Treatment of asthma patients with herbal medicine TJ-96: a randomized controlled trial

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Abstract  Alternative medicine use has increased at a remarkable pace all over the world in recent years. Although herbal medicine for the treatment of asthma is becoming the focus of public attention, randomized studies had not been performed, even in Eastern countries including Japan. This study was designed to investigate whether one of the Japanese government approved herbal complexes Saiboku-to (TJ-96) is effective for the treatment of atopic asthma, and to investigate whether this protective activity is associated with a reduction in eosinophilic inflammation. A double-blind, randomized, crossover design was used. Subjects received 2.5 g of TJ-96 or placebo orally 3 times daily for 4 weeks and then, after a washout period of at least 4 weeks, crossed over to receive the alternative treatment. We assessed the effects of pretreatment with TJ-96 on bronchoconstriction precipitated by inhalation of methacholine. Furthermore, eosinophil counts and measurement of eosinophilic cationic protein (ECP) were performed. After 4 weeks of treatment with TJ-96, values of PC20-methacholine significantly improved in the treatment with TJ-96. Also, patients’ symptoms, blood eosinophils, serum ECP, sputum eosinophils, and sputum ECP were significantly decreased. Our results suggest that TJ-96 has an antiinflammatory effect on bronchial eosinophilic infiltration. This study raises further interesting therapeutic possibilities and argues for further trials of new approaches to the treatment of asthma.

Keywords  bronchial asthma; eosinophilic cationic protein; TJ-96 (“Saiboku-to” or “Chai-pu-tang”); bronchial eosinophilic inflammation.

INTRODUCTION

Herbal therapy for the treatment of asthma has been established long time ago in Japan. Since Japanese Ministry of Health and Welfare officially approved traditional herbs for medical use, the governmental health insurance cover the cost of treatments with them. Surprisingly, randomized studies had not been performed for the approval, although the Japanese government authorized 161 complexes of traditional herb (1). Japanese herbal medicine is called “Kampo”, which was originated from ancient China, modified and sophisticated in the history of traditional Japanese medicine.

In Japanese National Guidelines for the Management of Asthma, six kinds of herbal complexes are included as recommended therapy for the treatment of asthma (2). Especially, “Saiboku-to” (“Chai-pu-tang” in Chinese, TJ-96) is one of the most popular herbal complexes for long-time management of asthma. A previous study showed that TJ-96 is effective in reducing bronchial hyperresponsiveness and allergen-induced eosinophilic infiltration in sensitized guinea pigs (3). The aims of the present study were (1) to confirm these observations of TJ-96’s antiasthmatic activity and other effects on bronchial eosinophilic infiltration and (2) to examine whether the protective activity of this drug against asthma is associated with a reduction in the eosinophilic inflammation that contributes to the pathogenesis of asthma. A double-blind, randomized, crossover design was used.

MATERIALS AND METHODS

Subjects

We studied 33 adult patients (15 males and 18 females; ages, 42 ± 7 (sd) years; FEV1, 81.5 ± 6.9 (sd) %pred.) with...
atopic asthma. The atopy was determined by skin test and serum IgE level. All patients were nonsmokers (never smokers only), neither were aspirin intolerant nor COPD, and all had mild or moderate asthma diagnosed according to the criteria of the American Thoracic Society (4). The patients were in stable condition and had been free of symptoms of respiratory infection for at least 6 weeks.

Patients using any herbal medicine including TJ-96 before, oral or inhaled corticosteroids, any antileukotriene drug, and chemical mediator inhibitor such as cromolyn sodium were excluded. All patients used a metered-dose inhaler of β-stimulants on demand and/or theophylline to control their asthma. The study was conducted according to the ethical standards of our medical center, which require informed consent from each patient.

**Study design**

A double-blind, randomized, crossover design was used. Subjects were randomly assigned to receive orally a 4-week course of daily dose of 2.5 g of readymade freeze-dried powdered Saiboku-to TJ-96 (Tsumura Co., Ltd., Tokyo, Japan) or a dose of placebo that was identical in appearance and taste. After a washout period of at least 4 weeks, the subjects crossed over to receive the alternative treatment for 4 weeks.

FEV1, FVC, and methacholine provocation testing were performed. Blood and induced-sputum, differential and total cell counts were determined before and after each 4-week treatment course.

**Blood count and serum eosinophilic cationic protein (ECP) measurement**

Blood samples for eosinophil counting and measurement of ECP were taken between 8:00 and 9:00 a.m. on the day of methacholine provocation testing (5). Blood was collected in heparinized tubes by direct venipuncture, and blood smears were made for differential staining (Diff quick; American Scientific Products, McGawPark, IL). Differential cell counts were calculated as the mean value of two slides, with 300 cells counted per slide. The total leukocyte count was determined with a hemocytometer (Neubauer Chamber: Hauser Scientific, Blue Bell, PA), and cell populations were expressed as number of cells per milliliter of blood.

To avoid interassay variations, all ECP measurements were performed in the same run. Blood 4 ml was collected in an SST tube (Becton Dickinson, Mountain View, CA), stored for 60 min at 25°C, and subsequently centrifuged at 1300 × g for 10 min at 4°C. Serum was stored at −80°C until ECP levels were measured with an ECP RIA (radioimmunoassay) kit (Pharmacia Diagnostics, Uppsal, Sweden). The detection limit of this assay is 2.0 μg/l.

**Sputum analysis**

Sputum was induced and processed as described by Popov et al. (5) Subjects inhaled 3, 4, and 5% saline for 7 min each. The induction was stopped when an adequate sample was obtained or if the FEV1 dropped ≥ 20% from baseline. Cell plugs with few or no squamous epithelial cells were selected from the sample with an inverted microscope, separated from saliva, and weighed. Samples were aspirated in twice their volume of 0.1% dithiothreitol (Sputolysin, Calbiochem Corp., San Diego, CA) and twice their volume of Dulbecco's phosphate-buffered saline (DPBS; Gibco, Grand Island, NY). The cell suspension was filtered through nylon gauze (mesh size is 52 μm; BN Sh Thompson, Scarborough, ON, Canada) to remove debris, then centrifuged at 1300 × g for 10 min at 4°C. Supernatants were collected and stored at −80°C for fluid-phase measurements of ECP.

The total cell count was determined with a hemocytometer (Neubauer Chamber) and expressed as the number of cells per milliliter of sputum. Cells were resuspended in DPBS at a concentration of 0.75−1.0 × 10^6/ml. Cell preparations for cytocentrifugation were made on glass slides from 50 μl of cell suspension and subjected to cytocentrifuge (Shandon III, Shandon Southern Instruments, Sewickeey, PA), at 300 × g for 5 min. Differential cell counts were obtained from the mean of two slides stained with Diff Quick with 400 cells counted per slide. Cell types were quantitated as the number of cells per milliliter of sputum. Cell preparations for cytocentrifugation were made on apex-coated slides and fixed for 10 min in periodate-sine-paraformaldehyde for immunocytochemical staining for ECP.

**Methacholine provocation testing**

Bronchial responsiveness was evaluated by methacholine provocation according to the standard method of the Japanese Society of Allergology (6,7) This test employed methacholine chloride (Sigma, St. Louis, MO), dissolved in saline to make solutions of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 mg/ml administrated by a jet nebulizer driven by compressed air (Nihon Shoji Co., Ltd. Osaka, Japan).

All patients were given a provocation test after a 4-week treatment course with either TJ-96 or placebo in a double-blind, randomized manner. Thereafter subjects were crossed over to receive alternative treatment, and following an identical procedure at the same time of day. To avoid the acute-phase effect of TJ-96, each provocation test was performed before taking TJ-96 in the morning. Each study day began with measurement of FEV1 and FVC for baseline, followed by inhalation of nebulized saline to rule out a nonspecific obstructive reaction; FEV1 was measured 5 min before and 5, 10, and 15 min after
completion of inhalation. If FEV$_1$ did not fall more than 10%, the subject entered the main part of study.

Methacholine was inhaled for 2 min by tidal breathing with a nose clip, immediately followed by spirometry. Methacholine of increasing concentration was successively inhaled until FEV$_1$ fell by 20% or more from baseline. The measured values were plotted on semilogarithmic graph paper, and the methacholine provocative concentration producing a 20% fall in the FEV$_1$ was calculated as PC$_{20}$-methacholine. The provocation test was terminated by the administration of an inhaled bronchodilator if FEV$_1$ fell by more than 35% from baseline. The higher of the first two satisfactory recordings was taken for analysis.

**Symptom score**

To evaluate the effect of TJ-96 on the improvement of symptoms in asthmatics, symptoms were assessed weekly by physicians using four grades: 0=asymptomatic more than 4 days/week; 1=mild symptom (aware of symptoms but easily tolerated) more than 4 days/week and nocturnal asthma symptoms less than 2 days/week; 2=moderate symptom (discomfort enough to interfere with daily activity) more than 4 days/week and/or nocturnal asthma symptoms more than 2 days/week; and 3=severe attack (unable to perform usual activity) more than 4 days/week and/or nocturnal asthma symptoms almost everyday. This method is modeled after that of Oosaki et al. (8).

**Statistical analysis**

Statistical evaluation employed StatView J-4.02 for Macintosh (Abacus Concepts, Inc., Berkeley, CA). All PC$_{20}$ values were logarithmically transformed before analysis, and summary statistics were, expressed as mean ± standard deviation (SD). Student's paired t-test was used to compare mean differences. Wilcoxon’s signed-rank test was used for comparison of the two treatment groups at the same time point regarding FEV$_1$ and ECP values. A $P$ value less than or equal to 0.05 (two-sided) was considered significant.

**RESULTS**

After the 4 weeks of treatment with TJ-96, none of the subjects reported any adverse effect, nor could they discriminate between TJ-96 and placebo. The FEV$_1$ was slightly improved but not significant by administration of TJ-96 after 4-weeks’ each treatment (Table I).

Twenty-seven of the 32 subjects improved in general symptoms, such as nocturnal cough, wheezing, and severity or frequency of asthmatic attack. Other subjects felt no change in their symptoms, since their conditions were stable. No subject got worse than before the beginning of this study. The symptom score significantly decreased after treatment with TJ-96 (Table I, 0.73 ± 0.25 after TJ-96 vs 1.69 ± 0.39 after placebo, $P < 0.001$).

Blood eosinophils, serum ECP, sputum eosinophils, and sputum ECP were significantly decreased by TJ-96 (Table 2). On the other hand, the number of blood and sputum neutrophils did not change significantly.

On methacholine provocation day, all subjects were followed for longer than 120 min after provocation test. After pretreatment with TJ-96 or placebo, challenge with methacholine caused an obstructive reaction in all patients. Data for PC$_{20}$-methacholine in all patients are displayed in Fig. 1. In the provocation challenge with methacholine, values of PC$_{20}$-methacholine significantly improved after TJ-96. Mean log PC$_{20}$-methacholine was 2.93 ± 0.54 after TJ-96, vs 2.52 ± 0.55 after placebo ($P < 0.01$).

In the present study, after 4-weeks’ treatment with either TJ-96 or placebo, there is no significant association between the improvement of PC$_{20}$-methacholine and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Mean ± SD of indicated parameter</th>
<th>TJ-96 Mean ± SD of indicated parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$_1$ (L)</td>
<td>2.88 ± 0.14</td>
<td>2.89 ± 0.11</td>
</tr>
<tr>
<td>%FEV$_1$</td>
<td>81.0 ± 6.0</td>
<td>82.1 ± 5.6</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.88 ± 0.24</td>
<td>3.84 ± 0.20</td>
</tr>
<tr>
<td>%FVC</td>
<td>93.0 ± 4.0</td>
<td>898 ± 3.6</td>
</tr>
<tr>
<td>Symptom score</td>
<td>1.66 ± 0.43</td>
<td>1.65 ± 0.39</td>
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Note: All values marked with asterisks were significantly greater than baseline: *$P < 0.001$ baseline.
ECP values, or between the decreasing of eosinophils and the improvement of PC20-methacholine. In addition, Wilcoxon’s signed-rank test showed no significant correlation between the maximum falls in FEV1 or the concentration of methacholine inhaled and the corresponding maximum decrease in ECP values after placebo. All the data for before placebo, after placebo, before TJ-96 were not different significantly.

**DISCUSSION**

Eosinophils are among the most important components of bronchial inflammation and bronchial hyperresponsiveness. Serum ECP is thought to be a useful marker of eosinophilic inflammation in bronchial asthma (9–12). Release of ECP and other granular proteins may contribute to epithelial damage and inflammation and thereby to an increase in bronchial hyperresponsiveness. Also, ECP has been reported to elevate bronchoalveolar lavage fluid, to play an active secretory role of the eosinophil in the late-phase asthmatic reaction, and play an active role in the late-phase asthmatic reaction (13). In this study, 4 weeks of treatment with TJ-96 lowered the symptom scores and decreased bronchial responsiveness to methacholine in patients with atopic asthma, although neither FVC nor FEV1 increased. Moreover, TJ-96 treatment decreased both blood and sputum ECP levels as well as blood and sputum eosinophil counts. Thus TJ-96 has an antiasthmatic activity which is not associated with bronchodilatation but with inhibition of eosinophil infiltration.

Japanese traditional herbal therapy is so systemic, but its theory is completely different from Western medicine. Doctors who want to use herbal medicine should learn how to evaluate patients’ conditions, and how to choose the best herbal medicine for the improvement of patients’ illness along with the theory of Oriental medicine. They also have to distinguish patients whether responders or nonresponders according to their expertise before beginning herbal therapy. The fundamental rules to choose appropriate herbal medicine are based on patients’ constitution, predisposition, stamina, and strength of response against illness. However, we did not distinguish the patients according to the diagnosis.
along with the theory of Oriental medicine, because this study was a randomized controlled study. Our study revealed that TJ-96 is significantly effective for the management of allergic asthma even without the diagnosis along with the theory of Oriental medicine.

TJ-96 is one of the most useful non-steroidal therapeutic Kampo drugs for the treatment of asthma. Although the inhibitory action of TJ-96 on antiasthmatic activity is well documented, its mechanism is less well defined. Previous studies showed several pharmacological effects in vivo and in vitro. Tohda et al. reported that TJ-96 inhibited both immediate asthmatic response and late asthmatic response, infiltration of eosinophils and T-lymphocytes into the lung parenchyma in sensitized guinea pigs (14). Interestingly, a remarkable steroid sparing effect of TJ-96 was noted within 6–12 months of treatment in steroid-dependent asthmatic patients (15, 16). TJ-96 spared the downregulation of glucocorticoid receptor of human lymphocytes, plasma ACTH, and cortisol levels. It also spared downregulation of $\beta_2$ receptor by $\beta_2$ agonists and suppressed mACH receptor at the same time. Furthermore, TJ-96 inhibited the induction of expression of IgE-Fc â€¢ R/CD23 by mite allergen in the lymphocytes of the patients with mite-allergic asthma (15). Nakajima et al. showed TJ-96 inhibited IgE production by mite allergens, and decreased the number of eosinophils in the bronchoalveolar lavage fluid during late asthmatic response. These findings suggest that TJ-96 may be effective in inhibiting both the expression of IgE-Fcâ€¢R2 and the induction of expression of IgE-Fcâ€¢R1. Also, it has been reported that TJ-96 also has a steroid-like action and polyhedral antiasthmatic activities (16). In vitro, TJ-96 modulates basophil growth and differentiation (17), eosinophilic infiltration (3), platelet-activating factor production in human neutrophils (18), and histamine release from mast cells (19). Konno et al. reported that TJ-96 selectively stimulates Na absorption across the tracheal epithelium through intracellular accumulation of cyclic AMP and NO generation (20,21). Moreover, it has been reported that TJ-96 inhibits 5-lipoxygene genase activity in leukotriene synthesis in rat basophilic leukemia-I cells (22). These properties indicate that TJ-96 can modulate immune responses both in vitro and in vivo.

Some previous reports suggested that one of the active ingredients of TJ-96, magnolol might contribute to the antiasthmatic effects and the inhibitory effects on prednisolone metabolism through inhibition of Ilb-hydroxy steroid dehydrogenase (23–25). Magnolol, isolated from Magnolia officinalis, has anti-inflammatory effect, reversed passive Arthus reaction (26), and inhibits leukotriene release from basophils (27). To determine the exact mechanisms of antiasthmatic effect of TJ-96, additional studies will be required.

In this study, our results provide clinical support for these previous investigations. This study raises further interesting therapeutic possibilities and supports the use for further trials of new approaches to the treatment of asthma.

REFERENCES


