

# Antiinflammatory effects of Bu-zhong-yi-qi-tang in patients with perennial allergic rhinitis

Sien-Hung Yang<sup>a,b,\*</sup>, Chia-Li Yu<sup>c</sup>

<sup>a</sup> School of Chinese Medicine, Chang Gung University, Taoyuan, Taiwan

<sup>b</sup> Department of Chinese Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC

<sup>c</sup> Institute of Molecular Medicine, National Taiwan University College of Medicine, Taipei, Taiwan, ROC

Received 19 August 2006; received in revised form 13 September 2007; accepted 13 September 2007

Available online 19 September 2007

## Abstract

Bu-zhong-yi-qi-tang, an ancient formula of Chinese medicine usually used in the treatment of allergic diseases, was evaluated in the treatment of patients with perennial allergic rhinitis. In this study, 60 patients allergic to house dust mite allergen confirmed by skin test and MAST test were recruited and randomized. An experimental group of 36 patients was treated with Bu-zhong-yi-qi-tang, whereas a control group of 24 patients was treated with a non-effective formula Ping-wei-san for 3 months. The nasal symptomatic scores and the responses of polymorphonuclear neutrophils (PMN) to IL-4-stimulation were measured after treatment. The nasal symptomatic scores in the experimental group were significantly improved ( $3.78 \pm 0.09$  before treatment vs.  $0.57 \pm 0.06$  after treatment). In contrast, no change was found in symptomatic scores in the control group ( $3.17 \pm 0.12$  before treatment vs.  $2.79 \pm 0.14$  after treatment). Moreover, total serum IgE and the IL-4-stimulated production of PGE<sub>2</sub> and LTC<sub>4</sub> by PMN was significantly suppressed in the experimental group after treatment compared to the control group. The COX-2 mRNA expression in IL-4-stimulated PMN was also significantly suppressed after Bu-zhong-yi-qi-tang treatment. These results suggest that Bu-zhong-yi-qi-tang but not Ping-wei-san was beneficial to the patients with perennial allergic rhinitis via suppressed nasal inflammation by an antiinflammatory effect.

© 2007 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Traditional Chinese medicine; Allergic rhinitis; Bu-zhong-yi-qi-tang; Immunoglobulin E; Prostaglandin E<sub>2</sub>; Leukotriene C<sub>4</sub>; Cyclooxygenase 2 mRNA

## 1. Introduction

Allergic rhinitis (AR) is an important disease in Taiwan with an estimated prevalence rate approximately 15%. Many patients in Taiwan choose the Chinese herbal medicine for the treatment of allergic rhinitis. Bu-zhong-yi-qi-tang (BZYQT), a formula of Chinese herbal medicine, has been used clinically to prevent the recurrent attack of allergic rhinitis. In our previous studies, we found that treatment of a mixed formula of Chinese herbal medicine significantly enhanced IL-10 but decreased IFN- $\gamma$  and IL-5 production by PHA-stimulated peripheral mononuclear cells (MNC). In addition, the COX-2 mRNA expression in stimulated PMN cells was also sig-

nificantly suppressed after mixed herbal formula treatment. These results suggest that the new mixed formula treatment is beneficial to the patients with perennial allergic rhinitis via modulating the function of lymphocytes and neutrophils (Yang et al., 2001). Further study demonstrated that the new mixed herb formula treatment suppressed nasal mucosa inflammation by normalizing stimulatory effects of allergic nasal discharge of patients with high-IgE allergic rhinitis (Yang et al., 2002). According to the theory of traditional Chinese medicine, individual prescription was effective for acute attacks and/or non-acute afflictions of allergic rhinitis. It is conceivable that BZYQT is usually used in non-acute afflictions, because it is considered a preventive medicine for patients with allergic rhinitis.

Recently, BZYQT has been reported with many immunomodulatory effects such as decreasing the recurrent attack rate of allergic rhinitis and asthma, improving semen quality with treatment (Furuya et al., 2004), suppressing IgE levels in animal models of atopic dermatitis (Kobayashi

\* Corresponding author at: Department of Chinese Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC. Tel.: +886 3 3196200x2611; fax: +886 3 3298995.

E-mail address: [dryang@adm.cgmh.org.tw](mailto:dryang@adm.cgmh.org.tw) (S.-H. Yang).

et al., 2003), antibacterial effect against *Helicobacter pylori* infection in mice (Yan et al., 2002), suppressing chronic contact hypersensitivity (Nakada et al., 2002), suppressive effect on collagen-induced arthritis in mice (Hai et al., 2002), radioprotective effects in mice (Kim et al., 2002), inhibiting proliferation of hepatoma cell lines (Kao et al., 2001), modulating allergic inflammation in a murine model for asthma (Ishimitsu et al., 2001), anti-aging effects in mice (Shih et al., 2000) and suppression of IgE production in mice (Kaneko et al., 1997). All of these effects are relevant to the immunomodulatory effects of BZYQT.

Based on these facts, this study was designed to answer the following two questions: first, whether BZYQT is beneficial to the patients of allergic rhinitis in the non-acute stage; secondly, whether BZYQT exhibits antiinflammatory effects in the treatment of allergic rhinitis. Accordingly, a study group on BZYQT and a control group on Ping-wei-san (PWS) that does not contain immunomodulatory effects was compared. The symptomatic score, serum total IgE levels, and IL-4-stimulated PGE<sub>2</sub>, LTC<sub>4</sub> and COX-2 gene expression of PMNs were evaluated for the antiinflammatory effects of BZYQT.

## 2. Materials and methods

### 2.1. Patients and subgroups

Sixty patients (20 females and 40 males, age range from 17 to 32 years) suffered from perennial allergic rhinitis were enrolled in this study. These patients were randomized into two groups. Those patients treated with BZYQT served as a study group (22 males and 14 females) and those treated with PWS served as a control group (18 males and 6 females).

### 2.2. Medications and interleukins

The medications were purchased from the Sun Ten Pharmaceutical Co., Ltd. Batch number of BZYQT and PWS were 064845 and 214743 with production date on 17 December 2004 of BZYQT and 26 November 2004 of PWS, this batch of BZYQT was analyzed by HPLC before the study in order to quarantine the quality of medication. The batched BZYQT contained glycyrrhizin 62.46 mg/day (range: 31.50–94.50 mg/day), hesperidin 55.67 mg/day (range: 23.00–69.00 mg/day) and less than 50 ppm of the total heavy metals. The compositions of BZYQT and PWS are listed in Tables 1 and 2, respectively. Each patient received BZYQT or PWS in powdered form in three times/day after meal in a package of 4 g for 3 months. No patient withdrew from the study due to intolerability or adverse events. The clinical trial was approved by Medical Ethics and Human Clinical Trial Committee of Chang Gung Memorial Hospital, Taipei, Taiwan (CGMH IRB No. 94-250B, issued date: 6 February 2006). Informed consent was obtained from each participant.

Interleukin-4 (IL-4) and interleukin-13 (IL-13) were purchased from R&D, Minneapolis, MN, USA.

Table 1

Components of Bu-zhong-yi-qi-tang (every 12 g of a water extract are derived from 27 g of the raw materials)

Plant name	Plant part	Ratio (g)
<i>Astragalus mongholicus</i> Bunge.	Root	6.0
<i>Panax ginseng</i> C.A.Mey.	Root	4.0
<i>Citrus reticulata</i> Blanco.	Pericarp	2.0
<i>Atractylodes macrocephala</i> Koidz.	Rhizome	2.0
<i>Angelica dah-rica</i> Fisch. ex Hoffm.	Root	2.0
<i>Cimicifuga foetida</i> L.	Rhizome	1.0
<i>Bupleurum chinense</i> DC.	Root	1.0
<i>Zingiber officinale</i> Rosc.	Rhizome	3.0
<i>Ziziphus jujuba</i> Mill. var. <i>inermis</i> Rehd.	Fruit	2.0
<i>Glycyrrhiza uralensis</i> Fisch.	Root and rhizome	4.0

Table 2

Components of Ping-wei-san (every 4.5 g of a water extract are derived from 8.5 g of the raw materials)

Plant name	Plant part	Ratio (g)
<i>Atractylodes lancea</i> Thunb.	Rhizome	4.0
<i>Magnolia officinalis</i> Rehd. Et Wils.	Bark	1.5
<i>Citrus reticulata</i> Blanco.	Pericarp	1.0
<i>Zingiber officinale</i> Rosc.	Rhizome	0.5
<i>Ziziphus jujuba</i> Mill. var. <i>inermis</i> Rehd.	Fruit	0.5
<i>Glycyrrhiza uralensis</i> Fisch.	Root and rhizome	1.0

### 2.3. Clinical evaluation

The nasal symptoms of the allergic rhinitis were evaluated according to the scoring system of Okuda and co-workers (1984), as listed in Table 3. The nasal symptoms including the number of sneezing attacks, the number of nose blowing, and the degree of nasal obstruction were recorded daily by patients using a recording card. The three major nasal symptoms (sneeze, rhinorrhea and obstruction) were scored in 0–3, depending on their severity. The percent improvement of nasal symptoms was calculated before and after 3-month treatment by the following formula:

$$\% \text{improvement} = \frac{\text{scores before treatment} - \text{scores after treatment}}{\text{scores before treatment}} \times 100$$

### 2.4. Isolation of polymorphonuclear neutrophils (PMN)

Heparinized venous blood obtained from patients was mixed with one-quarter volume of 2% dextran solution (molecular weight 500,000) and incubated for 30 min at room temperature. The leukocyte-enriched supernatants were aspirated and diluted with the same volume of Hanks' balanced salt solution (HBSS). After Ficoll-Hypaque (specific gravity 1.077) density gradient centrifugation at 150 × g for 25 min. After three washes, PMN were suspended in 10% fetal bovine serum (FBS) in RPMI-1640 (10% FBS-RPMI). The cell concentration of PMN was adjusted to 2 × 10<sup>6</sup> cells/ml. The viability of these cell suspensions was confirmed >95% by trypan blue dye exclusion.

Table 3  
Scoring of nasal symptoms (as proposed by Okuda et al.)

Scoring of sneeze
0: no sneezing attack
1: the number of sneezing attack is 1–5
2: the number of sneezing attack is 6–10
3: the number of sneezing attack is over 11
Scoring of rhinorrhea
0: no nasal blowing
1: the number of nasal blowing is 1–5
2: the number of nasal blowing is 6–10
3: the number of nasal blowing is over 11
Scoring of nasal obstruction
0: no nasal obstruction
1: nasal obstruction without mouth breathing
2: nasal obstruction with sporadic mouth breathing
3: nasal obstruction with predominant mouth breathing

### 2.5. Determination of serum total IgE level

The serum total IgE level was determined by a commercially available ELISA kit (Immunotech, Marseille, France). The results were denoted by KIU/L. One KIU represents 2.4 ng of IgE. The minimal detectable concentration of IgE by the kit was 5.75 KIU/L.

### 2.6. Measurement of prostaglandin $E_2$ ( $PGE_2$ ) and leukotriene $C_4$ ( $LTC_4$ ) in PMN-cultured supernatants by ELISA

The concentrations of  $PGE_2$  and  $LTC_4$  in IL-4-treated PMN-cultured supernatants were determined by commercially available ELISA kits (R&D). The minimal detectable amount was 0.1 pg/ml for  $PGE_2$  and 0.04 pg/ml for  $LTC_4$ , respectively.

## 3. Detection of COX-2 mRNA expression in PMN by reverse transcription-polymerase chain reaction (RT-PCR)

### 3.1. Total RNA extraction and cDNA synthesis

The total cellular RNA was extracted from PMN ( $5 \times 10^6$  cells/ml) after incubation with medium, IL-4 (10 ng/ml) or IL-13 (10 ng/ml) for 3 h at 37 °C in 5%  $CO_2$ –95% air according to the method of Chomczynski and Sacchi (1987). cDNA was synthesized by priming 1  $\mu$ g/ml of total RNA at 42 °C for 1 h in a final volume of 20  $\mu$ l containing 1  $\mu$ g of oligo-dT primer (Pharmacia Fine Chemicals, Piscataway, NJ, USA), 200 nmole of each dNTP (Pharmacia), and M-MLV reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD, USA) at 200 U/ $\mu$ g RNA.

### 3.2. Amplification of cDNA by PCR

Aliquots of cDNA were amplified by PCR using oligonucleotide pair primers specific for human COX-2 (Clontech Laboratories, Inc., Palo Alto, CA, USA) as shown below.

hCOX-2:	5'-TTC AAA TGA GAT TGT GGG AAA ATT GCT-3' (sense)
	5'-AGT TCA TCT CTG CCT GAG TAT CTT-3' (anti-sense)
hG3PDH:	5'-ACC ACA GTC CAT GCC ATC AC3' (sense)
	5'-TCC ACC ACC CTG TTG CTG TA3' (anti-sense)

Primers for human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were used to amplify the ubiquitous molecule in the cells as an internal control. A HYBAID OmniGene DNA Thermal Cycler (Teddington, Middlesex, TW, UK) was run for 26 cycles of denaturation at 94 °C for 1 min and annealing/extension at 65 °C for 2 min in the case of G3PDH. Thirty-five cycles of denaturation at 95 °C for 1 min and annealing/extension at 60 °C for 2 min were conducted for COX-2. The PCR products were electrophoresed in 1.8% agarose gel using  $\phi$ X174/Hae III as the calibration markers. The cDNA fragments amplified by these sets of primers were 305 bp for hCOX-2 and 452 bp for hG3PDH.

### 3.3. Statistical analysis

Results represent mean  $\pm$  S.D. Difference between the two groups was assessed by non-parametric Wilcoxon's signed-rank test. A  $p$  value  $<0.05$  was considered significant difference.

## 4. Results

### 4.1. The effects of both regimens on nasal symptom scores of AR patients after 3-month treatment

As demonstrated in Table 4, the nasal symptom scores were significantly decreased in the BZYQT group from  $3.21 \pm 0.08$  (before treatment) to  $0.56 \pm 0.09$  (after treatment) ( $p < 0.05$ ). No significant change found in the PWS group before ( $3.17 \pm 0.12$ ) and after treatment ( $2.79 \pm 0.14$ ).

### 4.2. Serum total IgE levels

The serum total IgE levels were significantly decreased in the BZYQT group after 3-month treatment from  $285.9 \pm 24.8$  KIU/L to  $132.7 \pm 26.2$  KIU/L ( $p = 0.028$ ) whereas there was no change in serum total IgE levels in the PWS group before ( $236.27 \pm 28.6$  KIU/L) and after ( $219.0 \pm 29.0$  KIU/L) treatment (Fig. 1).

Table 4  
Changes of nasal symptom scores before and after 3-month treatment in both groups

Group	Nasal symptom score	
	Before treatment	After treatment
Study group ( $n = 36$ ) <sup>a</sup>	$3.21 \pm 0.08$	$0.56 \pm 0.09$ <sup>c</sup>
Control group ( $n = 24$ ) <sup>b</sup>	$3.17 \pm 0.12$	$2.79 \pm 0.14$

<sup>a</sup> Study group is those patients treated with Bu-zhong-yi-qi-tang.

<sup>b</sup> Control group is those patients with Ping-wei-san.

<sup>c</sup> Denotes  $p < 0.05$ , compared to "before treatment".

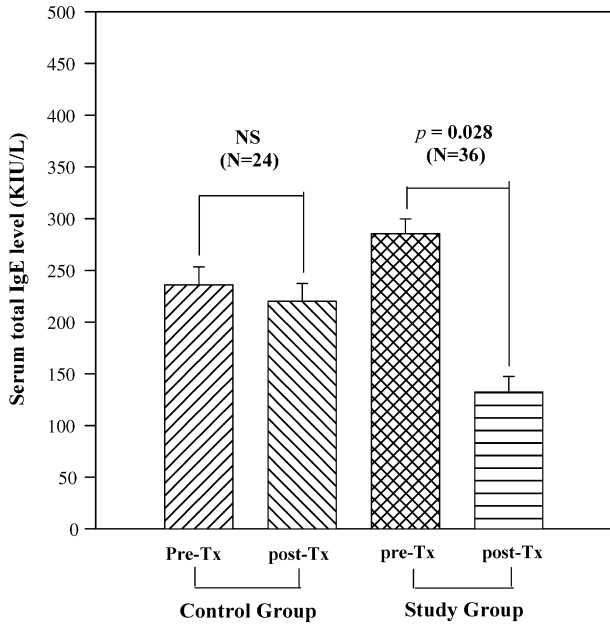


Fig. 1. Comparison of serum IgE levels in IL-13-stimulated MNC-cultured supernatants before and after treatment of BZYQT (study group) and PWS (control group) on AR patients for 3 months. IgE levels were attenuated after treatment of BZYQT, but not in the treatment of PWS.

4.3. The concentrations of PGE<sub>2</sub> and LTC<sub>4</sub> in IL-4-stimulated PMN-cultured supernatants of AR patients before and after treatment

The concentrations of PGE<sub>2</sub> in IL-4-stimulated PMN-cultured supernatant were significantly decreased in the BZYQT group after 3-month treatment from 2.13 ± 0.43 ng/μl to 1.43 ± 0.28 ng/μl (p=0.021) whereas no change was

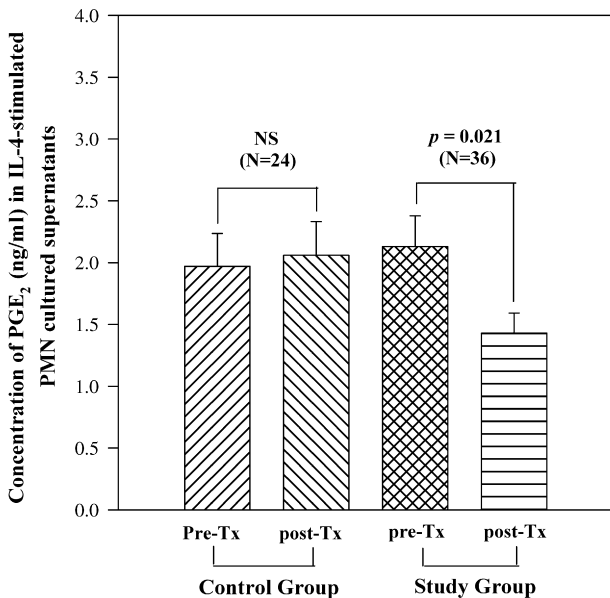


Fig. 2. Comparison of serum PGE<sub>2</sub> levels in PMN-cultured supernatants before and after treatment of BZYQT (study group) and PWS (control group) on AR patients for 3 months. PGE<sub>2</sub> levels were attenuated after treatment of BZYQT, but not in the treatment of PWS.

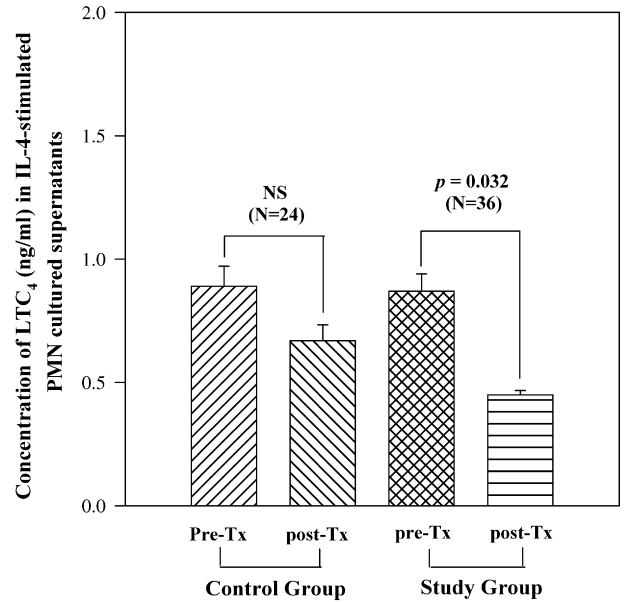


Fig. 3. Comparison of serum LTC<sub>4</sub> levels in PMN-cultured supernatants before and after treatment of BZYQT (study group) and PWS (control group) on AR patients for 3 months. LTC<sub>4</sub> levels were attenuated after treatment of BZYQT, but not in the treatment of PWS.

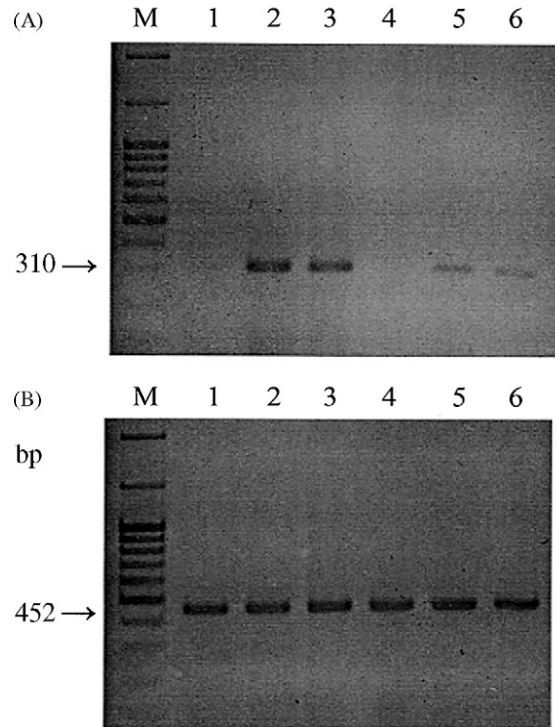


Fig. 4. A representative case demonstrating COX-2 mRNA expression in PMN after stimulated with IL-4 and IL-13 from AR patients before and after 3-month treatment with both groups. Lane 1: incubation with medium before treatment, lane 2: incubation with IL-4 before treatment, lane 3: incubation with IL-13 before treatment, lane 4: incubation with medium after treatment, lane 5: incubation with IL-4 after treatment, lane 6: incubation with IL-13 after treatment. The same experiment was conducted for three times with a similar tendency.

found in PWS group before ( $1.97 \pm 0.46 \text{ ng}/\mu\text{l}$ ) and after ( $2.06 \pm 0.47 \text{ ng}/\mu\text{l}$ ) treatment (Fig. 2). Similarly,  $\text{LTC}_4$  concentrations in PMN-cultured supernatant were significantly decreased in the BZYQT group after 3-month treatment from  $0.87 \pm 0.12 \text{ ng}/\mu\text{l}$  to  $0.45 \pm 0.03 \text{ ng}/\mu\text{l}$  ( $p = 0.032$ ) whereas no change was found in  $\text{LTC}_4$  levels in the PWS group before ( $0.89 \pm 0.14 \text{ ng}/\mu\text{l}$ ) and after ( $0.676 \pm 0.11 \text{ ng}/\mu\text{l}$ ) treatment (Fig. 3).

#### 4.4. Effects on COX-2 mRNA expression in cytokine-stimulated PMN after BZYQT treatment

COX-2 mRNA expression (310 bp) in PMN was measured by RT-PCR after stimulation with medium, IL-4 and IL-13 for 60 min in the BZYQT (A) before (lanes 1–3) and after (lanes 4–6) 3 months of BZYQT treatment. As shown in Fig. 4, the expression of COX-2 mRNA (305 bp) in PMN was markedly enhanced by stimulation with IL-4 (A) (lane 2) and IL-13 (A) (lane 3) before BZYQT treatment. This stimulating effect was attenuated after 3-month treatment (A) (lanes 5 and 6). Panel B is the mRNA expression of G3PDH (452 bp) as internal control. There is no change before and after treatment with PWS for 3 months.

## 5. Discussion and conclusion

Allergic rhinitis is an important disease with high prevalence in Taiwan. Chinese traditional herbs have been tried in the treatment of allergic diseases by many authors. Kaneko et al. (1997) reported that a Chinese popular herb, BZYQT was effective in decreasing serum IgE level in mice. However, the study was conducted in animals, we are the first group to evaluate the effects of BZYQT on human perennial allergic rhinitis.

Our previous studies have shown that mixed herbal medicine was beneficial for the patients with perennial allergic rhinitis (Yang et al., 2001, 2002) via the mechanism of modulating the function of lymphocytes and neutrophils. Nevertheless, by the theory of Chinese traditional medicine, medical treatment during the non-acute stage of allergic rhinitis is more important than treatment during acute attacks. It suggests that the prevention for acute attacks of allergic rhinitis is the key purpose for Chinese medicine treatment.

BZYQT, a well-known herbal medicine was traditionally used as preventive therapy in allergic rhinitis during non-acute stages. Nevertheless, the molecular basis for the anti-allergic effect of BZYQT in the treatment of patients with allergic rhinitis has not been elucidated.

We evaluated the nasal symptoms of the perennial AR patients in the same season to avoid the seasonal variation of nasal manifestations. We clearly demonstrated that the nasal score in BZYQT group significantly decreased after the 3-month treatment whereas no improvement in the PWS group after treatment (Table 4). In addition, a significant inhibition of serum total IgE production by BZYQT treatment was also found that is compatible with the report of Kaneko et al. (1997) in mouse experiment.

It is conceivable that chronic mucosa inflammation in nasal tissue plays an important role in the recurrent attacks of allergic rhinitis (Foresi et al., 2000). The major infiltrated cells in the nasal mucosa of patients with allergic rhinitis (AR) include eosinophils and neutrophils (Savill, 1997; Hans-Uwe, 1999). Foresi et al. (2000) have demonstrated that abnormal prolongation of leukocyte life span occurred in the site of acute mucosal inflammation in patients with allergic asthma and allergic rhinitis. In our previous studies, we reported that the inflammatory response of human neutrophils after incubation with nasal discharge collected from patients with perennial allergic rhinitis could be normalized by decreasing production of soluble intercellular adhesion molecule 1 (sICAM-1), interleukin 8 (IL-8),  $\text{PGE}_2$  and COX-2 mRNA expression after treatment with a mixed-herbal-formula (Yang et al., 2002). In line with our previous studies, the present study further showed that the end products of arachidonic acids  $\text{PGE}_2$  and  $\text{LTC}_4$  levels, was also ameliorated after treatment with BZYQT.

Recently, some authors have shown that COX-2 expression and activity were enhanced in different allergic diseases including asthma (Sousa et al., 1997; Maloney et al., 1998) and allergic rhinitis (Dubois et al., 1998). In this study, COX-2 mRNA expression was ameliorated by BZYQT treatment and supports the antiinflammatory effect of BZYQT for the treatment of allergic rhinitis. From this study, we clearly demonstrated that anti-allergic and antiinflammatory effects of BZYQT on non-acute stage of allergic rhinitis that is compatible with Price et al. (2006), Bellodi et al. (2006) and Failla et al. (2006). These authors suggest that an approach aims to treat the airway inflammation is beneficial for the large proportion of patients who suffer from AR.

In conclusion, long-term therapy with BZYQT was beneficial to the non-acute stage of allergic rhinitis via their antiinflammatory mechanisms.

## Acknowledgement

This study was supported by a grant from the Committee on Chinese Medicine and Pharmacy, Department of Health, (CCMP88-RD-036) Executive Yuan, Taiwan.

## References

- Bellodi, S., Tosca, M.A., Pulvirenti, G., Petecchia, L., Serpero, L., Silvestri, M., Sabatini, F., Battistini, E., Rossi, G.A., 2006. Activity of budesonide on nasal neutrophilic inflammation and obstruction in children with recurrent upper airway infections. A preliminary investigation. *International Journal of Pediatric Otorhinolaryngology* 70, 445–452.
- Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* 162, 156–159.
- Dubois, R.N., Abramson, S.B., Croford, L., Gupta, R.A., Simon, L.S., Van De Putte, L.B., Lipsky, P.E., 1998. Cyclooxygenase in biology and disease. *FASEB Journal* 12, 1063–1073.
- Failla, M., Biondi, G., Provvidenza Pistorio, M., Gili, E., Mastruzzo, C., Vancheri, C., Crimi, N., 2006. Intranasal steroid reduces exhaled bronchial cysteinyl leukotrienes in allergic patients. *Clinical & Experimental Allergy* 36, 325–330.

- Foresi, A., Teodoro, C., Leone, C., Pelucchi, A., D'Ippolito, R., Chetta, A., Oliveri, D., 2000. Eosinophil apoptosis in induced sputum from patients with seasonal allergic rhinitis and with asymptomatic and symptomatic asthma. *Annals of Allergy, Asthma & Immunology* 84, 411–416.
- Furuya, Y., Akashi, T., Fuse, H., 2004. Effect of Bu-zhong-yi-qi-tang on seminal plasma cytokine levels in patients with idiopathic male infertility. *Archives of Andrology* 150, 11–14.
- Hai le, X., Kogure, T., Niizawa, A., Fujinaga, H., Sakakibara, I., Shimada, Y., Watannabe, H., Terasawa, K., 2002. Suppressive effect of hochu-ekki-to on collagen induced arthritis in DBA/J mice. *Journal of Rheumatology* 29, 1601–1608.
- Hans-Uwe, S., 1999. Apoptosis in inflammatory diseases. *International Archives of Allergy & Immunology* 118, 261–262.
- Ishimitsu, R., Nishimura, H., Kawauchi, H., Kawakita, T., Yoshikai, Y., 2001. Dichotomous effect of a traditional Japanese medicine, Bu-zhong-yi-qi-tang on allergic asthma in mice. *International Immunopharmacology* 1, 857–865.
- Kaneko, M., Kishihara, K., Kawakita, T., Nakamura, T., Takimoto, H., Nomoto, K., 1997. Suppression of IgE production in mice treated with a traditional Chinese medicine, Bu-zhong-yi-qi-tang. *Immunopharmacology* 36, 79–85.
- Kao, S.T., Yeh, C.C., Hsieh, C.C., Yang, M.D., Lee, M.R., Liu, H.S., Lin, J.G., 2001. The Chinese medicine Bu-zhong-yi-qi-tang inhibited proliferation of hepatoma cell lines by inducing apoptosis via G0/G1 arrest. *Life Science* 69, 1485–1496.
- Kim, S.H., Lee, S.E., Oh, H., Kim, S.R., Yee, S.T., Yu, Y.B., Byun, M.W., Jo, S.K., 2002. The radioprotective effects of Bu-zhong-yi-qi-tang: a prescription of traditional Chinese medicine. *American Journal of Chinese Medicine* 30, 127–137.
- Kobayashi, H., Mizuno, N., Kutsuna, H., Teramae, H., Ueoku, S., Onoyama, J., Yamanaka, K., Fujita, N., Ishii, M., 2003. Hochu-ekki-to suppresses development of dermatitis and elevation of serum IgE level in NC/Nga mice. *Drugs under Experimental & Clinical Research* 29, 81–84.
- Maloney, C.G., Kutchera, W.A., Albertine, K.H., McIntyre, T.M., Prescott, S.M., Zimmerman, G.A., 1998. Inflammatory agonists induce cyclooxygenase type 2 expression by human neutrophils. *Journal of Immunology* 160, 1402–1410.
- Nakada, T., Watanabe, K., Matsumoto, T., Santa, K., Triixuka, K., Hanawa, T., 2002. Effect of orally administered Hochu-ekki-to, a Japanese herbal medicine, on contact hypersensitivity caused by repeated application of antigen. *International Immunopharmacology* 2, 901–911.
- Okuda, M., Ishikawa, T., Saito, Y., Shimizu, T., Baba, S., 1984. A clinical evaluation of N-5' with perennial-type allergic rhinitis. A test by multi-clinic, intergroup, double blind comparative method. *Annals of Allergy* 53, 178–185.
- Price, D.B., Swern, A., Tozzi, C.A., Philip, G., Polos, P., 2006. Effect of montelukast on lung function in asthma patients with allergic rhinitis: analysis from the COMPACT trial. *Allergy* 61, 737–742.
- Savill, J., 1997. Apoptosis in resolution of inflammation. *Journal of Leukocyte Biology* 61, 375–380.
- Shih, H.C., Chang, K.H., Chen, F.L., Chen, C.M., Chen, S.C., Lin, Y.T., Shibuya, A., 2000. Anti-aging effects of the traditional Chinese medicine bu-zhong-yi-qi-tang in mice. *American Journal of Chinese Medicine* 28, 77–86.
- Sousa, A.R., Pfister, R., Christie, P.E., Lane, S.J., Nasser, S.M., Schmitz-Schumann, M., 1997. Enhanced expression of cyclo-oxygenase isoenzyme 2 (COX-2) in asthmatic airways and its cellular distribution in aspirin-sensitive asthma. *Thorax* 52, 940–945.
- Yan, X., Kita, M., Minami, M., Yamamoto, T., Kuriyama, H., Ohno, T., Iwakura, Y., Imanishi, J., 2002. Antibacterial effect of Kampo herbal formulation Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang) on *Helicobacter pylori* infection in mice. *Microbiology & Immunology* 46, 475–482.
- Yang, S.H., Hong, C.Y., Yu, C.L., 2001. Decreased serum IgE level, decreased IFN- $\gamma$  and IL-5 but increased IL-10 production, and suppressed cyclooxygenase 2 mRNA expression in patients with perennial allergic rhinitis after treatment with a new mixed formula of Chinese herbs. *International Immunopharmacology* 1, 1173–1182.
- Yang, S.H., Hong, C.Y., Yu, C.L., 2002. The stimulatory effects of nasal discharge from patients with perennial allergic rhinitis on normal human neutrophils are normalized after treatment with a new mixed formula of Chinese herbs. *International Immunopharmacology* 2, 1627–1639.