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Anti-allergic Effect of Acteoside Derived from Arid Land Plant *Cistanche tubulosa*

Rieko IIJIMA[1], Parida YAMADA[1], Junkyu HAN[1], Hideyuki SHIGEMORI[1], Hiroko ISODA[1*]

Abstract: The immediate-type allergic reaction is involved in many allergic diseases such as asthma, allergic rhinitis, and sinusitis. The discovery of drugs for the treatment of immediate-type allergic disease is a very important subject in human health. The three kinds of phenylethanoid glycoside, acteoside, echinacoside and cistanoside A, were extracted from *Cistanche tubulosa* which thrives in arid land. In this report, we investigated the effect of acteoside, echinacoside and cistanoside A on the immediate-type allergic reaction, focusing on the basophilic cell-mediated allergic reaction. Acteoside, echinacoside and cistanoside A decreased *β*-hexosaminidase release from IgE-sensitized BSA-stimulated RBL-2H3 cells at the antigen–antibody binding stage.

Keywords: *β*-hexosaminidase, Basophil cells, *Cistanche tubulosa*, Phenylethanoid glycosides

1. Introduction

Cistanche tubulosa (Schrenk) R. Wight is a perennial parasitic plant growing on the roots of *Salvadora* or *Calotropis* species, and distributed in North Africa, Arabia, and Asian countries (Kobayashi *et al*., 1987). *Cistanche tubulosa* thrives in arid land where environment is too harsh for the most plants. However, *Cistanche tubulosa* can thrive in such environment, so they are thought to have some potent protective mechanisms against UV stress, lack of water and some environmental stress. *Cistanche tubulosa* cannot do photosynthesis, because they do not have roots and reefs, so they depend on nutrient elements as the products of their host plants to grow up themselves. People living near the Takla Makan Desert have who used *Cistanche tubulosa* as a traditional medical food are noted for the longer life span. *Cistanche tubulosa* seems to have effects on cerebral nerve, immunity and skin aging.

Type I allergy is induced by certain types of antigens such as foods, dust, mites, medicines, cosmetics, moldspores, and pollen. This class of antigens induces the production of antigen-specific IgE antibodies that bind to receptors on mast cells or basophilic cells. The early phase reaction in type I allergy occurs within minutes and then mediators such as histamine and serotonin are released from the cell. These mediators induce vasodilation, mucous secretion, and bronchoconstriction. Histamine, which is released from mast cells stimulated by antigen or degranulation inducers, is usually determined by using a degranulation marker in experiments on the immediate allergic reaction.

RBL-2H3 cells, a tumor analog of mast cells, display characteristics of mucosal-type mast cells and express several hundred thousand IgE receptors on the membrane surface. After sensitization with IgE, the cells respond to the antigen and release histamine. *β*-hexosaminidase, which is stored in the secretory granules of mast cells, is released concomitantly with histamine when mast cells are immunologically activated (Schiwartz *et al*., 1981). Thus, the *β*-hexosaminidase activity in the medium is used as a marker of mast cell degranulation (Mastuda *et al*., 2002). RBL-2H3 cells are therefore considered as good tool for studying the effect of unknown compounds on histamine release and *β*-hexosaminidase release activity.

In the course of our search for anti-allergic compounds against BSA-induced *β*-hexosaminidase release from RBL-2H3 cells, we found that the BuOH extract of *Cistanche tubulosa* has an inhibitory effect. By bio-activity-guided fractionation, we isolated three kinds of phenylethanoid glycosides, such as acteoside, echinacoside, and cistanoside A. Some of these phenylethanoid glycosides appear to have various biological activities, such as anti-inflammatory and anti-oxidant activity (Xiong *et al*., 2000, Sahpaz *et al*., 2002). And, acteoside as one of these compounds is contained in olive fruits.

There are no reports about anti-allergic effect of the three phenylethanoid glycosides derived from *Cistanche tubulosa* on IgE sensitized BSA-stimulated RBL-2H3 cells and A23187 plus PMA stimulated KU812 cells as type I allergy model.

[1]* Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan
*Corresponding author. Fax: +81 29 853 5776; *E-mail address*: isoda@sakura.cc.tsukuba.ac.jp (H. Isoda).

2. Materials and Methods

2.1. Reagents

Dinitrophenylated bovine serum albumin (DNP-BSA) was purchased from Cosmo Biotechnology Co.; anti-DNP-IgE, ketotifen, L-glutamine were purchased from Sigma (Sigma Aldrich Co., USA). Fetal bovine serum (FBS) was purchased from Hyclone Co. Ltd. Eagle's Minimum Essential Medium (MEM) was purchased from Nissui Pharmaceutical Co., Ltd. RPMI medium 1640 was purchased from Gibco (Gibco Co., USA.). Calcium ionophore A23187 and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma Japan and dissolved in DMSO at a concentration of 10 mM.

2.2. Cells and cell culture

RBL-2H3 cells were purchased from Riken Cell Bank, Japan. The cells were maintained in MEM supplemented with 10% FBS and 2 mM L-glutamine, and incubated at 37 ˚C in a 5% CO_2 incubator, while KU812 cells were maintained in a RPMI 1640 medium supplemented with 10 % FBS at 37 ˚C in a 5 % CO_2 incubator.

2.3. Plant Material

The stems of *Cistanche tubulosa* (Orobanchaceae) were purchased from Dojindo Pharmacy in Beijing.

2.4. Extraction and Isolation

The stems of *Cistanche tubulosa* (500 g) were extracted with MeOH (1.5 L), and evaporated to dryness *in vacuo* at 30 ˚C. The MeOH extract was partitioned between EtOAc (1.0 L × 3) and H_2O (1.0 L) and then the H_2O layer was partitioned with *n*-BuOH (1.0 L × 3). The *n*-BuOH-soluble portion (3.1 g) was subjected to a ODS column (Cosmosil ODS, 2.2 × 30 cm, MeOH/H_2O, 3:7→1:0) to separate twelve fractions (CT-BU-1~12) including echinacoside (CT-BU-9, 25.4 mg). CT-BU-10 (491 mg) was applied to a silica gel column (2.2 × 30 cm, $CHCl_3$/MeOH/H_2O, 80:25:3) to afford thirteen fractions (CT-BU-10-1~13) including cistanoside A (CT-BU-10-11, 86.2 mg) and CT-BU-10-9 was purified by a silica gel column (2.2 × 30 cm, $CHCl_3$/MeOH/H_2O, 80:25:3) to give acteoside (12.1 mg).

2.5. *β*-hexosaminidase inhibition assay

The *β*-hexosaminidase release inhibition assay with RBL-2H3 cells was performed according to the method described by Yamada (Yamada *et al*., 2008). Three phenylethanoid glycosides were added at the IgE sensitization stage. RBL-2H3 cells were seeded onto 96-well plates at 5.0×10^4 cells/well in 100 μl of medium. The cells were incubated and sensitized for 24 h at 37 ˚C with 0.3 μg/ml anti-DNP-IgE. After incubating the cells at 37 ˚C for 10 min in 60 μl per well of a releasing mixture containing 5 μl of three phenylethanoid glycosides (0.1, 1.0, 10.0 μg/ml), the cells were exposed to 5 μl per well of 4 μg/ml DNP-BSA in PBS (-), before being incubated at 37 ˚C for 1 h.

2.6. MTT assay

MTT assay was performed according to the method described by Yamada (Yamada *et al*., 2007). RBL-2H3 cells and KU812 cells were seed onto 96-well plate at 5.0×10^4 cells. And, then cells were treated with 100 μl of medium contained three phenylethanoid glycosides (0.1-100.0 μg/ml) at 37 ˚C, 5% CO_2 incubator for 48 h.

3. Results and Discussion

Cistanche tubulosa (Schrenk) R. Wight (shown in **Figs. 1A** and **B**) is a perennial parasitic plant in arid land of North Africa, Arabia, and Asian countries (Kobayashi *et al*., 1987). The environment of arid land is too severe to grow up for the most plans. So, *Cistanche tubulosa*

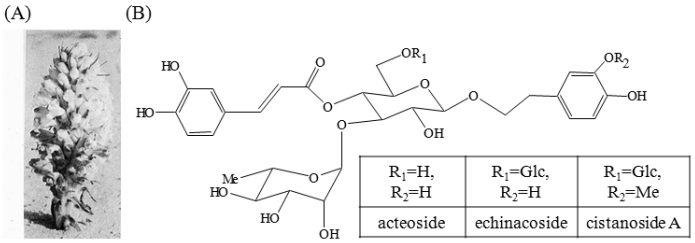

Fig. 1. (A) Cistanche tubulosa (Kotb *et al*., 1985) (B) Chemical structure of thee kinds of phenylethanoid glycosides.

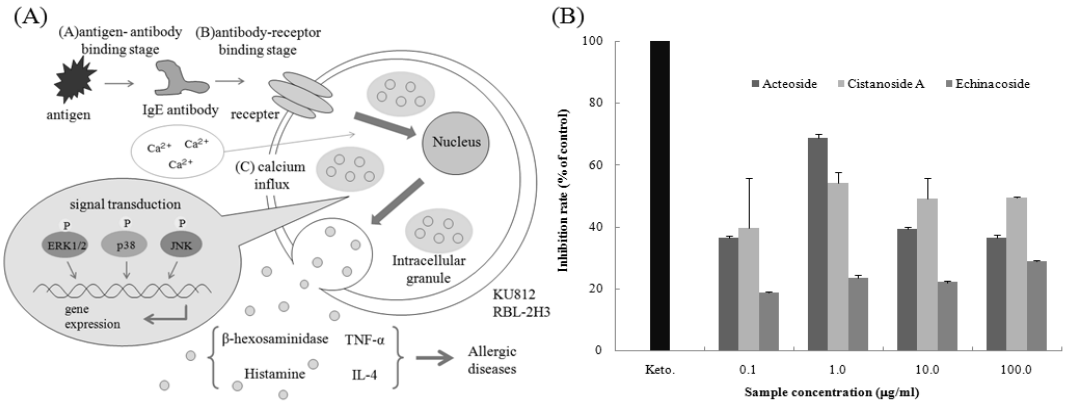

Fig. 2. Effects of phenylethanoid glycosides on *β*-hexosaminidase. (A) The model of Type I allergy. (B) Inhibitory effect of three phenylethanoid glycosides from *Cistanche tublosa* on *β*-hexosaminidase release from RBL-2H3 cells at the antigen-antibody binding stage.

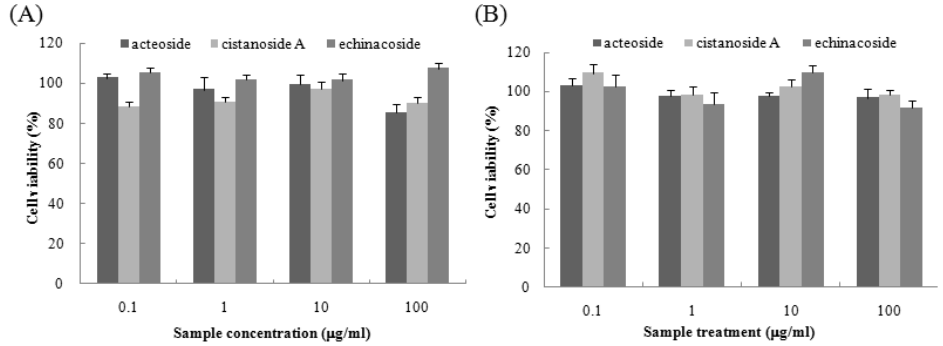

Fig. 3. Cytotoxic effect of phenylethanoid glycosides from *Cistanche tubulosa* on KU812 cells and RBL-2H3 cells. The percent cell viability was calculated relative to the untreated cells. (A) The cell viability of KU812 cells, (B) the cell viability of RBL-2H3 cells.

is considered to contain a lot of anti-oxidant compounds such as echinacoside, acteoside, and cistanoside A for protection against the environmental stress. hese compounds are phenylethanoid glycosides, whose structures are shown in **Figure 1C**.

3.1. Three phenylethanoid glycosides extracted from *Chistanche tubulosa* were screened for their inhibitory effect on *β*-hexosaminidase release by RBL-2H3 cells.

We can perform to screen anti-allergic effects at three different stages. The first is antigen-antibody binding stage (**Fig. 2A**), the second is antibody-receptor binding stage (**Fig. 2B**), and the last is calcium influx (**Fig. 2C**).

This paper reports inhibitory effects of *β*-hexosaminidase release from IgE-sensitized BSA stimulated RBL-2H3 cells at the antigen-antibody stage. The inhibition rate of *β*-hexosaminidase release from RBL-2H3 as affected by three samples was shown in **Figure 3B**. In the present study, we compared the effects of samples with the clinically available anti-allergic drug, ketotifen fumarate (Keto.), which is known as a mast cell stabilizer, H1- receptor antagoist and eosinophil inhibitor (Harish *et al*., 2001).

The acteoside at 1.0 μg/ml concentration had the most potent inhibitory effect among other phenylethanoid glycoside, tested followed by echinacoside and cistanoside A at the antigen–antibody binding stage. It suggests that R_1 site and R_2 site should be hydrogen group to exert inhibitory effect on *β*-hexosamindase release. Echinacoside has glucose group, and cistanoside A has not only glucose group but methyl group, instead of hydrogen group as acteoside.

3.2 Cytotoxic effect of three phenylethanoid glycosides on RBL-2H3 and KU812 cells.

We used the MTT assay to assess the cytotoxicity of three phenylethanoid glycosides on RBL-2H3 cells and KU812 cells. The cells were treated with samples for 48 h at final concentration of 0.1, 1.0, 10.0, and 100.0 μg/ml. The results showed that three phenylethanoid glycosides did not have cytotoxicity on both cells at 0.1-10.0 μg/ml. Echinacoside also did not show any cytotoxicity at 100.0 μg/ml on KU812 cells. The other treatmented cells at 100 μg/ml showed around 90 % viability.

These results suggest that the reduction of *β*-hexosaminidase release in Figure 2B is not caused by cell death. Three kinds of phenylethanoid glycosides did not show cytotoxity, and then, these compounds could inhibit *β*-hexosaminidase release.

4. Conclusions

In this study, we found that the three kinds of phenylethanoid glycoside extracted from *Cistanche tubulosa* have anti-allergic effect on BSA-stimulated RBL-2H3 cells. Although acteoside, cistanoside A and echinacoside have inhibitory effect on *β*-hexosaminidase release, they have some differences in terms of effect and concentration. These differences might be caused from the difference in structure, so we will elucidate the relationship between the structure and anti-allergic effect in the future.

Cytokines produced by basophilic cells, TNF-*α*, IL-4, IL-13, and IL-5 are key molecules for allergic inflammation such as IgE production, Th2 differentiation, and allergic inflammation (Borish *et al*., 2003). The reduction of pro-inflammatory cytokines from mast cells or basophil cells is one of the key indicators of reduced allergic symptom (Paul *et al*., 1993). So, the inhibitory effects of phenylethanoid glycosides on the cytokine production should be investigated in the future.

These findings provide evidence that the three kinds of phenylethanoid glycosides extracted from *Cistanche tubulosa* could be a candidate as an anti-allergic agent. Mitogen-activated protein kinase (MAPKs) pathways are said to modulate cytokine production. The detailed mechanism of phenyethanoid glycosides effects on signal pathways behind its anti-allergy effect as an anti-oxidant is the subject of a future study.

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