA binding-independent manner (13,14).

PPAR-α is highly expressed in liver, heart, muscle, kidney and cells of the arterial wall and it is activated by fibrate, fatty acids and eicosanoids. PPAR-α ligands inhibit interleukin-1-induced production of IL-6 and prostaglandin and inhibit expression of cyclooxygenase-2 (Cox-2) by negatively interfering with NF-κB transcriptional activity (15). PPAR-α ligands are thought to inhibit NF-κB activity by inducing IκBα which, in turn, inhibits NF-κB signaling (16). PPAR-γ is expressed at high levels in adipose tissue and is a critical regulator of adipocyte differentiation and reportedly plays a role in glucose homeostasis and insulin sensitivity (17,18). In addition, PPAR-γ has been suggested to be an important immunomodulatory factor (19). PPAR-γ is expressed in the immune system, specifically in the spleen (20), monocytes, bone-marrow precursors (21), and helper T-cells (22). Recent data have shown that endogenous prostaglandin, 15-deoxy-$\Delta^{12,14}$ -prostaglandin J_2 (15d-PGJ_2), and synthetic anti-diabetic thiazolidinedione, which are PPAR-γ ligands, inhibit phorbol ester-induced expression of nitric oxide and macrophage-derived cytokines (i.e., TNF-α, IL-1, and IL-6) (13,14). PPAR-γ ligands also reportedly inhibit the disease progression of inflammatory bowel diseases (IBD) (23), ischemic heart diseases (24,25), experimental autoimmune encephalomyelitis (EAE) (26) and RA(27). These PPAR-γ ligands inhibit gene expression by antagonizing the activity of the transcription factor, NF-κB, by inhibition of kinase activity of inhibition of IκB kinase (IKK), which leads to promote the inhibition of NF-κB by IκB (28-30). Taken together, these findings suggest that PPAR-

α and PPAR-γ may negatively regulate the inflammatory processes in RA.
In this study, we demonstrate the expression of PPAR-α in cultured synoviocytes from patients with RA. We also show that fenofibrate, a ligand for PPAR-α inhibits cytokine production in these cells. Moreover, we show that fenofibrate inhibits osteoclast differentiation from osteoclast progenitor cells in the peripheral blood mononuclear cell (PBMC) population. Finally, we demonstrate that fenofibrate inhibits the development of adjuvant-induced arthritis (AIA) in female Lewis rats. These results suggest that fenofibrate may have potential as an effective anti-rheumatic drug.

Methods

Materials

Pioglitazone was obtained from Takeda Co., Ltd. (Osaka, Japan) and rosiglitazone from GlaxoSmithKline Co., Ltd (Tokyo, Japan). PGJ2 and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) were purchased from Cayman Chemical (Ann Arbor, Michigan, USA). Fenofibrate was obtained from Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan) and bezafibrate from Kissei Pahrmaceutical Co., Ltd. (Nagano, Japan).

Synovial fibroblasts

Rheumatoid synovial fibroblasts (RSFs) were isolated from the fresh synovial tissue biopsy samples of three RA patients as previously reported (31). These patients had active RA as defined by the clinical criteria of the American Rheumatism Association (32). The average disease duration of these patients was 12 years and the average steroid dosage was 3.3mg/day; none of them took immunosuppressive agents. Tissue samples were minced into small pieces and treated with 1mg/ml collagenase/dispase (Boehringer Mannheim, Mannheim, Germany) for 10-20 min at 37°C. The cells obtained were cultured in RPMI medium (Nikken Bio Medical Laboratory, Kyoto, Japan) supplemented with 10% fetal calf serum (FCS) (Bioscience International Inc., Rockville, MD, USA). The culture medium was changed every 3-5 days, and non- adherent lymphoid cells were removed. Adherent cell subcultures were maintained in the same medium and harvested by trypsinization every 7-10 days before they reached cellular confluency. All experiments were carried out using cell cultures during the third to seventh passages.

Measurement of cytokine levels

Cytokine concentrations in RSF culture supernatants were determined using cytokine-specific ELISA kits for IL-6 (QuantiGlo Human IL-6 Immunoassay, R&D Systems, Minneapolis, USA), IL-8 (IL-8 Elisa Kit, Toray Fuji Bionics, Tokyo, Japan), and GM-CSF (Quantikine Human GM-CSF Immunoassay, R&D Systems, Minneapolis, USA). Assays were performed according to the manufacturers' instructions. Cytokine concentrations were measured 12 h after stimulation with 10 ng/ml IL-1β as described previously (33). The effects of pioglitazone, rosiglitazone, PGJ2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), fenofibrate and bezafibrate on induction of these cytokines were evaluated in the absence of serum. The concentrations of IL-6, IL-8 and GM-CSF were measured in culture supernatants after incubation with various concentrations (0-100 μM) of PPAR-α ligands (fenofibrate or bezafibrate) or PPAR-γ ligands (pioglitazone, rosiglitazone, PGJ_2 or 15d-PGJ_2) and subsequent stimulation with IL-1β for 12 h. The cytotoxic effects of these compounds were evaluated by measuring the number of viable cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (34). Experiments were performed three times with each of the three independent cultures.

Immunostaining

Cultured synovial fibroblasts were fixed with acetone for 5 minutes and immunohistochemical staining was performed using monoclonal antibodies against human PPAR-α and human PPAR-γ (1:100 dilution, in PBS). Normal goat serum was used as a control. The cells were then incubated with the secondary antibody, biotin-conjugated rabbit anti-goat immunoglobulin (1: 300 dilution) at room temperature for 3 hours with Histofine SAB-PO(G) kit (NICHIREI Corporation, Tokyo, Japan). Stained cells were examined by light microscopy. For the detection of the sub-cellular localization of NF-κB, cultured synovial fibroblasts were stimulated with 10 ng/ml IL-1β for 30 min and were fixed with acetone as described above. Fixed RSF were then incubated with a rabbit polyclonal antibody against the p65 subunit of NF-κB (Santa Cruz Biotechnology, Santa Cruz, CA) for 45 min at 37°C. After washing with PBS, the cells were incubated with biotin-conjugated goat anti-rabbit immunoglobulin (1:300 dilution) at room temperature for 3 hours with Histofine SAB-PO(R) kit (NICHIREI Corporation, Tokyo, Japan). Stained cells were examined by light microscopy.

Western blot analysis

Western blot analysis was performed by standard methods. All incubations with antibodies were for 1h at room temperature. Cells were pre-treated with 100μM of Pioglitazone, Fenofibrate, or Bezafibrate for 1h and then stimulated with 10 ng/ml IL-1β for 30min. An anti-IκBα antibody (SC-371; Santa Cruz Biotechnology) was used for detection of IκB.

Measurement of phosphorylated IκBα levels

Phosphorylated IκBα in RSF were determined using phosphorylated IκBα -specific ELISA kits (Human IκBα [pS32] Immunoassay Kit, BIOSOURCE, California, USA). Assays were performed according to the manufacturers'instructions. Phosphorylated IκBα concentrations were measured 30min after stimulation with 10 ng/ml IL-1β. The effects of pioglitazone, rosiglitazone, fenofibrate and bezafibrate on phosphorylation of IκBα were evaluated.

Detection of osteoclast formation

Human peripheral blood from healthy volunteers was collected in syringes containing 1,000 units/ml of preservative-free heparin. Informed consent was obtained in all cases before the blood was drawn. PBMC were isolated by centrifugation over Histopaque 1077 (Sigma, St. Louis, MO) density gradients, washed, and resuspended at 1.3 x 10^6 cells/ml in α-minimal essential medium (α-MEM) (Gibco BRL,

Gaithersburg, MD) supplemented with 10% fetal bovine serum (JRH Bioscience, Lenexa, KS). PBMC were then cultured for 3 days in 48-well plates ($5x10^5$ cells/0.3ml/well; Corning, Corning, NY) in the presence of M-CSF (100 ng/ml), and non-adherent cells were removed. The adherent PBMC were cultured in the presence of human sRANKL (100 ng/ml) with various concentrations of pioglitazone or fenofibrate. Adherent PBMC were used as monocytes in the co-culture system. Cultures were incubated in quadruplicate. Culture media were replaced every 2 or 3 days with fresh media supplemented with the agents described above. Osteoclast formation was identified by positive immunohistologic staining for vitronectin receptor (CD51/61, Sanbion Co., Am uden, Netherlands) and the absence of phagocytic activity using fluorescent microbeads (Polyscience Co., Warrington, PA, USA) after culture for 7 days as described elsewhere (35).

Adjuvant-induced arthritis in rats

Seven-week-old female Lewis rats were obtained from Charles River Japan (Yokohama, Japan). Complete Freund's adjuvant (CFA) was prepared by suspending heat-killed *Mycobacterium butyricum* (Difco Laboratories, Detroit, Michigan, USA) in liquid paraffin at 10 mg/mL. CFA-induced arthritis was stimulated by injection of 100 μL of the CFAemulsion intradermally at the base of the left paw. Treatment commenced at the onset of the disease: pioglitazone (once a day) and fenofibrate (once a day) were orally administered at the specified doses, until 7 days after the onset of arthritis. Pioglitazone and fenofibrate were freshly suspended in 1.0% methyl cellulose (MC) PBS. In each experiment, a group of rats was orally administered 1.0% MC PBS, which served as a control. At days 1, 7 (onset of arthritis), 8, 11 and 14 after immunization, rats were examined for adjuvant-induced arthritis (AIA) using two clinical parameters: paw swelling and a clinical score. The footpad volume was measured with a TK-101 plethysmometer (Unicom Japan, Tokyo, Japan). For the clinical evaluation of AIA, we used a scoring system as follows [as reported previously in (36)]: ear, forelimb, non-treated hind-limb and tail, were scored 0 to 5: 0, normal; 1, minimal swelling; 2, mild swelling; 3, moderate swelling; 4, severe swelling; 5, severe and non-weight-bearing arthritis. Each limb was graded, resulting in a maximal clinical score of 20 per animal. For the histological evaluation, we performed hematoxylin and eosin (H&E) staining of tissue specimens of the ankle.

Statistical analysis

The Mann-Whitney U test was used to compare non-parametric data for statistical significance. This test was used to evaluate the histological examination of rat ankles, paw volume and the clinical arthritis score.

Results

PPAR expression in cultured rheumatoid synovial fibroblasts (RSF)

To examine the expression of PPAR-α and -γ in RSF cultures, immunohistochemistry was performed. As demonstrated in Figure 1A, PPAR-α as well as PPAR-γ were expressed in these cells. PPAR-α was localized in the cytoplasmic region but not in the perinuclear region or in the nucleus. PPAR-γ was localized in the perinuclear and cytoplasmic region but not in the nucleus.

Effect of PPAR-α and γ ligands on cytokine production in cultured RSF

To examine the induction of IL-6, IL-8 and GM-CSF, RSF obtained from three different RA patients were stimulated with 10 ng/ml IL-1β. As shown in Figure 1B, IL-6, IL-8 and GM-CSF production from RSF was suppressed in a dose-dependent manner in the presence of PPAR-γ ligands. A PPAR-α ligand, fenofibrate, also inhibited the production of these cytokines from RSF in a dose-dependent manner; however a different PPAR-α ligand, bezafibrate, had no inhibitory effect up to 100 μM. Neither PPAR-alpha nor PPAR-gamma ligands inhibited basal level expression of these cytokines. PPAR-α and PPAR-γ ligands were not toxic to RSF at concentrations up to 100 μM as indicated by MTT assay (Fig. 1B). Therefore, the inhibiting effect of PPAR-γ ligands (pioglitazone, rosiglitazone, PGJ2, and 15d-PGJ2) and fenofibrate on the production of cytokines was not due to drug cytotoxicity.

Effects of PPAR-α and -γ ligands on nuclear translocation of NF-κB in cultured RSF

Induction of the inflammatory cytokines, IL-6, IL-8 and GM-CSF are known to be under the control of NF-κB. As demonstrated above, the production of these cytokines was inhibited by PPAR-α and -γ ligands. These ligands reportedly prevent the activation of NF-κB by obstructing IKK, which is a kinase of IκB, a cellular inhibitor protein of NF-κB (28-30). Therefore, it is plausible that PPAR ligands suppress NF-κB activation in RSF. To test this hypothesis, we examined whether PPAR-α and -γ ligands inhibit nuclear translocation of NF-κB by immunohistochemical assay or not. As shown in Figure 2, RSF were incubated in the presence of 10 ng/ml IL-1β to stimulate NF-κB nuclear translocation. As expected, without IL-1β stimulation, NF-κB remained localized in the cytoplasm (Figure 2A-a). However, after 30 min of IL-1β stimulation, NF-κB was mainly localized in the nucleus (Figure 2A-b). In the presence of 100 μM pioglitazone, rosiglitazone, or fenofibrate, nuclear localization of NF-κB was inhibited (Figures 2A-c, d and e). However, in the presence of 100 μM bezafibrate, NF-κB remained in the nucleus (Figure 2A-f). These results are consistent with the PPAR-induced suppression of cytokine expression described above and indicate that this suppression is due to the inhibition of NF-κB nuclear translocation in RSF. To further investigate the anti-NF-κB effects of these compounds, we performed western blots to detect IκBα degradation by IL-1β signal. As demonstrated, PPAR-γ ligand (pioglitazone) and fenofibrate inhibited the IL-1β -stimulated degradation of IκBα but bezafibrate had less inhibitory effect. (Figure 2B). These results indicated that PPAR-γ ligands and fenofibrate inhibit NF-κB activation more than bezafibrate.

Inhibition of osteoclast formation by PPAR-α and -γ ligands

NF-κB signaling is involved in osteoclast differentiation and activation of PPAR-γ reportedly inhibits osteoclast

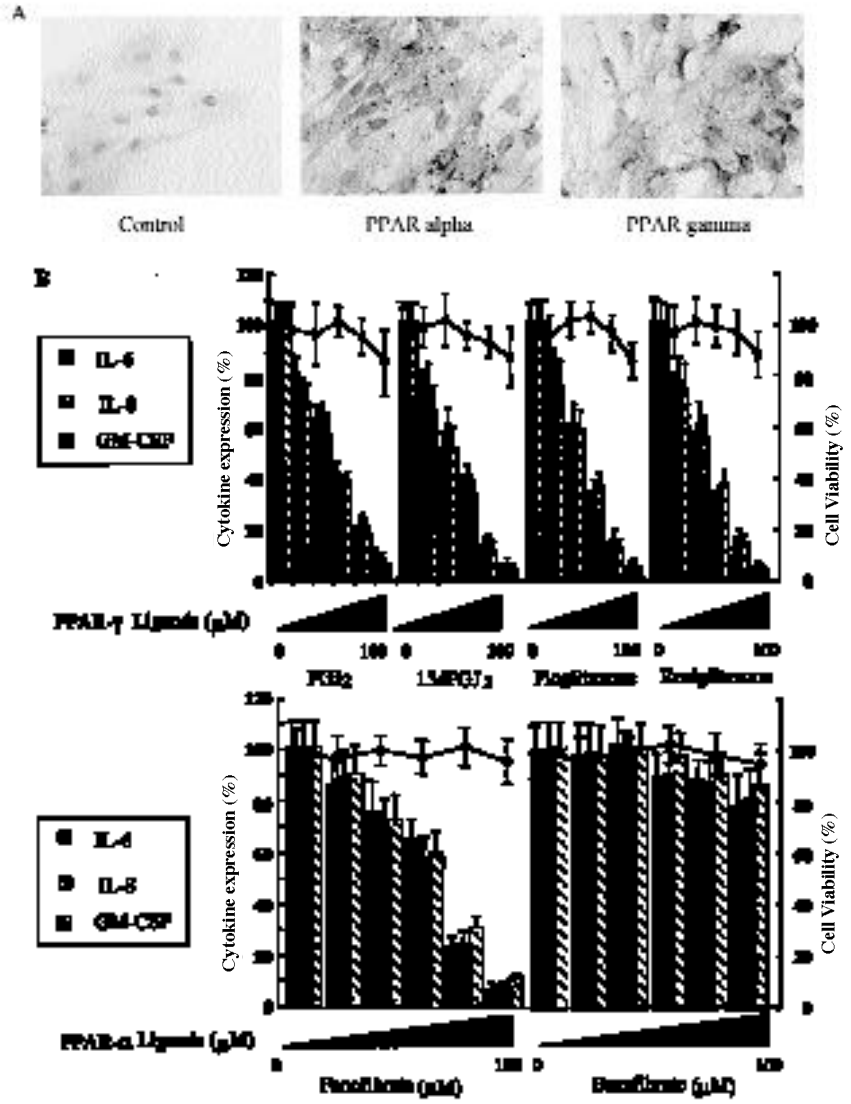

Fig. 1. PPAR expression in cultured rheumatoid synovial fibroblasts and inhibition of cytokine production by PPAR-α and -γ ligands. (**A**) Expression of PPAR-α and -γ were demonstrated by immunohistochemical staining. (**B**) PPAR-γ (PGJ2, 15d-PGJ_2, pioglitazone, rosiglitazone) and PPAR-α (fenofibrate) ligands inhibited cytokine expression from cultured rheumatoid synovial fibroblasts in a dose-dependent manner. One PPAR-α ligand (bezafibrate), however, did not inhibit cytokine expression. The cytotoxic effect of these compounds was evaluated by measuring the number of viable cell using the MTT assay (*diamond*). The level of the absorbance in the MTT assay obtained without ligands (PBC) was taken as 100%. The data are represented as the mean ± standard deviation.

differentiation (37,38). Therefore, we next studied the effects of PPAR-α and PPAR-γ ligands on the formation of osteoclasts from PBMC. The number of osteoclasts arising from PBMC was scored in the presence of various concentrations of fenofibrate or pioglitazone. As shown in Figure 3, formation of osteoclasts from PBMC was suppressed, in a dose-dependent manner, in the presence of pioglitazone or fenofibtrate. This result suggests that PPAR-α and -γ ligands inhibit differentiation of osteoclasts, which play a major role in joint destruction in RA.

Oral administration of PPAR-α and -γ ligands ameliorate adjuvant-induced arthritis

Our data showing that PPAR-α and -γ ligands inhibit NF-κB induced cytokine production, as well as osteoclast formation *in vitro*, strongly suggest that these ligands could play a role in the pathogenesis of RA. Therefore, we tested the effect of PPAR-α and -γ ligands *in vivo* on the progression and severity of AIA in female, Lewis rats. At the onset of arthritis on day 7, pioglitazone and fenofibrate were orally administered. This paradigm was chosen to approximate a clinical usage pattern. As shown in Figure 4, pioglitazone and fenofibrate suppressed the progression of clinical arthritis compared with control rats treated with PBS, as demonstrated by paw volume (Figure 4A) and arthritis score (Figure 4B). In both pioglitazone- and fenofibrate-treated rats, statistically significant effects were observed with higher doses ($P < 0.01$). By day 18, histological analysis of the ankle joint in pioglitazone-treated rats (100 mg/kg/day) (b), fenofibrate-treated rats (100 mg/kg/day) (c) and control rats (PBS) (a) show that both pioglitazone and fenofibrate inhibit mononuclear cell infiltration and pannus formation in synovial tissue (Figure 4C). There was no mortality and no body weight loss in either pioglitazone- or fenofibrate-treated rats. These data suggest that both PPAR-α and -γ ligands have anti-arthritis effects in vivo.

Discussion

In the present study, we demonstrated that immunoreactive PPAR-α are expressed in cultured RSF obtained from joint tissues of RA patients and that fenofibrate, a PPAR-α ligand, inhibited IL-1β-induced cytokine (i.e., IL-6, IL-8, GM-CSF) induction through suppression of NF-κB *in vitro*. We also showed that fenofibrate suppressed differentiation of osteoclasts; a major player in joint destruction in RA, from PBMC. Moreover, we demonstrated that fenofibrate is a potent inhibitors of arthritis progression and severity in AIA.

In this study, the induction of IL-6, IL-8 and GM-CSF were inhibited by PPAR-α and -γ ligands (Figure 1). Since gene expression of these cytokines is under the control of NF-κB, we hypothesized that the anti-inflammatory action of PPAR-α and -γ ligands could be mediated through NF-κB. PPAR-α and -γ ligands reportedly inhibit NF-κB action through suppression of IKK (in the case of PPAR-γ) and induction of IκBα (in the case of PPAR-α) (16, 28). In concordance with these mechanisms, our data show that nuclear translocation of NF-κB in RSF is inhibited by PPAR-α and -γ ligands (Fig. 2).

Osteoclasts are multinucleated cells with bone-resorbing activity that dif-

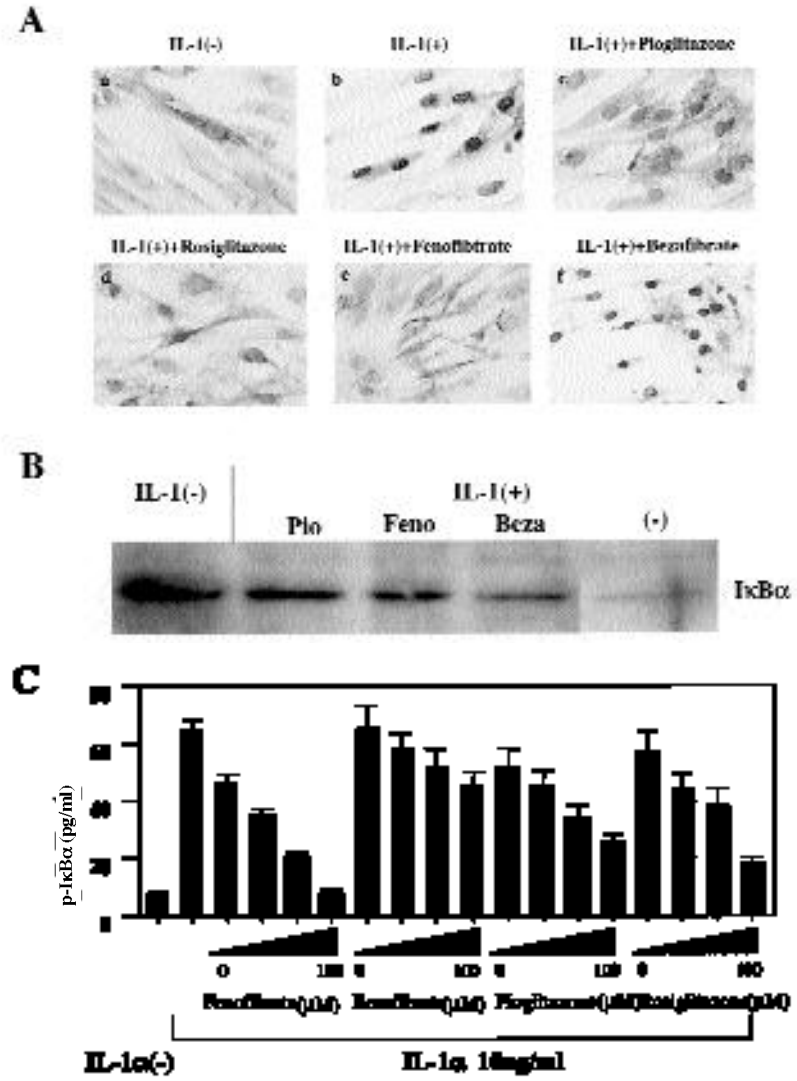

Fig. 2. Inhibition of NF-κB nuclear translocation by PPAR ligands. Subcellular location of NF-κB in rheumatoid synovial fibroblasts (RSF) was studied by immunohistochemical staining using a monoclonal antibody against the p65 subunit of NF-κB. After stimulation with IL-1β, NF-κB localized to the nucleus. In the presence of PPAR-α and -γ ligands (pioglitazone, rosiglitazone, fenofibrate), however, NF-κB nuclear translocation was inhibited and it remained in the cytoplasm. The PPAR-α ligand, bezafibrate, did not inhibit NF-κB translocation in RSF (**A**). PPAR-γ ligand and fenofibrate inhibit degradation of IκBα in IL-1β-stimulated RSF. Synovial fibroblasts were treated with pioglitazone (Pio), fenofibrate (Feno), or bezafibrate (Beza) for 1h and then stimulated with IL-1β for 30min, and whole cell extracts were assayed for IκBα by Western blotting (**B**).

ferentiate from cells of the monocyte-macrophage lineage. Osteoclast precursors express receptor activator of NF-κB (RANK). Differentiation of these precursor cells into osteoclasts is mediated by RANK binding to its ligand, receptor activator of NF-κB ligand (RANKL) during cell-to-cell interaction with osteoblasts/stromal cells, and in the presence of macrophage colony-stimulating factor (M-CSF). Mature osteoclasts also express RANK, and their bone-resorbing activity is also activated by RANKL on osteoblasts/stromal cells. A series of studies suggests that NF-κB is involved in the signal transduction pathway responsible for the osteoclast differentiation. For example, 1) expression of IL-1 and M-CSF are induced by NF-κB and 2) soluble RANKL, which stimulates osteoclast differentiation together with M-CSF, activates NF-κB signaling (37, 38). Consistent with these findings, mice with a deletion of the gene encoding the p50/p52 heterodimer of the NF-κB/Rel family exhibit osteoporosis due to a deficiency in osteoclast differentiation (10, 11). Inhibition of osteoclast differentiation by PPAR-α and -γ ligands in our study is likely to be mediated by the inhibition of NF-κB with these compounds (Fig. 3). One of the PPAR-α ligands, fenofibrate inhibited NF-κB activation and cytokine expression, whereas the other PPAR-α ligand, bezafibrate failed to suppress this. This might be explained by the fact that bezafibrate has less affinity for PPAR-α compared to fenofibrate (more than 5 fold) (39). However, other explanation on how fenofibrate inhibits stronger than bezafibrate could be involved.

Taken together, our results suggest that the mechanism of the anti-inflammatory effects of PPAR-α and -γ ligands in AIA can be explained, at least in part, by inhibition of NF-κB, which leads to the suppression of inflammatory cytokine production, osteoclast differentiation, and COX-2 production and the induction of apoptosis of several cell types, such as synovial cells. Kawahito *et al*. showed that the PPAR-γ ligand, 15-deoxy- delta$^{(12,14)}$-PGJ_2, induces synoviocyte apoptosis (27). This could be mediated by PPAR-γ ligand-induced inhibition of NF-κB since NF-κB possesses anti-apoptotic activity (40-42). Yet, we did not see any toxic effects towards synovial fibroblasts of PPAR-α and PPAR-γ ligands. And recently, Kalajdzic *et al*. showed that PPAR-α and PPAR-γ ligands stimulate COX-2 expression by PPAR-response element (PPRE) dependent activation (43). However, this report did not show the effects of these ligands on activated NF-κB dependent expression of COX-2 gene.

The action of other anti-rheumatic drugs is also reportedly due to inhibition of NF-κB. For example, aspirin and glucocorticoids block the activation of NF-κB by increasing the steady-state level of IκBα either by preventing IκB degradation or by inducing its gene expression (44-46). Considering the wide array of events under the control of NF-κB, such as cytokine and COX-2 expression, osteoclast differentiation and apoptosis (40-42), and the impact of these events on the pathogenesis of RA, NF-κB is an efficient and feasible therapeutic target for RA. Therapy with fenofibrate may serve as a new anti-NF-κB strategy for the treatment of RA.

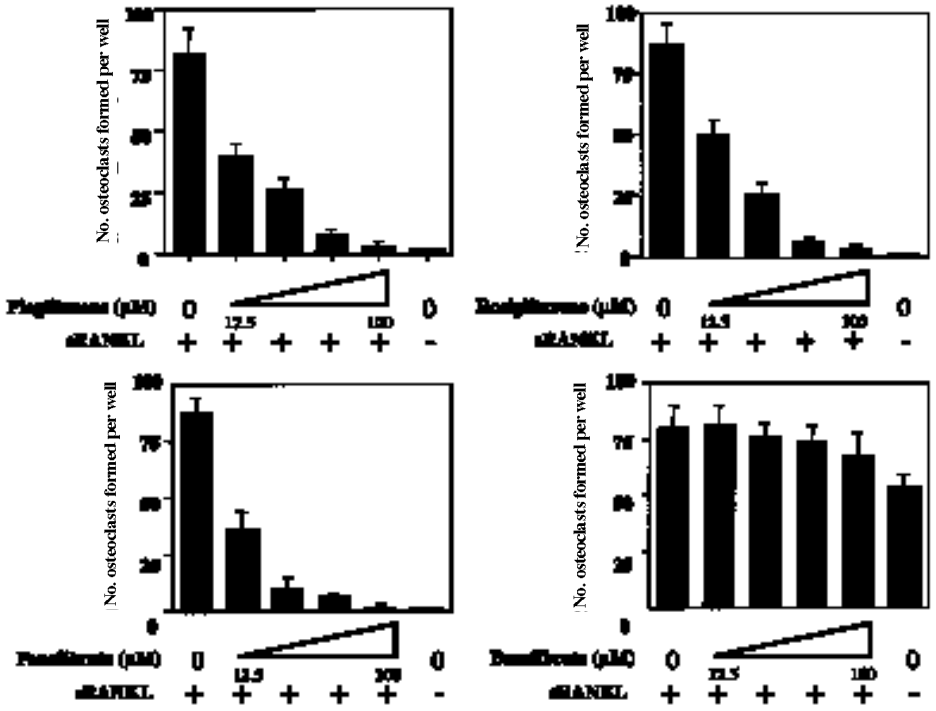

Fig. 3. Inhibition of osteoclast formation by PPAR ligands. Formation of osteoclasts from human peripheral blood mononuclear cells (PBMC) was inhibited in the presence of PPAR-α and -γ ligands. Adherent PBMC were cultured in the presence of human sRANKL (100ng/ ml) with various concentrations of pioglitazone or fenofibrate. Formation of osteoclasts from PBMC were suppressed in the presence of pioglitazone or fenofibtrate in a dose-dependent manner. The data are represented as mean ± standard deviation.

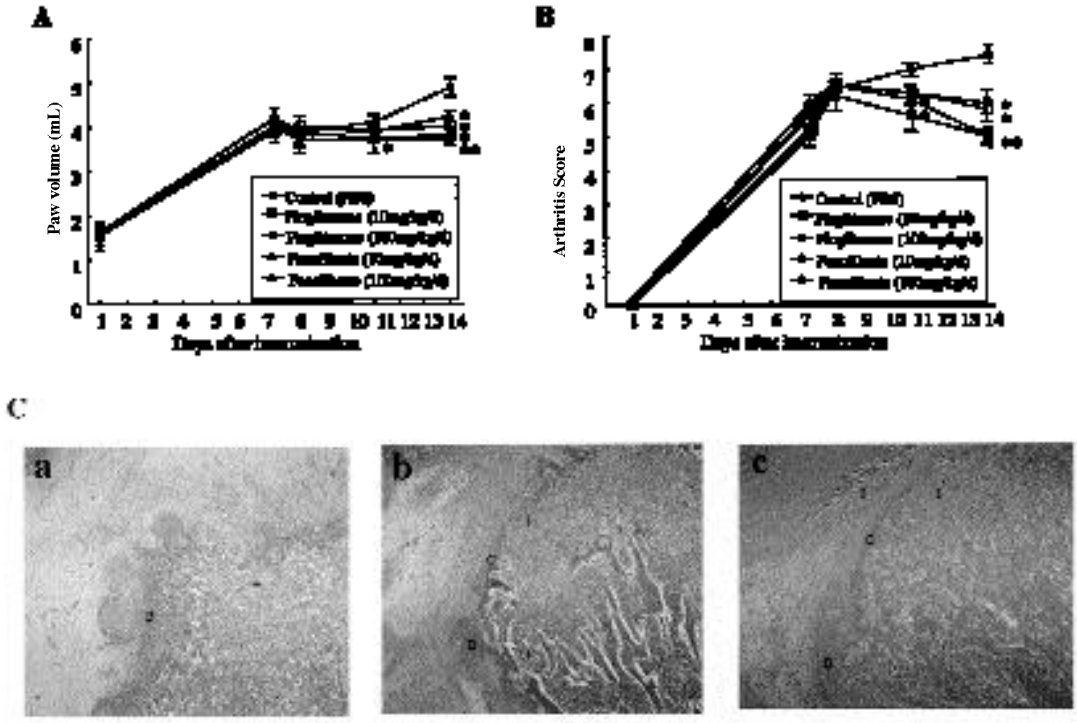

Fig. 4. Suppression of arthritis development by PPAR ligands in an adjuvant-induced arthritis model. Pioglitazone and fenofibrate suppressed the progression of clinical arthritis compared with control rats treated with PBS, as demonstrated by paw volume (**A**) and arthritis score (**B**). The data are represented as mean ± standard deviation. *: $p < 0.05$; **: $p < 0.01$.
(**C**) Histological findings of the foot joint in (**a**) control rats (PBS), (**b**) pioglitazone-treated rats (100 mg/kg/day) and (**c**) fenofibrate-treated rats (100 mg/kg/day).
B: bone, **C**: cartilage, **I**: infiltrates.

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