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Research paper

6-O-Veratroyl catalpol suppresses pro-inflammatory cytokines via regulation of extracellular signal-regulated kinase and nuclear factor-κB in human monocytic cells

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ABSTRACT

The compound *6-O-veratroyl catalpol* (*6-O*) is a bioactive iridoid glucoside that was originally isolated from *Pseudolysimachion rotundum* var. subintegrum. It has been demonstrated that catapol derivative iridoid glucosides including *6-O*, possess anti-inflammatory activity in carragenan-induced paw edema mouse model as well as bronchoalveolar lavage fluid of ovalbumin-induced mouse model. In the present study, we investigated whether *6-O* modulates inflammatory responses in THP-1 monocytic cells stimulated with phorbol12-myristate-13-acetate (PMA). Our data showed that *6-O* inhibited PMA induced interleukin (IL)-1β and tumor necrosis factor (TNF)-α expression in THP-1 monocytic cells. Mechanistic studies revealed that *6-O* suppressed the activity of protein kinase C (PKC), which further resulted in downstream inactivation of extracellular signal-regulated kinase (ERK) and nuclear factor-κB (NF-κB) inflammatory pathway. The results implied that *6-O* may protect against inflammatory responses that could be a potential compound in treating inflammatory diseases.

1. Introduction

Inflammation is a body normal defense induced by pathogens and toxic stimuli such as physical injury or chemicals [1,2]. Acute inflammation is an immediate response that usually results in healing while chronic inflammation is a long-term medical condition that is involved in active inflammation. Such persistent inflammation may lead to severe diseases such as allergy, cancer, arthritis, and autoimmune diseases. Although inflammatory responses exert in different diseases, the most important group controlling these phenomenon likely be inflammatory cytokines and mediators [3]. Protein kinase C (PKC) family members, consisting of 12 phospholipid-dependent serine/threonine kinases, have been demonstrated involving in cell migration and proliferation [4,5]. The tumor promoter phorbol-12-myristate-13-acetate (PMA) has been shown to induce the enzyme protein kinase C (PKC) as PMA can substitute for diacylglycerol (DAG) in promoting classical and non-conventional PKC isoforms [6]. It has been reported that PKCs are able to activate the mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) pathways which finally leads to the release of pro-inflammatory cytokines or mediators [7–10]. Therefore, PMA-induced inflammation is implicated in releasing of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, chemokine (C–C motif) ligand 5 (CCL5), and other mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2 [11–14]. Thus, in this study, applying PMA-stimulated human monocytic leukemia cell line THP-1, we investigated the signaling pathway activated by PKC and the anti-inflammatory effects of *6-O-veratroyl catalpol* (*6-O*).

Pseudolysimachion genus plant, and the catalpol derivatives isolated therefrom, possesses anti-inflammatory, antiallergic and anti-asthmatic activity. According to that, we isolated *6-O* from *Pseudolysimachion* genus plant to figure out its biological effect. 6-O ($C_{24}H_{30}O_{13}$) with molecular formula as described in Fig. 1A has been

Abbreviations: 6-O, 6-O-veratroyl catalpol; IL-1, Interleukin-1; ERK, Extracellular signal-regulated protein kinase; NF-κB, Nuclear Factor-κB; PMA, phorbol12-myristate-13-acetate; PKC, protein kinase C.

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previously mentioned as a bioactive compound for various inflammatory disorders. Studies have suggested that *6-O* shows inhibitory effect on elevated IgE, IL-4 and IL-13 levels and eosinophilia in the plasma and bronchoalveolar lavage fluid (BALF), and mucus overproduction in the lung tissues in an ovalbumin (OVA)-induced asthmatic mouse model [15]. However, the underlying mechanism of *6-O* on anti-inflammation is not well understood. In this study, we isolated *6-O* from *Pseudolysimachion rotundum* var. subintegrum, and investigated its inhibitory mechanism on the inflammatory reaction in PMA-stimulated THP-1 cells.

2. Materials and methods

2.1. Cell culture

We obtained the human THP-1 monocyte and A549 cell line from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in Roswell Park Memorial Institute (RPMI) medium (Welgene Incorporation, Daegu, Korea) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT, USA). The cells were incubated under an atmosphere of 5% CO_2 at 37 °C and were subjected to a maximum of 20 cell passages.

2.2. Reagents

The dried stems and leaves of *Pseudolysimachion rotundum* were washed with sterile water and then 2.0 kg was extracted with 200 mL of methanol (MeOH) at 30 °C for 3 days. The solvent was evaporated under reduced pressure to yield the crude extracts (198.7 g), which were used in determining the biological activities and in the ultra-performance liquid chromatography (UPLC) analysis.

2.3. MTS assay

Cell viability was quantified using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent. The MTS assay is a colorimetric assay that measures the activity of mitochondrial reductases that reduce tetrazolium to formazan [16]. The THP-1 cells (1×10^4 cells/well) were seeded in 100 μL of medium in 96-well plates and then treated with *6-O* at concentrations of 5–50 μM for 24 h with 10 nM PMA. The effect of *6-O* on cell viability was assessed using the Cell Titer 96 Aqueous One Solution Assay (Promega) containing MTS and phenazine methosulfate (PMS), an electron coupling reagent. An aliquot of 20 μL Aqueous One Solution reagent was added to each well, and the cells were incubated for another 1 h. The absorbance was measured at 492 nm using a microplate reader (Apollo LB 9110, Berthold Technologies GmbH, Bad Wildbad, Germany). The percentage of viable cells was estimated relative to the untreated controls. The viability assay was repeated thrice.

2.4. RNA extraction and mRNA expression analysis

The THP-1 cells (3×10^6 cells/well) were seeded in 6-well-plates and treated with *6-O* (1.25–5 μM) followed by PMA (10 nM) for 24 h. The treated cells were collected and lysed using the easy-BLUE

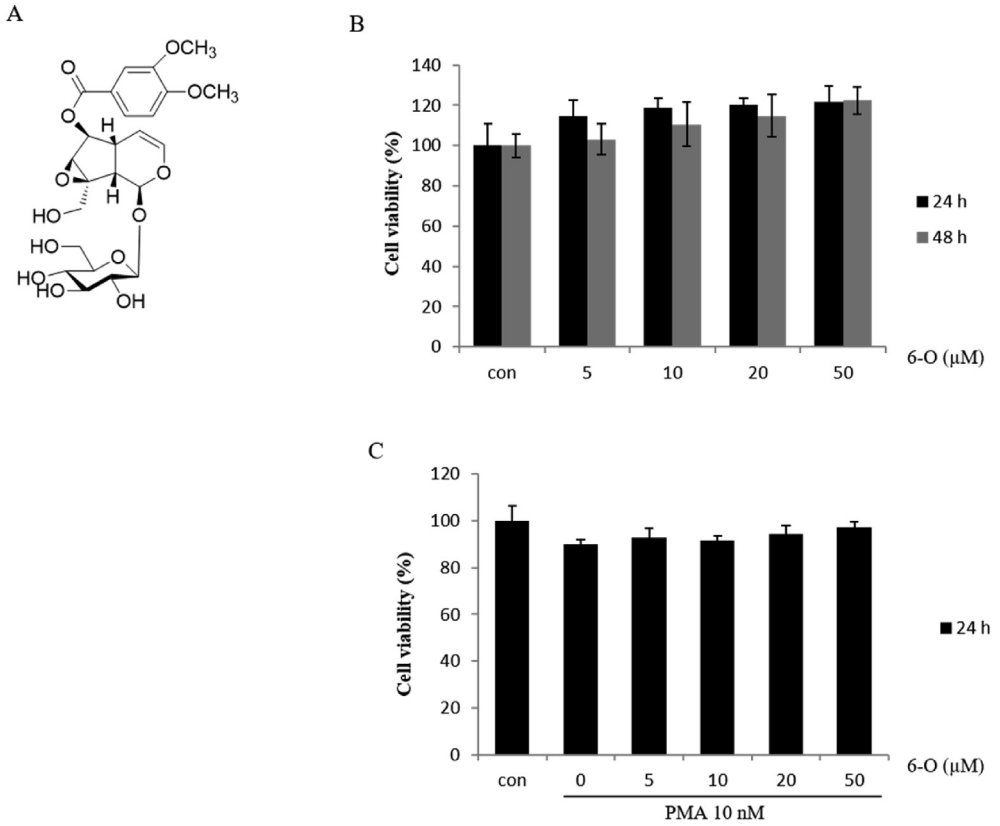

Fig. 1. Cytotoxic effect of *6-O*-veratroyl catalpol (*6-O*) in THP-1 cells. (A) The chemical structure of *6-O*. (B) Effect of *6-O* on the viability of THP-1 monocytic cells. THP-1 cells were incubated with various concentrations of *6-O* (5–50 μM) for 24 h and 48 h. Cell viability was measured using the MTS assay. (C) Effect of *6-O* on the viability of THP-1 macrophages. THP-1 cells were incubated with various concentrations of *6-O* (5–50 μM) and then stimulated with PMA (10 nM) for 24 h. Cell viability was measured using the MTS assay. Data are presented as three independent experiments and reported as the mean ± SD.