doi.org/10.36721/PJPS.2023.36.1.REG.205-210.1

Effects of qufeng xuanfei formula in guinea pig model of airway hyperergy

Kun Ji, Jian-Ling Ma, Li-Qing Shi*, Liang-Min Wang, Niu-Niu Li, Shang-Juan Dong, Bin Lin, Li-Yun Wang and Shao-Hui Wen
Department of Respiratory Medicine, Dongfang Hospital, Beijing University of Chinese Medicine, Beijing, China

Abstract: This work aimed to clarify the potential regulating effects of Qufeng Xuanfei formula (QFXF) on airway neurogenic inflammation and its underlying target signal pathway. Guinea pig model of airway hyperergy (AHR) was used. The relative susceptibility of major proteins to airway neurogenic inflammation was assessed using Western blot immunoassay followed by being separated by SDS-PAGE. Compared to the model group, QFXF of all concentrations effectively depressed the capsaicin enhanced cough in guinea pigs and the peak values of airway resistance significantly decreased. The results illustrated that QFXF alleviated cough symptom in guinea pigs and reduced airway neurogenic inflammation when compared to AHR model group. Airway inflammation and damage, as well as the levels of NGF, SP and c-Fos in QFXF decreased the most in the high-dose group. The mechanism of antitussive activity may be associated with reducing airway inflammation. QFXF displayed effect on chronic cough through reducing the levels of neuropeptides, attenuating airway inflammation and promoting recovery from disease to decrease the airway neuro sensitivity, suggesting that the potential mechanism may be related to Ras/ERK/c-Fos pathway.

Keywords: Chronic cough, Airway neurogenic inflammation, Qufeng Xuanfei formula, Nerve growth factor, Ras/ERK/c-Fos.

INTRODUCTION

Qufeng Xuanfei formula (QFXF), a representative traditional Chinese medicine (TCM), contains various ingredients including *Herba Ephedrae*, processed *Radix Stemonae, Aster tataricus, Radix Peucedani, Mangnolia officinalis*, and *Sinomenium acutum stem*is. It has been widely used in clinical for lung ventilation, removing heat, relieving cough, and dissolving phlegm (Jia and Feixia, 2014), especially for the treatment of post-infectious cough. Cough is one of the most common complaints for which patients seek medical attention. However, the potential mechanism of QFXF for the treatment of chronic cough remained uncertain.

Yet even the post-infectious cough pathogenesis is not clearly identified and is generally considered to be associated with chronic neurogenic inflammation in the airway (Chen *et al*., 2007, Folkerts and Nijkamp, 1995). It has been reported that persistent neurogenic airway inflammation can lead to airway hyper-reactivity, which is the main cause of chronic cough and asthma in clinical (Niimi and Chung, 2015, Lambrecht and Hammad, 2012, Decalmer *et al*., 2007). Airway hyperergy is a dramatic characteristic of asthma, which reflects the capacity of the airways to undergo exaggerated narrowing in response to stimuli (Lauzon AM and Martin JG, 2016). Airway neurogenic inflammation is mainly caused by tachykinin released by sensory C-fibers in the airway, which is characterized by excessive secretion and immune cell activation (Luo *et al*., 2013, Nassini *et al*., 2010, Zhe *et al*., 2018). It is mainly mediated by nerve growth factor (NGF), phosphor-extra cellular signal-regulated protein kinases (p-ERK), Substance P (SP), Pan-Ras Antibody (Pan-Ras) and c-Fos Antibody (c-Fos).

NGF is considered as the initiation factor of airway neurogenic inflammation (Weigand *et al*., 2015). The over expression of NGF in the airways can induce inflammation, hyperergy and asthma through the complex network of cells and cytokines (Wu *et al*., 2017). It has been proved that tyrosine kinase signaling pathway and downstream-related protein such as rat sarcoma (Ras) play an important role in NGF-induced neuropeptide production (Matsumoto and Shimizu, 2013). In addition, Mitogen-activated protein kinase pathway (MAPK) and downstream-related effector such as c-fos, which regulates the cell growth, cell difference and other important cellular physiological and pathological processes, also involved in NGF-mediated airway neurogenic inflammation (Chen *et al*., 2010, Qiao *et al*., 2010, Peng *et al*., 2016, Chan *et al*., 2008).

In the present study, airway hyperergy guinea pig model was used to investigate the effect and possible mechanism of QFXF on airway neurogenic inflammation. Specifically, the changes in cough symptom, lung pathology and airway inflammation as well as the levels of NGF, p-ERK, SP, pan-Ras and c-Fos were studied.

**Corresponding author:* e-mail: shilq_med@126.com
# Contributed equally to this work

MATERIALS AND METHODS

Reagents

Cyclophosphamide (CTX), ovalbumin (OVA), methacholine and capsaicin (CAP) were purchased from Sigma Aldrich (Louis, USA). RNAiso Plus and RT Reagent Kits were obtained from Takara (Takara, Japan).

QFXF was prepared by the Department of Pharmacy, Dongfang Hospital affiliated to Beijing University of Chinese Medicine (Beijing, China), which was composed of 8 Chinese medicine herbs: 6g of processed *Herba Ephedrae* (Zhi Mahuang), 12g of processed *Radix Stemonae* (Zhi Baibu), 12g of *Aster tataricus* (Ziyuan), 10g of *Radix Peucedani* (Qianhu), 6g of *Mangnolia officinalis* (Houpu), and 14g of *Sinomenium acutum stem* (Qingfengteng). The above herbs were mixed and extracted by boiling in ten volumes of water and simmering for 1h. The extraction was concentrated under reduced pressure and the resulting extract was stored for use. QFXF powder (15g extract equals to 60g crude drug) was diluted into 1g/mL with sterile water.

Animals and sensitization

Thirty male guinea pigs (200-250 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). They were maintained in a standard animal laboratory with temperature of 21±2ºC and humidity of 30-50% for 3 d prior to experiments. All the experimental procedures of animals were strictly conducted in accordance with the protocols approved by Ethics Committee for Animal Studies at Beijing University of Chinese Medicine, China.

All experimental animals were randomly assigned to 6 groups (n=5): control group, AHR model group, methoxyphenamine group (Azmi, 0.23mg/kg·d^{-1}), L-QFXF group (low dose: 0.25mg/kg·d^{-1}), M-QFXF group (middle dose: 0.5mg/kg·d^{-1}), and H-QFXF group (high dose: 1mg/kg·d^{-1}). The rat model of post-infectious cough was established according to Muraki's method (Muraki *et al*., 2008). That is, all animals but control group were first intraperitoneally administered with CTX (30mg/kg), followed by 2mg OVA together with 100mg aluminum hydroxide three days later. After three weeks, another intraperitoneal injection comprising of 10μg OVA and 100 mg aluminum hydroxide were conducted as an enhanced sensitization. Ten days later, all groups received treatment (saline, QFXF, Azmi) through intragastric administration daily for 10 days. Upon drug treatment, the sensitized animals were inspired to cough by aerosol inhalation of capsaicin (0.5mmol/L) with an ultrasonic nebulizer for 60 seconds. The control group was exposed to saline as a placebo. At 1 hour before inhalation of capsaicin, all animals were fasted but access to water. The frequency of cough within 3 minutes was counted and recorded.

Airway responsiveness model establishment

The airway reaction of anesthetized guinea pigs after injection of acetylcholine was measured though the method described by Lin *et al* (Liu *et al*., 2001). Guinea pigs were anesthetized by intraperitoneal injection of 12% ethyl carbamate (1.2mg/kg). After anesthesia, the guinea pigs underwent tracheotomy and intubation. The animals were then assisted with ventilation by small animal respiratory pump (AniRes 3020, Beijing Bestab high-tech Co. Ltd, China) with a tidal air flow of 6mL/kg at a rate of 60 blows/min. Airway resistance (cm H_2O·s/mL) was measured with intravenous saline and acetylcholine solution (400, 600, 800μg/kg). Airway resistance peaks within 5 minutes were selected as indictors to evaluate airway reactivity.

Assessment of pulmonary histopathology

After anesthesia, the tracheas of guinea pig were exposed and perfused with 10% formalin solution. The removed lung tissues were fixed in 4% formalin solution. The fixed tissues were embedded in paraffin and sliced to 5μm thick slices, and then stained with hematoxylin eosin (HE). All images were obtained by Olympus microscope and VS120-s6-W digital microscope observation system. All slides were examined by 2 independent investigators, and 10 view fields from 5 sections were randomly obtained and analyzed in each group.

Western blot

1g lung tissue sample was homogenized in 10mL lysis buffer (0.05M Tris-HCl, pH 7.4; 0.15M NaCl; 1mM EDTA; 1% Triton X-100) containing protease inhibitors on ice. Thirty minutes later, the homogenate was then centrifuged (12000 rpm) at 4°C for 5 minutes. The protein from supernatant was separated by SDS-PAGE and transferred by electro-blotting onto a nitrocellulose membrane. Then the membranes were blocked and incubated overnight at 4°C with the primary antibody (rabbit anti-Fos, 1:2,000, Zhongshan Golden Bridge Biotechnology Co. Ltd., Beijing, China). Then the membrane was washed with TBST and incubated for 1 hour with the appropriate horseradish peroxidase (HRP)-labeled anti-rabbit IgG for NGF, p-ERK and c-Fos (1:2000, Abcam, UK), anti-mouse IgG for pan-Ras and anti-mouse IgG for β-actin (1:5,000, Immunoway, USA). Finally, the protein blots were imaged by exposing the membranes using Bio-Rad Universal Hood II (Bio-Rad, Bossier City, LA).

Real-time PCR

The lung tissues were homogenized with a homogenator, and the total RNA from tissues was extracted using RNAVzol (Vigorous Biotechnology Beijing Co., Ltd., Beijing, China) according to manufactures' protocol. 1μg RNA was reversely transcribed to cDNA in 20μL reaction mixture by the Prime Script RT reagent Kit with gDNA Eraser (Takara, Japan) at 42°C for 2 minutes, 37°C for 15 minutes and 85°C for 5 seconds. Then real-time PCR was

performed using SYBR Premix Ex TaqTM (Takara, Japan). The reaction components contained 10μL of reaction mix, 0.5μL of forward primer (10μM), 0.5μL of reverse primer (10μM), 2μL of cDNA template and 7μL of ddH_2O. The following experimental run protocol was used: denaturation program (95°C for 10min), amplification and quantification program repeated 40 times (95°C for 5 s, 60°C for 40 s). The specific forward and reverse primer sequences for Real-time PCR reaction were described in table 1.

STATISTICAL ANALYSIS

All data were analyzed by SPSS 21.0 statistical software and presented as mean ± standard error. Differences between groups for peak value of airway resistance and the expression of related proteins were determined using *t* test. Analysis of variance (ANOVA) was followed by *t*-test for pairwise comparison Statistical significance was defined as $p<0.05$ for all tests.

RESULTS

QFXF alleviated cough symptom

The antitussive activity of QFXF was first evaluated by recording the frequency of capsaicin-induced cough of guinea pigs in 3 minutes. As shown in fig. 1, the cough symptom of animals in the AHR model group significantly was more serious than that in the normal group without any treatment ($p<0.05$), indicating the success of the modeling. Compared to model group, QFXF of all concentrations effectively depressed the capsaicin enhanced cough in guinea pigs, as well as methoxyphenamine (Azmi) ($p<0.05$).

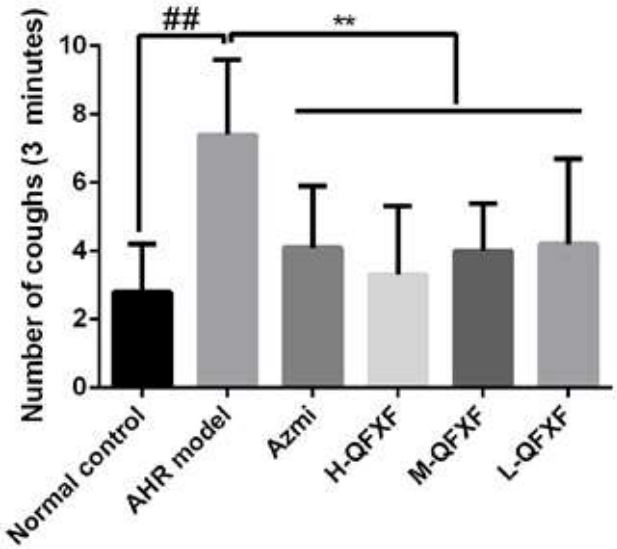

Fig. 1: Effect of QFXF on the capsaicin-induced cough in guinea pigs. Each bar represents the mean ± SD (n=5). $^{\#\#}p<0.05$ was significantly different from normal control group; $^{**}p<0.05$ was significantly different from model group.

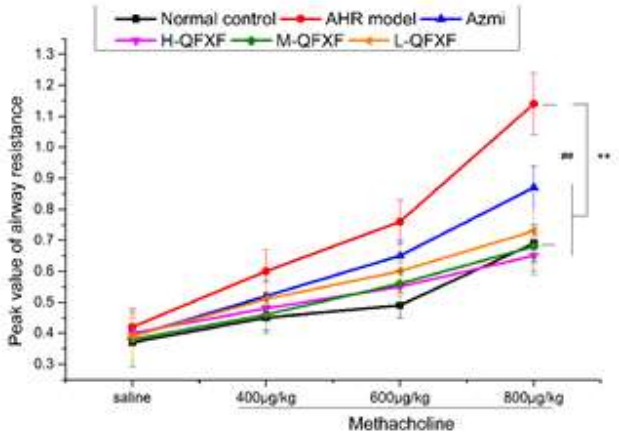

Fig. 2: Effects of QFXF on airway reactivity to methacholine. Data were shown as the mean ± SD (n=5). $^{\#\#}p<0.05$ was significantly different from normal control group; $^{**}p<0.05$ was significantly different from model group.

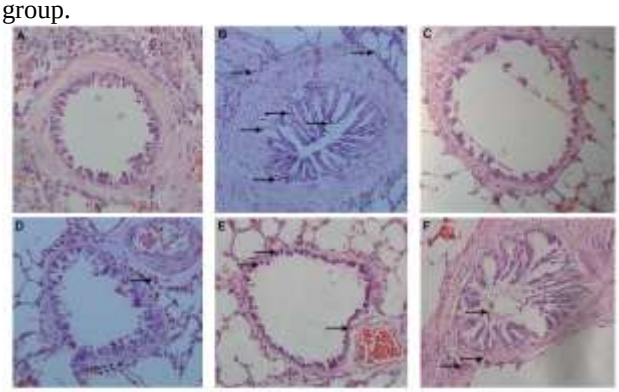

Fig. 3: Representative images of hematoxylin-eosin staining lung sections of each group, magnification ×200: (A) normal control group, (B) AHR model group, (C) Azmi control, (D) H-QFXF group, (E) M-QFXF group, and (F) L-QFXF group.

QFXF decreased airway hyperreactivity to methacholine

As shown in fig. 2, The airway resistance of guinea pigs increased gradually with the increase of the dose of methacholine. The statistical difference between AHR model group and the others was also more remarkable ($p<0.05$) during the increasing stimulation. Compared to model group, the peak value of airway resistance in QFXF group significantly decreased in a dose-dependent manner when the dosage of methacholine was 600 and 800μg/kg ($p<0.05$), and was even lower than that in Azmi group while at the high dose of methacholine. However, the down-regulation of QFXF group did not show a dose-dependent way when methacholine was at a lower dosage (800μg/kg). The result revealed that QFXF had obvious effect on decreasing the airway reactivity to methacholine even at low dosage, however, there was no significant difference among groups of various dosages.

Table 1: The specific forward and reverse primer sequences for RT-PCR reaction

Primer	Primer sequences
p-ERK forward primer	5'-TCCAACCTGCTGCTCAATACC-3
p-ERK reverse primer	5'-TTGCCACATACTCTGTCAGGAATC-3'
Pan-Ras forward primer	5'-AAGGCGGGAGTTAGCCAAGA-3'
Pan-Ras reverse primer	5'-CAAGACAAGGCACCCAGATTTT-3'
c-Fos forward primer	5'-CCGACCTGCCTGCAAGATTC-3'
c-Fos reverse primer	5'-GATGATGCGGGGAACATGAAG-3'
NGF forward primer	5'-GACAGTGTTGGCAGAGGTAAATG-3'
NGF reverse primer	5'-TGGGTTGTGGTGCAATATGAG-3'
SP forward primer	5'-AAATCCTCGTCGCTTTGGC-3'
SP reverse primer	5'-AAATCCTCGTCGCTTTGGC-3'
β-Actin forward primer	5'-TATGCTATGTTGCCCTGGACTT-3'
β-Actin reverse primer	5'-GGAGTTGAAGGTAGTTTCGTGGA-3'

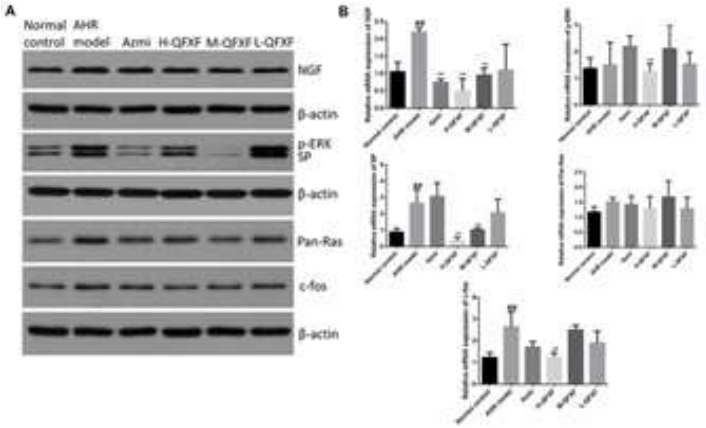

Fig. 4 Effect of QFXF the protein (A) and mRNA (B) levels of NGF, pan-RAS, p-ERK, C-FOS and SP in lung tissue. Each bar represents the mean ± SD (n=5). $^{\#\#}P<0.05$, was significantly different from normal group; ** $P<0.05$ was significantly different from model group.

Histopathological evaluation of lungs

In appearance, the lung tissues from control group were soft and elastic with normal size, smooth surface and light red color. While those from AHR model group were harder and bigger, showing dull red color with some hyperemia. After antitussive treatment of Azmi or QFXF, the abnormality of lung tissues signally relieved.

Under the microscope, the lung section from control group demonstrated intact structure of bronchial epithelium and alveolar epithelial without significant inflammatory cells infiltration (fig. 3A). By contrast, a marked infiltration of inflammatory cells into perivascular and peri bronchial connective tissues was observed in that of model group, for the bronchial lumen was narrower and alveolar wall was thicker. Further, it demonstrated multiple bronchial epithelial necrosis and shedding, as well as significant inflammatory cells infiltration and obvious hyperemia in pulmonary alveolus (fig. 3B). After antitussive treatment of Azmi or QFXF at either concentration, the degree of Bronchial lumen stenosis and mucosal fold thickening, epithelial necrosis and shedding, and inflammatory cells infiltration were all notably relieved (fig. 3C-F).

Effects of QFXF on expression of NGF, p-ERK, SP, pan-RAS and c-Fos

The mechanism of QFXF in regulating airway neurogenic inflammation was investigated by assessing the expression of NGF, p-ERK, SP, pan-RAS and c-Fos. Western blot was performed to analyze those protein levels. As displayed in fig. 4A, the level of all target proteins in model group obviously enhanced by comparison with the control group, especially NGF, p-ERK, SP and pan-RAS. In the positive intervened groups, there were down-regulation of protein level in different extent compared with the model group, especially the p-ERK, SP, and pan-RAS. In order to further verify the regulating effect of QFXF on NGF, p-ERK, SP, pan-Ras and c-Fos, real-time PCR was additionally proceeded. The result was almost consistent with that of western blot assay for the changes of the mRNA expression resembled that of the protein level (fig. 4B). Upon the treatment of QFXF, the expression of NGF, SP and c-Fos in QFXF groups dramatically declined in a dose-dependent manner compared with model group ($p<0.05$). However, the expression of p-ERK and pan-Ras showed no significant difference in animals in normal and model groups. These results indicated that NGF, SP and c-Fos play an

important role in airway neurogenic inflammation and QFXF may relieve airway inflammatory symptom by down-regulation of the protein levels of NGF, SP and c-Fos. The antitussive effect of QFXF may related to the concentration of those proteins. As for the dosage of QFXF, the group of high concentration down-regulated the expression of NGF, SP and c-Fos to lower extent than both the medium and low dosage.

DISCUSSION

The neurogenic inflammation is inflammatory response involving sensory nerves, which stimulates cascade reaction affecting the normal function and even causes damage to tissues or organs. There are abundant sensory nerves in the airway epithelium, mainly participating in airway neurogenic inflammation, which can secrete several neuropeptides including SP and neurokinin A (NKA) (Sekizawa *et al*., 1996, Millqvist and Bende, 2006). These factors can induce airway neurogenic inflammation by increasing vascular permeability and promoting inflammatory cells activation, resulting in airway hyperergy and eventually cough or other symptoms (Otsuka *et al*., 2011, Chung, 2007). Consistent with previous research (De Swert and Joos, 2006, Lee, 2009), the frequency of capsaicin-induced cough in AHR model group obviously increased by comparison with that of normal group. However, treatment with QFXF can alleviate cough symptom. Similar result was also observed in peak values of airway resistance and histological experiment. QFXF can markedly reduce enhanced airway resistance and histologic lesions. These results suggest that QFXF does have a significant inhibitory effect on airway neurogenic inflammation.

NGF play an important role in airway neurogenic inflammation. The biological effect of NGF in both nerve cell and non-nerve cell depends on the cell surface receptor tyrosine kinase receptor A (TrkA) (Millqvist and Bende, 2006). Tyrosine kinase signaling pathway (Ras-Raf-MAPK) is a necessary signal for NGF in regulating neuropeptide production. The binding of NGF to the extracellular domain of TrkA receptor induces TrkA receptor depolymerization, and leads to intracellular phosphorylation and signal transduction. Inactivated signals further activate membrane protein receptor tyrosine kinase (PTK), and then catalyze Ras into RasGTP, which participates in activating ERK-MAPK pathway. Phosphorylation of The ERK-MAPK signaling pathway is a remarkable intracellular signaling pathway regulating various intracellular functions (Joseph *et al*., 2018). It promote MAPK to enter the nucleus and induce the expression of transcription factors and nuclear protein such as c-Fos (Chaudhuri *et al*., 2005, de Vries *et al*., 1999). NGF promotes cell proliferation and differentiation, reclines the threshold of nerve cell impulse conduction, regulates the production of neuropeptides, and eventually induces airway neurogenic inflammation through Ras/Erk/c-Fos pathway.

In the present study, the increasing levels of NGF and SP were observed in animals of AHR model group, which decreased when treated with QFXF. To further investigate the potential mechanism of QFXF, Ras/ERK/c-Fos pathway related protein were examined. Animals in AHR model group exhibited higher protein level of c-Fos than that in normal group, corresponding with what was reported. Yet the other two proteins, p-ERK and pan-Ras, did not showed prominent changes. Meaningfully, QFXF can obviously down-regulate the protein level of c-Fos. These results indicated that QFXF exert beneficial effect on airway neurogenic inflammation by inhibiting Ras/ERK/c-Fos pathway.

CONCLUSION

An animal model of Guinea pig to investigate pathophysiology and treatment of pathological cough with increased cough sensitivity associated with allergy was established in this work. The inflammation was attenuated by either QFXF or methoxyphenamine with great effect, while the efficiency of QFXF in subsiding the inflammation was higher. It may be inferred that QFXF exerted the therapeutic effect on chronic cough by reducing airway neurogenic inflammation in Guinea pigs. These data could also be deemed as the “bridge link” between the understanding of TCM and modern medicine clinical practice in chronic cough. However, there are many problems to be resolved, such as what constituent or substance on earth plays the most important role in the antitussive effect, why the levels of p-ERK and pan-Ras did not show significant alteration.

ACKNOWLEDGMENTS

The study was supported by the National Natural Science Foundation of China (No. 81373588), Beijing Youth Natural Science Foundation (7184224) and Beijing Natural Science Foundation (No. 7192115).

REFERENCES

Chan CP, Chang MC, Wang YJ, Chen LI, Tsai YL, Lee JJ, Jia HW and Jeng JH (2008). Thrombin activates Ras-CREB/ATF-1 signaling and stimulates c-fos, c-jun and c-myc expression in human gingival fibroblasts. *J. Periodontol.,* **79**(7): 1248-54.

Chaudhuri R, Mcmahon AD, Mcsharry CP, Macleod KJ, Fraser I, Livingston E and Thomson NC (2005). Serum and sputum neurotrophin levels in chronic persistent cough. *Clin. Exp. Allergy.,* **35**(7): 949-53.

Chen RC, Liu CL and Luo W (2007). The cough reflex sensitivity and airway neurogenic inflammation in patients with cough post infectious. *Chin. J. Pract. Int.*

Med.

Chen YJ, Chen P, Wang HX, Wang T, Chen L, Wang X, Sun BB, Liu DS, Xu D, An J and Wen FQ (2010). Simvastatin attenuates acrolein-induced mucin production in rats: Involvement of the Ras/extracellular signal-regulated kinase pathway. *Int. Immunopharmacol.*, **10**(6): 685-693.

Chung KF (2007). Chronic cough: future directions in chronic cough: Mechanisms and antitussives. *Chron. Respir. Dis.*, **4**(3): 159-65.

De Swert KO and Joos GF (2006). Extending the understanding of sensory neuropeptides. *Eur. J. Pharmacol.*, **533**(1-3): 171-81.

De Vries A, Dessing MC, Engels F, Henricks PA and Nijkamp FP (1999). Nerve growth factor induces a neurokinin-1 receptor- mediated airway hyperergy in guinea pigs. *Am. J. Respir. Crit. Care Med.*, **159**(5 Pt 1): 1541-1544.

Decalmer S, Woodcock A, Greaves M, Howe M and Smith J (2007). Airway abnormalities at flexible bronchoscopy in patients with chronic cough. *Eur. Respir. J.*, **30**(6): 1138-1142.

Folkerts G and Nijkamp FP (1995). Virus-induced airway hyperergy. Role of inflammatory cells and mediators. *Am. J. Respir. Crit. Care. Med.*, **151**(5): 1666-1673.

Jia Z and Feixia L (2014). Effects of Qufeng Xuanfei decoction in animal model of post-infectious cough. *Cell. Biochem. Biophys.*, **70**(2): 881-885.

Joseph V., Joanna P. & Gary E. L. 2018. ERK/MAPK signaling and autism spectrum disorders. *Prog. Brain. Res.*, 241, 63-112.

Lambrecht BN and Hammad H (2012). The airway epithelium in asthma. *Nat. Med.*, **18**(5): 684-92.

Lauzon AM and Martin JG (2016). Airway hyperergy; smooth muscle as the principal actor. *Faculty. Rev.*, **5**: 1-9

Lee LY (2009). Respiratory sensations evoked by activation of bronchopulmonary C-fibers. *Respir. Physiol. Neurobiol.*, **167**(1): 26-35.

Liu Q, Fujimura M, Tachibana H, Myou S, Kasahara K and Yasui M (2001). Characterization of increased cough sensitivity after antigen challenge in guinea pigs. *Clin. Exp. Allergy.*, **31**(3): 474-84.

Luo YL, Li PB, Zhang CC, Zheng YF, Wang S, Nie YC, Zhang KJ and Su WW (2013). Effects of four antitussives on airway neurogenic inflammation in a guinea pig model of chronic cough induced by cigarette smoke exposure. *Inflamm. Res.*, **62**(12): 1053-61.

Matsumoto K and Shimizu N (2013). Activation of the phospholipase C signaling pathway in nerve growth factor-treated neurons by carbon nanotubes. *Biomaterials.*, **34**(24): 5988-94.

Millqvist E and Bende M (2006). Role of the upper airways in patients with chronic cough. *Curr. Opin. Allergy. Clin. Immunol.*, **6**(1): 7-11.

Muraki M, Iwanaga T, Haraguchi R, Kubo H and Tohda Y (2008). Continued inhalation of lidocaine suppresses antigen-induced airway hyperreactivity and airway inflammation in ovalbumin-sensitized guinea pigs. *Int. Immunopharmacol*, **8**(5): 725-31.

Nassini R, Materazzi S, Andre E, Sartiani L, Aldini G, Trevisani M, Carnini C, Massi D, Pedretti P, Carini M, Cerbai E, Preti D, Villetti G, Civelli M, Trevisan G, Azzari C, Stokesberry S, Sadofsky L, Mcgarvey L, Patacchini R and Geppetti P (2010). Acetaminophen, via its reactive metabolite N-acetyl-p-benzo-quinoneimine and transient receptor potential ankyrin-1 stimulation, causes neurogenic inflammation in the airways and other tissues in rodents. *Faseb. J.*, **24**(12): 4904-4916.

Niimi A and Chung KF (2015). Evidence for neuropathic processes in chronic cough. *Pulm. Pharmacol. Ther.*, **35**: 100-104.

Otsuka K, Niimi A, Matsumoto H, Ito I, Yamaguchi M, Matsuoka H, Jinnai M, Oguma T, Takeda T, Nakaji H, Chin K, Sasaki K, Aoyama N and Mishima M (2011). Plasma substance P levels in patients with persistent cough. *Respiration.*, **82**(5): 431-8.

Peng C, Zeng W, Su J, Kuang Y, He Y, Zhao S, Zhang J, Ma W, Bode AM, Dong Z and Chen X (2016). Cyclin-dependent kinase 2 (CDK2) is a key mediator for EGF-induced cell transformation mediated through the ELK4/c-Fos signaling pathway. *Oncogene.*, **35**(9): 1170-9.

Qiao X, Pham DN, Luo H and Wu J (2010). Ran overexpression leads to diminished T cell responses and selectively modulates nuclear levels of c-Jun and c-Fos. *J. Biol. Chem.*, **285**(8): 5488-96.

Sekizawa K, Jia YX, Ebihara T, Hirose Y, Hirayama Y and Sasaki H (1996). Role of substance P in cough. *Pulm. Pharmacol.*, **9**(5-6): 323-8.

Weigand LA, Kwong K and Myers AC (2015). The Effects of Nerve Growth Factor on Nicotinic Synaptic Transmission in Mouse Airway Parasympathetic Neurons. *Am. J. Respir. Cell Mol. Biol.*, **53**(4): 443-9.

Wu XR, Zhou X, Hu YX, Liu C and Wang J (2017). Neutralization of nerve growth factor (NGF) inhibits the Th2 response and protects against the respiratory syncytial virus (RSV) infection. *Immunol. Res.*, **65**(3): 721-8.

Zhe C, Sun L, Chen H, Gu D, Zhang W, Yang Z, Peng T, Dong R and Lai K (2018). Dorsal Vagal Complex Modulates Neurogenic Airway Inflammation in a Guinea Pig Model With Esophageal Perfusion of HCl. *Front. Physiol.*, **9**: 536.