Research Article

Ginsenoside Rf inhibits cyclooxygenase-2 induction via peroxisome proliferator–activated receptor gamma in A549 cells

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ABSTRACT

Background: Ginsenoside Rf is a ginseng saponin found only in Panax ginseng that affects lipid metabolism. It also has neuroprotective and antiinflammatory properties. We previously showed that Korean Red Ginseng (KRG) inhibited the expression of cyclooxygenase-2 (COX-2) by hypoxia via peroxisome proliferator–activated receptor gamma (PPARγ). The aim of the current study was to evaluate the possibility of ginsenoside Rf as an active ingredient of KRG in the inhibition of hypoxia-induced COX-2 via PPARγ.

Methods: The effects of ginsenoside Rf on the upregulation of COX-2 by hypoxia and its antimigration effects were evaluated in A549 cells. Docking of ginsenoside Rf was performed with the PPARγ structure using Surfex-Dock in Sybyl-X 2.1.1.

Results: PPARγ protein levels and peroxisome proliferator response element promoter activities were promoted by ginsenoside Rf. Inhibition of COX-2 expression by ginsenoside Rf was performed with the PPARγ-specific inhibitor, T0070907. The PPARγ inhibitor also blocked the ability of ginsenoside Rf to suppress cell migration under hypoxia. The docking simulation results indicate that ginsenoside Rf binds to the active site of PPARγ.

Conclusions: Our results demonstrate that ginsenoside Rf inhibits hypoxia induced-COX-2 expression and cellular migration, which are dependent on PPARγ activation. These results suggest that ginsenoside Rf has an antiinflammatory effect under hypoxic conditions. Moreover, docking analysis of ginsenoside Rf into the active site of PPARγ suggests that the compound binds to PPARγ in a position similar to that of known agonists.

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1. Introduction

The Panax genus of the Araliaceae family, commonly known as ginseng, is a representative medicinal herb in Asian countries that has been used for more than 2,000 years. Its recent use is not limited to Asia but has been extended to the market of Western countries and is estimated to be worth $2.084 million including ginseng root and its processed products [1]. The global popularity of ginseng consumption indirectly supports the efficacy of ginseng and has scientifically proven its pharmacology [1,2]. In particular, ginseng reduces inflammation, and both in vitro and in vivo studies have shown that ginseng has antiinflammatory and anticancer effects [3–7].

Saponins, known as ginsenosides, are widely regarded as the most highly bioactive compounds in ginseng [8]. Single or crude mixtures of saponins have been considered to be responsible for most of the pharmacological effects of ginseng over the years [9]. Based on their chemical structure, ginsenosides are divided into two groups: protopanaxatriol and protopanaxadiol. The protopanaxatriol group includes ginsenosides Rg1, Re, Rf, Rh1, and Rg2.
Ginsenoside Rf is a steroid-like compound linked to sugars. The content of ginsenoside Rf was 0.54 ± 0.26 mg/g (Rg1 contains 2.01 ± 0.65 mg/g) in fresh ginseng and 0.78 ± 0.25 mg/g (Rg1 contains 3.34 ± 0.98 mg/g) in red ginseng [10]. The content of ginsenoside Rf in Panax ginseng is shown as follows: roots, 1.8 ± 0.1 mg/g; stems and leaves, 0.3 ± 0.1 mg/g; and fruits, 3.6 ± 1.1 mg/g [11]. Ginsenoside Rf is a ginseng saponin that is only present in Panax ginseng [1]. Although the concentration of ginsenoside Rf is low, it is an important regulator of lipid metabolism with additional neuroprotective, antinociceptive, and antiinflammatory properties [12–15].

Hypoxia refers to an overall reduction in tissue oxygen, a characteristic of solid tumors, and causes cell metastasis and invasion [16]. The production of cyclooxygenase-2 (COX-2) is increased by diverse factors, such as hypertonicity, lipopolysaccharide, cytokines, and hypoxia [17–20]. The upregulation of COX-2 increases the risk of metastasis of cancer cells, and the suppression of COX-2 decreases tumor formation and metastasis [21,22]. Mammary epithelial cells contain peroxisome proliferator-activated receptor gamma (PPARγ), which is important for the formation of breast tumors and is associated with COX-2 [23]. According to these observations, COX-2 inhibition is important to prevent the invasion of cancer cells induced by hypoxia.

PPARγ is a key regulator of adipocytes and is primarily present in adipose tissue. PPARγ is also involved in regulating inflammation by controlling COX-2 expression using the PPAR response element within the COX-2 promoter [24–26]. However, the effects of PPARγ may vary depending on the cell type, and some studies have indicated that PPARγ activates or inhibits COX-2 via PPARα-dependent or -independent mechanisms [27–29].

We reported previously that Korean Red Ginseng (KRG) suppressed COX-2 expression under hypoxia via PPARγ [30]. In this study, we aimed to determine whether ginsenoside Rf is the active constituent of KRG leading to the inhibition of hypoxia-induced COX-2 expression via PPARγ.

2. Materials and methods

2.1. Materials

Ginsenoside Rf was supplied by the Korea Ginseng Cooperation (Daejeon, Korea). 17-β-estradiol, dihydrotestosterone, and bicalutamide were purchased from Sigma (St. Louis, MO, USA), T0070907 was purchased from Selleckchem (Houston, TX, USA), ICI 182,780 (ICI) was purchased from ZENeca Pharmaceutical (Tocris, UK). Fetal bovine serum and penicillin/streptomycin were purchased from Gibco Invitrogen (Grand Island, NY, USA). CCK-8 was bought from Enzo (Enzo LifeSciences, Lausen, Switzerland). Anti-COX-2 was used from Cayman Chemical (Certara Inc., Princeton, NJ, USA) using default parameters.
2.9. Statistical analysis

All data were analyzed and expressed as means and standard deviations. The two-tailed, unpaired Student t test was applied using SPSS software (version 23.0; IBM, Armonk, NY, USA).

3. Results

3.1. Ginsenoside Rf induces PPARγ transcriptional activity and its protein expression in A549 cells

We previously showed that KRG inhibited hypoxia-induced COX-2 activation via PPARγ [32]. In search of the active constituent of KRG that inhibits hypoxia-induced COX-2, ginsenoside Rf was chosen because previous studies suggested that ginsenoside Rf may modulate PPARγ [36]. To assess the effects of ginsenoside Rf on hypoxia-induced COX-2 activation, A549 cells were pretreated with ginsenoside Rf for 1 h and exposed to hypoxic conditions for 24 h. Ginsenoside Rf increased the protein levels of SIRT-1 and PPARγ compared with hypoxia (Fig. 1A). At the same time, 10 μM of ginsenoside Rf efficiently blocked the upregulation of COX-2 transcriptional activity and protein level under hypoxia (Fig. 1A and B).

Next, we wanted to examine whether ginsenoside Rf activated PPARγ luciferase reporter activity (Fig. 1C). Ginsenoside Rf significantly activated PPRE luciferase reporter activity. Ginsenoside Rf–induced PPRE luciferase gene activation was blocked by treatment with the PPARγ antagonist, T0070907, indicating that ginsenoside Rf-induced PPRE luciferase gene activation is PPARγ-specific. Cell viability was not affected at concentrations of 1–10 μM ginsenoside Rf for 24 h under normoxic or hypoxic conditions (Fig. 1D).

3.2. Ginsenoside Rf inhibits hypoxia-induced COX-2 protein expression and COX-2 transcriptional activity through PPARγ in A549 cells

To determine whether PPRE activation is related to COX-2 inhibition by ginsenoside Rf, COX-2 protein levels were measured using T0070907 (Fig. 2A). The suppression of hypoxia-induced COX-2 protein expression by ginsenoside Rf was blocked by T0070907, indicating that the response involves PPARγ. To further identify the involvement of PPARγ activation, COX-2 promoter activity was measured following treatment with T0070907 (Fig. 2B). These results suggest that the suppression of hypoxia-stimulated COX-2 by ginsenoside Rf in A549 cells is dependent on PPARγ.

3.3. Ginsenoside Rf repress hypoxia-induced cellular migration in A549 cells

To confirm whether ginsenoside Rf inhibits cellular migration via PPARγ, migration capability was examined using T0070907 in A549 cells. As shown in Fig. 3, ginsenoside Rf inhibited cellular migration under hypoxia. The inhibition of hypoxia-induced cellular migration by ginsenoside Rf was significantly blocked by...
T0070907. These results suggest that ginsenoside Rf inhibits hypoxia-induced cellular migration via PPARγ.

3.4. Ginsenoside Rf has no estrogen receptor or AR transcriptional activity

To determine whether ginsenoside Rf induces other nuclear hormone receptors, estrogen receptor (ER) and AR transcriptional activities were examined. As shown in Fig. 4A, 17-β-estradiol, as a positive control, induced ERE-transcriptional activity at a concentration of 10 nM in MCF-7 cells. However, ginsenoside Rf did not induce ER transcriptional activity. We also analyzed the anti-ERE transcriptional activity of ginsenoside Rf (Fig. 4B). Ginsenoside Rf did not exhibit anti-ER transcriptional activity. Moreover, ginsenoside Rf was examined for ARE transcriptional activity (Fig. 4C). Ginsenoside Rf did not show AR transcriptional activity. Lastly, we...
checked for anti-ARE transcriptional activity of ginsenoside Rf (Fig. 4D). Ginsenoside Rf did not exhibit anti-AR transcriptional activity. These results suggest that ginsenoside Rf specifically activates PPARγ, not ER or AR.

3.5. Docking modeling: ginsenoside Rf fits into the agonist binding site of PPARγ

To investigate whether ginsenoside Rf directly interacts with PPARγ, a molecular docking simulation of the interaction between ginsenoside Rf and PPARγ was performed (Fig. 5). For comparison, the binding pose of ginsenoside Rf was superimposed over the X-ray pose of BPR1H036, a known indole-based PPARγ agonist (Fig. 5B). The overall binding mode of ginsenoside Rf predicted by Surflex-Dock was similar to that of BPR1H036. In the X-ray structure, the carboxylate group in the polar head of BPR1H036 formed a hydrogen bond network with key residues of PPARγ, including SER289, HIS323, HIS449, and TYR473. The glucopyranoside group of ginsenoside Rf also occupied this site, forming two hydrogen bonds with SER289 and HIS449. This hydrogen bond pattern of the acidic polar head group is conserved in most PPARγ agonists, which is believed to be essential for the activity of the ligand. Moreover, the tetracyclic skeleton of ginsenoside Rf exhibited strong hydrophobic interactions with PPARγ, playing an important role in the binding of ginsenoside Rf to the protein. These results indicate that ginsenoside Rf may directly interact with PPARγ; however, further studies are required to confirm the binding mode of ginsenoside Rf to PPARγ.

4. Discussion

Many studies have shown that KRG has antioxidant and anti-inflammatory effects both in vitro and in vivo [37,38]. We previously demonstrated that KRG inhibits hypoxia-induced COX-2 activation.
via PPARγ [30]. In search of the active constituent of KRG that inhibits hypoxia-induced COX-2, we found that ginsenoside Rf activates PPARγ and suppresses hypoxia-induced COX-2 via PPARγ. PPARγ is a member of the nuclear receptor superfamily and is a ligand-dependent transcription factor [39]. PPARγ regulates glucose metabolism, fatty acid storage, and adipocyte differentiation and is a target of antidiabetic drugs [40,41]. Several studies have shown that the ginsenosides Rg3 and Rh2 and compound K inhibit adipogenesis via the regulation of PPARγ and CCAAT/enhancer-binding protein alpha expression in 3T3-L1 cells, human primary preadipocytes, and mice [42]. Indeed, it has been suggested that ginsenoside Rf binds directly to the active site of PPARγ [36]. However, these ginsenosides downregulate PPARγ and perilipin protein expression in 3T3-L1 cells, indicating an antiadipogenic effect [42]. It was recently recognized that PPARγ plays a fundamental role in the immune response through its ability to inhibit the expression of inflammatory cytokines and induce the differentiation of immune cells against antiinflammatory phenotypes [24]. Hesperetin, a flavonone from the fruit peel of Citrus aurantium L, exhibits strong antiinflammatory effects through the upregulation of PPARγ [43]. Moreover, fenretinide, a synthetic ligand of PPARγ, induces antiinflammatory activity, although its exact mechanism is not fully understood [44]. Our data are consistent with previous results demonstrating that ginsenoside RF modulates PPARγ and appears to have distinct functions, such as antiadipogenesis and antiinflammation, according to the cell type. In our study, ginsenoside RF specifically inhibited COX-2 expression via PPARγ activation under hypoxic conditions. The COX-2 suppression we observed may be because of the sum of the activities of each component and their interactions, as ginsenosides Rb1 and Rg1 elicited some response, albeit weaker than that of ginsenoside RF. Moreover, the inhibition of COX-2 expression and cellular migration under hypoxia were dependent on PPARγ activation. The docking simulation results indicated that ginsenoside RF binds to the active site of PPARγ; however, further studies are required to confirm the binding mode.

These findings provide a mechanical explanation for the effects of ginsenoside RF on metabolic disorders and cancer.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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References

[1] Chan TW, But PP, Cheng SW, Kwok IM, Lau FW, Xu HX. Differentiation and authentication of Panax ginseng, Panax quinquefolius, and ginseng products by using HPLC/MS. Anal Chem 2000;72:1261–7.
[2] Bhattacharya SK, Mitra SK. Anxiolytic activity of Panax ginseng roots: an experimental study. J Ethnopharmacol 1991;34:87–92.
[3] Jin Y, Kotakadi VS, Ying L, Hofseth AL, Cui X, Wood PA, Windust A, Matesic LE, Pena EA, Chiuza C, et al. American ginseng suppresses inflammation and DNA damage associated with mouse colitis. Carcinogenesis 2008;29:2351–9.
[4] Park JS, Shin JA, Jung JS, Hyun JW, Van Le TK, Kim DH, Park EM, Kim HS. Antiinflammatory mechanism of compound K in activated microglia and its neuroprotective effect on experimental stroke in mice. J Pharmacol Exp Ther 2012;341:59–67.
[5] Yang Y, Yang WS, Yu T, Sung CH, Park KW, Yoon K, Son YJ, Hwang H, Kwak YS, Lee CM, et al. ATF-2/CREB/IRF-3-targeted anti-inflammatory activity of Korean red ginseng water extract. J Ethnopharmacol 2014;154:218–28.
[6] Kim HJ, Kim P, Shin CY. A comprehensive review of the therapeutic and pharmacological effects of ginseng and ginsenosides in central nervous system. J Ginseng Res 2013;37:8–29.
Kaidi A, Qualtrough D, Williams AC, Paraskeva C. Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. Cancer Res 2006;66:683–91.

Lee JW, Choi BR, Kim YC, Choi DJ, Lee YS, Kim GS, Baek NI, Kim SY, Lee DY. Comprehensive profiling and quantification of ginsenosides in the root, stem, leaf, and berry of Panax ginseng by UPLC-QTOF/MS. Molecules 2017;22(12):E2147.

Ahn S, Siddiqi MH, Aceituno VC, Simu SY, Yang DC. Suppression of MAPKs/NF-kappaB activation induces intestinal anti-inflammatory action of Ginsenoside Rf in HT-29 and RAW264.7 cells. Inmunol Invest 2016;45:439–49.

Choi K, Kim M, Ryu J, Choi C. Ginsenosides compound K and Rh(2) inhibit tumor necrosis factor-alpha-induced activation of the NF-kappaB and JNK pathways in human astroglial cells. Neurosci Lett 2007;421:37–41.

Lee H, Gonzalez HJ, Yoon M. Ginsenoside Rf, a component of ginseng, regulates lipoprotein metabolism through peroxisome proliferator-activated receptor alpha. Biochem Biophys Res Commun 2006;339:196–203.

Li Y, Wang Q, Yao XM, Li Y. Induction of COX-2 expression in A549 airway epithelial cells by baicalin, baicalein, chlorogenic acid, and ginsenoside Rf through constitutive androstane receptor- and pregnane X receptor-mediated pathways. Eur J Pharmacol 2010;640:46–54.

Arsenault D, Brochu-Gaudreau K, Charbonneau M, Dubois CM. HDAC6 deacetylase activity is Required for hypoxia-invadopodia formation and cell invasion. Plos One 2013;8:

Fredensburgh LE, Ma J, Perrella MA. Cyclooxygenase-2 inhibition and hypoxia-induced pulmonary hypertension: effects on pulmonary vascular remodeling and contractility. Trends Cardiovasc Med 2009;19:31–7.

Kadi A, Qualtrough D, Williams AC, Parasekava C. Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. Cancer Res 2006;66:683–91.

Lee JJ, Natsuizaka M, Ohashi S, Wong GS, Takaoka M, Michaylira CZ, Budo D, Tohbi JS, Kanai M, Shirakawa Y, et al. Hypoxia activates the cyclooxygenase-2/2-prostaglandin E synthase axis. Carcinogenesis 2010;31:427–34.

Zhao L, Wu Y, Xu Z, Wang H, Zhao Z, Li Y, Yang P, Wei X. Involvement of COX-2/PGE2 signalling in hypoxia-induced angiogenic response in endothelial cells. J Cell Mol Med 2012;16:1840–55.

Stasinopoulos I, Shah T, Penet MF, Krishnamachary M, Bhujwalla ZM. COX-2 in cancer: Gordian knot or Achilles heel? Front Pharmacol 2013;4:34.

Tsuij M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A 1997;94:3336–40.

Apostoli AJ, Roche JM, Schneider MM, SenGupta SK, Di Lena MA, Rubino RE, Peterson RT, Nicol CJ. Opposing roles for mammmary epithelial-specific PPAR-gamma signalling and activation during breast tumour progression. Mol Cancer 2015;14:85.

Tyagi S, Gupta P, Saini AS, Kaulash C, Sharma S. The peroxisome proliferator-activated receptor: a family of nuclear receptors role in various diseases. J Adv Pharm Technol Res 2011;2:236–40.

Harza S, Dubinett SM. Ciglitazone mediates COX-2 dependent suppression of PGE2 in human non-small cell lung cancer cells. Prostaglandins Leukot Essent Fatty Acids 2007;77:51–8.

Park J, Shim MK, Jin M, Rhyu MR, Lee Y, Yet RK. Ginsenoside Rf with PPARgamma major transcriptional factor of adipogenesis. Bull Korean Chem Soc 2011;32:201–7.

Lee JW, Choi BR, Kim YC, Hong YC, Kim HJ, Lee JY, Bisphephenal Q, a component of ginseng, regulates COX-2 through the mitogen-activated protein kinase pathway and is associated with levels of inflammation-related markers in elderly populations. Environ Res 2017;158:490–8.

Mehnistroo N, Huang CF, Peng YH, Wang CC, Liao CC, Liw W, Chittimalka SL, Huang WJ, Chai CH, Prakash E, et al. Novel insulin-based peroxisome proliferator-activated receptor gamma agonists: design, SAR, structural biology, and biological activities. J Med Chem 2005;48:1894–208.

Sohn YS, Lee Y, Park C, Hwang S, Kim S, Kim B, Son M, Suh JK, Kim KH, Lee KW. Pharmacophore identification for peroxisome proliferator-activated Receptor Gamma agonists. Bull Korean Chem Soc 2011;32:201–7.

Siraj FM, Natarajan S, Hugh MA, Kim JY, Yang DC. Structural investigation of ginsenoside Rf with PPARgamma major transcriptional factor of adipogenesis and its impact on adipocyte. J Ginseng Res 2015;39:141–7.

Kim EH, Kim HJ, Lee MJ, Thach Nguyen C, Ha JA, Lee SC, Choi S, Choi KT, Pyo S, Rhee DK. Anti-inflammatory effect of red ginseng in the brain is mediated by peptidyl arginine deiminase type IV (PADI4) repression via estrogen receptor (ER) epsilon and repression of COX-2/PGE2 signalling in hypoxia-induced angiogenic response in endothelial cells. J Cell Mol Med 2012;16:1840–55.

Li M, Pascual G, Glass CK. Peroxisome proliferator-activated receptor gamma dependence of the inducible nitric oxide synthase gene. Mol Cell Bio 2000;20:4699–707.

Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WW, Wilson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 1995;270:12953–6.

Siersbaek R, Nielsen R, Mandrup S. PPARgamma in adipocyte differentiation and metabolism–novel insights from genome-wide studies. FEBS Lett 2010;584:3142–9.

Zhang L, Virgou S, C H Ginseng and obesity: observations and understanding in cultured cells, animals and humans. J Nutr Biochem 2017;44:1–10.

Chen X, Ding HW, Li HD, Huang HM, Li XF, Yang Y, Zhang YL, Pan XY, Huang C, Meng XM, et al. Hesperetin derivative-14 alleviates inflammation by activating PPAR-gamma in mice with CCI4-induced acute liver injury and LPS-treated RAW264.7 cells. Toxicol Lett 2017;274:51–63.

Lin CH, Lee SY, Zhang CC, Du YF, Hung HC, Wu HT, Ou HY. Fenretinide inhibits macrophage inflammatory mediators and controls hypertension in spontaneously hypertensive rats via the peroxisome proliferator-activated receptor gamma pathway. Drug Des Devel Ther 2016;10:3591–7.