



## Traditional Chinese medicine, Xin-yi-san, reduces nasal symptoms of patients with perennial allergic rhinitis by its diverse immunomodulatory effects

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### ABSTRACT

**Background:** Perennial allergic rhinitis (AR) is a common health problem with a high prevalence rate of 43.6% in Taiwan. In our previous study, a mixed formula of Chinese herbs consisted of Xin-yi-san, Xiao-ging-long-tang, and Xiang-sha-liu-jun-zi-tang, exerted diverse immunomodulatory effects in the treatment of patients with perennial allergic rhinitis.

**Objective:** The aim of the present study was to determine whether Xin-yi-san (XYS) alone is effective in the treatment of AR and tried to explore its molecular mechanism of anti-allergic activity.

**Methods:** In a randomized double-blind study, 108 patients with AR received either YYS or a placebo for 3 months. The effectiveness of YYS was evaluated by nasal symptoms, nasal airflow resistance, nostril dissection area, and serum titer of specific IgE antibodies against house dust mite allergens. In addition, the production of T helper (Th) 1 (represented by interferon- $\gamma$ ) and Th2 [represented by interleukin (IL)-4, IL-10, and IL-13] cytokines, the proinflammatory cytokine IL-8, soluble intercellular adhesion molecule (sICAM), and arachidonate metabolites prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and leukotriene C<sub>4</sub> (LTC<sub>4</sub>) by polymorphonuclear neutrophils (PMNs) were compared before and after treatment between the two groups.

**Results:** YYS attenuated nasal symptoms (sneezing and rhinorrhea) and nasal congestion through reduction of nasal airflow resistance and increase in nostril dissection areas. We also found that YYS exerted diverse immunomodulatory effects, including suppression of serum IgE levels and increased production of IL-10, sICAM-1, and IL-8 compared to placebo group. However, YYS treatment did not affect the release of PGE<sub>2</sub> and LTC<sub>4</sub> from PMNs.

**Conclusions:** This study originally provides the evidence that Xin-yi-san alone is an effective herb in the treatment of perennial allergic rhinitis.

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### 1. Introduction

Perennial allergic rhinitis (AR) is a common health problem in Taiwan. The prevalence rates were 42.0% and 34.0% in males and females respectively aged 13 to 18 years [1]. In urban areas, the prevalence rate was even high up to 43.6% in the age group of 13 to 14 years [2]. Several Chinese herbal formulae have long been used in the treatment of AR. Xin-yi-san (XYS), a popular traditional Chinese herb, in combination with other herbs has been used for hundreds of year in the treatment of AR for alleviating nasal obstruction and rhinorrhea [3,4]. We have reported that a mixed herbal formula

consisting of Xin-yi-san, Xiao-ging-long-tang, and Xiang-sha-liu-jun-zi-tang was beneficial for the treatment of AR [5,6]. We demonstrated that the mixed formula significantly enhanced IL-10 but decreased IFN- $\gamma$  and IL-5 production by phytohemagglutinin-stimulated peripheral mononuclear cells (MNC). In addition, the COX-2 mRNA expression in LPS-stimulated PMNs was significantly suppressed after treatment. These results suggest that the mixed formula treatment could modulate the functions of lymphocytes and neutrophils [5]. Further study by us demonstrated that the mixed herbal formula treatment suppressed nasal mucosa inflammation by normalizing the stimulatory effects of allergic nasal fluid from AR patients with high serum IgE [6]. Since YYS is an essential component of the mixed formula, we hypothesized that YYS alone should be effective for nasal symptom relief of AR patients. In the present study, a randomized double-blind clinical trial was conducted to answer the following two questions. First, whether YYS alone is really beneficial to the patients

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with AR. Second, whether XYs alone exhibits immunomodulatory effects on nasal allergic mechanisms. The AR patients were treated with XYs or placebo for 3 months. The nasal symptomatic score, nasal airflow resistance, serum specific IgE levels, helper T cell cytokine production, and inflammation-related molecules released from PMNs including soluble intercellular adhesion molecule 1 (sICAM-1), interleukin-8 (IL-8), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), and leukotriene C<sub>4</sub> (LTC<sub>4</sub>) were evaluated for understanding the molecular basis of XYs effectiveness on AR treatment.

## 2. Methods

### 2.1. Patient recruitment

Patients with AR were recruited based on the two criteria: a clinical history of perennial allergic rhinitis and positive reaction to multiple allergen simultaneous tests (MAST). All patients suffered from nasal symptoms with sneezing, rhinorrhea, mucosal congestion, and itching. Sera of the patients were proved reactive to house dust mite allergens Der p1 (100%) and Der p2 (100%), fungal allergens (12.4%), and animal danders (5.6%). One hundred and eight patients (60 males and 48 females, aged 18–64 years) with perennial allergic rhinitis were assessed for eligibility. Informed consent was obtained from each participant and the clinical protocol was approved by Medical Ethics and Human Clinical Trial Committee, Chang Gung Memorial Hospital, Taipei, Taiwan (CGMG IRB No. 94-250B, issue date: 2, Feb, 2009). Finally, a total of 100 patients (55 males and 45 females) were selected and randomized into two groups: 62 patients (34 males and 28 females) were treated for 12 weeks with XYs (XYs

group), and 38 patients (21 male and 17 females) were treated for 12 weeks with placebo (placebo group) (Fig. 1). The demographic and clinical/laboratory features of the two groups were quite compatible as shown in Table 1. Although one hundred patients in total entered the trial, eighty-six completed the trial including 56 patients (32 males and 24 females) in the XYs group and 30 patients (17 males and 13 females) in the placebo group (Fig. 1). Corticosteroid nasal sprays, decongestants, and anti-histamines were prepared as rescue medications in the entire course of study.

### 2.2. Medications

Xin-yi-san composes of 9 herbs: (1) dried flower of *Magnolia liliiflora* Desr, (2) dried roots of *Asarum heterotropoides*, (3) dried roots of *Saposhnikovia divaricata* Schischk, (4) dried roots of *Angelica dahurica* Benth. et Hook, (5) dried rhizoma of *Liquisticum sinense* Oliv, (6) dried rhizomas of *Liquisticum Wallichii* Franch, (7) dried rhizomas of *Cimicifuga foetida* L, (8) dried rhizomas of *Akebia quinata* Decne, and (9) dried roots and rhizome of *Glycyrrhiza uralensis* Fisch. The manufacturing processes start with decoction and separation of the decoction liquid through the sieve separator. Afterwards, the filtrate is concentrated and the excipients are added during granulation process. After granulation operation is completed, the samples were sent for composition analysis by HPLC. Only the samples proved good quality are used in the present study.

It is believed that the active ingredients of the formula are derived from *magnolia liliiflora* that may induce mitochondria- and caspase-dependent mast cell apoptosis [7]. We purchased XYs from Sun Ten Pharmaceutical Co., Ltd. (Taipei Taiwan) with batch number 104117.

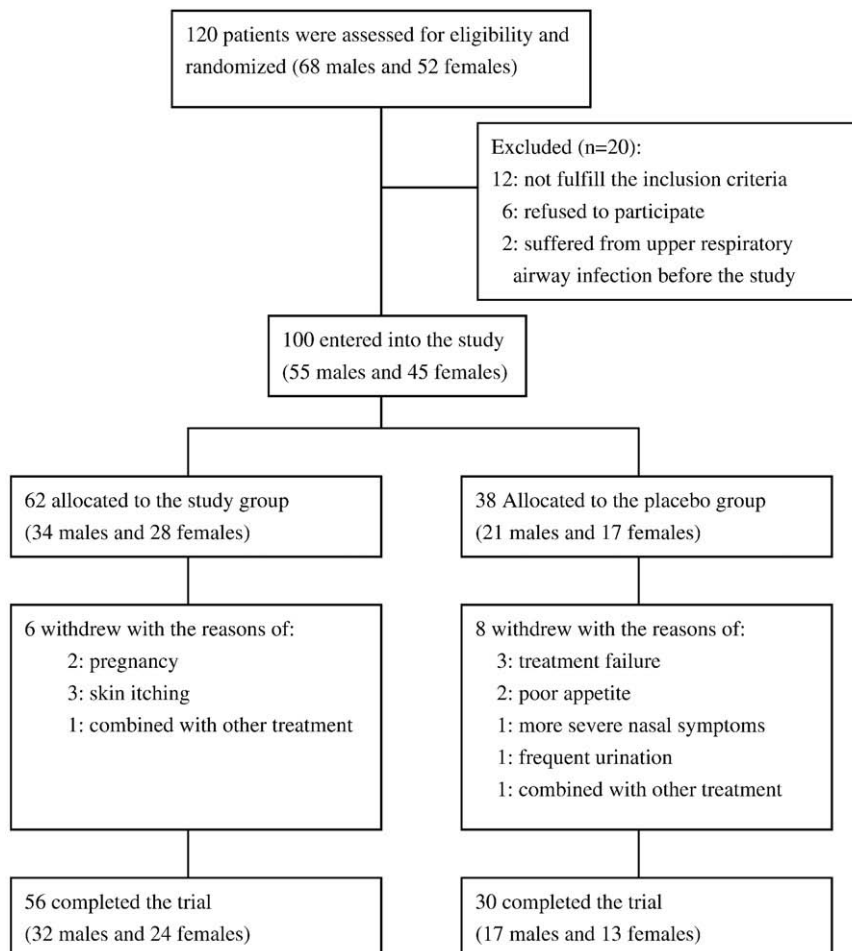


Fig. 1. Flow chart of participant recruitment and selection.

**Table 1**  
Demographic and clinical features of 100 patients entering into the study.

	XYS	Placebo
Number of patient	62	38
Male/female	34/28	21/17
Age (mean ± SD, years)	30 ± 6.8	29 ± 6.2
Positive MAST test (%)	62 (100%)	38 (100%)
Total IgE (Kua/L)	536.8 ± 282.3	522.0 ± 220.9
Non-smoker (%)	51 (82.3%)	31 (81.6%)
Asthma history (%)	6 (9.7%)	4 (10.5%)
Atopic dermatitis history (%)	3 (4.8%)	2 (5.3%)
Drug allergy history (%)	19 (30.6%)	11 (28.9%)
Food allergy history (%)	15 (24.2%)	9 (23.7%)
Comorbidity diseases during the study		
Asthma (%)	0 (0%)	0 (0%)
Urticaria (%)	4 (6.5%)	2 (5.3%)

Each 5 g of YYS powder in a capsule was prepared from the 9 herbs as mentioned above. The placebos were prepared by packing 5 g starch in the same-colored and same sized-capsules. Individual patient received capsules containing 5 g of either YYS or starch three times a day after meals. Seven patients (3 in the YYS group and 4 in placebo group) withdrew from the study due to intolerable but mild adverse effects as shown in Fig. 1.

### 2.3. Clinical evaluation

All patients were evaluated for nasal symptoms following the scoring system (Table 2) proposed by Okuda et al. [8]. Patients were requested to record a diary of nasal symptoms, including the number of sneezing attacks and nose blowing, and the degree of nasal obstruction. Depending on the degree of severity, the typical nasal symptoms such as sneezing, rhinorrhea, and obstruction were recorded by a scale of 0–3 (0 = none; 1 = mild; 2 = moderate and 3 = severe) (Table 2). The sum of the scores before and after treatment was used for calculating the percent improvement as follows:

$$\text{Percent of improvement} = \frac{\text{Scores before treatment} - \text{Scores after treatment}}{\text{Scores before treatment}} \times 100$$

### 2.4. Determination of specific IgE titers against house dust mite allergens

Serum specific IgE antibodies against house dust mite allergens *Dermatophagoides pteronyssinus* 1 (*Der p1*) and 2 (*Der p2*) were measured by MAST according to manufacturer's instructions (Pharmacia and Upjohn, Uppsala, Sweden).

**Table 2**  
Scoring of nasal symptoms suggested by Okuda et al. [8].

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. Measurement of nasal airflow and nostril dissection area by rhinomanometry

Nasal airflow and mean nostril dissection area were measured by active anterior rhinomanometry (DK-3540, RhinoMetrics Industry, Lyngø, Denmark) that can accurately evaluate the nasal resistance [9]. Patients wore a tightly fitted facemask and breathed through one nostril with the mouth closed. A sensor was placed in the contralateral nostril to record pre- and post-nasal pressures and the cross sectional area. The transnasal airflow and pressure signals were amplified, digitized, and saved for statistical analysis. Nasal airflow was reported as the sum of airflow through both nostrils in milliliter per second at a pressure difference of 150 Pa across the nasal passage. At least four measurements were made on each patient and the mean value was calculated when reproducible values were achieved. Normal values of less than 0.50 Pa/ml/s was considered significant. The values for cross sectional area measurement were greater than 0.88, with a mean interday coefficient of variation of less than 0.12. The minimal cross sectional area (MCA) of each nasal cavity between 22 mm and 54 mm from the anterior nostril, and the nasal volume between the MCA depth and 54 mm (VOL), were recorded. For comparison, the median value of the triplicates was calculated.

### 2.6. Isolation of mononuclear cells (MNCs) and polymorphonuclear neutrophils (PMNs) from peripheral blood

The isolation of MNCs and PMNs were conducted as the same procedures we reported before [5]. Heparinized venous blood obtained from patients was mixed with one-fourth volume of 2% dextran (molecular weight 467 000 Da) (Sigma-Aldrich Chemical Company, St. Louis, MO, USA) for 30 min at room temperature. The leukocyte-enriched supernatant was aspirated and diluted with an equal volume of Hanks' balanced salt solution. After Ficoll-Hypaque (specific gravity 1.077) density gradient centrifugation at 150 × g for 25 min, MNCs were aspirated from the interphase and PMN were obtained from the bottom. After three washes, the isolated MNCs and PMNs were suspended in RPMI-1640 culture medium containing 10% fetal bovine serum. The cell viability of MNCs and PMNs was ≥ 95% as confirmed by trypan blue dye exclusion.

### 2.7. Preparation of anti-CD3 + anti-CD28-stimulated MNCs and IL-4-stimulated PMNs culture supernatants

The isolated cells at a cell density of 1 × 10<sup>6</sup> cells/ml were stimulated with monoclonal antibodies against CD3 (1 μg/ml) and CD28 (0.5 μg/ml) for MNCs (R&D Systems, Minneapolis, MN, USA) and 10 ng/ml of IL-4 (R&D Systems) for PMNs, respectively. Following 24 h incubation at 37 °C in 5% CO<sub>2</sub>–95% air, cell-free culture supernatants were collected by centrifugation and stored at –20 °C until use.

### 2.8. Measurement of Th1/Th2 cytokine levels in MNC culture supernatants by ELISA

Th1 (represented by IFNγ) and Th2 (represented by IL-4, IL-10, and IL-13) cytokine levels in MNC culture supernatants were determined by individual commercially available ELISA kit (R&D Systems) according to the manufacturer's instructions. The detection range of IFN-γ, IL-4, IL-10, and IL-13 were 25.6–1000 pg/ml, 10.24–400 pg/ml, 15.36–600 pg/ml, and 62.5–4000 pg/ml, respectively.

### 2.9. Measurement of sICAM-1, PGE<sub>2</sub>, IL-8, and LTC<sub>4</sub> levels in PMN culture supernatants by ELISA

The levels of sICAM-1, PGE<sub>2</sub>, IL-8, and LTC<sub>4</sub> in IL-4 stimulated PMN culture supernatants were determined by individual commercially available ELISA kit (R&D Systems) according to the manufacturer's instructions. The minimum detection limits of sICAM-1, IL-8, PGE<sub>2</sub>, and LTC<sub>4</sub> were 0.49 pg/ml, 31.2 pg/ml, 0.1 pg/ml, and 0.04 pg/ml, respectively.

2.10. Statistical analysis

Results represent mean ± s.d. The data of symptomatic score was assessed by non-parametric Wilcoxon's signed-rank test, while the other data were analyzed by parametric statistics to compare difference between two groups. A value of  $p < 0.05$  was considered statistical significance.

3. Results

3.1. Effects of XYZ on nasal symptom scores in patients with AR

As demonstrated in Fig. 2A, XYZ significantly reduced nasal symptom scores from  $6.39 \pm 1.28$  (before treatment) to  $3.19 \pm 1.35$  (after treatment) ( $p < 0.05$ ). No significant change was found in the placebo group before ( $6.06 \pm 1.39$ ) and after ( $5.71 \pm 1.61$ ) 12 weeks treatment.

3.2. Effects of XYZ on nasal airflow resistance in patients with AR

Nasal airflow resistance in inspiration and expiration was compared before and after treatment between the two groups. As shown in Fig. 2B, the average nasal resistance of the nostrils was markedly decreased after

XYZ treatment in both inspiration ( $0.56 \pm 0.32$  Pa/ml/s before treatment vs.  $0.44 \pm 0.27$  Pa/ml/s after treatment;  $p = 0.02$ ) and expiration ( $0.63 \pm 0.31$  Pa/ml/s before treatment vs.  $0.53 \pm 0.34$  Pa/ml/s after treatment;  $p = 0.03$ ). In contrast, no significant change was observed in the placebo group in both inspiration ( $0.59 \pm 0.46$  Pa/ml/s before treatment vs.  $0.52 \pm 0.36$  Pa/ml/s after treatment;  $p = \text{NS}$ ) and expiration ( $0.68 \pm 0.34$  Pa/ml/s before treatment vs.  $0.69 \pm 0.42$  Pa/ml/s after treatment;  $p = \text{NS}$ ). Considering the highly inter-personal variability, the relative values were calculated by comparing to the baseline values. The results showed that an 18–20% reduction in airflow resistance in both inspiration ( $p = 0.02$ ) and expiration ( $p = 0.03$ ) in XYZ group. In contrast, no significant difference was seen in the placebo group (data not shown).

3.3. Effects of XYZ on the dissection area of nostrils in patients with AR

The average dissection area of both nostrils was compared between both groups before and after treatment. As demonstrated in Fig. 2C, a significant increase in the average nostril dissection area was observed in the XYZ group ( $0.56 \pm 0.18$  cm<sup>2</sup> before treatment vs.  $0.62 \pm 0.22$  after treatment;  $p = 0.038$ ). In the placebo group, however, no significant change after treatment was noted. Due to

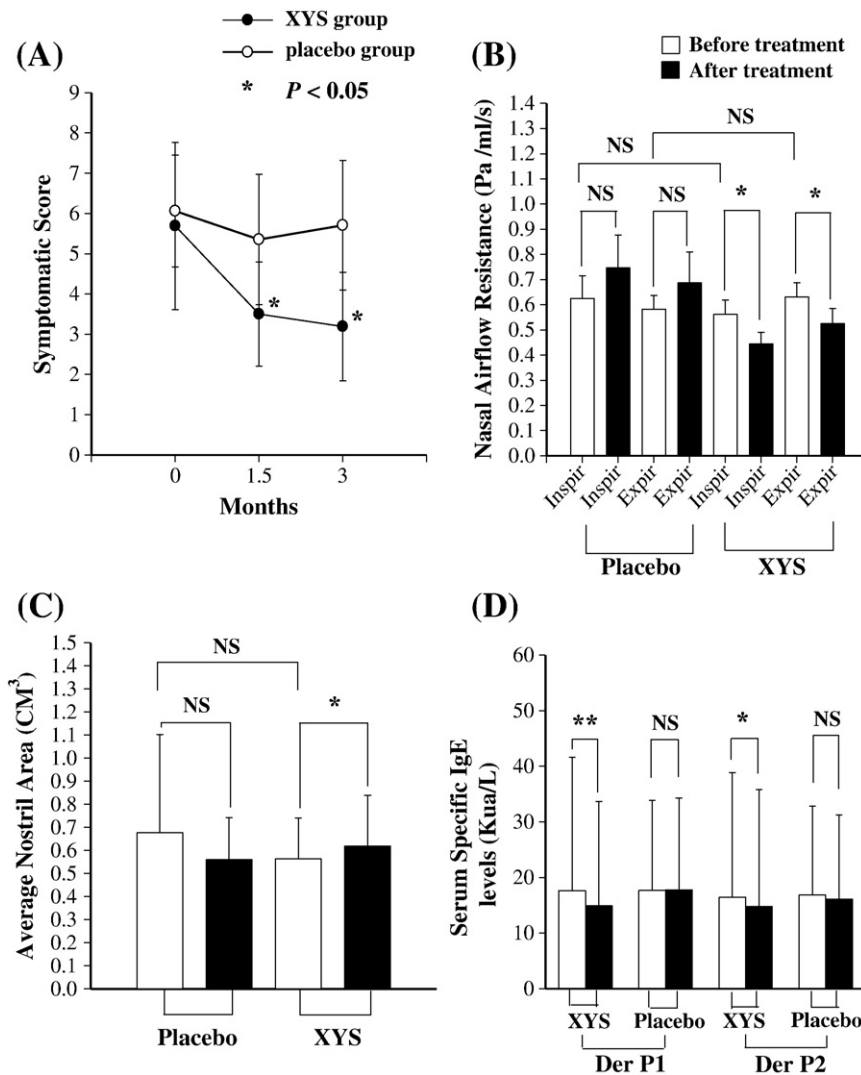


Fig. 2. Effects of XYZ on nasal symptoms, nasal functions and serum IgE levels in patients with perennial allergic rhinitis before and after 3 months treatment. (A) Changes of nasal symptomatic scores. (B) Changes of nasal airflow resistance in the inspiratory (Inspir.) and expiratory (Expir.) phases. (C) Average dissection areas in both nostrils. (D) Serum specific IgE levels against Der p1 and Der p2, as determined by MAST. □: before treatment, ■: after treatment. "\*" denotes  $p < 0.05$  and "\*\*" denotes  $p < 0.001$  compared to "before treatment".

highly inter-personal variability, the relative values were calculated by comparing to the baseline values. As a result, the average dissection area of the nostrils increased by 10–12% in the XY group ( $p = 0.03$ ), whereas there was no change in the placebo group after treatment (data not shown).

#### 3.4. Effects of XY on serum levels of house dust mite specific IgE antibodies

Since all patients were allergic to house dust mite allergens, the specific IgE titers were compared before and after XY treatment. While nothing changed in IgE titers against *Der p1* ( $17.7 \pm 16.2$  Kua/L before treatment vs.  $17.8 \pm 16.5$  Kua/L after treatment) and *Der p2* ( $16.8 \pm 16.0$  Kua/L before treatment vs.  $16.1 \pm 15.1$  Kua/L after treatment) in the placebo group, a significant decrease in the anti-*Der p1* ( $17 \pm 24.0$  Kua/L before treatment vs.  $14.9 \pm 18.8$  Kua/L after treatment;  $p = 0.008$ ) and anti-*Der p2* ( $16.4 \pm 22.4$  Kua/L before treatment vs.  $14.8 \pm 21.0$  Kua/L after treatment;  $p = 0.026$ ) IgE titers in the XY group was found (Fig. 2D). Due to highly inter-personal variability, the relative values were calculated by comparing to the baseline values. XY treatment resulted a 15–20% reduction in both *Der p1* ( $p = 0.007$ ) and *Der p2* ( $p = 0.001$ ) whereas no difference was observed in the placebo group (data not shown).

#### 3.5. Production of Th1/Th2 cytokines in MNC culture supernatants after XY treatment

To elucidate the molecular mechanism underlying the immunomodulatory effects of XY, the Th1/Th2 cytokine production by anti-CD3 + anti-CD28-activated MNC was measured by ELISA. Among the cytokines measured, only IL-10 was markedly elevated in the XY group ( $69.4 \pm 35.5$  ng/ml before treatment vs.  $83.7 \pm 30.2$  ng/ml after treatment;  $p = 0.025$ ) (Fig. 3A). Although XY treatment resulted in a tendency of increased IL-4, IL-13, and IFN- $\gamma$  production by activated MNC, no statistical significance could be found. (Fig. 3B, C, and D). In contrast, a tendency of increased IL-13 production was noted in both groups after treatment with either XY or placebo. However, only placebo group reached a statistical significance (Fig. 3C).

#### 3.6. Comparison of sICAM-1, IL-8, PGE<sub>2</sub>, and LTC<sub>4</sub> levels in culture supernatant of IL-4-stimulated PMN

Leukocyte emigration into tissue requires the involvement of adhesion molecules and chemotactic factors. Patients with AR exhibit a significant influx of eosinophils and neutrophils into the nasal cavity. To investigate the effects of XY on nasal inflammation, we assessed the release of inflammation-related molecules including sICAM-1, IL-8, and arachidonic acid metabolites PGE<sub>2</sub> and LTC<sub>4</sub>, from IL-4 stimulated PMN before and after treatment (Fig. 4A to D). In the XY group, the sICAM-1 levels increased from  $3.5 \pm 2.4$  ng/ml to  $5.3 \pm 3.6$  ng/ml ( $p = 0.007$ , Fig. 4A), and IL-8 increased from  $16.9 \pm 8.2$  ng/ml to  $22.6 \pm 9.5$  ng/ml ( $p = 0.002$ , Fig. 4B). No significant difference was found in PGE<sub>2</sub> and LTC<sub>4</sub> levels before and after treatment in both groups (Fig. 4C and D).

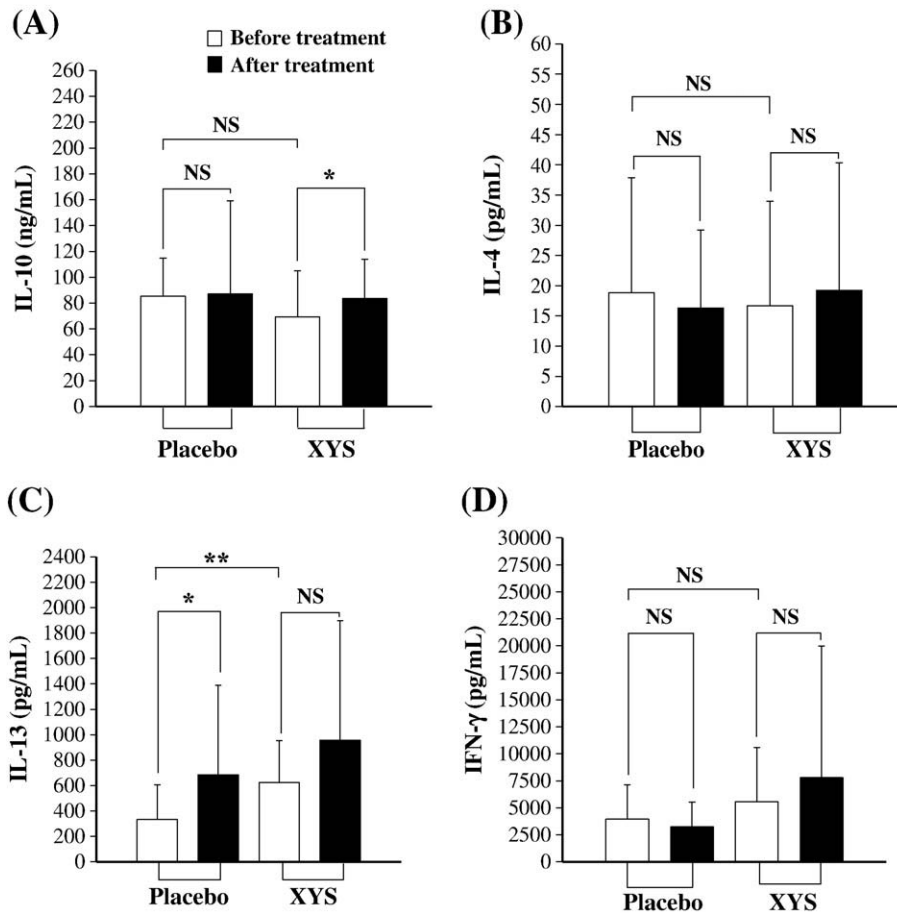
## 4. Discussion

Allergic rhinitis is a common health problem in Taiwan with a rapidly increasing prevalence rate in recent years. Many Chinese herbs have been proven effective in the treatment of allergic diseases. Kaneko et al. [10] have reported that a popular Chinese herb, Bu-zhong-yi-qi-tang, suppressed serum IgE levels in mice. Xue et al. [11] have demonstrated that many Chinese herbal medicines were not only effective in providing symptomatic relief but also in improving quality of life in some patients with allergic rhinitis. *In vitro* studies also suggest that different Chinese herbs are able to modulate immune cell function. For example, Xiao-chai-hu-tang is one Chinese

herb that modulates the immunological functions of B cells [12], NK cells [13], and antigen presenting cells [14]. Ren-shen-yang-rong-tang [15] is another Chinese herb that exerts immunomodulatory activities in the treatment of cytomegalovirus infection. Nevertheless, the abovementioned studies involved the use of a single herbal formula. In contrast, our previous studies have demonstrated that a mixed herbal formula consisting of Xin-yi-san, Xiao-gin-long-tang, and Xiang-sha-liu-jun-zi-tang exhibited beneficial effects in treatment of perennial allergic rhinitis through modulating the functions of lymphocytes and neutrophils [5,6]. In addition, we have found that Bu-zhong-yi-qi-tang exerted anti-inflammatory activities that suppressed nasal inflammation in patients with perennial allergic rhinitis in non-acute stage [16].

XY is a popular Chinese herb usually used in combination with other herbs for alleviating nasal obstruction and rhinorrhea since ancient time. In the present study, we demonstrated the effectiveness of XY alone in the treatment of allergic rhinitis and explored its molecular basis on anti-allergic inflammation. As expected, the patients who received XY alone for 3 months felt relief of nasal symptoms including sneezing, rhinorrhea, and congestion and reduced serum levels of specific IgE antibodies against *Der p1* and *Der p2*. For more accuracy, we measured the changes of nasal airflow resistance and the nostril dissection area. We clearly demonstrated that XY treatment resulted in reduced nasal airflow resistance and increased nostril dissection area whereas no change was found in the placebo group (Fig. 2B and C). Nasal congestion, the most disturbing symptom of allergic rhinitis, impairs the human natural nasal breath and is often associated with poor sleep quality. Subsequently, the somnolence may lead to a poor life quality in daytime. It is therefore quite important to resolve the nasal congestion at night to prevent daytime somnolence and improve the quality of life in patients with AR [17,18]. We have provided in the present study the evidence of XY alone in attenuating nasal congestion effectively by reducing airflow resistance and increasing the dissection area of the nostrils.

To investigate the molecular basis of the action of XY in allergic rhinitis, the production of Th1/Th2 cytokines and different inflammatory mediators were assessed. We showed that IL-10 plays an important role in the effectiveness of XY similar to the report of our previous observation that the mixed herbal formula shifted the immune responses towards anti-inflammatory pathway [5]. Robinson et al. [19] demonstrated the increased IL-10 levels in BAL fluid of patients with active bronchial asthma. It is conceivable that increased IL-10 production in patients with bronchial asthma has the potential to down-regulate Th1 cytokine production [19] and facilitate the migration of Th2 cells from peripheral blood to the bronchial tree after allergen exposure [20]. On the contrary, Borish et al. [21] have reported reduced expression of IL-10 in peripheral mononuclear cells of asthmatic patients. IL-10 has been shown to stimulate mast cell proliferation and also acts as an inhibitor of eosinophil survival and LPS-induced secretion of proinflammatory cytokines [21]. Our finding of enhancing IL-10 production in XY group is compatible with that of Jeannin et al. [22] that IL-10 has the potential to suppress IL-4-induced IgE production by peripheral mononuclear cells. It appears that IL-10 possesses pleiotropic and contradictory activities in allergic reactions. It is believed that IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ) are produced by regulatory T cells (Treg cells) that may potentially suppress IgE production and other different effector cells in allergic inflammation, such as mast cells, basophils, and eosinophils. Hence, it would be interesting to investigate whether Treg cells are activated in patients with AR after treatment with XY. It is equally interesting that a tendency of increased IL-13 production in both groups after treatment is shown in Fig. 3C. However, significantly elevated IL-13 levels were only found in placebo group, but not in XY group (Fig. 3C). Three possibilities are considered: (a) lower standard deviation in placebo group than XY group, (b) lower IL-13 baseline value in placebo group compared to XY group, and (c) unknown placebo effect in the



**Fig. 3.** Effects of YYS on Th1/Th2 cytokine production by anti-CD3 + anti-CD28-activated peripheral blood mononuclear cells isolated from patients with AR after 3 months treatment. (A) Interleukin-10, (B) interleukin-4, (C) Interleukin-13, and (D) interferon- $\gamma$ , by ELISA.  $\square$ : before treatment, and  $\blacksquare$ : after treatment. "\*" denotes  $p < 0.05$  and "\*\*" denotes  $p < 0.001$  compared to "before treatment".

placebo group. Obviously, more investigation is required to elucidate the real mechanism of it.

Contradictory immunopathological roles of IL-13 in allergic inflammation have been reported in the literatures. Miyahara et al., [23] reported that IL-13 is a major contributor to the development of a late nasal response with little influence on the early response whereas no affection on the nasal eosinophilic inflammation. Cheng et al., [24] reported no evidence of Arg110Gln variant at the *IL-13* locus as a genetic risk factor in the development of Japanese cedar pollinosis. However, Llanes et al., [25] reported that IL-13 polymorphisms were associated with specific allergy to olive pollen. *IL-13 C1112T* polymorphism is a protective factor but *IL-13 R130Q* polymorphism is a risk factor. The nasal discharge fluid obtained from AR patients contains IL-5, IL-8, eosinophils, and neutrophils that merely reflex the inflammatory nature of allergic rhinitis [6]. In the present study, we have found that the production of sICAM-1 and IL-8, but not  $PGE_2$  or  $LTC_4$ , are significantly increased by IL-4-stimulated PMN in AR patients following 3 months of treatment with YYS (Fig. 4). In our previous study, however, we have noted decreased production of  $PGE_2$  and  $LTC_4$  by activated PMN in patients with AR following treatment with the mixed herbal formula. It is likely that some uncharacterized ingredients in the mixed herbal formulae rather than YYS, exert different immunomodulatory effects on activated PMNs. The real mechanism responsible for this difference is now under investigation.

IL-5 is a key cytokine in regulating eosinophil maturation. In contrast, IL-8 plays an important role in recruitment and activation of neutrophils [26]. Ciprandi et al. [27] have demonstrated that the number of eosinophil runs parallel to total nasal symptom score and nasal airflow resistance. Although IL-8 plays a crucial role in

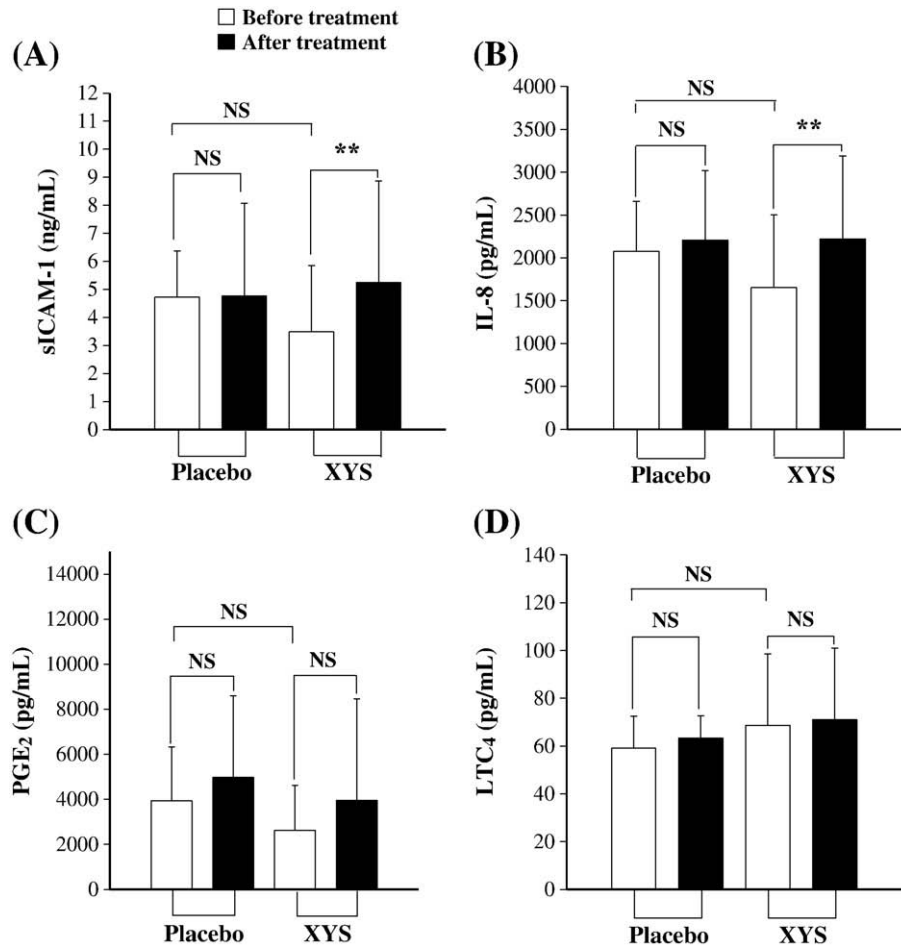
modulating the severity of allergic rhinitis [27], this chemokine acts as a histamine release inhibitory factor [28]. Furthermore, Nesterova et al. [29] have reported neutrophil dysfunction in allergic children. In the present study, we found increased IL-8 production after YYS treatment. These findings also suggest the pleiotropic effects of IL-8 in allergic inflammation. The role of neutrophil dysfunction in the pathogenesis of allergy is now under investigation.

Although YYS alone has been proven effective in relieving nasal symptoms and nasal congestion [3,4], it is rarely used singly in the treatment of allergic rhinitis. Instead, YYS is commonly used in combination with other herbs in most Chinese communities as reported in our previous studies [5,6]. Recently, Krouse et al. [30] proposed the concept of an integrative approach for the treatment of AR. This proposal is based on the fact that different combinations of Chinese herbs involve different mechanisms. It appears that the immunosuppressive and anti-inflammatory effects derived from the mixed formula of Chinese herbs are broader than YYS. Nevertheless, we found that YYS alone sufficiently exerts immunomodulatory effects on patients with AR.

In conclusion, this study provides evidence to support that YYS, when used singly, is beneficial in nasal allergic inflammation through enhancing IL-10 and IL-8 production.

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**Fig. 4.** Effects of YYS on the production of inflammation-related factors by IL-4 activated polymorphonuclear neutrophils (PMNs) isolated from patients with allergic rhinitis after 3 months treatment. (A) soluble intercellular adhesion molecule-1, (B) interferon-8, (C) prostaglandin E<sub>2</sub>, and (D) leukotriene C<sub>4</sub>, by ELISA. □: before treatment and ■: after treatment. “\*\*” denotes  $p \leq 0.001$  compared to “before treatment”.

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