



Research Article

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Modulation effect of diarylheptanoids on interleukin 1 β -related immune responses of bone-marrow macrophage

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ABSTRACT

Pro-inflammatory cytokine IL-1 β is produced as inactive precursor form in inflammatory process, it needs to be cleaved to active form, and mediates acute inflammatory responses. The inflammasome is cytosolic protein complex regulating maturation of pro-IL-1 β and IL-18 peptide, and composed of a NALP that acts as a sensor for danger signal and ASC, which recruits caspase-1 in the complex. For contact dermatitis in the skin, sensitizing allergen promotes activation of inflammasome and causes delayed-type hypersensitivity. As selectively blocking of inflammasome activity or IL-1 β secretion may be useful to control contact dermatitis or related diseases, here we investigated suppressive activities of Oregonine and Hirsutenone purified from *Alnustinctoria Sarg.* on IL-1 β secretion. Results of this study shows that Oregonine and Hirsutenone are effectively suppressed IL-1 β secretion; ED50 of Hirsutenone is lower than that of Oregonine. These results suggest that Hirsutenone and Oregonine are nice candidates for immune disease including atopic dermatitis and psoriasis.

Keywords: *Alnustinctoria*, Oregonin, Hirsutenone, Interleukin 1 β

INTRODUCTION

Interleukin-1 is a typical multifunctional cytokine and a general name for two distinct cytokines, IL-1 α and IL-1 β . Along with IL-1 receptor antagonist (IL-1R-a), IL-1 α and IL-1 β play an important role in up- and down-regulation of acute inflammation. The precursor of IL-1 β is 269 amino acids length protein having a MW of 31kDa and is cleaved into a 116 amino acids length pro-segment and a 153-amino acids length, 17kDa mature segment. The cleavage of the precursor of IL-1 β is a complex process and is suggested to occur through the action of 45kDa cysteine protease named IL-1 β -converting enzyme (ICE). After the pro-segment is cleaved, the 17kDa mature segment is released. The process of activating IL-1 β precursor includes the cleavage by the action of NALP protein (NACHT, LRR and Pyrin domain containing protein), ASC (apoptosis-associated speck-like protein containing a CARD domain) and Caspase-1 [1,2]. It is well known that IL-1 β plays a critical role in the onset of immunological disease and dermatitis including atopic dermatitis and contact dermatitis. It is reported that in the ASC deficient mouse, the incidence of contact dermatitis has been remarkably reduced. The released IL-1 β stimulates the biosynthesis of inflammatory mediators such as various chemokines, prostaglandin and inducible nitric oxide (iNOS)[3], and thus induces early stage of an inflammatory response. IL-1 β along with TNF- α is generally acknowledged to be a target for the treatment of inflammatory disease.

Alnustinctoria Sarg. is a member of the Family Betulaceae, and is colloquially referred to as *Jeok Yang* among practitioners of oriental medicine, who believe that it “clears body heat” and “reduces body heat”[4]. Recently, we isolated several diarylheptanoids such as oregonin and hirsutenone contained mainly in the *Alnus* genus [5]. In our previous study, diarylheptanoid effectively scavenged free radicals [6], inhibited production of nitric oxide and prostaglandin E₂ synthesis [7], downregulated cyclooxygenase-2 expression[8], suppressed the synthesis of oxygen radicals, reactive nitrogen species (RNS) and multiple pro-inflammatory molecules and also reduced elevations in

calcium level [9] *in vitro*. In addition, diarylheptanoid reduced the atopic dermatitis-like state in NC/Nga mice *in vivo* [10,11].

This paper describes a biological evaluation of diarylheptanoid from the stems *Alnustinctoria* Sarg. on interleukin-1 β level for the purpose of developing new anti-inflammatory and immunologic skin disease agents.

EXPERIMENTAL SECTION

2.1. Phytochemical

2.1.1. Extraction and isolation of Oregonin(ORE)

Fresh, chopped stems (350 g) of *Alnustinctoria* Sarg. were extracted using 80% aqueous MeOH at room temperature for 3 days. The filtrate was concentrated and applied to a Sephadex LH-20 column (10-25 μ m, GE Healthcare Bio-Science AB, Uppsala, Sweden) containing increasing proportions of MeOH (30~100%) afforded 4 fractions (1-4). Repeated column chromatography of fraction 2 on MCI-Gel CHP 20P (75-150 μ m, Mitsubishi Chemical, Tokyo, Japan) using a H₂O: methanol gradient has resulted in oregonin(1) (2.72g) [12].

2.1.2. Preparation of Hirsutenone(HIR) by the Enzymatic Hydrolysis of Oregonin(ORE)

The ORE (1g, 1%, w/w) was diluted in distilled water (940ml, 94%, w/w) and Pectinex[®] AFP-L4 (polygalacturonase from *Aspergillus aculeatus* or *Aspergillus niger*) (Nobozymes[®] Co. Ltd, Bagsvaerd, Denmark) (50ml, 5%, w/w) was added [13,14]. The mixture was then shaken aerobically at 150 rpm for 18 hours at room temperature, heated at 85°C for 5 min to inactivate the enzyme, and then centrifuged (3000 rpm) for 30 min and filtered. The filtrate was fractionated with ethyl acetate, and the ethyl acetate layer (0.61g) was applied to a Sephadex LH-20 column and eluted with 60% MeOH to yield HIR (0.251g).

2.1.3. ORE (1,7-bis-(3,4-dihydroxy-phenyl)-heptane-3-on-5-O- β -D-xylopyranoside)

Brown amorphous powder, Negative FAB-MS m/z : 477 [M-H]⁻, ¹H-NMR and ¹³C-NMR data [15].

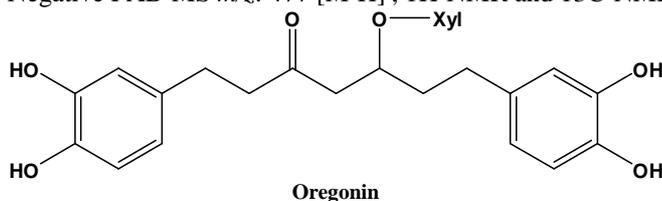


Fig. 1. Chemical structures of oregonin (ORE)

2.1.4. HIR (1,7-bis-(3,4-dihydroxyphenyl)-4-heptene-3-one)

Brown oil, Negative EI-MS m/z : 328 [M]⁺, ¹H-NMR and ¹³C-NMR data [15].

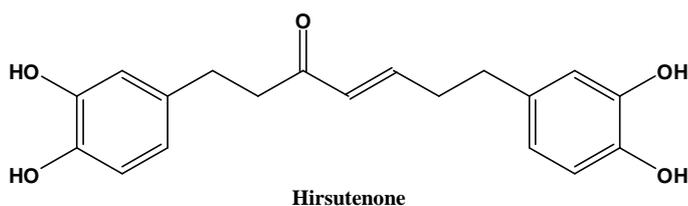


Fig. 2. Chemical structures of hirsutenone (HIR)

2.2. Experimental of Interleukin-1 β

2.2.1. Differentiation of Macrophage from Bone Marrow

BMDM (Bone Marrow Derived Macrophage) was prepared by differentiating the bone-marrow cells. The femoral region was excised from Balb/c mouse and bone-marrow cells are separated by washing it with phosphate buffer solution and removing the red blood cells. The recovered bone-marrow cells were resuspended with DMEM media containing 30% of L-9292 media, added into 96-well plate with the concentration of 5×10^5 cells/well, and incubated for 5-7 days.

2.2.2. Treatment with Test Materials

The differentiated BMDM cells were treated with LPS (500 ng/ml) and incubated for 3 hours. Then, the test materials (hirsutenone or oregonin) were added at the concentrations of 5 μ M, 10 μ M, 20 μ M, and 30 μ M, and incubated for 1 hour. Afterwards, ATP was added with the concentration of 5 mM and incubated for 6 hours. The media is recovered after 6 hours incubation, and the level of IL-1 β released from cells was analyzed quantitatively

with standard curve by using ELISA.

RESULTS

3.1. Inhibition of IL-1 β secretion by Oregonin(ORE)

Oregonin inhibited the IL-1 β secretion in a concentration dependent manner as evidenced by graph of figure 1 below. At the lowest concentration of 5 μ M of oregonin, the rate of reduction in IL-1 β secretion is about $42.30 \pm 4.31\%$.

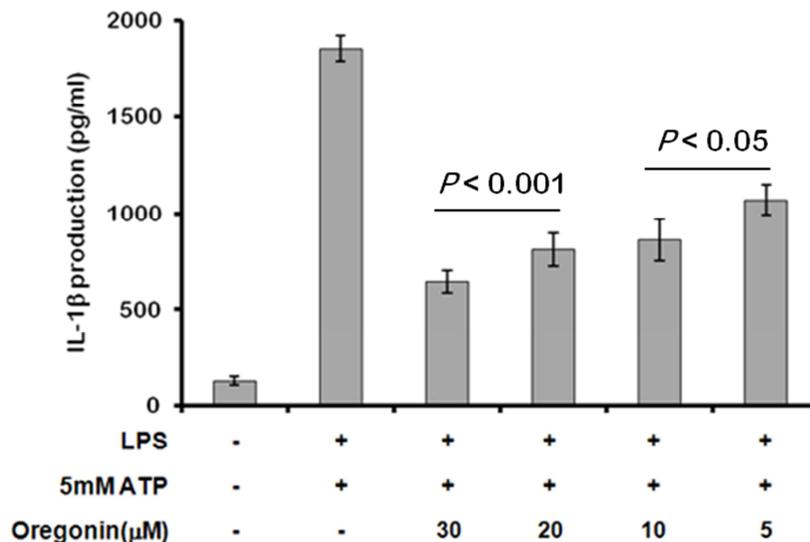


Fig.3. Inhibition effect on IL-1 β secretion by Oregonin (ORE)

Data were represented as means \pm SD of triplicate experiments. P-values were calculated by paired Student t test.

3.2. Inhibition of IL-1 β secretion by Hirsutenone(HIR)

Hirsutenone also inhibited the IL-1 β secretion in a concentration dependent manner as evidenced by below graph. At the lowest concentration of 5 μ M of hirsutenone, the rate of reduction in IL-1 β secretion is about $73.45 \pm 2.53\%$.

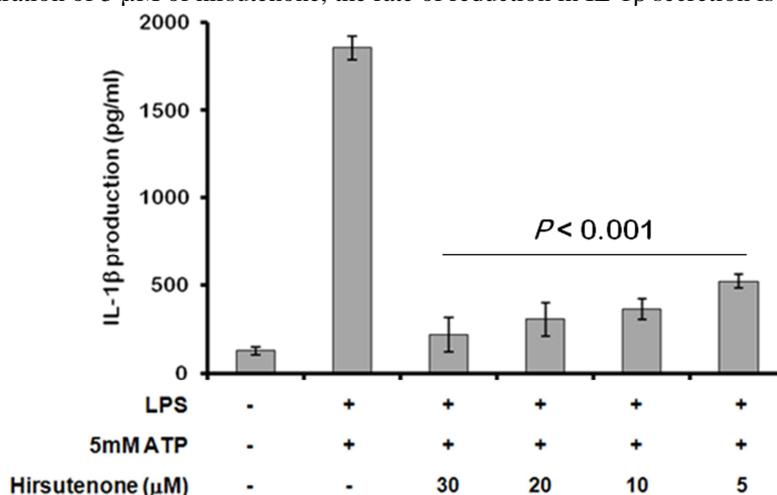


Fig.4. Inhibition effect on IL-1 β secretion by Hirsutenone (HIR)

Data were represented as means \pm SD of triplicate experiments. P-values were calculated by paired Student t test.

DISCUSSION

IL-1 β along with TNF- α is a representative inflammatory cytokine, which is secreted from macrophages and dendritic cells when activated by foreign antigen. IL-1 β is released in the earlier stage and induces immune responses. The released IL-1 β recruits macrophage to the site of inflammation and stimulates the differentiation of T cells residing in the tissue. Thus, the suppression of IL-1 β secretion is a critical target for the control of inflammation. According to the above mentioned experiment, it is evidently clear that hirsutenone has more potent ability to inhibit the secretion of IL-1 β compared to oregonine at the concentration of 5 μ M ($73.45 \pm 2.53\%$ vs.

42.30±4.31%).

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