INTRODUCTION:
Collagenase-3, a matrix metalloproteinase (MMP-13) plays an important role in the degradation of cartilage in pathologic conditions. In addition, inflammation-stimulated synovial fibroblasts are able to release MMP-13 and other cytokines in patients with rheumatoid arthritis (RA). The peroxisome proliferator-activated receptor-γ (PPARγ) is reported to inhibit the inflammatory response in RA. However, the endogenous PPARγ ligand, 15-deoxy-A12,14-prostaglandin-J2 (15d-PGJ2) was reported to be more potent than other synthetic PPARγ compounds in the inhibition of inflammatory arthritis (1, 2). In this study, we found that 15d-PGJ2 inhibited TNF-α-induced activation of IKK and the translocation of NF-κB, which is strongly involved in the increase of MMP-13 in synovial fibroblasts.

METHODS:
Cell Cultures
Synovial fibroblasts were obtained from patients with rheumatoid arthritis (RA) undergoing total knee replacement surgeries (Taichung Veterans General Hospital, Taichung, Taiwan) and were approved by institutional review board (IRB). Cultured synovial fibroblasts passages of four to nine were used in this study.

RT-PCR for mRNA Analysis
RNA was analyzed by using two-step MMLV-RT and Taq polymerase and primers specific for the metalloproteinase-13 (MMP-13) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Amplification was accomplished with 30–35 cycles. PCR products were then separated electrophoretically in a 2% agarose DNA gel and stained with ethidium bromide.

Western Blotting
For the separation of cytoplasmic extracts (CE) and nuclear extracts, cells were cultured onto 10 cm dish. After reaching confluence, cells were treated with test substances, cytoplasmic extracts and nuclear extracts were separated by NE-PER (Thermo Scientific-Pierce, Rockford, IL, USA). The blots were probed with rabbit antibodies against ERK, phospho-ERK, p38, phospho-IKKα/β, IkBa or NF-κB p50 (1:1000) and visualized by enhanced chemiluminescence.

Immunofluorescent staining
For immunolabeling studies, synovial fibroblasts were stained with primary rabbit antibody against NF-κB-p50 (1:500) or NF-κB-p65 (1:500) and then with Alexa-543-conjugated goat anti-rabbit secondary antibody (Invitrogen, Carlsbad, California). The nucleus was stained by DAPI (4',6-diamidino-2-phenylindole) and the confocal images were obtained using excitation wave length of 543 nm (for Alexa-543) (model SP2 TCS; Leica, Heidelberg, Germany).

RESULTS:
It has been reported that NF-κB is involved in the induction of MMP-13 by TNF-α. In this study, 15d-PGJ2 inhibited TNF-α-induced MMP-13 expression in a PPARγ-independent manner (Fig. 1). NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) significantly inhibited TNF-α-induced MMP-13 mRNA expression in synovial fibroblasts (Figs. 2A and 2B) and it was found that treatment of TNF-α activated IKKα/β (Fig. 2C), which can phosphorylate IkBa at Ser32 and Ser36 (3) to produce ubiquitination of IkBa at lysine residues and to be degraded by proteosome. In addition, phosphorylation and degradation of IkBa were also observed after stimulation by TNF-α. However, 15d-PGJ2 directly inhibited the activation of IKKα/β and the further phosphorylation and degradation of IkBa were also attenuated by 15d-PGJ2. Pretreatment of PPARγ antagonist GW9662 did not antagonize these inhibitory actions of 15d-PGJ2. It was also found that NF-κB-p50 (Fig. 2B) and p65 (Fig. 3) translocated from cytosol to nucleus after stimulation by TNF-α and 15d-PGJ2 attenuated this translocation. GW9662 also could not antagonize these inhibitory actions of 15d-PGJ2.

DISCUSSION:
It has been reported that the induced production of cytokines and MMPs, especially MMP-13 by inflammatory signals are NF-κB-dependent in synovial fibroblasts (4). In this study, treatment of 15d-PGJ2 but not ciglitazone quickly inhibited the activation of IKKα/β after TNF-α stimulation. 15d-PGJ2 also attenuated the downstream steps of activation of IKK such as the phosphorylation of IkBa and the translocation of NF-κB p50 and p65 from cytosol into nucleus. These data indicate that 15d-PGJ2 inhibited TNF-α-induced MMP-13 expression by the inhibition of NF-κB activation in a PPARγ-independent manner.

REFERENCES:
2. Lin TH et al, ORS 2009

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