

Experimental Research

Effect of “three-acupoint and five-needling method” on the expression level of p-ERK 1/2 protein in lung tissue of rats with allergic bronchial asthma*

“三穴五针法”对过敏性支气管哮喘大鼠肺组织p-ERK 1/2蛋白表达的影响*

ZHAO Ye (赵 叶), YANG Jin-hua (杨金华), HAN Jun-ping (韩君萍), LI Shuang (李 双),
YU Yue-yue (虞跃跃), FAN Zhi-zhong (樊志忠), CUI Jian-mei (崔建美)✉

1. School of Traditional Chinese Medicine, North China University of Science and Technology, Tangshan 063000, Hebei Province, China (华北理工大学中医学院, 河北唐山 063000, 中国)

ARTICLE INFO

First author: ZHAO Ye (1990-), female, graduate student.

Research field: Study on the mechanism of acupuncture-moxibustion in prevention and treatment of asthma.

✉ Corresponding author: CUI Jian-mei (1978-), female, professor. Research field: the study on acupuncture-moxibustion effect.

E-mail: cjm2188@126.com.

*Supported by Project of National Natural Science Foundation of China: 81303046; Graduate innovation project of North China University of Science and Technology: 2016542.

Accepted on September 1, 2016

ABSTRACT

Objective To explore the effect of “three-acupoint and five-needling method” on the expression level of p-ERK 1/2 protein in lung tissue of rats with allergic bronchial asthma. **Methods** Forty SPF-grade male SD rats were selected and randomly divided into four groups, including control group (group C), model group (group M), acupuncture group (group A) and blocker group (group PD), with 10 rats in each group. After corresponding intervention was given, cell counting was conducted, and the expression level of p-ERK 1/2 protein in the lung tissue of rats was detected via immunohistochemical method and Western blot method. **Results** The number of leukocyte and eosinophil in the bronchoalveolar lavage fluid of rats in group A and group PD was smaller than that in group M, but larger than that in group C. It was shown based on the immunohistochemical test and Western blot test that the expression level of p-ERK1/2 protein in the lung tissue of rats in group M was higher than that in group C ($P<0.05$), while the expression level of p-ERK1/2 protein in group A and group PD was lower than that in group M ($P<0.05$). **Conclusion** “Three-acupoint and five-needling method” can effectively relieve the inflammatory response of rats with allergic bronchial asthma, and inhibit the expression level of p-ERK 1/2 protein in the lung tissue of rats with asthma. Acupuncture may alleviate the symptom of allergic bronchial asthma through inhibiting the expression level of p-ERK 1/2 protein.

KEY WORDS: Acupuncture; allergic bronchial asthma; p-ERK 1/2; immunohistochemical test; Western blot test

INTRODUCTION

Asthma is a kind of heterogeneous disease which is characterized by chronic airway inflammation, with the manifestations of such respiratory symptoms as wheezing, shortness of breath, chest oppression and cough. Pathologically, bronchial wall thickening, proliferation of airway smooth muscle cells and plenty of eosinophil infiltration may appear. By taking Fèishū (肺俞 BL 13), Dàzhū (大椎 GV 14) and Fēngmén (风

门 BL 12) as the main acupoints in order to diffuse and unblock the lung *qi* and dispel pathogen to relieve panting, “three-acupoint and five-needling method” can treat asthma effectively. The signal pathway of extracellular signal-regulated kinase (ERK) was the first cell signaling transduction pathway found by researchers. ERK kinase family belongs to tyrosine protein kinase^[1] which may give play to its activity after phosphorylation. Under normal condition, ERK 1/2 is located inside the cytoplasm. After being

activated, p-ERK 1/2 may enter into the cell nucleus, and phosphorylate a series of transcription factors, such as Elk-1, c-Myc, etc., which may change the expression and activity of specific proteins, thus regulating cell metabolism and functions^[2-4]. In order to further explore the regulatory effect of acupuncture on p-ERK 1/2 protein in treatment of asthma, in this study, the inflammatory response of rats with asthma was observed via cell counting, immunohistochemical test and Western blot test, and the expression level of p-ERK 1/2 protein in the lung tissue of rats was detected.

MATERIALS AND METHODS

Laboratory animals

Forty SPF-grade male SD rats, with the weight of 100–130 g, were purchased from Tianjin Shanchuanhong Laboratory Animal Science and Technology Co., Ltd., and the license number of animal use was SYXK 2015–0038. All the animals were fed in the ten-thousand-level barrier laboratory of Animal Experiment Center, North China University of Science and Technology (use certificate: MY10DXK07) with common feedstuff [batch No.: SCXK- (J) 2014-001], and they can drink water freely. The environmental temperature was 20–25 °C, and the humidity was 40%–60% with good ventilation. “3R” principle was adopted for the feeding and use of laboratory animals, and humanitarianism concern was given.

Main reagents and instruments

Ovalbumin (A5503, U.S. Sigma Company), aluminium hydroxide (Tianjin Oubokai Chemical Industry Co., Ltd.), PD98059 (S1177, Selleckchem), p-ERK1/2 rabbit anti-rat primary antibodies (Affinity), GAPDH rabbit anti-rat primary antibodies (bioworld), PV6000 general kits (K146819D, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.), DAB (Kit-0031, Fuzhou Maixin Biotech. Co., Ltd.), PVDF membrane (U.S. Millipore Company), TBST (10×, 20151107, Solarbio), ECL color-developing agent (Beyotime), horseradish peroxidase-labeled goat anti-rabbit secondary antibodies (Beyotime), antibody diluent (Beyotime P0023), disposable sterile acupuncture needles (*Hwato*), microtome (RM2235 model, German LEICA), nebulizer (085G3005, German PARI), light microscope (BX51 model, Japan Olympus), refrigerated centrifuge (GL-20G-II model, Shanghai Anting Scientific Instrument Factory), multi-function electrophoresis apparatus (Beijing Liuyi Instrument Factory), chemiluminescence imager (721BR08455, U.S. Bio-Rad).

Methods

(1) Grouping

Forty SD rats were divided into 4 groups according to the random number table after adaptive feed for 1 week, with 10 rats in each group. In order to ensure that there was only one different factor among the groups, the rats were grouped as follows:

a. Control group (group C): without any treatment.

b. Model group (group M): establishing models based on the establishment method of rat asthma model.

c. Acupuncture group (group A): “three-acupoint and five-needling method” was adopted for acupuncture since the next day after establishment and sensitization strengthening.

d. Blocker group (group PD): aerosol inhalation with blocker PD98059 was conducted since the next day after establishment and sensitization strengthening.

(2) Animal models establishment

1 mL of normal saline containing 1 mg of ovalbumin (OVA) and 10 mg of Al(OH)₃ gel was injected intraperitoneally to the SD rats in group M, group A and group PD. After 1 week, sensitization was strengthened for once, and aerosol inhalation with 1% OVA suspension was conducted since the next day after sensitization strengthening for excitation. Aerosol inhalation was conducted for once every other day, 30 min per time, and 7 times were needed in total. Thus SD rat asthma models were established. Normal saline in corresponding dosage was injected intraperitoneally to and inhaled by SD rats in group C.

(3) Acupuncture intervention with “three-acupoint and five-needling method”

Before aerosol inhalation with 1% OVA suspension, GV 14, BL 12 (bilateral) and BL 13 (bilateral) were selected in the rats in group A, and even reinforcing and reducing method was carried out. The needles were retained for 20 min at each time, with needling manipulation for once every other 5 min (twirling speed: 200 times/min, and twirling for 20 times were considered as needling manipulation for once). Acupuncture was conducted for once every other day, and 7 times were needed in total.

(4) Aerosol blocker intervention

Before aerosol inhalation with 1% OVA suspension in group PD, aerosol inhalation with blocker PD98059 was conducted for 30 min in the dosage of 1 mg/kg. Aerosol inhalation was conducted for once every other day, and 7 times were needed in total.

(5) Cell counting

10% chloral hydrate was intraperitoneally injected in the dosage of 3.5 mL/kg. The trachea and esophagus were cut for intubation, and the left lung bronchus was ligatured. 3×10 mL of normal saline was injected to collect the bronchoalveolar lavage fluid (BALF).

The total number of leukocyte: 210 µL of BALF was extracted into a 1.5 mL EP tube, and the cover slip was tiled on the cell counting chamber. 10 µL of BALF was added on the edge of cell counting chamber. The number of leukocyte in the 4 large squares under the microscope was counted and recorded, and the average number was calculated.

Eosinophil (EOS) counting: the remaining 200 µL of BALF was centrifuged (4 °C, 3500 r/min, 15 min), then the supernatant was removed and 100 µL of PBS was added, blending evenly. 20 µL of the abovementioned liquid was dropped to the glass slide for preparation of cell smear. After drying of the glass slide, 2–3 drops of anhydrous ethanol were added for fixation for 1–2 min, then 50 µL of Swiss blue liquid A was added on the smear, and the dye solution needed to cover the whole smear for 1 min. 100–150 µL liquid B was added on the surface of liquid A, and staining was conducted for 5 min after the two kinds of dye solutions were blended fully. The smear was rinsed in water. Then anhydrous ethanol was dropped. The smear was rinsed by xylene I and II for 10 min, respectively. The smear was sealed with neutral balata, and the proportion of eosinophil in 200 leucocytes was calculated.

(6) Slice preparation

After collection of BALF, the middle lobe of the right lung was ligatured. Sterile normal saline was adopted for pulmonary perfusion till both lungs were pale. The middle lobe of the right lung was cut and rinsed in normal saline, then it was dried by water absorption. After fixed with 4% paraformaldehyde for 24 h, the lobe was rinsed in water overnight. Dehydration with gradient ethanol, transparency with xylene and wax impregnation were carried out, and then conventional paraffin embedding was conducted.

(7) Detection of the expression level of p-ERK1/2 protein in lung tissue

After conventional paraffin embedding, the lung tissue was sliced continuously with the thick of the slice of 4 µm. The tissue was dewaxed in water, and rinsed in PBS for 5 min×3 times; high-pressure repair was performed with citrate, and the tissue was rinsed

in PBS for 5 min×3 times; the tissue was incubated in 3% H₂O₂ for 15 min, and rinsed in PBS for 5 min×3 times; p-ERK1/2 primary antibody was added, the tissue was rewarmed at room temperature after staying overnight at 4 °C, and rinsed in PBS for 5 min×3 times; general secondary antibody (PV6000) was added, the tissue was incubated at room temperature for 20 min, and rinsed in PBS for 5 min×3 times; DAB solution was added for color development for 1–3 min, and the staining degree was monitored under the microscope; the tissue was rinsed in tap water for 10 min and then stained again with hematoxylin for 1 min, and hydrochloric acid alcohol differentiation and back-to-blue were conducted; finally, dehydration, transparency, sealing and microscopic examination were performed successively.

(8) The expression level of p-ERK1/2 protein in lung tissue through Western blot test

1 mL of Western and IP cell lysis buffer was added into 100 g of lung tissue, then the tissue was broken through ultrasonication at 4 °C, and centrifuged at 12 000 r/min for 15 min, finally, supernatant was subpackaged. BCA protein kit was adopted for detection of protein level. 12% separation gel and 5% spacer gel were prepared, and the loading quantity of sample of total protein in each track was 50 µg, the total protein and buffer were blended evenly in the ratio of 4:1, boiling water bath was performed for 8 min, and the sample was loaded after cooling, at the same time, colorized prestain Marker was added. Electrophoresis (80 V, 20 min, turn to 120 V, 1 h after the Marker running off) was carried out. After bromophenol blue entered into the bottom of gel, the protein was blotted on the PVDF membrane (90 V, 30 min). p-ERK1/2 primary antibody (1:2 000) was incubated, and GAPDH primary antibody (1:5 000) was referred internally, staying overnight at 4 °C. Horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1:5 000) was added for incubation at room temperature for 1 h. ECL color-developing agent was added, after film exposure, color development and color fixation, the positive band in the position of 42/44 kD molecular mass was just the p-ERK1/2 protein band, the positive band approximately in the position of 37 kD molecular mass was GAPDH protein band. The protein bands were disposed with Image J, and the result was expressed as the ratio with internally referred GAPDH gray value.

(9) Statistical analysis

SPSS 17.0 statistical software package was adopted for analysis, and all the data were expressed as mean±deviation ($\bar{x} \pm s$). After the tests of normal

distribution and homogeneity of variance, one-way analysis of variance (*ANOVA*) was carried out for testing its heterogeneity. The result conformed to the data of normal distribution and homogeneity of variance, *LSD* method was adopted in intergroup comparison, and the difference was statistically significant when $P < 0.05$.

RESULTS

Cell counting results

According to statistics, the number of leukocyte in the bronchoalveolar lavage fluid of rats in group M was more than that in group C, group A and group PD ($P=0.001$, $P=0.001$, $P=0.001 < 0.05$), and the differences between group C and group A as well as group C and group PD were not statistically significant ($P=0.956$, $P=0.967 > 0.05$). The number of EOS in the bronchoalveolar lavage fluid of rats in group M was more than that in group C, group A and group PD ($P=0.000$, $P=0.001$, $P=0.002 < 0.05$), and the differences between group C and group A as well as group C and group PD were not statistically significant ($P=0.061$, $P=0.041 > 0.05$). (Table 1)

Table 1 Cell counting results in BALF of rats in each group ($\bar{x} \pm s$)

Groups	Leukocyte	EOS/Leukocyte
C	$4.6389 \pm 1.2507^{2)}$	$0.7778 \pm 0.6667^{2)}$
M	$11.8889 \pm 7.2329^{1)}$	$3.8889 \pm 1.7638^{1)}$
A	$4.7500 \pm 1.2055^{2)}$	$1.8889 \pm 1.1667^{2)}$
PD	$4.7222 \pm 4.0223^{2)}$	$2.0000 \pm 1.0000^{2)}$
<i>F</i>	6.499	10.135
<i>P</i>	0.001	0.000

Notes: Compared with group C, $^{1)}P < 0.05$; compared with group M, $^{2)}P < 0.05$.

The expression level of p-ERK1/2 protein in lung tissue through immunohistochemical test

As shown in Figure 1, the expression of p-ERK1/2

protein showed pale brown, mainly in the bronchial epithelial cells. The expression level of p-ERK 1/2 protein in group C was lower, the positive expression in group M was obviously higher than that in group C ($P=0.00 < 0.05$), and the positive expression in group A and group PD was also higher than that in group C ($P=0.00 < 0.05$). However, the positive expression in group A and group PD was lower than that in group M ($P=0.00 < 0.05$), and the positive expression in group A was higher than that in group PD ($P=0.00 > 0.05$). The difference was statistically significant. (Table 2)

Table 2 Immunohistochemical test results of p-ERK1/2 in lung tissue ($\bar{x} \pm s$)

Groups	p-ERK (OD value)
C	$15.3433 \pm 2.1713^{2)}$
M	$61.1212 \pm 8.3374^{1)}$
A	$40.0205 \pm 10.3394^{1)2)}$
PD	$25.9677 \pm 4.6128^{1)2)}$
<i>F</i>	77.334
<i>P</i>	0.000

Note: Compared with group C, $^{1)}P < 0.05$; compared with group M, $^{2)}P < 0.05$.

The expression level of p-ERK 1/2 protein in lung tissue through Western blot test

According to the ratio between IOD value of p-ERK 1/2 protein band and internally referred GAPDH, the expression level in group M was higher than that in group C, group A and group PD ($P=0.003$, $P=0.011$, $P=0.005 < 0.05$), and there was no difference upon the comparison between group A and group C as well as group A and group PD ($P=0.630$, $P=0.868 > 0.05$), indicating that the expression level of p-ERK 1/2 protein in group M obviously increased, and acupuncture and blocker intervention inhibited the expression level of p-ERK 1/2 protein. The difference in protein expression between the two groups and group C was less. (Figure 2, Table 3)

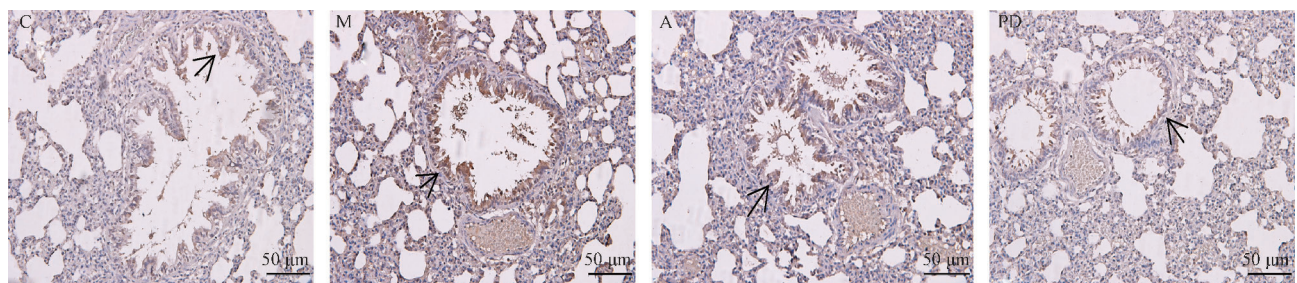


Figure 1 The expression level of p-ERK1/2 in lung tissue of rats with asthma(Immunohistochemical test $\times 400$)

Note: In bronchial epithelial cells, the expression level of p-ERK1/2 protein in group C was lower, the positive expression in group M was obviously higher than that in group C, and the positive expression in group A and group PD was also higher than that in group C. However, the positive expression in group A and group PD was lower than that in group M, and the positive expression in group A was higher than that in group PD.

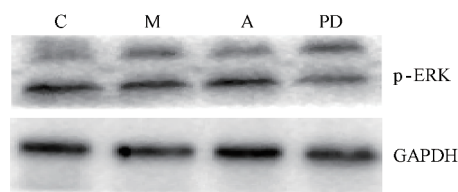


Figure 2 p-ERK1/2 expression in the lung tissue of rats

Note: The expression level of p-ERK1/2 protein in group M was higher than that in group C, group A and group PD, and there was no difference upon the comparison between group A and group C as well as group A and group PD.

Table 3 The ratio of IOD value of p-ERK1/2 and IOD value of GAPDH protein in the lung tissue of rats ($\bar{x} \pm s$)

Grouping	p-ERK/GAPDH (IOD value)
C	$0.6450 \pm 0.5699^{23)}$
M	$2.3583 \pm 1.1831^{1)}$
A	$1.2283 \pm 0.9216^{2)}$
PD	$0.4983 \pm 0.2281^{23)}$
F	6.525
P	0.003

Note: Compared with group C, ¹⁾ $P < 0.05$; compared with group M, ²⁾ $P < 0.05$; compared with group A, ³⁾ $P > 0.05$.

DISCUSSION

The clinical effect of acupuncture in treatment of asthma is significant. Professor SHAO Jing-ming, from Henan University of Chinese Medicine, has summarized the “three-acupoint and five-needling method” with BL 13, GV 14 and BL 12 as the main acupoints. As an intersecting point of governor vessel and several *yang* meridians, GV 14 not only can be used for treatment of cold, malaria and other heat diseases, but also is a key acupoint for diffusing and unblocking the lung qi and relieving panting. BL 13 is the location of lung meridian *qi* infusion, which can be used for treatment of various internally and externally-contracted diseases of respiratory system. BL 12 is a bladder meridian acupoint on the back, which can be used for dispelling pathogen to relieve panting and prevention from cold. According to clinical observation, the combination use of the three points can give play to the role of relieving panting and prevention from asthma relapse.

As a kind of serine/threonine protein kinase existing extensively in the mammals, MAPK (Mitogen-Activated Protein Kinases) can be activated by a series of extracellular signal stimuli, such as physical stress, inflammatory cytokines, growth factors, and etc. ERK pathway, an important pathway of the MAPK family, belongs to the tyrosine protein kinase^[1], which can be activated after phosphorylation. It was reported in some literature that the expression

level of p-ERK 1/2 and ERK2 mRNA in lung tissue of rats with asthma up-regulated, which was closely related to airway remodeling, indicating that ERK kinase may be involved in airway smooth muscle proliferation^[5]. In the airway of patients with asthma, the sustained activation of ERK 1/2 may aggravate airway inflammatory response and airway remodeling, thus providing molecular basis for airway hyperresponsiveness of asthma^[6]. XU et al. found that curcumin can inhibit the chronic airway inflammation of asthma, and improve airway remodeling, and the mechanism may be related to the inhibition of the phosphorylation of ERK 1/2 in rats with asthma^[7]. FANG et al. found on the basis of study of *Pingchuanning* (平喘宁) that it can regulate the ERK signal transduction pathway through inhibiting the expression level of p-ERK 1/2 mRNA, thus treating asthma via inhibition of airway remodeling^[8].

It was shown from the previous studies that according to immunohistochemical test and Western Blot test, the expression level of ERK 1/2 protein in group M was significantly higher than that in group C, and the expression level of ERK 1/2 protein in group A was lower than that in group M, indicating that acupuncture intervention can inhibit the expression of ERK 1/2 protein in the lung tissue of rats with asthma, and “three-acupoint and five-needling method” gave play to a certain role in treatment of asthma. ERK is located in the cytoplasm under normal condition. After ERK is activated, it transforms into p-ERK and enters into the cell nucleus, and phosphorylates a series of transcription factors, such as Elk-1, Jun, Fos, and Max, etc., which will transcribe their corresponding target genes to change the expression or activity of specific proteins, eventually regulating the cellular metabolism and functions, and influencing cells on producing specific biological effects^[2-4]. PD98059 is a specific blocker of ERK signal transduction pathway, which can specifically inhibit the upstream kinase MEK1 (MEK1 is the main activator of ERK^[9]). By combining with the non-active MEK1, it can inhibit the activation and phosphorylation of MEK1^[10], thus blocking the signaling pathway of ERK. Therefore, in order to further explore the role of ERK 1/2 phosphorylation in the process of acupuncture treatment of asthma, this study was designed, so as to explore the mechanism of “three-acupoint and five-needling method” for treatment of asthma.

It was shown from this study that the number of leukocyte and EOS in the bronchoalveolar lavage fluid of rats in group M was higher than that in group C, group A and group PD. it was shown according

to the immunohistochemical test and Western blot test that the expression level of p-ERK 1/2 protein in group M increased ($P < 0.05$), and the expression level of p-ERK 1/2 protein in group A and group PD reduced after acupuncture and blocker intervention ($P < 0.05$), indicating that the difference between the two groups and the differences between group A and group C as well as group PD and group C were not statistically significant ($P > 0.05$). The above results showed that the intervention of “three-acupoint and five-needling method” on rats with allergic bronchial asthma may relieve the inflammatory response, inhibit the proliferation of airway smooth muscle, and alleviate the attack of asthma in rats by inhibiting ERK 1/2 phosphorylation. MEK 1/2 kinase is the upstream kinase of ERK1/2 kinase, and it is the major activator of ERK 1/2^[9]. In order to further explore the relationship between ERK pathway and allergic bronchial asthma, the upstream and downstream kinases of ERK1/2 need to be studied in order to define the regulatory role of ERK pathway in the process of “three-acupoint and five-needling method” for treatment of allergic bronchial asthma.

REFERENCES

- [1] Kiyokawa E, Takai S, Tanaka M, Toshio Iwase, Suzuki M, Xiang YY, et al. Overexpression of ERK, an EPH family receptor protein tyrosine kinase, in various human tumors. *Cancer Res* 1994; 54(14): 3645-3650.
- [2] Widmann C, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* 1999; 79(1): 143-180.
- [3] Zuber J, Tchernitsa OI, Hinzmann B, Schmitz AC, Grips M, Hellriegel M, et al. A genome-wide survey of RAS transformation targets. *Nat Genet* 2000; 24(2): 144-152.
- [4] Schulz A, Nicke B, Warne PH, Tomlinson S, Downward J. The transcriptional response to Raf activation is almost completely dependent on mitogen-activated protein kinase kinase activity and shows a major autocrine component. *Mol Biol Cell* 2004; 15(7): 3450-3463.
- [5] Fan XY, Zhang HP. The change of expression levels of p-ERK1/2 and ERK2 mRNA in the airway of rats with asthma. *Chin J Comp Med (Chin)* 2008; 18(3): 14-18.
- [6] Xie M, Liu X.-S, Xu Y.-J, Zhang Z.-X, Bai J, Ni W, Chen S.-X. ERK1/2 signaling pathway modulates the airway smooth muscle cell phenotype in the rat model of chronic asthma. *Respiration* 2007; 74(6): 680-690.
- [7] Xu LD, Wang KX, Ji XM, Wang DY, Jiang XY. Effect of curcumin on the airway inflammation and the expression level of p-ERK1/2 in rats with chronic asthma. *Chin Arch Tradit Chin Med (Chin)* 2015; 33(2): 419-421.
- [8] Fang XM, Wang ZX, Wu XQ. Effect of Pingchuanning on the expression levels of TGF- β 1, CyclinD1 and p-ERK1/2 mRNA in the lung tissue of rats with asthma. *China J Tradit Chin Med Pharm (Chin)* 2016; 31(1): 296-299.
- [9] Shama J, Garcia-Medina R, Pouysseur J, Vial E. Major contribution of MEK1 to the activation of ERK1/ERK2 and to the growth of LS174T colon carcinoma cells. *Epub* 2008; 372(4): 845-849.
- [10] Sengupta TK, Talbot ES, Scherle PA, Lvashkiv LB. Rapid inhibition of interleukin-6 signaling and Stats activation mediated by mitogen activated protein kinase. *Proc Natl Acad Sci USA* 1998; 95(19): 11107-11112.

ABSTRACT IN CHINESE

[摘要] 目的：探讨“三穴五针法”对过敏性支气管哮喘大鼠肺组织p-ERK1/2蛋白表达的影响。方法：取SPF级雄性SD大鼠40只，随机分为空白组（C组）、模型组（M组）、针刺组（A组）及阻断剂组（PD组），每组10只，给予相应干预后，进行细胞计数，采用免疫组织化学法及Western blot法检测大鼠肺组织中p-ERK1/2蛋白的表达程度。结果：A组及PD组大鼠肺泡灌洗液中白细胞及嗜酸性粒细胞较M组少而较C组多，免疫组化法及Western blot法均显示M组哮喘大鼠肺组织p-ERK1/2蛋白的表达高于C组（ $P < 0.05$ ），而A组及PD组大鼠肺组织p-ERK1/2蛋白表达低于M组（ $P < 0.05$ ）。结论：“三穴五针法”可明显改善过敏性支气管哮喘大鼠气道炎性反应，抑制哮喘大鼠肺组织p-ERK1/2蛋白的表达，针刺可能通过抑制p-ERK1/2蛋白的表达而达到减轻过敏性支气管哮喘症状的目的。

[关键词] 针刺 过敏性支气管哮喘 p-ERK1/2 免疫组化 Western blot