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Basic Study

Effect of electroacupuncture on expressions of acetylcholine and mucin 5AC in the lungs of rats with chronic obstructive pulmonary disease

电针对慢性阻塞性肺疾病大鼠肺中乙酰胆碱及黏蛋白5AC表达的影响

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Abstract

Objective: To observe the effect of electroacupuncture (EA) on the expressions of acetylcholine (ACh) and mucin 5AC (MUC5AC) in the lungs of rats with chronic obstructive pulmonary disease (COPD), and explore the mechanism of EA in treating COPD.

Methods: Thirty Sprague-Dawley (SD) rats were randomly divided into a control group, a COPD group, and an EA group, with 10 rats in each group. The control group was a group of normal rats. The COPD rat model was induced by cigarette smoke combined with lipopolysaccharide (LPS). The COPD rats were treated with EA at bilateral Feishu (BL 13) and Zusanli (ST 36) in the EA group, 30 min each time, once a day, successively for 14 d. The lung function was tested. The contents of ACh and MUC5AC in lungs and bronchoalveolar lavage fluid (BALF) were detected by enzyme-linked immunosorbent assay (ELISA). Pearson method was used to analyze the correlation between pulmonary function and the content of MUC5AC in lungs. The mRNA and protein expressions of MUC5AC in lung tissues were detected by real-time polymerase chain reaction (RT-PCR) and Western blot (WB), respectively. The immune response of MUC5AC was observed by immunohistochemistry.

Results: Eight rats were left in each group, and the other two died. Compared with the control group, the total airway resistance (Raw) increased significantly and dynamic compliance (Cdyn) decreased significantly in the COPD group (P<0.01); compared with the COPD group, the Raw level declined significantly and Cdyn increased significantly in the EA group (P<0.01). The contents of ACh and MUC5AC in the lungs and BALF were remarkably higher in the COPD group compared with those in the control group (P<0.01, P<0.001); compared with the COPD group, the contents of ACh and MUC5AC were significantly lower in the EA group (P<0.05, P<0.001). There was a negative correlation between MUC5AC content and lung function (P<0.001). The mRNA and protein expressions of MUC5AC in the lungs were significantly higher in the COPD group than in the control group (P<0.001); compared with the COPD group, the expressions were significantly lower in the EA group (P<0.01). Compared with the control group, the immune response of MUC5AC in the airway epithelium significantly increased in the COPD group (P<0.001); the immune response of MUC5AC was significantly lower in the EA group compared with that in the COPD group (P<0.001).

Conclusion: EA treatment can improve the lung function of COPD rats, which may be related to its effect in the down-regulation of ACh and MUC5AC contents in the lungs as well as the inhibition of mucus hypersecretion.

Keywords: Acupuncture Therapy; Electroacupuncture; Pulmonary Disease, Chronic Obstructive; Point, Feishu (BL 13); Point, Zusanli (ST 36); Acetylcholine; Mucin 5AC

【摘要】目的：观察电针对慢性阻塞性肺疾病(COPD)大鼠肺中乙酰胆碱(ACh)及黏蛋白5AC(MUC5AC)表达的影响，探讨针刺治疗COPD的作用机制。**方法：**将30只Sprague-Dawley (SD)大鼠随机分为对照组、COPD组和电针组，每组10只。采用香烟烟雾联合脂多糖诱导方法制备COPD模型。电针组大鼠予以电针双侧肺俞和足三里，每日1次，每次30 min，连续治疗14 d。干预结束后检测肺功能，应用ELISA法检测肺组织及支气管肺泡灌洗液(BALF)中ACh及MUC5AC的含量，采用Pearson法分析肺功能与MUC5AC含量的相关性，实时聚合酶链反应(RT-PCR)检测肺组织MUC5AC mRNA的表达，蛋白免疫印迹(WB)及免疫组化方法检测MUC5AC的蛋白表达及免疫反应性。**结果：**每组均

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有2只大鼠死亡，剩余8只。与对照组比较，COPD组总气道阻力(Raw)显著升高且动态顺应性(Cdyn)显著降低(P＜0.01)；与COPD组比较，电针组Raw显著下降，Cdyn显著升高(P＜0.01)。与对照组比较，COPD组大鼠肺组织及BALF中ACh及MUC5AC含量均显著升高(P＜0.01，P＜0.001)；与COPD组比较，电针组ACh及MUC5AC含量均显著降低(P＜0.05，P＜0.001)。肺功能与MUC5AC含量呈负相关(P＜0.001)。与对照组比较，COPD组大鼠肺组织中MUC5AC的mRNA及蛋白表达均显著升高 (P＜0.001)；与COPD组比较，电针组MUC5AC的mRNA及蛋白表达均显著下降(P＜0.01)。与对照组比较，气道上皮MUC5AC免疫反应活性显著升高(P＜0.001)；与COPD组比较，电针组气道上皮MUC5AC免疫反应活性显著下降(P＜0.001)。**结论：**电针可改善COPD大鼠肺功能，其机制可能与电针下调肺组织中ACh及MUC5AC的表达及降低气道黏液高分泌有关。

【关键词】针刺疗法；电针；肺疾病，慢性阻塞性；穴，肺俞；穴，足三里；乙酰胆碱；黏蛋白 5AC

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Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable chronic inflammatory disease, characterized by persistent airflow limitation and accompanied by a progressive aggravation of chronic inflammatory response of airway and lungs to harmful particles or gases. World Health Organization (WHO) predicts that the ranking of COPD among the global fatal diseases will increase to the third place in 2020 from the sixth place in 1990[1]. Clinical and experimental studies have demonstrated the therapeutic effects of electroacupuncture (EA) on COPD. Patients with COPD experience improvement in dyspnea and quality of life (QOL) after receiving traditional acupuncture[2-4]. An experimental study also showed that EA can reduce airway resistance and increase lung compliance in COPD rats, indicating that acupuncture can improve pulmonary ventilation function in COPD[5]. However, the action mechanisms of acupuncture are still unclear.

Recently, mucous hypersecretion in airway has become the hot spot of research on COPD pathogenesis. Clinical research has shown that mucous hypersecretion has close relations with rapid pulmonary function decline[6]. Hence, it has become an important target of COPD treatment to suppress airway mucous hypersecretion[7].

Mucin 5AC (MUC5AC), the main component of airway mucus, plays a crucial role in the elasticity and adhesion of airway mucus and has become the indicator of mucous secretion of airway epithelium[8]. In the acute phase and stable phase of COPD, MUC5AC secretion in airway epithelium increases sharply, and the expression of MUC5AC appears a significantly negative relation with the pulmonary function indexes in COPD patients, indicating that the hypersecretion of MUC5AC in mucus is an important factor contributing to the chronic progressive development of COPD[9-10].

Therefore, in this experiment we established a COPD rat model to observe the changes in the expressions of acetylcholine (ACh) and MUC5AC in the lungs after EA at bilateral Feishu (BL 13) and Zusanli (ST 36), and to look into the action mechanism of EA in treating COPD.

1 Materials

1.1 Animals

Thirty clean-grade male Sprague-Dawley (SD) rats, weighing 220-250 g, three months old, were obtained from the Experimental Animal Center of Cavens Technology Services, Changzhou, China [certificate number: SCXK(SU)2014-0003]. Before the experiment began, the rats stayed for one-week adaptation under controlled laboratory conditions at 25 ℃ and humidity 50%-70%, with free access to food and water.

1.2 Main reagents

Cigarette, containing 8 mg tar and 0.4 mg nicotine each (Hongsanhuan Brand, China Tobacco Anhui Industrial Co., Ltd., China); lipopolysaccharide (LPS, Sigma, USA); enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Yuanye Biotechnology Co., Ltd., China); primary antibody of MUC5AC (Abcam, UK); β-actin and secondary antibodies (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., China); polymerase chain reaction (PCR) primer (Invitrogen, USA); fluorescent quantitative kit (QIAGEN, Germany).

1.3 Laboratory equipments

SDZ-V EA instrument (Huatuo Brand, Suzhou Medical Appliance Factory, China); stainless steel acupuncture needle (0.25 mm in diameter and 13 mm in length, Suzhou Hualun Medical Appliance Co., Ltd., China); AniRes2005 lung function system (Beijing Bestlab High-Tech Co., Ltd., China); Real-time PCR instrument (Thermo, USA); BX53 microscope (Olympus, Japan).

2 Methods

2.1 Animal grouping

The rats were randomly allocated to one of the following groups: a control group, a COPD group, and an EA group. The whole experiment conformed to the Guidelines for Care and Use of Laboratory Animals stipulated by the National Institutes of Health and all efforts were made to minimize the number of animals and sufferings to them.

2.2 COPD model preparation

COPD model was established by exposure to cigarette

smoke (CS) plus infusion with LPS[5]. Rats were placed in a self-made 1 m^3 box and exposed to CS from 20 ignited cigarettes for 1 h per day and for 8 weeks in total. On the 1st and 15th days during the course of CS exposure, each rat was infused with 200 μg LPS intratracheally.

2.3 Acupoint locations

Acupoints: Feishu (BL 13) and Zusanli (ST 36).

The acupoints were located according to the *Laboratory Animal Acupoint Atlas* and anatomical and anthropomorphic methods for rat's acupoints from the *Experimental Acupuncture Science*[11]. Feishu (BL 13) was located below and 3 mm lateral to the third thoracic vertebra on the back and Zusanli (ST 36) was 5 mm below and lateral to the anterior tubercle of the tibia in an adult rat.

2.4 EA method

The rats in the EA group were fixed on a self-made rat board in a prone position. Acupuncture needles were inserted 5 mm into the bilateral points, and the homolateral points were connected to the electrodes as one pair. The EA instrument was then turned on, with sparse-dense wave, 4 Hz/20 Hz and 1-3 mA. The stimulation should produce slight vibration of limbs or local skin. The treatment lasted 30 min each time, once a day for successive 14 d. The EA treatment was performed by a well-trained acupuncturist.

2.5 Items for detection

2.5.1 Lung function

The rats were anesthetized with 3% pentobarbital sodium [70 mg/(kg·bw)] for tracheal intubation, and put in a volume tracing box which was connected to the respirator and signal processors. Total airway resistance (Raw) and pulmonary dynamic compliance (Cdyn) of rats were recorded by AniRes2005 lung function system.

2.5.2 Contents of ACh and MUC5AC

After pulmonary function test, the rats bled to death from the abdominal aorta. The left side of the main bronchus was tied. The right lungs were lavaged three times with 3 mL normal saline solution which was pre-cooled at 4 ℃ via the tracheal cannula, and the bronchoalveolar lavage fluid (BALF) was collected. One-hundred-milligram lung tissue was separated from the upper left lung and homogenated. All BALF samples and homogenated lung tissues were immediately centrifuged at 3 500 r/min for 10 min at 4 ℃. The supernatants were obtained for ELISA. The contents of ACh and MUC5AC were detected according to the manufacturer instructions of ELISA kits.

2.5.3 Expression of MUC5AC mRNA

The mRNA expression of MUC5AC was detected by real-time polymerase chain reaction (RT-PCR). The 100 mg tissues, taken from right and middle lungs, were homogenated. The total RNA was then extracted and reverse-transcribed into cDNA. Took 1 μL cDNA as the sample, and the gene GAPDH was taken as the value of housekeeping gene to normalize the target gene. The primers of MUC5AC and GAPDH were set as follows: 5'-GTTTGGGAACTTTCAGAAGATGGAC-3' (forward) and 5'-AAGCCTCCAGGTAGCTGCTGA-3' (reverse) for MUC5AC; 5'-CAACGGGAAACCCATCACCA-3' (forward) and 5'-ACGCCAGTAGACTCCACGACAT-3' (reverse) for GAPDH. The total reaction volume was 10 μL and amplification condition was 95 ℃ for 5 min, 95 ℃ for 10 s, and 60 ℃ for 30 s, repeated for 40 cycles. After PCR, the products were analyzed by relative quantitative analysis with $2^{-\triangle\triangle Ct}$ as the indicator.

2.5.4 Expression of MUC5AC protein

The protein expression of MUC5AC was evaluated by Western blot (WB). One-hundred-milligram tissues from the right lower lung were lysed in RIPA lysis buffer, and the total protein was extracted. Protein samples, 10 μL, were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (on stacking gel at 80 V for 30 min and on separation gel at 120 V for 1 h) and transferred onto polyvinylidene difluoride (PVDF) membrane. The membranes were blocked for 2 h at 25 ℃ with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20, and then were incubated with the primary antibody (MUC5AC 1:1 000; β-actin 1:1 000) for 2 h at 25 ℃. After washed three time by TBST, the membranes were incubated with the secondary antibody for 1.5 h at 25 ℃, and transferred into the gel image system after electrochemiluminescence (ECL). The gray level was evaluated by Quantity One analysis software.

2.5.5 Immunoreaction of MUC5AC

The immunoreaction of MUC5AC in the lungs was tested by immunohistochemistry method. The remaining left lung tissues were fixed in 4% buffered formaldehyde, paraffin-embedded and cut into slices. The slices were dewaxed in dimethylbenzene, heated for repairing antigen, and treated with 3% hydrogen peroxide to block the activity of endogenous peroxidase at 25 ℃ followed by blocking antigen with 50 μL goat serum. The sections were incubated with the MUC5AC primary antibody for 1 h at 37 ℃, and then with the second antibody marked with biotin for 10 min at 25 ℃. After rinsing, the sections were incubated with the streptomyces and biotin protein-peroxidase solution for 10 min, and treated with diaminobenzidine (DAB), counterstained with hematoxylin, and observed under a light microscope. Six different fields of vision of each slice were randomly chosen under BX53 microscope. Integral optical density (IOD) was used to analyze the positive response by Image Pro Plus 6.0 analysis software.

2.6 Statistical processing

The SPSS version 24.0 statistical software was used for statistical analysis. All the data were presented as mean ± standard deviation ($\bar{x} \pm s$) and compared by

one-way ANOVA when the normal distribution and variance were qualified. The least significant difference (LSD) method was used to examine the inter-group difference, and $P<0.05$ was considered statistically significant.

3 Results

3.1 Effect of EA on the lung function

Compared with the control group, the Raw increased significantly ($P<0.01$) and the Cdyn decreased significantly in the COPD group ($P<0.01$). Compared with the COPD group, the Raw level declined significantly and Cdyn increased significantly in the EA group ($P<0.01$), indicating that EA therapy can improve lung function in COPD rats (Table 1).

3.2 Effect of EA on the contents of ACh and MUC5AC in lungs and BALF in COPD rats

The contents of ACh and MUC5AC in the lung homogenates and BALF were remarkably higher in the COPD group than in the control group ($P<0.01$, $P<0.001$), but significantly down-regulated after EA ($P<0.05$, $P<0.001$), (Table 2).

Table 1. Comparison of lung function ($\bar{x}$ ±s)

Group	n	Raw [cm H_2O/(mL·s)]	Cdyn (mL/cm H_2O)
Control	8	0.242±0.023	0.234±0.022
COPD	8	0.291±0.031[1)]	0.201±0.014[1)]
EA	8	0.249±0.019[2)]	0.233±0.019[2)]

Note: Compared with the control group, 1) $P<0.01$; compared with the COPD group, 2) $P<0.01$

Table 2. Comparison of ACh and MUC5AC contents in lungs and BALF of the rats ($\bar{x}$ ±s)

Group	n	ACh (U/mL)		MUC5AC (ng/mL)	
		Lung	BALF	Lung	BALF
Control	8	68.31±5.08	32.22±8.41	4.65±0.91	5.61±1.05
COPD	8	85.44±7.25[2)]	43.62±8.47[1)]	12.41±2.04[2)]	9.80±1.43[2)]
EA	8	72.83±5.89[4)]	35.16±5.96[3)]	8.11±0.87[4)]	6.75±0.92[4)]

Note: Compared with the control group, 1) $P<0.01$, 2) $P<0.001$; compared with the COPD group, 3) $P<0.05$, 4) $P<0.001$

3.3 Effect of EA on the expressions of MUC5AC mRNA and protein in lungs of the COPD rats

We observed the mRNA and protein expressions of MUC5AC in the lungs of six rats per group. There was a significant increase in the COPD group than in the control group ($P<0.001$), but a decrease in the EA group ($P<0.01$), (Table 3, Figure 1 and Figure 2).

Table 3. Comparison of the expression of MUC5AC mRNA in lungs of the rats ($\bar{x}$ ±s)

Group	n	MUC5AC
Control	6	1.0±0.0
COPD	6	210.08±50.32[1)]
EA	6	23.70±4.12[2)]

Note: Compared with the control group, 1) $P<0.001$; compared with the COPD group, 2) $P<0.01$

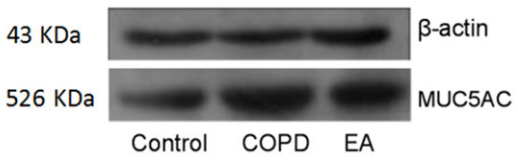


Figure 1. The image of WB strip

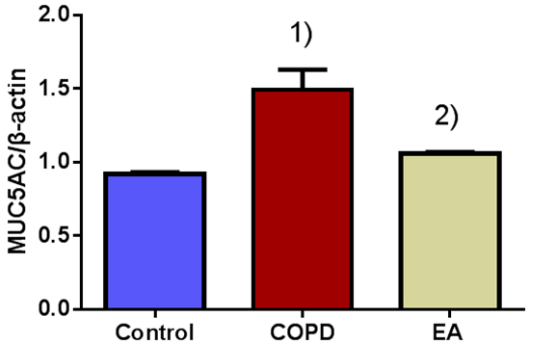
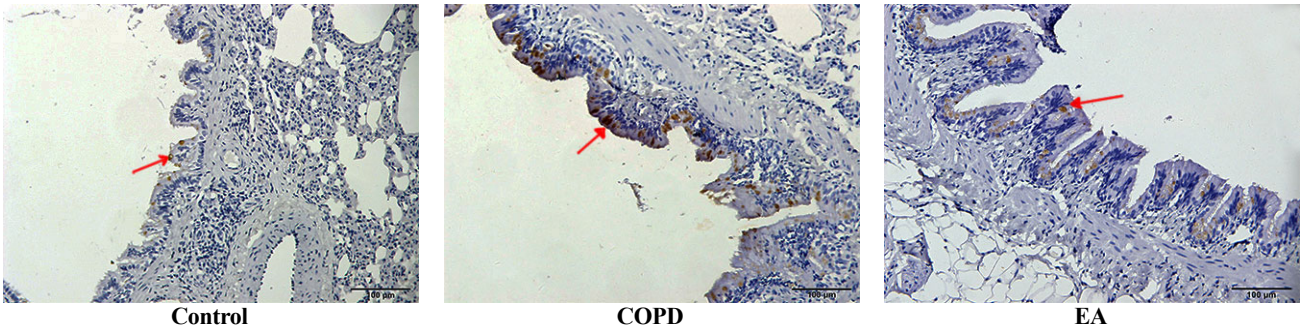


Figure 2. The expression of MUC5AC protein in lung

Note: Compared with the control group, 1) $P<0.001$; compared with the COPD group, 2) $P<0.01$ (n=6)

3.4 Effect of EA on the immunoreaction of MUC5AC in lungs of the COPD rats

We evaluated the immune responses of six rats in each group. In lungs of the rats, the immunoreactive cells of MUC5AC were mainly distributed on the apical wall of the goblet cells. The IOD of positive immunoreaction of MUC5AC was significantly higher in the COPD group than in the control group ($P<0.001$), but significantly lower after EA ($P<0.01$), (Figure 3 and Table 4).



Control COPD EA

Figure 3. Positive immunoreactions of MUC5AC in lungs of the rats (red arrows)

Table 4. Comparison of the IOD of MUC5AC in lungs of the rats ($\bar{x}$ ±s)

Group	n	IOD
Control	6	18.19±1.52
COPD	6	43.96±6.48[1)]
EA	6	23.40±4.25[2)]

Note: Compared with the control group, 1) P<0.001; compared with the COPD group, 2) P<0.001

3.5 The correlation between the lung function and the content of MUC5AC in lungs of the rats

Pearson method was used to analyze the correlation between the pulmonary function and the content of MUC5AC in the lungs. For the correlation coefficient, the content of MUC5AC showed a strong positive correlation with the level of Raw (r=0.822, P<0.001), and a strong negative correlation with the level of Cdyn (r=−0.725, P<0.001), (Figure 4).

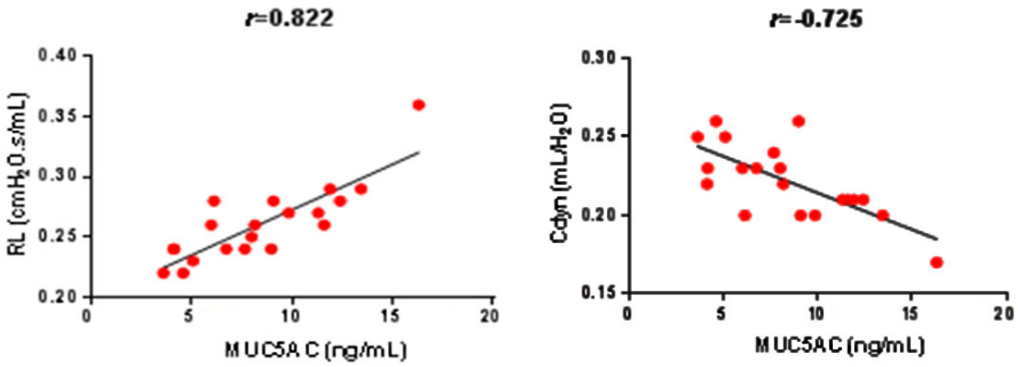


Figure 4. The relation between the lung function indicators and the content of MUC5AC in the lungs

4 Discussion

Clinical studies have shown that EA can reduce the airway resistance and increase lung compliance, and enhance the QOL in COPD patients[10-11], yet the underlying mechanism is not clear.

A lot of studies have reported that there is a certain correlation between the regulatory function of acupuncture and the excitation of vagus nerves[12]. A study showed that efferent fiber discharge of vagus nerve was increased by EA at bilateral Zusanli (ST 36) in rats[13]. The abscission of vagus nerve significantly decreased or eliminated the effect of EA, escalated the level of tumor necrosis factor, and aggravated organic damage[14]. Vagus nerve is a pair of cranial nerves with the longest circulation and widest distribution, dominating a majority of human visceral organs. Its efferent fiber can release ACh after the activation to adjust organ function. In the respiratory system, vagus nerve adjusts airway tension, blood supply, breathing mode and even mucous secretion.

In the body, as the first barrier of respiratory system, airway mucosa prevents the invasion of pathogenic microorganisms and harmful substances, and maintains the stability of airway microenvironment. Mucous hypersecretion in the air passage caused by airway goblet cell hyperplasia is one of the crucial factors contributing to COPD. Its molecular basis is the expression and increased secretion of airway mucoprotein MUC5AC[15]. Persistent inflammatory response in COPD causes hyperactivity of airway vagus nerve, brings about airway mucous hypersecretion to block the air passage, and causes the restriction of airflow[16]. Persistent mucous hypersecretion in the air passage is an important factor causing the progressive decline of pulmonary function in COPD patients[17]. Hence airway mucous hypersecretion is considered as an independent indicator of exacerbation of COPD[18], and reducing airway mucous secretion has been taken as the target in treating COPD. MUC5AC protein, the

main component of airway mucus, plays a crucial role in the elasticity and adhesion of airway mucus, so the transcriptional level and content of MUC5AC are regarded as the major index of airway mucous secretion degree.

In this experiment, by establishing the COPD rat model with an integrated modeling method of cigarette smoke and LPS[19], we observed the decline of pulmonary function and the increase of ACh content and MUC5AC expression, which indicated that the COPD rats presented a certain degree of airway mucous hypersecretion. But after EA, the content of ACh and the expression of MUC5AC in the lungs decreased. In addition, there was a correlation between the lung function indicators and the content of MUC5AC in the lungs[20-22]. Therefore, down-regulating the ACh release from vagus nerve could be one of the mechanisms for EA to intervene airway mucous hypersecretion. The pathway from ACh to MUC5AC in the airway could be a potential target of curing airway mucous hypersecretion in COPD. However, considering the complexity of nerve conduction and signal transduction, the specific mechanism in the action that vagus nerve intervenes MUC5AC should be further discussed, besides, the reason of the died rats also remained a question.

In conclusion, this experiment has suggested that EA at bilateral Feishu (BL 13) and Zusanli (ST 36) can suppress airway mucous hypersecretion by lowering the expressions of ACh and MUC5AC in lungs of COPD rats.

Conflict of Interest

There is no potential conflict of interest in this article.

Acknowledgments

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

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References

[1] Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, López Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agustí A. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report. GOLD executive summary. Am J Respir Crit Care Med, 2017, 195(5): 557-582.

[2] Liu X, Fan T, Lan Y, Dong S, Fu J, Mao B. Effects of transcutaneous electrical acupoint stimulation on patients with stable chronic obstructive pulmonary disease: a prospective, single-blind, randomized, placebo-controlled study. J Altern Complement Med, 2015, 21(10): 610-616.

[3] Coyle ME, Shergis JL, Huang ET, Guo X, Di YM, Zhang A, Xue CC. Acupuncture therapies for chronic obstructive pulmonary disease: a systematic review of randomized, controlled trials. Altern Ther Health Med, 2014, 20(6): 10-23.

[4] Feng J, Wang X, Li X, Zhao DJ, Xu LQ, Cao CX. Acupuncture for chronic obstructive pulmonary disease (COPD): a multicenter, randomized, sham-controlled trial. Medicine (Baltimore), 2016, 95(40): e4879.

[5] Zhang XF, Zhu J, Geng WY, Zhao SJ, Jiang CW, Cai SR, Cheng M, Zhou CY, Liu ZB. Electroacupuncture at Feishu (BL 13) and Zusanli (ST 36) down-regulates the expression of orexins and their receptors in rats with chronic obstructive pulmonary disease. J Integr Med, 2014, 12(5): 417-424.

[6] Allinson JP, Hardy R, Donaldson GC, Shaheen SO, Kuh D, Wedzicha JA. The presence of chronic mucus hypersecretion across adult life in relation to COPD development. Am J Respir Crit Care Med, 2016, 193(6): 662-672.

[7] Martin C, Frija-Masson J, Burgel P R. Targeting mucus hypersecretion: new therapeutic opportunities for COPD? Drugs, 2014, 74(10): 1073-1089.

[8] Kesimer M, Ford AA, Ceppe A, Radicioni G, Cao R, Davis CW, Doerschuk CM, Alexis NE, Anderson WH, Henderson AG, Barr RG, Bleecker ER, Christenson SA, Cooper CB, Han MK, Hansel NN, Hastie AT, Hoffman EA, Kanner RE, Martinez F, Paine R 3rd, Woodruff PG, O'Neal WK, Boucher RC. Airway mucin concentration as a marker of chronic bronchitis. N Engl J Med, 2017, 377(10): 911-922.

[9] Dijkstra AE, Boezen HM, van den Berge M, Vonk JM, Hiemstra PS, Barr RG, Burkart KM, Manichaikul A, Pottinger TD, Silverman EK, Cho MH, Crapo JD, Beaty TH, Bakke P, Gulsvik A, Lomas DA, Bossé Y, Nickle DC, Paré PD, de Koning HJ, Lammers JW, Zanen P, Smolonska J, Wijmenga C, Brandsma CA, Groen HJ, Postma DS. Dissecting genetics for chronic mucus hypersecretion in smokers with and without COPD: genome wide analysis on mucus hypersecretion. Eur Respir J, 2015, 45(1): 60-75.

[10] Liu H. Acupuncture combined with acupoint application improves symptoms, daily life quality and lung function in chronic obstructive pulmonary disease patients during acute exacerbation. Zhen Ci Yan Jiu, 2016, 41(3): 251-254.

[11] Liu LJ, Shi MY, Song XM, Zhang W, Jiang CJ. Clinical effect observation on acupuncture for chronic obstructive pulmonary disease. J Acupunct Tuina Sci, 2015, 13(5): 306-311.

[12] Zhu B. Biological significance of bidirectional regulation of acupuncture. Shijie Zhongyiyao, 2013, 8(3): 241-244.

[13] Ke H, Cheng Q. Effect of autonomic discharge of EA different acupoints on the small intestine Dyskinesia's rats. Nanjing Zhongyiyao Daxue Xuebao, 2014, 30(1): 50-52.

[14] Lim HD, Kim MH, Lee CY, Namgung U. Anti-inflammatory effects of acupuncture stimulation via the vagus nerve. Plos One, 2016, 11(3): e0151882.
[15] Kim V, Oros M, Durra H, Kelsen S, Aksoy M, Cornwell WD, Rogers TJ, Criner GJ. Chronic bronchitis and current smoking are associated with more goblet cells in moderate to severe COPD and smokers without airflow obstruction. Plos One, 2015, 10(2): e0116108.
[16] Boucherat O, Boczkowski J, Jeannotte L, Delacourt C. Cellular and molecular mechanisms of goblet cell metaplasia in the respiratory airways. Exp Lung Res, 2013, 39(4-5): 207-216.
[17] Li J, Wang Q, Cheng J, Zhang LS, Qiu ZJ, Wu J, Luo H, Dong FL, Liu FD, Zhao FC, Qi Y, Xue GW. Correlative research between the high secretion of airway mucus and lung function in AECOPD rat model. Shijie Zhongxiyi Jiehe Zazhi, 2013, 8(4): 397-399.
[18] Kesimer M, Ford AA, Ceppe A, Radicioni G, Cao R, Davis CW, Doerschuk CM, Alexis NE, Anderson WH, Henderson AG, Barr RG, Bleecker ER, Christenson SA, Cooper CB, Han MK, Hansel NN, Hastie AT, Hoffman EA, Kanner RE, Martinez F, Paine R, Woodruff PG, O'Neal WK, Boucher RC. Airway mucin concentration as a marker of chronic bronchitis. N Engl J Med, 2017, 377(10): 911-922.
[19] Jones B, Donovan C, Liu G, Gomez HM, Chimankar V, Harrison CL, Wiegman CH, Adcock IM, Knight DA, Hirota JA, Hansbro PM. Animal models of COPD: what do they tell us? Respirology, 2017, 22(1): 21-32.
[20] Ehre C, Worthington EN, Liesman RM, Grubb BR, Barbier D, O'Neal WK, Sallenave JM, Pickles RJ, Boucher RC. Overexpressing mouse model demonstrates the protective role of Muc5ac in the lungs. Proc Natl Acad Sci USA, 2012, 109(41): 16528-16533.
[21] Ni ZH, Tang JH, Chen G, Lai YM, Chen QG, Li Z, Yang W, Luo XM, Wang XB. Resveratrol inhibits mucus overproduction and MUC5AC expression in a murine model of asthma. Mol Med Rep, 2016, 13(1): 287-294.
[22] Guo X, Pace RG, Stonebraker JR, Commander CW, Dang AT, Drumm ML, Harris A, Zou F, Swallow DM, Wright FA, O'Neal WK, Knowles MR. Mucin variable number tandem repeat polymorphisms and severity of cystic fibrosis lung disease: significant association with MUC5AC. PLoS One, 2011, 6(10): e25452.