Introduction

Matrix metalloproteinases (MMPs) consist of Zn⁺-containing matrix degrading enzymes, which regenerate and degrade matrix proteins in the body. To date, 23 MMP proteins have been identified in humans, and these are closely related to pathological conditions such as joint inflammation and joint degenerative diseases (1). Among various MMPs, collagenases such as MMP-1 and -13 are known to degrade collagens in cartilage and bone. Chondrocytes are important cells that reside in the cartilage and are primarily responsible for degrading extracellular matrix (ECM) in joint space, especially under the conditions of arthritis. Chondrocytes synthesize ECM-like collagen type II and aggrecan and synthesize and secrete proteolytic enzymes, MMPs. MMP-13 is largely responsible for degradation of the cartilage collagen matrix in articular joints (2, 3). Moreover, immunohistochemistry has revealed the presence of MMP-13–specific type II collagen degradation products and MMP-13 enzymes in osteoarthritis (OA) cartilage (4, 5). These findings suggest that inhibition of MMP-13 expression may show some beneficial effects of chondroprotection against pathological conditions such as OA (6).

Flavonoids of plant origin are well-known anti-inflammatory agents (7). Of their cellular action mechanisms, a down-regulating capacity of inflammation-related molecule expression at the transcriptional level is most im-

Full Paper

Effects of Flavonoids on Matrix Metalloproteinase-13 Expression of Interleukin-1β–Treated Articular Chondrocytes and Their Cellular Mechanisms: Inhibition of c-Fos/AP-1 and JAK/STAT Signaling Pathways

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Abstract. To identify the therapeutic potential for cartilage degradation and its action mechanisms, the effects of naturally-occurring flavonoids on matrix metalloproteinase-13 (MMP-13) induction were examined in the human chondrocyte cell line SW1353. Flavones including apigenin and wogonin strongly inhibited MMP-13 induction in interleukin (IL)-1β–treated SW1353 cells, while flavonols such as kaemperol, quercetin, and flavanone (naringenin) did not at 5 – 25 μM. Apigenin and wogonin primarily inhibit MMP-13 by blocking the c-Fos / activator protein-1 (AP-1) and Janus kinase 2 (JAK2) / signal transducer and activator of transcription 1/2 (STAT1/2) pathways, but not nuclear factor-κB (NF-κB) signaling. Apigenin was also shown to inhibit extracellular matrix degradation in rabbit cartilage culture. The following study using some synthetic flavones demonstrated that A-ring C-5,7-dihydroxyl and B-ring dihydroxyl substitution at C-2,3, C-2,4, or C-3,4 are important for the suppression of MMP-13 expression. Among these flavones, 2',3',5,7-tetrahydroxyflavone also inhibited both the c-Fos/AP-1 and STAT1/2 pathways. Taken together, these results indicate that certain flavonoids, especially flavones, inhibit MMP-13 expression in IL-1β–treated chondrocytes, at least in part, by suppressing the c-Fos/AP-1 and JAK2/STAT1/2 pathways. Furthermore, these findings suggest that some flavonoids have the potential for protecting against collagen matrix breakdown in the cartilage of diseased tissues such as those found in arthritic disorders.

Keywords: flavonoid, apigenin, 2',3',5,7-tetrahydroxyflavone, matrix metalloproteinase-13, signal transducer, activator of transcription
portant (8). The target molecules suppressed by flavonoids include cyclooxygenase-2, inducible nitric oxide synthase, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and MMPs. Certain flavonoids inhibit MMPs and/or suppress the induction of MMPs. For example, delphinidine (anthocyanidin); several flavonols including quercetin, kaempferol, and hyperoside; and catechins with a galloyl moiety have previously been found to inhibit gelatinases (MMP-2 and -9) and neutrophil elastase (MMP-12) activities (9 – 11). Moreover, several flavonoids such as quercetin and nobiletin have been reported to down-regulate MMP-1 expression in human vascular endothelial cells, human synovial fibroblasts, and UVA-irradiated human dermal fibroblasts (HDFs) (12, 13). We also investigated the suppressive mechanisms of MMP-1 down-regulation by naturally-occurring flavonoids in human dermal fibroblasts (14). Recently, it was also found that quercetin and kaempferol reduced MMP-3 activity in breast carcinoma cells (15).

However, only a few studies concerning the effects of flavonoids on collagenases (MMP-1 and -13) of chondrocytes or cartilage are available. Epigallocatechin-3-gallate (EGCG) has been reported to suppress MMP-1 and -13 induction in chondrocytes (16). The same compound was found to down-regulate MMP-1, -3, and -13 induction in human tendon fibroblasts (17). EGCG also inhibited MMP-1, -3, and -13 induction in IL-1β-treated articular chondrocytes only at high concentrations of 50 μM (18). Recently, icariin was shown to inhibit the induction of MMP-1, -3, and -13 in lipopolysaccharide (LPS)-treated murine chondrocytes (19). However, no other studies of flavonoids have been conducted to identify inhibitory potential or to elucidate their cellular action mechanisms to date. The possibility of flavonoid therapy for protection against cartilage loss is significant since inflammatory stimuli as well as the aging process provoke an induction of MMPs as noted above. Therefore, to establish the therapeutic potential against OA and other joint inflammatory diseases, down-regulation of MMP-13 and the suppressive cellular mechanisms of flavonoids were investigated in this study using the human chondrocyte cell line SW1353. Structural-activity relationships of flavonoids were also demonstrated.

Materials and Methods

Chemicals
Human IL-1β, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and anti-MMP-13 antibody were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DMEM and other cell culture reagents including FBS, were products of Gibco BRL (Grand Island, NY, USA). The protein assay kit was purchased from Bio-Rad Lab. (Hercules, CA, USA). The ELISA kit for human MMP-13 was purchased from R&D Systems (Minneapolis, MN, USA). All antibodies relating to mitogen-activated protein kinase (MAPK) and Janus kinase (JAK) / signal transducer and activator of transcription (STAT) signaling were purchased from Cell Signaling Technologies (Dancers, MA, USA). Lamin B1 antibody was purchased from Bioworld Technology (Minneapolis, MN, USA). The gel shift assay system and nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) consensus oligo were obtained from Promega (Madison, WI, USA). [γ-32P]ATP was purchased from IZOTOP (Budapest, Hungary). Flavonoids including naringenin, apigenin, kaempferol, and quercetin (Fig. 1) were purchased from Sigma. Wogonin was isolated according to the previously published methods (20) and TLC analysis gave one spot.

Animals
Male New Zealand white rabbits (5-weeks-old) were purchased from Orient-Bio Ltd. (Seongnam, Korea). Animals were fed with standard laboratory chow and water was freely available. The animals were maintained in the animal facility (Kangwon National University) at 20°C – 22°C under 40% – 60% relative humidity and a 12 h/12 h (light/dark) cycle for at least 7 days prior to the experiment. The experimental design using the animals was approved by the local committee for animal experimentation of Kangwon National University (KIACUC-10-0028). The animals were handled accord-

Fig. 1. Chemical structures of the naturally-occurring flavonoids used in this study.
ing to the guideline described in the Food and Drug Administration (Korea) Guide for the Care and Use of Laboratory Animals throughout the experiments.

**SW1353 cell culture and MMP-13 induction**

SW1353 cells (human chondrosarcoma cell line) were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were maintained in DMEM with 10% FBS, glutamine, and penicillin/streptomycin. To induce MMP-13, IL-1β (10 ng/ml) with/without test compounds was added to the cells in serum-free DMEM. After 24-h incubation, media was collected and the concentration of MMP-13 released was measured by ELISA according to the manufacturer’s recommended procedure. From the same media, MMP-13 expression was also examined by Western blotting analysis. The optimum expression time of MMP-13 and other signaling molecules was determined in the previous study (21). In brief, media was concentrated with TCA and acetone. Protein samples were separated on SDS-PAGE and blotted to a PVDF membrane. The blot was incubated with anti-MMP-13 antibody in 5% skim milk in TBST and visualized with an ECL system (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Test compounds were dissolved in DMSO and diluted with serum-free DMEM. Cell viability was checked using MTT bioassay (22). MMP-13 expression was measured at non-cytotoxic concentrations of the test compounds, unless otherwise specified.

**Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis**

Total RNA was isolated using an RNeasy mini kit (Qiagen, Hilden, Germany) from the cells treated with IL-1β for 6 h according to the manufacturer’s instructions. RNA concentration was determined by absorbance at 260 and 280 nm. cDNA was synthesized using the RT reaction at 42°C for 50 min and 99°C for 5 min in a Gene Cycler thermal cycler (Bio-Rad). Primers were synthesized based on the repeated human cDNA sequences for MMP-13 and G3PDH. The primer sequences used for PCR were: MMP-13: 517 bp, Tm (60°C), 27 cycles, 5′-GCT TAG AGG TGA CTG GCA AC-3′; 5′-CCG GTG TAG GTG TAG ATA GGA AC-3′; G3PDH: 604 bp, Tm (60°C), 27 cycles, 5′-GAA GCC CAT GCC AGT GAG CTT CC-3′; 5′-CCA TCA ACG ACC CCT TCA TTG ACC-3′. PCR was carried out under saturation, in a 25-μl reaction mixture. After amplification, 5 μl of reaction mixture was analyzed on 1.5% agarose gel electrophoresis. The bands were visualized by ethidium bromide staining for 10 min.

**Electrophoretic mobility shift assay (EMSA)**

EMSA was used to detect nuclear NF-κB and AP-1. Oligos were radio-labeled by T4 polynukleotid kinase with 10 μCi of [γ-32P]ATP (3,000 Ci/mmol) at 37°C for 10 min. Unincorporated nucleotides were removed by a Micro-spin G-25 column (GE Healthcare). Nuclear extract containing 5 μg protein was incubated with 32P-labeled oligo nucleotide in gel shift binding buffer at room temperature for 20 min. The incubation mixture was subjected to electrophoresis on a 4% polyacrylamide gel in 0.5 x TBE buffer at 250 V. The gel was dried and exposed to X-ray film overnight at −70°C.

**Cellular mechanisms of inhibition of MMP-13 induction**

Expression and phosphorylation of MAPKs and JAK/STAT were investigated in total cellular lysates. Total cellular proteins were extracted with Pro-Prep solution (iNtRON Biotechnology, Seongnam, Korea) containing 1 mM PMSF, 1 mM sodium orthovanadate, and 1 mM sodium fluoride. Expression of NF-κB p65, c-Jun, and c-Fos were identified in nuclear fractions. For an extraction of nuclear proteins, cells were resuspended in 400 μl of buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) and incubated on ice for 10 min. After 25 μl of 10% NP-40 was added, cells were vortexed for 10 s and centrifuged at 5,000 rpm for 2 min. The nuclear pellet was vigorously vortexed in buffer B (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) and centrifuged at 13,000 rpm for 10 min. The BCA protein assay (Pierce, Rockford, IL, USA) was used to determine protein concentration in the nuclear fraction. Proteins were separated, blotted, and visualized as described above.

**Effects of apigenin on glycosaminoglycan (GAG) release from rabbit cartilage culture**

Articular cartilages were excised from the femoral chondyles of rabbit knee and incubated in DMEM containing 5% FBS for 1 – 2 days. Cartilages were cut into small fragments with a scalpel and weighed under sterile condition. Approximately 30 mg cartilage fragments per well were seeded on 48-well plates, and media were changed to DMEM containing 1% FBS in 400 μl/well. Cartilages were treated with 10 μg/ml of human IL-1α (Sigma) and test compounds for 3 days. The amounts of released GAG in the supernatant were measured with a Blyscan sulfated glycosaminoglycan assay kit (Biocolor, Northern Ireland, UK) based on the dimethylmethyle blue (DMMB) assay according to manufacturer’s protocol.
Effects of the synthetic flavones on MMP-13 induction in chondrocytes and cellular mechanisms

For elucidating the structural-activity relationships, the effects of 14 synthetic flavones derivatives on MMP-13 induction were examined. The synthetic flavones used in this study were prepared based on the previously described synthetic procedures (23, 24) and they showed one spot in TLC. These synthetic flavones were added to IL-1β–treated SW1353 cells at 10 μM under the same experimental conditions described above. The released MMP-13 in the media was checked by ELISA. For elucidating cellular action mechanisms of MMP-13 down-regulation, c-Fos/AP-1 and STAT1/2 pathways were examined as described above.

Statistical analysis

Experimental values are represented as the arithmetic mean ± S.D. Statistical analysis was evaluated using one-way ANOVA followed by Dunnett’s analysis. P-values less than 0.05 were considered as significantly different.

Results

Effects of naturally-occurring flavonoids on MMP-13 expression

MMP-13 production in SW1353 cells was induced by IL-1β treatment for 24 h. When flavonoids were added simultaneously with IL-1β, flavone derivatives (apigenin and wogonin) strongly inhibited MMP-13 expression at concentrations up to 25 μM, while flavonols (quercetin and kaempferol) and naringenin (flavanone) did not (Fig. 2a). Quercetin and wogonin showed some cytotoxic effects against SW1353 cells at 25 μM (data not shown). Thus, the highest concentration of these two flavonoids tested was limited to 10 μM. Apigenin showed concentration-dependent inhibition of MMP-13 induction at 5 – 25 μM as demonstrated by Western blotting and RT-PCR (Fig. 2b). To confirm these results, IL-1β–treated SW1353 cells were treated with flavonoids at 10 μM and the MMP-13 concentration in the media was then measured by ELISA. As shown in Fig. 2c, apigenin and wogonin almost completely abrogated MMP-13 production, while quercetin and naringenin did not, as expected. Kaempferol slightly inhibited MMP-13 production (22.3%), but this inhibition was not significant. Moreover, apigenin inhibited MMP-13 production at 5 – 25 μM in a concentration-dependent manner. These results are well correlated with the results obtained by Western blotting and RT-PCR analysis, indicating that some flavones such as apigenin and wogonin down-regulate MMP-13 expression in IL-1β–treated chondrocytes. Based on these findings, apigenin was selected for further analysis of its mechanism of action.

*Fig. 2. Effects of flavonoids on MMP-13 expression in IL-1β–treated SW1353 cells. a) Inhibition of MMP-13 expression by flavonoids (Western blot): Cells were treated with flavonoids and IL-1β (10 ng/ml) for 24 h and MMP-13 was detected from the media. b) Concentration-dependent inhibition of MMP-13 expression by apigenin: Upper lane (Western blot), lower lanes (RT-PCR analysis). For semi-quantitative RT-PCR analysis, the cells were incubated with apigenin (5, 10, and 25 μM) and IL-1β for 6 h. c) Inhibition of MMP-13 production by flavonoids: Cells were treated with flavonoids and IL-1β (10 ng/ml) for 24 h, and MMP-13 in the media were detected by ELISA. **P < 0.01, significantly different from the IL-1β–treated control group (n = 3). Q: quercetin, A: apigenin, W: wogonin, K: kaempferol. N: naringenin.
Effects of apigenin on transcription factor activation (NF-κB and AP-1)

Since NF-κB and AP-1 are transcription factors known to be involved in the IL-1β activation pathway leading to MMP-13 induction (25, 26), the effects of apigenin on the activation of these transcription factors were examined. Figure 3, a and b, showed the EMSA results of NF-κB and AP-1 activation. Apigenin did not reduce NF-κB activation. In addition, translocation of p65 (component of NF-κB) to the nucleus was not reduced by apigenin treatment (Fig. 3c), suggesting that apigenin has little effect on NF-κB activation in IL-1β–treated chondrocytes.

Conversely, apigenin clearly inhibited AP-1 binding to the corresponding DNA site (Fig. 3b). Importantly, c-Fos translocation was almost completely abrogated by apigenin (Fig. 3c), while c-Jun translocation to the nucleus (downstream of JNK) was only slightly reduced (10.8% at 25 μM), indicating that apigenin blocked AP-1 activation to some degree via inhibition of c-Fos activation.

Effects of apigenin on the MAPK pathway

It is well known that the MAPK pathway participates in AP-1 activation and that p38 MAPK is critically in-

Fig. 3. Effects of apigenin on nuclear transcription factor activation in IL-1β–treated SW1353 cells. a) EMSA analysis of NF-κB activation. b) EMSA analysis of AP-1 activation: Cells were incubated with apigenin (5, 10, and 25 μM) for 2 h before IL-1β treatment for 30 min. Nuclear extract was prepared and EMSA was analyzed using consensus oligo; C: competitor (1.75 μM). c) Inhibition of apigenin on nuclear transcription factor activation (Western blot): Cells were incubated with apigenin (5, 10, and 25 μM) for 2 h before IL-1β treatment for 30 min. Expressions of c-Jun, c-Fos, NF-κB p65, and lamin B1 in nuclear extract were determined. *P < 0.05, **P < 0.01, significantly different from the IL-1β–treated control group (n = 3).
volved in MMP-13 expression (27, 28). When the effects of apigenin on MAPK activation by IL-1β were examined, it did not inhibit p38 MAPK or extracellular signal-regulated protein kinase (ERK)1/2 activation at 5 – 25 μM (Fig. 4a). Rather, apigenin weakly inhibited JNK activation. This inhibition was also observed in the time-course study (Fig. 4b). However, the inhibitory action of JNK activation is not likely a major inhibitory mechanism of apigenin against MMP-13 expression because the potency of inhibition is very weak and the JNK pathway is not primarily involved in AP-1 activation and MMP-13 expression in IL-1β–treated SW1353 cells (21). Taken together, these results demonstrated that the inhibitory action of apigenin on c-Fos/AP-1 activation is not related to the major MAPKs examined, and the upstream effector molecule of c-Fos/AP-1 activation affected by apigenin was not identified.

Effects of apigenin on the JAK/STAT signaling pathway
Along with NF-κB and AP-1 activation, the STAT activation pathway is also involved in MMP-13 expression of the IL-1β–treated chondrocytes (29). In particular, STAT1/2 activation is involved in IL-1β–treated SW1353 cells (21). When the cells were treated with apigenin, STAT1/2 activation was considerably inhibited in a concentration-dependent manner (Fig. 5a).

Because one possible upstream effector molecule of STAT1/2 activation is JAK2 in IL-1β–treated SW1353 cells, the effects on JAK2 activation were examined under the same experimental conditions. Total JAK2 was detected as shown in Fig. 5b. p-JAK2 was found to be inhibited by apigenin, indicating that apigenin inhibits STAT1/2 activation via inhibition of JAK2 activation, leading to MMP-13 down-regulation.

Effects of other naturally-occurring flavonoids on c-Fos/ AP-1 and STAT signaling pathways
To elucidate the inhibitory action mechanisms of other naturally-occurring flavonoids, their effects on the c-Fos/AP-1 and STAT signaling pathways were examined. At 10 μM, apigenin, wogonin and kaempferol inhibited c-Fos activation (67.8%, 67.0%, and 62.3%, respectively), while quercetin and naringenin did not (Fig. 6a). As expected, apigenin, and wogonin also inhibited AP-1 activation, while kaempferol showed weak inhibition of AP-1 activation. Moreover, apigenin strongly inhibited STAT1/2 activation (52.8% and 53.3%, respectively), followed by wogonin (15.2% and 23.4%, respectively). Quercetin slightly inhibited STAT activation (4.8% and 19.0%, respectively); however, this inhibition was not statistically significant (Fig. 6b).

As shown above, apigenin strongly inhibits c-Fos and STAT1/2 activation. Moreover, wogonin, another flavone that inhibits MMP-13 expression, also inhibits both pathways, being less active on the STAT pathway. Con-
versely, quercetin and kaempferol, which showed weak or no inhibitory action against MMP-13 expression, only inhibited one of the two pathways. Specifically, Kaempferol inhibited c-Fos/AP-1 activation, while quercetin weakly reduced the STAT2 activation pathway. Flavanone (naringenin) did not inhibit the AP-1 or STAT pathway.

**Effects of apigenin on ECM degradation of rabbit cartilage**

To confirm the MMP-13 inhibitory action of apigenin, the effects on the ECM breakdown of rabbit cartilage explant cultures were examined. When treated with IL-1β, the cartilage explants released $0.19 \pm 0.01 \mu g$ GAG/mg cartilage from the basal level of $0.10 \pm 0.04 \mu g$ GAG/mg cartilage. Under this condition, apigenin inhibited the release of GAG in a concentration-dependent manner.

**Fig. 6.** Effects of flavonoids on c-Fos and STAT1/2 activation in IL-1β-treated SW1353 cells. a) Inhibition of flavonoids on c-Fos translocation to the nucleus (Western blot) and EMSA analysis of AP-1 activation: Cells were incubated with flavonoids (10 μM) for 2 h before IL-1β treatment for 30 min and nuclear extracts were used; C: competitor (1.75 μM). b) Inhibition of STAT1/2 activation: Cells were treated with flavonoids (10 μM) for 2 h before IL-1β treatment for 4 h. Activations of STAT1/2 in total cell lysate were detected by Western blotting. *$P < 0.05$, significantly different from the IL-1β–treated control group (n = 3). Q: quercetin, A: apigenin, W: wogonin, K: kaempferol, N: naringenin.

**Fig. 7.** Effect of apigenin on GAG release in rabbit cartilage culture. A 30-mg explant culture was treated with apigenin (A, 5 and 10 μM) or dexamethasone (D, 10 μM) along with IL-1α (10 ng/ml) for 3 days. The amount of GAG in the supernatant was measured with a GAG assay kit. *$P < 0.05$, **$P < 0.01$, significantly different from the IL-1α–treated control group (n = 3).
Fig. 8. Effects of the synthetic flavones on MMP-13 expression and activation of nuclear transcription factor in IL-1β–treated SW1353 cells. a) Chemical structures of the 14 synthetic flavones used in this study. b) Inhibition of MMP-13 expression by 14 synthetic flavones (ELISA analysis): Cells were treated with the synthetic flavones (10 μM) and IL-1β (10 ng/ml) for 24 h, and MMP-13 was detected from the media by ELISA; A: Apigenin. c) Inhibition of c-Fos translocation to the nucleus (Western blot), AP-1 activation (EMSA analysis), and STAT1/2 activation (Western blot) by compound 7 and apigenin (10 μM): Cells were treated with IL-1β (10 ng/ml) for 30 min after pretreatment with compound 7 and apigenin for 2 h, and nuclear extracts were used for c-Fos detection and activation of AP-1. Cells were treated with IL-1β (10 ng/ml) for 4 h after pretreatment with compound 7 and apigenin for 2 h, and total cell lysates were used for measuring STAT1/2 activation. *P < 0.05, **P < 0.01, significantly different from the IL-1β–treated control group (n = 3).
(Fig. 7). The reference drug, dexamethasone, also inhibited the release of GAG (67.6% at 10 μM).

**Structural-activity relationships of the flavone derivatives and action mechanisms**

When 14 synthetic flavones with diverse chemical structures (Fig. 8a) were examined, compounds 7, 8, 9, and 12 showed strong inhibitory action (more than 40% inhibition) against MMP-13 induction at 10 μM (Fig. 8b). Specifically, compound 7 (2',3',5,7-tetrahydroxy-flavone) showed the most potent down-regulatory effect, being comparable to apigenin. To confirm the cellular action mechanisms, compound 7 was added to IL-1β–treated SW1353 cells and the c-Fos and STAT activation pathways were examined. As shown in Fig. 8c, compound 7 clearly inhibited both pathways at 10 μM, as did apigenin.

**Discussion**

This study showed for the first time that certain flavonoids (flavones) of plant origin inhibit MMP-13 induction in IL-1β–treated human chondrocytes, at least in part, by blocking the c-Fos/AP-1 and JAK2/STAT1/2 signaling pathways.

The naturally-occurring flavonoids tested in this study were naringenin, apigenin, wogonin, kaempferol, and quercetin. Among the numerous structural diversities, these flavonoids were selected based on their anti-inflammatory potential and abundance in nature. Quercetin and kaempferol are the most abundant flavonoids in plants, and these compounds down-regulate the expression of certain inflammation-related molecules (8). Apigenin and wogonin are potent down-regulators of cyclooxygenase-2, inducible nitric oxide synthase, and MMP-1. Naringenin is a representative flavonoid of flavanone type devoid of C-2,3-double bonds.

The results of this study indicated that certain flavones such as apigenin and wogonin strongly inhibited MMP-13 expression, whereas flavonols and naringenin showed no or weak inhibition of the MMP-13 induction in IL-1β–treated SW1353 cells. The inactive nature of quercetin was correlated with the results of a previous study (30). Despite the small numbers of flavonoids initially tested, our results suggest that the C-2,3-double bond in the flavonoid structure is essential, but that the 3-hydroxyl moiety abolished or reduced MMP-13 down-regulatory activity. For further characterization of structural requirements in flavone structures, 14 synthetic flavone derivatives were tested and the following structure–activity relationships were found. C-3-hydroxyl substitution reduced the suppressive activity (luteolin vs. quercetin). A-ring 5,7-dihydroxyl substitution enhanced the activity (3 vs. 7, 4 vs. 8, 5 vs. 9). B-ring hydroxyl substitution at C-4', C-2', C-3', C-2',4', or C-3',4' increased the suppressive action of MMP-13 expression (1 vs. 2, 3, 4).

Collectively, it is believed that the A-ring C-5,7-dihydroxyl groups or C-5-hydroxyl-7-methoxyl groups are essential and B-ring C-4-hydroxyl residue or the dihydroxyl substitutions at C-2,3, C-2,4 or C-3,4 are important. This is the first observation of structure–activity relationships of flavonoids possessing MMP-13–suppressive action.

EGCG (one specific flavanol-type flavonoid) and icariin (flavonol glycoside) are the only flavonoids that have previously been reported to down-regulate collagenase induction in chondrocytes. EGCG was reported to inhibit MMP-1, -3, and -13 expression in IL-1β–treated chondrocytes, and it was claimed that this suppression was mediated by inhibition of transcription factor activation (NF-κB and AP-1) (16, 18). Icariin down-regulated MMP-1, -3, and -13 induction in LPS-treated chondrocytes (19). Our study is distinct from these previous studies in that the MMP-13–suppressive action of flavonoids was generalized and new inhibitory mechanisms were found. Furthermore, some structural-activity relationships of flavonoids were demonstrated for the first time. During the present study, it was found that apigenin and wogonin also inhibited MMP-1 expression from IL-1β–treated SW1353 cells, while quercetin, kaempferol, and naringenin did not (data not shown). However, the signaling pathway to MMP-1 induction appears to be different from those of MMP-13 induction. Further study is needed in this area.

IL-1β treatment of SW1353 chondrocytes is known to induce activation of NF-κB, AP-1, and STATs (28, 31–33). Of these activation pathways, the active flavone derivatives (apigenin, wogonin, and compound 7: 2',3',5,7-tetrahydroxylflavone) strongly inhibited both the c-Fos/AP-1 and STAT1/2 activation pathways, while the inactive flavanone (naringenin) did not. Inactive flavonol (quercetin) only weakly inhibited STAT activation, and kaempferol inhibited one of the pathways, c-Fos/AP-1 activation. These results suggest that inhibition of either of these pathways may not be sufficient to strongly inhibit MMP-13 expression. Dual inhibition of AP-1 and STAT activation by flavonoids may potentiate MMP-13 down-regulatory action in SW1353 cells.

Many flavonoid derivatives have previously been shown to inhibit activation of the transcription factors, NF-κB (34) and AP-1, and some flavonoids such as apigenin and quercetin inhibit the STAT activation pathway in T lymphocytes and microglia (35, 36). However, STAT inhibition by flavonoids in chondrocytes has not been reported to date. To the best of our knowledge, this is the first report of flavonoids inhibiting the JAK/STAT activation pathway in chondrocytes.
The concentration ranges of flavonoids showing MMP-13 down-regulation in this study were 5 – 25 μM, which may not be easily obtained by normal ingestion of flavonoid-rich foods. However, these concentration ranges could be obtained in the body via pharmacological treatment. Moreover, it is well-known that proinflammatory and catabolic cytokines including IL-1β play an important role in enhancement of inflammatory response as well as degradation of ECM in the cartilage under arthritic conditions. Recently, low innate production of IL-1β and IL-6 were found to be related to the absence of OA in old age (37). This background information may further strengthen our research rationale in that the cytokine pathway appears to be important to the development of OA and the flavones mentioned in the present investigation block the IL-1β activation pathway of chondrocytes, suggesting that they have the potential to prevent OA development as well as inhibit cartilage degradation in arthritis.

In summary, certain flavonoids, especially apigenin, wogonin, and some synthetic flavones, were shown to down-regulate MMP-13 expression in IL-1β–treated SW1353 cells. Our study provides strong evidence that these flavonoids inhibit MMP-13 induction, at least in part, via inhibition of the c-Fos/AP-1 and JAK2/STAT1/2 pathways. These results suggest that certain flavonoids, especially flavones, may protect against collagen matrix breakdown by MMP-13 down-regulation in the cartilage of diseased tissues such as those found in arthritic disorders.

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