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PPARγ ligands inhibit nitrotyrosine formation and inflammatory mediator expressions in adjuvant-induced rheumatoid arthritis mice

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Abstract

Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor, whose activation has been linked to several physiologic pathways including those related to the regulation of insulin sensitivity. Here, we investigate effects of PPARγ specific ligands, rosiglitazone and pioglitazone, on formation of nitrotyrosine and increased expression of inflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 and intercellular adhesion molecule-1 (ICAM-1) in adjuvant-induced murine arthritis. Administration of rosiglitazone or pioglitazone (30 mg/kg, p.o.) significantly inhibited the adjuvant-induced increase in formation of nitrotyrosine and expression of iNOS on both ankle and temporomandibular joints. Rosiglitazone also inhibited the adjuvant-induced expression of M30 positive cells, as a marker of apoptosis, in the joint tissues. In addition, treatment with rosiglitazone or pioglitazone (30 μM) inhibited lipopolysaccharide plus tumor necrosis factor (TNF)-α-induced protein expression of iNOS, cyclooxygenase-2, ICAM-1 and nitrotyrosine formation in RAW 264 cells, a murine macrophage-like cell line. Rosiglitazone or pioglitazone inhibited increase in phosphorylated I-κB (pI-κB) expression, as an index of activation of nuclear factor (NF)-κB, in both joint tissues and RAW264 cells. Furthermore, in PPARγ-transfected HEK293 cells, rosiglitazone inhibited the TNF-α-stimulated response using NF-κB-mediated transcription reporter assay. These results indicate that PPARγ ligands may possess anti-inflammatory activity against adjuvant-induced arthritis via the inhibition of NF-κB pathway.

Keywords: Peroxisome proliferator-activated receptor (PPARγ); Nitrotyrosine; Inflammation; Rheumatoid arthritis; Temporomandibular joint

1. Introduction

Many reports indicated formation of nitrotyrosine in proteins during inflammation such as arthritis, nephritis, sepsis and ischemia–reperfusion injury, because nitrotyrosine is formed by a reaction of tyrosine residue with peroxynitrite generated from superoxide and nitric oxide (NO) radicals (Wada et al., 1998; Mapp et al., 2001; Noiri et al., 2001; Pfeiffer et al., 2001). Pathological roles of nitrotyrosine are still unknown. However, it is required for normal physiological conditions to reduce or metabolize the nitrated proteins, since the nitrated proteins may affect physiological regulation systems by phosphorylation of tyrosine. Few reports indicated inhibitors on the formation of nitrotyrosine, although there might be specific enzymes that recover function of the nitrated proteins (Kamisaki et al., 1998).

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of nuclear receptor superfamily of ligand-activated transcriptional factors including steroid hormone, thyroid hormone, vitamin D, and retinoic acid (Michalik and Wahli, 1999; Kersten et al., 2000). PPARγ is highly expressed in adipose tissue and plays a key role in adipocyte differentiation and insulin sensitivity. Synthesized ligands, thiazolidinedione derivatives, such as rosiglitazone, pioglitazone and troglitazone, are used as oral antihyperglycemic agents in the therapy of non-insulin-dependent diabetes mellitus (Vamecq and Latruffe, 1999; Kadowaki,

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2000). In addition, recent studies have shown that PPARγ may participate in control of inflammation, especially, in modulating the production of inflammatory mediators (Jiang et al., 1998; Ricote et al., 1998). Actually, we have reported that the stimulation of endogenous PPARγ pathway causes anti-inflammatory responses in experimental bowel disease inflammation, such as dextran sodium sulfate and ischemia-induced colitis (Nakajima et al., 2001; Saubermann et al., 2001; Wada et al., 2001). According to those observations, PPARγ ligand therapy is also suggested to suppress other inflammatory diseases such as rheumatoid arthritis, sepsis, nephritis and pancreatitis.

Rheumatoid arthritis is a chronic, destructive polyarticular joint disease, being characterized massive synovial proliferation and subintimal infiltration of inflammatory cells (Feldmann, 2001). In this report, we applied PPARγ specific ligands, rosiglitazone and pioglitazone, for adjuvant-induced murine arthritis model. We investigated the effects of PPARγ ligands on the nitrotyrosine formation and inflammatory mediator expression, such as inducible nitric oxide (iNOS), cyclooxygenase-2 and intercellular adhesion molecule-1 (ICAM-1), in ankle and temporomandibular joint tissues. First, we revealed that administration of PPARγ ligands inhibits expression of inflammatory mediators in arthritis tissues, and that suppression of nuclear factor (NF)-κB pathway may be involved in the inhibitory mechanisms of PPARγ ligands on the nitrotyrosine formation and inflammatory mediator expressions in ankle and temporomandibular joints.

2. Materials and methods

2.1. Adjuvant-induced arthritis

All animal experiments were performed in accordance with the guideline for animal experimentation of Graduate School of Dentistry, Osaka University. Adult male Balb/c mice were purchased from SLC (Shizuoka, Japan). Under slight ether anesthesia, adjuvant-induced arthritis was induced by injection of complete Freund's adjuvant (CFA, Difco Laboratories; 0.2 ml 1:1 saline suspension) in mice back skin (Nozawa-Inoue et al., 1998). The animals were sacrificed, Ankle and temporomandibular joints were collected 10 days after the injection of CFA.

2.2. Administration of PPARγ ligands on CFA-induced arthritis mice

PPARγ specific ligands, rosiglitazone and pioglitazone, were kind gifts from Glaxo SmithKline (Tokyo, Japan) and Takeda Pharmaceutics (Osaka, Japan), respectively. The mice were orally administrated with rosiglitazone or pioglitazone (30 mg/kg each) once a day for 10 days from 1 day before the injection of CFA. Control group was only administered with vehicle (milli-Q water).

2.3. Cell culture and ligands treatment

RAW 264 cells, murine peritoneal macrophage-like cell line, were purchased from Riken Gene Bank (Tokyo, Japan). The cells were cultured in the medium of Dulbecco's modified eagle's medium (DMEM; Sigma) supplemented with penicillin (5 unit/ml), streptomycin (5 μg/ml) and amphotericine B (250 ng/ml) to confluence in 10-cm dishes at 37 ° containing 5% CO_2. The treatment with rosiglitazone or pioglitazone (30 μM each) was performed for 2 h and followed the stimulation lipopolysaccharide (1 μg/ml) and tumor necrosis factor (TNF)-α (10 ng/ml). Cells were harvested 24 h after the stimulation.

2.4. Western blot analysis for inflammatory mediators

For the samples of in vivo study, tissues were crushed to powder in liquid nitrogen and homogenized in lysis buffer (20 mM Tris–HCl, 150 mM NaCl, 4 mM EGTA, 1% Triton X-100) containing a cocktail of protease inhibitors (Sigma, Tokyo, Japan). Similarly, harvested cells were homogenized in lysis buffer. Samples (20 μg protein per well) were applied to sodium dodesyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred onto a polyvinylidene difluoride membrane. Anti-nitrotyrosine antibody (mouse monoclonal, Zymed Laboratories, CA), anti-iNOS, anti-cyclooxygenase-2, anti-ICAM-1 or anti-phosphorylated I-κB (pIκ-B) antibody (rabbit polyclonal, Santa Cruz, CA) was applied for overnight at 4°. Visualization of proteins was performed with an ECL-plus kit (Amersham, London, UK). Visualized bands were digitized, and the net band intensities were normalized and expressed as arbitrary unit.

2.5. Immunohistochemistry

Joint tissues were immediately removed and immersed in 3.7% paraformaldehyde fixative for overnight. Then, samples were embedded in paraffin and 8-μm sections were prepared. Staining for nitrotyrosine or M30 was performed using anti-nitrotyrosine antibody or M30 antibody (mouse monoclonal, Japan Roche Diagnostics, Tokyo, Japan) with standard immunohistochemical techniques. Vectastain ABC kit (Vector Laboratories, Burlingame, CA) was used for detection.

2.6. Reporter assay

HEK293 cells were co-transfected with PPARγ$_1$ expression plasmid (pHMCMV6-PPARγ1) or rsGFP expression plasmid (pHMCMV6-rsGFP) and the reporter plasmid of pNF-κB-Luc (Mercury Pathway Profiling System, Clontech Laboratories Japan, Tokyo, Japan) by Effectene (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. Cells were treated with either vehicle (0.005% dimethylsulfoxide) alone, TNF-α (2 μg/ml) or TNF-α + rosiglitazone

(30 μM) for 2 h. The cell extracts were subsequently assayed for luciferase activity (Luciferase reporter assay system, Promega, Tokyo, Japan) and results are shown as fold induction over vehicle alone.

2.7. RT-PCR for detection of iNOS and COX-2 mRNA levels on RAW264

RAW264 cells were treated with rosiglitazone or pioglitazone (30 μM) for 2 h and were subsequently treated with TNF-α (10 ng/ml) plus lipopolysaccharide (1 μg/ml) for 2 h. RNA was extracted by Trizol (Gibco BRL) and cDNA were synthesized using the Super Script First-Strand Synthesis System for PCR (Gibco BRL) according to the manufacturer's instructions. Cyclooxygenase-2 and iNOS were amplified 34 cycles using relative RT-PCR kit (Ambion) by Gene Amp PCR System 9700 (PE Biosystem). Amplification products were resolved by agarose gel electrophoresis, digitized and analyzed by Kodak Digital Science system (Kodak, Tokyo, Japan). The net band intensities were normalized to 18S for each sample and expressed as arbitrary unit.

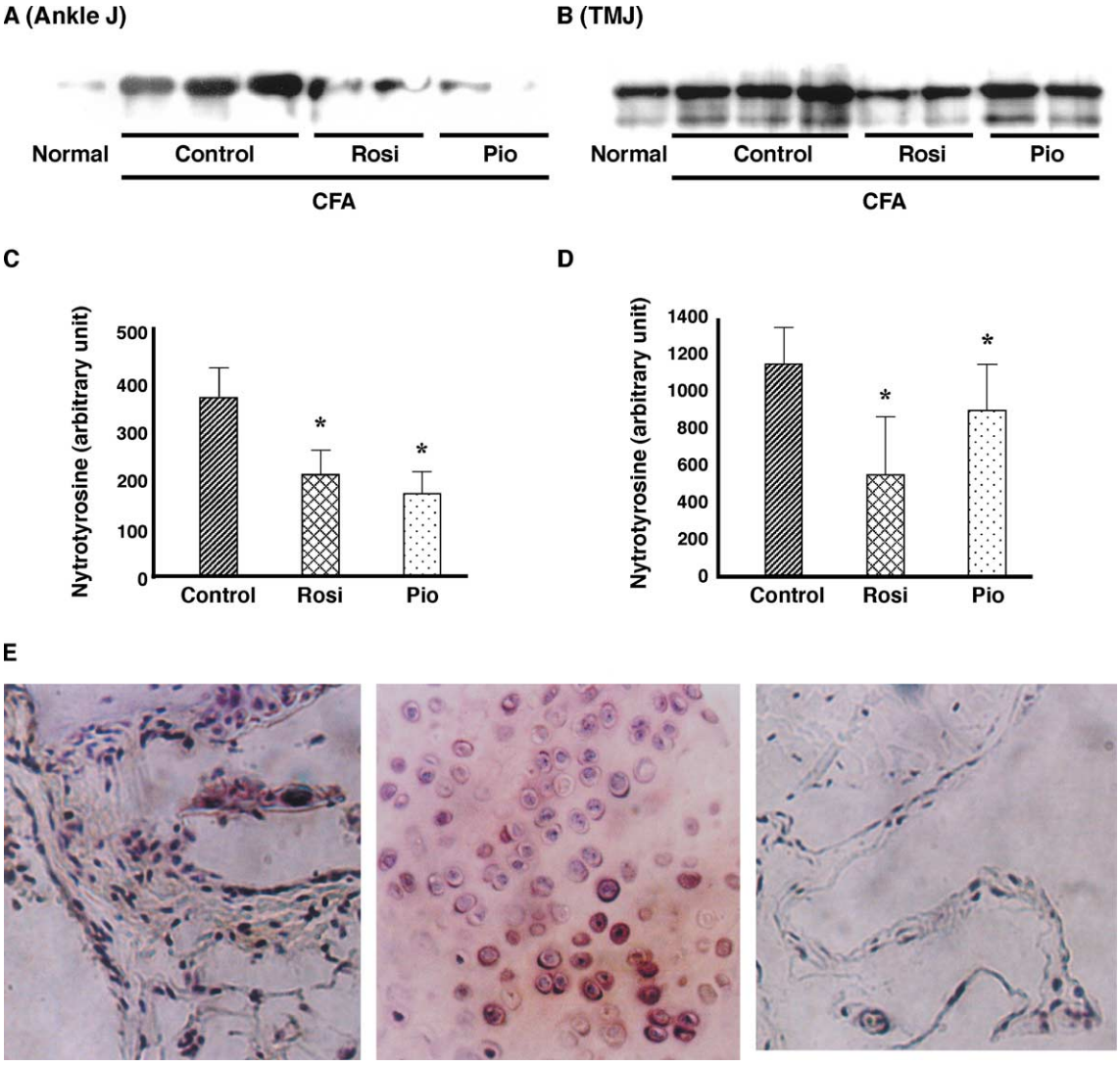

Fig. 1. Inhibition by PPARγ ligands of adjuvant-induced nitrotyrosine formation in joint tissues from adjuvant-induced arthritis mice; typical Western blot analysis on ankle (A) and temporomandibular joints (TMJ, B). CFA groups were administered complete Freund's adjuvant. Rosiglitazone (Rosi) or pioglitazone (Pio) (30 mg/kg/day, respectively) was orally administered for 10 days, and control group was administered vehicle. Net band intensities of nitrated proteins in ankle (C) and temporomandibular joints (D). Data are expressed as arbitrary unit. Each column represents mean ± SD from six to eight different animals. * $P < 0.05$ vs. control. Immunohistochemical stainings of nitrotyrosine in temporomandibular joint from adjuvant-induced arthritis mice (E). Connected tissues around cartilage were prepared from CFA-treated control (left panel) and Rosi group (right panel). Nitrotyrosine positive stainings were observed in control group, but weak stainings were in Rosi-treated group. Positive stainings were also observed in synoviocytes in cartilage from adjuvant-induced arthritis mice (middle panel). Magnifications are × 100 in left and right panels and × 200 in middle panel.

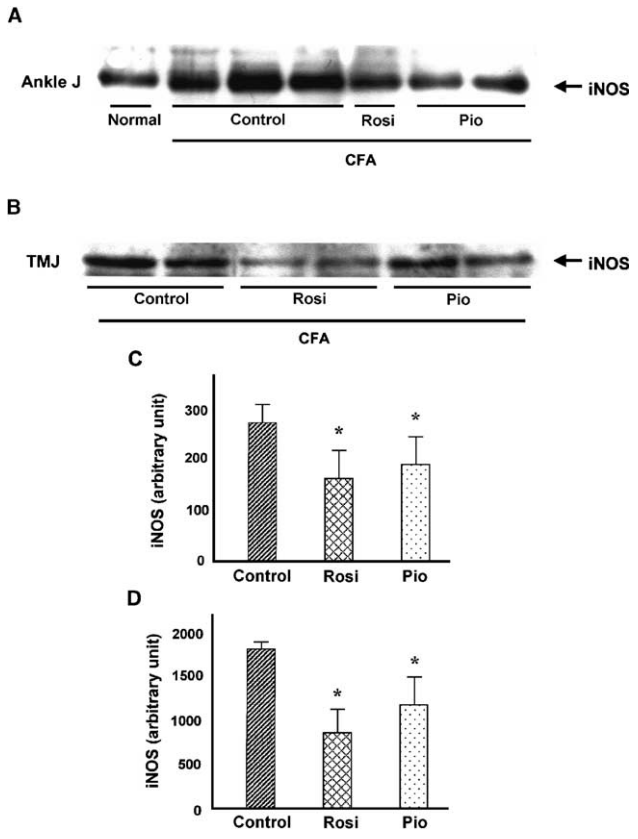

Fig. 2. Effects of PPARγ ligands, rosiglitazone (Rosi) or pioglitazone (Pio), on the increased expression of iNOS proteins in ankle (A and C) and temporomandibular joints (B and D) of CFA-treated mice; typical Western blot analysis (A and B) and net band intensities of iNOS proteins (C and D). Data are expressed as arbitrary unit. Each column represents mean ± SD from six to eight different animals. * $P<0.05$ vs. control. Rosiglitazone or pioglitazone inhibited the increase in iNOS, compared to those of vehicle control group.

2.8. Statistical analysis

Statistical comparisons were made using Scheffe's method after analysis of variance (ANOVA). The results were considered significantly different when $P<0.05$.

3. Results

3.1. PPARγ ligands inhibit adjuvant-induced nitrotyrosine formation in joint tissues

Administration of adjuvant (CFA) increased nitrotyrosine formation in ankle and temporomandibular joints (Fig. 1). By Western blot analysis, although several proteins of various molecular weights were nitrated, the protein of 90–100 kDa is most extensively nitrated (shown in Fig. 1A and B, respectively). Normalized net band intensities of nitrated proteins were also shown in Fig. 1C and D, respectively. We also confirmed the formation of nitrotyrosine in CFA-treated mice by immunohistochemical stainings with anti-nitrotyrosine antibody. Positive stainings were observed in inflammatory cells, endothelial cells and synovial lining cells in ankle joint of CFA-treated mice (Fig. 1E, left and middle panels). Similar results were also observed in temporomandibular joint (data not shown). Treatment with rosiglitazone or pioglitazone (30 mg/kg/day, p.o.) inhibited the increase in nitrotyrosine formation by both Western blot analysis (Fig. 1A, B, C and D) and immunohistochemistry (Fig. 1E, right panel).

3.2. PPARγ ligands inhibit adjuvant-induced increases in iNOS protein

Since nitrotyrosine is one of the products of NO, we investigated NO-generating enzyme, the protein

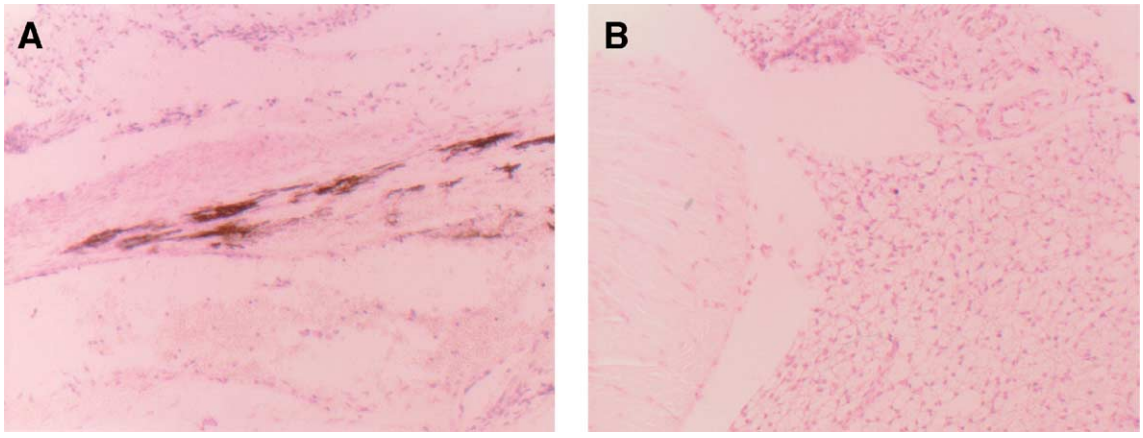

Fig. 3. Immunohistochemical stainings of M30, a marker of early apoptosis, around temporomandibular joint of control (A) or rosiglitazone-treated (B) temporomandibular joint in CFA-induced arthritis mice. Positively stained cells were observed in control tissue around temporomandibular joint, but not in that of rosiglitazone-treated mice. Magnifications are × 100.

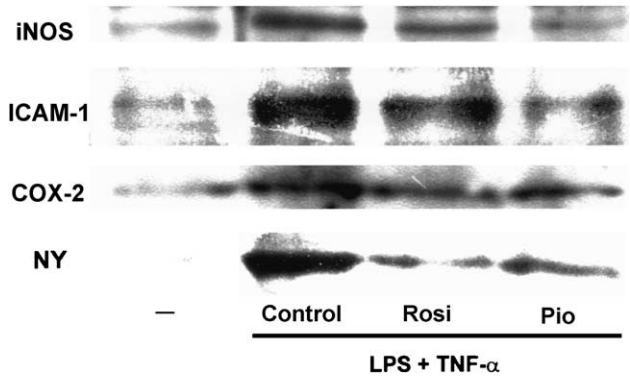

Fig. 4. Inhibition by PPARγ ligands of increased expression of inflammatory mediators in RAW264; Western blot analysis. Cells were stimulated with lipopolysaccharide + TNF-α for 24 h. Treatment with rosiglitazone (Rosi) or pioglitazone (Pio) was performed 2 h before the stimulation. —, no treatment. Control, stimulated with lipopolysaccharide + TNF-α but no ligands. Anti-iNOS, ICAM-1, cyclooxygenase (COX)-2 or nitrotyrosine (NY) antibody was applied for each Western blot analysis.

level, iNOS, in joint tissues of PPARγ treated and non-treated mice by Western blot analysis. CFA administration increased iNOS protein levels in both ankle (Fig. 2A and C) and temporomandibular joints (Fig. 2B and D). The increased level of iNOS was significantly inhibited by the treatment with rosiglitazone or pioglitazone.

3.3. PPARγ ligand inhibits adjuvant-induced apoptosis around temporomandibular joint

We adopted anti-M30 antibody for immunohistochemistry of inflamed joints to detect the early stage of apoptotic cells (Kusama et al., 2000; Song et al., 2001). M30 positive cells were observed around temporomandibular joint of CFA-treated mice (Fig. 3A). The positive cells were fibroblasts in the connected tissues around the joint. Administration of rosiglitazone completely inhibited the expression of M30 positive cells in temporomandibular joint (Fig. 3B). There were no positive stainings in inflammatory cells, synovial cells and osteochondrocytes.

3.4. PPARγ ligands inhibit increased expression of inflammatory mediators in RAW264 cells

We also investigated the effects of PPARγ ligands on cytokine-induced expression of inflammatory mediators in RAW264 to confirm the effects in mice. Stimulation of TNF-α + lipopolysaccharide increased protein levels of inflammatory mediators such as iNOS, ICAM-1, cyclooxygenase-2 and nitrated proteins in RAW264 (Fig. 4). Those increased levels of inflammatory mediators were attenuated by the treatment with rosiglitazone or pioglitazone.

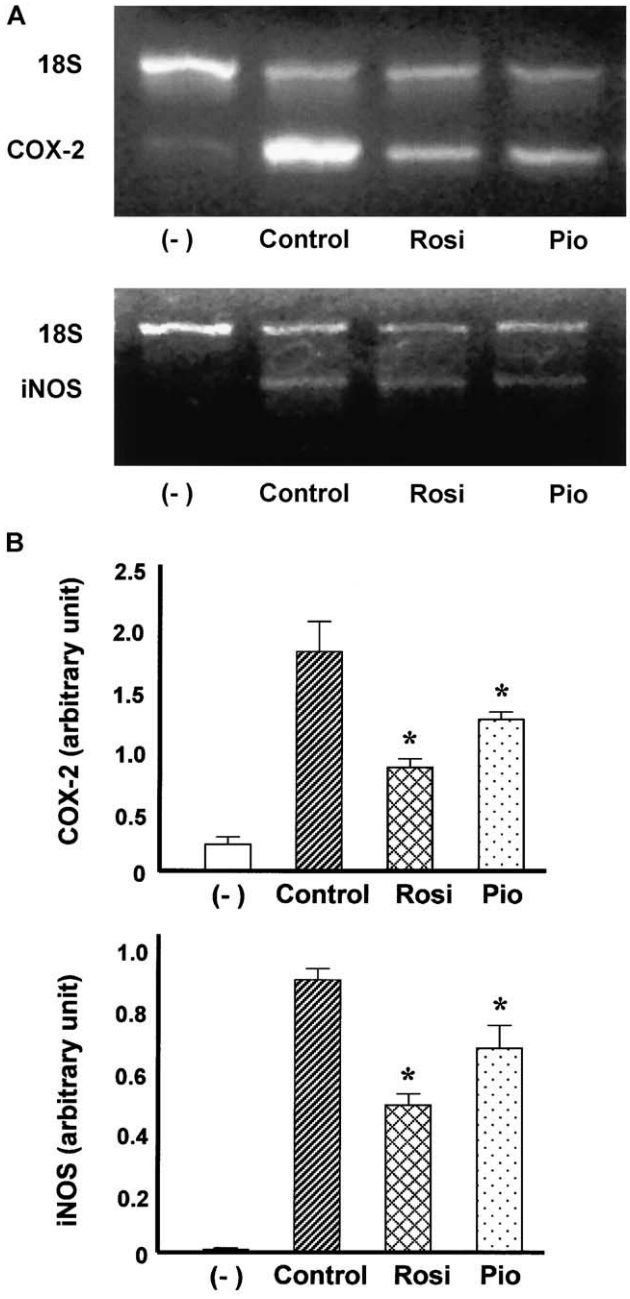

Fig. 5. Effects of PPARγ ligands on mRNA expressions of iNOS and cyclooxygenase (COX)-2 in the stimulated RAW264; RT-PCR analysis. Typical RT-PCR results (A) and summaries of semi-quantitative RT-PCR for COX-2 and iNOS (B). Cells were treated with rosiglitazone (Rosi) or pioglitazone (Pio) for 2 h, subsequently stimulated by lipopolysaccharide + TNF-α for 2 h. Total RNAs were prepared and cDNA were synthesized as described in Materials and methods. After the PCR reaction, products were applied to agarose gel electrophoresis and visualized. The 18S bands were also amplified as an internal standard. Each band was digitized, and the net band intensities were normalized to 18S for each sample. Bars and brackets represent mean ± SD of three to six independent experiments. * $P < 0.05$ vs. control.

3.5. iNOS and cyclooxygenase-2 mRNA expressions are inhibited by PPARγ ligands in TNF-α stimulated RAW264 cells

We also investigated the effects of PPARγ ligands on TNF-α-induced iNOS and cyclooxygenase-2 mRNA expressions in RAW264 cells using RT-PCR method. TNF-α-treated cells showed the elevation of iNOS and cyclooxygenase-2 mRNA levels (Fig. 5A and B). The increased mRNA levels were inhibited by the treatment with rosiglitazone or pioglitazone, indicating that the reduced protein levels of inflammatory mediators are due to the reduction in mRNA levels.

3.6. Detection of PPARγ protein levels in joint tissues

To investigate the levels of PPARγ protein in joint tissues, we applied tissue samples for Western blot analysis. As shown in Fig. 6A (upper panel), expressions of PPARγ protein were observed in ankle joint tissues. Administration of rosiglitazone or pioglitazone caused slightly decreases in PPARγ protein levels. Similar result was observed in temporomandibular joints (data not shown).

3.7. Effects of PPARγ ligands involve NF-κB activation pathway

NF-κB pathway is well known as a major transcription factor for most inflammatory mediators such as iNOS, cyclooxygenase-2, ICAM-1, e.g. (Barnes and Karin, 1997; Yamamoto and Gaynor, 2001; Ye, 2001). We, therefore, investigated the effects of PPARγ ligands on the activation of NF-κB pathway. Phosphorylation of I-κB by I-κB kinase is a key step for the activation of NF-κB, and level of pI-κB is considered a marker of NF-κB activation. Fig. 6B showed the increases in pI-κB in ankle and temporomandibular joints of CFA-treated mice and RAW264 cells treated with lipopolysaccharide + TNF-α. The increases of pI-κB were suppressed by administration with rosiglitazone or pioglitazone in both joint tissues and RAW264 cells. These results indicate that effects of PPARγ ligands may involve the inhibition of NF-κB pathway.

Furthermore, we examined whether PPARγ ligands affect the transcription levels that were regulated by NF-κB pathway during inflammation using co-transfected HEK293 cells with PPARγ gene and luciferase-reporter gene. As shown in Fig. 6A (lower panel), PPARγ-transfected HEK293 cells showed increased level of PPARγ proteins. Treatment with rosiglitazone significantly inhibited TNF-α-induced NF-κB activation in PPARγ-transfected HEK293 cells (Fig. 6C), however not in rsGFP-transfected cells (data not shown). These results clearly showed that the effects of PPARγ ligands are mediated through the regulation by NF-κB in transcription of inflammatory mediator proteins.

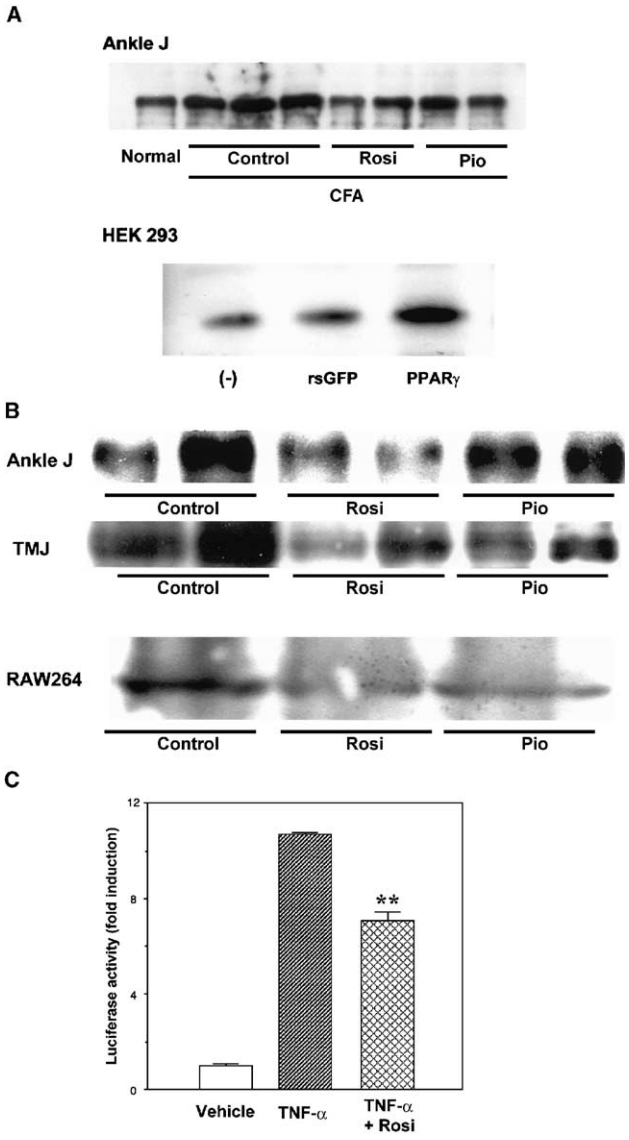

Fig. 6. Effects of PPARγ ligands on the NF-κB activation; Western blot analysis of PPARγ on ankle joint and HEK293 cells (A). Joint tissue samples were homogenized and applied for Western blot analysis by anti-PPARγ antibody (Ankle J, upper panel in A). Samples from non-transfected (−), rsGFP-transfected (rsGFP) and PPARγ-transfected (PPARγ) HEK 293 cells were also applied for Western blot analysis (HEK 293, lower panel in A). Western blot analysis of pI-kB on ankle (Ankle J), temporomandibular joint (TMJ) and RAW264 (B). Proteins from Ankle J, TMJ and RAW264 cells were analyzed by anti-pI-κB antibody to compare the activation of NF-κB by the indicated treatments. Rosiglitazone (Rosi) or pioglitazone (Pio), ligand treatment on CFA- or LPS + TNF-α treated samples; control, vehicle alone on CFA- or LPS + TNF-α treated samples. Reporter assay of NF-κB-mediated transcriptions in HEK293 cells (C). HEK293 cells were co-transfected with PPARγ$_1$ expression plasmid and the reporter plasmid of pNF-κB-Luc. Cells were treated with either vehicle alone (0.005% DMSO), TNF-α (2 μg/ml) or TNF-α + Rosi (30 μM) for 2. The cell extracts were subsequently assayed for luciferase activity and shown as fold inductions over vehicle alone. Each column represents mean ± SD from six different experiments. ** $P < 0.01$ vs. TNF-α control.

4. Discussion

It is well known that superoxide and NO radicals rapidly react with each other to form peroxynitrite, and the peroxynitrite attacks to tyrosine residues in proteins, resulting in the formation of nitrotyrosine residues in proteins (Xia and Zweier, 1997; Greenacre and Ischiropoulos, 2001; Knapp et al., 2001). Therefore, increased nitrotyrosine formation is due to a result of inflammation (Kamisaki et al., 1997; Wada et al, 1998; Greenacre and Ischiropoulos, 2001; Mapp et al., 2001). In this study, marked increases in nitrotyrosine-containing proteins were observed in the joint tissues of adjuvant-induced arthritis mice. The increased formation of nitrotyrosine was suppressed by the treatment with PPARγ specific ligands, rosiglitazone and pioglitazone. Although many reports described the increased formation of nitrotyrosine, no one mentioned the reduction in the nitrotyrosine formation in vivo (Greenacre and Ischiropoulos, 2001; Mapp et al., 2001; Noiri et al., 2001; Pfeiffer et al., 2001). Therefore, this is the first report that PPARγ ligands inhibit the nitrotyrosine formation in joint tissues. The ligands also ameliorated the elevated protein levels of iNOS in ankle and temporomandibular joints. The suppression of iNOS by PPARγ ligands is reasonable to explain the decrease in nitrotyrosine formation in adjuvant-induced inflammatory tissues, because iNOS is the enzyme to produce NO during inflammatory conditions (Wada et al., 1998; Greenacre and Ischiropoulos, 2001).

The inhibitions by PPARγ ligands of nitrotyrosine formation and inflammatory mediator proteins were also observed in TNF-α and lipopolysaccharide-stimulated cultured macrophage-like cell line. The effects of PPARγ ligands were also confirmed by the decreases in mRNA levels from the results of RT-PCR experiment. These results indicate that the inhibition by PPARγ ligands of the increased inflammatory mediators are due to the reduction in their mRNA levels in cytokine-stimulated macrophage-like cells.

To clarify more detail mechanisms of PPARγ ligands to inhibit the expression of inflammatory mediators, we investigated the effects of PPARγ ligands on the level of pI-κB protein, the marker of NF-κB activation, by Western blot analysis. It is well known that stimulation of cytokines, lipopolysaccharide, oxidative stress and ischemia–reperfusion cause the activation of I-κB kinase to produce phosphorylated I-κB (pI-κB), and then pI-κB dissociate from p50 and p65 subunits, resulting in the regulation of transcription of various proteins (Barnes and Karin, 1997; Yamamoto and Gaynor, 2001; Ye, 2001). In the present study, we detected the increase in protein levels of pI-κB in joint tissues of arthritis mice and the stimulated RAW264 cells. In addition, administration with PPARγ ligands inhibited the increases in pI-κB level. These results suggest that the activation of PPARγ by its specific ligands inhibits the NF-κB activation and results in the inhibition of the expression of inflammatory mediators. We also confirmed the mechanism of the effect of PPARγ ligands on the suppression of NF-κB mediated transcription of inflammatory mediators by reporter assay using PPARγ-transfected HEK293 cells. Because basal level of PPARγ in HEK293 cells is extremely low, they are good tools to investigate the effect of PPARγ ligands by the comparison between those in cells transfected with or without PPARγ gene. Rosi significantly suppressed the increase in NF-κB mediated transcription levels in PPARγ-transfected, but not in GFP-transfected cells. Again, these data support that the mechanism of PPARγ ligands to inhibit the expression of inflammatory mediators is attributed to the inhibition of transcription via the NF-κB pathway. The detail mechanisms of PPARγ pathway to inhibit the NF-κB activation are still unknown, and several possibilities were reported (Barnes and Karin, 1997; Yamamoto and Gaynor, 2001; Ye, 2001). In our present study, it is indicated that PPARγ might inhibit the step of phosphorylation of I-κB. Further investigations are required.

In the present study, treatment with PPARγ ligands ameliorated the apoptosis of cells around joint tissues from adjuvant-induced arthritis mice. However, it was reported that PPARγ ligands caused the apoptosis on cultured synovial cells and macrophage-like cell lines (Chinetti et al., 1998; Kawahito et al., 2000). The discrepancy may be due to the differences between experimental conditions of in vitro and in vivo, the differences of concentrations of PPARγ ligands, or the differences of target cell types. M30 positive apoptotic cells were observed in connected tissues around the joints of the arthritis mice. Furthermore, treatment with PPARγ ligands caused the complete reduction in the apoptotic cells. PPARγ ligands may suppress the apoptosis of connected tissues around joints and result in the inhibition of further inflammations and damages, although it may be possible that high concentrations of PPARγ ligands cause apoptosis on cultured macrophages or synoviocytes in vitro. Further investigations are required to clarify the mechanisms of PPARγ ligands on the inhibition (or induction) of apoptosis in inflammatory tissues.

In conclusion, the treatment with PPARγ specific ligands inhibited the formation of nitrotyrosine and increase in inflammatory mediators in joint tissues of adjuvant-induced arthritis mice. The mechanism that PPARγ ligands reduce the inflammation of arthritis may involve the suppression of the activated NF-κB to regulate the transcription of various inflammatory mediators. Therapy with PPARγ ligands may be useful for suppression of inflammatory mediators in chronic inflammatory disorders, such as rheumatoid arthritis.

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