The Interleukin-18 Inhibitory Activities of Echinocystic Acid and its Saponins from *Impatiens pritzellii* var. *hupehensis*

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Echinocystic acid (**1**), an echinocystic acid saponin, **2**, and four of its ester saponins, **3**–**6**, obtained from the active fraction of *Impatiens pritzellii* var. *hupehensis*, an traditional Chinese medicine for rheumatoid arthritis, were investigated for their effects on lipopolysaccharide (LPS)-induced interleukin (IL)-18 in human peripheral blood mononuclear cells. Three of them, **1**, **2** and **6**, showed obvious activity to inhibit the production of IL-18, especially the ester saponins with a sugar chain at C-28, **6**. Structure-activity relationships are discussed in brief.

Key words: Echinocystic Acid, *Impatiens pritzellii* var. *hupehensis*, Interleukin-18

Introduction

Interleukin (IL)-18, which was formerly called "IFN-γ-inducing factor", has been found both in synovial tissue and serum of patients with treatment of rheumatoid arthritis (RA). IL-18 is a novel cytokine with pleiotropic activities that is critical to the development of T helper cell type 1 (Th1) responses, and is considered to act at the epicentre of the inflammatory process of RA (Wei *et al.*, 2001). It can induce many other cytokines or components, which play important roles in RA, including IFN-γ, TNF-α, IL-1, GM-CSF, NO, and PGE2 (Gracie *et al.*, 1999). It has been reported that IL-18 is a potential therapeutic target in RA (Bessis and Boissier, 2001).

Impatiens pritzellii Hook. f. var. *hupehensis* Hook. f. (Balsaminaceae) has long been known and used by local people in China as an anti-RA herb. Our previous study has revealed that the administration of the *n*-butanol (*n*-BuOH) fraction of *I. pritzellii* could significantly inhibit the development of collagen-induced arthritis (CIA) in mice, which is one of the most widely used models for studying RA, decrease the levels of IgG, INF-γ, IL-18, and increase the concentration of IL-10 in the serum of CIA mice (Zhou *et al.*, 2007a).

In order to search for new biologically active substances in this traditional Chinese medicine, echinocystic acid, an echinocystic acid saponin, and four of its ester saponins were obtained after chemical investigation in the *n*-BuOH fraction of *I. pritzellii*. Their structures were determined by means of spectroscopy (Zhou *et al.*, 2007b). Echinocystic acid and its saponins have been reported to show anti-inflammatory, cytotoxic and anti-HIV activities (Lee *et al.*, 2002; Navarro *et al.*, 2001; Konoshima *et al.*, 1995), but their activities to RA are unknown. Because of the key role of IL-18 in RA progress, the function to inhibit IL-18 is important to search for anti-RA substances. The present study reports the effects to lipopolysaccharide (LPS)-induced IL-18 in human peripheral blood mononuclear cells (PBMCs) of six compounds.

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Material and Methods

Compounds and reagents

Compounds **1**–**6** were isolated from the *n*-BuOH fraction of the rhizomes of *I. pritzellii*. Their stuctures were determined as echinocystic acid (**1**), 3-*O*-*β*-D-glucuronopyranosyl echinocystic acid (**2**), 3-*O*-[(6-*O*-methyl)-*β*-D-glucuronopyranosyl] echinocystic acid (**3**), 3-*O*-[(6-*O*-ethyl)-*β*-D-glucuronopyranosyl] echinocystic acid (**4**), 3-*O*-[(6-*O*-*n*-butyl)-*β*-D-glucuronopyranosyl] echinocystic acid (**5**), and 3-*O*-[(6-*O*-*n*-butyl)-*β*-D-glucuronopyranosyl]-28-*O*-[*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl] echinocystic acid (**6**) by means of spectroscopy (Zhou *et al*., 2007b). Azothioprine [AZP, 6-(3-methyl-5-nitroimidazol-4-yl)-sulfanyl-7*H*-purine], used as positive control, was purchased from Jiufu Drug Company (Shanghai, China). LPS from *Escherichia coli* was purchased from Sigma (St. Louis, MO, USA). Human IL-18 instant enzyme-linked immunosorbent assay (ELISA) kits were purchased from BMS (Vienna, Austria).

Preparation of monocytes

Human PBMCs were obtained from 3 healthy volunteers after informed consent. 20 mL of peripheral blood were withdrawn from the vein of the forearm. PBMCs were isolated from the buffy coat of human peripheral blood by centrifugation on a NycoPrep 1.077 (AXIS-SHIELD, Oslo, Norway) instrument, and then washed three times in RPMI 1640 medium (Nissui Pharm. Co. Ltd., Tokyo, Japan). Monocytes were suspended at a final concentration of $1 \cdot 10^6$ cells/mL in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum.

Cytokine assays

Monocytes ($1 \cdot 10^6$ cells/mL) were incubated with compounds **1**–**6** (1, 10, and 100 μM) and LPS in 5% CO_2/air at 37 °C. After 24 h of culture, the cell suspensions were transferred into Eppendorf tubes and centrifuged. The cell-free supernatant fractions were assayed for IL-18 protein. IL-18 was measured using commercially available ELISA kits; the detection limit was 10 pg/mL.

Statistical analysis

The results are presented as mean ± SD. The statistical significances were evaluated using ANOVA, followed by Dunnett's *t*-test (Takahashi *et al*., 2004). P values < 0.05 were considered significant.

Results and Discussion

The effects of LPS with two concentrations (500 and 1000 ng/mL) on the changes in the production of IL-18 in the supernatant at 12, 24 and 48 h were examined (Fig. 1). The concentration of LPS and the culture time in following experiments were determined to be 1000 ng/mL and 24 h, as reported in the literature (Takahashi *et al*., 2004). The levels of IL-18 in PBMCs co-cultured with compounds **1**–**6** (Fig. 2) and LPS were determined by ELISA kits after standardization of the protein concentrations (Table I).

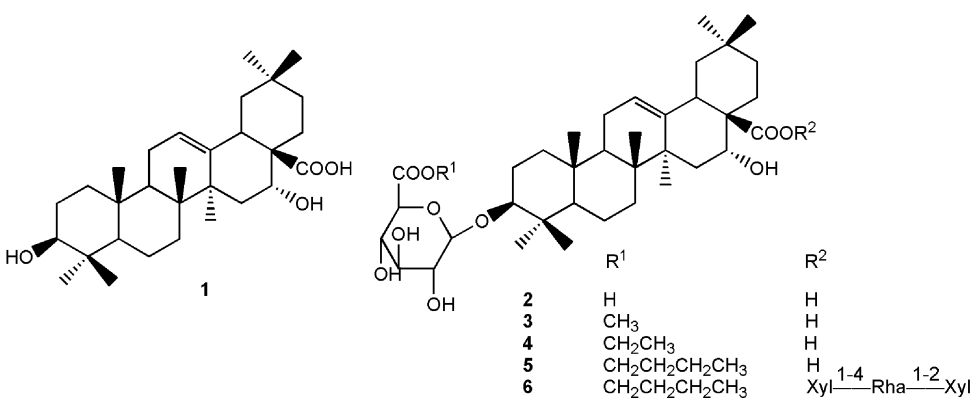

Fig. 1. Chemical structrues of compounds **1**–**6** isolated from *Impatiens pritzellii* var. *hupehensis*.

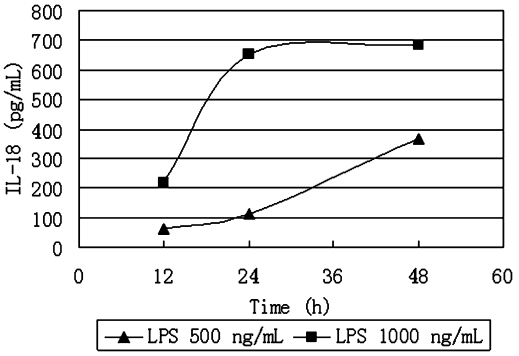

Fig. 2. Time-course and concentration-response relationship for the effects of lipopolysaccharide (LPS) on the IL-18 production in monocytes.

It is reported that IL-18 could inhibit CIA in mice (Plater-Zyberk *et al.*, 2001). In our research, compounds **1** and **2** inhibited the production of IL-18 *in vitro* at concentrations of 100 and 10 μM, and compound **6** could be considered to be a potent IL-18 inhibitor because of its significant activity at 1 μM. This result indicated that **1**, **2** and **6** might be the active substances in *I. pritzellii*, but their anti-RA activities should be further confirmed.

From the results of our research, the presence of glucuronic acid did not modify the activity of echinocystic acid because only some slight differences in the potency between **1** and **2** were observed. Compounds **3**–**5** were esterified with glucuronic acid, which showed no activity to IL-18. This suggested that esterification, which increased the lipophilicity of the saponin, could create a different interaction with the cell membrane. Compound **6**, with a three-sugar chain at C-28, improved the potency remarkably. This result indicate that the sugar moiety at C-28 was significant to improve the activity. Our previous study revealed that **6** has no cytotoxic activity (Zhou *et al.*, 2007b), and it was also reported that linkage of enough sugar moieties at the C-28 site is essential for noncytotoxicity (Lee *et al.*, 2002). That is to say, **6** is assumed to be of high potency and low toxicity, and may therefore be considered as a lead compound of new anti-RA drugs.

Table I. Effects to IL-18 produced on PMBCs induced by LPS of compounds **1**–**6**.

Group	Dose [μM]	IL-18 concentration [ng/mL]
Model		0.620 ± 0.064
AZP	100	0.042 ± 0.011 [a]
	10	0.108 ± 0.032 [a]
	1	0.267 ± 0.080 [a]
1	100	0.105 ± 0.020 [a]
	10	0.309 ± 0.078 [a]
	1	0.525 ± 0.122
2	100	0.189 ± 0.070 [a]
	10	0.369 ± 0.094 [a]
	1	0.516 ± 0.142
3	100	0.534 ± 0.096
	10	0.530 ± 0.113
	1	0.525 ± 0.124
4	100	0.534 ± 0.106
	10	0.552 ± 0.074
	1	0.554 ± 0.090
5	100	0.534 ± 0.101
	10	0.696 ± 0.122
	1	0.792 ± 0.232
6	100	0.165 ± 0.075 [a]
	10	0.171 ± 0.062 [a]
	1	0.288 ± 0.101 [a]

Values represent means ± SD (n = 8).
One-way ANOVA revealed significant effects between model and treatment groups ($P < 0.001$).
[a] $P < 0.01$ compared with model (Dunnett's t-test).

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