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Influence of acupuncture on the expression of VIP, SP, NKA and NKB, cAMP/cGMP and HE content and treatment of bronchial asthma in rats

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Abstract: This research was set up to explore the neural mechanisms of acupuncture in the treatment of bronchial asthma in rats by detecting the content of substance P(SP), vasoactive intestinal peptide (VIP), neurokinin A(NKA), neurokinin B (NKB), cyclic adenosine monophosphate/cyclic guanosine monophosphate ratio (cAMP/cGMP) and hematoxylin-eosin (HE) staining for the pathological changes of lung tissue, in order to Institute Certain Experimental and Theoretical Foundation for Traditional Chinese Medicine (TCM) Prevention and Treatment of Bronchial Asthma. For this purpose, fifty healthy adult Wistar male rats, weighing 200-250 g, were randomly divided into 5 groups: normal control group A, asthma control group B, asthma acupuncture group C, adrenalectomy (ADX)-asthma group D, adrenalectomy (ADX)-asthma acupuncture group E. Group A was raised with other groups at the same period; Group B was induced asthma by ovalbumin; Group C was induced asthma as Group B and then acupuncture on the basis of group D. HE staining was performed in the lung tissue of rats from each group, and histopathologic changes were observed. SP, VIP, NKA, NKB in each rat lung tissue were measured by immunohistochemistry. cAMP/cGMP was measured with ELISA to speculate the neural mechanisms of acupuncture in the treatment of bronchial asthma. The results were as: decrease of cAMP/cGMP and VIP and increase of SP, NKA, NKB in the lung tissue are the neural mechanisms of an asthma attack. The increase of cAMP/cGMP and decrease of NKA, NKB, SP and VIP in the lung tissue of SP and VIP in group E were related to the reduction of glucocorticoid after ADX which influenced the effect of acupuncture. The neural regulation mechanisms of acupuncture, and related to the release of inflammatory mediators.

Key words: Acupuncture; Bronchial asthma; Neural mechanism.

Introduction

Bronchial asthma, though a common disease, cause great trouble to people's life and work. Statistically, the occurrence ranges 0.1-32% among different countries of the world. The mechanisms of bronchial asthma include immune response, airway inflammation, nerve-receptor imbalance, genetic factors and so on (1). In recent years, the theory of nerve-receptor imbalance in bronchial asthma has attracted the attention of researchers. Airway smooth muscle contraction; mucus production, secretion and elimination; release and inhibition of allergic media; as well as the maintenance of normal function of mucosal movement are all mediated by the autonomic nerve receptors (2, 3). The regulation of the autonomic nervous system is very complex, and the nerve-receptor function in the human body is in a state of dynamic equilibrium under normal circumstances (4, 5).

The acupuncture treatment of bronchial asthma is attracting increasing attention recently, for the confirmation of its therapeutic effect and without side effects of glucocorticoids and other antiasthmatic drugs (5, 6). Treating bronchial asthma by acupuncture originated from the Qin Dynasty, and after the accumulation of experience and improvement of the asthma symptoms for such a long time, its antiasthmatic clinical efficacy has been verified. Based on the clinical effect, our study focused on the mechanism of acupuncture on the neural regulation mechanisms of bronchial asthma in rats by evidence-based medicine. The aim of this research is an investigation of the effects of acupuncture on expression of VIP, SP, NKA and NKB, cAMP/cGMP and HE content and treatment of bronchial asthma in rats.

Materials and Methods

Asthma model preparation and assessment

The rats received a peritoneal injection of 1 ml Ovalbumin (OVA; 1 mg/ml) on days 8 and 15 to induce anaphylaxis. They placed in a semi-enclosed container from day 16, they were inhaled atomized OVA (10%) every other day until they develop the asthma symptoms.

Assessment Criteria

Sneezing, difficult breathing, strengthened abdominal contraction, Musset's sign, twisting the body and scratching were present in rats after atomization.

Grouping

Fifty healthy adult male Wistar rats, weighing 200-250 g, were randomly divided into 5 groups. Group A, as the control group, was raised with other groups in the same period without operation or induced bronchial asthma by OVA. The saline was used to replace OVA. Group B, underwent asthma model preparation. Group C, underwent asthma model preparation and then were acupunctured five acupoints (bilateral Feishu, bilateral Fengmen, and Dazhui) for 30 min a day on every other day. Group D, underwent adrenalectomy and asthma model preparation. The rats were anesthetized by 20% ethylurethanm (5 ml/kg) intraperitoneal injection, fixed on the operating table with supine position, shaved abdominal hair, cut a 3-4cm incision along the abdomen midline below the xiphoid, and removed the bilateral adrenal glands. They drank 0.9% NS continuously and after a week they were injected and atomized as group B. Group E was treated with acupuncture on the basis of group D. They were acupunctured on five acupoints for 30 min a day on every other day.

For all the rats, the samples were taken on day 45. Electric acupuncture:

The standard of acupoints selection refers to Experimental Acupuncture, and five acupoints (bilateral Feishu, bilateral Fengmen, and Dazhui) were acupunctured Feishu was located on the bilateral intercostal space under the third thoracic vertebrae; Fengmen was on the bilateral intercostal space under the second thoracic vertebrae, and Dazhui was between the second thoracic vertebrae, and Dazhui was between the seventh cervical and first thoracic vertebra on the midline of the back of rats. Electric acupuncture was performed after each atomization. Fixing on a wooden bench at 9 am, the rats were connected with the electric acupuncture apparatus then punctured into 3-5mm. The wave of condensation and rarefaction was applied and the strength was that the needle could fibrillate slightly. The acupuncture lasted for 30 minutes each time.

Sampling

Lung tissue

The rats underwent thoracotomy after anesthesia by 20% ethylurethanm (5ml/kg). The lung was taken as soon as possible and put into PBS solution (0 °C) to remove the extra blood. Two samples of lung tissue were clipped for every rat, each weighing 50mg. One sample was soaked temporarily in formalin for paraffin embedding. Paraffin Microtome was used for continuous section and 10 sections were obtained, each of which is 5um in thickness. Another sampling was ground in a mortar, put into a disinfected EP tube, which is centrifuged for 15min (3000r/min). The supernatant was sucked by a pipette and reserved in a refrigerator at a temperature of-20 °C.

Measurement

cAMP, cGMP

We detected the expression of cAMP and cGMP in lung tissues by ELISA. uPA, PAI-1 ELISA kits were

bought from Shanghai Westing-Bio Science Co. Follow the instructions to operate.

VIP, SP, NKA, NKB, Immunohistochemistry

Tissues were sectioned into 4 μ m sections for hematoxylin and eosin (H&E) staining. We used immunohistochemistry to detect the expression of VIP, SP, NKA, NKB. Immunohistochemical kits for them were purchased from Wuhan Boster Bio-Engineering Co, Ltd. Follow the instructions to operate.

Data analysis

SPSS17.0 statistical software was used for data analysis, numerical data by Q test. All data were expressed by the mean + standard deviation ($\bar{x}\pm$ SD). p>0.05 means no significant difference, p<0.05 was considered to be statistically significant, and p<0.01 means significant difference.

Results

Influence of acupuncture on the pathological changes of lung tissue of rats (HE staining) Normal control group A

Orderly arrangement of the epithelial cells of the bronchiole wall; noedema under the mucosa; normal inflation of the alveolar lumen; no eosinophils and other inflammatory cells; no stenosis and incrassation of the small arteries.

Asthma control group B

Partialdesquamation of the epithelial cells from the bronchiole wall; edema under the mucosa; more goblet cells; thickened airway smooth muscle; ruptured alveolar septum and visible pulmonary bulla; more eosinophils; stenosis and incrassation of the small arteries.

Asthma acupuncture group C

No apparent desquamation of the epithelial cells from the bronchiole wall; fewer goblet cells and eosinophils than those in group B; minute quantities of secretion in the lumen; no apparent thickening of the airway smooth muscle; normal inflation of the alveolar lumen; unobstructed small arteries.

ADX-asthma group D

Local desquamation of the epithelial cells from the bronchiole wall; edema under the mucosa; more goblet cells and eosinophils; visible pulmonary bulla; increased vascular permeability and incrassation of the small arteries.

ADX-asthma acupuncture group E

Rather an orderly arrangement of the epithelial cells of the bronchiole wall; fewer goblet cells and eosinophils than those in group B, no apparent edema under the mucosa; no apparent thickening of the airway smooth muscle; occasionally visible pulmonary bulla; unobstructed small arteries (As shown in Fig 1).

Measurement of the indices of the lung tissue cAMP/cGMP of the lung tissue in different groups

cAMP/cGMP of the asthma group was decreased compared with that of the normal control group(p<0.01), which indicated that the cAMP/cGMP decreased in an

Cell Mol Biol (Noisy le Grand) 2020 | Volume 66 | Issue 5



asthma attack. cAMP/cGMP of the asthma acupuncture group was higher than that of the asthma group(p<0.05), which indicated that acupuncture could relieve the asthma symptoms by increasing cAMP/cGMP. cAMP/ cGMP of the ADX-asthma acupuncture group was higher than that of the asthma group(p<0.05), which indicated that the acupuncture was effective. cAMP/ cGMP of the ADX-asthma acupuncture group was higher than that of the ADX-asthma group(p<0.05), which indicated that the acupuncture was effective. (As shown in Table 1).

Table 1. cAMP/cGMP in different	groups.
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The average absorbance value of VIP of the lung tissue in different groups

VIP of the asthma group was decreased significantly compared with that of the normal control group(p<0.01), which indicated that an asthma attack was related to the decrease of VIP. VIP of the asthma acupuncture group was higher than that of the asthma group(p<0.05), which indicated that acupuncture could increase VIP, relax the bronchus to relieve the asthma symptoms.VIP of the ADX-asthma acupuncture group was higher than that of the asthe ADX-asthma group (p>0.05), which indicated that the adrenal gland removal might reduce the release of glucocorticoid and affect the acupuncture (As shown in Table 2).

The average absorbance value of NKA of the lung tissue in different groups

NKA of the asthma group was increased significantly compared with that of the normal control group(p<0.01), which indicated that NKA increased in an asthma attack. NKAin asthma acupuncture group and ADX-asthma acupuncture group was decreased significantly compared with that of the asthma group(p<0.01), which indicated that the acupuncture could relieve the asthma symptoms by increasing NKA. NKA of the ADX-asthma acupuncture group was much lower than that of the ADX-asthma group(p<0.05), which indicated that the acupuncture (As shown in Table 3 and Fig. 2).

The average absorbance value of NKB of the lung tissue in different groups

NKB of the asthma group was increased significantly compared with that of the normal control group (p<0.01), which indicated that NKA increased in an asthma attack. NKB in the asthma acupuncture group was decreased significantly compared with that of the asthma group (p<0.01), which indicated that the acupuncture was effective. NKB of the ADX-asthma acupuncture group was lower than that of the asthma group(p<0.05), which indicated that the acupuncture

Group (n)	cAMP/cGMP (nmol/L)
A Normal control group (10)	0.40±0.10
B Asthma group (10)	$0.23{\pm}0.11^{*}$
C Asthma acupuncture group (10)	0.36±0.14▲
D ADX-asthma group (10)	$0.22{\pm}0.07$
E ADX-asthma acupuncture group (10)	0.36±0.15▲•

Notes: *Significant difference compares with the normal group. A significant difference compares with the asthma group; *Significant difference compares with the ADX-asthma group.

Table 2. The average absorbance value of VIF	of the lung tissue in differe	ent groups ($x \pm SD$).
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Group (n)	The average absorbance value of VIP
A Normal control group (10)	61.87±7.33
B Asthma group (10)	$50.19{\pm}5.95^{*}$
C Asthma acupuncture group (10)	58.98±10.78▲
D ADX-asthma group (10)	43.88±13.23
E ADX-asthma acupuncture group (10)	49.08±6.43



E. ADX-asthma acupuncture group 10×40

Figure 2. The expression of NKA in lung tissues of different groups of rats was detected by immunohistochemistry.

was effective. NKB of the ADX-asthma acupuncture group was lower than that of the ADX-asthma group (p<0.05), which indicated that the acupuncture was effective (As shown in Table 4).

The average absorbance value of SP of the lung tissue in different groups

SP of the asthma group was increased significantly compared with that of the normal control group(p<0.01), which indicated that an asthma attack was related to the increase of SP. SP of the asthma acupuncture group was lower than that of the asthma group(p<0.05), which



A. Normal control group 10×40



40 D. ADX-asthma group10×40

B. Asthma group 10×40



C. Asthma acupuncture group 10×40



E. ADX-asthma acupuncture group 10×40

Figure 3. The expression of SP in lung tissues of different groups of rats was detected by immunohistochemistry.

indicated that acupuncture could decrease SP. The decease of SP of the ADX-asthma acupuncture group was not significant compared with that of the asthma group as well as the ADX-asthma group (p>0.05), which indicated that the adrenal gland removal might reduce the release of glucocorticoid and affect the acupuncture (As shown in Table 5 and Fig. 3).

Discussion

The neural mechanism is an important part of the pathogenesis of bronchial asthma, which includes the

Table 3. The average absorbance value of NKA of the lung tissue in different groups ($\overline{x} \pm SD$).

Group (n)	The average absorbance value of NKA
A Normal control group (10)	12.02±3.22
B Asthma group(10)	$62.53{\pm}7.07^{*}$
C Asthma acupuncture group(10)	38.68±10.08▲
D ADX-asthma group(10)	59.49±7.19
E ADX-asthma acupuncture group (10)	52.36±5.38▲•

Table 4. The average absorbance value of NKB of the lung tissue in different groups($x \pm SD$).

Group (n)	The average absorbance value of NKB
A Normal control group (10)	13.02±4.28
B Asthma group(10)	$41.08{\pm}5.88^{*}$
C Asthma acupuncture group(10)	29.93±4.06▲
D ADX-asthma group(10)	39.53±4.89
E ADX-asthma acupuncture group (10)	34.14±4.81▲•

Table 5. The average absorbance value of SP of the lung tissue in different groups ($x \pm SD$).

C. ELISA kit

Group (n)	The average absorbance value of SP
A Normal control group (10)	24.24±5.01
B Asthma group(10)	$49.66{\pm}8.57^*$
C Asthma acupuncture group(10)	41.09±6.45▲
D ADX-asthma group(10)	52.43±10.15
E ADX-asthma acupuncture group (10)	55.96±10.09





A. Acupuncture process

Figure 4. Pictures of rat modeling and acupuncture process, ELI-SA kit.

B. Adrenalectomy in rats

traditional theory of sympathetic/parasympathetic imbalance and the discovery of non-adrenal non-cholinergic nerve (NANC).

According to the traditional view, asthma is associated with sympathetic inhibition and vagal-tone hyperactivity, but in recent years there appear different views. Generally, there are very few sympathetic nerves in the airway, but a wealth of β 2- adrenergic receptors (β 2-R). In asthma patients, the number of β 2-R decreases associated with dysfunction. The main consequence of the activation of β 2-R is the activation of the adenosine cyclase, increasing the synthesis of cAMP, and the relaxation of airway smooth muscles. In patients with bronchial asthma, hyperactivity and increased number of receptors of M1 and M3, hypofunction of M2 and decreased number of receptors cause the contraction of airway smooth muscles and more secretion of mucus.

Balance of secondary messengers(cAMP/cGMP): clinically-validated drug research found that maintenance of the tension stability of bronchial smooth muscle cells relies on the balance and antagonism of cAMP and cGMP and normal function of cell membrane receptors (2). The function of cAMP is to relax bronchial smooth muscles, while cGMP induces contraction and spasm of them. cAMP in mast cells activates protein kinase to change the cell membrane permeability, inhibit removal particles of mast cells and block the release of bioactive substances, which prevents bronchial asthma. Nevertheless, the effect of cGMP is reversed. It promotes mast cell to remove particles and release bioactive substances, which causes bronchial asthma. At present, bronchodilators, as effective antiasthmatic drugs, mainly includes \beta2 agonists, anticholinergic drugs and theophylline drugs. B2 receptor agonists act on the adrenergic nervous system and β 2 receptors of the bronchial smooth muscle, activate the adenosine cyclase, and increase the synthesis of cAMP, therefore, the airway smooth muscles relax. Theophylline drugs relax airway smooth muscles by inhibiting phosphodiesterase activity and increasing cAMP concentration.

The results show that the ratio of cAMP/cGMP of the asthma group decreased significantly compared with that of the normal group, which indicated the mechanism of rat asthma was related to their balance. The different increases of them in the asthma acupuncture group and the ADX-asthma acupuncture group suggest that acupuncture treatment of bronchial asthma plays a role by regulating the balance of cAMP/cGMP.

A growing number of studies have found that NANC and its released neurotransmitters play an important role in the regulation of airway responsiveness and neurogenic inflammation. NANC contains non-adrenergic nerve (iNANC), which belongs to the inhibitory nerve, and non-cholinergic nerve (eNANC), which belongs to the excitatory nerve. Many substances, including allergen, histamine, prostaglandin and leukotriene, can induce pulmonary sensory nerves to release neuropeptide. It is generally believed that in an asthma attack, the release of the excitatory neuropeptide increases while the release of inhibitory neuropeptide decreases. But some scholars are skeptical of this view. Van der Velden et al (3) thought that although eNANC in asthma patients was apparently excitatory, people still could not find the difference in iNANC nerves in asthmatics as well as the healthy persons.

So far, non-adrenergic nerve (iNANC) is known as the only inhibitory nerve in the airway smooth muscles. Neurotransmitters include VIP and NO. VIP, which acts on the trachea to relax airway smooth muscles and regulate mucus secretion, is the most important endogenous bronchial dilator. It is widely distributed in the airway and pulmonary vessels, and its nerve endings eventually dominate the airway smooth muscle layer, the outer membrane of the pulmonary vascular wall and the submucosal glands of the bronchial mucosa (4). VIP can combine with the receptors (via the C- terminal binding) to activate cyclase and increase the level of cAMP, so the function of VIP is very similar to that of the Breceptor agonist. VIP, one of the most important airways smooth muscle relaxants, is released by the parasympathetic nerve. It can block the cholinergic receptors in synergy with NO to weaken the Ach in airway constriction. VIP and Ach are both in cholinergic fibers, and VIP can regulate the release of Ach or change its biological effect to weaken the role of Ach in airway contraction (5). In addition, VIP in synergy with NO can inhibit the release of presynaptic Ach. The study showed that relaxation of VIP on the airway smooth muscles is 50 times that of norepinephrine (6). VIP can inhibit airway smooth muscle contraction caused by the metabolic products of arachidonic acid and histamine, and regulate the release of histamine from mast cells.

The results show that VIP of the asthma group decreased significantly compared with that of the control group, and VIP of the asthma acupuncture group increased significantly compared with that of the control group. These suggest that the asthma attack in rats is related to the decrease of VIP, and acupuncture can relieve the asthma symptoms by regulating VIP in lung tissues. The increase of VIP of the ADX-asthma acupuncture group was not significant compared with that of the asthma group, which indicated that the adrenal gland removal might reduce the release of glucocorticoid and affect the acupuncture.

Non-cholinergic nerve (eNANC) is an excitatory nerve, and its neurotransmitters are NKA, NKB, SP, CGRP and others. Neuropeptides are mainly distributed under the mucous membrane, in smooth muscles, in the endothelium, around the blood vessels, in submucosal glands, and ganglion cells of the trachea and bronchi. All kinds of stimulating factors transmit up along the axon, to stimulate airway sensory nerve endings. If part of these factors transmits to the sensory nerve branches along with the axis reflection, Non-cholinergic nerve transmitters, such as SP, CGRP and NKA, are released from sensory nerve endings. These neurotransmitters may lead to contraction, inflammatory exudation, more secretion of mucus, mucosal edema, vasodilation, and increased permeability of bronchial smooth muscles. The inflammation caused by axis reflection is called neurogenic inflammation (7).

The results show that SP, NKA, NKB of the asthma group increased significantly compared with that of the control group, suggesting the asthma attack of rats is related to the increase of the three tachykinins, which can cause airway inflammatory reactions. SP, NKA, NKB of asthma acupuncture group decreased to varying degrees. It shows that the improvement of asthma symptoms is related to the decrease of the three tachykinins. The decease of SP of the ADX-asthma acupuncture group was not significant compared with that of the asthma group, which indicated that the adrenal gland removal might reduce the release of glucocorticoid and affect the acupuncture.

The standard of acupoints selection: Feishu, Fengmen, and Dazhui are selected in the present experiment.

Selection of Dazhui: Dazhui is located between the seventh cervical and first thoracic vertebra on the midline of the back of rats. Dazhui is the convergent acupoint of the three Yang meridians of hand and foot, where Yang of the body gathers together. It belongs to Governor meridian. Yang is in charge of exterior and defense. It can dredge the whole body's yang qi, supplement deficiency and strengthen vital qi. It is a very important acupoint for ventilating lung and relieving asthma. Chen et al (8) observed the efficiency of acupuncture on Dazhui against bronchial asthma. In 52 patients with bronchial asthma who were in the acute exacerbation, 94.23% reported being effective, which suggested that acupuncture on Dazhui could relieve the acute attack of asthma effectively. Li et al (9) found that acupuncture on Dazhui could improve the immunity of guinea pigs with bronchial asthma, and its mechanisms were related to the regulation of IL-4, TNF, PLA2, IgE to reduce or relieve inflammatory reactions in an asthma attack. Zhang et al (10) found that suspended moxibustion on Dazhui could stimulate the immune reaction mechanism by inhibiting the secretion of IL-4, reducing the inflammatory reactions of IgE, and increasing $INF-\gamma/IL-4$.

Selection of Fengmen: Fengmen is located on the bilateral intercostal space under the second thoracic

vertebrae. According to Hui Yuan Acupuncture, Fengmen is where the wind comes in and out. Fengmen can also be called Refu, a convergent acupoint of the meridian of Foot-Tai Yang and Governor meridian. It has the function of dispel the wind, aster exterior syndrome, so it is named. Governor meridian gathers Yang Qi of the whole body; meridian of Foot-Tai Yang governs the exterior of the whole body; and Fengmen, as the door of wind coming in and out, connects with the lung, thus the treatment on Fengmen can clear away heat and ventilate the lung, dredge defensive yang. Treatment on Fengmen can also relieve asthma and cough, regulate Qi. Just as Compendium of Acupuncture and Moxibustion, Fengmenis in charge of lifting qi and panting. Contemporary acupuncture is often used to treat flu, chronic bronchitis, and asthma. Recently it is reported that acupuncture on Fengmen combined with cupping therapy is clinically effective (11) Cao et al (12) treated bronchial asthma patients with apitherapy on Fengmen as well as Feishu, Chize, Lieque, Dingchuan, to regulate the immune function and prevent a recurrence. Their treatment is proved to be effective.

Selection of Feishu: Feishu is located on the bilateral intercostal space under the third thoracic vertebrae (13), which is connected with the lung. According to Qijie theory meridian of Hand-Tai Yin goes along the chest and its Qi transmits into the prothorax and back Yu. It is the acupoint where the lung's Qi transfers in and out of the body. It is the back-shu transport point of the lung. It has significant curative effects for external infection and internal injury diseases of Lung Meridian of Hand-Taiyin, it also has significant curative effects for the distension in chest and shortness of breath (chest full of shortness of breath), cough and asthma with the adverse rising of lung Qi. Modern research proves that acupuncture on Feishu can greatly improve lung functions. Retaining the needle for 20min can show the acupuncture effect, for 40min shows the best effect (14). Treatment on Feishu can treat the diseases related to cough, wheezing and the dispersive and descending disorder of qi. Li et al (15) observed that acupuncture on Feishu can greatly relieve difficult breathing symptoms as cough, wheezing of the patients with bronchial asthma, improving their quality of life. Acupuncture on Feishu can also greatly improve the Lung function indices, such as peak expiratory flow (PEF), Forced expiratory volume in one second (FEV1), forced vital capacity (FVC), which provides the objective indices to measure the remission of asthma symptoms.

Speculative neural mechanism of acupuncture in the treatment of bronchial asthma: Acupuncture treatment of bronchial asthma is mainly to stimulate the spinal nerve roots of C7-T5 on the back of patients with bronchial asthma. Stimulation of Feishu improves local tissue metabolism, and effects on sympathetic nerve endings, somatic sensory nerve endings and its concomitant vessels. Segmental reflex and axon reflex generated by stimulation act on the corresponding autonomic center of the spinal cord. Neuroregulation of Feishu on the lung the whole body is achieved by connecting with the conducting fibers of the brain (15). It is speculated that acupuncture on the acupoints alleviates neurogenic inflammation and hyper-responsiveness of the airway by decreasing NKA, NKB and SP, and by increasing VIP and cAMP/cGMP in the lung tissue (16). Today, genome editing technology (17) can be used for more detailed studies.

Only limited to the immune mechanism cannot explain that acupuncture and moxibustion treatment of bronchial asthma is often selected point c7-t5 section of back shu points the characteristic of selecting acupuncture points at the root of spinal nerve indicates that it is a good research direction to apply the neural mechanism to explain acupuncture treatment of bronchial asthma.

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Conflict of interest

The authors declared that there is no conflict of interest.

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