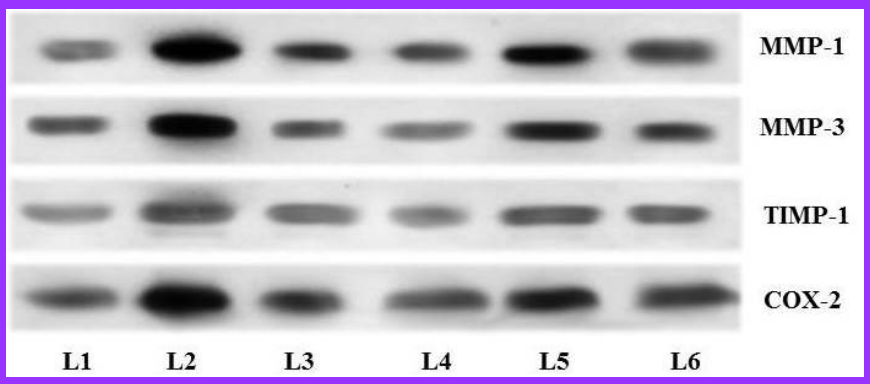

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Tangeretin inhibits IL-1β induced proliferation of rheumatoid synovial fibroblasts and the production of COX-2, PGE2 and MMPs via modulation of p38 MAPK/ERK/JNK pathways

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Abstract

The study aimed to determine the effect of tangeretin in inhibiting the interleukin-1β (IL-1β)-induced proliferation of RASFs and suppression of the production of inflammatory mediators. RASFs were isolated from synovial tissue obtained from patients with RA during knee arthroscopy. The cells were exposed to IL-1β (1.0 ng/mL) and/ or tangeretin (50 or 100 μM). Cell viability was assessed following treatments. Expressions of MMPs and COX-2 were analysed by real-time PCR and western blotting. Production of prostaglandin E2 (PGE2) by RASFs were analysed by ELISA. Expressions of mitogen activated protein kinases (MAPKs) and nuclear factor-kB (NF-kB) were assessed by Western blotting. Tangeretin significantly inhibited the proliferation of RASFs, as well as down-regulated the expression of MMP-1, MMP-3 and COX-2 mRNA and protein and also the phosphorylation of ERK, p38 and JNK. Raised level of expression of NF-κB and PGE2 induced by IL-1β was reduced by tangeretin. Results indicate that tangeretin was effective in inhibiting the synovial fibroblast proliferation, as well regulated MMPs, COX-2, PGE2 via modulation of p38 MAPK, ERK and JNK pathways.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory systemic disease of unknown etiology. It is characterized by high infiltration of leukocytes into the synovium, leading to hyperplasia of the synovial lining, which causes progressive destruction of the cartilage consequently resulting in erosion of the underlying bone (Pope, 2002). The activated rheumatoid synovial fibroblasts (RASFs) aggressively participate in RA synovitis. RASFs in RA joints vigorously proliferate forming a pannus, which produce inflammatory mediators such as cytokines, matrix metalloproteinases (MMPs), and cyclooxygenase-2 (COX-2) eventually leading to the destruction of articular bone and cartilage (Cawston, 1995; Han et al., 2003).

Interleukin 1β (IL-1β) is considered as the most important cytokine in the pathogenesis of inflammatory events in RA. IL-1β induces the proliferation of RASFs and also causes the production of high levels of inflammatory mediators- MMPs and prostaglandin E2 (PGE2) (Choy and Panayi, 2001). While the pathogenesis of RA is not completely understood, the process is reported to involve cellular infiltration into the synovial tissue with marked increase of inflammatory cytokines-tumor necrosis factor (TNF) α, interleukin (IL)-1β and IL-6, that eventually contribute to cartilage and bone erosion (Arend, 2001; Choy, 2012). These mediators activate major signalling pathways such as the nuclear factor (NF)-κB and mitogen activated protein kinases (MAPKs) (Tas et al., 2005).

MAPKs are a family of serine/threonine kinases that are involved in various cellular events such as inflammation, transcription of pro-inflammatory factors and

phosphorylation of transcription factor NF-κB (Su and Karin, 1996; Dong et al., 2002). Further research have demonstrated the activation of the members of signaling cascades such as NF-κB p65, and extra cellular signal-regulated kinase (ERK), JunN-terminal kinase (JNK) and p38MAPKs in RA synovial tissue. Upon activation ERK, JNK and p38MAPK play vital roles in synovial inflammation and proliferation of synovial cells (Luo et al., 2010; Yang et al., 2010). Present day treatment strategies towards RA are targeted predominantly against inflammatory media-tors. Owing to the adverse effects of the chronic usage of anti-inflammatory drugs in the treatment, exploring alternative means is indispensible.

Flavonoids are widely present in fruit and vegetables. Citrus flavonoids have a wide range of biological activities including anticarcinogenic and antitumor. Tangeretin (5,6,7,8,4′-pentamethoxy flavone) is present in oranges and in other citrus peels (Dong et al., 2014). It has been reported to exhibit various pharmacological activities: antioxidant (Chen et al., 2012), neuroprotective (Datla et al., 2001), anti-inflammatory activity (Ho and Kuo, 2014; Shu et al., 2014) and inhibition of cancer cell proliferation (Dong et al., 2014; Periyaswamy et al., 2015). Tangeretin has been reported to reduce the production of nitric oxide (NO) by inhibition of LPS-induced expression of nitric oxide synthase (iNOS) and COX-2 in microglial cells (Shu et al., 2014). Considering the biological activities of tangeretin, we investigated its effects in proliferation of rheumatoid synovial fibroblasts and production of chemokines by RASFs. Influence of tangeretin on various signal transduction pathways involved in inflammation were also evaluated.

Materials and Methods

Reagents and chemicals

Tangeretin was obtained from Sigma-Aldrich (USA) and dissolved in DMSO with a concentration of 100 mM stock solution. Fetal bovine serum (FBS) was obtained from Sigma-Aldrich (USA). Recombinant human IL-1β was purchased from Cell Signaling Technology (USA). Antibodies against IkBα, NF-κB (p65), ERK, JNK, p38, p-ERK, p-JNK, p-p38, β-actin (Cell Signaling Technology, USA), MMP-1, MMP-3 and tissue inhibitors of metalloproteinases (TIMP), COX-2 (Santa Cruz Biotechnology, Inc., USA) were used in Western blot analysis. All other chemicals used in the investigation were purchased from Sigma-Aldrich, (USA) unless otherwise specified.

Isolation and culture of RASFs

This study was approved by the national university hospital ethical committee. Synovial tissues were obtained from patients at the total knee arthroplasty in accordance with the American College of Rheumatology Criteria for RA (Arnett et al., 1998) and as previously described by Lee et al. (2006) and informed consent was obtained from all patients. Cells were incubated at 37°C in 5% CO_2 in RPMI 1640 medium supplemented with 10 % (v/v) FBS, 100 units/mL of penicillin, and 100 µg/mL of streptomycin. The RASFs were isolated after 3-7 passages. The synovial cells were homogeneous in morphology and had the manifestation of RASFs with distinctive fibroblastoid configuration. Purity of the cells were tested using phycoerythrin (PE)-conjugated anti-Thy-1 (CD90) or anti-CD14 and fluorescein isothiocyanate (FITC)-conjugated anti-CD3 mAb (BD Pharmingen, USA).

Determination of cell viability

Cell viability of the RASFs was determined using CCK-8 kit (Dojindo Laboratories, Japan). CCK-8 kit employs Dojindo's tetrazolium salt, WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H -tetrazolium, monosodium salt] for determining viable cells. WST-8 in the living cells is reduced by cellular dehydrogenases to an orange formazan product that is directly proportional to the viable cell counts. Briefly, RASFs (2 x $l0^4$ cells/well) were treated with various concentrations of tangeretin (50 and 100 µM) and incubated for 24 or 48 h with/without IL-1β (1.0 ng/mL). Following incubation, the cells were washed twice with PBS and CCK-8 (20 µL) was added to each well and incubated for about 2-3 hours. Formazan crystals formed were dissolved by adding DMSO (100 µL/well). The absorbance was read at 450 nm using a microplate reader (Model 3550, Bio-Rad, USA).

Analysis of apoptosis by annexin V assay

Following incubation with tangeretin with/without IL-1β (1.0 ng/mL) for 24 and 48 hours, cells were then trypsinized and collected for detection of apoptosis with annexin V-FITC Apoptosis Detection kit (Santa Cruz Biotechnology, USA) according to the manufacturer's instructions. Briefly, 1 × 10^6 cells that were pretreated with tangeretin were subjected to annexin V staining. The treated cells were washed in PBS, resuspended in 100 µL of binding buffer containing a FITC-conjugated anti-annexin V antibody. The cells were then analyzed for flurosence using a flow cytometer (FACS Calibur, BD Biosciences).

RNA isolation and semi-quantitative RT-PCR

To evaluate the expression of MMP-1, MMP-3, TIMP-1 and COX-2 mRNA, RASFs (1 × 10^6 cells) were cultured for 12, 24 or 48 hours with/without IL-1β (1.0 ng/mL) and/or tangeretin (50 or 100 µM). Total RNA was extracted from cultured RASFs after incubation with tangeretin using TRIsol reagent (Invitrogen, USA) following the manufacturer's instructions. cDNA derived from the reverse transcription of RNA using Maxime RT Premix Kit (iNtRON Biotechnology, South Korea) was amplified using the following primer sets:

TIMP-1 (forward) 5′-CCT TCT GCA ATT CCG ACC TCG TC-3′ (reverse) 5′-CGG GCA GGA TTC AGG CTATCT GG-3′, MMP-1 (forward) 5′-GAA GGA GAT GAA GCA GCC CAG ATG T-3′ (reverse) 5′-CAG TTG TGG CCA GAA AAC AGA AGT GAA A-3′, MMP-3 (forward) 5′GAC ACC AGC ATG AAC CTT GTT-3′ (reverse) 5′-GGA ACC GAG TCA GGA CTATG-3′, COX -2 (forward) 5′-TCC TTG CTG TTC CCA CCC ATG-3′ (reverse) 5′-CAT CAT CAG ACC AGG CAC CAG-3′, GAPDH (forward) 5′-AAA TCA AGT GGG GCG ATG CT-3′ (reverse) 5′-AGC TTC CCG TTC AGC TCA GG-3′. The products were subjected to electrophoresis in 1% agarose gel. The bands were visualized by staining with ethidium bromide. Densitometric analysis was performed on the relative intensity of each band (MultiGauge program, version 3.0, Fuji film, Japan).

Western blotting

RASFs were cultured with/without IL-1β (1.0 ng/mL) and/or tangeretin (50 or 100 µM). RASFs (1×10^6 cells) were seeded on 100-mm culture dishes and harvested in PBS. After washing with PBS, cell pellets were lysed with lysis buffer (20 mM HEPES, pH 7.2, 150 mM NaCl, 1% Triton X-100, 0.1 mM phenyl-methylsulfonyl fluoride, 1 µg/mL aprotinin, 1 mM EDTA). After incubition for 30 min at 4°C, cellular debris were removed by centrifugation at 100,000 g for 30 min, and supernatants were analyzed by SDS-PAGE. For COX-2 immunoblotting the cell membranes were prepared from isolated RASFs as described previously (Hodgkin et al., 1990). To determine the cytoplasmic IkB, cytoplasmic extracts were prepared (Lee et al., 2006). To analyze NF-kB (p65), nuclear extract was prepared using a previously described method (Lee et al., 2006). Protein concentration was determined by Bio-Rad protein assay kit (Bio-Rad Laboratories, USA). Proteins were then fractionated by SDS-PAGE, electro transferred to nitrocellulose membranes, blotted with respective antibodies (MMP-1, MMP-3, TIMP, COX-2, ERK, p-ERK- 1/2, p-38, p-p38 MAPK, JNK, p-JNK, NF-kB (p65), IkBα, and β-actin) and the immunoreactive bands were detected by enhanced chemiluminescence (GE Healthcare).

Assay of PGE2 production

To analyse PGE2 production in RASFs, the cells (1×10^4 cells) were grown in 25 cm^2 tissue culture flasks for 48 hours before treatment and starved serum overnight before stimulation with IL-1β. After washing with PBS (pH 7.4), RASFs were pre-treated with IL-1β (1.0 ng/mL) and/ or tangeretin (50 or 100 µM) for 48 hours at 37°C in DMEM supplemented with 10% (v/v) FCS in an atmosphere of 5% CO_2 (Sung et al., 2012). The supernatant was collected after incubation for 48 hours. The levels of PGE2 in the medium were determined by ELISA kit (R&D Systems, USA).

Statistical analysis

All data were expressed as the mean ± SD of results of three or six individual experiments. The data were analyzed using SPSS (free version). Group mean values were compared by one-way ANOVA. The values at $p<0.05$ were considered significant.

Results

IL-1β induced proliferation and apoptosis of RASFs

IL-1β is a well-known potent growth-promoting factor of synovial fibroblasts. IL-1β induced the proliferation of RASFs significantly ($p<0.05$) as compared with the control cells cultured in DMSO without tangeretin (Figure 1a). Exposure to tangeretin (50 or 100 µM) significantly ($p<0.05$) inhibited the proliferation of RASFs treated with or without IL-1β ($p<0.05$). The decrease in cell proliferation and viability was almost multifold as compared to IL-1β induction alone. Further, the effects of tangeretin on apoptotic counts of RASFs were examined by flow cytometry. IL-1β induced increased proliferation is accompanied with a marked decrease in apoptotic cell counts. However, the percentage of annexin V-positive cells was observed to be significantly increased in the RASFs treated with tangeretin at both the doses as compared with the cells cultured without tangeretin ($p<0.05$) (Figure 1b;1c). Tangeretin at 100 µM dose was observed to be more effective in reducing cell viability and inducing apoptosis than at 50 µM.

IL-1β induced MMP-1, MMP-3 and TIMP-1 mRNA expression

Studies have reported the expression of COX- 2 and MMPs in human RASFs is enhanced by pro-inflammatory cytokines such as IL-1β (Crofford et al., 1994; Tolboom et al., 2002). In our study to evaluate the effects of tangeretin on expression of MMP-1, MMP-3 and TIMP-1 genes under the influence of IL-1β, RT-PCR was performed. RASFs were stimulated with IL-1β (1.0 ng/mL). IL-1β significantly ($p<0.05$), enhanced the mRNA levels of MMP-1 and MMP-3 in RASFs nearly twice. However much changes were not observed in TIMP-1 mRNA levels. Co-treatment with tangeretin with IL-1β caused a marked ($p<0.05$) decline in the mRNA levels of MMP-1 and MMP-3 (Figure 2). The inhibition of mRNA expression was more obvious on exposure to 100 µM. Further, inhibition of the expression of MMPs was in a dose-dependent manner in both unstimulated and IL-1β-stimulated RASFs, indicating the anti-inflammatory potential of tangeretin.

IL-1β-induced COX-2 expression

COX-2 is a major enzyme that catalyzes the synthe-sis of various PGs including PGE2 (Smith et al., 1996). We determined the effect of tangeretin on IL-1β-induced COX-2 expression in synovial fibroblasts. RASFs were co-treated with 1.0 ng/mL IL-1β and/or tangeretin (50

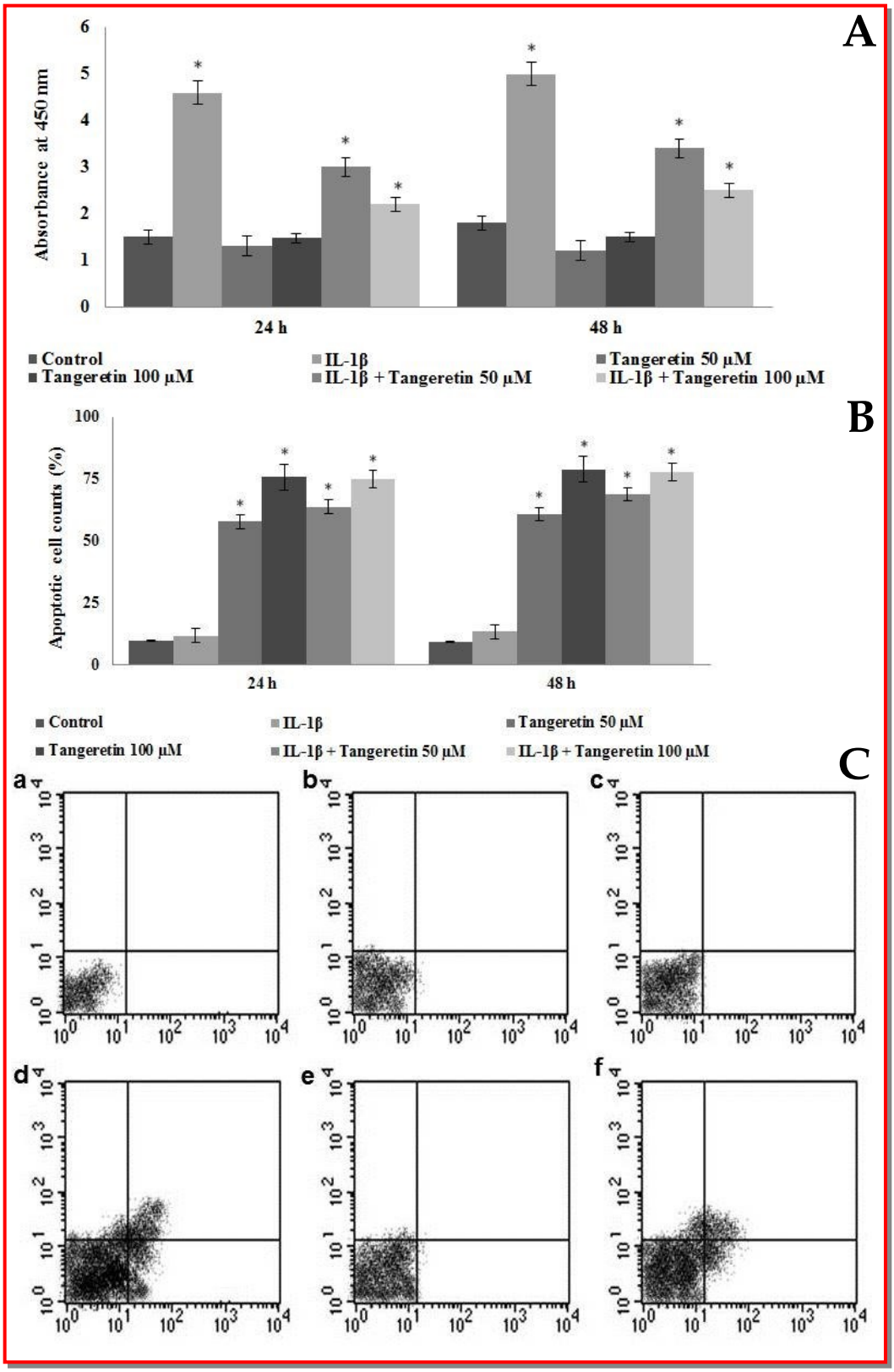

Figure 1: Influence of tangeretin on IL-1β induced proliferation of RASFs

Exposure to tangeretin (50 or 100 μM) significantly ($p<0.05$) inhibited the proliferation of RASFs in a dose-dependent manner (A) and as well significantly induced apoptosis as seen by increased apoptotic cell counts by annexin V staining (B and C); [a-Control; b-IL-1β; c-tangeretin 50 μM; d-tangeretin 100 μM; e-IL-1β + tangeretin 50 μM ; f-IL-1β + tangeretin 100 μM]; Values are represented as mean ± SD; n=6; *represents statistical significance at $p<0.05$ compared against control as determined by one-way ANOVA

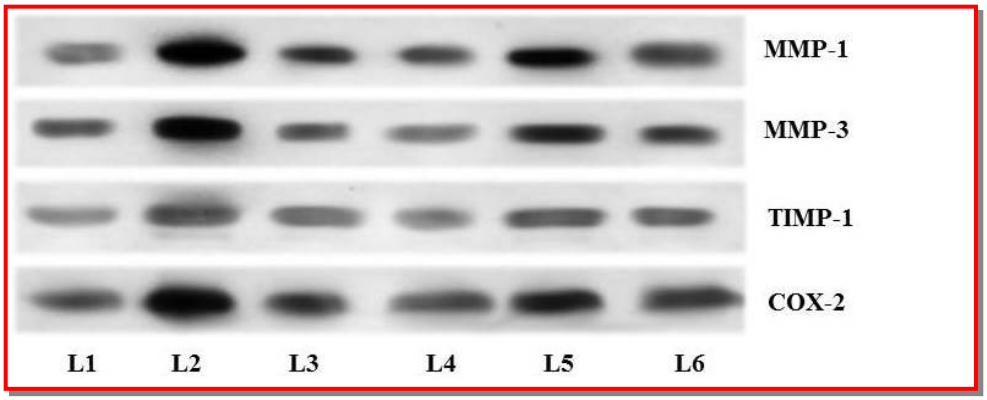

Figure 2: Influence of tangeretin on IL-1β induced MMP-1, MMP-3 and TIMP-1 mRNA expression

L1-Control; L2-IL-1β; L3-Tangeretin 50 μM; L4-Tangeretin 100 μM; L5-IL-1β + Tangeretin 50 μM; L6-IL-1β + Tangeretin 100 μM

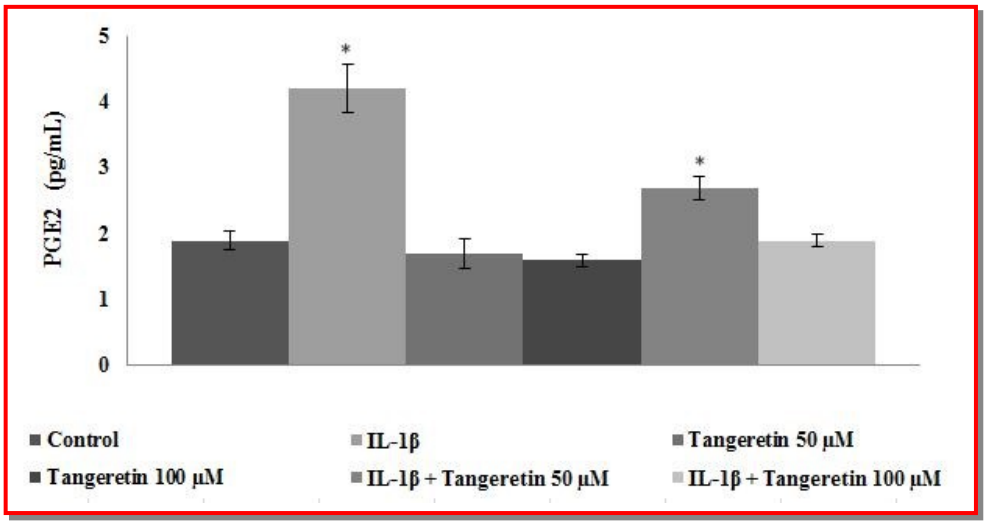

Figure 3: Tangeretin inhibits IL-1β-induced PGE2 production in RASFs

Values are represented as mean ± SD; n=6; *represents statistical significance at p<0.05 compared against control as determined by one-way ANOVA

or 100 μM) for 12, 24 or 48 hours, and COX-2 mRNA levels were detected by RT-PCR. Tangeretin significantly attenuated the IL-1β-induced COX-2 mRNA expression on 12, 24 or 48 hours. Exposure to tangeretin at 100 μM caused a more significant inhibition of COX-2 mRNA expression. The cells revealed a dose-dependent inhibitory effect of tangeretin on the IL-1β-induced increase in COX-2 mRNA levels (Figure 2). In addition, immunoblotting showed that tangeretin could attenuate the IL-1 β -induced COX-2 protein increase ($p<0.05$).

IL-1β-induced PGE2 production in RASFs

PGE2 is a pleiotropic mediator of inflammation and its excessive production is associated with many pathologic processes. PGE2 plays a vital role in eliciting signs and symptoms of inflammation (Martel-Pelletier et al., 2004). We investigated the effects of tangeretin on PGE2 production by RASFs. RASFs were cultured with IL-1β (1.0 ng/mL) for 48 hours. A multifold increases in PGE2 production occurred after IL-1β treatment ($p<0.05$) in comparison to cells not exposed to IL-1β. However, tangeretin treatment markedly inhibited PGE2 production in a dose-dependent manner. The levels of PGE2 were in line with the results of COX-2 expression, indicating that suppression of COX-2 decreased PGE2 production (Figure 3).

IL-1β-induced signal pathways in RASFs

The influence of tangeretin on the expression of MAPK pathway proteins and NF-κB were determined by Western blotting. MAPK pathway is involved in regulation of cell proliferation, apoptosis, cytokine expression and MMP production (Kim et al., 2006). NF-κB and MAPKs have been reported to participate in the pathogenic mechanisms of inflammation and the destruction of joints in RA. IL-1β at 1.0 ng/mL caused activation of the intracellular MAPKs including ERK, p-38, and JNK resulting in marked increase in the phosphorylation status of these proteins as well increase in MMP levels (Figure 4 a-h).

However, tangeretin significantly down-regulated IL-1β-induced phosphorylation of JNK, ERK, and p38. The inhibition of phosphorylation by tangeretin was obser-

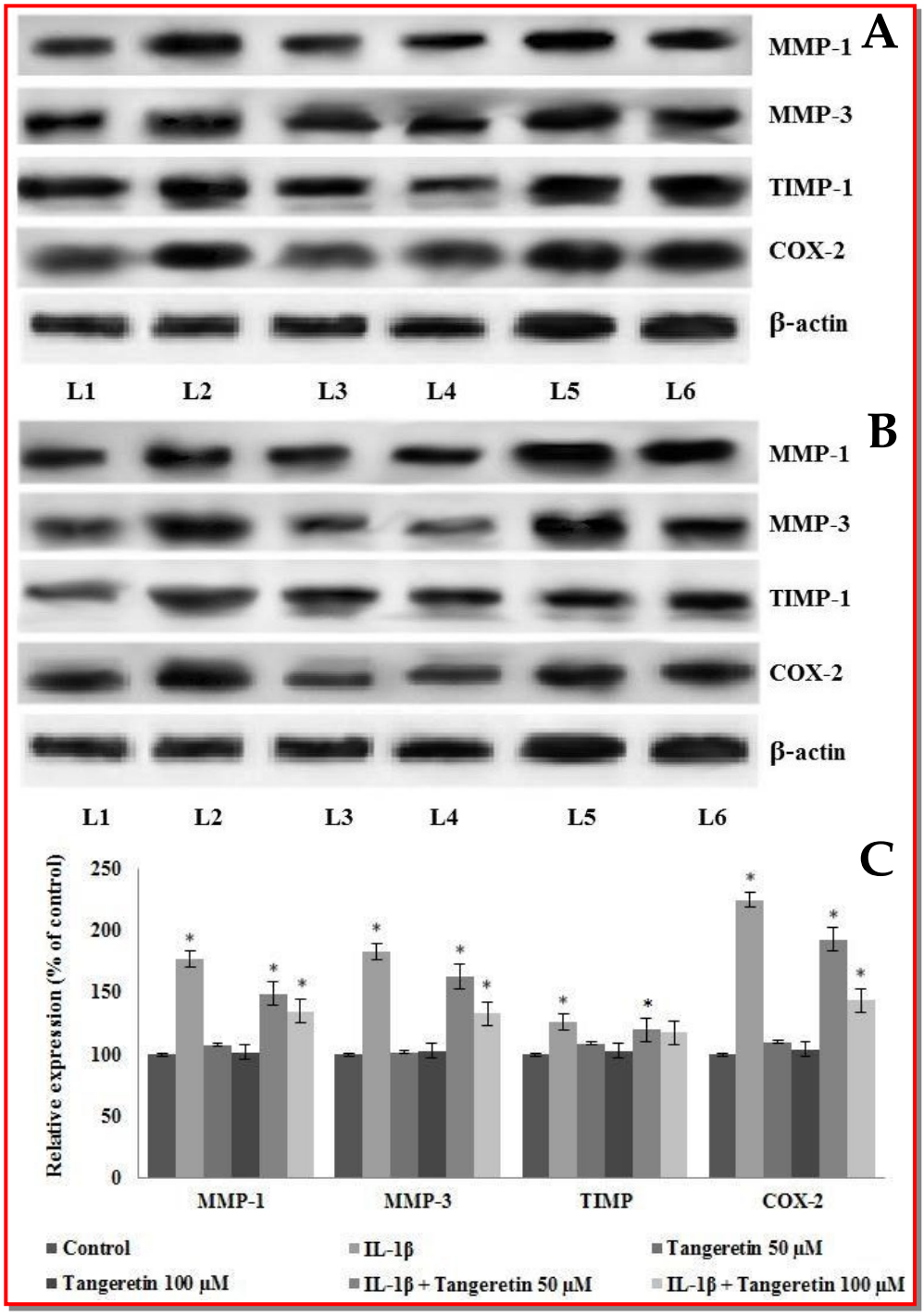

Figure 4: Influence of tangeretin on IL-1β-induced protein expressions

Tangeretin significantly reduced the IL-1β-induced raised expressions of MMPs and COX-2 following 24 hours (A and C) and 48 hours (B and D) of exposure. IL-1β-induced marked increases in the phosphorylation status and as well increased expressions of MAPK pathway proteins were regulated by tangeretin at both the doses (50 and 100 µM) and at 24 hours (E and G) and 48 hours (F and H); L1-control; L2-IL-1β; L3-tangeretin 50 µM; L4-tangeretin 100 µM; L5-IL-1β + tangeretin 50 µM; L6-IL-1β + tangeretin 100 µM; Values are represented as mean ± SD; n=3; *represents statistical significance at $p<0.05$ compared against control as determined by one-way ANOVA

ved to be time and dose-dependent. Furthermore, tangeretin reduced the expressions of MMPs and COX-2. Nuclear translocation and activation of NF-kB is known to be dependent on phosphorylation and subsequent degradation of IkBα (Adcock, 1997). While IL-1β caused marked activation of NF-κB and p65, significant decrease of cytoplasmic IkBα was observed in RASFs. NF-κB activation was observably inhibited by

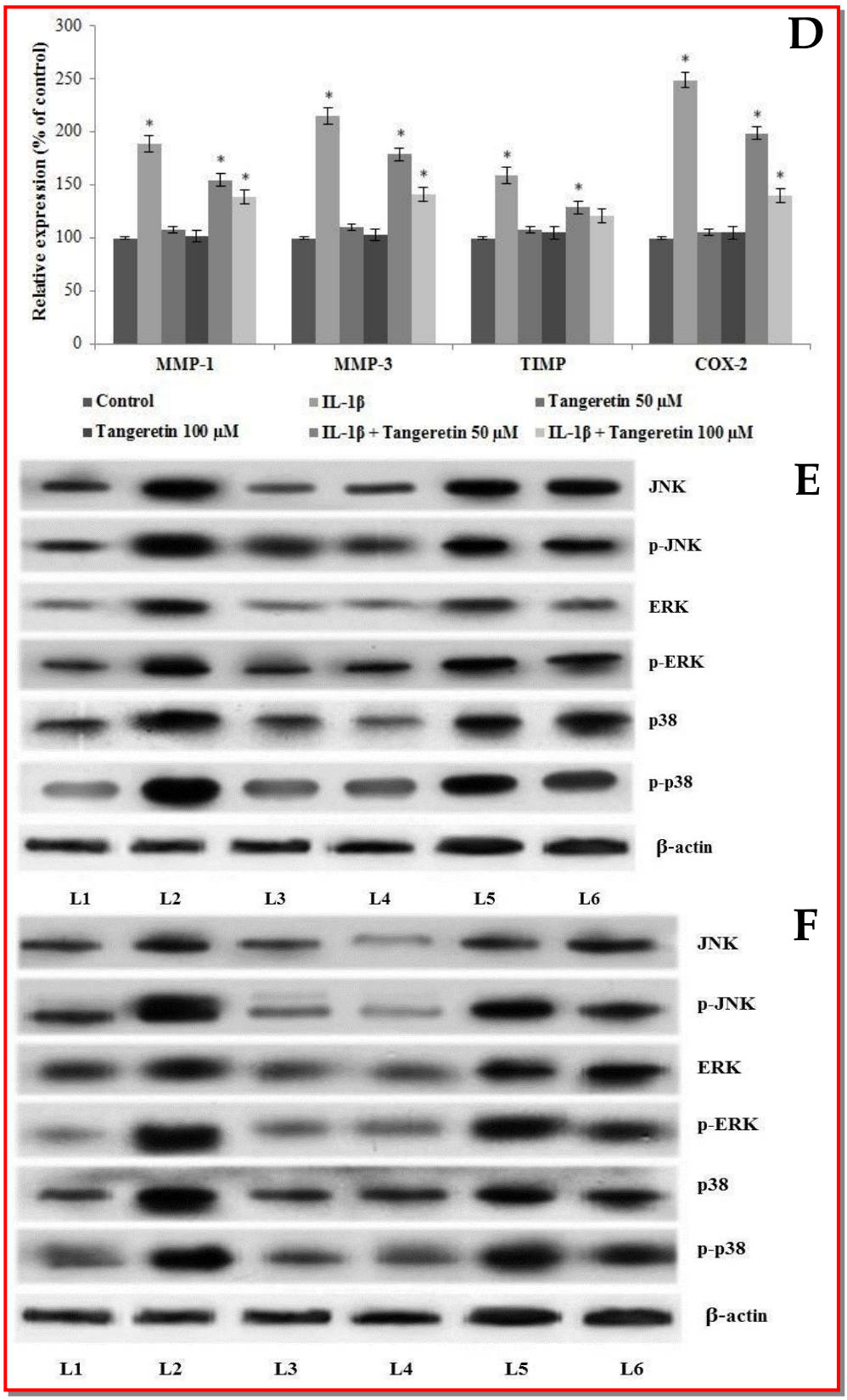

Figure 4: Influence of tangeretin on IL-1β-induced protein expressions (Cont.)

Tangeretin significantly reduced the IL-1β-induced raised expressions of MMPs and COX-2 following 24 hours (A and C) and 48 hours (B and D) of exposure. IL-1β-induced marked increases in the phosphorylation status and as well increased expressions of MAPK pathway proteins were regulated by tangeretin at both the doses (50 and 100 µM) and at 24 hours (E and G) and 48 hours (F and H); L1-Control; L2-IL-1β; L3-Tangeretin 50 µM; L4-Tangeretin 100 µM; L5-IL-1β + Tangeretin 50 µM; L6-IL-1β + Tangeretin 100 µM; Values are represented as mean ± SD; n=3; *represents statistical significance at $p<0.05$ compared against control as determined by one-way ANOVA

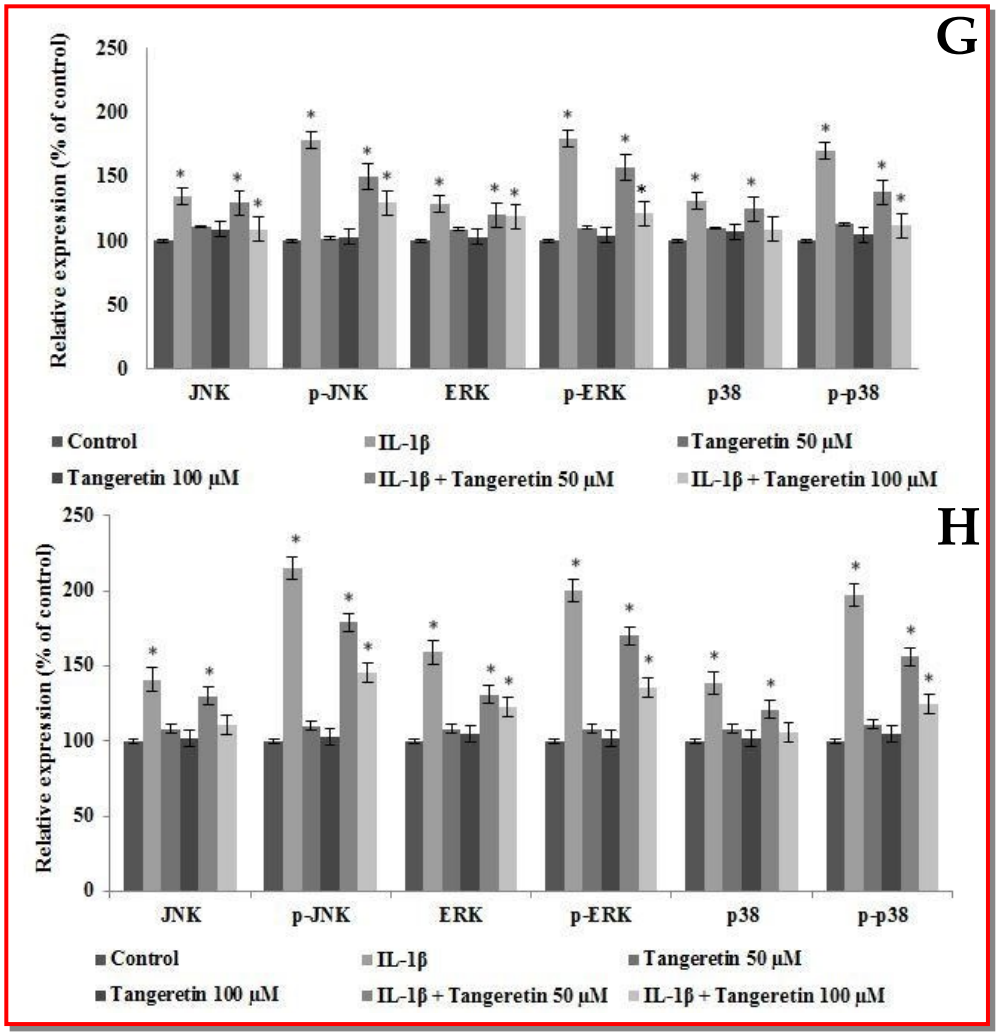

Figure 4: Influence of tangeretin on IL-1β-induced protein expressions (Cont.)

Tangeretin significantly reduced the IL-1β-induced raised expressions of MMPs and COX-2 following 24 hours (A and C) and 48 hours (B and D) of exposure. IL-1β-induced marked increases in the phosphorylation status and as well increased expressions of MAPK pathway proteins were regulated by tangeretin at both the doses (50 and 100 µM) and at 24 hours (E and G) and 48 hours (F and H); L1-Control; L2-IL-1β; L3-tangeretin 50 µM; L4-tangeretin 100 µM; L5-IL-1β + tangeretin 50 µM; L6-IL-1β + tangeretin 100 µM; Values are represented as mean ± SD; n=3; *represents statistical significance at $p<0.05$ compared against control as determined by one-way ANOVA

tangeretin with considerable increase in cytoplasmic IkBα (Figure 5 a-d). These results indicate that tangeretin potentially inhibited IL-1β-induced, expression of COX-2 and regulated the intracellular MAPKs and NF-κB pathways.

Discussion

Pathogenesis of RA involves cellular infiltration into the synovium and increase of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 consequently leading to cartilage and bone erosion (Arend, 2001; Choy and Panayi, 2001; Pratt et al., 2009; Choy, 2012). RASFs play critical role in the pathogenesis of RA by involvement in angiogenesis and in regulation of the inflammatory cells (Cawston, 1995; Han et al., 2003). Strategies that aim in blocking either the proliferation of RASFs or production of the inflammatory mediators are possible means of therapy against RA.

In our study, tangeretin potentially reduced the proliferation of RASFs and also raised the apoptotic cell counts. The aggressive proliferation of RASFs is the main mechanism for the hyperplasic growth of the RA synovium. Cytokine -IL-1β induces the proliferation of RASFs and plays an vital role in the pathogenesis of inflammatory synovitis and joint destruction (Gitter et al., 1989; Williams et al. 2007; Tanida et al., 2009; Choy, 2012). The results indicate that tangeretin (50 and 100 µM) significantly inhibits IL-1β-induced proliferation of RASFs in a dose- and time-dependent manner. Further tangeretin was also able to reduce proliferation of synovial fibroblasts not exposed to IL-1 β as well.

Studies have shown that pro-inflammatory cytokines including IL-1β could enhance the expression of COX-2

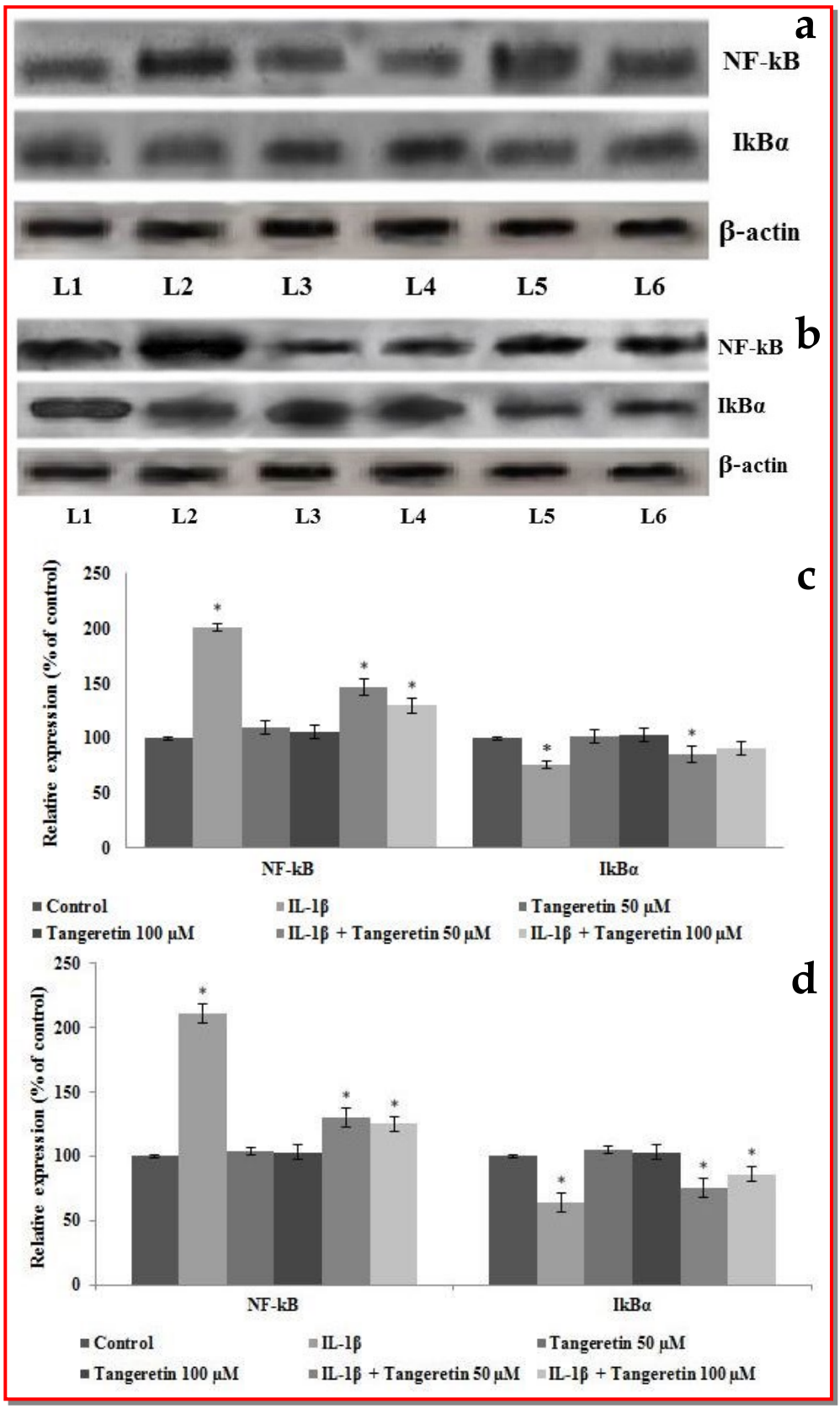

Figure 5: Tangeretin regulates the expressions of NF-kB and IkBα

Tangeretin significantly reduced the NF-kB expression and regulated IkBα; Following 24 hours (a and c) and 48 h (b and d) of exposure; L1-control; L2-IL-1β; L3-tangeretin 50 µM; L4-tangeretin 100 µM; L5-IL-1β + tangeretin 50 µM; L6-IL-1β + tangeretin 100 µM; Values are represented as mean ± SD; n=3; *represents statistical significance at $p<0.05$ compared against control as determined by one-way ANOVA

and MMPs in human RASFs (Tolboom et al., 2002). MMPs play a key role in the destruction of the extracellular matrix in articular structures. The cartilage destruction in RA is mainly caused by the activation of MMPs (Feldmann et al., 1996). MMP-1 and -3 have been shown to be the major enzymes produced by the synovial fibroblasts (Konttinen et al., 1999). MMP-1 preferentially degrades fibrillar collagens, whereas MMP-3 degrades a broad array of extracellular matrix substrates (Ogata et al., 1992; Knauper et al., 1996; Jackson et al., 2001). COX-2 is involved in the synthesis of inflammatory mediators and is responsible for the conversion of the free arachidonic acid to prostaglandins and variety of bioactive products. PGE2, a pleiotropic mediator of inflammation, is involved in various pathological processes and further when in excess, it also elicits the signs and symptoms of inflammation (Martel-Pelletier et al., 2004). In this study we observed that tangeretin at both the doses were able to down-regulate the IL-1β-induced expressions of MMP-1,3 and COX-2 both at the mRNA and protein levels. Down-regulation of COX-2 observed in tangeretin treatment also reflected in the levels of PGE2. We found that tangeretin caused marked decreases in the levels of PGE2 in a dose-dependent manner.

The pro-inflammatory cytokines, upon binding to the respective receptors initiate various signalling pathways. Cytokines, IL-1 induce the activation of NF-kB in RA (Morel and Berenbaum, 2004; Rannou et al., 2006; Lie et al., 2012). NF-κB is an important transcriptional factor complex that regulates the expression of various genes involved in the process of inflammatory response (Karin and Lin, 2002; Shaulian and Karin, 2002). In the cell under normal conditions, NF-kB is maintained in an inactive state within the cytoplasm sequestered by IkB. Upon stimulation chemokines like IL-1, the IkB kinase complex gets activated leading to phosphorylation and degradation of IkBα. This further causes a transitory increase in unbound NF-kB molecules that get activated and translocate to the nucleus, resulting in transcription of inflammatory responsive genes (Tak and Firestein, 2001; Aggarwal, 2004). Thus, the increase in NF-kB observed following IL-1β treatment in our study is correlating with the concurrent decrease in expression of IkBα. Tangeretin was able to considerably regulate the expressions of IkBα and NF-kB. At both the doses (50 and 100 µM) tangeretin significantly down-regulated NF-kB. The results suggest that tangeretin was able to inhibit IL-1β- induced inflammatory responses as evident from marked decrease in the cell proliferation and production of PGE2.

MAPKs are involved in a wide variety of cellular processes such as inflammation (Su and Karin, 1996; Dong et al., 2002). The major members of MAPK families - ERK, JNK, and p38 kinases, are expressed in active forms in synovial tissue and in cultured RASFs (Schett et al., 2000; Han et al., 2001; Sweeney and Firestein, 2004). Several reports have demonstrated that inhibitors of NF-κB or MAPKs reduce synovial inflammation, bone destruction and cartilage damage in animal models of arthritis (McIntyre et al., 2003; Nishikawa et al., 2003). In our study, increased activated forms of MAPK kinases (JNK, ERK, p38) were seen upon IL-1β exposure suggesting the activation of the signal transduction pathways. However, significant down-regulation in the phosphorylated forms of ERK, JNK and p38 kinases were observed following tangeretin exposure, suggesting that tangeretin was able to effectively regulate the alterations in the MAPK signalling cascades in RASFs.

Conclusion

Tangeretin was found effective in inhibiting IL-1β-induced RASFs proliferation and also regulates the inflammatory responses. It also reduced the signalling pathways, blocked the expressions of transcription factors, reduced the generation and effects of chemokines.

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Conflict of Interest

Authors declare no conflict of interest

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