

Original Article

Aqueous nasal spray of chitosan oligosaccharide ameliorates perennial allergic rhinitis by affecting serum levels of interleukin-6 and interleukin-10

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Abstract: *Background and objective:* Chitosan oligosaccharide (COS) is widely used to attenuate inflammation. However, its potential to suppress allergic rhinitis remains unknown. *Methods:* A 24-week study of patients between 3 and 9 years old with perennial allergic rhinitis (AR) was conducted to assess the effects of COS on AR. Ninety-eight subjects were evenly assigned into two groups: CG group (nasal administration of 100 mg COS daily) or PG group (nasal administration of placebo daily) for 24 weeks. The levels of interleukin-6 (IL-6) and interleukin-10 (IL-10) were measured by qRT-PCR, Western Blot and ELISA. Baseline demographic characteristics were similar between two groups ($P>0.05$). *Results:* After 24-week intervention, 93 subjects completed the study (47 patients from CG and 46 patients from PG). The patients had lower incidence of AR in CG group than in PG group after eight-week therapy ($P<0.05$). The serum level of IL-6 was lower in CG group than in PG group ($P<0.05$). In contrast, the blood level of IL-10 was higher in CG group than in PG group ($P<0.05$). The severity of AR was positively related with the blood levels of IL-6 and negatively related with the levels of IL-10. Furthermore, 100 mg COS was found to be well tolerated daily, with fewer side effects in AR subjects. *Conclusions:* Aqueous nasal spray of COS ameliorates perennial allergic rhinitis by affecting serum levels of IL-6 and IL-10. COS should be developed a potential drug for rhinitis therapy.

Keywords: Allergic rhinitis, chitosan oligosaccharide, side effects, interleukin-6, interleukin-10

Introduction

Allergic rhinitis (AR) is very common rhinologic disorder that affects most children and adolescents [1, 2]. Most The prevalence of AR has increased worldwide and the predisposing and risk factors that affect AR prevalence [3]. Much work is needed to be done on risk factors, specifically in developing countries [4]. Intranasal corticosteroid is mostly considered as first-line medicine for AR therapy. The medicine is well tolerated and effective for AR therapy. The medicine is mostly considered for perennial AR subjects who are treated with inhaled corticosteroids for a long term. Although the medicine is the main therapy option, perfect resolution cannot be obtained yet. The side effects are still significant, including nasal irritation and epistaxis [5]. Furthermore, the administration of the drug is still controversial in special popu-

lation, such as pregnant [6]. Long-term use of intranasal corticosteroids will result in systemic adverse events, such as osteoporosis [7], open-angle glaucoma [8], cataracts [9], adrenal suppression [10] and impaired growth of children [11]. Furthermore, the children with chronic renal failure will have growth retardation, which can be aggravated by corticosteroids [12]. Thus, it is extremely demanded to explore new medicine for AR therapy.

Chitosan oligosaccharide (COS) is well known as a biosafe and biocompatible natural product [13]. The biological function of COS is its anti-inflammation [14], anti-tumor [15] and anti-bacteria activities [16]. The pathogenesis of AR is complex, and most cases are associated with inflammatory responses [17]. Anti-inflammatory effects of AR therapy have been approved by reducing the levels of cytokines, such as TNF,

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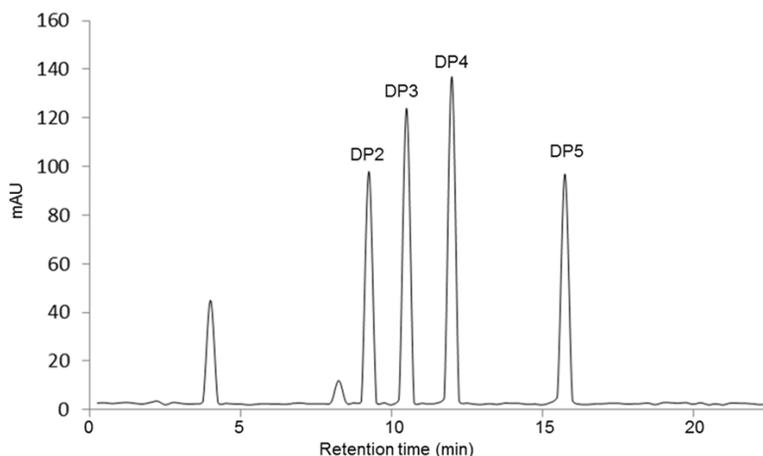


Figure 1. HPLC analysis of Chitosan oligosaccharide (COS) mixture. COS mixture fractionated using HPLC and UV detection (340 nm). The eluting time of degrees of polymer (DP) was 9.3, 10.5, 12.0 and 15.8 min for DP2, 3, 4 and 5, respectively.

IL-4, IL-5, and IL-13 [18]. Previous work suggests that COS inhibits lipopolysaccharide-induced up-regulation of IL-6 in human umbilical vein endothelial cells [19]. The plasmatic concentrations of IL-6, IL-10 and IL-2 are associated with the risk of chronic AR [20]. COS may ameliorate AR by affecting the levels of interleukin and was explored here by investigating the changes of IL-6 and IL-10.

Methods

Preparation of COS

COS was purchased from Qingdao Yunzhou Biochemistry Co., Ltd (Qingdao, China). COS was made by enzymatic digestion according to an earlier report with slight modification [21]. One-microliter chitosanase (1 mg/mL) was mixed with 100 μ L of soluble chitosan (pH 6.0), and the reaction mixture was incubated at 39°C for one hour. One mL of the mixture (50:50) of acetonitrile and ddH₂O was added. The mixture was filtered by a 3 kDa NMWL (nominal molecular weight limits) membrane (Millipore, Billerica, MA, USA). Ten- μ L filtrated aliquot was injected into an Agilent 1100 series HPLC. Mobile phase was 20 mM PBS (pH 7.5) + 0.25 mM NaCl and the flow rate was 0.5 mL/min. The final products were detected by absorbance at 235 nm. According to the peak time, each identifiable peak was collected via a detector. The collected fraction was further identified by following experiments.

Mass spectrometry

MALDI-TOF-MS (Bruker, Billerica, MA, USA) with Compass 1.3 control and software were used. Ionization was obtained by one-kHz smart beam-II solid state laser with an initial laser power of 60% and measured by the Flash Detector. The voltages of reflector mode were optimized for reflector-1 (27.8 kV) and reflector-2 (14.2 kV), ion sources (IonSource-1: 25.8 kV, IonSource-2: 23.8 kV) and the extraction of pulsed ion (315 ns). Ubiquitin was used to calibrate mass spectrometer.

The mass spectrometer was calibrated over the m/z 0-1500.

Ethics approval and consent to participate

The study and all experimental protocols were approved by the Ethic Committee of Shanghai Jiao Tong University School of Medicine, which complies with the World Medical Association Declaration of Helsinki. All patients and their parent or guardian were required to provide signed informed consent before joining our test. Ninety-eight AR patients at age of between 3 and 9 years of age were enrolled in the study from 2 March, 2015 to 24 June, 2015.

Including criteria

All children were at no more than stage I according to the Tanner Classification of Sexual Maturity. The whole period of experiment was 24 weeks. The skeletal ages were tested by a radiograph of the left wrist before the recruitment. All subjects were diagnosed with more than one-year AR, which needs therapy in past one year. All patients had positive skin responses to perennial allergen. AR is classified as Mild-Intermittent, Moderate-Severe intermittent, Mild-Persistent, and Moderate-Severe Persistent. Intermittent is defined as <4 per week or <4 consecutive weeks. Persistent is defined as >4 d/week and >4 consecutive weeks. The symptom is regarded as mild if the child had normal sleep, no trouble for daily

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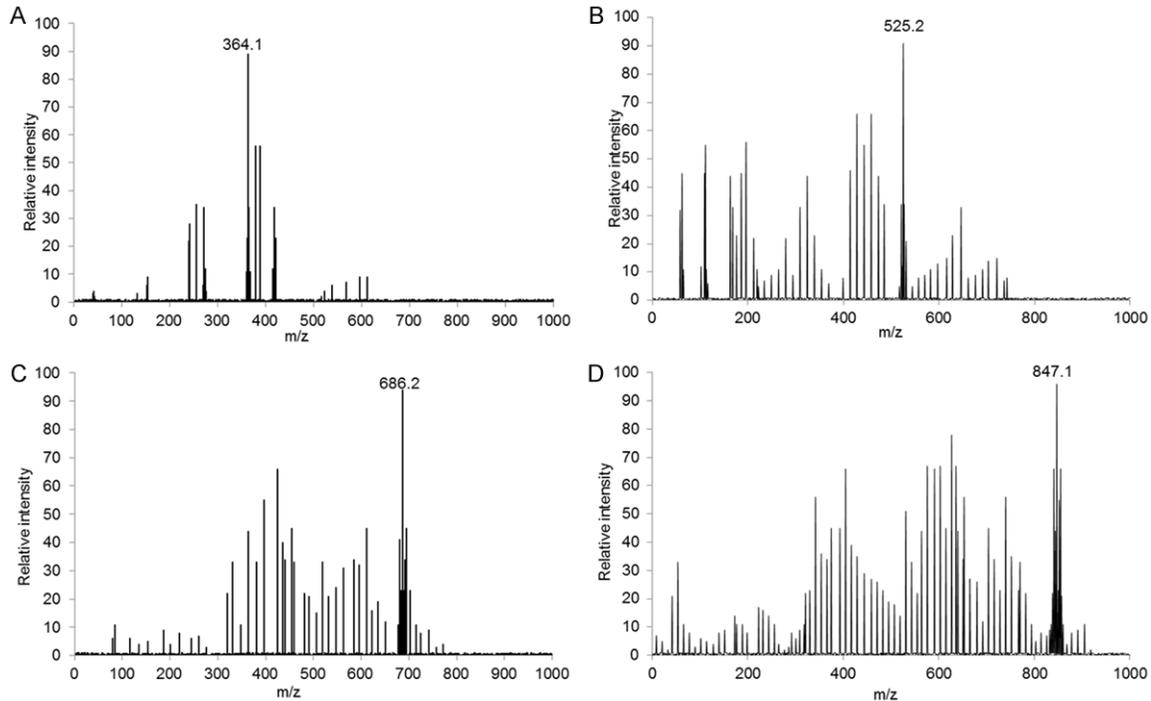


Figure 2. MALDI-TOF MS spectrometry analysis of COS. A. Mass spectra were visualized following the separation of DP2 ($[M + Na]^+ = 364$ Da). B. Mass spectra were visualized following the separation of DP3 ($[M + Na]^+ = 525$ Da). C. Mass spectra were visualized following the separation of DP4 ($[M + Na]^+ = 686$ Da). D. Mass spectra were visualized following the separation of DP5 ($[M + Na]^+ = 847$ Da).

activities, or study. The symptom is regarded as severe symptoms if the child had sleep disturbance, trouble for daily activities, at school or work [22].

Excluding criteria

The following excluding criteria were performed: 1) the children had asthma; 2) the children had an abnormal growth or gross malnutrition; 3) the children had multiple medicine allergies; 4) the children had infection diseases within one month before present study; 5) the children had no motivation joining present study.

Quantitative RT-PCR (qRT-PCR)

Five-ml blood was obtained from each child before COS or placebo intervention, and after 8-, 16- and 24-week intervention. RNA was isolated from blood samples by RNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesized by cDNA Synthesis Kits from Thermo Fisher Scientific. SYBR Green Real-Time PCR Master Mixes (Thermo Fisher Scientific) was used for qRT-PCR. The following primers were synthesized: IL-6, F: 5'-ccccaca-

cagacagccactc-3', R: 5'-ttctgccagtgccctctttgc -3' (140 bp); IL-10, F: 5'-cttcgagatctccgagatgc -3', R: 5'-gccttgatgtctgggtcttg-3' (200 bp); GAPDH, F: 5'-GGAAAAGCTGTGGCGTGAT-3', R: 5'-AAGGTGGAAGAATGGGAGTT-3'; The values of cycle time for the interest genes were normalized with GAPDH.

Western blot analysis

Total proteins were extracted by using total protein isolation kit (ITSI Bioscience, Johnstown, PA, USA). Total proteins were separated SDS-PAGE and transferred to PVDF (RTP company, Winona, MA, USA). The PVDF was incubated with mouse anti-IL-6 antibody (ab66231, Abcam), mouse Anti-IL-10 antibody (ab9969, Abcam) and mouse polyclonal GAPDH antibody (ab8245, Abcam) overnight at 4°C. Subsequently, the sample was incubated with peroxidase- and conjugated goat anti-rabbit IgG (ab97023, Abcam). The immunoreactive bands were stained with DAB (Sigma-Aldrich, St Louis, MO, USA) and band density was quantified by Image J software (NIH, Bethesda, MD, USA).

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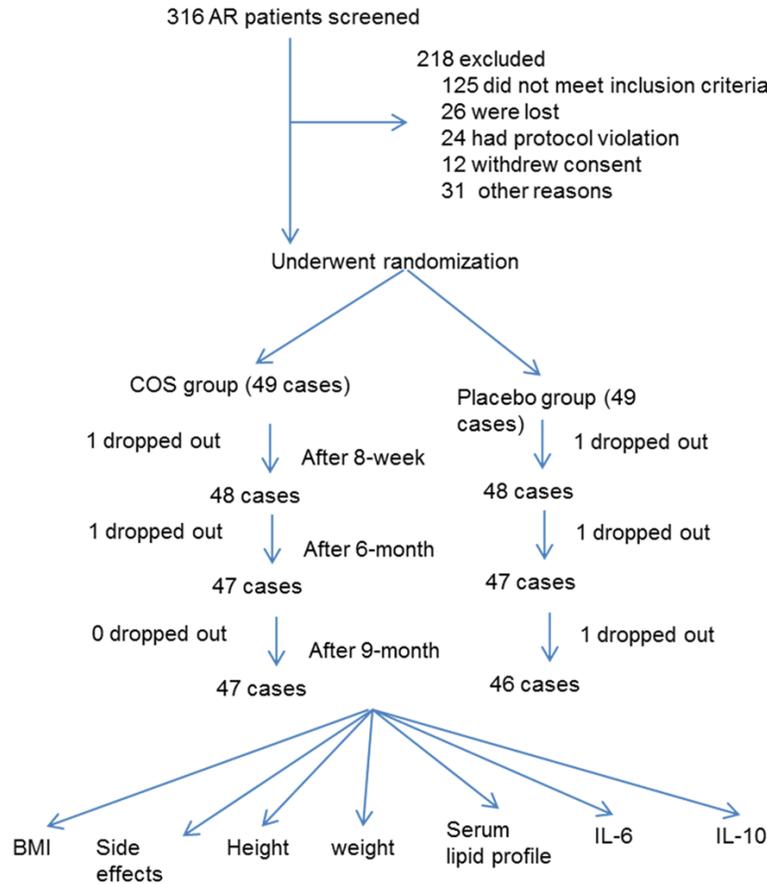


Figure 3. The flowchart of present study. AR, Allergic rhinitis. The whole period of follow-up is 24 weeks.

ELISA analysis

AR is closely associated with Interleukin-6 and IL-10. Five-ml blood samples were obtained from all children before this study, and after 8-, 16- and 24-week intervention. Thus, the levels of these cytokines were measured by using human ELISA kits for IL-6 (Cat. No. ab100572) and IL-10 (Cat. No. ab108871) from Abcam (Shanghai) Ltd. (Shanghai, China).

COS intervention

Aqueous nasal spray of run-in phase was performed for half a month between two groups. The following indexes were measured: rhinitis indication scores, associated complications, and side effects. In CG, all children were instructed to use the nasal spray devices and receive 100-mg COS daily, administered intranasally one spray (50 mg COS) per nostril in the morning. In PG, all children were instructed to use the nasal spray devices and receive 100-

mg placebo daily, administered intranasally one spray (50 mg placebo) per nostril in the morning. The whole period was 24 weeks, in which none was permitted to use other medicine.

Side effects evaluation

The height of all children was measured from their bare-foot to heads in natural position at the same time daily. The growth retardation was evaluated after 24-week intervention when compared with PG. The following side effects were examined during 24-week intervention: Epistaxis, Headache, Nasal irritation, Pharyngitis, Sneezing, Urticarial, Conjunctivitis, Uncomfortable feeling and Sleepy.

Statistical analysis

A paired t-test is used to compare two related means of response variables between two groups. Chi-square analysis is used to compare the significance for the numbers of two groups. Spearman rank correlation is used to test the relationship between two variables. All the data were analyzed by SPSS Statistics 20 (IBM Corporation, Armonk, NY, USA). There are significantly statistical differences if $P < 0.05$.

Results

COS ingredients

HPLC analysis showed that COS from DP2 to DP5 were produced by the enzymatic hydrolysis of chitosan. The contents of DP2, 3, 4 and 5 were 21%, 25%, 27% and 20% of COS, respectively (**Figure 1**).

DP2, 3, 4 and 5 were further confirmed by MALDI-TOF MS under the conditions that produced mass spectra with $[M + Na]^+$. **Figure 2** showed that the masses predicted for DP2 (**Figure 2A**), DP3 (**Figure 2B**), DP4 (**Figure 2C**) and DP5 (**Figure 2D**) were 341 Da, 502 Da, 663

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Table 1. Baseline clinical characters of AR patients in this study

		CG (n = 49)	PG (n = 49)	t/ χ^2	P value
Age (years)		6.1±2.4	6.5±2.3	0.25	0.61 ^a
Boys/Girls		29/20	27/22	0.17	0.68 ^b
Race, n (%)	HAN ZHU	45	46	0	1 ^b
	MANCHU	2	2	0	1 ^b
	MONGOLIANS	2	0	0	1 ^b
	TIBETANS	0	1	0	1 ^b
Weight (kg)		21.6±10.5	22.8±11.1	0.27	0.52 ^a
BMI (kg/m ²)		22.4±3.6	22.1±2.9	0.63	0.61 ^a
Mean height (cm)		123.8±23.7	120.5±24.6	0.71	0.45 ^a
TG (mmol/L)		2.5±1.1	2.4±1.5	0.66	0.17 ^a
TC (mmol/L)		4.8±1.4	4.9±1.6	0.21	0.48 ^a
HDL (mmol/L)		1.3±0.3	1.5±0.5	0.24	0.56 ^a
LDL (mmol/l)		2.8±1.0	3.0±1.2	0.28	0.71 ^a
Severity of AR	Mild intermittent	23	21	0.16	0.68 ^b
	Moderate-severe intermittent	16	17	0.05	0.83 ^b
	Mild persistent	5	6	0.10	0.75 ^b
	Moderate-severe persistent	5	5	0	1.00 ^b

Note: ^at-test and ^bchi-square test. There is no significant statistic difference at P>0.05. Mild-intermittent, Moderate-Severe intermittent, Mild-persistent, and Moderate-severe persistent.

Table 2. Biochemical indices after 24-week intervention

		CG (n = 47)	PG (n = 46)	t/ χ^2	P value
Age (years)		6.5±2.4	6.9±2.3	0.21	0.67 ^a
Boys/Girls		28/19	26/21	0.17	0.68 ^b
Race, n (%)	Han zhu	43	43	0	1 ^b
	Manchu	2	2	0	1 ^b
	Mongolians	2	0	0	1 ^b
	Tibetans	0	1	0	1 ^b
Weight (kg)		22.6±10.1	23.1±12.0	0.21	0.62 ^a
BMI (kg/m ²)		23.5±3.1	24.0±2.8	0.55	0.72 ^a
Mean height (cm)		124.9±23.7	121.2±24.8	0.45	0.63 ^a
TG (mmol/L)		2.6±1.2	2.5±1.4	0.61	0.19 ^a
TC (mmol/L)		4.4±1.3	4.8±1.5	0.98	0.06 ^a
HDL (mmol/L)		1.6±0.3	1.4±0.4	1.43	0.03 ^a
LDL (mmol/l)		2.6±1.1	2.9±1.0	1.18	0.05 ^a

Note: ^at-test and ^bchi-square test. There is no significant statistic difference at P>0.05.

COS for more than 7 days and three children dropped out from PG because of treatment failure. Thus, 47 patients in CG and 46 patients in PG completed the study (**Figure 3**). There was no significantly statistical difference between two groups for baseline characters including age, gender, race, lipid profile, BMI, height and AR severity (**Table 1**, P>0.05). The results suggested that the clinical baseline characters will not affect the results of COS intervention.

Changes of clinical characters after 24-week intervention

Da and 824 Da, respectively. Since the chromatographic and MASS analysis showed that the properties of COS were identical with theoretic values, subsequent work was performed by using the COS mixture.

Baseline characters

The whole period of study was 24 weeks. Two children dropped out from CG without using

The mean height for the patients in COS group was 123.8±23.7 cm, compared with 120.5±24.6 cm in placebo group. The differences were not significant (**Table 2**, P>0.05). Furthermore, COS intervention improved the lipid profile by increasing HDL-c level (P<0.05). For most parameters, there was no significantly statistical difference between two groups (**Table 2**, P>0.05).

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Table 3. Side effects during 24-week intervention

	CG (n = 47)	PG (n = 46)	χ^2	P value
Epistaxis, n (%)	0 (0)	6 (13.04)	4.57	0.03
Headache, n (%)	1 (2.13)	5 (10.87)	1.67	0.20
Nasal irritation, n (%)	0 (0)	3 (6.52)	1.42	0.23
Pharyngitis, n (%)	1 (2.13)	7 (15.22)	3.54	0.06
Sneezing, n (%)	1 (2.13)	5 (10.87)	1.67	0.20
Urticarial, n (%)	0 (0)	1 (2.17)	0.00	0.99
Conjunctivitis, n (%)	0 (0)	1 (2.17)	0.00	0.99
Uncomfortable feeling, n (%)	2 (4.26)	4 (8.7)	0.20	0.65
Sleepy, n (%)	1 (2.13)	4 (8.7)	0.89	0.35

Note: chi-square test. There is no significant statistical difference at $P > 0.05$.

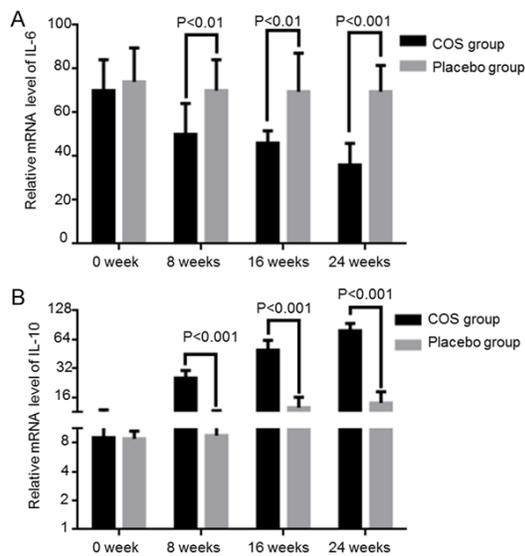


Figure 4. qRT-PCR analysis of relative mRNA levels of IL-6 and IL-10. A. Relative mRNA level of IL-6. B. Relative mRNA level of IL-10. All data were presented as mean values \pm S.D. There was significantly statistical difference if $P < 0.05$.

Side effects

After 24-week COS intervention, most patients (45) were at least 80% compliant with the natural products, which was higher than in PG group (13 patients were at least 80% compliant with placebo intervention) ($P < 0.05$). From above results, COS intervention did not retard growth when compared with PG (Table 2, $P > 0.05$). The patients with epistaxis were lower in CG than in PG ($P < 0.05$). The incidences of other side effects were similar between two groups (Table 3).

COS intervention reduces mRNA levels of IL-6 and increases the level of IL-10

As Figure 4 showed, there was no significantly statistical difference for mRNA levels of IL-6 and IL-10 between two groups before this study ($P > 0.05$). Comparatively, mRNA levels of IL-6 were significantly reduced in CG and there was significantly statistical difference for mRNA levels of between two groups after 8-week intervention ($P < 0.01$). The mRNA levels of

IL-6 were further reduced in CG and there was significantly statistical difference for mRNA levels of IL-6 between two groups since 16-week intervention (Figure 4A, $P < 0.01$). Much difference for mRNA levels of IL-6 was observed between two groups after and 24-week intervention (Figure 4A, $P < 0.001$). The results suggest that COS intervention reduces mRNA levels of IL-6. In contrast, mRNA levels of IL-10 were significantly increased in CG and there was significantly statistical difference for mRNA levels of between two groups after 8-week intervention ($P < 0.001$). The mRNA levels of IL-10 were further increased in CG and there was significantly statistical difference for mRNA levels of IL-10 between two groups since 16-week intervention (Figure 4B, $P < 0.001$). Much difference for mRNA levels of IL-10 was observed between two groups after and 24-week intervention (Figure 4B, $P < 0.001$). The results suggest that COS intervention increases mRNA levels of IL-10.

COS intervention reduces protein levels of IL-6 and increases the level of IL-10

As Figure 5 showed, there was no significantly statistical difference for protein levels of IL-6 and IL-10 between two groups before this study, ($P > 0.05$). Comparatively, protein levels of IL-6 were significantly reduced in CG and there was significantly statistical difference for protein levels of between two groups after 8-week intervention ($P < 0.05$). The protein levels of IL-6 were further reduced in CG and there was significantly statistical difference for protein levels of IL-6 between two groups since 16-week

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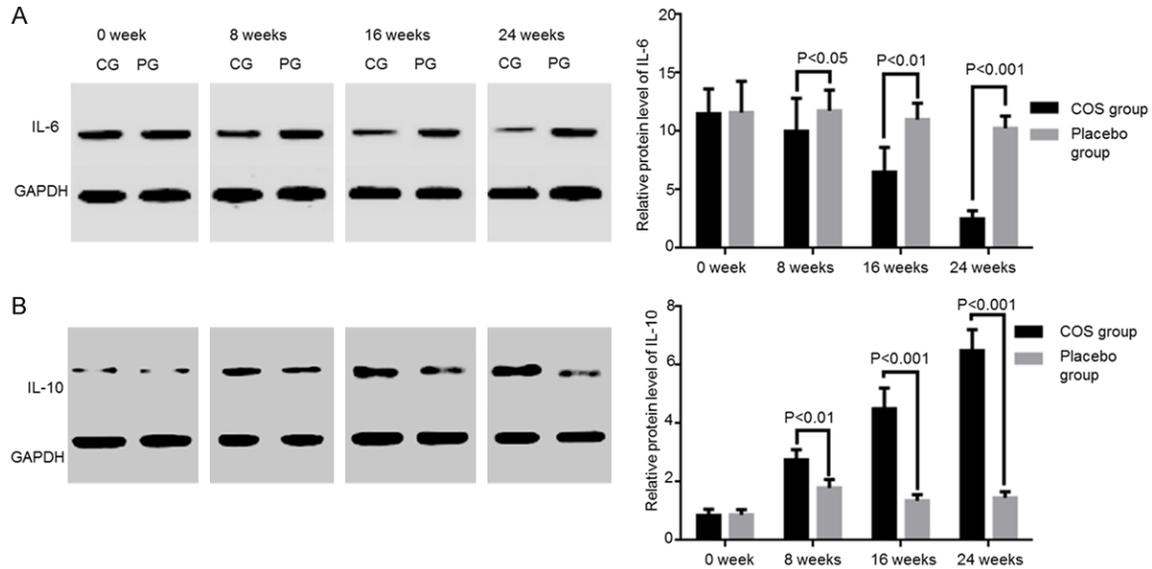


Figure 5. Western Blot analysis of relative protein levels of IL-6 and IL-10. A. Relative protein level of IL-6. B. Relative protein level of IL-10. All data were presented as mean values \pm S.D. There was significantly statistical difference if $P < 0.05$.

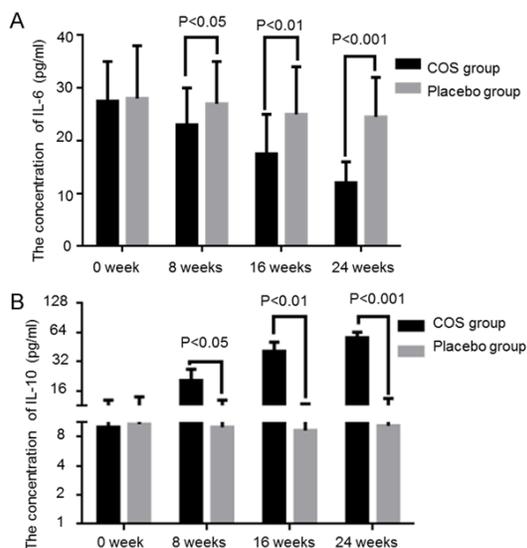


Figure 6. Measurement of concentrations of IL-1 beta and IL-6 by ELISA in blood samples. A. The concentration of IL-1 beta. B. The concentration of IL-6. All data were presented as mean values \pm S.D. There was significantly statistical difference if $P < 0.05$.

intervention (**Figure 5A**, $P < 0.01$). Much difference for protein levels of IL-6 was observed between two groups after 24-week intervention (**Figure 5A**, $P < 0.001$). The results suggest that COS intervention reduces protein levels of IL-6. In contrast, protein levels of IL-10 were significantly increased in CG and there was signifi-

cantly statistical difference for protein levels of between two groups after 8-week intervention ($P < 0.01$). The protein levels of IL-10 were further increased in CG and there were significantly statistical difference for protein levels of IL-10 between two groups since 16-week intervention (**Figure 5B**, $P < 0.001$). Much difference for protein levels of IL-10 was observed between two groups after and 24-week intervention (**Figure 5B**, $P < 0.001$). The results suggest that COS intervention increases the levels of IL-10.

COS intervention reduces blood concentrations of IL-6 and increases the concentration of IL-10

ELISA analysis showed that there was no significantly statistical difference for the concentration of IL-6 and IL-10 between two groups before this study ($P > 0.05$). Comparatively, the concentration of IL-6 were significantly reduced in CG and there was significantly statistical difference for the concentration of IL-6 between two groups after 8-week intervention ($P < 0.05$). The blood concentration of IL-6 were further reduced in CG and there was significantly statistical difference for concentration of IL-6 between two groups since 16-week intervention (**Figure 6A**, $P < 0.01$). Much difference for blood concentration of IL-6 was observed between two groups after and 24-week inter-

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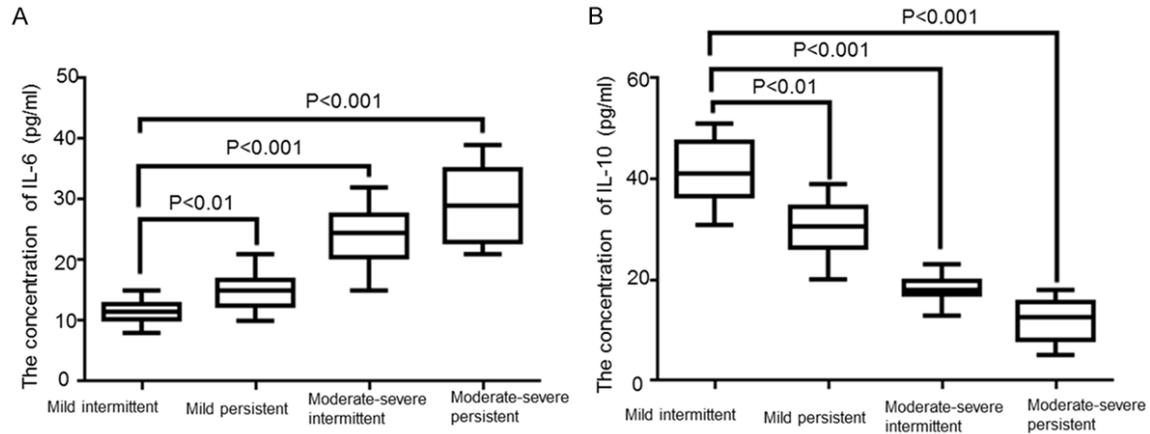


Figure 7. The relationship between AR severity and the levels of IL-6 or IL-10. A. The level of IL-6 in different-severity AR patients. B. The level of IL-10 in different-severity AR patients. There was significantly statistical difference if $P < 0.05$.

vention (**Figure 6A**, $P < 0.001$). The results suggest that COS intervention reduces blood concentration of IL-6. In contrast, blood concentration of IL-10 was significantly increased in CG and there was significantly statistical difference for concentration of IL-10 between two groups after 8-week intervention ($P < 0.05$). The blood concentration of IL-10 was further increased in CG and there were significantly statistical difference for blood concentration of IL-10 between two groups since 16-week intervention (**Figure 6B**, $P < 0.01$). Much difference for blood concentration of IL-10 was observed between two groups after and 24-week intervention (**Figure 6B**, $P < 0.001$). The results suggest that COS intervention increases blood concentration of IL-10.

The association of the levels between severity of AR and blood concentration of IL-6 or IL-10

Figure 7 showed that the levels of IL-6 were increased when AR severity was changed from mild to moderate and severe. The levels of IL-6 were the lowest in the patients with mild intermittent AR and reached highest level in the patients with moderate-severe persistent AR ($P < 0.01$) (**Figure 7A**). In contrast, the levels of IL-10 were reduced when AR severity was changed from mild to moderate and severe. The levels of IL-10 were highest in the patients with mild intermittent AR and reached the lowest level in the patients with moderate-severe persistent AR ($P < 0.01$) (**Figure 7B**). The results suggest that lower level of IL-6 and higher level of IL-10 will result in lower severity of AR.

Discussion

Previous Meta-analytic trials suggested that short-term intranasal topical corticosteroid for AR therapy caused lower growth rate in the patients as assessed by knemometry. Its effects on longer-term growth rate remain unknown [23]. Another report showed that inhaled corticosteroid but not intranasal corticosteroids resulted in one cm/y difference of growth velocity [24]. Compared with these reports that the use of intranasal corticosteroids will result in the growth retardation of children, present findings showed that intranasal spray of 100-mg COS daily didn't cause the growth retardation in all subjects. The heights of COS-treated patients were similar with those using placebo.

Spraying COS is a better method for AR therapy in children since there are the risks of systemic exposure to corticosteroids from inhaled and growth retardation. Present study also showed biosafe and biocompatible of COS with a fewer common side effects. Recurrent epistaxis is mostly related to AR cases. Many children usually have nosebleeds because of nasal disease and complications in the atopic state. Hemostasis disease has been considered as a main reason for causing the symptoms [25]. Present results showed that COS inhibited epistaxis completely when compared with placebo group. The general improvement in the patients from CG indicated that COS was beneficial to prevent the disorder of hemostasis.

COS intervention can improve functions of antioxidant and anti-inflammation status of AR patients by affecting the blood levels of inflammatory cytokines (Figures 2-4). Previous study reported that the IL-6 rs1800795 polymorphism was associated with an increasing risk of AR [26]. Another earlier report showed that two promotor variants of IL-6, especially rs1800795, were predisposing factors for AR progression. The SNPs of IL-6 also affected the clinical indices, the nature of sensitivity and persistency of AR subjects [27]. AR has been reported to be an inflammatory disorder with the high level cytokines and tissue eosinophilia. Recent research suggests that IL-17, chemokine (C-C motif) ligand (CCL) 26/eotaxin-3, and CCL13/monocyte chemoattractant protein-4 (MCP-4) are also associated with the pathogenesis of AR [28]. IL-4, IL-10, IL-17, eotaxin-3, and MCP-4 are also associated with nasal allergen and their release during natural pollen exposure. The comparison showed that IL-10 alleviated nasal mucosal allergy [29]. Our results showed that COS decreased the levels of IL-6 and increased the levels of IL-10 in blood sample. Therefore, COS may ameliorate AR severity by affecting the levels of IL-6 and IL-10. The reasons for the functions of COS intervention are complicated. To make sure the interaction between the pathway and these molecules, much work is needed to be done in future work.

There were some limitations for present study: 1) COS intervention should be performed in a larger population since COS has a fewer adverse events; 2) The detail molecular mechanism for the association between inflammatory cytokines and COS remains unclear; 3) Increase antioxidant enzymes and decreasing oxidative stress is a potential way for AR therapy. COS is often considered as important functional materials for many applications due to its antioxidant bioactivities. Thus, COS can be recommended to treat AR by decreasing oxidative stress in AR. However, further work is highly demanded to confirm present results in the future.

Beneficial effects of COS were proved without a fewer side effects, and can be a potential therapeutic candidate for AR therapy. The rehabilitant functions of COS may be related to the changes for the levels of IL-6 and IL-10. The decrease in serum level of IL-6 will reduce the inflammatory symptoms of AR while the increase in serum level of IL-10 is beneficial to

inhibit pro-inflammatory cytokines. Thus the ratio of IL-6 and IL-10 plays an important role in anti-inflammation activities in AR patients. COS as non-pharmaceutical intervention should be developed a new way to prevent the risk of AR.

Disclosure of conflict of interest

None.

Abbreviations

COS, Chitosan oligosaccharide; AR, allergic rhinitis; IL-6, interleukin-6; IL-10, interleukin-10; ELISA, Enzyme-Linked ImmunoSorbent Assay; CCL, chemokine (C-C motif) ligand 26/eotaxin-3; MCP-4, monocyte chemoattractant protein-4.

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