ANti-allergic activity of ethanol extract of Chinese Petasites tatewakianus Kitam in preventive treatment of allergic rhinitis

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ABSTRACT

Objective: This study aims to evaluate potential protective effects of the ethanol extract of Chinese Petasites tatewakianus Kitam (PTK) on rats with induced AR.

Methods: Sprague-Dawley rats were sensitized and randomly allocated into two groups: control (n = 10) and PTK (n = 10). One hr prior to intra-nasal challenge with the antigen, rats received either the PTK or saline vehicle per os. Nasal symptoms and quality of life were compared between the rats in the two groups over the course of 20 min post-challenge. Nasal fluids and in serum were collected 1 hr after the antigen challenge and then evaluated for the levels of several key inflammatory mediators. Lipid mediator production was also evaluated ex vivo using PBMC (peripheral blood mononuclear cells) and granulocytes from the sensitized rats.

Results: The results showed that a single administration of PTK extract resulted in significantly decreased histamine and LTs levels (LTB4 and cysteinyl-LTs) in the blood and nasal fluids of the rats. Further, the synthesis of cysteinyl-LTs and LTB4 were all significantly inhibited ex vivo by the PTK extract. Overall, the treatment with PTK extract significantly alleviated the nasal symptoms and improved the quality-of-life of the challenged hosts.

Conclusion: Taken together, these results indicated to us that PTK extract was effective in treating AR, in part, by decreasing levels of nasal inflammatory mediators and the synthesis of LTs and, in doing so, ameliorated allergy symptoms and improved the quality of life of the antigen-challenged sensitized hosts.

Keywords: butterbur extract, anti-allergic activity, histamine, leukotrienes.

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Introduction

Allergic rhinitis (AR) is a common allergic disease characterized by sneezing, nasal congestion and rhinorrhea. People with AR are at risk to develop asthma, bronchitis, eustachian tube inflammation, otitis media and other complications. In many instances, patients also suffered from headache, impaired nocturnal sleep and decreased quality of life. Several studies have shown that the pathophysiology of allergic rhinitis is based on the infiltration of allergen into the inflammatory cells located in the nasal mucosa, such as lymphocytes, mast cells and eosinophils. While the best treatment for AR is obviously to avoid the triggering allergens; however, this is often very difficult to achieve for most patients, particularly those exposed to outdoor allergens on a regular basis. In most cases, drug treatment is required to control symptoms, and there are many clinically prescribed and over-the-counter medications currently on the market.
However, some of these medications have undesirable side-effects (such as drowsiness) and they have variable effectiveness in most patients. Therefore, almost half of patients with AR try a natural product to relieve symptoms\(^{(12)}\).

Petasites tatewakianus Kitam (PTK), perennial herb, is widely distributed in Northeast China, Japan and the Korea peninsula\(^{(13,14)}\). In Northeast China, PTK is used as a wild vegetable and cultivated widely\(^{(15)}\). Some of petasites species have been used as folk medicine in treatment of several diseases. Petasites hybridus was reported to help to alleviate gastrointestinal pain\(^{(16)}\). Another form, Petasites japonicus, has been used for allergy and asthma therapy\(^{(14)}\). To date, there are no reports of applications of Chinese PTK in medicine or its pharmacological activity. Accordingly, the study reported here investigated the anti-Type I allergic effects of an ethanol extract of PTK in a rat model of AR. It was expected that these studies might not only be able to ascertain what, if any, effect the PTK might have on this pathology, but also shed some light on potential immuno-modulatory mechanisms underlying any such effect.

Method

**Chemicals**

Egg albumin (ovalbumin, OVAS, Grade V, Sigma, St. Louis, MO), aluminum hydroxide (Al(OH)\(_3\)), alum and inactivated Bordetella pertussis were of analytical grade and purchased from Tianjin Chemical Reagent Co., Ltd (Tianjin, China).

**Animals**

Pathogen-free male Sprague-Dawey (SD) rats (6 wk-of-age, 200-240 g) were obtained from Experimental Animal Center (Aiermaite technology Co., Ltd., Suzhou, China). Rats were maintained on a 12-hr light/dark cycle in a controlled environment at 21 ± 2°C and with a relative humidity of 50-60%. All experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animal and approved by the Animal Care and Use Committee of Linyi people’s hospital.

**Extraction of PTK**

Samples of Chinese Petasites tatewakianus Kitam (PTK) were cultivated in Fangzheng County of Heilongjiang Province. The voucher specimen was deposited at the Department of Pharmacognosy in Yantai University (voucher specimen #1404002). The PTK were washed with distilled water, dried in an oven, and then ground with a mortar and pestle. Samples of the powdered PTK (3.6 kg) were extracted with 60% ethanol for 6 hr (at 70°C). The solvent was evaporated under vacuum; the extract recovered was then powdered by spray-drying and weighed. The yield of PTK extract was 10.32%.

**Sensitization**

Rats were sensitized by an injection (into the four footpads) with a total of 1ml of physiological saline solution containing egg albumin (1 mg), alum (2 mg), and 1010 Bordetella pertussis on Day 1.17 Five days later, the rats received a subcutaneous injection of 1 ml of physiological saline containing egg albumin (0.5 mg) into a total of 10 sites on the back. Fourteen days later, local sensitization was performed daily until Day 42 by dripping the egg albumin in saline (1 mg/ml) into the bilateral nasal cavities (10 μl/nostril) using a micropipette\(^{(18)}\).

**Evaluation of nasal symptoms and quality of life**

On Day 42, the sensitized rats were randomly allocated into two groups: control (n = 10) and PTK (n = 10). PTK extract (20 mg/kg) was administered per os 1 hr before antigen injection in PTK group; control rats received no treatment before antigen administration. Before instillation of albumin, all rats were acclimated at least 10 min in observation cages. For the antigen exposure, rats received a nasal instillation of 10 μl egg albumin (1 mg/ml) into the bilateral nasal cavities. Thereafter, the numbers of sneezing and nasal rubbing events were counted for 30 min. The quality of life evaluated three domains, i.e., activities, behavior and sleep, using a ten-point scale (0, very much better; 10, very much worse). figure

**Quantification of Inflammatory Mediators in nasal fluids and serum**

At 20 min after the albumin exposure above, to permit measures of nasal inflammatory mediators, nasal fluids were collected by the delivery of normal sodium solution (0.9%; 3 ml) syringe into the cavities and collection of the fluid. Levels of eosinophil cationic protein (ECP), tryptase, and total-IgE in the fluids were measured using commercial fluoro-immunoassays kits (Pharmacia & Upjohn, Stockholm, Sweden) according to manufacturer instructions.
Levels of Cysteinyl-LTs, LTB4 and PGE2 were measured using commercial ELISA kits (Amersham Pharmacia Biotech Europe, Freiburg, Germany) according to manufacturer protocols.

At the same time the rats were being lavaged, blood was collected from the suborbital vein. A portion of the samples were allowed to clot for 1 min at 25°C and the resultant serum collected after centrifugation (2500 × g, 10 min, 4°C). The serum samples were stored at -20°C until also undergoing assay for ECP, tryptase, total-IgE, cysteinyl-LTs, LTB4 and PGE2.

**Lipid mediator production assays**

From the remaining blood collected, PBMC (Peripheral Blood Mononuclear Cell) and granulocytes were purified as previously described(19,20,21) and then counted in a optical microscope. Thereafter, each population was separated placed in wells of 96-well plates at 1 × 106 cells/ml in DMEM (GIBCO, USA) containing 10% heat inactivated fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen, USA) and cultured at 37°C in a humidified incubator containing 5% CO2. After 24 hr, the cells were stimulated with 10nM anaphylatoxin C5a (Sigma, St. Louis, USA) for 25 min. After this period, culture supernatants were collected and stored at -20°C until used for analysis of cysteinyl-LTs, LTB4, and PGE2.

**Statistical Analysis**

All data were expressed as mean ± SD. Analysis were conducted using one-way analysis of variance (ANOVA). A Statistical Analysis System Version 9.0 software package (SAS, NC, USA) was used for all analyses. A p-value < 0.05 was considered to be statistically significant.

**Results**

**The effects of PTK extract on the nasal symptoms and quality of life**

Figure-I depicted the differences between the control and PTK groups with respect to nasal symptoms after treatment with sensitizing antigen, egg albumin. Events, including sneezing, nasal rubbing, rhinorrhea, and nasal congestion, were evaluated on a 10-point scale (0, very much better; 10, very much worse). PTK extract significantly (P < 0.01) decreased the scores for sneezing, nasal rubbing and rhinorrhea (2.55 [± 0.30], 3.36 [± 0.39] and 1.75 [± 0.27], respectively) from the corresponding control scores of 8.03 [± 0.45], 6.86 [± 0.41] and 6.12 [± 0.32]. Use of the PTK extract also significantly (P < 0.05) attenuated the nasal congestion score to 5.21 [± 0.36] from 6.37[± 0.52] with the control rats.

Figure-II shows the effects of PTK extract on quality of life, also evaluated on a 10-point scale (0, very much better; 10, very much worse). Compared with the controls, PTK extract-treated rats had significantly (P < 0.01) improved the quality of life with respect to activity, behavior and sleep. Scores for activity, behavior and sleep were raised in PTK rats (5.73 [± 0.39], 6.69 [± 0.42] and 6.32 [± 0.50], respectively)from the corresponding control value of 2.38 [± 0.21], 3.84 [± 0.43] and 1.96 [± 0.17].

**The effects of PTK extract on the inflammatory mediators in serum and in nasal fluids**

The levels of select inflammatory mediators in serum and in nasal fluids are shown in Table-I and Table-II. In nasal fluids, PTK treatment resulted in significant decreases from control values in histamine (from 15.32 [± 2.02] to 8.75 [± 0.97] ng/ml, 42.9%), cysteiny-LTs (from 149.37 [± 25.62] to 109.17 [± 20.71] ng/ml, 27.1%), and PGE2 (from 73.57 [± 12.85] to 49.83 [± 7.62] ng/ml, 32.1%).
76.71 [± 18.33] pg/ml, 48.6%) and tryptase (from 2.73 [± 0.22] to 1.80 [± 0.17] µg/ml, 34.1%) (all P < 0.01; Table 1). Levels of LTB4, ECP and PGE2 were also all significantly inhibited by PTK extract (P < 0.05; Table-I).

In serum, the PTK extract resulted in significantly attenuated levels (vs. Control values) of cysteinyl-LTs (from 123.51 [± 21.08] to 70.40 [± 17.11] pg/ml, 43.0%, P < 0.01), LTB4 (from 205.27 [± 34.81] to 158.39 [± 23.49] pg/ml, 22.8%, P < 0.05), ECP (from 17.32 [± 2.01] to 12.58 [± 1.65] µg/ml, 27.4%, P < 0.05), PGE2 (from 297.53 [± 36.37] to 211.59 [± 30.05] µg/ml, 28.9%, P < 0.05), tryptase (from 6.86 [± 1.03] to 3.27 [± 1.16] µg/ml, 52.3%, P < 0.01), and total IgE (from 201.32 [± 25.45] to 139.85 [± 26.72] kU/l, 30.5%, P < 0.05) (table 2).

TABLE 1: The effects of PTK extract on the inflammatory mediators in nasal fluids. Data were expressed as mean ± SD (N = 10/group). *p < 0.05, **p < 0.01 versus the control group.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>PTK group</th>
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<tbody>
<tr>
<td>Histamine (ng/ml)</td>
<td>15.32 ± 2.02</td>
<td>8.75 ± 0.97 **</td>
</tr>
<tr>
<td>Cysteinyl-LTs (pg/ml)</td>
<td>149.37 ± 25.62</td>
<td>76.71 ± 18.33 **</td>
</tr>
<tr>
<td>LTB4 (pg/ml)</td>
<td>352.49 ± 39.08</td>
<td>267.57 ± 31.28 *</td>
</tr>
<tr>
<td>ECP (µg/ml)</td>
<td>2.39 ± 0.24</td>
<td>1.83 ± 0.19 *</td>
</tr>
<tr>
<td>PGE2 (pg/ml)</td>
<td>317.83 ± 30.55</td>
<td>251.29 ± 25.41 *</td>
</tr>
<tr>
<td>Tryptase (µg/ml)</td>
<td>2.73 ± 0.22</td>
<td>1.80 ± 0.17 **</td>
</tr>
</tbody>
</table>

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TABLE 2: The effects of PTK extract on the inflammatory mediators in serum. Data were expressed as mean ± SD (N = 10/group). *p < 0.05, **p < 0.01 versus the control group.

<table>
<thead>
<tr>
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<th>Control group</th>
<th>PTK group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteinyl-LTs (pg/ml)</td>
<td>123.51 ± 21.08</td>
<td>70.40 ± 17.11 **</td>
</tr>
<tr>
<td>LTB4 (pg/ml)</td>
<td>205.27 ± 34.81</td>
<td>158.39 ± 23.49 *</td>
</tr>
<tr>
<td>Total IgE (kU/l)</td>
<td>201.32 ± 25.45</td>
<td>139.85 ± 26.72 *</td>
</tr>
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<tr>
<td>Tryptase (µg/ml)</td>
<td>6.86 ± 1.03</td>
<td>3.27 ± 1.16 **</td>
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</table>

The effects of PTK extract on lipid mediator generation

Functional tests of C5a-induced lipid mediator production in monocytes and granulocytes were conducted (Table-III). PTK extract could significantly (all P < 0.05) decreased the synthesis of cysteinyl-LTs, LTB4 and PGE2 to 1142.8 [± 237.5], 1783.1 [± 268.9] and 2367.2 [± 319.6] pg/ml in PBMC (monocytes) compared with the corresponding control monocyte levels of 1824.3 [± 246.7], 2079.6 [± 318.3] and 2948.3 [± 353.0] pg/ml. These corresponded to decrements of 37.3%, 14.3%, and 19.7%, respectively.

Similarly outcomes were noted with the granulocytes (Table 3). The synthesis of lipid mediators (Table 3). The synthesis of lipid mediators cysteinyl-LTs, LTB4 and PGE2 was also significantly attenuated by PTK extract to, respectively, 1096.4 [± 194.8] (p < 0.01), 3739.7 ± 328.4 (p < 0.05), and 2970.9 [± 228.3] (p < 0.01) pg/ml from corresponding control values of 1675.7 ± 229.4, 4897.4 [± 458.6], and 3905.7 [± 348.0] pg/ml. These corresponded to decrements of 34.6%, 23.6%, and 23.9%, respectively.

The effects of PTK extract on ex vivo lipid mediator generation in blood cells types

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival rate of skin flaps (%)</th>
</tr>
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<tbody>
<tr>
<td>Experiment group</td>
<td>96.76±3.87*</td>
</tr>
<tr>
<td>Control group</td>
<td>40.48±7.83</td>
</tr>
<tr>
<td>Blank group</td>
<td>48.97±5.68</td>
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</table>

Table 3: d Survival rate of abdominal island skin flap of rats.

*p <0.01 compared to the control group, # p <0.01 compared to the blank group

Discussion

AR is inflammation of the nasal mucous membranes due to an Ig E mediated response to allergens such as dust mites or pollen. Allergen could cross the epithelial barrier in the nasal mucosa and activate the production of allergen-specific Ig E by local immune cells. The antibody, in turn, can subsequently bind to the FcεRI receptor on the surface of mast cells, effectively arming them for any subsequent exposure to the allergen. Upon such an exposure, the mast cell FcεRI receptors become cross-linked [by the multivalent antigen] and this activates mast cell degranulation and the release a
vast variety and quantity of mediators, such as histamine and LTs.

Histamine has been implicated as a major mediator of AR, causing sneezing, nasal itching and rhinorrhea. Recent studies also indicated that cysteinyl-LTs and LTB4 may be important mediators. Cysteinyl-LTs have been shown to cause nasal congestion and rhinorrhea. LTB4 could stimulate lysosomal enzyme release and amplify leukocyte adhesion in inflamed or infected nasal tissues.

Petasites tatewakianus Kitam (PTK) is a type of butterbur (Petasites hybridus) that is becoming an increasingly popular herbal treatment for AR. In this study reported here, administration of an PTK extract significantly decreased the histamine levels in nasal fluids of sensitized hosts (rats) challenged intra-nasally with the triggering antigen. Cysteinyl-LTs and LTB4 levels were also significantly decreased in nasal fluids; this suggested to us that the PTK extract was effective in treating AR, in part, by decreasing levels of nasal inflammatory mediators.

These results were confirmatory of those from a clinical study on the efficacy of butterbur tablets in treating AR symptoms and showed that butterbur could significantly reduce histamine and LT concentrations and improve quality of life scores. In the present research, the specific composition in the extract was not ascertained, however, researches showed that the active component in butterbur is petasin, a sesquiterpene that functioned as an inhibitor of cysteinyl-LTs biosynthesis, ECP release and cytoplasmic phospholipase A2 (cPLA2) activity. Also, a research in Japan demonstrated that butterbur extract could inhibit IgE-sensitized RBL-2H3 cell degranulation, LTC4/D4/E4 synthesis. Because LT synthesis depends on cPLA2 activity, a blockade of cPLA2 would block LT production; this could be one of the effects of PTK extract seen here.

Apart from its effects on formation of various LT forms, the Chinese PTK was also able to impact the levels of ECP, tryptase, and PGE2 in the sensitized host rats. The decreases in levels of these inflammatory mediators enhanced the potential for efficacious use of PTK extract against AR. Indeed, large-scale physiologic measures, including measures of overall nasal symptoms (sneezing, nasal rubbing, rhinorrhea, and nasal congestion) and host the quality of life evaluated in the present study indicated that PTK extract could significantly alleviate the nasal symptoms and permit the sensitized hosts to have a better quality of life compared to that of control non-PTK counterparts.

Although the preventive effects of PTK extract on AR were studied here, yet further researches need to be done about the precise components in the PTK extract.

**Conclusion**

Administration of the PTK ethanol extract produced here could alleviate nasal symptoms and improve the rats’ quality of life by mitigating the onset/intensity of any inducible allergic rhinitis. In these extract-treated hosts, after intranasal challenge with the test antigen, levels of inflammatory mediators in serum and nasal fluids were all significantly decreased. Moreover, the synthesis of cysteinyl-LTs, LTB4 and PGE2 ex vivo were all significantly inhibited by the extract, suggesting to us that PTK extract might act as LTs or LTs receptor inhibitor and/or a blocker of LT biosynthesis.

**References**

Expansion of cytokine-producing CD4-CD8-T cells


Model of allergic rhinitis in rats by topical sensitization


WL and FYG: designed the research, performed the experiments, interpreted the data and wrote the manuscript.

HDJ: supervised, headed and financed the work and helped editing the manuscript.

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