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Ginsenosides from Korean red ginseng inhibit matrix metalloproteinase-13 expression in articular chondrocytes and prevent cartilage degradation

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ABSTRACT

Among the mammalian matrix metalloproteinases (MMPs), MMP-1, -3 and -13 are collagenases. Particularly, MMP-13 is important for the degradation of major collagens in cartilage under certain pathological conditions such as osteoarthritis. To establish a potential therapeutic strategy for cartilage degradation disorders, the effects of 11 ginseng saponins (ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg3, Rg5, Rk1 and F4) on MMP-13 induction were examined in a human chondrocyte cell line, SW1353. Among these, several saponins including ginsenoside Rc, Rd, Rf, Rg3 and F4 were found to inhibit MMP-13 expression in IL-1β-treated SW1353 cells at non-cytotoxic concentrations (1–50 μM). The most prominent inhibitors were ginsenosides F4 and Rg3. Ginsenoside F4 inhibited MMP-13 expression 33.5% ($P<0.05$), 57.9% ($P<0.01$) and 90.0% ($P<0.01$) at 10, 30 and 50 μM, respectively. Significantly, ginsenoside F4 was found to strongly inhibit activation of p38 mitogen-activated protein kinase in signal transduction pathways (86.6 and 100.0% inhibition at 30 and 50 μM, $P<0.01$). The MMP-13 inhibitory effect was also supported by the finding that ginsenosides F4 and Rg3 reduced glycosaminoglycan release from IL-1α-treated rabbit joint cartilage culture to some degree. Taken together, these results indicate that several ginsenosides inhibit MMP-13 expression in IL-1β-treated chondrocytes. Ginsenoside F4 and Rg3 blocked cartilage breakdown in rabbit cartilage tissue culture. Thus, it is suggested that certain ginsenosides have therapeutic potential for preventing cartilage collagen matrix breakdown in diseased tissues such as those found in patients with arthritic disorders.

1. Introduction

The extracellular matrix (ECM) consists of collagens, elastin, proteoglycans and their large aggregates. In particular, collagens and aggrecans are the main ECM materials in joint cartilage (Roughley, 2006). These cartilage materials provide support and tensile strength for the joint. The aging process and many physical stresses are known to induce progressive loss of integrity and strength of the cartilage principally due to the denaturation and degradation of ECM materials in the cartilage and synovial fluid, leading to the symptoms of osteoarthritis (OA). In the joint cartilage, matrix metalloproteinases (MMPs) synthesized and secreted by the residing chondrocytes are largely responsible for degrading ECM (Hadler-Olsen et al., 2011). MMPs at the basal level have an important role in the normal turn-over of ECM materials. However, under special pathological conditions, such as inflammation or OA, chondrocytes highly induce MMPs and aggrecanases. Among these, MMP-13 is important for degrading cartilage collagens (Hadler-Olsen et al., 2011; Mitchell et al., 1996; Takaishi et al., 2008). Thus, it can be reasonably assumed that inhibitors/down-regulators of MMPs may have the potential to prevent and/or treat osteoarthritic symptoms and several other ECM-degradation diseases to some extent. Indeed, one MMP inhibitor, Periostat®, is currently available in clinics for treating periodontitis (Caton and Ryan, 2011).

To obtain potential therapeutic agents, several plant extracts and marine natural products have been evaluated for their inhibition of MMP-1 or -13 (Kim et al., 2011; Shakibaei et al., 2012). Recently, flavonoids, which are the major plant constituents, were found to strongly down-regulate MMP-13 expression in chondrocytes (Lim et al., 2011). In line with these efforts, the effects of ginsenosides were examined in the present investigation to find natural collagenase down-regulators.

Korean red ginseng (the steamed roots of *Panax ginseng* C.A. Meyer, family Araliaceae) is frequently used as a tonic to enhance the vitality and strengthen the immune response. As major constituents,

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many ginsenoside saponins (ginsenosides) have been isolated and structurally identified from white ginseng and Korean red ginseng (Nag et al., 2012). Ginsenosides have been shown to exhibit various pharmacological activities, including vitality enhancement, immune modulation and anticancer activity (Wu et al., 1992; Mochizuki et al., 1995; Nag et al., 2012). However, few studies have examined the therapeutic potential of ginsenosides or ginseng products for cartilage degradation. Ginsenoside Rg3 was shown to inhibit MMP-13 induction in human chondrocytes in osteoarthritic cartilage (So et al., 2013), and ginsenoside Rh2 was shown to inhibit MMP-1 expression in human astroglioma cells (Kim et al., 2007). Additionally, ginsenoside Rh1 was found to inhibit MMP-1 expression in human hepatocellular carcinoma cells (Yoon et al., 2012). In the present investigation, we isolated 11 ginsenosides from Korean white and red ginseng. The potential of these compounds to down-regulate MMP-13 expression was examined using a human chondrocyte cell line, SW1353, to establish their therapeutic potential for OA and other joint inflammatory diseases, and we also studied their cellular mechanisms of action. This is the first thorough investigation of ginsenosides (dammarane-type triterpenoids) that may inhibit MMP-13 expression and directly inhibit the cartilage degradation.

2. Materials and methods

2.1. Chemicals

Human IL-1α, IL-1β, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dexamethasone, diclofenac and anti-MMP-13 antibody were purchased from Sigma Chem. (St. Louis, MO). DMEM and other cell culture reagents including FBS, were the products of Gibco BRL (Grand Island, NY). The protein assay kit was purchased from Bio-Rad Lab. (Hercules, CA). All antibodies related to nuclear transcription factor-κB (NF-κB) p65, mitogen-activated protein kinases (MAPK), c-Fos, c-Jun and signal transducer and activator of transcription (STAT) signaling were purchased from Cell Signaling Technologies (Dancers, MA). Lamin B1 antibody was purchased from Bioworld technology (Minneapolis, MN).

2.2. Preparation of ginsenosides

Korean red ginseng was obtained from Korean Ginseng Corporation (Daejon, Korea) and *Panax ginseng* roots (white ginseng) were purchased from Kyungdong Market (Seoul, Korea). Dried Korean red and white ginseng samples were extracted twice with 70% EtOH by sonication for 3 h, followed by rotary vaporization at 40 °C under reduced pressure. The dried extracts were then dissolved in distilled water and fractionated with *n*-butanol three times. The butanol fractions were again centrifuged to remove the traces of high polar particles (sugar), evaporated and freeze-dried. This fraction was subject to high-speed counter-current chromatography for the separation of ginsenosides following the previous report (Shehzad et al., 2012a, 2012b). They include ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg3, Rg5, Rk1 and F4 (Fig. 1). The purity of each ginsenoside was determined to be over 98% by HPLC analysis.

2.3. Animals

Male New Zealand white rabbits (6 week old) were purchased from Nara Biotech. (Seoul, Korea). The animals were maintained in an animal facility (KNU) at 20–22 °C under 40–60% relative humidity and a 12 h/12 h (light/dark) cycle. The experimental design using the animals was approved by the local committee for animal experimentation of Kangwon National University (KIACUC-12-0012). The animals were handled according to the guideline described in the Food and Drug Administration (Korea) Guide for the Care and Use of Laboratory Animals throughout the experiments.

2.4. SW1353 cell culture and MMP-13 induction

SW1353 cells (human chondrosarcoma cell line) purchased from American type culture collection (Manassas, VA) were cultured and treated with IL-1β according to the previously described procedures (Lim and Kim, 2011). Briefly, the cells were normally maintained in DMEM with 10% FBS, glutamine and penicillin/streptomycin. For an induction of MMP-13, IL-1β (10 ng/ml) with/without test compounds was added to the cells in serum-free DMEM. After 24 h incubation, MMP-13 expressed and released in the media was examined by Western blotting analysis. After separating on SDS-PAGE, the blots were treated with anti-MMP-13 antibody in 5% skim milk in TBST. An ECL system (GE Healthcare, UK) and ImageQuant LAS4000 mini (GE Healthcare, UK) were used to visualize the band. The densities of the bands were analyzed with Image J (NIH, USA). All test compounds were initially dissolved in DMSO and diluted with serum-free DMEM to adjust the final DMSO concentration to 0.1% (v/v). Cell viability was checked using MTT bioassay (Mossman, 1983). Cell viability and the levels of MMP-13 expression were not changed by the treatment of 0.1% DMSO.

2.5. Cellular mechanisms of inhibition of MMP-13 induction

Using total cellular lysates, expression and phosphorylation of MAPKs and STAT were examined. Total cellular proteins were extracted with Pro-Prep solution (iNtRON Biotechnology) 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. Later 25 μl of 10% NP-40 was added, cells were vortexed for 10 s and centrifuged at 5000 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. BCA protein assay (Pierce, IL, USA) was used to determine the protein concentration in the nuclear fraction. Proteins were separated, blotted and visualized as described above.

2.6. Effects of ginsenosides on glycosaminoglycan (GAG) release from rabbit cartilage culture

According to the previously described procedures (Lim et al., 2011), articular cartilages were excised from the femoral chondyles of rabbit knee and incubated in DMEM containing 5% FBS for 1–2 days. Cartilages were then cut into small fragments with scalpel and weighed under sterile condition. Approximately 30 mg cartilage fragments per well were seeded on 48 well plates and media was changed to DMEM containing 1% FBS in 400 μl/well. Cartilages were treated with 10 ng/ml of human IL-1α (Sigma Chem.) in the presence or absence of test compounds for 3 days. Ginsenosides F4 (2 and 10 μM) and Rg3 (0.5 and 2 μM) were added. The amounts of released GAG in the supernatant were measured with Blyscan sulfated glycosaminoglycan assay kit (Biocolor, Northern Ireland, UK) based on dimethylmethylene blue (DMMB) assay according to manufacturer's protocol.