Chen L, Zhao JM, Li YW, *et al*. Comparative study of electroacupuncture and moxibustion in influencing Tianshu (ST 25) regions mast cells in visceral hyperalgesia rats. J Acupunct Tuina Sci, 2016, 14(4): 242-249
DOI: 10.1007/s11726-016-0932-4

Special Topic for 973 Program

Comparative study of electroacupuncture and moxibustion in influencing Tianshu (ST 25) regions mast cells in visceral hyperalgesia rats

电针与艾灸对内脏高敏感大鼠天枢穴区肥大细胞影响的对比研究

Chen Liu (陈柳)[1], Zhao Ji-meng (赵继梦)[1,2], Li Yu-wei (李雨薇)[1], Shang Hai-xia (商海霞)[1], Wu Lu-yi (吴璐一)[1], Shi Yin (施茵)[1,2], Dou Chuan-zi (窦传宇)[1,2], Wu Huan-gan (吴焕淦)[1,2]
1 Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
2 Shanghai Research Institute of Acupuncture and Meridian, Shanghai 200030, China

Abstract

Objective: To evaluate and compare electroacupunctures (EA) with different parameters and moxibustion at different temperatures influencing the activation of mast cells (MC) in Tianshu (ST 25) regions of visceral hyperalgesia model rats.

Methods: Rats (except for model group) respectively accepted 1 mA or 3 mA EA or moxibustion at 43 ℃ or 46 ℃ to stimulate Tianshu (ST 25) points after randomization of the fifty visceral hyperalgesia model rats, and then were compared with that in model and normal groups. Number, degranulation numbers, degranulation rates in Tianshu (ST 25) regions MC of rats in each group were observed using toluidine blue staining. Abdominal withdrawl reflex (AWR) score was used to evaluate the rat visceral hyperalgesia reactions.

Results: Compared with the normal group and the model group, MC numbers ($P<0.05$, $P<0.01$, $P<0.01$, $P<0.01$), degranulation numbers and degranulation rates ($P<0.01$, $P<0.01$, $P<0.05$, $P<0.01$) of Tianshu (ST 25) MC in regions tissues in 43 ℃ and 46 ℃ moxibustion groups, and 1 mA and 3 mA EA groups all increased significantly. Compared with the model group, AWR scores were significantly lower in 43 ℃ and 46 ℃ moxibustion groups, and 1 mA and 3 mA EA groups under the stimulation of 20 mmHg, 40 mmHg, 60 mmHg or 80 mmHg colorectal distension (CRD) ($P<0.05$ in 1 mA and 3 mA EA groups under the stimulation of 20 mmHg, $P<0.01$ in the other groups). AWR scores in 43 ℃ and 46 ℃ moxibustion groups under the stimulation of 20 mmHg, 40 mmHg, 60 mmHg or 80 mmHg CRD were not significantly different from those in the normal group (all $P>0.05$); AWR scores in 1 mA EA group under the stimulation of 60 mmHg or 80 mmHg were significantly higher than that in the normal group ($P<0.01$); AWR score in 3 mA EA group under the stimulation of 60 mmHg was significantly higher than that in the normal group ($P<0.01$), and AWR scores in 3 mA EA group under the stimulation of 20 mmHg or 80 mmHg were also higher than that in the normal group ($P<0.05$). AWR scores were higher in 1 mA EA group under the stimulation of 40 mmHg or 80 mmHg than that in 46 ℃ moxibustion group ($P<0.05$); AWR score was higher in 3 mA EA group under the stimulation of 40 mmHg than that in 46 ℃ moxibustion group ($P<0.05$).

Conclusion: There are differences among EA of different parameters and moxibustion of different temperatures in activating on Tianshu (ST 25) regions MC of visceral hyperalgesia model rats, as well as in improving the visceral hyperalgesia reaction. The effect of 46 ℃ moxibustion is the most significant.

Keywords: Electroacupuncture; Moxibustion Therapy; Point, Tianshu (ST 25); Mast Cells; Visceral Hyperalgesia; Rats

【摘要】目的：观察并比较不同参数电针和不同温度艾灸对内脏高敏感模型大鼠穴区肥大细胞(mast cells, MC)活化的影响。**方法：**将50只内脏高敏感模型大鼠随机分组后分别给予1 mA、3 mA电针和43 ℃、46 ℃艾灸刺激天枢穴，并与模型组和正常大鼠进行对照，采用甲苯胺蓝染色法观察各组大鼠天枢穴区肥大细胞MC数量、脱颗粒数、脱颗粒率情况，同时采用腹部撤回反射(abdominalwithdrawl reflex, AWR)评分评价各组大鼠的内脏高敏感反应。**结果：**与正常组和模型组比较，艾灸43 ℃组、艾灸46 ℃组、电针1 mA组及电针3 mA组大鼠穴区组织MC个数有显著性增加($P<0.05$, $P<0.01$, $P<0.01$, $P<0.01$)，大鼠穴区组织MC脱颗粒数和脱颗粒率均有显著性增加($P<0.01$, $P<0.01$, $P<0.05$, $P<0.01$)。与模型组相比，在20 mmHg、40 mmHg、60 mmHg、80 mmHg结直肠扩张(colorectal distension, CRD)刺激下，艾灸43 ℃组、艾灸46 ℃组、电针1 mA组和电针3 mA组的AWR评分均显著降低(20 mmHg刺激下电针

Co-first Authors: Chen Liu, master degree candidate; Zhao Ji-meng, doctor degree candidate
Corresponding Author: Shi Yin, chief physician, doctoral supervisor.
E-mail: flysy0636@163.com

1 mA 和电针 3 mA组, $P<0.05$, 其余均$P<0.01$); 艾灸 46 ℃组、艾灸 43 ℃组大鼠在CRD刺激强度为 20 mmHg、40 mmHg、60 mmHg、80 mmHg时与正常组的AWR评分均无统计学差异(均$P>0.05$); 电针 1 mA组在 60 mmHg和80 mmHg时AWR评分均显著高于正常组(均$P<0.01$); 电针 3 mA组在 60 mmHg时AWR评分亦显著高于正常组($P<0.01$), 在 20 mmHg和 80 mmHg时亦高于正常组(均$P<0.05$); 在 40 mmHg和 80 mmHg时, 电针 1 mA组AWR评分高于艾灸 46 ℃组(均$P<0.05$); 电针 3 mA组在 40 mmHg时AWR评分高于艾灸 46 ℃组($P<0.05$)。**结论**：不同参数电针和不同温度艾灸刺激对内脏高敏感模型大鼠穴区肥大细胞活化的影响以及改善其内脏高敏感反应存在差异, 其中以46 ℃的艾灸效应最显著。

【关键词】电针; 艾灸; 穴, 天枢; 肥大细胞; 内脏高敏感; 大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Modern studies have suggested that acupuncture is a mechanical stimulation. A metal needle was inserted into the body from different points by a certain depth. Acupuncture exerts the effect by provocative maneuvers of lifting, thrusting and twirling[1-2]; moxibustion belongs to thermal stimulation. It places ignited moxa wool or other herbs on points over the body surface thus exerts an effect via the moxibstion fire and medicinal effect[3-6]. The two stimulating methods of acupuncture and moxibustion convert the stimulation into neuronal signaling by activating different receptors, and transmit it through nerve fibers to the central nervous system[7-9]. Researchers have found that in addition to stimulating the perception, acupuncture or moxibustion can also induce activation reaction of some bioactive substances in local tissues surrounding the points, such as master cells. The activation reaction of master cells is associated with the perception and conduction of acupuncture or moxibustion stimulation information and plays an essential role in their biological effects[10-11]. This study was arranged to explore and perform the comparative study of the two different stimulating methods of electroacupuncture (EA) and moxibustion on MC activation in Tianshu (ST 25) regions and its target organ effects (visceral hyperalgesia) of visceral hyperalgesia model rats. The intention was to provide experimental evidence for investigating different effector mechanisms of acupuncture and moxibustion.

1 Materials and Methods

1.1 Experimental animals

Sixty Sprague-Dawley (SD) male suckling, 8-day old (per 6-8 suckling rats with a maternal rat), and clean grade rats with free access to food and water were kept at room temperature. The rats were provided by Fudan University Laboratory Animal Science [animal license number: SYXK-(Shanghai) 2009-0082]. Animal treatment during the experiment was in line with the 'instructive notions with respect to caring for laboratory animals'.

1.2 Reagents and Instruments

Toluidine blue (Sinopharm Chemical Reagent Co., Ltd., China); glacial acetic acid (Sinopharm Chemical Reagent Co., Ltd., China); HANS-100 pain management apparatus (Nanjing Jisheng Medical Technology Co., Ltd., China); Testo 905-T2 surface thermometer (Testo AG, Germany); BX53 optical microscope (Olympus, Japan); CANON A640 camera (Olympus, Japan); MIQAS medical image analysis system and medical image quantitative analysis software (Shanghai Qiuwei biological Technology Co., Ltd., China).

1.3 Preparation of models

By referring to the relevant literatures, we used a saccule to stimulate the suckling rat's colorectum to prepare chronic visceral hyperalgesia rat model[12]. Ten of the 60 suckling rats (8-day old) were randomly selected as normal group and given perineal region stimulation by grasping and kneading. The other 50 rats only accepted distention stimulation with colorectal saccule. Saccule (about 3 cm in length) was made using a disposable condom and immobilized to a latex tube (about 4 mm in diameter and 10 cm in length). The end of the latex tube was connected to a syringe. Appropriate amount of liquid paraffin was smeared on the surface of saccule before operation. Then the saccule was slowly inserted from the anus along the physiological curvature of the rat's rectum to the site of the rat's descending colon (about 2 cm). The saccule was inflated with 0.2 mL air using a syringe and kept for 1 min, then the air was slowly released and the saccule was pulled out. The same operation was repeated 30 min later. Stimulation above was performed once daily and continuously conducted for 14 d to prepare the models. Further experiments were started at the 6th week.

General conditions of all the rats were observed after modeling. Five rats in each of the normal group and the experimental groups were randomly selected. Abdominal withdrawl reflex (AWR) scores were used to assess the sensitivity of rats to rectal distension. If AWR scores in the experimental groups were significantly higher than that in the normal group, the visceral hyperalgesia rat model was successfully made.

1.4 Experimental grouping and treatment

The fifty successfully prepared visceral hypersensitivity model rats were randomly divided into a model group, 1 mA EA group, 3 mA EA group, 43 ℃ moxibustion group and 46 ℃ moxibustion group, with 10 rats in each group. Bilateral Tianshu (ST 25) points were stimulated accordingly for each acupuncture group and moxibustion group. Positioning of Tianshu (ST 25) point was based on the *Experimental Acupuncture Science*[12] and analogy with the bone-length measurement of human body points: on the rat's abdominal midline, 5 mm away from the intersection point of the upper 2/3 and the lower 1/3 of the connection line between xiphoid and superior margin of the pubic symphysis [Shenque (CV 8) point].

1.4.1 Normal group

Rats in the normal group, without any intervention, were only given the same indicator examinations as the rats in the treatment groups.

1.4.2 Model group

Rats in the model group, without any intervention, were only given the same indicator examinations as the rats in the treatment groups.

1.4.3 43 ℃ moxibustion group

Dedicated thin moxa sticks for animal experiments (12 cm in length, 7 mm in diameter) were lit and moxibustion was performed over the points by a distance of 2-2.5 cm from the surface of the points (hair on the rat points was shaved). The point surface temperature was controlled at 43 ℃ by surface thermometer, 10 min for each moxibustion, once a day, continuously for 10 d.

1.4.4 46℃moxibustion group

Same moxa sticks as those used in 43 ℃ moxibustion group were lit and moxibustion was performed over the points by a distance of 1.5-2 cm from the surface of the points. The point surface temperature was controlled at 46 ℃ by surface thermometer, 10 min for each moxibustion, once a day, continuously for 10 d.

1.4.5 1 mA EA group

Acupuncture needles (Huatuo brand, 0.25 mm in diameter, 25 mm in length) were rapidly pierced subcutaneously (5 mm by depth) and connected to a HANS-100 pain management apparatus with continuous wave, 0.5-1 ms pulse width, 2 Hz frequency and 1 mA intensity. 10 min for each stimulation, once a day, continuously for 10 d.

1.4.6 3 mA EA group

Needles, EA apparatus and its waveform, pulse width and frequency used were same as those in 1 mA EA group. Stimulatory intensity was 3 mA, 10 min for each stimulation, once a day, continuously for 10 d.

1.5 Observation items and detection methods

1.5.1 Observing the activity changes of MC in Tianshu (ST 25) regions tissues in each group

Detection with toluidine blue staining was carried out by following steps.

① 4 μm paraffin sections were routinely dewaxed to water. 20 min for xylene Ⅰ and 20 min for xylene Ⅱ; 5 min for absolute ethanol, 90% ethanol, 80% ethanol, 70% ethanol individually. ② Stained with 1% toluidine blue for 20 min. ③ Washed with distilled water for 10 s. ④ Rapidly differentiated with 0.5% acetic acid, controlled by microscopic examination until the cytoplasm appeared purple. ⑤ Dehydrated with 95% ethanol for 1 min. ⑥ Dehydrated with absolute ethanol for 1 min × 2. ⑦ Transparent treatment: 20 min for xylene Ⅰ and xylene Ⅱ individually. ⑧ Mounting: added a drop of neutral gum and coverslip to seal. ⑨ Observation: observed changes of MC in Tianshu (ST 25) regions tissues under an optical microscope.

Image analysis: MC morphous observation and counting of the sections were performed under the light microscope (10×40 times). Under light microscopy, the particles in MC cytoplasm were purple and nuclei were blue. Those with smooth and complete membrane, and clear nuclear staining were recognized as being stable states of the MC; those with membranolysis and blue-stained-like particles were recognized as degranulated MC.

Cell count method of MC: 3 unique visual fields of each section were randomly selected under the 10 × 40 times light microscope. Total number and degranulation number rate of MC were counted. The means were designated as the total number and degranulation number of MC; MC degranulation rate was then calculated according to the following equation: MC degranulation rate = Degranulation number of MC ÷ Total number of MC × 100%.

1.5.2 AWR scores of rats in each group before and after treatments

After treatment, AWR scores of rats in each group were observed by referring the relevant literatures using saccule to stimulate the rat colorectum. Sensitivities of rats to intrarectal distension stimulation were evaluated according to the rat AWR scores[13]. Saccule (about 2 cm in length) was made using a disposable condom and immobilized to a latex tube (about 4 mm in diameter and 10 cm in length). The end of the latex tube was connected to a triple valve，two of the valves were connected to mercury sphygmomanometer, and the third valve was connected to a syringe. In the formal experiment, the saccule smeared with liquid paraffin was slowly inserted from the anus along the physiological curvature of rat's colorectum to the

site of rat's descending colon (about 2 cm). When the rats got acclimatized, four different intensities of colorectal distention (CRD) stimulation with 20 mmHg, 40 mmHg, 60 mmHg or 80 mmHg were produced by injection of different amount of air. Duration of each CRD stimulation was about 20 s. Rats were scored by the non-experimenter based on the stress performance of rats. Stimulating intensity of each time was triplicated and the mean values were used as the final scores. Here is the AWR score standard.

0 point: Rats showed no behavioral response to CRD.

1 point: The body was stationary with reduced head movement.

2 points: A slight contraction of the rat's abdominal muscles with abdomen on the plane.

3 points: Strong contraction of the rat's abdominal muscles with abdomen off the plane.

4 points: Pelvis was lifted with arched body.

1.6 Statistical methods

Statistical analyses for the experimental data were performed using SPSS 16.0 statistical software. Data with normal distribution were described by mean ± standard deviation ($\bar{x} \pm s$) and compared using One-way ANOVA. Least significant difference (LSD) was used for multiple comparison analysis among groups. Non-normal distribution data were described by median and interquartile (lower quartile, upper quartile), [M (Q_{25}, Q_{75})], and performed non-parametric test (Mann-Whitney test). $P<0.05$ was considered statistically significant.

2 Results

2.1 Observing MC activity changes in Tianshu (ST 25) regions tissues in each group

2.1.1 Comparing MC numbers in Tianshu (ST 25) regions tissues in each group

Compared with the normal group and the model group, MC numbers in Tianshu (ST 25) regions tissues in 43 ℃ moxibustion, 46 ℃ moxibustion, 1 mA EA, and 3 mA EA groups were all significantly increased ($P<0.05$, $P<0.01$, $P<0.01$, $P<0.01$); MC number in Tianshu (ST 25) regions tissues in 46 ℃moxibustion group was significantly higher than that in 43 ℃ moxibustion, 3 mA EA, and 1 mA EA groups (all $P<0.01$). The results suggested that 46 ℃ moxibustion group was better in increasing the MC number in Tianshu (ST 25) regions tissues than the other treatment groups.

2.1.2 Comparing MC degranulation numbers in Tianshu (ST 25) regions tissues in each group

Compared with the normal and model groups, MC degranulation numbers in Tianshu (ST 25) regions tissues in 43 ℃ moxibustion, 46 ℃ moxibustion, 1 mA EA, and 3 mA EA groups were all significantly increased ($P<0.01$, $P<0.01$, $P<0.05$, $P<0.01$); MC degranulation number in Tianshu (ST 25) regions tissues in 46 ℃ moxibustion group was significantly higher than that in 43 ℃ moxibustion, 3 mA EA, and 1 mA EA groups ($P<0.01$, $P<0.01$, $P<0.01$). The results suggested that 46 ℃ moxibustion group was better in increasing the MC degranulation number in Tianshu (ST 25) regions tissues than the other treatment groups.

Table 1. Comparing MC numbers in Tianshu (ST 25) regions tissues in each group ($\bar{x} \pm s$)

Group	n	MC total number
Normal	10	5.60±1.96
Model	10	5.50±0.69
43 ℃ moxibustion	10	7.83±4.23[2)4)5)]
46 ℃ moxibustion	10	12.47±2.62[1)3)]
1 mA EA	10	8.67±1.99[1)3)5)]
3 mA EA	10	8.77±0.96[1)3)5)]

Note: Compared with the normal group, 1) $P<0.01$, 2) $P<0.05$; compared with the model group, 3) $P<0.01$,4) $P<0.05$; compared with the 43 ℃ moxibustion group, 5) $P<0.01$

Table 2. Comparing MC degranulation numbers in Tianshu (ST 25) regions tissues in each group ($\bar{x} \pm s$)

Group	n	MC degranulation number
Normal	10	1.63±0.66
Model	10	1.73±0.95
43 ℃ moxibustion	10	4.30±3.05[1)2)4)]
46 ℃moxibustion	10	7.07±1.90[1)2)]
1 mA EA	10	3.53±1.60[1)3)4)]
3 mA EA	10	4.03±0.91[1)2)4)]

Note: Compared with the normal group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$, 3) $P<0.05$; compared with the 46 ℃ moxibustion group, 4) $P<0.01$

2.1.3 Comparing MC degranulation rates in Tianshu (ST 25) regions tissues in each group

Compared with the normal group and the model group, MC degranulation rates in Tianshu (ST 25) regions tissues in 43 ℃ moxibustion, 46 ℃ moxibustion, 1 mA EA, and 3 mA EA groups were all significantly increased ($P<0.01$, $P<0.01$, $P<0.05$, $P<0.01$); MC degranulation rate in Tianshu (ST 25) regions tissues in the 46℃moxibustion group was significantly higher than that in the 1 mA EA and 3 mA EA groups ($P<0.01$, $P<0.05$). This suggested that 46 ℃ moxibustion group was better in increasing the MC degranulation rate in Tianshu (ST 25) regions tissues than the other treatment groups (Table 3).

Table 3. Comparing MC degranulation rates in Tianshu (ST 25) regions tissues in each group ($\bar{x}$ ±s, %)

Group	*n*	MC degranulation rate
Normal	10	28.9±7.0
Model	10	30.9±16.3
43 ℃ moxibustion	10	51.8±9.3[1)3)5)]
46 ℃ moxibustion	10	55.9±7.5[1)3)5)6)]
1 mA EA	10	39.8±12.4[2)4)]
3 mA EA	10	45.8±10.2[1)3)]

Note: Compared with the normal group, 1) $P<0.01$, 2) $P<0.05$; compared with the model group, 3) $P<0.01$, 4) $P<0.05$; compared with the 1 mA EA group, 5) $P<0.01$; compared with the 3 mA EA group, 6) $P<0.05$

Under light microscopy, the particles in MC cytoplasm were purple and nuclei were blue. MC numbers in dermis and subcutaneous tissues in Tianshu (ST 25) regions in the normal and model groups were less and mainly distributed in perivascular area; MC number in 46 ℃ moxibustion group was significantly higher than that in 3 mA EA and 1 mA EA groups (Figure 1).

2.2 Comparing AWR scores of rats in each group after treatment

Compared with the model group, AWR scores were significantly lower in the 43 ℃ and 46 ℃ moxibustion, 1 mA and 3 mA EA groups under the stimulation of 20 mmHg, 40 mmHg, 60 mmHg or 80 mmHg CRD ($P<0.05$ in 1 mA and 3 mA EA groups under the stimulation of 20 mmHg, $P<0.01$ in all the other groups); compared with the normal group, AWR scores, in the 46 ℃ and 43 ℃ moxibustion groups under the stimulation of 20 mmHg, 40 mmHg, 60 mmHg or 80 mmHg CRD, were not statistically significant (all $P>0.05$); AWR scores in 1 mA EA group under the stimulation of 60 mmHg or 80 mmHg were significantly higher than that in normal group (all $P<0.01$); AWR score in 3 mA EA group under the stimulation of 60 mmHg was significantly higher than that in the normal group ($P<0.01$), AWR scores in 3 mA EA group under the stimulation of 20 mmHg or 80 mmHg were also higher than that in normal group (all $P<0.05$). AWR scores were higher in 1 mA EA group under the stimulation of 40 mmHg or 80 mmHg than that in 46 ℃ moxibustion group (all $P<0.05$); AWR score was higher in 3 mA EA group under the stimulation of 40 mmHg than that in the 46 ℃ moxibustion group ($P<0.05$). These results indicated that EA and moxibustion could differently improve the visceral hyperalgesia of rat models, and increase the pain threshold. Effect of 46 ℃ moxibustion treatment was superior to 1 mA or 3 mA EA treatment (Table 4).

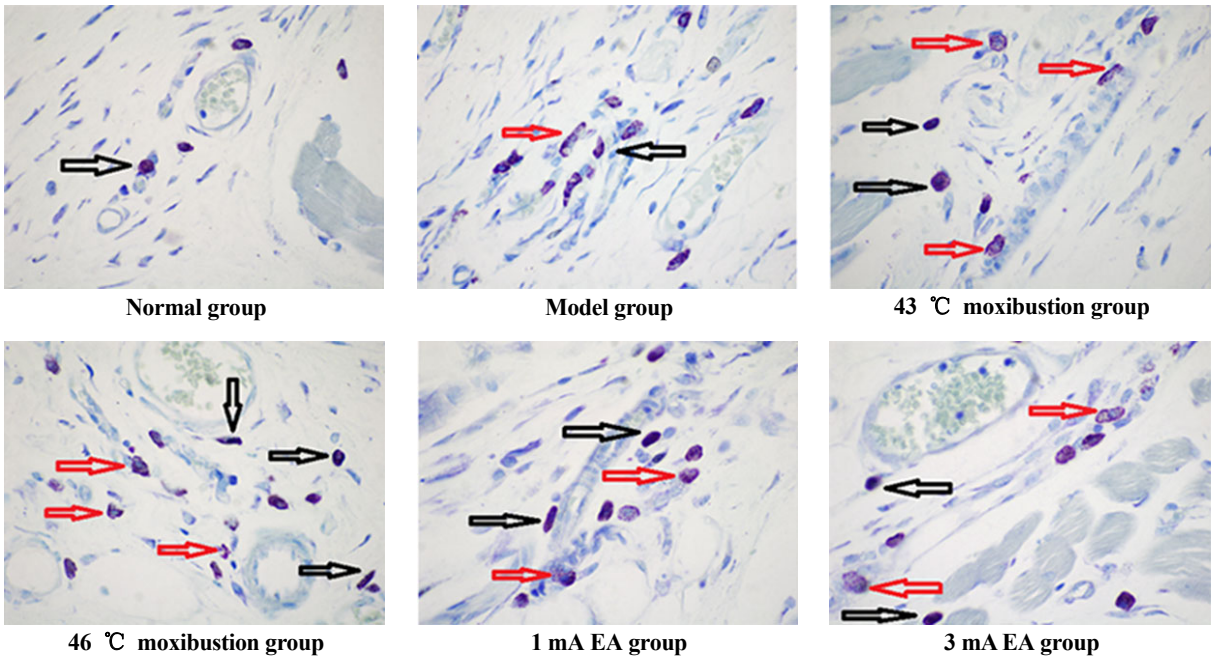

Figure 1. MC degranulation of rats in each group (HE staining, ×400) (Note: The black arrow indicates the MC, the red arrow indicates the degranulation of MC)

Table 4. Comparing AWR scores of rats in each group after treatment [M (Q_{25}, Q_{75})]

Group	*n*	20 mmHg	40 mmHg	60 mmHg	80 mmHg
Normal	10	0.00 (0.00, 0.00)$^{3)}$	1.00 (0.00, 1.00)$^{3)}$	1.00 (1.00, 1.25)$^{3)}$	2.00 (2.00, 2.00)$^{3)}$
Model	10	1.00 (0.75, 1.00)$^{1)}$	2.00 (1.75, 2.00)$^{1)}$	3.00 (2.00, 3.25)$^{1)}$	4.00 (3.00, 4.00)$^{1)}$
43 ℃ moxibustion	10	0.00 (0.00, 0.25)$^{3)}$	1.00 (1.00, 1.00)$^{3)}$	2.00 (1.00, 2.00)$^{3)}$	2.00 (2.00, 2.25)$^{3)}$
46 ℃ moxibustion	10	0.00 (0.00, 0.00)$^{3)}$	0.50 (0.00, 1.00)$^{3)}$	1.00 (1.00, 2.00)$^{3)}$	2.00 (2.00, 2.00)$^{3)}$
1 mA EA	10	0.00 (0.00, 1.00)$^{4)}$	1.00 (1.00, 1.25)$^{4)5)}$	2.00 (1.00, 2.00)$^{1)3)}$	3.00 (2.00, 3.00)$^{1)4)5)}$
3 mA EA	10	0.00 (0.00, 1.00)$^{2)4)}$	1.00 (1.00, 1.25)$^{4)5)}$	2.00 (1.75, 2.00)$^{1)3)}$	2.50 (2.00, 3.00)$^{2)3)}$

Note: Under the same stimulating intensity, compared with the normal group, 1) $P<0.01$, 2) $P<0.05$; compared with the model group, 3) $P<0.01$, 4) $P<0.05$; compared with the 46 ℃ moxibustion group, 5) $P<0.05$

3 Discussion

Acupuncture and moxibustion act to unblock meridians, harmonize yin and yang, supplement anti-pathogenic qi and remove pathogenic factors. However, their action principle and mechanism remains further study and exploration. Since mechanical stimulation by acupuncture and thermal stimulation by moxibustion both target the skin around the points, they also activate some bioactive substances in the local area. These substances are probably involved in conduction of acupuncture and moxibustion signals. Consequently, changes in bioactive substances around the points such as mast cells are of great significance to study the effects of acupuncture and moxibustion.

MC are common cells in loose connective tissues and widely distributed in submucous, around the small blood vessels or lymphatic vessels[14-15]. Histological studies have found that MC numbers in Tianshu (ST 25) regions tissues are significantly more than non-regions, showing the distribution characteristics through or along the meridian[16]. Many scholars explored a lot on meridian phenomenon, correlations between the effects of acupuncture and moxibustion and MC[1,17]. Research results indicated that stimulation of acupuncture and moxibustion could increase MC numbers and degranulation within the meridian regions tissues. This reaction should be closely related to the along-meridian vascular nerve reaction and acupuncture analgesia[18-20], which indicates that the change of MC activity in regions is an important cytological indicator reflecting the effects of acupuncture. Some experimental results showed that acupuncture at Zusanli (ST 36) could promote MC degranulation in point region; while the degranulation decreased after pretreatment with sodium cromoglicate or collagenase Ⅰ. Sodium cromoglicate is a MC stabilizer and can inhibit MC activation and degranulation by inhibiting influx of calcium ion[21-22]. Acupuncture can activate MC in point region, induce MC degranulation and the production of histamine and other biologically active substances, increase capillary penetration, further accelerate the flow of tissue fluid and activate MC, and spread the secretory products of MC together with the tissue fluid flow to activate the mast and nerve cells distributed along the meridian line, and then directionally transfer the information of acupuncture stimulating effect along the meridians for a long distance[23-24]. Likewise, Cheng K and others used far-infrared light with a wavelength of 10.6 μm to irradiate rat Zusanli (ST 36). This far-infrared light is easily to be absorbed by biological tissues and produce powerful nonpenetrated surface heat, which can produce not only moxibustion treatment role but also a strong analgesic effect. The experimental results found that MC degranulation numbers and rates in points' regions tissues were significantly higher. MC degranulation rates in points' regions were thought to be positively correlated with the analgesic effect and play an important role in producing laser acupuncture analgesia effect[25-26]. Luo MF and others observed the influence of EA and suspended moxibustion at rat's Dazhui (GV 14) on the MC in points' regions. The results found that EA and moxibustion could increase the numbers, distribution, as well as cell degranulation of MC in the points' regions tissues. Effect of moxibustion on cell degranulation was stronger than that of EA[27].

In this experiment, compared with the model group, AWR scores were significantly different in the 43 ℃ and 46 ℃ moxibustion, 1 mA and 3 mA EA groups under the stimulation of 20 mmHg, 40 mmHg, 60 mmHg or 80 mmHg CRD, which indicated that both EA and moxibustion treatments could produce analgesic effects; compared with the normal group, AWR scores in 46 ℃ and 43 ℃ moxibustion groups under the stimulation of 20 mmHg, 40 mmHg, 60 mmHg or 80 mmHg CRD, were not significantly different; AWR scores in 1 mA and 3 mA EA groups under the stimulation of 60 mmHg or 80 mmHg were still significantly different from that in the normal group; AWR scores were higher in 1 mA and 3 mA EA groups under the stimulation of 40 mmHg than that in the 46 ℃ moxibustion group. Results in this study indicated that 46 ℃ moxibustion treatment was better than 1 mA or 3 mA EA treatment with regard to

improving visceral hyperalgesia reactions and increasing the pain threshold of rats.

In this experiment, we respectively observed the effects of EA with different parameters (1 mA and 3 mA) and moxibustion at different temperatures (43 ℃ and 46 ℃) on numbers, degranulation numbers and degranulation rates in Tianshu (ST 25) regions MC in visceral hyperalgesia model rats. The results showed that, compared with the model group, the numbers, degranulation numbers and degranulation rates in Tianshu (ST 25) regions MC in the four treatment groups were significantly increased, and the numbers and degranulation numbers in Tianshu (ST 25) regions MC increased with the increase of EA intensity and moxibustion temperature. Meanwhile, compared with the model group, AWR scores of rats in each group after EA and moxibustion treatment were significantly reduced, which indicated that the changes of rat regions MC were closely related to the effects of EA and moxibustion. 46 ℃ moxibustion showed the most significant effect on MC. This suggested that, by activating responses of MC in regions tissues, EA and moxibustion stimulation could participate in mediating afference of acupuncture and moxibustion stimulating information, so as to improve visceral hyperalgesia reaction in model rats. This result also provided some experimental basis to demonstrate that the activation of MC in regions tissues, involved in transduction (afference) of the acupuncture or moxibustion stimulating information, plays an important role in the biological effects of acupuncture and moxibustion.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

Acknowledgments

This work was supported by National Basic Research Program of China (973 Program, 国家重点基础研究发展计划, No. 2015CB554500); National Natural Science Foundation of China (国家自然科学基金项目, No. 30973784).

Statement of Human and Animal Rights

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

Received: 5 January 2016/Accepted: 25 January 2016

References

[1] Pan P, Guo Y. Mast cells are one of key factors of amplifying acupuncture effect signal. Liaoning Zhongyi Zazhi, 2009, 36(12): 2066-2068.

[2] Wang SJ. Preliminary discussion on relationship between acupuncture-moxibustion effect and cell signal transduction. Zhongguo Zhongyi Jichu Yixue Zazhi, 2004, 10(11): 31-33.

[3] Wang LL. Characteristic of moxibustion and its warming-dredging effect. Zhongguo Zhen Jiu, 2011, 31(10): 865-868.

[4] Yang HY, Hu ZC. Biophysical characteristic of moxibustion. Zhongguo Zhen Jiu, 2009, 29(11): 897-899.

[5] Han JS. Neuroscience. 3rd Edition. Beijing: Peking University Medical Press, 2009: 676-677.

[6] Yang HY, Liu TY. Primary explore on the biophysical mechanism of moxibustion therapy. Zhongguo Zhen Jiu, 1996, 16(10): 17-18.

[7] Zhu B. System Acupuncture: Renaissance of Body Surface Medicine. Beijing: People's Medical Publishing House, 2015: 115.

[8] Deng HP, Shen XY, Ding GH. Characteristics of infrared radiation of moxibustion and meridian-acupoints. Zhongguo Zhen Jiu, 2004, 24(2): 105-107.

[9] Yao W, Yang HW, Yin N, Ding GH. Mast cell-nerve cell interaction at acupoint: modeling mechanotransduction pathway induced by acupuncture. Int J BiolSci, 2014, 10(5): 511-519.

[10] Pan P. Experimental study on different functional conditions mast cells affecting acupuncture effect information. Doctor Thesis of Tianjin Zhongyiyao Daxue, 2009.

[11] Zhang XY. Study on the actions of histamine and mast cells in acupuncture effect. Master Thesis of Tianjin Zhongyiyao Daxue, 2008.

[12] Li ZR. Experimental Acupuncture Science. Beijing: China Press of Traditional Chinese Medicine, 2007: 32.

[13] AL-chaer ED, Kawasaki M, Pasricha PJ. A new model of chronic visceral hyperalgesia in adult rats induced by colon irritation during postnatal development. Gasteoenterology, 2000, 119(5): 1276-1285.

[14] Gao Y. Research on the effect of mast cells in correlation of acupoins and Zang-fu organs. Master Thesis of Tianjing Zhongyiyao Daxue, 2007.

[15] Metz M, Siebenhaar F, Maurer M. Mast cell functions in the innate skin immune system. Immuno Biology, 2008, 213(3): 251-260.

[16] Luo Q, Li SJ, Liu WJ, Zhang W, Ji MQ, Xie JP. Comparing the effects of electro-acupuncture and medicinal vesiculation on mast cells activation in rats with hepatic fibrosis. JCAM, 2015, 31(4): 59-63.

[17] Gao S. The effect of electroacupuncture on mast cell ultrastructure in rats' acupoint area. Liaoning Zhongyi Zazhi, 1981, 24(5): 40-42.

[18] Song XJ, Luo MF, Jiang J, Zhang JL. Effects of electroacupuncture stimulation of 'Zusanli' (ST 36) on the regional vascular intercellular adhesion molecule-1 m RNA expression and mast cell activity in normal rats. Zhen Ci Yan Jiu, 2014, 39(6): 461-465.

[19] Luo MF, Dong XT, Song XJ, Jiang J, Zhan JL, Han Y. Study on the dynamic compound structure composed of mast cells, blood vessels, and nerves in rat acupoint. Evid Based Complement Alternat Med, 2013: 160651.

[20] Huang H, Zhan R, Yu XJ, Zhang D, Liu WM, Ding GH. Different effects and peripheral mechanism between manual-acupuncture and electroacupuncture on mast cell function and acupuncture analgesia by nerve block in acupionts. J Acupunct Tuina Sci, 2008, 6(5): 273-275.

[21] Sui PS, Liu CM, Yu YP, Ma YX. The effects of herbal cake-partitioned moxibustion over navel on Shenque (CV 8) regions mast cells of diarrhea-predominant irritable bowel syndrome model rat. Zhejiang Zhongyi Zazhi, 2015, 50(9): 649-650.
[22] Sa ZY, Huang M, Zhang D, Ding GH. Relationship between regional mast cell activity and peripheral nerve discharges during manual acupuncture stimulation of 'Zusanli' (ST 36). Zhen Ci Yan Jiu, 2013, 38(2): 118-122.
[23] Song XJ, Luo MF, Jiang J, Zhang JL, Yang ZX. Influence of point injection with sodium cromoglicate on numbers and degranulation rates of regions mast cells and electroacupuncture analgesia effect. Zhongguo Zhongyi Jichu Yixue Zazhi, 2012, 18(5): 543-544, 554.
[24] Luo MF, He JN, Guo Y, Li CH, Zhang JL. Effect of electroacupuncture and moxibustion of 'Dazhui' (GV 14) on the number and distribution of degranulated mast cells in GV 14 region Zhen Ci Yan Jiu, 2007, 32(5): 327-329.
[25] Ding GH, Shen XY, Yao W, Dang RS, Yang J, Chen EY. Dynamics mechanism of tissue fluid directional flow and human body meridian phenomenon. Ziran Kexue Jinzhan, 2005, 15(1): 61-70.
[26] Kang JQ, Zhang S, Guo Y, He WJ. Study of the roles of acupoint substance P and mastocytes in starting the acupuncture effect. Shanghai Zhenjiu Zazhi, 2014, 33(11): 1054-1058.
[27] Cheng K, Shen XY, Ding GH, Wu F. Relationship between laser acupuncture analgesia and the function of mast cells. Zhongguo Zhen Jiu, 2009, 29(6): 478-483.

Translator: Yang Yan-ping (杨燕萍)