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Anti-inflammatory activity of ginsenosides in LPS-stimulated RAW 264.7 cells

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Abstract Ginsenosides, the main active constituents of *Panax ginseng* Meyer (*P. ginseng*), have potential therapeutic effects. All tested ginsenosides except ginsenoside F1 have previously been reported in inflammation studies using the RAW 264.7 macrophage cell line. We examined the anti-inflammatory effects of single sugar moiety ginsenosides such as compound K (CK), Rh2, Rh1, and F1 that were isolated from *P. ginseng* through in silico docking studies. We investigated their biological activity predictions, including absorption, distribution, metabolism, excretion, and toxicity and PASS properties, on the suppression of NF-κB, followed by in vitro validation in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. The molecular docking results of our study showed that all treated ginsenosides are non-toxic and may be drug-like molecules. The molecular binding interactions of these ginsenosides with the active residues of NF-κB noticeably support their anti-inflammatory activity. CK and Rh1 significantly reduced the production of nitric oxide, cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines such as prostaglandin E2 and tumor necrosis factor alpha (TNF-α) in a dose-dependent manner. Real-time PCR and Western blot analyses further confirmed that protopanaxadiols (PPDs) and protopanaxatriols (PPTs) inhibitory effects may have been due to the down-regulation of TNF-α, inducible nitric oxide synthase, COX2, nuclear factor kappa B (NF-κB), and I kappa B kinase. The expression of co-stimulatory molecules such as ROS was also inhibited by CK and Rh1 in a dose-dependent manner. Furthermore, activation of NF-κB in LPS-stimulated RAW 264.7 macrophages was significantly suppressed by CK and Rh1. Taken together, these results provide evidence that PPD- and PPT-type ginsenosides including CK and Rh1 may exhibit strong anti-inflammatory effects by inhibiting pro-inflammatory mediators through down-regulation of NF-κB.

Keywords Ginsenosides · Protopanaxadiol · Protopanaxatriol · Inflammation · NF-κB/IKK · RAW 264.7

1 Introduction

Inflammation is a complex biological process involving the response of several vascular tissues to injurious stimuli, such as damaged cells, irritants, and pathogens. Inflammation plays a critical role in various human diseases, including cancer, neurological disorders, metabolic syndrome, cardiovascular disease, inflammatory bowel disease, arthritis, and infectious diseases [1–3]. In the pathogenesis of inflammation, the overexpression of different cytokines such as COX-2, inducible nitric oxide synthase (iNOS), PGE2, and TNF-α produced by activated macrophages plays an important role in the induction of acute and chronic inflammatory diseases [4, 5]. Lipopolysaccharide (LPS), a well-known component of the cell wall of gram-negative bacteria, stimulates a number of signaling pathways, including NF-κB/IKK signaling in macrophages [6]. NF-κB plays a significant role in the pathogenesis and regulation of inflammatory responses [7].

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During normal conditions, NF-κB dimers (p50 and p65 subunits) exist in the cytoplasm, complexed with the inhibitor IκB. After cell activation, specific kinases phosphorylate IκB, leading to its degradation and the release of NF-κB from IκB. This allows translocation of NF-κB into the nucleus, where it binds to specific promoter regions of target genes and induces different transcription factors for inflammatory disease, such as iNOS and COX-2 [8, 9]. Therefore, NF-κB is considered to play a vital role in the up-regulation of several inflammatory mediators such as pro-inflammatory cytokines, including interleukin-1 beta (IL-1β) and TNF-α [10]. Thus, agents that restrain the activation of the IKK/NF-κB complex could potentially be used to manage inflammatory disorders [11, 12]. As NF-κB activation is commonly involved in the pathogenesis of inflammatory diseases, it is a common target in the development of anti-inflammatory drugs. Many therapeutic agents have been studied for anti-inflammatory activity, but are associated with diverse side effects. Thus, the discovery of anti-inflammatory therapeutic agents that down-regulate NF-κB and pro-inflammatory cytokines with few side effects could be very beneficial.

Korean ginseng (*Panax ginseng* Meyer) is the most common traditional medicinal plant and has been used for the treatment of several diseases for over 2000 years. The active components of ginseng are collectively known as ginsenosides. On the basis of structure, these ginsenosides are divided into two main groups, the protopanaxadiols (PPD) and the protopanaxatriols (PPT). These ginsenosides have several pharmacological and biological effects, including anticancer, anti-diabetic, anti-osteoporosis, and anti-inflammatory activities [13]. However, the combined comparison of PPD- and PPT-type ginsenosides with single sugar moiety from *P. ginseng* in inflammatory diseases is unknown. We aimed to identify effective ginsenoside(s) that can play a preventive and protective role against inflammatory disorders, with the goal of developing a ginsenoside-containing anti-inflammatory diet. NF-κB inhibitory activity became our key focus for preliminary screening. As a part of our ongoing screening project of ginsenosides to assess anti-inflammatory properties, we selected single sugar molecules from *P. ginseng* such as Rh2, CK (PPD), Rh1, and F1 (PPT; Fig. 1) and studied their anti-inflammatory effects by using an in silico docking study followed by in vitro validation in LPS-stimulated RAW 264.7 cells.

2 Materials and methods

2.1 Computational study

2.1.1 Selection of compounds/ligands preparation

The set of tested ginsenosides were collected from *P. ginseng* plants based on pharmacological activity, mainly anti-inflammatory behavior. The list of PPD- and PPT-type ginsenosides includes Rh2, CK, Rh1, and F1. The structures of the given ginsenosides were collected from our own in-house database. The two-dimensional structure (2D) of these ginsenosides was drawn with the ChemSketch program (http://

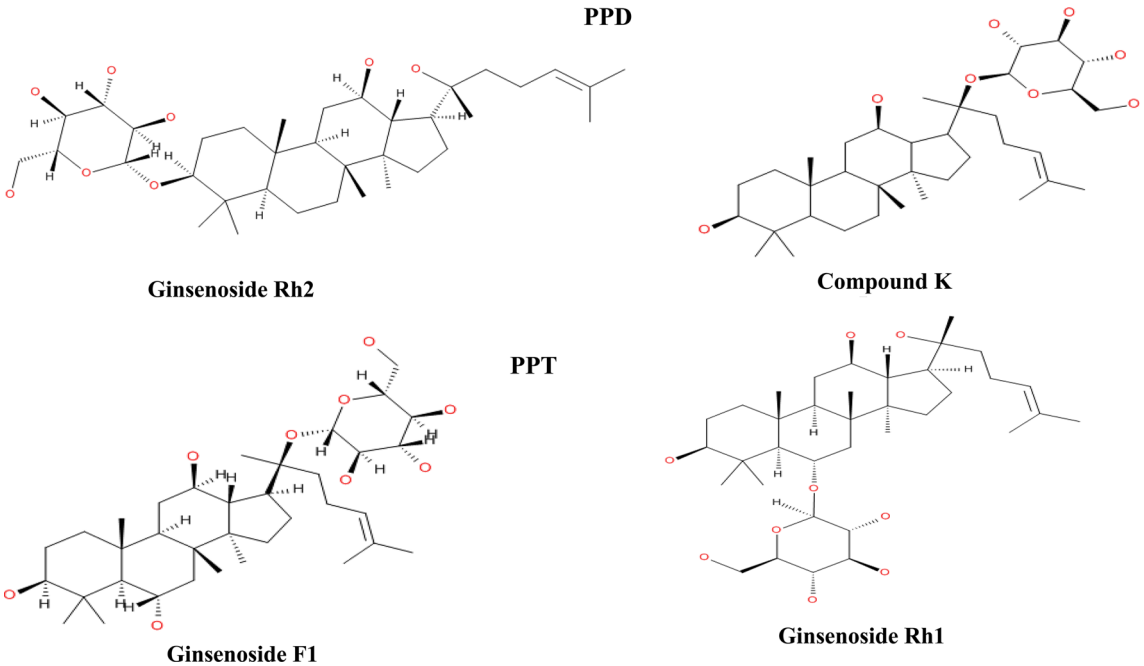

Fig. 1 Structures of PPD- and PPT-type ginseng saponin