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Gentiopicroside prevents interleukin-1 beta induced inflammation response in rat articular chondrocyte

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ABSTRACT

Ethnopharmacological relevance: In traditional Chinese medicine, *Gentiana macrophylla* Pall have been prescribed for the treatment of pain and inflammatory conditions. In addition, it is a common Tibetan medicinal herb used for the treatment of tonsillitis, urticaria, and rheumatoid arthritis (RA), while the flowers of *G. macrophylla* Pall have been traditionally treated as an anti-inflammatory agent to clear heat in Mongolian medicine. The secoiridoid glycosides and their derivatives are the primary active components of *G. macrophylla* and have been demonstrated to be effective as anti-inflammatory agents.
Materials and methods: Solvent extraction and D101 macroporous resin columns were employed to concentratethe gentiopicroside. Gentiopicroside cytotoxicity was assessed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; the toxicity of gentiopicroside in chondrocytes was reconfirmed using Hoechst staining. Western blotting, reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry were utilized to explore the protective effects and mechanisms of gentiopicroside prevents interleukin-1 beta induced inflammation response in rat articular chondrocyte.
Results: The MTT assay demonstrated that 50, 500, and 1500 μg/mL of gentiopicroside exhibited no significant toxicity to chondrocytes ($P > 0.05$) after 24 h. Using immunohistochemistry, ELISA, RT-PCR, Western blot method to explore the protective effect and mechanism of gentiopicroside on chondrocytes induced by IL-1β. The results showed some pathways of IL-1β signal transduction were inhibited by gentiopicroside in rat chondrocytes: p38, ERK and JNK. Meanwhile, gentiopicroside showed inhibition in the IL-1β-induced release of MMPs while increasing Collagen type II expression.
Conclusions: The current study demonstrated that gentiopicroside exhibited a potent protective effect on IL-1β induced inflammation response in rat articular chondrocyte. Thus, gentiopicroside could be a potential therapeutic strategy for treatment of OA.

1. Introduction

Osteoarthritis (OA) is one of the most prevalent age-related degenerative joint disorders including knee osteoarthritis, cervical osteoarthritis and lumbar osteoarthritis. Cartilage degradation is one of the pathological changes in OA, and is characterized by extracellular matrix (ECM) damage and tissue cellularity loss (Martel-Pelletier, 1998). Chondrocytes are the only cells of articular cartilage, which play a central role in the degradation of ECM. In this regard, the chondrocyte is of clinical importance in the context of pathogenesis of OA, which results from a failure to maintain a balance between synthesis and degradation of the cartilage extracellular matrix (ECM). The models which mimic the circumstances leading to in vivo cartilage degradation are valuable in the evaluation of pathogenesis and therapeutic methods of OA. Interleukin-1 (IL-1β) has been shown to induce chondrocytes degradation in vitro and treatment of chondrocytes with IL-1β serve as a model for experimental inflammation seen in OA (Yasuhara et al., 2005).

IL-1β is a major pro-inflammatory cytokine implicated in arthritic joint damage. it alters chondrocytes anabolism by repressing the synthesis of proteoglycan and proteoglycan degradation could be enhanced through stimulation of chondrocytes to induce MAPKs signaling pathway, which finally results in the expression of a number of inflammatory mediators including matrix metalloproteinases (MMPs) such as MMP-1, MMP-3, MMP-13 and the aggrecanases, NO, PGE2 and cyclooxygenase-2 (COX-2) (Aida et al., 2005). Overproduction of NO, PGE2 and COX-2 leads to clinical

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manifestations of OA (Chen et al., 2014). It is worth noting that MMP-1 and MMP-13 can directly inhibit progeoglyean synthesis, cleave different components of the cartilage ECM, induce OA in end (Tehetina et al., 2005; Tchetverikov et al., 2005; Knäuper et al., 1996; Bluteau et al., 2001). Based on it, inhibition of these factors may potentially be a therapeutic target in OA.

Recently, natural products of plant origin with immense ethnopharmacological importance have been given top priority as treatments for inflammatory diseases (Talhouk et al., 2007; Li et al., 2010). *Gentiana macrophylla* Pall have been used for the treatment of arthralgia, stroke, hemiplegia, pains, jaundice, and infantile malnutrition, also has been an important traditional Chinese medicinal (TCM) herb for fighting OA, since ancient times in China (Cai et al., 2010). According to the Pharmacopoeia of the People's Republic of China (edited in 2010), QinJiao, Gentianaceae gentian is the dry root of *G. macrophylla* Pall, *Gentiana straminea* Maxim, *Gentiana crassicaulis* Duthie, or *Gentiana dahurica* Fisch (China Pharmacopoeia committee, 2010). To date, *G. macrophylla*, *Gentiana crassicaulis*, and *Gentiana straminea* have been chemically and biologically investigated by several groups (Lv et al., 2012; Singh, 2008; Tan et al., 1996; Xu et al., 2009a; Liu et al., 1994). The results suggested that those plants contained mainly secoiridoid glycosides and the water extracts from roots of *G. macrophylla* displayed a significant inhibitory effect on acute treatment of rheumatoid rats (Yu et al., 2004). Gentiopicroside (The chemical structure is shown in Fig. 1), is the important active component of total secoiridoid glycosides and has significant biological activities, such as protecting the liver, as well as anti-inflammatory and antibacterial activities. The anti-inflammatory effect of gentiopicroside has been illustrated by significant inhibition of xylene-induced mouse ear swelling model, rat foot swelling induced by carrageenan or zymosan A, and acetic acid-induced abdominal capillary permeability, it is confirmed that acute inflammatory reactions in albumin-induced rat paw edema and the edema of rat granulomas were suppressed by gentiopicroside to some extent (Chen et al., 2003). While the activity of gentiopicroside inhibited COX-2 was detected. Wistar rats in the treatment group were administered with gastric perfusion of gentiopicroside and various polar extracts, and drug-containing serum was obtained from rats. With the use of the drug-containing serum in vitro, gentiopicroside and various polar extracts inhibited PLA_2 (phospholipase A_2) activity in RAW 26417 and lipopolysaccharide-induced NO production, but only gentiopicroside inhibited COX-2 activity (An and Jin, 2007).

These findings demonstrate that gentiopicroside can be used as potential drug for the treatment of osteoarthritis. Gentiopicroside has anti-inflammatory, analgesic, antibacterial, and free radical scavenging activities (Kondo et al., 1994; Jiang et al., 2005; Chen et al., 2008; Kumarasamy et al., 2003). However, to the best of our knowledge, the effect of gentiopicroside on OA chondrocytes has not been reported. In the present study, we reported the cytotoxicity of gentiopicroside on chondrocytes evaluated the protective effect of gentiopicroside on chondrocytes through investigating the proliferation and the protein expression of MMP1, -3, -13, PGE_2, COX-2, and type II collagen production in IL-1β-induced rat chondrocytes. We also analyzed the activation of MAPK pathways, namely, ERK, JNK, and P38.

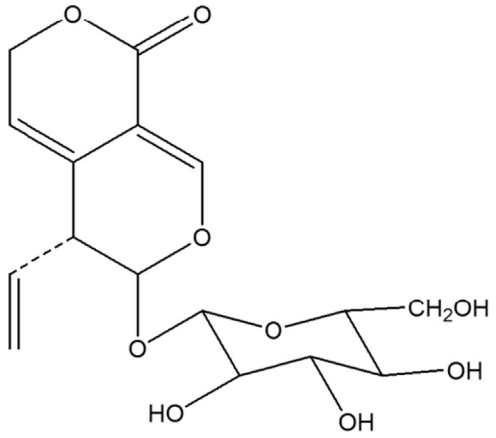

Fig. 1. The chemical structure of gentiopicroside.

2. Materials and methods

2.1. Preparation of gentiopicroside

G. macrophylla Pall was obtained from Gan Nan (Gansu, China), and identified by Dr. Ling Jing from Gansu University of Traditional Chinese Medicine. A voucher specimen (Reference number 120910-11) has been deposited in the herbarium stock room of the College of Pharmacy, Gansu University of TCM, Lanzhou, China. The extraction and purification of gentiopicroside was carried out according to previous reports (Hao, 2002). The whole *G. macrophylla* Pall plant (10 kg) was powdered and extracted with boiling water three times. The extract solution was subjected to chromatography on D101 macroporous resin column and the 30% alcohol elution was concentrated under low pressure. Next, a column-chromatography on silica gel was performed with EtOAc:MeOH:H_2O (20:2:1) as the mobile phase to give a slightly yellow crystal. The crystal was examined by TLC, HPLC, NMR, MS and IR. All of the data obtained were in accord with previous research. Thus, the crystal was identified as gentiopicroside and the purification determined by HPLC was 99% (a standard was provided by the National Institute on Drug Abuse of China with the Batch number of 0770-200008).

2.2. Rats

Rat articular chondrocytes and chondrocyte growth medium were purchased from Tianjin WeikaiBioeng Ltd. Recombinant IL-1β was obtained from Peprotech GmbH (Hamburg, Germany). Hoechst 33342, dimethylsulfoxide (DMSO) and Rabbit anti-collagen was purchased from Boster Co., Ltd., Wuhan. The antibodies for P38 MAPK (#8690), phospho-P38 MAPK (#4511), P44/42 MAPK (ERK1/2) (#4695), phospho-P44/42 MAPK (ERK1/2) (#4370), stress activated protein kinase (SAPK) /JNK (#9252), and phospho-SAPK/JNK (#4668) used in the western blot experiments were from Cell Signaling Technology, USA. The rat PGE_2 enzyme-linked immunosorbent assay (ELISA) kit was purchased from Nanjing Jian cheng Bioeng Ltd. The primers for MMP-1, MMP-3, MMP-13, 18s, and COX-2, as well as the reverse transcription kits and amplification kit, were purchased from Invitrogen (Carlsbad, CA, USA).

2.3. Cell culture

Rat chondrocytes were incubated in chondrocyte growth medium at 37 °C in a humidified 5% CO_2 atmosphere. The medium was then replaced with a serum-free medium overnight and then treated with IL-1β (10 ng/mL) with or without gentiopicroside for 15 min to 120 min. The cells were subsequently collected for western blot analysis. In another study, cells were incubated in the absence or presence of gentiopicroside for 24 h. The conditioned media was collected for PGE_2 measurement, and the cells were collected for reverse transcription-polymerase chain reaction. Rat chondrocytes cultured without IL-1β and gentiopicroside were the controls.