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Ginsenoside Rg1 exerts synergistic anti-inflammatory effects with low doses of glucocorticoids in vitro

Yanqin Song [1], Feng Zhao [1], Leiming Zhang, Yuan Du, Tian Wang, Fenghua Fu *

Key Laboratory of Molecular Pharmacology and Drug Evaluation, Ministry of Education of China, School of Pharmacy, Yantai University, Yantai 264005, PR China

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ABSTRACT

Glucocorticoids (GCs) are usually used to treat inflammatory diseases. However, they cause severe and irreversible side effects, which limit the use of these compounds. Ginsenoside Rg1 had been demonstrated to possess anti-inflammatory and anti-cancer effects. The present study was designed to investigate whether Rg1 exhibits synergistic anti-inflammatory effects when combined with glucocorticoids. After stimulated by lipopolysaccharide (LPS), murine macrophagic RAW264.7 cells were treated with Rg1, corticosterone (Cort) or Rg1 and Cort. Then nitric oxide (NO), tumor necrosis factor-α (TNF-α) and glucocorticoid receptor (GR) expression were measured. The results showed that Rg1 or Cort could reduce the production of NO and TNF-α, and Rg1 dose-dependently up-regulated GR expression, while Cort dose-dependently down-regulated GR expression. The combination of low concentrations of Rg1 with Cort, which alone could not markedly inhibit the release of inflammatory factors, inhibited the secretion of NO and TNF-α in LPS-stimulated RAW264.7 macrophage cells, and up-regulated the expression of GR. The findings suggested Rg1 can synergize with glucocorticoid to enhance its anti-inflammatory effect.

1. Introduction

Glucocorticoids (GCs) are the most potently used anti-inflammatory and immunosuppressive drugs presently. The anti-inflammatory and immunosuppressive effects of GCs are mediated predominantly by glucocorticoid receptor (GR), a member of the nuclear receptor superfamily. Unfortunately, long-term and/or high-dose glucocorticoid administration is commonly associated with side effects, such as hypertension, disorders of glucose and lipid metabolism, osteoporosis, and especially femoral head necrosis [1–3]. One of the methods of minimizing the undesirable side effects of corticotherapy is through their association with other pharmaceuticals, especially with other more specific anti-inflammatories or immunosupressors, aiming at synergistic effects in order to avoid the use of glucocorticoids or to reduce the dosage and duration of corticotherapy [4].

Ginseng is a traditional Chinese medicine which consists of the dried roots of Asiatic ginseng, *Panax ginseng* C.A. Meyer. [5], and has been used for more than 1000 years. It is now one of the most extensively used alternative medicines in the treatment of inflammation. Ginsenosides (GSS), the major active components of ginseng, which are triterpene saponins that have a rigid steroidal skeleton with sugar moieties and produce multiple pharmacological responses [6]. Based on their chemical structure, GSS are generally divided into 2 groups: protopanaxadiols (PD) and protopanaxatriols (PT). The sugar moieties in the PT group are attached to a 6-position

* Corresponding author at: Department of Pharmacology, School of Pharmacy, Yantai University, 30 Qingquan Road, Yantai, Shandong 264005, PR China.
E-mail address: fufenghua@sohu.com (F. Fu).
[1] Contributed equally.

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of GSS including Re, Rf and Rg1, etc. However the sugar moieties of 6-position are not the same. Each ginsenoside may have different effects in pharmacology and mechanisms due to their different chemical structures. As to the anti-inflammatory effects of GSS, it has been showed that ginsenoside Rd could inhibit the expressions of iNOS and COX-2 by suppressing NF-κB, ginsenoside Rb1 and its metabolite compound K inhibit IRAK-1 activation—the key step of inflammation [7,8]. Many studies reported that Rg1 could inhibit LPS-induced cytokine production in vitro [9–13]. Furthermore, Rg1 showed the glucocorticoid-like effects, and to be a functional ligand of GR [14]. But it was not clear whether there is an interaction between Rg1 and glucocorticoids.

In this study, the aim was to investigate whether the ginsenoside Rg1 could exert synergistic anti-inflammatory effects with low concentrations of glucocorticoids in vitro and the mechanism of the synergistic effect.

2. Materials and methods

2.1. Reagents

Lipopolysaccharide (LPS), DMSO, MTT, and corticosterone (Cort) were obtained from Sigma-Aldrich. Rg1 (Fig. 1) was purchased from Yantai Science & Biotechnology Co., Ltd. (HPLC ≥ 98%). Rabbit polyclonal antibody to GR and β-actin antibody were purchased from Santa Cruz Biotechnology, Inc.

RPMI 1640 medium and fetal bovine serum were purchased from Invitrogen. Penicillin–streptomycin stock solution, mouse TNF-α ELISA kit, and Bradford protein assay kit were purchased from Yantai Science & Biotechnology Co., Ltd.

2.2. Cell culture

The mouse monocyte-macrophage cell line RAW 264.7 was purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Cells were kept at 37 °C with 5% CO_2 in a fully humid atmosphere. The medium was routinely changed every day.

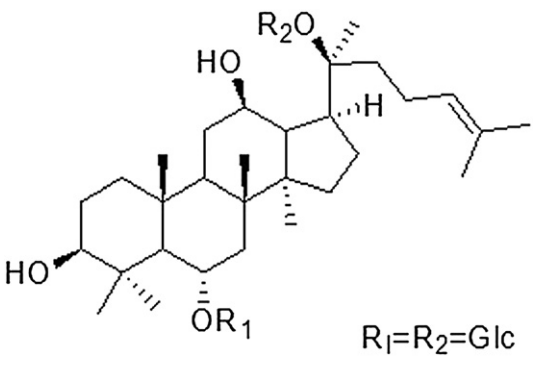

Fig. 1. The structure of ginsenoside Rg1.

2.3. Cell viability assay

Cells were treated with Re, Rg1 or Cort at the concentrations of 1–100 μM, respectively. The control group received an equal amount of DMSO, which resulted in a final concentration of 0.2% DMSO in the culture medium. The mitochondrial-dependent reduction of 3-(4,5-dimethylthizaol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan was used to measure cell respiration as an indicator of cell viability [15]. Briefly, after 24 h incubation, an MTT solution (final concentration is 200 μg/ml) was added, and the cells were incubated for another 4 h at 37 °C. After removing the supernatant, 150 μl of DMSO were added to the cells to dissolve the formazan. The absorbance of each group was measured by using a microplate reader at a wavelength of 570 nm. The control group, consisting of untreated cells, was considered as having 100% of viable cells. Results are expressed as percentage of viable cells when compared with the control group.

2.4. Analysis of NO

RAW 264.7 cells were treated by LPS (1 μg/ml) with or without drugs (1–25 μM) for 24 h. The concentration of nitrite in the cell culture supernatant corresponds to the production of NO and was measured using the Griess reagent. Briefly, 100 μl supernatant of medium was mixed with an equal volume of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride). The absorbance at 540 nm was measured, and concentrations of nitrite were calculated in accordance to the standard curve obtained from sodium nitrite [16].

2.5. Measurement of TNF-α

Cells were treated by LPS (1 μg/ml) with or without drugs (10–50 μM) for 6 h. 100 μl of the culture supernatant were taken out to determine the level of TNF-α using ELISA assay kits according to the manufacturer's instructions. The ELISA data representing mean values ± SD were obtained in duplicate from at least three independent experiments.

2.6. Western blot of GR

After the treatment with LPS (1 μg/ml) and respective drugs (0.1–100 μM) for 24 h, RAW 264.7 cells were washed with cold PBS and lysed in ice-cold lysis buffer (Beyotime, China) plus 1:100 volume of phenylmethylsulfonyl fluoride (PMSF). Cell debris was removed after centrifugation (12,000 *g*, 4 °C, 10 min). After the protein concentration for each aliquot was determined by the Bradford method, suspensions were boiled in SDS-PAGE loading buffer. 30 μg of the proteins obtained were separated using 10% SDS-PAGE gels. Blots were transferred to nitrocellulose membranes and incubated in blocking buffer (5% nonfat dried milk in Tris-buffered saline with Tween 20) at room temperature for 2 h, and then incubated with rabbit polyclonal antibody against GR in diluent buffer (Beyotime, China) overnight at 4 °C (1:1000 dilution) and anti-β-actin antibody respectively. The membranes were washed with TBS T and incubated with HRP-conjugated secondary antibody solution for 1 h at room temperature. The blots were washed thrice in TBS T