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Effects of *Viola yedoensis* Makino anti-itching compound on degranulation and cytokine generation in RBL-2H3 mast cells

Hai-Rong Zeng [a,c], Bing Wang [a,b,**], Zhen Zhao [a], Qi Zhang [a,b], Mei-Yun Liang [a], Ya-Qi Yao [a], Ka Bian [c,d], Wei-Rong Zhang [a,*]

[a] Experiment Center for Teaching and Learning, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Rood, Pudong New District, Shanghai 201203, China
[b] School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 1200, Cailun Road, Pudong New District, Shanghai 201203, China
[c] Murad Research Center for Modernisized Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Rood, Pudong New District, Shanghai 201203, China
[d] Department of Biochemistry and Molecular Biology, George Washington University of USA, WA 20052, USA

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ABSTRACT

Ethnopharmacological relevance: The Chinese herb compound prescription *Viola yedoensis* Makino Anti-itching Compound (VYAC), which consists of *Viola yedoensis* Makino, herb, *Sophora flavescens* Aiton, root, and *Dictamnus dasycarpus* Turcz, root and rhizome, has been traditionally used to treat various skin allergic inflammatory diseases in clinic.

Aim of the study: The aim of this study is to investigate the effects of VYAC on degranulation and to determine its anti-inflammatory mechanism in RBL-2H3 mast cells.

Materials and methods: VYAC was extracted with water-coction extraction (Shufen et al., 2012). The aqueous extracts were concentrated in vacuum under reduced pressure and lyophilized using a freeze dryer, and lyophilized powder was obtained. MTT was used to evaluate the cytotoxic of VYAC on RBL-2H3 cells. Degranulation was carried out with RBL-2H3 cell model, which was stimulated with A23187 plus PMA. β-Hexosaminidase and histamine were measured to evaluate degranulation. The mRNA levels of inflammation cytokines (IL-1β, TNF-α, IL-6, and iNOS) were investigated by RT-PCR to explain the anti-inflammatory mechanism of VYAC.

Results: VYAC did not show cytotoxic effect on RBL-2H3 cells in the range of 25–400 μg/mL. A higher dose of VYAC (800 μg/mL) showed significant cytotoxicity ($P < 0.05$). VYAC could significantly inhibit β-hexosaminidase and histamine release when treated with 100, 200, and 400 μg/mL ($P < 0.05$), but could not significantly inhibit β-Hexosaminidase and histamine release when treated with 25 and 50 μg/mL ($p > 0.05$). The mRNA levels of inflammatory cytokines (TNF-α, IL-1β, IL-6, and iNOS) could significantly decrease when treated with 200 and 400 μg/mL ($P < 0.05$) of VYAC, which were associated with the development of inflammation.

Conclusions: Results showed that VYAC inhibited β-hexosaminidase and histamine release, which was inhibit A23187 plus PMA stimulated RBL-2H3 cell degranulation and downregulated inflammatory cytokines (IL-1β, TNF-α, IL-6, and iNOS) expression to block inflammatory development.

1. Introduction

Allergic dermatitis (AD) is caused by an exaggerated or hypersensitivity reaction to the immune system when a person is exposed to normal harmless environment substance (Xi et al., 2015; Thanh et al., 2010), such as pollen, insects, chemical agents, animal dander, and house dust mites, which caused a disorder of the immune system and lead to allergic skin disease (Atarashi, 2008). Mast cells are effectors of the allergic responses and are commonly found at antigen-mediated allergic response (Askenase et al., 1983). Mast cells are well known for their important role in the pathogenesis of allergic skin diseases and inflammatory processes (Brown et al., 2008; Galli et al., 2005). Allergen caused allergen-specific $CD4^+$ Th2 cell generation and motivated Th2 cell

* Corresponding author.
** Corresponding author at: Experiment Center for Teaching and Learning, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Pudong New District, Shanghai 201203, China.
E-mail addresses: annabel_cn@163.com (B. Wang), zwr885@hotmail.com (W.-R. Zhang).

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effectors to produce interleukin, which leads to the production of allergen-specific IgE by B cells (Akdis et al., 2011; Jae et al., 2012). Mast cell surface has high-affinity receptor for IgE (FcεRI) (Von et al., 2003; Thanh et al., 2012), which caused intracellular events by aggregation of IgE and FcεRI (Siraganian, 2003). Mast cell surface also increased intracellular Ca^{2+} levels and degranulation, which caused the secretion of bioactive substances such as histamine, β-hexosaminidase, leukotrienes, prostaglandins, proinflammatory cytokines, and chemokines (Broide, 2001; Zhang et al., 2012). Mast cell-derived histamine and proinflammatory cytokines such as TNF-α and interleukin (IL-1β, IL-6) contribute to itching and inflammation, which plays a key role in the pathogenesis of allergic skin disease (Oyoshi et al., 2009). Moreover, mast cells can be activated by phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 in vitro and are widely used as model cells (Shin et al., 2009, 2005). AD is one of the most common dermatoses. Current first-line therapy for AD included glucocorticosteroids, non-steroid anti-inflammatory drugs, oral anti-histamines, and emollients (Cohen and Heidary, 2004). However, patients are worried about long-term use of the drugs and its severe adverse effects (Kobayashi et al., 2010). Thus, new and effective drugs should be found.

Many effective herb medicines for AD are available in Eastern countries. *Viola yedoensis* Makino Anti-itching Compound (VYAC) is an original prescription from the traditional Chinese medicine in clinics in China, and it had been used to cure hundreds of patients who suffered skin pruritus by Professor Zhang for decades (Wang et al., 2013). Under the guidance of traditional Chinese medicine theory, many herbs are widely and longtime used for treating various skin diseases including AD (Kim et al., 2012).

Many bioactive chemical constituents have been reported in this compound. The types of constituents in *Viola yedoensis* Makino including flavonoids (Du et al., 2015) and coumarin: esculetin, dicoumarin: euphorbetin (Hai et al., 2009). The types of constituents in *Sophora flavescens* Aiton including alkaloids: matrine and oxymatrine (Noriko and Jun, 2012), flavonoids: trifolirhizin (Nan et al., 2013), kuraridin, kurarinone, kurarinone and Sophoraflavanone G (Jeong et al., 2010). The types of constituents in *Dictamnus dasycarpus* Turcz including alkaloids: dictamine and skimmianine (Hyungwoo et al., 2013), limonoids (Jian et al., 2013a, 2013b), sesquiterpenes and glycosides (Jian et al., 2013b). Studies showed that have effects of anti-inflammatory (Hyungwoo et al., 2013), anti-allergic (Hyungwoo et al., 2012; Myung et al., 2009).

However, its mechanism of treatment has not yet been elucidated. Therefore, the aim of this study is to evaluate the effects of VYAC on degranulation of rat basophilic leukemia (RBL-2H3) cells and to determine the herb's potential anti-inflammatory mechanism.

2. Materials and methods

2.1. Chemicals and reagents

RBL-2H3 mast cell line was obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Calcium ionophore A23187, phorbol 12-myristate13-acetate (PMA), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and 4-nitrophenyl-N-acettyl-β-D-glucosaminide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbeco's Modified Eagle's Medium (DMEM/high glucose), fetal bovine serum (FBS), penicillin-streptomycin liquid, and trypsin-EDTA (0.25%) were purchased from Gibco (BAL, Co. Ltd, USA). A histamine assay kit was purchased from Labor Diagnostika, Nord GmbH & Co. KG (Nordhorn, Germany). Trizol reagent (Invitrogen, California, USA) and RevertAid First Stand cNDA Synthesis kit (ThermoFisher Scientific, Waltham, USA) were used in the paper.

2.2. Sample preparation

The compound consists of *Viola yedoensis* Makino, *Sophora flavescens* Aiton, and *Dictamnus dasycarpus* Turcz from the traditional Chinese herbs. All the herbs were purchased from Kang Qiao Herb Materials Co., Ltd. (Shanghai, China) and identified by Senior Experimentalist Jun-song Li, Experiment Center for Teaching and Learning, Shanghai University of Traditional Chinese Medicine, Shanghai, China. The Chinese herbal compound can heat-clearing and detoxifying, wind-dispelling and itching-arresting. *V. yedoensis* Makino and *S. flavescens* Aiton were the key herbs of this formula. A main active ingredient of *V. yedoensis* Makino is aesculetin, and the active ingredient of *S. flavescens* Aiton is matrine(Zhou et al., 2014). The preparation of herbal extracts was as follows: 30 g of *V. yedoensis* Makino, 15 g of *S. flavescens* Aiton, and 15 g of *D. dasycarpus* Turcz were mixed and immersed with 600 mL of distilled water for 30 min and then extracted twice with reflu under reduced pressure using vacuum evaporator (Rotavapor R-220, BUCHI) for 1 h (Shufen et al., 2012). The aqueous extracts were collected and filtered with filter paper. The filtrate was then concentrated under reduced pressure. The condensed extract was lyophilized using a freeze dryer (Labconco, Kansas City, MO, USA) (Gang et al., 2014). Finally, 13.08 g of lyophilized powder was obtained (yield, 21.8%). The dry powder was stored in an −80 °C refrigerator until use.

2.3. HPLC analysis

High-performance liquid chromatography (HPLC) analysis was conducted using an Agilent 1100 Series HPLC System composed of a vacuum degasser, a quaternary pump, a standard auto-sampler, a column compartment, and an ultraviolet detector (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was carried on an Ultimate XB-C18 Column (250 mm × 4.6 mm, 5 μm, Welch). For aesculetin detection, an isocratic elution was carried out using the following solvent systems: mobile phase A was 0.1% phosphoric acid; and mobile phase B was acetonitrile, in which A: B was 82:18. The sample injection volume was 10 μL, and the solvent flow rate was 1 mL/min. Detection was performed at UV 348 nm. For matrine detection, an isocratic elution was carried out using the following solvent systems: mobile phase A was methanol and mobile phase B was phosphate buffer (potassium dihydrogen phosphate dissolved in 1000 mL pure water, regulated at pH 3 with phosphoric acid), in which A:B was 12:88. The sample injection volume was 10 μL, and the solvent flow rate was 1 mL/min. Detection was performed at UV 220 nm. The extracted powder was dissolved in methanol at a concentration of 0.02 g/mL and filtered with 0.45 μm filter membrane.

2.4. Cell culture

Rat basophilic leukemia cell line (RBL-2H3) was grown in EMDM/high glucose medium supplemented with 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Cells were maintained in a humidified 5% CO_2/95% air atmosphere at 37 °C. The medium was replaced every day. The cells were extended every 2–3 days.

2.5. Measurement of cell viability

Cell viability was determined by the previously published MTT methods (Chen et al., 2011) with little modifications. Briefly, the cells were seeded in 96-well plates (1×10^5 cells/well) until