

Evaluation of efficacy of traditional Chinese medicines in the treatment of childhood bronchial asthma: clinical trial, immunological tests and animal study

Hsieh K-H. Evaluation of efficacy of traditional Chinese medicines in the treatment of childhood bronchial asthma: clinical trial, immunological tests and animal study.

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Traditional Chinese medicines (TCM) have been used to treat bronchial asthma for several centuries and a certain degree of clinical benefit has been observed; however, scientific substantiation is lacking. A multicenter, double-blind and placebo-controlled study was therefore conducted to evaluate the clinical efficacy in terms of symptom score, medication score, morning and evening PEFs, and changes of immunoregulatory function, such as distribution of lymphocyte subsets and *in vivo* and *in vitro* production of lymphokines (IFN- γ and IL-4) and inflammatory mediators (histamine, PGE₂ and LTC₄). Furthermore, the protective effect of TCM on the late asthmatic reaction (LAR) was evaluated by using asthmatic guinea pigs. Three hundred and three asthmatic children were classified by Chinese doctors, according to a standardized questionnaire designed on the basis of basic logic of Chinese medicine, into three groups of specific constitution (group A, B and C). Group A consisted of 32 herb A-treated patients and 34 placebo-treated; group B, 74 herb B-treated and 64 placebo-treated; and group C, 55 herb C-treated and 44 placebo-treated. The study period was six months. The results were: 1) Both treatment group and placebo group showed an improvement in all clinical parameters, thus demonstrating a placebo effect. However, the improvement was usually greater in the former than the latter, although only the difference in PEF was significant; 2) Herb A could increase total T cell and decrease B cell; 3) Herb A and B enhanced production of PGE₂ but not LTC₄, IFN- γ and IL-4; 4) There was a general tendency for *in vivo* and *in vitro* production of histamine to decrease at the end of study in both treatment group and placebo group; however, the decrease was significantly greater in the former than the latter; 5) In asthmatic guinea pigs, 10-day's pretreatment with Chinese herbs could reverse the decrease of sGaw, suppress eosinophilia in bronchoalveolar lavage fluid (BALF), prevent the eosinophil infiltration of airways, increase PGE₂ production and decrease LTC₄ production in serum and BALF. Thus, traditional Chinese medicines did show a certain degree of clinical efficacy. The decreased production of histamine and LTC₄, increased production of PGE₂ that were found in both asthmatic children and asthmatic guinea pigs, and prevention of occurrence of LAR by suppressing eosinophil infiltration of airways and preserving airway conductance that were observed in asthmatic guinea pigs after allergen challenge might be used to account partly for the effectiveness.

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Traditional Chinese medicines (TCM) have been used in the treatment of airway diseases, including bronchial asthma, for several centuries. Clinical observations by both doctors of modern medicine and

Chinese doctors show a certain degree of efficacy in a certain portion of asthmatic patients (1). It has been well known that ephedrine, the prototype of β agonist now used widely in modern medicine for the

treatment of bronchial asthma, has been extracted and purified from Ma Huang, which is the main component of a medicinal herb of traditional Chinese medicines used in treating acute asthmatic attacks for hundreds of years (2). Moreover, a few pure compounds possessing anti-inflammatory effect, such as PAF antagonists, have been isolated from medicinal herbs in TCM that have been used in the treatment of bronchial asthma (3–7). As TCM have been accepted by most Chinese, and the cost of TCM accounts for a large portion of medical expense in Taiwan, it is therefore mandatory, by using scientific methods of modern medicine, to study its clinical efficacy on the one hand and explore its working mechanisms on the other hand.

Acute asthma is a potential life-threatening condition and TCM are not suitable for this condition; therefore in this study, a multicenter, double-blind, and placebo-controlled clinical trial was conducted to evaluate three regimens of TCM commonly used by Chinese doctors to “normalize the altered reactivity” after asthma has been brought under control and become stable. In addition to measurement of the production of inflammatory mediators and lymphocyte function before and after treatment, the effect of TCM on the late asthmatic reaction of asthmatic guinea pigs were also studied.

Material and methods

Patients

During the 6-month period from November 1993 to April 1994, a total of 2130 asthmatic children (aged from 6 to 15 years) were recruited from five medical

centers throughout the country. The diagnosis of bronchial asthma was made on the basis of American Thoracic Society standards (8), and only 1543 patients whose asthmatic symptoms have been brought under control and become stable (by regular or sometimes rescue medications) were enrolled. The patients were then referred by allergists to Chinese doctors who were working at the same allergy clinics and were responsible for classifying the patients into 3 groups of particular constitution, i.e., deficiency of kidney energy (Group A), deficiency of spleen energy (Group B), and deficiency of kidney and spleen energy (Group C). The constitution was defined according to a standardized questionnaire containing information obtained from “observation, hearing and smelling, history taking and pulsating pattern of radial artery”, which has been the basic logic used by Chinese doctors for thousands of years in diagnosing and treating patients. Only 334 patients could be fitted into these three constitution groups and were included for study. For each group, the medications (active or placebo, see below) were distributed to patients by pharmacists, according to the order of visiting. Therefore, neither the doctors nor the parents/patients knew the nature of medications the patients were taking. This study has been approved by Human Research Committee of each medical center and informed consent was obtained from all parents.

Traditional Chinese medicines

For each group of particular constitution, a specific regimen was prescribed. The active drug used for

Table 1. Compositions of the three Chinese medicines used in this study

Prescription	Chinese medicinal plants		Prescription	Chinese medicinal plants	
	Name	Dose		Name	Dose
Herb A	Rehmannia root	15 gm	Herb B	Ginsheng root	7.5 gm
	Alisma rhizome	7.5 gm		Rhizomeatractylodes	7.5 gm
	Dioscorea rhizome	11.25 gm		Ovata	7.5 gm
	Poria, Hoelen	7.5 gm		Licorice root	7.5 gm
	Moutan root bark	7.5 gm		Dioscorea rhizome	3.25 gm
	Shan zhu yu	11.25 gm		Lablab seed	7.5 gm
Herb C	Ginsheng root	7.5 gm		Coix seed	3.25 gm
	Attractylodes ovata rhizome	7.5 gm		Lolus fruit	3.25 gm
	Poria, Hoelen	7.5 gm		Lolus fruit	3.25 gm
	Licorice root	3.25 gm		Tangerine peel	3.25 gm
	Ginger root (fresh)	3.25 gm		Amomn seed	3.25 gm
	Jujube (Chinese dale)	3.25 gm		Platycodon	
	Ophiopogon tuber	7.5 gm			
	Schisandra fruit (Chinese)	3.75 gm			
	Psoralea seed	7.5 gm			

Herb A: Liu-Wei-D; Huang-Wan; herb B: Shen-Ling-Bai-Shu-San; herb C: Jia-Wei-Si-Jun-Zi-Tang. The medicinal plants (daily dose) were extracted with water twice, lyophilized, cooked and dried starch added, divided into 3 packs and were given 1 pack t.i.d. Placebo was cooked and dried starch only.

Table 2. Demographic data of asthmatic children

	Deficiency of kidney energy		Deficiency of spleen energy		Deficiency of kidney and spleen energy	
	Herb A	Placebo	Herb B	Placebo	Herb C	Placebo
Number of cases	32	34	74	64	55	44
Age (year)	9.2±2.2	9.6±3.2	8.3±2.2	8.9±2.5	9.0±2.6	9.1±2.4
Male/Female	24/8	25/9	59/15	50/14	43/12	33/11
Duration of disease	6.8±2.0	7.0±1.9	5.9±1.8	6.1±2.1	6.8±2.2	6.5±2.1
Symptom scores	1.8±1.2	1.2±0.9	2.0±1.5	1.5±1.2	1.5±1.4	1.9±1.7
Medication scores	2.1±1.4	1.8±1.2	2.1±1.7	1.8±0.9	1.8±0.9*	3.2±2.3*
PEFR (L/min)						
Morning	200±61	222±95	187±63	198±67	214±62	216±66
Evening	216±64	222±95	200±62	211±69	231±66	230±75
Serum IgE level (IU/ml)	1645±792	1733±845	1836±881	1724±819	1698±825	1596±749
Number of patients receiving immuno-therapy with mite (> 3 months)	11	9	22	17	14	13

All data are expressed as mean±SD, *p<0.01.

group A was Liu-Wei-Di-Huang Wan (herb A) which was composed of six kinds of medicinal herbs; for group B Shen-Ling-Bai-Shu-San (herb B) which was composed of 11 kinds of herbs; and for group C Jia-Wei-Si-Jun-Zi-Tang (herb C) which was composed of nine kinds of herbs (Table 1). All three prescriptions have been used for hundreds of years in China and approved by the Ministry of Health of government for marketing. However, for convenience and standardization of drugs, the total amount of medicine required for the entire study was prepared at one time, aliquoted and packed. The quality of each component herb in the regimen was checked by high pressure liquid chromatography (HPLC) and fulfilled the government requirement. Both the active drugs and placebo were brown in color and packed in an undistinguishable appearance.

Protocol

After a run-in period of 2 weeks to obtain the baseline data and train the patients/parents to fill up the diary card and use the peak flow meter (Vitalograph Ltd., England), the treatment was started with one pack t.i.d. (4.5 gm/day) and was continued for 6 months. The patients came back every 2 weeks to get medications and to be followed up by both Chinese doctors and allergists, to check compliance and adverse effects of medications. At the completion of study, the diary cards were analysed by a biostatistician, who did not participate in the design of the study and the parents, allergists and Chinese doctors rated the overall effectiveness of treatment with a 4-point scale (1=very effective to 4=asthma worsening) before the codes were opened.

Patients/parents recorded the severity of daytime asthma, night-time asthma, morning chest tightness and cough with a 5-point scale (0=none to 4=sev-

Table 3. Changes of lymphocyte subsets after treatment

	Deficiency of kidney energy			Deficiency of spleen energy			Deficiency of kidney and spleen energy		
	0	3m	6m	0	3m	6m	0	3m	6m
CD3 ⁺ T cell	68.2±6.6*	65.0±4.9 ^a	77.8±14.0 ^a	68.9±6.7	67.6±7.6	67.0±7.6	69.8±10.3	69.8±8.3	68.4±8.3
	67.3±4.8 [#]	69.2±6.0	67.5±4.7 ^a	60.9±8.1	67.0±8.9	68.1±9.7	69.2±7.6	69.3±7.7	67.3±4.8
CD19 ⁺ B cell	23.2±5.7	24.6±8.6	11.7±4.9 ^b	20.3±6.1	19.5±5.6	18.0±5.6	19.2±9.5	19.5±7.1	19.5±8.9
	25.2±3.5	19.2±5.3	21.8±5.7 ^b	21.6±6.9	19.5±7.3	19.8±6.3	20.3±6.7	19.0±7.1	21.7±31.8
NK cell	6.3±3.2	9.0±4.9	6.0±5.3	8.3±4.8	9.4±5.2	10.6±5.4	8.4±4.4	8.6±4.1	9.0±4.0
	5.7±1.8	7.0±2.2	6.0±3.5	7.1±4.0	10.4±5.5	9.5±5.8	7.9±2.6	9.3±5.3	8.9±2.4
CD8 ⁺ T cell	24.5±5.8	23.4±6.1 ^c	32.7±10.5 ^c	26.2±5.4	26.9±5.9	27.8±5.0	25.8±5.7	26.1±5.0	26.5±6.7
	25.0±5.5	25.8±5.7	28.3±4.9	26.8±6.5	26.2±5.7	26.6±7.0	26.1±5.8	26.2±5.9	29.2±3.4
CD4 ⁺ T cell	38.3±4.8	35.2±4.4 ^d	40.3±6.8 ^d	37.0±6.6	33.1±9.1	37.4±7.0	35.4±7.1	38.7±6.6	36.2±10.1
	35.3±7.9	37.6±4.9	37.5±7.9	35.5±6.1	35.7±6.6	37.4±4.8	36.7±6.6	37.2±5.6	39.6±4.2
CD45RA ⁺ CD4 ⁺ memory T cell	12.0±2.1	14.6±3.8 ^e	22.3±8.5 ^e	11.8±3.6	14.0±4.1	16.4±3.9	13.6±3.6	14.9±4.0	16.7±4.6
	11.0±2.8	12.6±3.5	17.5±5.1	12.6±4.7	14.3±3.9	18.6±6.7	12.4±2.9	14.5±3.8	15.9±2.7
CD25 ⁺ CD4 ⁺ T cell	8.7±1.8	8.8±2.5	9.3±2.5	6.9±2.4	6.5±1.6	8.1±2.5	7.2±1.9	7.3±2.2	8.4±3.2
	7.2±2.0	8.7±1.2	8.3±3.5	7.8±2.7	7.0±2.2	10.2±6.5	7.1±2.7	7.7±1.8	8.0±2.0
HLA-DR ⁺ CD4 ⁺ T cell	4.5±1.1	3.8±1.6 ^f	5.1±1.0 ^f	4.0±2.1	3.8±1.3	5.1±1.7	3.7±1.4	4.0±1.6	4.8±2.2
	3.5±1.1	5.4±1.8	6.0±2.6	4.0±1.3	3.9±1.3	6.4±2.9	4.6±2.4	4.7±1.8	5.6±2.1

*Herbal group (mean±SD) # Placebo group (Mean ±SD) a, b, c, d, e, f: p < 0.001.

Table 4. Changes of serum histamine and *in vitro* production of histamine after treatment

		Serum histamine (ng/ml)			Supernatant histamine (ng/ml)		
		0	3m	6m	0	3m	6m
Deficiency of kidney energy							
Herbal group	(n=32)	142.0±73.1 ^{*a}	95.9±92.6	52.0±123.0 ^a	99.8±71.0 ^f	84.0±91.8	45.2±39.7 ^f
Placebo group	(n=34)	114.2±164.9	108.4±124.4	75.0±83.3	120.8±102.7 ^g	82.7±65.7	61.8±47.9 ^g
Deficiency of spleen energy							
Herbal group	(n=74)	170.3±251.8 ^b	235.7±227.6	43.6±81.3 ^b	102.9±145.6 ^h	58.2±101.0	22.5±44.9 ^h
Placebo group	(n=64)	145.4±222.2 ^c	228.8±192.5	61.8±46.9 ^c	110.7±167.1 ⁱ	87.5±126.0	42.0±69.6 ^j
Deficiency of kidney and spleen energy							
Herbal group	(n=55)	165.4±222.0 ^d	134.7±127.4	18.8±38.1 ^d	119.9±162.7 ^m	51.6±81.1	11.2±17.1 ⁿ
Placebo group	(n=44)	137.3±195.2 ^e	162.4±178.3	22.8±43.2 ^e	205.7±209.6 ^k	110.7±125.1	25.7±45.5 ^k

*Mean±SD, a, b, d, e, f, g, h, j: p<0.001; i: p<0.01; c, k: p<0.02; l, m, n: p<0.05.

ere), and the best of the three tests of morning and evening peak expiratory flow rates (PEFR) was recorded. The increase of PEFR was calculated by subtracting the absolute value of PEFR at a given time point after treatment from that of baseline.

The use of all antiasthmatic medications was recorded. The patients continued medications during the run-in period that were able to control their asthma before being enrolled into this study; however, immunotherapy was discontinued. After starting the study, the patients were encouraged, based on PEFR, to decrease their daily medications as much as possible until the earliest sign of discomfort was experienced. The primary rescue medication was inhaled β_2 agonists; however, any kind of antiasthmatic medication was used for various periods of time to control the acute exacerbation as soon as possible and to regain the stability of the disease. The medication score was calculated according to report of Bouquet et al. (9), with slight modification, e.g., a score of 3 for intravenous theophylline for one day and a score of 6 for intravenous steroid for one day.

Laboratory tests

Thirty ml of heparinized whole blood was drawn from each patient before, and 3 months and 6 months after the study. Blood samplings were done at around 4 p.m. each time. Peripheral blood mononuclear cells (MNCs) were isolated by Ficoll/Hypaque density gradient centrifugation and polymorphonuclear leukocytes (PMNs) by 6% dextran sedimentation. Lymphocyte subsets including total T cells (CD3), B cells (CD19), helper T cells (CD4), suppressor T cells (CD8), memory T cells (CD45RA-CD4+), activated T cells (CD25+CD4+ and HLA-DR+CD4+) and natural killer cells (CD3-CD16+ CD56+) were enumerated by staining with respective monoclonal antibodies (Becton-Dickin-

son, Mountain View, CA, USA) and counted by FACscan flow cytometry. Lymphokine (IFN- γ and IL-4) production was done by stimulating MNCs (2×10^6 cells/ml) with 2 μ g/ml PHA (Wellcome, UK) for 24 h; IFN- γ concentration was measured by IFN- γ ELISA kits (Medgenix Diagnostics SA, Belgium); and IL-4 by Quantikine IL-4 ELISA kits (R & C System, Minneapolis, MN, USA). Inflammatory mediator (histamine, PGE2, LTC4) production was done by stimulating PMNs (2×10^6 cells/ml) with 8 μ g/ml of calcium ionophore A23187 for 30 min and histamine concentration in supernatants was measured by Histamine ELISA kits (IBL, Hamburg), PGE2 by PGE2 ELISA kits (Cayman Chemical Co., Ann Arbor, MI, USA) and LTC4 by LTC4 ELISA kits (Cayman Chemical Co., Ann Arbor, MI, USA). In addition to immunological study, hepatic, renal and hematological functions were also followed-up.

Sensitization of guinea pigs with ovalbumin (OVA)

Sensitization of guinea pigs was done according to the method of Hutson et al. (10). Male guinea pigs weighing 250–300 gm were sensitized by exposure to aerosolized 1% OVA once a week for consecutive two weeks. The aerosols were generated by Pulmoaid (nebulizer driven by compressed air at 8 L/min, De Vilbiss, model 5610D, USA). The aerosols were generated into a 3.9 L perspex chamber in which the animals were placed for 10 min on each exposure. The guinea pigs were then challenged with 2% OVA one week later. A decrease in sGaw of greater than 50% was considered successful sensitization. About 75% of guinea pigs showed an immediate asthmatic reaction (IAR); and among them, 50% showed a late asthmatic reaction (LAR). As a whole, 55%–75% sensitized guinea pigs survived the challenge. The sGaw was measured by a body plethysmography (PMR-2, Shizume Medical Co., Ltd., Japan).

Table 5. The effect of traditional Chinese medicines on the distribution of leukocytes six hours after allergen challenge in ovalbumin-sensitized asthmatic guinea pigs

Guinea pigs	Blood	Bronchoalveolar lavage fluid				
	eosinophil (%)	WBC (/mm ³)	Total eosinophil count (/mm ³)	Eosinophil (%)	Neutrophil (%)	Mononuclear cells (%)
Not Sensitized not challenged (n=8)	1.0±0.8	15.6±5.3	0.6±0.7	3.5±4.1	10.3±4.1	86.2±5.0
Not Sensitized OVA challenged (n=6)	0.8±0.6	22.8±8.5	1.7±0.9	9.0±1.3	17.7±4.5	73.3±4.3
Sensitized, saline challenged (n=6)	3.0±1.2	36.7±5.9	5.7±1.6	16.3±3.1	3.3±1.2	80.3±3.1
Sensitized, challenged						
Pretreated 10 days with placebo (n=8)	16.5±8.0	206.3±15.0	77.9±23.7a	37.5±5.4b	13.6±1.9	48.9±5.7
Pretreated 10 days with herb A (n=9)	11.4±2.2	25.8±10.4	1.7±1.2	3.8±1.5	5.9±1.2	90.3±2.2
Pretreated 10 days with herb B (n=11)	14.1±3.9	132.7±29.3	17.5±5.0	13.3±1.5	7.0±1.5	79.7±2.7
Pretreated 10 days with herb C (n=8)	15.8±2.7	142.5±32.3	21.5±4.0a	15.5±1.3b	6.1±1.8	78.4±2.1

All guinea pigs were fed medicines for 10 days before challenge.

All data are expressed as mean±SE. a, b: $p < 0.0001$.

Study of LAR of asthmatic guinea pigs

Ten mg/kg of diphenhydramine was given intraperitoneally to guinea pigs, 30 min before challenge, to suppress IAR. The guinea pigs were then killed at 6 h, 12 h, 24 h, 48 h and 4 weeks after challenge, respectively. Bronchoalveolar lavage fluid (BALF) was obtained by bronchial washing and pathological examinations of lung tissue were done, with particular emphasis on the eosinophil infiltration. Eosinophils, PGE₂ and LTC₄ in BALF were measured.

Study of TCM on LAR of asthmatic guinea pigs

To study the therapeutic effect of TCM on LAR of guinea pigs, those animals showing LAR were divided into four groups, and then fed orally with 10 gm of placebo (n=8), herb A (n=9), B (n=11), and C (n=8) daily, respectively, for 10 days. Then OVA challenge was performed to study the protective effect of TCM on the occurrence of LAR. Three control groups were included: not sensitized and not challenged (n=8), not sensitized and OVA challenged (n=6), and OVA sensitized and saline challenged (n=6).

Statistical analysis

Diary card data were analysed as daily means for every month over the 6 months of treatment and the data were expressed as mean±SEM. Paired *t* test was used for comparison between baseline and post-treatment data, two sample *t* test for drug and placebo

groups, and Wilcoxon rank sum test for analysis of difference in symptom score and medication score between drug and placebo groups.

For animal studies, one way analysis of variance (ANOVA) was used to compare the changes between different groups. Two sample *t* test was used to analyse the difference among placebo- and herb-treated groups. A *p* value of less than 0.05 was considered significant.

Results

Three-hundred and three asthmatic children had completed the 6-month clinical trial. Thirty-one patients (13 in the treatment group and 18 in the placebo group) withdrew during the study period. The most common causes for dropout were intercurrent illness, lack of effectiveness and moving away. Only three patients reported abdominal pain after taking active medication (one in each group) and two disliked the taste of the medication. No abnormal hepatic, renal and hematological function was found. The demographic data are shown in Table 2. The age, sex, duration of illness, serum IgE levels, number of patients receiving mite immunotherapy, symptom score, medication score and morning and evening PEFs were comparable between treatment and placebo groups in all three groups of different constitution, except the medication score for placebo group in group C was higher than that of drug group (3.2±2.3 vs 1.8±0.9, $p < 0.01$). All of the patients were strong positive to *D. pteronyssinus* (6+ in CAP system, Pharmacia).

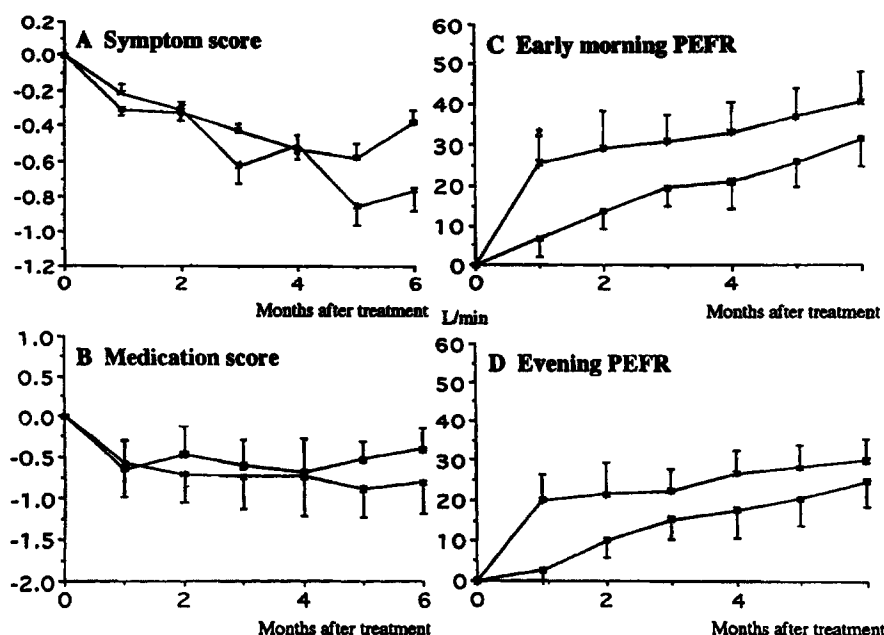


Fig. 1. Clinical evaluation of Liu-Wei-Di-Huang-Wan (herb A) in asthmatic children with deficiency of kidney energy. Thirty-two patients were treated with active drug (*) and 34 with placebo (■). The drug group showed a greater improvement in symptom score, medication score and increased PEFRs than the placebo group, but the difference was significant only in morning PEFR at one month after starting treatment ($p < 0.05$). Data are expressed as mean $\pm$ SE.

Efficacy

Fig. 1 shows the clinical efficacy of Liu-Wei-Di-Huang-Wan (herb A) in the treatment of patients with deficiency of kidney energy. The symptom score started to decrease in both drug and placebo groups eight weeks after treatment. Although there was no difference between the two groups, when compared to baseline the decrease was more prominent in the drug group and continued to the end of study. The medication score also decreased one month after treatment in both groups. Although there was no difference in total medication score, the requirement for the use of steroid was significantly less in drug group than in placebo group at 3 months of treatment ($p < 0.05$, data not shown). After treatment, the morning and evening PEFRs in both groups began to increase until the end of study; however, the difference in PEFR between drug and placebo group was significant only at one month of treatment, ($p < 0.05$ for morning PEFR, Fig. 1C).

Fig. 2 shows the results of treatment with Shen-Ling-Bai-Shu-San (herb B) in patients with deficiency of spleen energy. The symptom score began to decrease significantly one month after treatment and then continued until the end of study in both groups. The drug group tended to have fewer symptoms than the placebo group at 4 ($p = 0.06$) and 5 ($p = 0.07$) months after treatment. The medication score also decreased after treatment, and although there was no difference between drug and placebo group, the use of β_2 inhaler was much decreased in drug group ($p < 0.05$, data not shown). The morning and evening PEFRs increased in parallel for both groups, but no difference was noted.

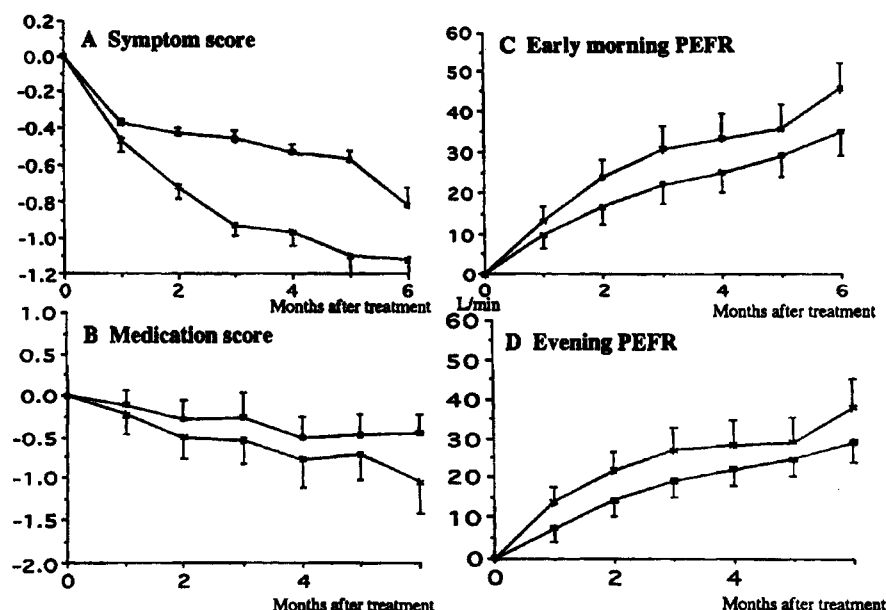
The efficacy of Jia-Wei-Si-Jun-Zi-Tan in the treatment of asthmatic children is shown in Fig. 3. Both symptom score and medication score decreased in drug and placebo groups after starting treatment, and the placebo group tended to show a greater, but not significant, benefit. To the contrary, the drug group showed a persistent greater improvement in morning and evening PEFRs than the placebo group, and the difference was significant for morning PEFR at 2 ($p < 0.01$) and 4 ($p < 0.05$) months after starting treatment (Fig. 3C).

The changes of lymphocyte subsets after treatment are shown in Table 3. Herb B and herb C had no effect on the distribution of lymphocyte subsets; however, herb A significantly decreased B cells ($p < 0.001$) and increased total (CD3+) T cell ($p < 0.001$) at 6 months of treatment, and the increase in CD3+ T cell was due to the simultaneous increase of both CD4+ and CD8+ T cell. Furthermore, the increased CD4+ T cell belonged mainly to the CD45RA-CD4+ memory T cell ($p < 0.001$) and HLA-DR+ CD4+ activated T cells ($p < 0.001$).

Serum IFN- γ could not be detected in the vast majority of patients, and serum IL-4 and *in vitro* production of IFN- γ and IL-4 were not changed after treatment in all patient groups (data not shown). There was no change in total serum IgE in all three constitutional groups at the end of treatment, although a slight, but not significant, decrease in total eosinophil count in drug groups was found (data not shown).

Table 4 shows the serum histamine and *in vitro* production of histamine before and after treatment. There was a general tendency for serum histamine to decrease significantly at the end of treatment for

Fig. 2. Clinical evaluation of Shen-Ling-Bai-Shu-San (herb B) in asthmatic children with deficiency of spleen energy. Seventy-four patients were treated with active drug (*) and 64 with placebo (■). The drug group showed a greater improvement in symptom score, medication score and increased PEFs, but no difference was found between the two groups. Data are expressed as mean $\pm$ SE.



both drug and placebo groups, and there was no difference between the two groups. There was also a general tendency for *in vitro* production of histamine to decrease in groups A, B and C, no matter whether treated with active drug or placebo ($p < 0.001$), but the decrease was much greater in the drug group than in the placebo group ($p < 0.05$, for groups B and C, respectively). It is interesting to point out that in group C, the *in vitro* production of histamine before treatment was much greater in the placebo group than that of the ex-drug group ($p < 0.05$) and this result might explain the higher medication score in the placebo group ($p < 0.01$, Table 2). There was no difference in the production of LTC₄ before and after treatment in all three constitutional groups, but in groups A and B, PGE₂ production was increased after treatment with herbals (4349.9 ± 1759.8 pg/ml vs 5279.4 ± 1719.2 pg/ml, $p < 0.02$ for group A and 3901.6 ± 2035.7 pg/ml vs 5273.8 ± 1543.8 pg/ml, $p < 0.001$ for group B).

Due to the ethical consideration (mainly young children in this study), asthmatic guinea pigs instead

of patients were used to study the working mechanisms of TCM. The changes of distribution of leukocytes in BALF after allergen challenge in OVA-sensitized asthmatic guinea pigs were followed up serially. The percentage of eosinophil in BALF increased from $16.3 \pm 3.1\%$ before challenge ($n=6$), to $37.5 \pm 5.4\%$ at 6 hours ($n=6$), to $37.7 \pm 1.2\%$ at 12 hours ($n=3$), and to $40.0 \pm 3.7\%$ at 24 hours ($n=3$), then started to decrease to $25.0 \pm 4.7\%$ at 48 hours ($n=3$) and finally to $17.0 \pm 1.9\%$ at 4 weeks ($n=4$) which was comparable to the prechallenge value. There was a parallel change in peripheral blood. Pretreatment of guinea pigs with three kinds of TCM markedly decreased the total number of WBC and eosinophilia in BALF (Table 5). The effect was particularly prominent for herb A, and the BALF eosinophilia in herb A-treated guinea pigs was even lower than that of OVA-sensitized and saline challenged guinea pigs (equivalent to asymptomatic asthmatics).

Treatment with TCM was able to prevent the bronchoconstriction after allergen challenge. Before

Table 6. The effect of traditional Chinese medicines on the serum and BALF level of PGE₂ and LTC₄ of ovalbumin-sensitized guinea pigs after allergen challenge

Guinea pigs	Serum PGE ₂ (pg/ml)	Serum LTC ₄ (pg/ml)	BALF PGE ₂ (pg/ml)
Not sensitized, OVA challenged (n=6)	163.05 $\pm$ 2.14	45.11 $\pm$ 0.99	93.96 $\pm$ 4.27
Sensitized, saline challenged (n=6)	185.05 $\pm$ 19.87	65.23 $\pm$ 21.11	116.75 $\pm$ 18.52
Sensitized, challenged			
Pretreated with placebo (n=8)	728.09 $\pm$ 16.82 _{a,b}	347.93 $\pm$ 23.76 _{f,g,h}	924.04 $\pm$ 135.29 _{k,l}
Pretreated with herb A (n=9)	646.07 $\pm$ 206.33 _{c,e}	197.34 $\pm$ 27.29 _{f,j}	853.86 $\pm$ 227.02 _{m,n}
Pretreated with herb B (n=11)	971.95 $\pm$ 199.35 _{a,c,d}	205.44 $\pm$ 14.85 _{g,i}	1058.68 $\pm$ 102.75 _{k,m}
Pretreated with herb C (n=8)	1118.46 $\pm$ 71.18 _{b,d,e}	242.98 $\pm$ 29.10 _{h,i,j}	1115.15 $\pm$ 88.77 _{l,n}

All data are expressed as Mean $\pm$ SE (pg/ml).
a, b, c, e, f, g, h, i, j, k, l, m: $p < 0.01$; d, n: $p < 0.05$

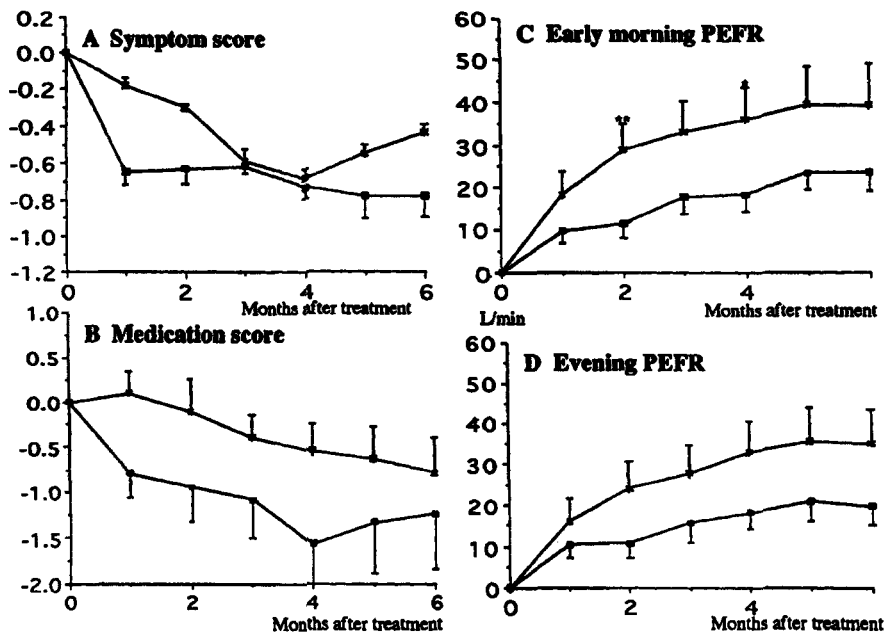


Fig. 3. Clinical evaluation of Jia-Wei-Si-Jun-Zi-Tang (herb C) in asthmatic children with deficiency of kidney and spleen energy. Fifty-five patients were treated with active drug (*) and 44 with placebo (■). The pre-treatment medication score for the placebo group was much higher than for the drug group ($p < 0.01$). After treatment, the placebo group showed a greater, but not significant, improvement in symptom score and medication score. To the contrary, the drug group showed a greater increase in PEFRs and the difference in morning PEFR was significant at 2 months ($p < 0.01$) and 4 months ($p < 0.05$) after treatment. Data are expressed as mean $\pm$ SE.

treatment, the sGaw of the placebo group ($n=8$) decreased to $35.7 \pm 9.5\%$ of baseline after allergen challenge; the figure for herb A ($n=9$) was $30.4 \pm 5.7\%$, $37.0 \pm 8.6\%$ for herb B ($n=11$) and $28.0 \pm 8.2\%$ for herb C ($n=8$). After treatment, the figure was nearly the same for the placebo group ($36.6 \pm 8.8\%$); however, the sGaw was increased to $86.1 \pm 5.1\%$ for herb A group ($p < 0.01$), to $69.0 \pm 6.2\%$ for herb B group ($p < 0.05$) and to $54.9 \pm 5.5\%$ for herb C group ($p < 0.05$).

Eosinophil infiltration of airways was examined serially in guinea pigs sacrificed at a particular given time after allergen challenge. Fig. 4a ($200 \times$ magnification) shows that only a few cells were present in the adventitia of large airways of OVA sensitized but not-challenged animal. There was no cell infiltration but markedly dilated alveoli in guinea pigs died immediately (within one minute) after allergen challenge (Fig. 4b). The type of infiltrating cells was mainly neutrophil at 30 min (Fig. 4c) and at 2 h (Fig. 4d). Eosinophil started to increase at 4 h (Fig. 4e) and increased steadily (6 h, Fig. 4f and 12 h, Fig. 4g) and was still present in large amount at 2 days after allergen inhalation (Fig. 4h).

The capacity of TCM to suppress the eosinophil infiltration of airways of asthmatic guinea pigs after allergen challenge is shown in Fig. 5. Only a few cells were present in normal guinea pigs (Fig. 5a). There were several cell nests containing eosinophils in the sensitized but not challenged animals (Fig. 5b, equivalent to asymptomatic asthmatics). At 6 h after allergen challenge, there was a marked eosinophil infiltration in the adventitia with destruction of mucosa (Fig. 5c). Treatment with herb A (Fig. 5d)

could markedly suppress the eosinophil infiltration. Herb B (Fig. 5e) and herb C (Fig. 5f) showed a much smaller effect.

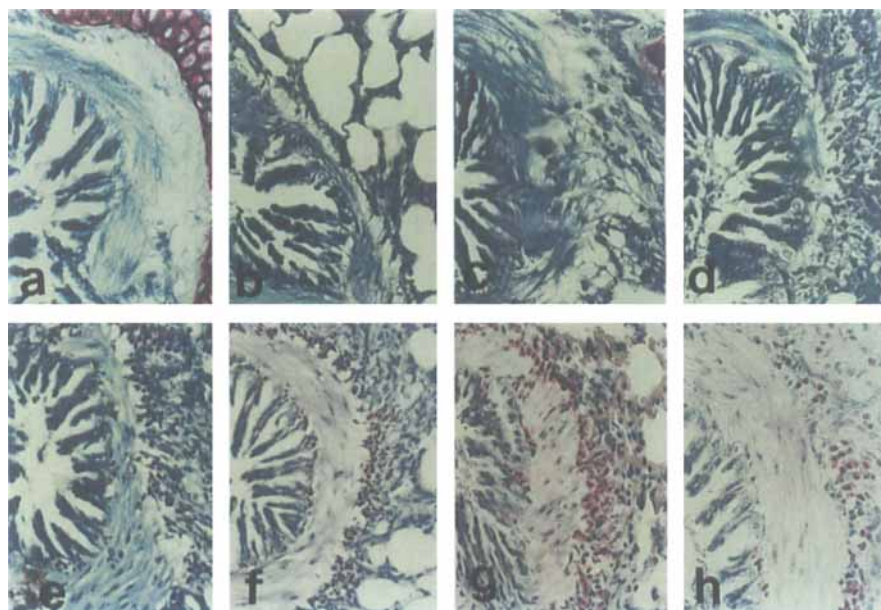
Table 6 shows that allergen challenge resulted in increased concentration of serum PGE 2 and LTC₄, and BALF PGE₂. While treatment with herb A suppressed only the allergen-induced increase of serum LTC₄ concentration, treatment with herb B and C increased the serum and BALF PGE₂ and decreased the serum LTC₄ simultaneously.

Discussion

Traditional Chinese medicines (TCM) have been used for the treatment of bronchial asthma for thousands of years, and a certain degree of benefit has been observed clinically (1). However, all of those reports usually included only a few cases, evaluated subjectively and were lacking scientific substantiation. Up to now, there is still no report on the treatment of bronchial asthma with traditional Chinese medicine in the literature that adapts the multicenter, double-blind and placebo-controlled method which has been used as a standard model in modern medicine to evaluate the efficacy of a new therapeutic modality. Although reports using this model in studying the treatment of atopic dermatitis with traditional Chinese medicine have been published recently (11, 12), no attempt was made to elucidate the working mechanism in those studies.

According to the principle of Chinese medicine, the prescription of medication for a patient should be based on both the constitution of the patient and the symptoms and signs at presentation. During the

Fig. 4. Time course of eosinophil infiltration of asthmatic guinea pig airways after allergen challenge. Only a few eosinophils were present before challenge (a). No cell infiltration but markedly dilated alveoli were found in guinea pig that died immediately after challenge (b). The type of infiltrating cells at 30 min (c) and 2 h (d) after challenge was mainly neutrophils. Eosinophil started to increase at 4 h (e), dominated at 6 h (f), 12 h (g) and even at 48 h (h) after challenge. ($\times 200$.)

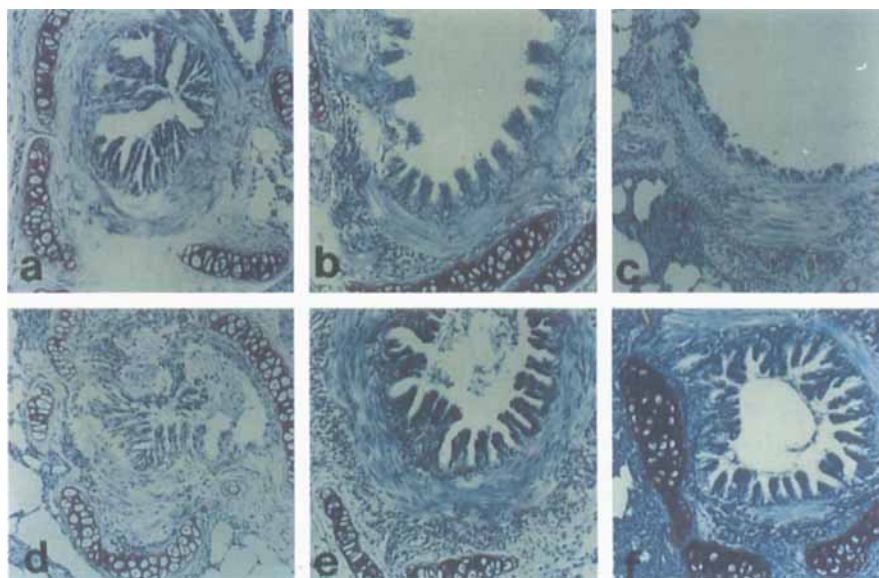


course of treatment, the component herbs in the prescription should be modified, depending upon the change of clinical manifestations after treatment. In this study, in order to observe the efficacy of a particular prescription, the composition of herbals was kept unchanged throughout the study, and this policy was different from the usual practice of Chinese medicine. However, the constitution of patients was classified by Chinese doctors using a standardized questionnaire to avoid individual bias and then the most appropriate regimen was prescribed for the patients with a particular constitution. The decision to choose these regimens was also made by a panel of experienced Chinese doctors. Thus, this was the first study in the world combining the multicenter,

double-blind and placebo-controlled method of modern medicine with the basic logic of Chinese medicine to treat patients with bronchial asthma. Moreover, detailed immunological testing and asthmatic guinea pig modeling were performed to elucidate the working mechanism of traditional Chinese medicine.

The symptom score for all three constitutional groups, no matter whether drug or placebo treated, began to decrease after starting treatment. In patients with deficiency of kidney energy and those with deficiency of spleen energy, the decrease in symptom score was usually greater in the drug group than in the placebo group, but in patients with deficiency of kidney and spleen energy, the result

Fig. 5. The effect of traditional Chinese medicines on the eosinophil infiltration of asthmatic guinea pig airways at 6 h after allergen challenge. Only a few cells were present in normal animal (a); there were cell nests containing eosinophils in the sensitized but not challenged animals (b); marked eosinophil infiltration was found at 6 h after challenge (c); treatment with herb A markedly suppressed the eosinophil infiltration (d); herb B (e) and herb C (f) showed a much smaller effect. ($\times 100$.)



was just the reverse. However, there was no statistically significant difference between the drug and the placebo group in all three constitutional groups. There was a similar tendency for medication score. The greater, although not significant, improvement in both symptom score and medication score in patients with deficiency of kidney and spleen energy treated with placebo might be due to more severe disease, as reflected in the higher *in vitro* histamine production ($p < 0.05$, Table 4) and greater amount of medication consumption at the beginning of the study ($p < 0.01$ for medication score, Table 2), and therefore had a greater space to improve. It is worth noting that the requirement for steroid in patients treated with herb A and the requirement for β_2 inhaler in patients treated with herb B was much less ($p < 0.05$ and $p < 0.05$, respectively) than their counterpart placebo group. There was a consistent finding that both the morning and evening PEFs began to increase after starting treatment, and again, the improvement was seen in both the drug and the placebo group, although the improvement was significantly greater in patients treated with herb A (Fig. 1C) and herb C (Fig. 3C).

It is interesting to note that total (CD3+) T cell was increased ($p < 0.001$) and B (CD19+) cell was decreased ($p < 0.001$) after treatment with herb A in patients with deficiency of kidney energy (Table 2). The increase in CD3+ T cell was due to a proportionately simultaneous increase of CD4+, CD8+, CD45RA-CD4+, and HLA-DR+CD4+ T cells. No such kind of change was seen in the other two groups. The clinical implication and significance needs further study as the changes in lymphocyte distribution in group A did not result in alteration in *in vivo* and *in vitro* production of lymphokines such as IFN- γ and IL-4, and serum IgE levels remained the same (data not shown).

A number of inflammatory mediators has been shown to be involved in the early and late asthmatic reaction (13); therefore, histamine, PGE2 and LTC4 were measured to see whether TCM has any effect on the production of inflammatory mediators. After treatment, the *in vivo* (serum) and *in vitro* production of histamine tended to decrease in all patients no matter whether treated with TCM or placebo (Table 4); however, only both drug groups of groups B and C showed a greater decrease of *in vitro* production of histamine than did their counterpart placebo groups ($p < 0.05$ and $p < 0.05$, respectively). Furthermore, the placebo group of group C that showed the highest serum histamine also had a higher medication score. There was no change in LTC4 production before and after treatment, but TCM treatment significantly enhanced PGE2 production in groups A ($p < 0.02$) and B ($p < 0.0001$). Thus, the decreased histamine production and enhanced PGE2 production

may be used to explain partly the clinical efficacy of TCM, although a placebo effect is present.

The global effectiveness was assessed independently by allergists, Chinese doctors and parents at the completion of study. In the opinion of allergists, 85% of group A patients responded to TCM and 50% to placebo; for group B, the figures were 75% and 40%, respectively; and for group C, 70% and 40%, respectively. In the opinion of Chinese doctors, the figures for group A were 85% and 50%, respectively; for group B, 80% and 50%, respectively; and for group C, 75% and 40%, respectively. In the opinion of parents, the figures for group A were 90% and 55%, respectively; for group B, 85% and 50%, respectively; and for group C, 80% and 50%, respectively. Therefore, the TCM did show a satisfactory therapeutic effect; however, a rather great placebo effect was also noted.

Recent studies strongly suggest that late phase asthmatic reaction with predominant eosinophil infiltration of airways is the main clinical and pathologic characteristics of human bronchial asthma (13), and this hypothesis is strongly supported by the animal models of bronchial asthma, particularly in guinea pigs (5, 10, 14–18). It is very important to note that in asthmatic guinea pigs, both peripheral blood and particularly BALF eosinophilia and infiltration of airways with eosinophil were markedly suppressed by administration of Chinese herbs, especially herb A. Moreover, the increased production of PGE2 after treatment with herb A and B in human, and herb B and C in guinea pigs (in serum and BALF) and decreased production of LTC4 after treatment with herb A in guinea pigs, in conjunction with decreased eosinophil infiltration of the airways, may be used to explain the clinical efficacy of the Chinese herbs in the treatment of bronchial asthma. The working mechanisms of TCM on the prevention of LAR looks very much like that of nedocromil (19) and PAF antagonist WEB 2086 (5), and the study of active principles in the TCM has already been underway.

The effectiveness of TCM to treat bronchial asthma was further proved by its capacity to reverse the decreased sGaw after allergen challenge. A pitfall of the method used in this study to measure the sGaw was that the decrease of sGaw after allergen challenge might not reflect the actual change of sGaw of the bronchial airways, because the change in conductance of the nose must contribute, to a certain degree, to the total change of sGaw. However, our studies analysing the bronchial lavage and lung tissue supported the changes occurring in the lower airways.

In conclusion, traditional Chinese medicines do show a therapeutic effect on childhood bronchial asthma. The decreased histamine and LTC4 produc-

tion and increased PGE₂ production that occurred in a certain portion of patients may be used to account partly for the clinical efficacy of TCM. Furthermore, a similar change in the production of inflammatory mediators, the markedly diminished pulmonary eosinophil infiltration, and preserved airways conductance after allergen challenge that was observed in asthmatic guinea pigs after treatment with TCM will further provide a rationale for Chinese herbs in the treatment of bronchial asthma. Finally, a significant placebo effect should be taken into consideration in evaluating a new therapeutic modality, including TCM, in childhood asthma.

Acknowledgements

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