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Total Glucosides of Paeony in Combination with Mizolastine Decreased Serum Concentrations of Il-17 And Il-25 in Patients with Chronic Autoimmune Urticaria: A 12-Week Study

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Abstract

Objective: This study investigated total glucosides of paeony (TGP)'s effect on serum IL-17 and IL-25 concentrations in patients with chronic autoimmune urticaria (CAU).

Methods: 71 autologous serum skin test (ASST)+CAU patients were randomized into two groups: 35 patients orally received 0.6g TGP t.i.d. plus 10 mg mizolastines once daily for 12 weeks (Treatment Group). The remaining 36 patients orally received 10 mg mizolastines once daily for 12 weeks (Control Group). Additionally, 60 ASST- chronic spontaneous urticaria patients were used for baseline comparison. Serum IL-17 and IL-25 concentrations were measured and compared between the Treatment Group, Control group and the ASST- patients at baseline; they were further measured and compared between the Treatment and Control groups at weeks 4 and 12.

Results: Baseline serum IL-17 and IL-25 concentrations in the ASST+ patients (173.40 ± 76.33 pg/ml, 22.07 ± 11.41 pg/ml, respectively) were significantly higher than those in the ASST- patients (76.15 ± 91.04 pg/ml, 8.69 ± 10.00 pg/ml, respectively). Significantly lower concentrations of IL-17 (102.60 ± 42.39 *vs.* 149.00 ± 76.61, P=0.003) and IL-25 (10.92 ± 5.45 *vs.* 16.74 ± 9.34, P=0.003) were associated with the Treatment Group *vs.* the Control Group at week 12, but not at baseline or week 4. Finally, the Treatment Group had significantly decreased IL-17 and IL-25 concentrations *vs* baseline at week 12 but not week 4, whereas the Control Group's IL-17 and IL-25 concentrations didn't change significantly over the 12 weeks.

Conclusion: TGP significantly decreased serum IL-17 and IL-25 concentrations in patient with CAU at week 12. This provided basis for exploring TGP as an alternative treatment for CAU.

Keywords: Chronic autoimmune urticaria; Total glucosides of paeony; IL-17; IL-25; Th1; Th2; Th17

Introduction

Chronic Urticaria (CU) is a skin disorder affecting up to 1% of general population [1]. Chronic spontaneous/idiopathic urticaria (CSU or CIU), a common subtype of CU, is characterized by rapid, spontaneous and recurrent pruritic wheals without obvious stimulus that last<24 h for ≥ 6 weeks [1-4]. An autoimmune etiology could be found in about 30-40% of CSU patients (chronic autoimmune urticaria ([CAU]), the autoimmune etiology involves histamine-releasing autoantibodies against the α subunit of high-affinity IgE receptor (Fc∈RIα) or IgE [1,2]. By binding to Fc∈RIα or IgE on the surface of mast cells and basophiles, these autoantibodies activate mast cells and basophiles and lead to their degranulation and release of histamine and other inflammatory mediators, causing symptoms such as itching, skin swelling and local vasodilation [1,2]. The autologous serum skin test (ASST) is an *in vivo* screening test for serum auto reactivity in patients with CSU [2,3]. Genetic predisposition to CU and CAU was observed [4]. It has been found that some human leukocyte antigen (HLA) class II molecules were associated with CU, for an example, DR4 and its genetically associated allele DQ8 were significantly more prevalent in CU patients as a whole and also in ASST+patients alone *vs.* healthy population [5]. Second-generation H1-antihistamines such as mizolastine are the first-line treatment for symptomatic CU, however, some patients remain unresponsive to antihistamines even in high dose, in such cases, and alternative treatments are needed [6].

It has been reported that total glucosides of paeony (TGP) in combination with citirizine was more effective in treating CU than citirizine alone [7]. Compared to citirizine alone, TGP-citirizine combination led to significantly greater decrease in total symptom score and significantly lower relapse rate, additionally, TGP-citirizine combination decreased serum IL-4 and IgE significantly in patients with CU while citirizine alone did not [7]. It seemed that TGP could potentially have therapeutic efficacy for CU by regulating patients' immune functions [7]. TGP is an active anti-inflammatory and immunoregulatory compound extracted from the roots of Paeonia lactiflora pall and contains more than 90% paeoniflorin (PF) [8]. Besides CU, TGP has shown therapeutic efficacy for other autoimmune diseases by ameliorating inflammatory reactions and immune response in these patients [8-10].

It has been reported that TGP decreased mRNA and protein levels of the inflammatory cytokine Interleukin-17 (IL-17) or shrank IL-17 producing cell population in autoimmune patients or animal models of various autoimmune diseases [9-13]. Numerous studies reported elevated serum IL-17 level in ASST+ CAU patients *vs.* ASST- CSU patients, indicating it played a role in the pathogenesis of CAU [2,3,14], although Degirmenci et al. reported comparable IL-17 level in ASST+ vs. ASST- CSU patients [4].

Chen et al. observed mixed T helper cell (Th) 1 (Th1)/Th2 and Th17 immune responses in CSU patients, and these patients had increased circulating levels of T helper (Th)1-, Th2- and Th17-related cytokines [3]. Moy et al. also observed a Th2 and Th17 skewed lymphocyte infiltrate in skin lesions of CU, indicating Th2-associated cytokines' role in CU [15]. Increased number of IL-25+ cells was found in skin lesions of CSU vs. non-skin lesion or normal control, indicating IL-25, a Th2-initiating cytokine, played a role in mast cell activation and degranulation, inflammation and vascular leakage in CSU [16]. Additionally, it has been reported that TGP was able to adjust Th1/Th2 cytokine polarization and help restore Th1/Th2 balance [17,18], this finding is meaningful as Th1/Th2 imbalance could be a possible mechanism for CU [3], Up till now, there has been no report on TGP's effects on circulating IL-25.

This study compared serum IL-17 and IL-25 levels in CAU patients vs. ASST- CSU patients, and also evaluated TGP's effects on IL-17 and IL-25 in patients with CAU.

Materials and Methods

Ethical approval

This study was approved by the Independent Ethics Committee of Hospital for Skin Diseases, Chinese Academy of Medical Sciences, and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice. Written informed consent was obtained from each patient before all trial-related activities.

Study population

Patients diagnosed with CU were recruited from the outpatient clinic of Department of Allergy and Rheumatology, Hospital for Skin Diseases, Chinese Academy of Medical Sciences from January 1, 2017 to March 31, 2017. Inclusion criteria included: recurrent daily or almost daily spontaneous, scattered skin wheals lasting <24 h for >6 weeks; hadn't taken any antihistamines for 7 days before the study; and hadn't taken any corticosteroids or immunosuppressant drug(s) during the one month before the study. Exclusion criteria included: patients with a definite drug or food allergen, mental trigger(s) or infection(s) including chronic worm infestation; patients with history of physical urticaria or urticarial vasculitis; patients with concurrent systemic diseases or severe liver or renal diseases; pregnant or lactating women. Patients who met the above criteria underwent ASST. 71 ASST+ CAU patients were enrolled in the study. An additional 60 ASST-CAU patients were randomly chosen from the outpatient clinic of the same institute to serve as basis for some of the comparisons in the study.

Study design

The 71 ASST+ CAU patients were randomized into two groups: 35 patients in the Treatment Group orally received 0.6g TGP (Miran Pavlin capsules (0.3 g TGP/capsule), Ningbo Lihua Pharmaceutical Co., Ltd, Ningbo, China) three time a day plus one 10 mg mizolastine tablet (Mizollen, Xian Janssen, Xi'an, China) once daily for 12 weeks. The remaining 36 patients in the Control Group orally received one mizolastine tablet (10 mg) (Mizollen, Xian Janssen, Xi'an, China) once daily for 12 weeks.

Upon their enrollment, peripheral blood samples were collected from the patients. 5 ml of peripheral blood was further collected from every ASST+ patients at weeks 4 and 12. Serum samples were subsequently prepared from the blood samples. The serum samples collected at baseline were used to perform ASST (detailed description below), and the remaining serum samples were kept at -20°C. All of the serum samples were stored at -20°C for later measurements. The 60 ASST-CSU patients from the outpatient clinic of the same institute had their blood samples taken only once upon their enrollment.

ASST

Intradermal ASST were performed [2]. Intradermal injections of 50 µl fresh autologous serum and 50 µl 0.9% weight/volume NaCl solution as a negative control were performed on the flexor forearm skin of each participating patient, one injection site being 3-5 cm from the other. The wheal and flare reaction was read at 30 minutes. A positive ASST result was defined as the appearance of a red serum-induced wheal with a diameter at least 1.5 mm greater than that of 0.9% NaCl-induced reaction at 30 minutes (Figure 1). The ASST tests for all of the patients were carried out by one person and the reaction was read by at least 2 persons.

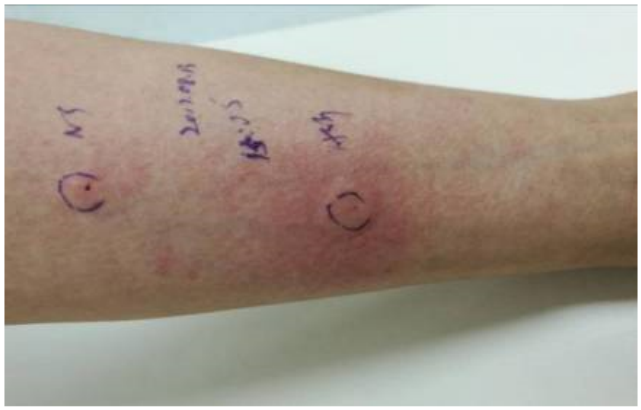

Figure 1: The flexor forearm skin of an ASST+ patients. A positive ASST test result was defined as the appearance of a red serum-induced wheal with a diameter at least 1.5mm greater than that of saline-induced reaction at 30 minutes.

Measurements of serum IL-17 and IL-25

Enzyme-linked immunosorbent assay (ELISA) was used to measure serum IL-17 and IL-25 for all ASST+ and ASST- patients at baseline and for all ASST+ patients at weeks 4 and 12. ELISA for IL-17 and IL-25 were performed with an IL-17 and an IL-25 testing kit, respectively (Jiangsu KeyGEN Bio TECH Corp., Ltd, Nanjing, China. Product No. KGEHC170[H]-1, KGEHC180-1, respectively) strictly according to the manufacturer's instructions.

Adverse events (AEs)

Incidence of AEs were recorded for the ASST+ patients in the Treatment Group and the Control Group to assess safety and tolerability of TGP

Statistical analysis

Data were expressed as mean±standard deviation (SD). Independent-sample t test and $\chi 2$ test were used to compare continuous variables and categorical variables, respectively. Repeated measure analysis of variance (AVOVA) followed by simple effects analysis were performed to test the statistical significance of difference between baseline and post-treatment serum IL-17 and IL-25 levels in the ASST+ patients; while independent-sample t test was used to compare baseline IL-17 and IL-25 levels between the ASST+ patient vs the ASST- patients. All statistical analyses were based on Intention-to-treat (ITT) population to reduce statistical bias. The ITT population included all enrolled patients who had at least one measurement of serum IL-17 and IL-25, and any missing data was processed using last observation carried forward (LOCF). All statistical analyses were performed using SAS9.4 software and statistically significant difference was accepted with a $P<0.05$.

Results

Patients

From January 1, 2017 to March 31, 2017, 71 ASST+ CAU patients were enrolled in the study. Among them, 35 patients were in the Treatment Group (TGP plus mizolastines) and the remaining 36 patients were in the Control Group (mizolastines only). The study lasted 12 weeks. During the study, 3 patients (2 males and 1 female) in the Treatment Group and 2 patients (1 male and 1 female) in the Control Group were lost to follow-up, a final 66 ASST+ patients completed the study. Gender distribution, age and CAU duration were comparable between the Treatment Group and the Control Group for the ASST+ patients and also comparable between the ASST+ patients and the ASST- patients (Table 1).

Variable	ASST+ patients		ASST- patients N=60		
	Treatment Group (TGP +mizolastine) N=35	Control Group (mizolastine alone) N=36		P^a	P^b
Male n (%)	20 (57.14%)	20 (55.56%)	34 (56.67%)	0.89	0.97
Age (years), mean ± SD	38.29 ± 9.12	37.47 ± 8.50	36.92 ± 8.65	0.7	0.53
Disease duration (months), mean ± standard deviation	10.06 ± 6.28	10.22 ± 6.66	9.78 ± 6.40	0.91	0.75

Note: P^a, difference between the ASST+ patients in the Treatment Group vs Control group;
P^b, differences between the ASST+ and ASST-patients.

Table 1: Baseline demographic and disease duration of the ASST+ and ASST- patients

Baseline serum IL-17 and IL-25 concentrations in the ASST+ and ASST- patients

The ASST+ patients had significantly higher baseline serum IL-17 and IL-25 levels (173.40 ± 76.33 pg/ml, 22.07 ± 11.41 pg/ml, respectively) than the ASST- patients (76.15 ± 91.04 pg/ml, 8.69 ± 10.00 pg/ml, respectively), with their P values both <0.0001 (Table 2).

	ASST+ patients N=71	ASST- patients N=60	P value
IL-17 (pg/ml)	173.40 ± 76.33	76.15 ± 91.04	<0.0001
IL-25 (pg/ml)	22.07 ± 11.41	8.69 ± 10.00	<0.0001

Note: All of values were expressed as mean ± standard deviation.

Table 2: Baseline serum IL-17 and IL-25 concentrations in the ASST+ and the ASST- patients

Significantly lower serum concentrations of IL-17 and IL-25 associated with patients in the Treatment Group vs. the Control Group at week 12

Repeated measure ANOVA revealed a significant time-effect ($F=262.40$, $P<0.0001$) and a significant group-time interaction ($F=69.63$, $P<0.0001$) for IL-17 concentration in the ASST+ patients. A simple effects analysis revealed comparable serum IL-17 concentrations for the patients in the Treatment Group and the Control Group at baseline and week 4, but significantly lower serum IL-17 concentration for the patients in the Treatment Group vs. the Control Group at week 12 (102.60 ± 42.39 *vs.* 149.00 ± 76.61, $P=0.003$) (Table 3).

As for IL-25 concentration, repeated measure ANOVA showed a significant time-effect ($F=111.63$, $P<0.0001$) and a significant group-time interaction ($F=16.57$, $P<0.0001$) in the ASST+ patients. A simple effects analysis showed comparable serum IL-25 concentrations for the patients in the Treatment Group and the Control Group at baseline and week 4, but significantly lower serum IL-25 concentration in patients in the Treatment Group vs. the Control Group at week 12 (10.92 ± 5.45 *vs.* 16.74 ± 9.34, $P=0.003$) (Table 3).

Significant decrease in serum concentrations of IL-17 and IL-25 at week 12 vs baseline for the ASST+ patients in the Treatment Group but not for patients in the Control Group

At week 4, there were no significant changes from baseline in serum IL-17 and IL-25 concentrations in the ASST+ patients in either the Treatment or the Control Group (Table 4). However, at week 12,

compared to baseline (175.90 ± 69.21 pg/ml for IL-17 and 22.02 ± 12.14 pg/ml for IL-25), significant decreases in the serum concentrations of IL-17 (102.60 ± 42.39 pg/ml, P<0.0001) and IL-25 (10.92 ± 5.45 pg/ml, P<0.0001) were found in the ASST+ patients in the Treatment Group but not in the patients in the Control Group (Table 4).

		Baseline		Week 4		Week 12	
		Concentration (pg/ml), mean ± SD	P value*	Concentration (pg/ml), mean ± SD	P value*	Concentration (pg/ml), mean ± SD	P value*
IL-17	Treatment Group (TGP +mizolastine) N=35	175.90 ± 69.21	0.79	151.60 ± 56.01	0.6	102.60 ± 42.39	0.003
	Control Group (mizolastine alone) N=36	171.00 ± 83.42		160.40 ± 81.91		149.00 ± 76.61	
IL-25	Treatment Group (TGP +mizolastine) N=35	22.02 ± 12.14	0.98	19.02 ± 9.20	0.64	10.92 ± 5.45	0.003
	Control Group (mizolastine alone) N=36	22.10 ± 10.82		20.15 ± 10.29		16.74 ± 9.34	

Note: SD: Standard Deviation
*Comparison between the Treatment Group and the Control Group.

Table 3: Comparison of serum IL-17 and IL-25 concentrations between the ASST+ patients in the Treatment Group (TGP +mizolastine) and the Control Group (mizolastine alone) at baseline, weeks 4 and 12.

	Treatment Group (TGP+mizolastine) N=35					Control Group (mizolastine alone) N=36				
	baseline	week 4	week 12	P^a	P^b	baseline	week 4	week 12	P^c	P^d
IL-17 (pg/ml)	175.90 ± 69.21	151.60 ± 56.01	102.60 ± 42.39	0.0975	<.0001	171.00 ± 83.42	160.40 ± 81.91	149.00 ± 76.61	0.5917	0.2556
IL25 (/pg/ml	22.02 ± 12.14	19.02 ± 9.20	10.92±5.45	0.2567	<.0001	22.10 ± 10.82	20.15 ± 10.29	16.74 ± 9.34	0.4389	0.0301

Note: All of the values were in mean±standard deviation (SD)

P^a, difference between week 4 vs. baseline for the ASST+ patients in the Treatment Group;

P^b, difference between week 12 vs. baseline for the ASST+ patients in the Treatment Group;

P^c, difference between week 4 vs. baseline for the ASST+ patients in the Control Group;

P^d, difference between week 12 vs. baseline for the ASST+ patients in the Control Group

Table 4: Change of serum IL-17 and IL-25 concentrations for ASST+ patients in the Treatment Group and Control Group

Adverse events

Of the 35 patients in the Treatment Group, 2 reported abdominal discomforts, 1 of them also developed mild diarrhea. Additionally, 1 other patient in the Treatment Group also reported mild diarrhea. Both symptoms resolved without any intervention. As to the Control group, 1 of the 36 patients reported drowsiness and another one felt thirsty, both symptoms resolved without any intervention.

Discussion

Our study evaluated TGP's effect on IL-17 and IL-25 in CAU patients and found significantly higher baseline serum IL-17 and IL-25 concentrations in the ASST+ patients vs. the ASST- patients. Significantly lower serum IL-17 and IL-25 levels were associated with the Treatment Group (TGP+mizolastine) vs. the Control Group (mizolastine alone) at week 12, but not at baseline or week 4. Also, patients in the Treatment Group had significantly decreased IL-17 and IL-25 concentrations vs. baseline at week 12 but not week 4, whereas concentrations of IL-17 and IL-25 of the patient in the Control group didn't change significantly over the 12 weeks. Additionally, TGP was well tolerated. Since it was the combination of TGP and mizolastine but not mizolastine alone that significantly decreased serum IL-17 and IL-25 levels at week 12, the IL-17 and IL-25 decreasing effect was due to TGP but not mizolastine, and that said effect was relatively slow acting.

Both IL-17 (IL-17A) and IL-25 (IL-17E) are members of the IL-17 family [2,15], and both are produced by activated CD4+T cells [2,19]. IL-17 could also be produced by neutrophils, eosinophiles and CD8+T cells [2,20-22]. As a T helper cell 17 (Th17)-related cytokine, IL-17 could evoke wide pro-inflammatory response from tissues/cells

expressing IL-17 receptors complex and lead to production of various pro-inflammatory cytokines, chemokines and proteins involved in acute phase response [2,3]. Elevated level of IL-17 has been found in many autoimmune diseases such as RA, psoriasis and multiple sclerosis, suggesting an important role for IL-17 in these autoimmune diseases [2]. Our study found higher serum IL-17 level in ASST+ patient vs. ASST- patients, this result was consistent with several previous reports, indicating a role of this cytokine in the pathogenesis of CAU [2,3,14]. Additionally, Atwa et al. found significant correlation between IL-17 concentration and disease severity [2], also pointing to a key role for IL-17 in CAU.

IL-25 is produced by activated Th2 cells and mast cells, and it could promote Th2 immune response [5,19-23]. Additionally, Moy et al. found more Th2 and Th17 cells in CU vs. healthy controls [15], Th2 mediated immune pathway produces cytokines that lead to IgE antibody production and the IgE antibody binds to Fc∈RIα on mast cells and induces mast cell activation and degranulation [15]. Our study found significantly higher serum IL-25 level in ASST+ patients *vs.* ASST- patients, and we hypothesize that such elevated IL-25 and IL-17 levels in the ASST+ patients indicated increased Th2 and Th17 cells in CAU patients to release more Th2 and Th17-related cytokines such as IL-4, IL-10 and IL-17, and these cytokines could inhibit Th1 cytokines production, and lead to Th1/Th2/Th17 imbalance which was a possible underlying mechanism of CU [3,14,16,23]. Such imbalance led to immuno-dysregulation in CAU and a disturbed T-cell induced cytokine production [14], resulting in symptomatic pathological injuries in CAU [2,14]. Of note was the fact that our study was the first to report elevated IL-25 level in ASST+ patient vs. ASST- patients. Besides Fc∈RIα, mast cells also express Toll-like receptors (TLRs) and complement 5a receptor, these receptors allow mast cells to degranulate and release histamine, leukotrienes, cytokines and other inflammatory mediators in response to various autoimmune conditions and infections [2,24,25], leading to vascular changes, wheals and angioedema [2].

TGP is an active anti-inflammatory, pain-relieving and immunoregulatory compound that has shown therapeutic efficacy for other autoimmune diseases such as primary Sjögren's syndrome and rheumatoid arthritis [7-9,26-28]. TGP is extracted from the roots of Paeonia lactiflora pall and contains more than 90% paeoniflorin [8]. TGP is mainly composed of 8 ingredients; they are paeoniflorin, albiflorin, benzoylpaeoniflorin, oxypaeoniflorin, benzoyloxypaeoniflorin, oxybenzoyl-paeoniflorin, paeoniflorigenone and lactiflorin [29]. Paeoniflorin is a main bioactive component of TGP [29]. The TGP capsules used in the current study (Miran Pavlin capsules, Ningbo Lihua Pharmaceutical Co., Ltd, Ningbo, China) contained 0.3 g TGP/capsule. It has been reported that TGP could restore immune balance such as Th17/Treg balance and suppresses inflammatory reactions by inhibiting the production of leukotriene B4, nitric oxide, prostaglandin E2, pro-inflammatory cytokines and chemokines [26,28,30]. Our study found that TGP treatment decreased serum IL-17 and IL-25 concentrations in CAU patients over time. Our finding was consistent with previous reports that TGP could decrease mRNA expression and protein level of IL-17 or shrank population of IL-17 producing cells in autoimmune patients or animal models of various autoimmune diseases [9-13]. Again as our finding that TGP could significantly decrease IL-25 level over time has not been reported before, more studies are needed to confirm this finding. Based on our findings and Long et al., findings that TGP had curative effect for CU [7], we hypothesize that TGP played a role in restoring balance of different Th cells. By reducing IL-17 and IL-25 levels, TGP suppressed Th2 and Th17 cell mediated immune response and re-establish Th1/Th2/Th17 balance, it further disrupted various signal transduction pathways, therefore inhibited the release of various pro-inflammatory cytokines and chemokines and reduced symptoms such as wheals, vasodilation, vascular leakage and angioedema [2,7-9,27,28,30], Additionally, it has been reported that TGP could block TLR4/5 activation and signaling, and thus could inhibit dendritic cells (DC) maturation and function, arrest DC's antigen-presenting capacity and decrease the expression of MHC2 and co-stimulator molecules CD80 and CD86, resulting in impaired Th1 and Th17 differentiation [8,31]. As IL-25 plays a role in late mast cell activation and prolongs presence of wheals beyond a histamine skin response [16], reducing IL-25 level by TGP could at least suppress late mast cell activation and degranulation and reduce the release of histamine, leukotrienes and other pro-inflammatory cytokines and chemokines [16]. Similarly, by reducing IL-17, TGP could impair IL-17's role in Th1/Th17 immune response mediated by antibodies against Fc∈RIα or IgE present on cells such as mast cells, dendritic cells and T cells [2,3,20-22], and could thus inhibit the production of various pro-inflammatory cytokines and chemokines and alleviate symptoms of CAU. Exact mechanism underlying TGP's efficacy needs to be further studied.

We noticed that patients receiving TGP did not have significantly decreased serum IL-17 and IL-25 concentrations at week 4, however, at week 12, the decreases in serum IL-17 and IL-25 concentrations were statistically significant. This finding suggested that when treating CAU, TGP was slow acting and had possible cumulative effect and its efficacy could potentially increase with prolonged use. As low incidence of mild AEs was associated with TGP use, TGP's ability to decrease IL-17 and IL-25 concentrations in patients with CAU and Long et al. [6] finding of TGP's curative effect for CU [7], TGP could potentially be developed into an alternative treatment for CAU suitable for long-term use. More long-term studies of larger sample sizes are needed to further explore this topic. As CAU patients need long-term medication, and the current first-line medications such as antihistamines have various side effects and could potentially cause liver or renal impairments [32], alternative treatments are always welcome.

Our repeated measure ANOVA was not adjusted for any covariates since baseline demographic for the ASST+ patients in the Treatment Group and the Control Group were comparable (Table 1).

Our study was limited by the fact that it lasted for only 12 weeks, therefore, it is unknown how the concentrations of serum IL-17 and IL-25 would change beyond the first 12 weeks of treatment. Additionally, this study had a relatively small sample size. Further, ideally, including a group of healthy subjects with no treatment would be helpful in assessing whether TGP treatment could reduce IL-17 and IL-25 levels back to normal, however, as the current study was a short-term pilot study focusing only on CAU patients, and also due to limited resources, we felt that it was too early to add normal subjects into the current study. Additionally, studying the clinical and/or immunological effects of a treatment by comparing patients receiving the treatment vs. those not without including normal healthy subjects was common, such as Long et al. and Zhou et al. [7,8]. Another point to note was that more than half (about 56%) of the 71 ASST+ CAU patients and the 60 ASST- CU patients enrolled in our study were male (Table 1), although generally CU is more prevalent in adult women than adult men [33]. A hospital based multicenter epidemiological study including 3,027 CU patients in China found a female/male ratio of 1.46:1 [34]. As our study enrolled patients visiting our outpatient

clinic from January 1, 2017 to March 31, 2017, and included only 131 patients, it was possible that more male patients than female patients visited our clinic during that 3 months. Preparation for a longer-term study of a larger sample size with a female preponderance more closely resembling Chinese CU population that also studies TGP's therapeutic effect for CAU is currently underway, and this study would include normal subjects as another control. Finally, as we did not measure serum IL-17 and IL-25's concentrations between weeks 4 and 12, we did not know when the significant decreases in serum concentrations of IL-17 and IL-25 began. Future studies will make more frequent measurements to address this question.

Conclusion

In conclusion, TGP significantly decreased serum IL-17 and IL25 in patient with CAU at week 12, this finding could provide basis for future clinical studies on TGP's therapeutic effect for CAU.

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References

1. Saini SS (2014) Chronic spontaenous urticaria: etiology and pathogenesis. Immunol Allergy Clin North Am 34: 33-52.
2. Atwa MA, Emara AS, Youssef N, Bayoumy NM (2014) Serum concentration of IL-17, IL-23 and TNF-α among patients with chronic spontaneous urticaria: association with disease activity and autologous serum skin test. J Eur Acad Dermatol Venereol 28: 469-474.
3. Chen Q, Zhong H, Chen WC, Zhai Z, Zhou Z, et al. (2018) Different expression patterns of plasma Th1-, Th2-, Th17- and Th22-related cytokines correlate with serum autoreactivity and allergen sensitivity in chronic spontaneous urticaria. J Eur Acad Dermatol Venereol 32: 441-448.
4. Degirmenci PB, Kırmaz C, Vatansever S, Onur E, Nal E, et al. (2017) Analysis of the association of chronic spontaneous urticaria with interlekin-4, -10, transforming growth factor-β1, interferon-γ, interleukin-17A and -23 by autologous serum skin test. Postepy Dermatol Alergol 34: 70-76.
5. Konstantinou GN, Asero R, Ferrer M, Knol EF, Maurer M, et al. (2013) EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. Allergy 68: 27-36.
6. Criado PR, Criado RF, Maruta CW, Reis VM (2015) Chronic urticaria in adults: state-of-the-art in the new millennium. An Bras Dermatol 90: 74-89.
7. Long JW, Wang YY, Pi XM, Tu YT (2010) Clinical observation on the treatment of chronic urticaria with total glucosides of paeony capsule combined with citirizine. Chin J Integr Med 16: 353-356.
8. Zhou Y, Jin L, Kong F, Zhang H, Fang X, et al. (2016) Clinical and immunological consequences of total glucosides of paeony treatment in Sjögren's syndrome: A randomized controlled pilot trial. Int Immunopharmacol 39: 314-319.
9. Lin J, Xiao L, Ouyang G, Shen Y, Huo R, et al. (2012) Total glucosides of paeony inhibits Th1/Th17 cells via decreasing dendritic cells activation in rheumatoid arthritis. Cell Immunol 280: 156-163.
10. Chen T, Fu LX, Zhang LW, Yin B, Zhou PM, et al. (2016) Paeoniflorin suppresses inflammatory response in imiquimod-induced psoriasis-like mice and peripheral blood mononuclear cells (PBMCs) from psoriasis patients. Can J Physiol Pharmacol 94: 888-894.
11. Sun J, Wu J, Xu C, Luo Q, Li B, et al.(2015) Paeoniflorin attenuates allergic inflammation in asthmatic mice. Int Immunopharmacol 24: 88-94.
12. Wang C, Yuan J, Wu HX, Chang Y, Wang QT, et al. (2015) Total glucosides of paeony inhibit the inflammatory responses of mice with allergic contact dermatitis by restoring the balanced secretion of pro-/anti-inflammatory cytokines. Int Immunopharmacol 24: 325-334.
13. Zhao J, Di T, Wang Y, Wang Y, Liu X, et al. (2016) Paeoniflorin inhibits imiquimod-induced psoriasis in mice by regulating Th17 cell response and cytokine secretion. Eur J Pharmacol 772: 131-143.
14. Dos Santos JC, Azor MH, Nojima VY, Prearo E, Maruta CW, et al. (2008) Increased circulating pro-inflammatory cytokines and imbalanced regulatory T-cell cytokines production in chronic idiopathic urticaria. Int Immunopharmacol 8: 1433-1440.
15. Moy AP, Murali M, Nazarian RM (2016) Identification of a Th2- and Th17-skewed immune phenotype in chronic urticaria with Th22 reduction dependent on autoimmunity and thyroid disease markers. J Cutan Pathol 43: 372-378.
16. Kay AB, Clark P, Maurer M, Ying S (2015) Elevations in T-helper-2-initiating cytokines (interleukin-33, interleukin-25 and thymic stromal lymphopoietin) in lesional skin from chronic spontaneous ('idiopathic') urticaria. Br J Dermatol 172: 1294-1302.
17. Zhang Y, Zhou R, Zhou F, Cheng H, Xia B (2014) Total glucosides of peony attenuates 2,4,6-trinitrobenzene sulfonic acid/ethanol-induced colitis in rats through adjustment of TH1/TH2 cytokines polarization. Cell Biochem Biophys 68: 83-95.
18. Wu G, Wu N, Li T, Lu W, Yu G (2016) Total glucosides of peony ameliorates Sjögren's syndrome by affecting Th1/Th2 cytokine balance. Exp Ther Med 11: 1135-1141.
19. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, et al. (2001) IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity 15: 985-995.
20. Teunissen MB, Koomen CW, de Waal Malefyt R, Wierenga EA, Bos JD (1998) Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. J Invest Dermatol 111: 645-649.
21. Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A (2003) IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. J Immunol 170: 2106-2112.
22. Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, et al. (2003) Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. J Allergy Clin Immunol 111: 1293-1298.
23. Kim MR, Manoukian R, Yeh R, Silbiger SM, Danilenko DM, et al. (2002) Transgenic overexpression of human IL-17E results in eosinophilia, B-lymphocyte hyperplasia, and altered antibody production. Blood 100: 2330-2340.
24. Agier J Żelechowska P, Kozłowska E, Brzezińska-Błaszczyk E (2016) Expression of surface and intracellular Toll-like receptors by mature mast cells. Cent Eur J Immunol 41: 333-338.
25. Jin M, Yu B, Zhang W, Zhang W, Xiao Z, et al. (2016) Toll-like receptor 2-mediated MAPKs and NF-κB activation requires the GNAO1-dependent pathway in human mast cells. Integr Biol (Camb) 8: 968-975.
26. Zhang H, Qi Y, Yuan Y, Cai L, Xu H, et al. (2017) Paeoniflorin Ameliorates Experimental Autoimmune Encephalomyelitis via Inhibition of Dendritic Cell Function and Th17 Cell Differentiation. Sci Rep 7: 41887.
27. Dai X, Wang LW, Jia XY, Chang Y, Wu XH, et al. (2015) Paeoniflorin regulates the function of human peripheral blood mononuclear cells stimulated by rhIL-1β by up-regulating Treg expression. Immunopharmacol Immunotoxicol 37: 252-257.
28. Zhang W, Dai SM (2012) Mechanisms involved in the therapeutic effects of Paeonia lactiflora Pallas in rheumatoid arthritis. Int Immunopharmacol 14: 27-31.

29. Xia LL, Zhu QJ, Wu YG (2017) Hepatoprotective effect of peony total glucosides and the underlying mechanisms in diabetic rats. Pharm Biol 55: 2178-2187.
30. Chen T, Guo ZP, Jiao XY, Jia RZ, Zhang YH, et al. (2011) Peoniflorin suppresses tumor necrosis factor-α induced chemokine production in human dermal microvascular endothelial cells by blocking nuclear factor-κB and ERK pathway. Arch Dermatol Res 303: 351-360.
31. Zhou Z, Lin J, Huo R, Huang W, Zhang J, et al. (2012) Total glucosides of paeony attenuated functional maturation of dendritic cells via blocking TLR4/5 signaling in vivo. Int Immunopharmacol 14: 275-282.
32. Kozel MM, Sabroe RA (2004) Chronic urticaria: aetiology, management and current and future treatment options. Drugs 64: 2515-2536.
33. Fine LM, Bernstein JA (2016) Guideline of Chronic Urticaria Beyond. Allergy Asthma Immunol Res 8: 396-403.
34. Zhong H, Song Z, Chen W, Li H, He L, et al. (2014) Chronic urticaria in Chinese population: a hospital-based multicenter epidemiological study. Allergy 69: 359-364.