

Original article

Effects of xiao-qing-long-tang (XQLT) on bronchoconstriction and airway eosinophil infiltration in ovalbumin-sensitized guinea pigs: *in vivo* and *in vitro* studies

Background: Xiao-qing-long-tang (XQLT sho-seiru-to), a traditional Chinese medicine, has been used to treat patients with bronchial asthma in Oriental countries for several centuries. However, the therapeutic mechanisms of this Chinese medicine remain a matter of considerable debate. Therefore, a series of experiments using ovalbumin-sensitized guinea pigs was performed to elucidate the possible antiasthmatic effect of XQLT.

Methods: The effect of XQLT on ovalbumin-induced airway inflammation in a guinea pig model of allergic asthma was examined, and early and late asthmatic responses were measured in terms of airway resistance and extent of eosinophil infiltration. Furthermore, the bronchorelaxing effect of XQLT was measured in isolated guinea pig trachea.

Results: XQLT significantly inhibited the antigen-induced immediate asthmatic response (IAR) and late asthmatic response (LAR) in actively sensitized guinea pigs. Cumulative administration of XQLT caused concentration-dependent relaxation of the carbachol-precontracted guinea pig trachea. The broncho-relaxing effect of XQLT was reversed by ICI-118551, a selective β_2 -adrenoceptor antagonist. Furthermore, examination of bronchoalveolar lavage fluid (BALF) revealed that XQLT significantly suppressed the increase in eosinophils (24 h after antigen challenge) in the airway. In addition, XQLT significantly attenuated the increase in eosinophils at 1, 6, 24, 48, and 72 h after antigen challenge when it was administered once daily from the day of sensitization to the day of challenge. Histopathologic examination results showed that XQLT suppressed eosinophil infiltration into lung tissue.

Conclusions: These results demonstrate that the antiasthmatic effects of XQLT appear to be partly mediated by stimulation of β_2 -adrenoceptors, leading to bronchorelaxation, and that XQLT inhibits the infiltration of eosinophils into the airway. Thus, XQLT may be useful for the prevention or treatment of asthma.

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Bronchial asthma was long considered a disease of the bronchial smooth muscle. However, it has been recently recognized that chronic inflammation of the airways plays an important role in the pathogenesis of asthma (1, 2). In atopic asthma, provocation of the airway by inhaled allergen causes immediate bronchoconstriction or an immediate asthmatic response (IAR). This bronchoconstrictor response is mediated by the actions of preformed and newly generated inflammatory mediators released after the IgE-dependent activation of mast cells associated with the bronchial mucous membrane (3, 4). The IAR reaches a maximum 15–30 min after challenge and lasts for as long as 2 h. In approximately 60% of atopic asthmatic patients, the IAR is followed by a late asthmatic response (LAR) that begins 4–12 h after allergen

challenge and lasts for several hours (5). A later LAR (LLAR) may occur with diminishing intensity over the following 2–3 days (6). The LAR is characterized by airway eosinophil infiltration and airway hyperresponsiveness, and this is thought to reflect more closely chronic natural asthma (7). Characteristically, sodium cromoglycate and β_2 -adrenoceptor agonist inhibit the IAR, whereas both the LAR and associated airways hyperresponsiveness are inhibited by sodium cromoglycate and corticosteroids, but not by β_2 -adrenoceptor agonists (8–10).

Recently, a number of animal models have been developed in which the LAR occurs in the airway after exposure to antigens. Guinea pigs have generally been used as a model of human asthma because similarities exist between the two species in the response to a variety

of contractile and relaxant substances (11–13). They also putatively involve chemical mediators in antigen-induced airway response and in the target organ in anaphylactic shock (14).

Xiao-qing-long-tang (XQLT; Japanese name: sho-seiryu-to), a traditional Chinese medicine, has been used to treat bronchial asthma and allergic rhinitis for several centuries. In a previous study, we found that XQLT exhibits an immunomodulation activity in allergen-sensitized mice (15). In this study, we investigated the effects of XQLT on the development of the ovalbumin-induced IAR, the LAR, the LLAR and eosinophil infiltration in a guinea pig model, and on bronchoconstriction in isolated guinea pig trachea.

Material and methods

Drugs and chemical preparations

Ovalbumin (albumin, chicken egg, Grade VI, Cat. No. A-2512), carbachol (carbamylcholine chloride, Cat. No. C-4382), and other chemicals were obtained from Sigma-Aldrich, Inc. ICI-118551 (Cat. No. I-127) was obtained from RBI/Sigma-Aldrich, Inc. Bosmin® (epinephrine hydrochloride, Inj., 1 mg/ml) was obtained from Daiichi Pharmaceutical, Taiwan Ltd. XQLT was supplied by Min Tong Pharmaceutical Co. (Taipei, Taiwan). This preparation is a freeze-dried powder of the aqueous-extract mixtures of eight common Chinese herbal medicines: *Ephedrae herba* (stem of *Ephedrae sinica* Stapf, 3.0 g), *Paeoniae radix* (root of *Paeoniae lactiflora* Pallas, 3.0 g), *Schizandrae fructus* (fruit of *Schizandra chinensis* Baill, 3.0 g), *Zingiberis siccaturum rhizoma* (root and rhizome of *Zingiber officinale* Roscoe, 1.0 g), *Glycyrrhizae radix* (root and rhizome of *Glycyrrhiza uralensis* Fischer, 2.0 g), *Cinnamonomi ramulus* (cortex of *Cinnamomum cassia* Blume, 3.0 g), *Pinelliae tuber* (root of *Pinellia ternata* Breitenbach, 6.0 g), and *Asari herba cum radice* (whole plant of *Asarum sieboldii* Miq, 3.0 g).

Ovalbumin sensitization and challenge

Specific pathogen-free male Dunkin-Hartley guinea pigs, weighing 350–450 g, were purchased from the National Laboratory Animal Breeding and Research Center, Taipei ROC. They were 8 weeks of age at the start of the experiments. Sensitization of the guinea pigs was performed, with minor modifications, as described previously (16). Male guinea pigs were sensitized by exposure to aerosolized 1% ovalbumin once every 8 days. The aerosols were generated by an ultrasonic nebulizer (driven by compressed air at 8 l/min) (De Vilbiss, Model 5610D, USA) in a 3.9-l perspex chamber, in which the animals were sensitized for 10 min. After 1 week, the guinea pigs were subjected to antigen inhalation challenge at day 16. One hour before the challenge, the animals were injected intraperitoneally with Bosmin® (2 ml/kg), a sympathomimetic drug which activates α_1 -, β_1 -, and β_2 -adrenoceptors (17), to avoid fatal anaphylactic reactions. The guinea pigs were then challenged with aerosols of 1% ovalbumin.

Experimental design and administration

Freeze-dried XQLT powder was dissolved (1 g/ml) and mixed at 37°C for 30 min in distilled water, and prepared for administration by a gastric tube. Respiratory resistance (*sRaw*) was measured 1 h before and at 5 min and 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after antigen challenge. Bronchoalveolar lavage and histologic examination were

performed at 1, 6, 24, 48, and 72 h after ovalbumin challenge. Exactly 180 male Dunkin-Hartley guinea pigs were randomly divided into six groups:

- 1) *treated group I*: treated with 10 g/kg of XQLT 30 min before challenge
- 2) *treated group II*: treated with 10 g/kg of XQLT 5 h after challenge
- 3) *treated group III*: treated with 10 g/kg of XQLT 30 min before challenge and 5 h after challenge
- 4) *treated group IV*: treated with 5 g/kg once daily from the day of sensitization to the day of challenge (the last XQLT administration 2 h before challenge)
- 5) *immunized group*: treated with 10 ml/kg 0.9% saline;
- 6) *nonimmunized group*: no sensitization, challenge, or treatment.

Measurement of respiratory resistance (*sRaw*)

sRaw was measured by a modification of the forced oscillation technique described by Mead (18). The measurements were carried out in inhalation-sensitized guinea pigs. Conscious guinea pigs were placed in a body plethysmography chamber (PMR-2, Shizume Medical Co., Japan). Their heads were left outside the chamber while a doughnut-shaped rubber balloon restrained their necks. A 25-Hz sine-wave oscillation was applied to their body surfaces; the oscillating pressure was generated with a 24-cm woofer speaker derived by a sine-wave generator and power amplifier. A rubber mask with a 200-mesh screen was snugly applied to their faces in order to measure the flow rate. The flow rate through the mask was measured by detecting the pressure difference across the 200-mesh screen with a differential pressure transducer. The box pressure was measured with a pressure transducer. The 25-Hz component of the mask flow rate and box pressure was extracted with a 25-Hz band-pass filter. *sRaw* was calculated from the ratio of the amplitude of the oscillating box pressure to the flow-rate fluctuation (19). The change of *sRaw* was expressed by the ratio of *sRaw* before to that after challenge at each time point.

Guinea pig tracheal contraction

Trachea from guinea pigs were dissected out and cut transversely between the segments of cartilage by the method of Bernareggi et al. (20). Briefly, male guinea pigs were killed by exsanguination after asphyxia with carbon dioxide. Tracheal strips were rapidly removed and placed in oxygenated modified Krebs-Henseleit solution of the following composition (mM): NaCl, 118; KCl, 5.9; MgSO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25.5; and glucose, 5.05. After careful dissection of connective tissue, the trachea was cut transversely with each segment containing three to four cartilaginous rings. For the bronchial preparations, bronchi were separated into main and hilar sections, respectively.

As for the *in vitro* studies in guinea pig tracheal contraction, XQLT was dissolved (100 mg/ml) and mixed at 37°C for 30 min in Krebs-Henseleit solution. This solution was centrifuged (12 000 g, 30 min) to remove insoluble ingredients. The supernatant was sequentially passed through 0.45- μ m and 0.22- μ m filters for sterilization. Tracheal and bronchial strips were suspended in 5-ml organ baths containing Krebs-Henseleit solution bubbled continuously with a mixture of 95% O₂ and 5% CO₂ at 37°C at pH 7.4. The resting tension was set at 1 g during a 1-h equilibration period. This resting tension was previously found to be optimal for measuring changes in isometric tensions. Each tracheal strip was prepared and suspended between a fixed base and strain gauze for measurement of isometric circumferential force. Once basal tension was established, the length of the strips was not altered thereafter. ICI-118551, a specific β_2 receptor antagonist (21), was administered 10 min before carbachol, and then exposed to increasing half-long cumulative

concentrations of XQLT. Isometric contractile responses were measured with Grass FT.03 force displacement transducers and recorded on a Gould 3200 polygraph (Gould, USA).

Bronchoalveolar lavage fluid (BALF)

At the time points of 1, 6, 24, 48, and 72 h after ovalbumin challenge, the lungs of guinea pigs were lavaged as follows. Each animal was anesthetized with urethane (1.5 g/kg), intraperitoneally. trachea was cannulated, and the lung lavage was done gently, using 5 ml of PBS (phosphate-buffered saline) at 37°C, followed by three subsequent aliquots of 5 ml of PBS. The tracheal cannula was clamped, and the thorax was massaged for 30 s before the BALF was recovered. The recovered lavage samples were cooled on ice, and centrifuged at 150 g for 10 min at 4°C. The pellets were obtained and resuspended in a final volume of 1 ml in PBS, and total cell numbers were counted and stained with Leu's solution. A total of 500 cells were counted and classified as mononuclear cells, neutrophils, or eosinophils according to standard morphologic criteria.

Histopathologic examination

For histopathologic examination of the lungs, guinea pigs were killed after collection of BALF at 1, 6, 24, 48, and 72 h after ovalbumin challenge. The lung and trachea were removed and fixed 10% of buffered formalin for 1 week. After the tissues were fixed, specimens of trachea and lung lobes were embedded in paraffin wax, and 5- μ m sections were cut and stained with hematoxylin-eosin. Lung tissues were prepared from two identical lobes (middle and lower) for each guinea pig and sectioned transversely through the mid-portion across the pulmonary bronchioles and bronchiolar branches. Thus, the trachea and sections from two lung lobes were examined histologically for each guinea pig.

Statistical analysis

Results are expressed as mean $\pm$ SEM. One-way ANOVA was used for multiple group comparisons and the Student's *t*-test for others. Differences with $P < 0.05$ were judged to be significant.

Results

Effect of XQLT on allergen-induced respiratory resistance

Respiratory resistance (*sRaw*) was monitored at regular intervals for up to 72 h after aerosol inhalation of saline or antigen (Fig. 1). Allergen provocation of ovalbumin-sensitized guinea pigs induced a pronounced IAR and LAR in all animals used. A dual asthmatic response was shown in the immunized group, and inhalation of ovalbumin caused immediate bronchoconstriction in the IAR, which reached a maximum at 5 min and lasted for as long as 1 h after ovalbumin challenge. After the immediate response, a second wave of bronchoconstriction (LAR) began 8 h after challenge and reached maximum at around 24 h after ovalbumin challenge; the maximum in the LAR was lower than in the IAR. Further bronchoconstriction then lasted for 2–3 days (LLAR). At any point in time after challenge, the *sRaw* of the immunized group was significantly higher than in the nonimmunized group ($P < 0.001$ at 5 min, and 1, 2, 4, 6, 8, 12, 24, and 48 h; $P < 0.01$ at 72 h) (Fig. 1, Table 1).

Treated group I. *sRaw*, at each time point from 5 min to 48 h after challenge, was significantly lower in treated group I than the immunized group ($P < 0.001$ at 5 min, and 1, 2, and 24 h; $P < 0.01$ at 4, 6, and 12 h; $P < 0.05$ at 8 and 48 h) (Fig. 1, Table 1). When given 30 min before ovalbumin challenge, XQLT almost completely inhibited both the early and late responses.

Treated group II. XQLT administered 5 h after antigen challenge did not reduce the late responses.

Treated group III. *sRaw*, at each time point throughout the course, was significantly lower in treated group III than the immunized group ($P < 0.001$ at 5 min, and 1, 2, 4, 6, 8, 12, 24, and 48 h; $P < 0.05$ at 72 h) (Fig. 1, Table 1). These results showed that in both treated groups I and III were significantly inhibited both the early and late responses.

Treated group IV. The decrease of *sRaw* in treated group IV was similar to treated group III, but it was more obvious in treated group IV. *sRaw*, at each time point throughout the course, was significantly lower in treated group IV than the immunized group ($P < 0.001$ at 5 min and 1, 2, 12, 24, and 48 h; $P < 0.01$ at 4, 6, and 72 h) ($P < 0.05$ at 8 h). This result indicated that XQLT administered from the beginning of sensitization until challenge completely inhibited both the early and late responses.

Effect of XQLT on carbachol-induced bronchoconstriction

In strips of guinea pig trachea contracted submaximally with 1 μ M of carbachol, XQLT (administered from 0.0625 to 3 mg/ml) caused concentration-dependent relaxations. The IC_{50} value of XQLT on carbachol-induced bronchoconstriction was 0.78 ± 0.13 mg/ml ($n = 8$, (Fig. 2A). These relaxations were competitively antagonized by ICI-118551. In the guinea pig trachea, ICI-118551 (0.1 and 1.0 μ M) produced competitive antagonism of XQLT-induced relaxations, yielding a Schild plot with a slope not significantly different from

Table 1. Effect of XQLT on ovalbumin-challenge increase of respiratory resistance (*sRaw*)

Treatment	Ratio of <i>sRaw</i> (before/after challenge)		
	5 min (IAR)	24 h (LAR)	72 h (LLAR)
Nonimmunized group	1.1 $\pm$ 0.1 [†]	1.1 $\pm$ 0.2 [†]	1.1 $\pm$ 0.1 ^{**}
Immunized group	1.9 $\pm$ 0.3	1.6 $\pm$ 0.2	1.5 $\pm$ 0.3
Treated groups			
I	1.4 $\pm$ 0.3 [†]	1.3 $\pm$ 0.2 [†]	1.4 $\pm$ 0.3
II	1.9 $\pm$ 0.3	1.5 $\pm$ 0.3	1.5 $\pm$ 0.3
III	1.5 $\pm$ 0.3 [†]	1.2 $\pm$ 0.2 [†]	1.2 $\pm$ 0.1 [*]
IV	1.4 $\pm$ 0.2 [†]	1.1 $\pm$ 0.2 [†]	1.1 $\pm$ 0.1 ^{**}

Values are presented as mean $\pm$ SEM ($n = 6$). * $P < 0.05$, ** $P < 0.01$ and [†] $P < 0.001$ as compared with immunized group.

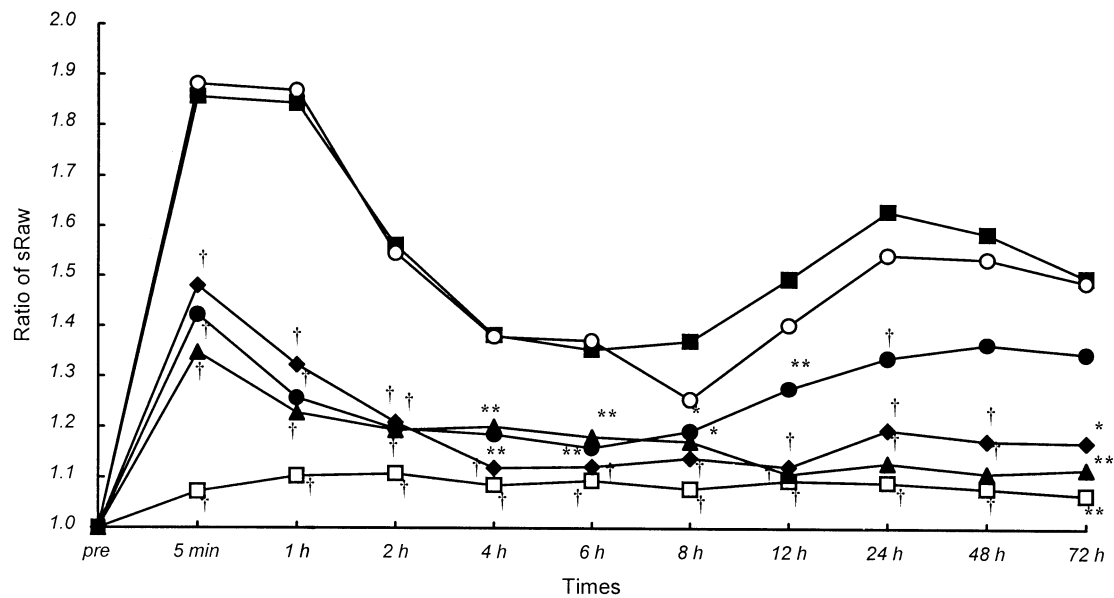


Figure 1. Effect of XQLT on allergen-induced increase of respiratory resistance (*sRaw*). Immunized group: ■; nonimmunized group: □; treated group I: •, group II: ○; group III: ◆; and group IV: ▲. Each point represents mean of six guinea pigs. **P* < 0.05, ***P* < 0.01 and †*P* < 0.001 as compared with immunized group.

unity (Fig. 2B). The inhibition by the selective β_2 -antagonist, ICI-118551, of the relaxations of trachea caused by XQLT demonstrates that the relaxation was mediated by stimulation of β_2 -adrenoceptors.

Effect of XQLT on ovalbumin-induced eosinophil infiltration in BALF

In each group, recovery of lavage fluid was consistently 80–90% of that instilled. When compared with the non-immunized group, ovalbumin caused a significant increase of neutrophils at 1 and 6 h in the immunized group (data not shown). There was also a significant increase of eosinophils at each time point after ovalbumin challenge, reaching a maximum at 24 h (Table 2).

The effects of XQLT on eosinophil infiltration in BALF are shown in Table 2. When compared with the immunized group, treated group I showed that XQLT significantly reduced the increase in eosinophils at 1 and 24 h (*P* < 0.001) after challenge. In treated group II, XQLT significantly reduced the increase in eosinophils only at 24 h (*P* < 0.001) after challenge. In treated group III, XQLT significantly reduced the increase in eosinophils at 1 h (*P* < 0.001) and 24 h (*P* < 0.01) after challenge. In treated group IV, XQLT significantly inhibited the increase of eosinophils in BALF at each time point (*P* < 0.001 at 1 h, 24 h, and 72 h; *P* < 0.01 at 48 h and *P* < 0.05 at 6 h) after challenge.

Histologic examination of effect of XQLT on eosinophil infiltration in lung tissue

Examination of tracheal and lung sections under light microscopy revealed an infiltration of neutrophils and

eosinophils into the airway, around the bronchi, bronchioles, and alveoli, of ovalbumin-challenged guinea pigs in the immunized group. At 1 h after challenge, neutrophils were observed beneath the airway smooth muscles, and were tracked through the muscle toward the epithelium (Fig. 3B). At 24 and 72 h, leukocyte infiltration was dominated by eosinophils (Fig. 3E and H), which infiltrated the peribronchial and peribronchiolar smooth muscles, mucosal epithelium, and alveoli. Treatment with XQLT (treated groups III and IV) suppressed the neutrophil infiltration at 1 h (Fig. 3C and D) and the eosinophil infiltration at 24 h (Fig. 3F and G) and 72 h (Fig. 3I and 3J) into the peribronchial and peribronchiolar smooth muscles and submucosal area after ovalbumin challenge.

Table 2. Effect of XQLT on eosinophil infiltration of BALF in ovalbumin-sensitized guinea pigs

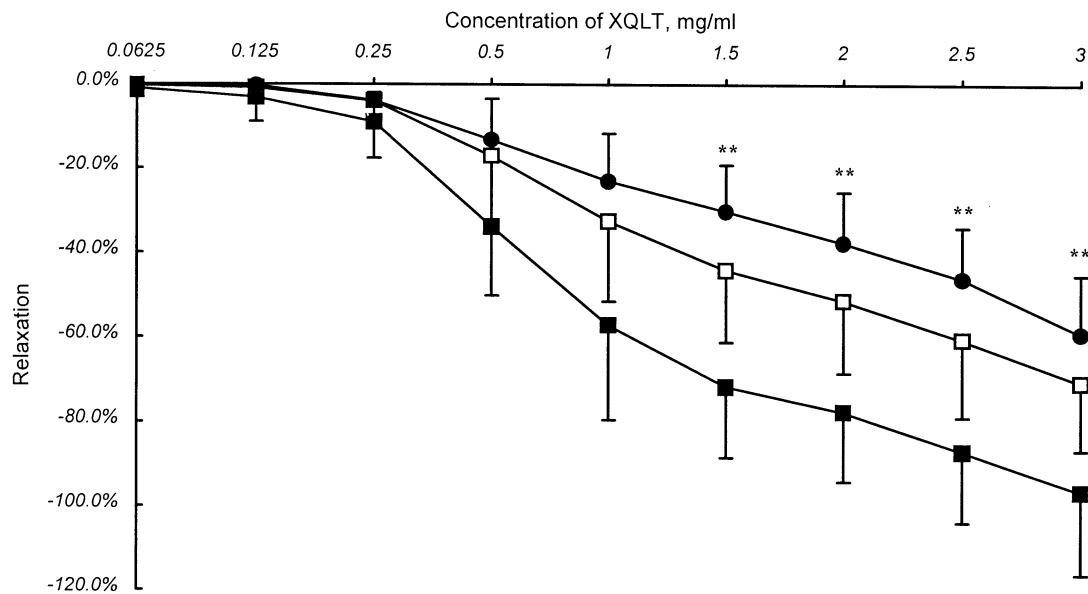
Treatment	Cell numbers ($\times 10^5$ cells in BALF)				
	1 h	6 h	24 h	48 h	72 h
Nonimmunized	3.9 $\pm$ 2.0 [†]	4.2 $\pm$ 1.6 [†]	3.5 $\pm$ 0.8 [†]	4.9 $\pm$ 2.3 [†]	4.4 $\pm$ 2.8 [†]
Immunized	17.5 $\pm$ 2.9	14.4 $\pm$ 3.1	31.9 $\pm$ 3.7	24.1 $\pm$ 4.2	22.1 $\pm$ 4.6
Treated group					
I	8.8 $\pm$ 3.1 [†]	12.7 $\pm$ 3.9	22.3 $\pm$ 4.7 [†]	21.1 $\pm$ 5.4	19.6 $\pm$ 5.2
II	17.0 $\pm$ 3.2	15.3 $\pm$ 5.6	21.3 $\pm$ 3.7 [†]	24.3 $\pm$ 8.6	19.4 $\pm$ 5.3
III	8.3 $\pm$ 3.4 [†]	10.3 $\pm$ 2.1	24.0 $\pm$ 3.2 ^{**}	18.3 $\pm$ 5.4	19.7 $\pm$ 5.7
IV	10.1 $\pm$ 4.3 [†]	8.2 $\pm$ 2.4 [*]	13.0 $\pm$ 3.6 [†]	11.8 $\pm$ 6.3 ^{**}	7.3 $\pm$ 2.9 [†]

Values were presented as mean $\pm$ SEM (*n* = 6), * *P* < 0.05, ** *P* < 0.01 and †*P* < 0.001 as compared with immunized group.

Discussion

XQLT has been widely used to treat patients with

(A)



(B)

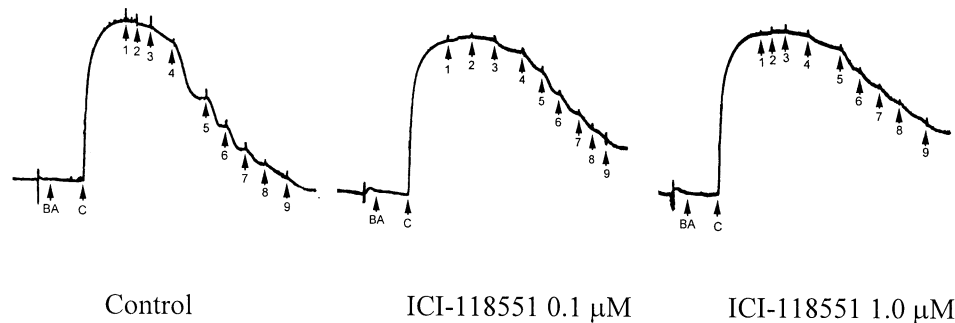


Figure 2. Effect of XQLT on carbachol-induced contractile responses in guinea pig trachea. Rings of tracheal smooth muscle were contracted with carbachol (1 μ M) for 15 min, and then various concentrations of XQLT (■) were added. ICI-118551 (0.1 μ M □; 1.0 μ M •) was administered 10 min before carbachol. Then various concentrations of XQLT were added. Each point represents mean $\pm$ SEM of eight guinea pigs (A). As typical tracing of ICI-118551 antagonist, XQLT administration from 0.0625 to 3 mg/ml (arrows 1–9) is presented. “BA” indicates pretreatment with or without ICI-118551, and “C” indicates carbachol-induced bronchoconstriction (B). Values are presented as means, and differences were judged significant at $**P < 0.01$ compared with control group (A).

bronchial asthma and allergic diseases (22, 23). The present study is the first demonstration that XQLT has both bronchodilator and anti-inflammatory activity in ovalbumin-sensitized guinea pigs. In the present study, the two-phase bronchoconstrictor response, the IAR and the LAR, to ovalbumin challenge in guinea pigs was observed. In our animal model, the time course for IAR and LAR was similar to that in a model of Santing

et al. (2). During the LAR, the sequence of inflammatory events within the airway, i.e., eosinophil preceded by neutrophil infiltration, is similar to that reported after antigen challenge in patients with asthma. When antiasthmatic drugs were administered before antigen challenge, cromolyn inhibited both the IAR and the LAR but showed no effect on eosinophil accumulation in the LAR (24). Albuterol, a β_2 -agonist, blocked the

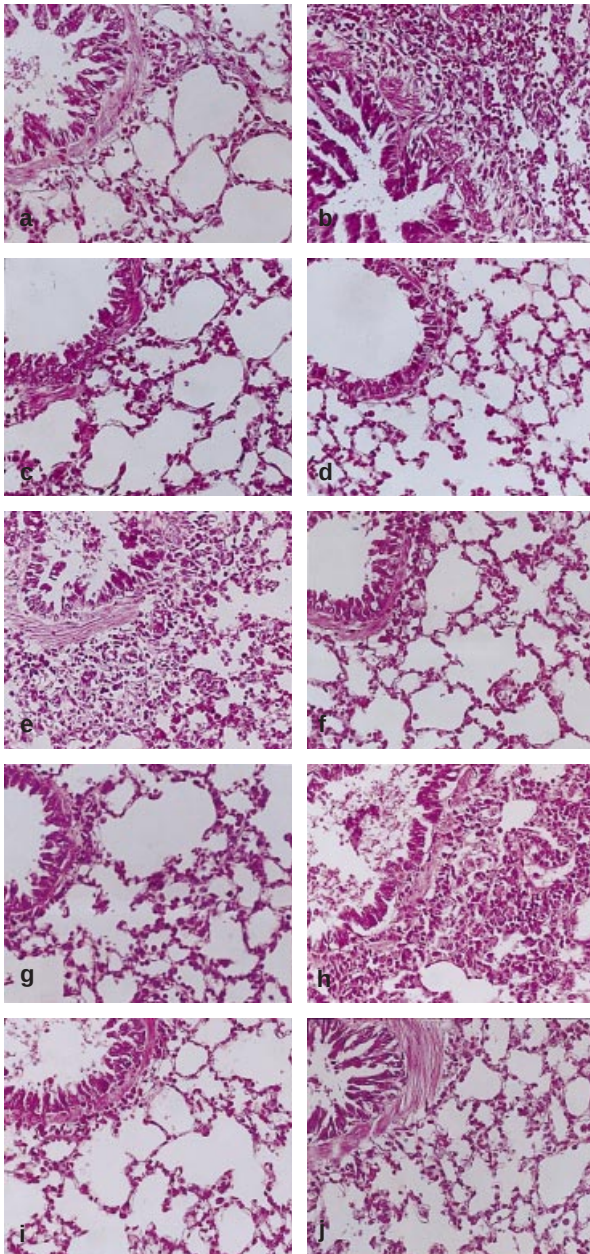


Figure 3. Microscopic evaluation of effect of XQLT on leukocyte infiltration of asthmatic airway at 1, 24, and 72 h after ovalbumin challenge in guinea pig. Few leukocytes were seen around airway in normal guinea pig (A). There was marked leukocyte infiltration at 1 h (B), 24 h (E), and 72 h (H) in immunized group when compared to treated group III at 1 h (C), 24 h (F), and 72 h (I), and to treated group IV at 1 h (D), 24 h (G), and 72 h (J). Treatment with XQLT markedly suppressed neutrophil infiltration at 1 h in treated groups III (C) and IV (D); and eosinophil infiltration at 24 h, in treated groups III (F) and IV (G); and at 72 h in treated groups III (I) and IV (J) after ovalbumin challenge. Hematoxylin-eosin stain; $\times 200$.

IAR but neither inhibited the LAR nor affected eosinophil infiltration (24). Dexamethasone inhibited the LAR, but not the IAR, and inhibited the increase in eosinophils in the BALF 24 h after antigen challenge. (25). When antiasthmatic drugs were administered both

before and after antigen challenge, cromolyn inhibited the IAR, the LAR, and the LLAR, and the increase of eosinophils at 72 h (24). Albuterol and salbutamol inhibited the IAR, but not the LAR, and had no effects on eosinophil infiltration (25). Administration of XQLT both 30 min before challenge (treated group I) and 30 min before and 5 h after challenge (treated group III) significantly inhibited the IAR, the LAR, the LLAR, and the increase of eosinophils. These findings suggest that XQLT is an antiasthmatic agent, useful to prevent or treat asthma.

Many investigators have reported that the development of the LAR is associated with recruitment into the airway of eosinophils that presumably cause cellular activation and bronchoconstriction. Because eosinophils release cytotoxic granular-associated proteins, including major basic protein, eosinophil cationic protein, and eosinophil peroxidase, they have a profound effect on the airway (26, 27). Furthermore, eosinophil activation results in the release of other mediators, such as leukotriene C_4 and platelet-activating factor, which can cause contraction of airway smooth muscles (28, 29). When XQLT was administered at half-dose from the beginning of sensitization until challenge (treated group IV), it not only completely inhibited the IAR, LAR, and LLAR, but also inhibited the increase of eosinophils throughout the time course. Furthermore, histopathologic examination of lung tissue also showed that XQLT inhibited eosinophil infiltration into the airway after ovalbumin challenge. These results demonstrated that XQLT exerts a prophylactic effect on allergen-induced airway inflammation. In a previous study, we have shown that XQLT exhibits an immunomodulating activity in allergen-sensitized mice (15). In the present study, we demonstrated that when XQLT is administered from the beginning of sensitization until before challenge (treated group IV), the antiasthmatic activity was more significantly increased than in the other groups. Recent studies have found that dehydroepiandrosterone (DHEA) has important immunomodulating effects on T-cell differentiation (30). Administration of DHEA before and during allergic sensitization was found to attenuate the subsequent allergic responses elicited by challenge, and the suppressive effect of DHEA was associated with downregulation of the TH2 response. Whether the immunomodulating effect of XQLT can influence the TH2 response is still under investigation.

The mechanism of action of the bronchodilator effect of XQLT was also investigated *in vitro* in guinea pig trachea. XQLT caused concentration-dependent relaxation of trachea precontracted with carbachol in guinea pigs. The relaxation of XQLT was significantly attenuated by ICI-118551, a specific β_2 -antagonist. This result demonstrated that the relaxation of XQLT was partially mediated by the stimulation of β_2 -adrenoceptors. Ephedrine is one of the active components of

Ephedrae sinica Stapf, and it has weak agonist activity at the α - and β -adrenoceptors. Both the β_2 -agonists, albuterol and salbutamol, inhibited only the IAR but neither blocked the LAR nor affected eosinophil infiltration (24, 25). Our results confirm that XQLT has β_2 -adrenergic agonist activity; however, XQLT not only inhibited the IAR, but also inhibited the LAR and the increase in eosinophils. XQLT differs from albuterol and salbutamol, in having other effects in treating asthma besides β_2 -adrenergic agonist activity. Therefore, ephedrine is not the only component to explain the efficacy of the antiasthmatic effect of XQLT.

In conclusion, we show for the first time that XQLT is effective against early and late phase airflow obstruction in conscious sensitized guinea pigs challenged by inhalation of ovalbumin, and we associated these changes

with the number of eosinophils in BALF. We suggest that there may be two antiasthmatic mechanisms of XQLT, a bronchodilator effect resulting from its stimulation of β_2 -receptors on bronchial smooth muscles, and an ability to inhibit the eosinophil infiltration into the airway. However, the precise mechanism of action of XQLT in asthma remains to be elucidated.

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