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BRIEF ARTICLE

Effects of moxibustion on dynorphin and endomorphin in rats with chronic visceral hyperalgesia

Hui-Rong Liu, Li Qi, Lu-Yi Wu, Xiao-Peng Ma, Xiu-Di Qin, Wen-Yan Huang, Ming Dong, Huan-Gan Wu

Hui-Rong Liu, Li Qi, Lu-Yi Wu, Xiao-Peng Ma, Xiu-Di Qin, Wen-Yan Huang, Ming Dong, Huan-Gan Wu, Key Laboratory of Acupuncture-Moxibustion and Immunological Effects, Shanghai University of Traditional Chinese Medicine, Shanghai 200030, China

Author contributions: Liu HR and Qi L contributed equally to this work; Wu HG, Liu HR and Qi L designed the research; Qi L, Wu LY and Dong M performed the experiments; Liu HR, Qi L and Wu LY analyzed the data; Liu HR, Qi L, Ma XP, Qin XD and Huang WY wrote and revised the manuscript; Wu HG supervised the whole research and edited the manuscript.

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Correspondence to: Huan-Gan Wu, Professor, Key Laboratory of Acupuncture-Moxibustion and Immunological Effects, Shanghai University of Traditional Chinese Medicine, 650 South Wanping Road, Xu Hui District, Shanghai 200030, China. wuhuangan@126.com
Telephone: +86-21-64644238 Fax: +86-21-64390339

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Abstract

AIM: To observe the analgesic effects of moxibustion in rats with chronic visceral hyperalgesia and its influence on the concentration of dynorphin (Dyn) and endomorphin (EM) in spinal cord.

METHODS: The rat model of chronic visceral hyperalgesia was established by colorectal distention (CRD). In moxibustion (MX) group, moxibustion was applied once daily for 7 d; in sham moxibustion (SM) group, moxibustion was given to the same acupoints but with the non-smoldered end of the moxa stick. Model control (MC) group and normal control group were also studied. The scoring system of abdominal withdrawal reflex was used to evaluate visceral pain for behavioral assessment. Enzyme linked immunosorbent assay was performed to determine the concentrations of Dyn and EM in spinal cord.

RESULTS: Moxibustion significantly decreased visceral pain to CRD in this rat model, and no significant difference was detected between the SM group and the MC group. In MX group, moxibustion also increased the concentrations of Dyn and EM in spinal cord, and no significant difference was found between the SM group and the MC group.

CONCLUSION: Moxibustion therapy can significantly enhance the pain threshold of rats with chronic visceral hyperalgesia, and the effect may be closely related to the increased concentration of Dyn and EM in spinal cord.

Key words: Moxibustion; Analgesia; Hypersensitivity; Dynorphins; Endomorphin

Peer reviewer: Guang-Yin Xu, MD, PhD, Assistant Professor, Division of Gastroenterology, Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX 77555-0655, United States

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INTRODUCTION

Acupuncture-Moxibustion is an ancient therapy with a history of 3000 years in China, and it has spread to more than 160 countries for its good effects in management

of pain, nausea induced by radiotherapy/chemotherapy, vomiting, *etc.*[1]. Many studies have proved the analgesic effect of acupuncture from the view point of neurophysiology, neurochemistry, molecular biology, and brain functional imaging[2-6]. As a twin therapy of acupuncture, moxibustion has shown its effects in treatment of irritable bowel syndrome (IBS)[7], ulcerative colitis[8], Crohn's disease[9] and chronic/acute gastritis[10], especially in alleviating visceral pain. Some studies believed that acupuncture could relieve pain by increasing the concentration or expression of dynorphin (Dyn) and endomorphin (EM) in spinal cord[11,12]. It has been reported that visceral sensory nerves are closely associated with the spinal cord fragments[13,14], so that moxibustion might achieve its analgesic effect in treating visceral pain by modulating the concentrations of Dyn and EM in the spinal cord fragments.

Our previous studies have revealed the analgesic effects of moxibustion in reducing abdominal pain in IBS patients[15] and IBS rat models[16,17]. However, the analgesic mechanism of moxibustion has not been clearly elucidated. In this study, a rat model of chronic visceral hyperalgesia was established by colorectal distention (CRD), and abdominal withdrawal reflex (AWR) scoring system was adopted for behavioral assessment in the evaluation of visceral pain after moxibustion intervention. The analgesic effect of moxibustion and increase of the concentration of Dyn and EM in spinal cord were demonstrated, which partially explained the mechanism of the analgesic effect of moxibustion in management of visceral pain.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (5 d old) were obtained from the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine (TCM). They were maintained in a plastic cage containing corn chip bedding with controlled temperature (22 ± 2℃), 60% ± 5% humidity and light-dark cycle (12:12 h) with a maximum of five rats per cage. Studies were performed in accordance with the proposals of the Committee for Research and Ethical Issues of the Council for International Organizations of Medical Sciences and approved by the Committee on the Use of Human and Animal Subjects in Teaching and Research, Shanghai University of TCM.

Study design

Neonatal rats were given daily mechanical colon distention beginning 8-21 d after their birth. After the distention was finished, the rats were kept until they reached adulthood (at least 6 wk old), and then experiments were conducted using behavioral test for visceral pain by acute CRD stimulus. Moxibustion (MX) group (n = 10): moxibustion was given to the acupoints of bilateral Tianshu (ST 25) and Shangjuxu (ST 37) using fine moxibustion stick with the smoldered end 2 cm away from the acupoints, once daily, 10 min each time, 7 times in total (Figure 1A). Sham moxibustion (SM) group (n = 10): intervention was given to bilateral Tianshu (ST 25) and Shangjuxu (ST 37) points

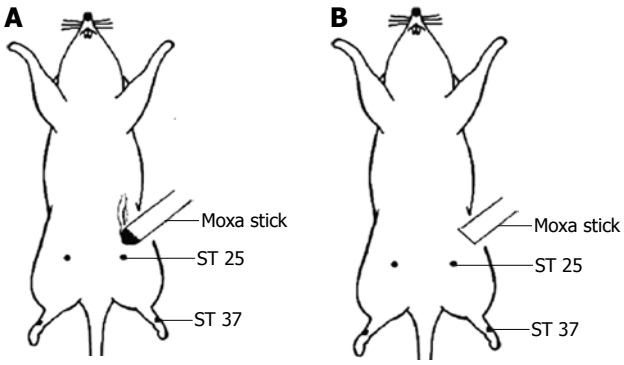

Figure 1 Moxibustion (A) and sham moxibustion (B).

Table 1 Abdominal withdrawal reflex scoring criteria

Score	
0	No behavioral response to colorectal distention
1	Immobile during colorectal distention and occasionally clicked the head at the onset of the stimulus
2	A mild contraction of abdominal muscles, but not lifting the abdomen off the platform
3	A strong contraction of the abdominal muscles and lifting the abdomen off the platform, not lifting the pelvic structure off the platform
4	Arching body and lifting the pelvic structure and scrotum

using fine moxibustion stick with the non-smoldered end 2 cm away from the acupoints, once daily, 10 min each time, 7 times in total (Figure 1B). Normal control (NC) group (n = 10) and model control (MC) group (n = 10): received no treatment except for constraining. After seven treatments, AWR was performed within 90 min, and a segment of spinal cord (L_4-S_1) was harvested and Dyn/EM concentration in spinal cord tissue was detected by enzyme linked immunosorbent assay (ELISA) (Figure 2).

Neonatal CRD irritation

Neonatal rats received CRD daily (the procedure was modified from previous reports[18,19]. Mainly, balloon (constructed from a condom; length: 20.0 mm; diameter: 3.0 mm) was inserted rectally into the descending colon. The balloon was distended with 0.5 mL air for 1 min and then deflated and withdrawn. The distention was repeated twice daily at a 30-min interval.

AWR scores

The AWR was assessed within 90 min after intervention using CRD based on semi-quantitative analysis. Prior to CRD, the rats were gently touched around anus for activating defecation. When the balloon was inserted into the descending colon, CRD was produced by rapidly inflating the balloon at strengths of 20, 40, 60, and 80 mmHg for a period of 20 s. Each score was tested three times, and each rat was tested by two people who were not involved in this research. There was a 3-min intervals between the two tests to allow the rats to adapt. The scoring criteria of AWR were referred to the method of Al-Chaer *et al*[18] (Table 1).

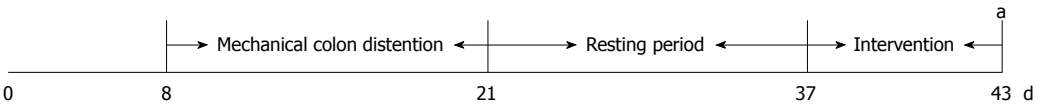

Figure 2 Experimental protocol of the study. [a]Abdominal withdrawal reflex sores after seven treatments.

ELISA for Dyn and EM

The dissected spinal cord tissue (L_4-S_1) was homogenized and weighed (10%), centrifuged for 30 min at 4℃, 4000 r/min. The supernatant was separated for assessment, and 100 μL standards and 100 μL dilution were mixed with 100 μL biotin, respectively. After incubation for 20 min at 20-25℃, 100 μL horse radish peroxidase was added. Followed by another 20 min of incubation at 20-25℃, 100 μL 3,3',5,5'-Tetramethylbenzidine substrate was added. Then 100 μL stop solution was put in after 20 min of incubation at 20-25℃. Calibration curve was drawn with OD value as the Y-coordinate and sample concentration as the X-coordinate. The concentration could be read according to the corresponding OD value. Rat Dyn and EM ELISA kits (THERMO MULTISKAN-MK3) were obtained from Finland.

Dyn in spinal cord (ng/L) = concentration × dilution times of the sample.

EM in spinal cord (ng/L) = concentration × dilution times of the sample.

Statistical analysis

The statistical analysis was done using SPSS 10.0 (SPSS Inc., USA). All data were expressed as mean ± SE for normally distributed continuous variables and as median (QL-QU) for abnormal variables. The differences in the mean values of the AWR score among the four groups (groups NC, MC, MX and SM) at each pressure of CRD were compared using the one-way analysis of variance (ANOVA, $P < 0.05$ as significant in differences). The differences in the median values of the concentration of Dyn and EM among the four groups were compared using the Kruskal-Wallis one-way analysis of variance on ranks. If the Kruskal-Wallis test result was significant ($P < 0.05$), we performed pairwise comparisons using a Wilcoxon rank sum test with a Bonferroni correction at 0.05/4 to correct for multiple comparisons. P value of < 0.05/4 was considered significant in differences.

RESULTS

Analgesic effects of moxibustion on chronic visceral hyperalgesia

At different levels of CRD stimuli (20, 40, 60 and 80 mmHg), the AWR scores in the MC group were significantly higher than in the NC group ($P < 0.01$); the AWR scores of MX group were significantly lower than that of the MC group ($P < 0.01$). There was no significant difference in the AWR scores between the MC group and SM group. This indicated that moxibustion treatment had a beneficial effect in chronic visceral hyperalgesia (Figure 3).

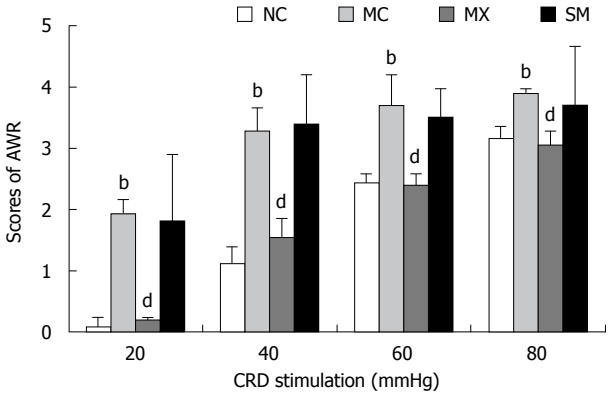

Figure 3 Analgesic effects of moxibustion on chronic visceral hyperalgesia. [b]$P < 0.01$ *vs* normal control (NC); [d]$P < 0.01$ *vs* model control (MC). AWR: Abdominal withdrawal reflex; MX: Moxibustion; SM: Sham moxibustion; CRD: Colorectal distention.

Influence of moxibustion in the Dyn concentration in spinal cord

The statistical analysis for the concentration of Dyn in spinal cord demonstrated a significant difference among the four groups, $F = 25.172$, $P = 0.000$. The concentration of MC group was significantly lower than that of NC group ($P < 0.01$). Compared with the MC group, the concentration of Dyn was significantly higher in the MX group ($P < 0.01$). No significant difference was detected in Dyn concentration between MC group and SM group (Figure 4A).

Influence of moxibustion on the EM concentration in spinal cord

Statistical analysis for the concentration of EM in spinal cord demonstrated a significant difference among the four groups, $F = 43.370$, $P = 0.000$. The concentration of MC group was significantly lower than that of NC group ($P < 0.01$). Compared with the MC group, the concentration of EM was significantly higher in the MX group ($P < 0.01$). No significant difference was detected in the EM concentration between the MC group and SM group (Figure 4B).

DISCUSSION

Visceral pain is commonly encountered by patients with functional intestinal disorders, leading to a miserable life and financial burden of the patients. Mertz *et al*[20] hold that the alterations of rectal sensitivity could be a biological indicator of IBS as IBS is featured by chronic abdominal pain. Alleviating abdominal pain is considered to be the main target in the management of IBS.

Moxibustion has been adopted as an analgesic method

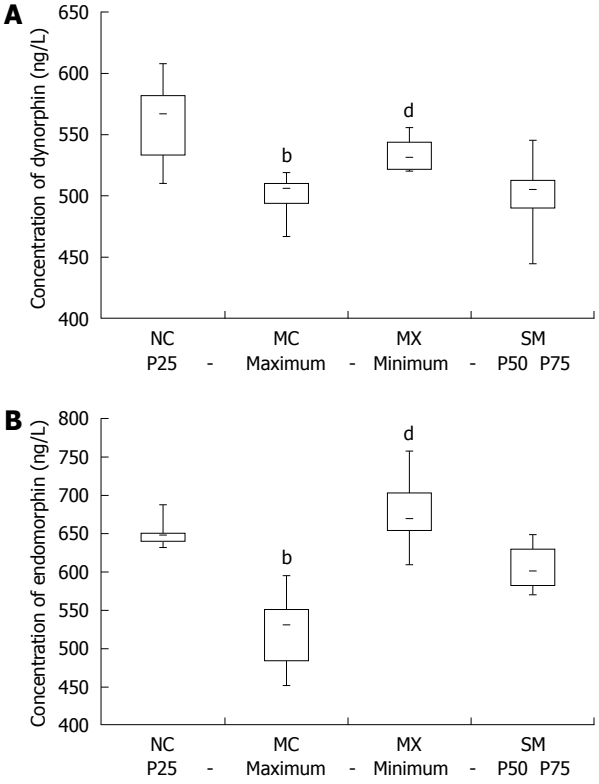

Figure 4 Influence of moxibustion on the concentration of dynorphin (A) and endomorphin (B) in spinal cord. $^{b}P < 0.01$ *vs* normal control (NC); $^{d}P < 0.01$ *vs* model control (MC). MX: Moxibustion; SM: Sham moxibustion.

for thousands of years in China, and is still frequently used in the present clinical practice. Many researches have shown its analgesic effect in treatment of primary dysmenorrhea[21], knee osteoarthritis[22], rheumatoid arthritis[23] and cancer pain[24]. Our previous studies also revealed that moxibustion could alleviate abdominal pain induced by IBS[16,17]. Although moxibustion has been practiced for thousand years, it is still difficult to establish its biological basis.

In the present study, CRD was adopted to establish a rat model of visceral hyperalgesia, and AWR was used for the behavioral assessment. The results showed that the AWR scores in the MC group were significantly higher than in the NC group at various CRD pressure levels (20, 40, 60 and 80 mmHg). Compared with the MC group, a marked reduction in AWR score was detected in the MX group ($P < 0.01$), and no significant difference was found in comparison with the SM group. It indicates that moxibustion has analgesic effects in management of visceral hyperalgesia, which is consistent with the results of our previous studies[16,17]. According to the previous studies adopting the same visceral pain model, herb-partitioned moxibustion could significantly inhibit the increase of AWR score and pain threshold induced by CRD. It has been also found that moxibustion could lower the expression of 5-HT in colon and modulate the expression of 5-HT in spinal cord, indicating a possible relationship between analgesic effect of moxibustion and central nervous system. Rats could keep quiet during the intervention of moxibustion, suggesting that modulating 5-HT was not the only way to reduce visceral hypersensitivity, some endogenous analgesic substances could also play a role in the process.

The analgesic effect of acupuncture has been widely accepted, especially in the study on chronic pain[25]. The endogenous opioid peptides (EOP) have been considered as important fundamental substances in acupuncture analgesia. According to Han JS[6,11,12,26], electro-acupuncture could activate the generation of EOP in spinal cord, such as orphanin, enkephalin, endomorphin, endorphin, and dynorphin.

The present study showed that the concentrations of Dyn and EM in spinal cord of the MC group were significantly lower than that of the NC group ($P < 0.01$). Compared with the MC group, the concentrations were significantly higher in the MX group ($P < 0.01$), and no significant difference was found from the SM group. It suggests that moxibustion could enhance the concentrations of Dyn and EM in spinal cord. Moxibustion may achieve its analgesic effect through multiple pathways and levels. Spinal cord may be the primary integrating center of moxibustion signal, increasing the concentrations of Dyn and EM in spinal cord, inducing a fragmental inhibition (including post-synaptic inhibition and pre-synaptic inhibition), and then blocking the further transmission of pain signal.

It has been shown that midbrain periaqueductal gray descending inhibitory system includes at least three transmitters: EOP, 5-HT and NA. Our findings indicate that moxibustion stimulation accelerates the synthesis and release of central EOP endorphin (dynorphin and endomorphin) and other neurotransmitters (5-HT) in the spinal dorsal horn neurons or nociceptive primary afferents, exerting analgesic effects.

In a word, moxibustion can significantly reduce AWR score and enhance the pain threshold of rats with chronic visceral hyperalgesia, and the analgesic effect may be closely related to the increased concentrations of Dyn and EM in spinal cord.

COMMENTS

Background

Previous studies into the mechanism of acupuncture analgesia have focused on the dynorphin (Dyn) and endomorphin (EM) in spinal cord. Whether analgesic effect of moxibustion is related to Dyn and EM in spinal cord remains unknown. In the previous studies, the authors have demonstrated the analgesic effect of moxibustion in reducing abdominal pain in irritable bowel syndrome (IBS) rats. However, the analgesic mechanism of moxibustion has not been clearly elucidated.

Research frontiers

More and more data have shown that the analgesic effect of moxibustion is closely related to the spinal cord fragments, which has become a hot spot of study.

Innovations and breakthroughs

Moxibustion is found effective against visceral pain. Moxibustion therapy exerts its effect on IBS by increasing the concentration of Dyn and EM in spinal cord

Applications

The experimental data can be used in further studies on moxibustion therapy for visceral pain.

Peer review

This is a good experimental investigation in which authors evaluate the effect of moxibustion, a Traditional Chinese Medicine, and possible involvement of endogenous dynorphin and endomorphin in the spinal cord in rats.

REFERENCES

1 NIH Consensus Conference. Acupuncture. *JAMA* 1998; **280**: 1518-1524
2 **Ma SX**. Neurobiology of Acupuncture: Toward CAM. *Evid Based Complement Alternat Med* 2004; **1**: 41-47
3 **Kim HY**, Koo ST, Kim JH, An K, Chung K, Chung JM. Electroacupuncture analgesia in rat ankle sprain pain model: neural mechanisms. *Neurol Res* 2010; **32** Suppl 1: 10-17
4 **Qu F**, Zhou J. Electro-acupuncture in relieving labor pain. *Evid Based Complement Alternat Med* 2007; **4**: 125-130
5 **Liu HX**, Tian JB, Luo F, Jiang YH, Deng ZG, Xiong L, Liu C, Wang JS, Han JS. Repeated 100 Hz TENS for the Treatment of Chronic Inflammatory Hyperalgesia and Suppression of Spinal Release of Substance P in Monoarthritic Rats. *Evid Based Complement Alternat Med* 2007; **4**: 65-75
6 **Han JS**. Acupuncture and endorphins. *Neurosci Lett* 2004; **361**: 258-261
7 **Lim B**, Manheimer E, Lao L, Ziea E, Wisniewski J, Liu J, Berman B. Acupuncture for treatment of irritable bowel syndrome. *Cochrane Database Syst Rev* 2006; CD005111
8 **Joos S**, Wildau N, Kohnen R, Szecsenyi J, Schuppan D, Willich SN, Hahn EG, Brinkhaus B. Acupuncture and moxibustion in the treatment of ulcerative colitis: a randomized controlled study. *Scand J Gastroenterol* 2006; **41**: 1056-1063
9 **Joos S**, Brinkhaus B, Maluche C, Maupai N, Kohnen R, Kraehmer N, Hahn EG, Schuppan D. Acupuncture and moxibustion in the treatment of active Crohn's disease: a randomized controlled study. *Digestion* 2004; **69**: 131-139
10 **Ouyang H**, Chen JD. Review article: therapeutic roles of acupuncture in functional gastrointestinal disorders. *Aliment Pharmacol Ther* 2004; **20**: 831-841
11 **Huang C**, Wang Y, Chang JK, Han JS. Endomorphin and mu-opioid receptors in mouse brain mediate the analgesic effect induced by 2 Hz but not 100 Hz electroacupuncture stimulation. *Neurosci Lett* 2000; **294**: 159-162
12 **Han Z**, Jiang YH, Wan Y, Wang Y, Chang JK, Han JS. Endomorphin-1 mediates 2 Hz but not 100 Hz electroacupuncture analgesia in the rat. *Neurosci Lett* 1999; **274**: 75-78
13 **Hwang SL**, Lin CL, Lieu AS, Kuo TH, Yu KL, Ou-Yang F, Wang SN, Lee KT, Howng SL. Punctate midline myelotomy for intractable visceral pain caused by hepatobiliary or pancreatic cancer. *J Pain Symptom Manage* 2004; **27**: 79-84
14 **Honda CN**. Visceral and somatic afferent convergence onto neurons near the central canal in the sacral spinal cord of the cat. *J Neurophysiol* 1985; **53**: 1059-1078
15 **Wu HG**, Shi Y, Zhang W, Zhou S, Liu HR. [Advances and thinking about prevention and treatment of inflammatory bowel diseases by acupuncture and moxibustion] *Zhongguo Zhenjiu* 2006; **26**: 454-458
16 **Zhou EH**, Liu HR, Wu HG, Shi Y, Wang XM, Tan LY, Yao LQ, Zhong YS, Jiang Y, Zhang LL. Suspended moxibustion relieves chronic visceral hyperalgesia via serotonin pathway in the colon. *Neurosci Lett* 2009; **451**: 144-147
17 **Zhou EH**, Liu HR, Wu HG, Shi Y, Wang XM, Yao LQ, Zhong YS, Yang Y. Herb-partition moxibustion relieves chronic visceral hyperalgesia and 5-HT concentration in colon mucosa of rats. *Neurol Res* 2009; **31**: 734-737
18 **Al-Chaer ED**, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; **119**: 1276-1285
19 **Cui KM**, Li WM, Gao X, Chung K, Chung JM, Wu GC. Electro-acupuncture relieves chronic visceral hyperalgesia in rats. *Neurosci Lett* 2005; **376**: 20-23
20 **Mertz H**, Naliboff B, Munakata J, Niazi N, Mayer EA. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. *Gastroenterology* 1995; **109**: 40-52
21 **Li WL**, Liu L, Sun LH. [Analysis on therapeutic effect of substance-partitioned moxibustion at Guanyuan (CV 4) and shenque (CV 8) for treatment of primary dysmenorrhea of cold-damp type] *Zhongguo Zhenjiu* 2006; **26**: 481-482
22 **Su JC**, Cao LH, Li ZD, Wang SC, Zhang QJ, Ma YH, Fu XM, Yu BQ, Du N, Zhang CC. [Controlled clinical trials of initial observation on therapeutic effects of moxibustion for osteoarthritis of the knee: multi-center clinical effect] *Zhongguo Gushang* 2009; **22**: 914-916
23 **Xie XX**, Lei QH. [Observation on therapeutic effect of the spreading moxibustion on rheumatoid arthritis] *Zhongguo Zhenjiu* 2008; **28**: 730-732
24 **Chen ZJ**, Guo YP, Wu ZC. [Advances of clinical study on acupuncture and moxibustion for treatment of cancer pain] *Zhongguo Zhenjiu* 2008; **28**: 392-394
25 **Park J**, Linde K, Manheimer E, Molsberger A, Sherman K, Smith C, Sung J, Vickers A, Schnyer R. The status and future of acupuncture clinical research. *J Altern Complement Med* 2008; **14**: 871-881
26 **Tian JH**, Zhang W, Fang Y, Xu W, Grandy DK, Han JS. Endogenous orphanin FQ: evidence for a role in the modulation of electroacupuncture analgesia and the development of tolerance to analgesia produced by morphine and electroacupuncture. *Br J Pharmacol* 1998; **124**: 21-26

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