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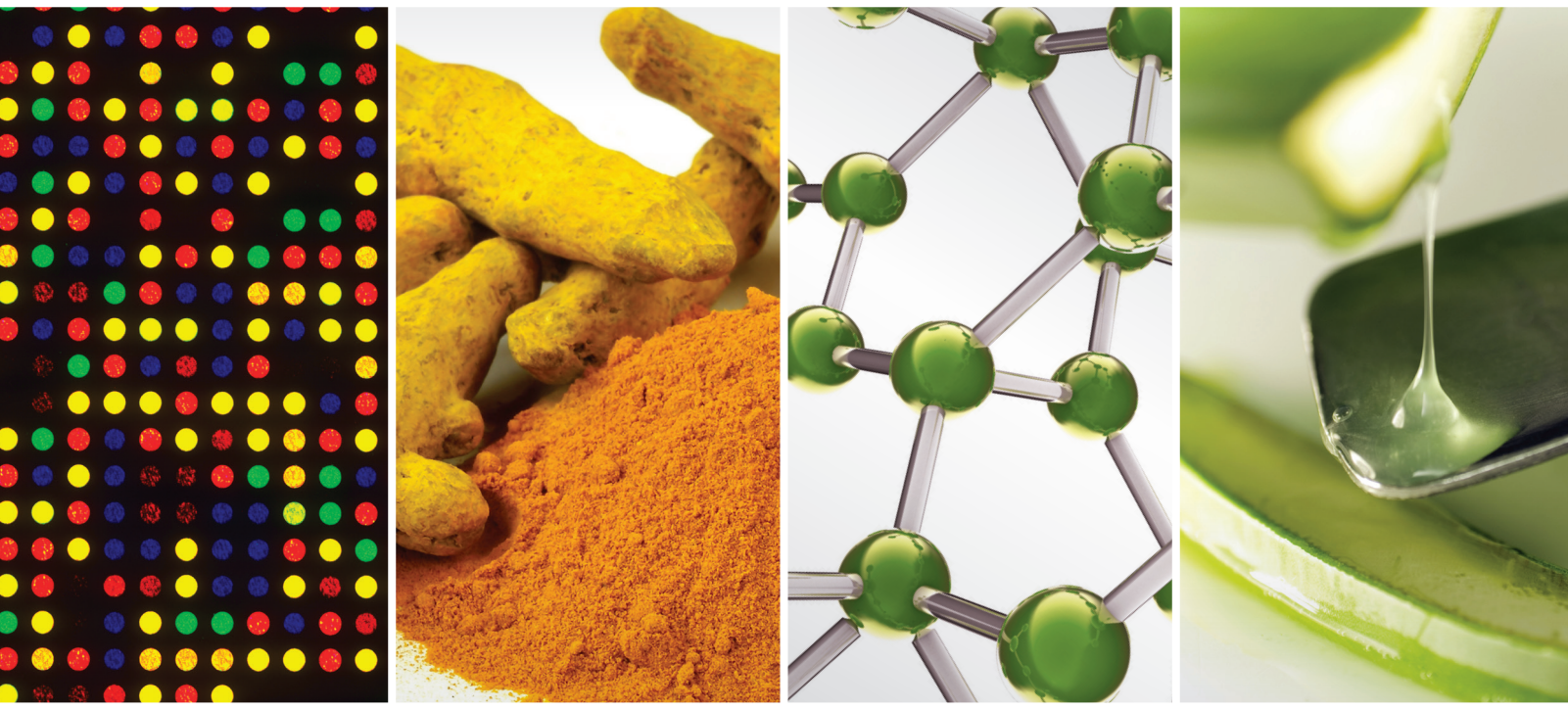
Biophysical and Clinical Research on Acupuncture and Moxibustion

Guest Editors: Xueyong Shen, Lixing Lao, Yan Zhang, and Ke Cheng



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Editorial

Biophysical and Clinical Research on Acupuncture and Moxibustion

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Acupuncture has been used for thousands of years in treating diseases. Meridians and acupoints, on which acupuncture is practiced, have also attracted many research interests. Moxibustion is often used in conjunction with acupuncture as a thermal stimulation on acupoints. Laser, which generates extremely pure light of single wavelengths, is now used widely to stimulate acupoints, because it is noninvasive and easy to manipulate.

This special issue contains 1 human research investigating the electrical characteristics of the acupoints, 2 randomized controlled trials (RCTs) evaluating the effect of traditional moxibustion on patients with knee osteoarthritis and lumber disc herniation, respectively, 3 animal studies exploring the effect and mechanism of the traditional moxibustion and laser moxibustion on irritable bowel syndrome (IBS), cyclophosphamide-induced leucopenia, and knee osteoarthritis (OA), respectively, 1 systematic review reviewing the safety of moxibustion, 1 paper describing the design of an electrical thermal stimulation system comparable to moxibustion, and 2 animal researches studying the effect and mechanism of electroacupuncture on disc degeneration and hepatic blood perfusion respectively.

The results of these researches showed that acupoints may have special electrical characteristics at pathological status; moxibustion may relieve back pain and improve quality of life in patients with knee OA. Although the two RCTs did not report severe adverse events related to moxibustion, the systematic review found many factors may affect the safety of the moxibustion. The results of these researches also enhanced our knowledge of how traditional moxibustion, laser acupuncture-moxibustion, and electrical-acupuncture work in treating IBS, knee OA, cyclophosphamide-induced leucopenia, disc degeneration, and hepatic blood perfusion.

The encouraging findings in this special issue will promote further investigations in the fields of biophysical and clinical research of acupuncture and moxibustion and may encourage more use of moxibustion in clinic. The electrical thermal moxibustion and laser moxibustion might be good substitutes for traditional moxibustion, because they produce thermal effect but with no chocking smoke and smell, thus being more suitable in modern medical environment. However, in this current special issue, researchers only investigated the effect of laser moxibustion on animal and introduced a design of electrical thermal moxibustion device; further clinical research on both moxibustion devices is needed in the future.

Acknowledgments

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Institute for Women's Health, Texas Tech University Health Sciences Center, Lubbock, TX, USA), and Dr. Ke Cheng (Lecturer, College of Acupuncture and Moxibustion, Shanghai University of Traditional Chinese Medicine, Shanghai, China) for their cooperation. The guest editors thank all authors for their excellent contributions and thank all reviewers for their honorable work for this special issue.

Xueyong Shen
Lixing Lao
Yan Zhang
Ke Cheng

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Research Article

Effectiveness of Moxibustion Treatment in Quality of Life in Patients with Knee Osteoarthritis: A Randomized, Double-Blinded, Placebo-Controlled Trial

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Objective. To observe the effects of traditional Chinese moxibustion, compared with sham moxibustion, on the quality of life (QOL) in patients with chronic knee osteoarthritis (KOA). *Methods.* This is a randomized double-blinded, placebo-controlled trial. 150 patients with KOA were randomly allocated to either a true moxibustion treatment ($n = 77$) or a sham moxibustion treatment ($n = 73$) three times a week for six weeks. The QOL of patients was evaluated with SF-36 at baseline and 3, 6, and 12 weeks after baseline. *Results.* 136 patients were available for analysis. Participants in the true moxibustion group experienced statistically significantly greater improvement in GH (general health) scores than the sham group at week 6 ($P = 0.015$) and week 12 ($P = 0.029$). Participants in the true moxibustion group experienced statistically significantly greater improvement in VT (vitality) scores than the sham group at week 12 ($P = 0.042$). No significant adverse effects were found during the trial. *Conclusion.* A 6-week moxibustion treatment seems to improve general health and vitality, which are associated with physical and mental quality of life, in patients with KOA up to 12 weeks, relative to credible sham moxibustion. This trial is registered with Clinicaltrials.gov ISRCTN68475405.

1. Introduction

The mode of medicine has changed, and so has people's view of their health. The WHO has proposed a new concept of health, which includes not only physical health but also social, mental, and psychological health. While the former method of determining whether certain treatments were effective was plain and had a single focus on physical health, the new method takes multiple influences on patients' social life and mental status into account. Thus, the Social Medical Index of Quality of Life (QOL) has received great attention [1]. In 1993, the WHO defined QOL as "the feeling of an individual about his/her social status, targets, anticipation, standards, and concern in his/her own cultural background and value system. It includes physical health, mental status, degree of independence, social relationship, personal belief and the relativity of their surroundings. It involves a wide range of complex concepts" and this definition is still in use [2].

Knee osteoarthritis (KOA) is commonly seen in middle-aged and elderly people. It causes pain and dysfunction that greatly interfere with patients' quality of life [3, 4]. There is no cure for KOA, and the current methods of treatment are limited to relief of the symptoms. Moxibustion is a common method of treating KOA in East Asia countries, and it is backed by thousands of years of clinical practice. There are many current reports on the clinical efficacy of moxibustion, but reports on the quality of life of KOA patients treated with moxibustion are rarely seen. The current work describes a randomized and strictly controlled clinical trial evaluating the efficacy of moxibustion in the treatment of KOA. The findings on pain and function after moxibustion have been reported in our previously published paper [5]; in this paper,

we focus on the evaluation and report of patients' QOLs after moxibustion.

2. Methods

2.1. Research Design and Setting. This was a double-blinded, placebo-controlled randomized controlled trial (RCT). The study protocol was approved by the Institutional Ethic Review Committee of Chinese Clinical Trials Registry based in Chengdu, China. This RCT was also registered in the Chinese Clinical Trials Registry (ChiCTR-TRC-11001408) on July 6, 2011. Patients were recruited from March 2010 to May 2012 from the Acupuncture and Moxibustion Department of Traditional Chinese Medicine Hospital of Pudong New District, Shanghai; the Community Service Center of Chuansha, Pudong, Shanghai; and the Outpatient Clinic of Acupuncture and Moxibustion of Nantong University's affiliated hospital.

2.2. Criteria for Diagnosis, Inclusion, Exclusion, Elimination, Dropping Out, and Termination of Treatment

2.2.1. Diagnostic Criteria. According to the KOA diagnostic criteria issued by the American College of Rheumatology (ACR) in 1986 [6], the following are characteristic of knee osteoarthritis: (1) pain in the knee most days of a month; (2) X-rays showing osteophytes on the sides of the joints; (3) synovial test indicating osteoarthritis; (4) age over 40 years; (5) morning stiffness less than 30 minutes; (6) clicking sound occurring when joint moves. Patients with (1) + (2) or (1) + (3) + (5) + (6) or (1) + (4) + (5) + (6) were diagnosed with knee osteoarthritis.

2.2.2. Inclusion Criteria. Patients were included if they met the diagnostic criteria given above and had (1) knee pain lasting longer than 3 months; (2) moderate or severe knee pain most days of the past month; (3) age under 78 years; (4) willingness to enter a randomized study and to sign the informed consent form.

2.2.3. Exclusion Criteria. Patients were excluded if they (1) had received corticosteroids or intra-articular hyaluronic acid treatment within the past three months; (2) had received joint irrigation or arthroscopy within the past year; (3) had diseases that could interfere the trial and affect the results, such as myocardial infarction, stroke, congestive heart failure, or other severe systemic disease; (4) had been diagnosed with inflammatory arthritis, gout, acute knee damage or other types of arthritis, meniscus, ligament injury, or intrajoint bone fracture; (5) were pregnant; (6) were incapable of filling the scales or who were not willing to be randomized.

2.2.4. Criteria for Rejection, Dropping Out, and Termination. (1) Patients who failed to follow the regimen or who underwent other treatments or drug regimens that could interfere with this trial's final outcome were rejected. (2) Patients who did not have any adverse reaction during the trial but still failed to complete the whole session for other reasons (such as the emergence of other diseases or lost connection) were rejected.

2.3. Randomization, Allocation Concealment, and Blinding

Randomization and Allocation Concealment. One of the personnel generated random numbers using the RAND function in Excel 2003. The true and sham moxibustion group was assigned with different codes, respectively, and the codes of grouping changed constantly during the RCT. The personnel who recruited the participants were blinded with the group assignment and are only aware of the name and grouping code of each patient. True and sham moxa pillar packs were assigned with the corresponding grouping codes. The practitioners who performed the treatment picked the moxa pillar pack according to the grouping code of the patients when applying treatment.

Blinding. During the treatment, the two groups of patients were treated separately by different practitioners. The personnel responsible for the evaluation, recording, and statistical analysis were all blinded to the allocation.

2.4. Moxibustion Devices. A commercially available moxibustion device was used (Nanyang Hanyi Moxa Company, Ltd.). It had a cylindrical opening to hold a pillar of moxa and it had an adhesive membrane at the base. During treatment, the device was placed at an acupoint, and the moxa was burned about 8 mm above the skin.

The sham moxibustion device looked exactly the same as the true devices, underwent the same burning process, and produced the same residues, but they had insulated layer in their bases to prevent heat and smoke from coming into contact with the patient's skin. The reliability of this device has been previously tested and validated by Zhao et al. [7].

2.5. Treatment Procedure

(1) True Moxibustion Treatment. The participant lay supine with the knees slightly flexed with the affected knee(s) exposed. Patients in both groups were treated at three local points, ST 35, EX-LE4, and an Ashi point (the most painful point around knee joint under palpation), in the area of the affected knee(s). Three consecutive moxa pillars were burned at each point. Once the pillar was affixed to a point, the first pillar was lit. When the patient felt burning hot, the pillar was removed and another pillar was affixed and lit. Each treatment session lasted about 20 min. Three pillars were used per acupoint. Treatment was performed three times per week for six weeks. If the patient missed one session he or she was given a session within one week as a makeup.

(2) Sham Moxibustion Treatment. The procedure was similar to the true moxibustion group. Because the sham moxibustion device provided insulation from the heat, the patients did not feel hot, and the 3 moxa pillars were allowed to burn out consecutively.

2.6. Outcome Measures. SF-36 (Chinese version) was used to assess patients' quality of life (QOL) [8, 9]. This scale covers 8 dimensions (35 items): physical functioning (PF), physical role functioning (RP), bodily pain (BP), general health (GH),

Table 1: Participant demographics and baseline characteristics.

Characteristics	Moxibustion ($n = 69$)	Sham moxibustion ($n = 67$)	P value
Age, mean ± SD, y	65.61 ± 7.42	64.06 ± 8.65	0.264
Sex (%)			
Men	20 (29%)	23 (34%)	0.814
Women	49 (71%)	44 (66%)	
Affected knees (%)			
Single knee	55 (80%)	45 (67%)	0.581
Both knees	14 (20%)	22 (33%)	
Duration, mean ± SD, y	6.50 ± 5.86	6.23 ± 7.17	0.121
Quality of life score, mean ± SD, 8 dimensions of SF-36			
PF	57.83 ± 16.88	60.15 ± 17.21	0.428
RP	33.70 ± 37.33	42.91 ± 41.70	0.177
RE	49.76 ± 44.14	56.22 ± 41.52	0.381
VT	53.55 ± 18.19	52.99 ± 18.55	0.858
MH	71.54 ± 14.41	68.78 ± 15.84	0.289
SF	78.62 ± 18.70	75.00 ± 16.71	0.236
BP	58.55 ± 12.82	57.69 ± 18.49	0.751
GH	50.29 ± 15.31	47.99 ± 15.88	0.390

BP: bodily pain; GH: general health; MH: mental health; RE: emotional role functionality; PF: physical functioning; RP: physical role functioning; SD: standard deviation; SF: social role functionality; VT: vitality; y: year.

vitality (VT), social role functionality (SF), emotional role functionality (RE), and mental health (MH). The 36th item was a self-evaluation of health changes (HT), which was analyzed separately. Each item was scored and the scores were standardized and transformed into final scores on a scale of 0–100, with 0 the worst and 100 the best. The 8 dimensions of SF-36 scale measure physical and mental health. Among these, the dimensions of PF, RP, and BP were mainly found to be associated with physical health, and the dimensions of MH and RE were mainly associated with mental health. The dimensions of SF, VT, and GH were associated with both physical and mental health [10].

The measurements were done before the treatment, at week 3 (midterm), week 6 (end of treatment), and week 12 (6 weeks after the end of treatment). The questionnaire was filled out by the patients with the assistance of the trained practitioners who were blinded with their assignment.

2.7. Statistical Analysis. SPSS 17.0 software was used in analysis. Histograms and Q-Q plots were used to assess the distribution of the quality of life outcomes. The outcome data were expressed as mean ± standard deviation (mean ± SD) when the data showed a normal distribution. Otherwise, they were expressed as median (mix, max, and IQR). Chi-square testing was used for analysis of between-group knee conditions and gender distribution. The t-test was used for analysis of age and duration of disease. To identify a trend in SF-36 scores, a repeated measure analysis of variance was used for multiple comparisons, and MANOVA was used for between-group assessment. The test level was $\alpha = 0.05$.

3. Results

A total of 221 potentially eligible patients were screened and enrolled between March 2010 and May 2012. Seventy-one patients were excluded because they did not meet the inclusion criteria. Of the 150 randomly assigned patients, 136 completed the 6-week course of treatment and were assessed at 12 weeks, in which 69 formed the true group and 53 the sham group (Figure 1). The two groups were comparable in demographic characteristics such as gender, age, duration of disease, and the 8 aspects of quality of life at baseline ($P > 0.05$). Baseline characteristics of the patients are presented in Tables 1 and 2.

The sham group's PF at week 6 and BP at both week 6 and week 12 showed statistically significant improvement from baseline ($P < 0.001$, $P < 0.01$). The true moxibustion group's PF, VT, and GH scores at both week 6 and week 12 showed significant improvement from baseline ($P < 0.01$, $P < 0.05$); the true moxibustion group's BP score at all time points (i.e., weeks 3, 6, and 12) showed statistically significant improvement from baseline ($P < 0.001$, $P < 0.01$) (Table 2).

Participants in the true moxibustion group experienced statistically significantly greater improvement in GH (general health) scores than those in the sham moxibustion group at week 6 ($P = 0.015$) and week 12 ($P = 0.029$). Participants in the true moxibustion group experienced statistically significantly greater improvement in VT (vitality) scores than the sham group at week 12 ($P = 0.042$). No significant differences were shown between the two groups for scores in other dimensions (Table 2).

Table 2: Comparison of SF-36 scores (mean ± SD).

8 dimensions of SF-36	Time points	True moxibustion group	Sham moxibustion group	*P* value
PF	Baseline	57.83 ± 16.88	60.15 ± 17.21	0.428
	Week 3	60.65 ± 15.29	61.87 ± 16.87	0.661
	Week 6	63.99 ± 18.24[b]	66.04 ± 14.42[b]	0.467
	Week 12	63.62 ± 17.06[b]	64.10 ± 17.47	0.871
RP	Baseline	33.70 ± 37.33	42.91 ± 41.70	0.177
	Week 3	43.12 ± 41.32	42.16 ± 42.02	0.894
	Week 6	43.48 ± 40.60	45.15 ± 40.88	0.811
	Week 12	41.67 ± 39.22	38.81 ± 40.89	0.678
RE	Baseline	49.76 ± 44.14	56.22 ± 41.52	0.381
	Week 3	59.42 ± 41.96	58.21 ± 44.70	0.871
	Week 6	57.49 ± 43.87	50.25 ± 44.71	0.342
	Week 12	54.11 ± 42.82	48.76 ± 42.37	0.465
VT	Baseline	53.55 ± 18.19	52.99 ± 18.55	0.858
	Week 3	56.16 ± 16.50	56.87 ± 14.40	0.791
	Week 6	59.42 ± 15.87[b]	56.49 ± 14.62	0.265
	Week 12	61.30 ± 16.22[b]	55.52 ± 16.68	0.042[d]
MH	Baseline	71.54 ± 14.41	68.78 ± 15.84	0.289
	Week 3	69.33 ± 13.35	66.27 ± 15.26	0.214
	Week 6	68.81 ± 12.75	66.99 ± 14.43	0.435
	Week 12	68.81 ± 13.95	66.33 ± 17.80	0.366
SF	Baseline	78.62 ± 18.70	75.00 ± 16.71	0.236
	Week 3	78.26 ± 15.99	76.49 ± 17.21	0.536
	Week 6	79.17 ± 13.33	78.54 ± 15.05	0.799
	Week 12	76.45 ± 14.93	75.00 ± 17.13	0.600
BP	Baseline	58.55 ± 12.82	57.69 ± 18.49	0.751
	Week 3	63.62 ± 12.46[b]	61.72 ± 15.72	0.434
	Week 6	67.54 ± 14.45[a]	65.93 ± 13.53[a]	0.506
	Week 12	66.56 ± 14.68[a]	65.60 ± 16.83[b]	0.723
GH	Baseline	50.29 ± 15.31	47.99 ± 15.88	0.390
	Week 3	49.20 ± 15.35	46.87 ± 14.30	0.360
	Week 6	54.42 ± 15.92[c]	48.43 ± 12.10	0.015[d]
	Week 12	54.57 ± 14.67[c]	48.88 ± 15.44	0.029[d]

BP: bodily pain; GH: general health; MH: mental health; RE: emotional role functionality; PF: physical functioning; RP: physical role functionality; SF: social role functionality; VT: vitality.

[a]Compared with pretreatment: $P < 0.001$; [b]compared with pretreatment: $P < 0.01$; [c]compared with pretreatment: $P < 0.05$; [d]b-group comparison: $P < 0.05$.

3.1. Safety Analysis. In the treatment group, 22 patients experienced blisters of varying sizes from the moxibustion, and 2 patients' blisters were relatively big, but they healed after 1 week. The other 20 patients' blisters all healed within 3 days. All patients considered the blisters nondebilitating and acceptable. No patient experienced discomfort during the moxibustion treatment. Most of the patients regarded moxibustion as a safe procedure and wanted to purchase some moxa pillars and perform moxibustion at home.

3.2. Dropouts. The dropout rate was low. By the end of the trial it was 9% (14/150). In the true moxibustion group, 8 of the 77 patients were lost to followup: 1 was allergic to the adhesive glue at the base of the pillar, 1 underwent knee replacement, 1 was hospitalized due to dizziness, 3 discontinued treatment and could not be contacted, and 2 were too busy to comply with the treatment and followup. In the sham moxibustion group, 6 of the 73 patients were lost to followup: 2 discontinued treatment due to travel, 2 could not be contacted, and 2 were too busy to comply with the treatment and followup (Figure 1).

4. Discussion

Guidelines for treating KOA regarded relieving pain, maintaining and improving joint's function, and improving patient's life quality as the first priority [11–13]. Our previously published article reported that in this trial, moxibustion reduced pain, improved function, and decreased stiffness for KOA compared with the sham moxibustion procedure [5]. Here, the effect of moxibustion on the quality of life of KOA patients was assessed and reported.

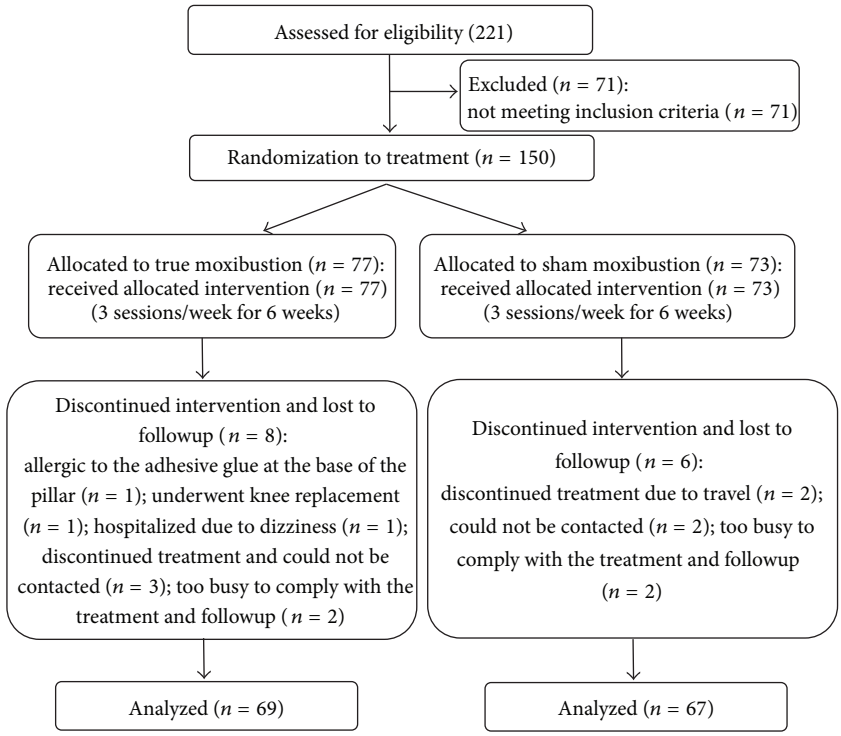

Figure 1: Flow diagram.

In this double-blind RCT, we found a 6-week course of moxibustion treatment significantly improved vitality and general health, which were both associated with physical and mental quality of life, relative to a believable sham control. Although the effectiveness of moxibustion treatment has been reported in other studies, one recent systematic review showed that the evidence of the larger effect associated with moxibustion is very poor [14]. This was due to small sample size, inadequate use of controls, inadequate followup, and lack of placebo control in previous studies. Reports of quality of life have been very rare for moxibustion treatment [14].

In the present RCT, these limitations were overcome by using rigorous double-blinded, placebo-controlled methods with adequate randomization and a longer followup. The compliance rate of the current RCT was high (91%). In order to facilitate blinding, a validated sham moxibustion device first reported by Zhao et al. in 2006 was used [7]. The appearance, burning process, and residue were the same in the sham and real devices; the only difference was the insulated layer in the base of the sham device. This minimized moxa-produced heat and smoke. The sham control device did produce some warmth, but less than the true moxibustion device. Local skin temperature was measured after each treatment, and active moxibustion produced a temperature of 49.8° on the skin versus the 40.9° produced by the sham treatment [15]. This probably made it difficult for patients to distinguish the true procedure from the sham. However, some physiological effect caused by this lower temperature heat cannot be precluded. To facilitate blinding, only patients who were naïve to moxibustion treatment were included. Communications regarding treatment experiences between the two groups were prevented by treating them on different days. Different groups of practitioners treated the two sets of patients; they had no chance to compare the two devices, saw feedback only from patients of the same group, and were instructed not to discuss the patients' experiences.

The mechanisms of action of moxibustion therapy are still largely unknown. Factors such as temperature, infrared radiation, smoke, odor, and the type of moxa are likely to be involved in the mechanisms by which moxibustion may work [16]. Heat is believed to be the most important factor involved in the effects of moxibustion. Mounting evidence shows that acupuncture relieves pain and improves function in KOA [17–19]. Moxibustion might play a role similar to that of acupuncture stimulation, although its effect on the sensory nerve would be more superficial, due to the heat stimulation on the superficial tissue.

5. Conclusion

Moxibustion can be a useful adjunctive treatment for improving physical and mental quality of life in patients with KOA. The findings of the present work should be confirmed and generalized in a larger RCT using a double-blinded, placebo-controlled, multicentered approach.

Conflict of Interests

The authors declare that there is no conflict of interests of any kind.

Authors' Contribution

Xiumei Ren and Chang Yao contributed equally to this study and should be considered as co-first authors.

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Research Article

A 3-Arm, Randomized, Controlled Trial of Heat-Sensitive Moxibustion Therapy to Determine Superior Effect among Patients with Lumbar Disc Herniation

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Systematic reviews of moxibustion for LDH have identified ponderable evidence, especially for heat-sensitive moxibustion (HSM). Therefore, we designed and carried out the large sample trial to evaluate it. 456 patients were recruited from 4 centers in China and were randomly divided into three groups by the ratio of 1 : 1 : 1 to HSM (152) group, conventional moxibustion (152) group, and conventional drug plus acupuncture (152) group. Compared with usual care, there was a statistically significant reduction in mean M-JOA score at 2 weeks and 6 months for HSM (3.8 ± 2.6 versus 8.5 ± 2.9; 3.7 ± 2.2 versus 10.1 ± 2.9) and conventional moxibustion (7.9 ± 3.0 versus 8.5 ± 2.9; 8.9 ± 3.1 versus 10.1 ± 2.9). Compared with conventional moxibustion group, HSM group showed greater improvement in all the outcomes. The mean dose of moxibustion was 41.13 ± 5.26 (range 21–60) minutes in the HSM group. We found that HSM was more effective in treating patients with LDH, compared with conventional moxibustion and conventional drug plus acupuncture. This finding indicated that the application of moxibustion on the heat-sensitive points is a good moxibustion technique in treating disease.

1. Background

Therapies to strengthen the motor function and relieve low back pain are the most commonly recommended treatment for lumbar disc herniation (LDH), such as acupuncture and moxibustion [1]. They have the advantage better than other therapies (especially surgery) that they have no physical side-effects or adverse reactions [2, 3]. Moxibustion is a traditional oriental therapy that treats diseases through thermal stimulation of burning herbs, primarily *Artemisia vulgaris*, at specific acupuncture and moxibustion point on the skin [4]. Traditional Chinese medicine (TCM) considers LDH to be the result of an unbalanced state among interfunctioning organs or a block vital energy (called Qi) condition with characteristic blood symptoms [5]. A large number of clinical studies have shown positive results of moxibustion remedies on LDH [6]. And moxibustion therapy has been important treatment in China. In particular, moxibustion treatment is effective for functional limitation and pain symptom because it provides warm energy, expels Qi-blood stagnation, and enhances local blood circulation [7]. Experimental studies showed moxibustion had anti-inflammatory or immunomodulatory effects against chronic inflammatory conditions in humans [8].

For moxibustion therapy, many factors influenced the therapeutic effect. However, the first thing to think about is the selection of location for manipulating moxa [9]. Conventional moxibustion applied moxibustion on fix acupuncture points based on pattern differentiation. Different patients received treatments on the same acupuncture points. However, heat-sensitive moxibustion (HSM) selected location that received moxibustion differently [10]. Heat-sensitive

moxibustion administered moxibustion on heat-sensitive acupuncture points, which are extremely sensitive to the heat stimulation of burning moxa [11]. By using such acupuncture points, it is easier for channel Qi to transmit and to allow a strong response to be produced by weak stimulation. Patients felt heat-sensitive sensation on these acupuncture points [12].

According to acupuncture point sensitized theory, there are two kinds of state in acupuncture points in human body: stimulated state and resting state. When people get sick, the acupoints on the body surface area are activated and sensitized. Our research found that the heat-sensitive phenomenon to acupuncture point or an area is a new type of reaction in a pathological state. The sensitive areas are susceptible to heat stimulation and called "heat-sensitive acupuncture points." A feature of these areas is that these areas are specific or closely relevant to acupuncture points and produce the same clinical effect as "a small stimulation induces a large response." This heat-sensitive acupuncture point is not only the pathological phenomenon reflection of the diseases but also an effective stimulating location with acupuncture and moxibustion. These heat-sensitized locations are not fixed, but may, during the progression of disease, dynamically change within a certain range centered on acupuncture points [13]. Our empirical evidence engaged us in formulating the following hypothesis: moxibustion at the heat-sensitive acupuncture points showed better efficacy than that at fixed acupuncture points.

However TCM theory in China agreed that the best place to apply moxibustion was on heat-sensitive acupuncture points, because using them led to better stimulation and transmission of channel Qi. When Qi arrives at one part of the body, it can treat the diseases nearby. In the part of *Miraculous pivot*, *the chapter of nine needles and twelve sources* said: "The key point of acupuncture is the arrival of Qi, it ensures therapeutic effect." However, there is little high-quality clinical evidence of its effectiveness. Therefore, we designed and carried out the large sample trial to evaluate it.

The results of a recent meta-analysis of six randomized controlled trials (RCTs) on moxibustion for LDH manifested that heat-sensitive therapy presented a favorable effect on LDH symptom scores compared with that of the drug [RR = 1.91, 95% CI (1.01, 3.60)] [14]. However, because of the number of eligible RCTs and the high risk of bias in the assessment of the available RCTs, the evidence supporting this conclusion is limited. Therefore, this well-designed and big sample RCT was needed to establish the efficacy of heat-sensitive moxibustion for LDH.

2. Methods

2.1. Objective. The aim of this study is to assess the effectiveness of heat-sensitive moxibustion for treating LDH compared with conventional drug plus acupuncture as well as conventional moxibustion.

2.2. Sample Size. An effect size on the M-JOA was sought when comparing the heat-sensitive with conventional moxibustion. In our previous pilot study, the effective rate in heat-sensitive moxibustion group is 65% and 45% in the other groups. An allocation ratio of 1 : 1 : 1 was chosen in order to increase power to detect statistically significant differences between the three groups. With 90% power and a two-sided significance level of 5%, the required group sizes were 126. Allowing for 20% attrition, the total sample size required was 456 (i.e., groups of 152, 152, 152, resp.):

$$n = \frac{p_1 \times (1 - p_1) + p_2 \times (1 - p_2)}{(p_2 - p_1)^2} \times f(\alpha, \beta). \quad (1)$$

2.3. Design. We performed a multicenter (four centers in China), randomized, assessor blinded, and positive controlled trial. Our trial was carried out in four hospitals in China, including the Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine (TCM) in Nanchang, the first Affiliated Hospital of Anhui University of TCM in Hefei, Jiangsu TCM Hospital in Nanjing, and Shanxi TCM Hospital in Xian. Patients were recruited through hospital-based recruitment and newspaper advertisements. After a baseline phase of one week, we used a central randomization system (random list generated with computer telephone integration by the statistician from China Academy of Chinese Medical Sciences) to randomize patients [15]. All study participants provided written, informed consent, and the study conformed to common guidelines for clinical trials (Declaration of Helsinki, ICH-GCP, including certification by external audit). The evaluation of participants and the analysis of the results were performed by professionals blinded to the group allocation.

2.4. Participants

2.4.1. Recruitment. Patients were recruited in China from December 30, 2011, to January 30, 2013. Informed consent was obtained from each subject, and the Ethics Committee of Affiliated Hospital of Jiangxi Institute of Traditional Chinese Medicine, China, approved the study protocol, authorization number: 2008(11).

2.4.2. Inclusion Criteria. Inclusion criteria were a diagnosis of LDH according to the guiding principle of clinical research on new drugs (GPCRND) [16], at least 10 scores in M-JOA in the baseline period, age 18–65 years, pain occurring in lower back and radiating to the lower limb, completed baseline LDH diary, and written informed consent. Meanwhile, heat-sensitive acupuncture points were found in the triangle region formed with bilateral Dachangshu (BL25) and Yaoshu (Du2) of patients (Dachangshu-Yaoshu-contralateral Dachangshu intraregion).

2.4.3. Exclusion Criteria. Main exclusion criteria were patients with serious life-threatening disease, such as disease of the heart and brain, blood, vessels, liver, kidney, and hematopoietic system, pregnant or lactating female, and psychotic patients. We also excluded patients with a single nerve palsy, or cauda equina nerve palsy, manifested as muscle paralysis or having rectum or bladder problems; complicated with lumbar spinal canal stenosis and space-occupying lesions or for

other reasons; complicated with lumbar spine tumors, infections, tuberculosis; complicated with moxibustion syncope and unwilling to be treated with moxibustion; patients do not sign informed consent.

2.5. Study Interventions. We developed the study interventions in a consensus process with China acupuncture experts and societies. Physicians trained and experienced (at least five years) in acupuncture delivered the interventions. All treatment regimens were standardized between four centers practitioners via video, hands-on training, and internet workshops. In the moxibustion groups, 22 mm (diameter) × 120 mm (length) moxa-sticks (Jiangxi Traditional Chinese Medicine Hospital, China) were used. The patient was usually in the comfortable supine position for treatment, with 24°C to 30°C temperature in the room.

2.5.1. Heat-Sensitive Moxibustion Group. For the heat-sensitive moxibustion group, moxibustion treatment was defined as burning a moxa-stick with the patient lying on his or her back. The moxa-sticks were lit by the therapist and held over the region among two Dachangshu (BL25) and Yaoshu (Du 2) of patients. The moxa-stick suspended at an approximate distance of 3 cm was used to search for acupuncture points showing the heatsensitisation phenomenon. The following patients sensation suggested the special heat-sensitization acupuncture points: heat penetration, patients reporting heat penetrating from the skin into subcutaneous tissues; heat expansion, heat expanding away from the stimulation site to surrounding cutaneous and subcutaneous tissues; heat transmission, patients perceiving a stream of heat conducting in certain directions or perceiving heat in some body regions or into the joint cavity; nonthermal sensations, instead of thermal sensations, some patients perceiving aching, heaviness, pain, numbness, pressure, or cold in local or distant locations of stimulation. When such an acupuncture point was found, the therapists marked the point. We tried our best to seek all the special acupuncture points in each patient by the repeated manipulation.

The therapists began to treat patients from the most heat-sensitive intensity acupoint. Treatment sessions ended when patients felt the acupoint heat-sensitization phenomenon had disappeared. Generally speaking, one point was selected each time. One point was treated 30~60 minutes. Patients received the treatment for two times daily in the first four days and for one time daily in remaining ten days. The whole treatment contained 18 sessions over 14 days.

2.5.2. Conventional Moxibustion Group. A licensed doctor performed fixed acupuncture point moxibustion. Common practices were similar to the first group. The different manipulation was that the therapists carry out warming moxibustion in traditional acupuncture point, selecting Dachangshu (BL25), Weizhong (BL40), and A-shi Xue. One point is treated 15 minutes a time. The whole process of moxibustion took about 45 minutes for each session. Patients usually felt local warmth without burning pain and might experience mild hyperemia in the local region. The sensation of acupuncture point heat-sensitization phenomenon was not pursued and not avoided in the treatment. Patients received the treatment for two times daily in the first four days and for one time daily in the remaining ten days. The whole treatment contained 18 sessions over 14 days.

2.5.3. Conventional Drug Plus Acupuncture Group. For conventional drug, patients received the 20% mannitol (250 mL, intravenously) and Voltaren tablets (75 mg, 2 times a day) in the first 3 days. Voltaren tablets were continued to be used in the subsequent 11 days. At the same time, acupuncture needles were used and acupuncture points selected from Bladder Meridian of Foot-Taiyang and Gallbladder Meridian of Foot-Shaoyang. Acupoints included Dachangshu (BL25), Yaojiaji (EX-B2), Huantiao (GB30), Weizhong (BL40), Yanglingquan (GB34), Xuanzhong (GB39), and Qiuxu (GB40). We selected bilateral acupoints located in waist and ipsilateral acupoints located in lower limbs. Needles remained in acupuncture point for 30 minutes. Patients received the acupuncture needle treatment one time/day in two weeks for a total of 14 sessions over 14 days.

2.6. Outcome Measures. Our primary outcome measure was the M-JOA. The JOA has proposed a series of criteria to define patient response in the context of clinical trials of LDH. M-JOA scale is a modified edition of JOA Back Pain Evaluation Questionnaire. According to these criteria, a patient with LDH is assessed for pain, the ability to conduct daily life and work, functional impairment, and particular clinical examinations. M-JOA scores range from 0 to 24, with LDH considered mild (0–9), moderate (10–20), or severe (21 and above). The M-JOA was used as a preference-based measure of health outcome. All patients were assessed before randomization (baseline phase), 2 weeks after randomization, and 6 months after the last treatment. This trial also recorded adverse effects reported by patients during treatment.

We ensured assessor blinding in this trial. Patients were informed not to tell outcome assessors the treatment they received. The outcome assessor was not involved in treatment administration.

2.7. Statistical Methods. Data were analysed on an intention-to-treat (ITT) basis including all randomised participants with at least one measurable outcome report. The statistician conducting the analyses remained blinded to treatment groups. All analyses were conducted using SPSS 11.5. The groups were compared on 2 weeks, with t-tests used to assess changes between baseline and 2 weeks within each arm. ANOVA was used to compare these changes among the three treatment arms of the trial. Where a significance difference was found among the three groups, pair-wise tests were used to determine specifically which groups differed significantly. Student-Newman-Keuls was used for pairwise comparison. All adverse reactions manifested were listed with detailed explanations. A significance level of 5% was used in all analyses.

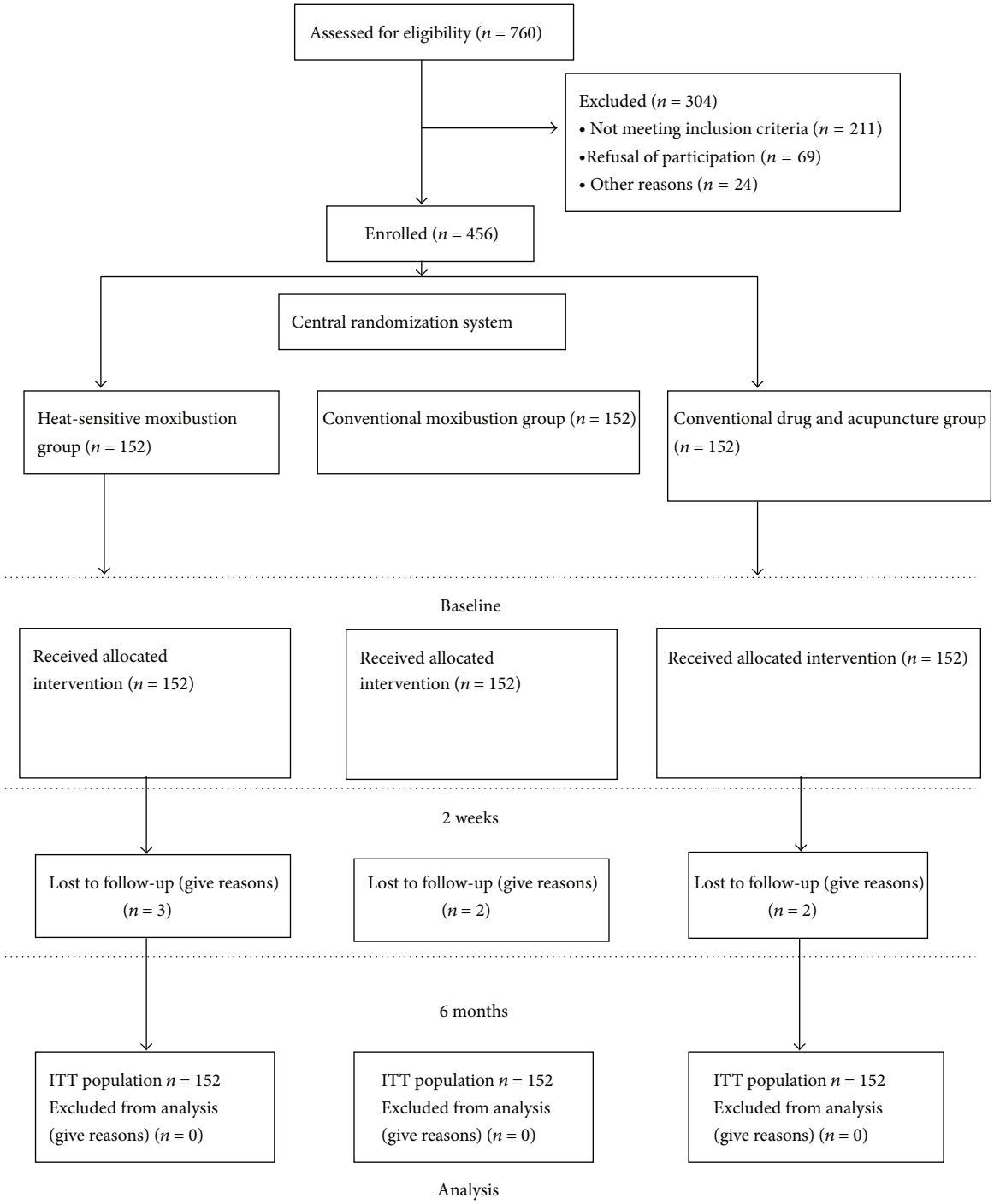

Figure 1: A flowchart of the study process.

3. Results

3.1. Population and Baseline. Participants were recruited from outpatients and inpatients in the four study centers. Patient flow in the trial was presented in Figure 1. After screening 760 patients, 456 were randomly assigned to treatment. 304 could not be included in the study, mainly because they did not meet all eligibility criteria. After six months, 7 patients missed. Reasons for missing follow-up data were not contactable. Participants had a mean age of 46.3 years, and 52.4% were female. Table 1 presented the history of LDH of the subjects. The mean M-JOA score was 17.6. Baseline patient characteristics were balanced between the trial arms. There was no difference in attrition rate among the groups at 6-month follow-up ($P > 0.05$, Fisher exact test).

Table 1: Baseline characteristics of participants.

Items	Heat-sensitive moxibustion group	Conventional moxibustion group	Conventional drug plus acupuncture group
Age, mean (SD), years	45.5 (10.6)	47.3 (11.2)	46.6 (10.5)
Age, min~max, years	18~59	20~58	18~59
Age, >60 y, *n* (%)	9 (5.92)	10 (6.6)	9 (5.92)
Sex *n* (%)			
Female	78 (51.3)	80 (52.6)	81 (53.3)
Male	74 (48.7)	72 (47.4)	71 (46.7)
Duration of pain *n* (%)			
<1 m	32 (21.1)	30 (19.7)	30 (19.7)
2~6 m	40 (26.3)	42 (27.6)	43 (28.2)
7~12 m	40 (26.3)	33 (21.7)	31 (20.3)
1~5 y	33 (21.7)	38 (25.1)	40 (26.3)
>5 y	7 (4.6)	9 (5.9)	8 (5.2)
BMI, mean (SD), kg/m′	22.2 (3.3)	22.4 (3.1)	21.1 (4.0)
BMI, min~max, kg/m′	14.3~30.1	16.2~29.2	13.1~28.9
M-JOA score *n* (%)			
Severe	115 (75.6)	113 (73.4)	119 (78.3)
Moderate	37 (24.4)	39 (25.6)	33 (21.7)
M-JOA score, mean (SD)	18.6 (3.8)	17.5 (3.3)	17.2 (4.4)

BMI, Body Mass Index; M-JOA, Improvement Japanese Orthopedic Association (M-JOA) Lumbago Score Scale; SD, standard deviation; LDH, lumbar disc herniation.

3.1.1. Total M-JOA Score. There was a significant reduction in mean M-JOA score from baseline in all three groups ($P < 0.01$). ANOVA test showed significant difference in the three groups at both time points. Mixed-effects model analysis (q-test) showed that subjects in the heat-sensitive moxibustion group had significantly greater reduction in M-JOA scores than those in conventional moxibustion group or conventional drug plus acupuncture group at 2 weeks and 6 months; however, there was no significant difference between conventional moxibustion and conventional drug plus acupuncture at both time points (Table 2).

3.2. Moxibustion Time in the Heat-Sensitive Moxibustion Group. Different from the conventional moxibustion group, moxibustion dose was individual in the heat-sensitive moxibustion group. According to the record of individual moxibustion time, the dose differed in terms of patients' conditions and moxibustion sensation, which had been measured about 21~60 minutes in the treatment of LDH. The range of mean moxibustion dose was about 41.13 ± 5.26 minutes in the conventional moxibustion group. We used a linear correlation to measure the strength of a relationship between change in M-JOA score and stimulation duration in the conventional moxibustion group. The Pearson coefficient $r = 0.0006$, showing a poor correlation between the two values.

3.3. Safety. No adverse events were reported in the 456 participants.

4. Discussions

The heat-sensitive moxibustion intervention tested in this study was significantly more effective than conventional moxibustion treatment and significantly more effective than the conventional drug plus acupuncture intervention in patients with LDH. No serious cases of adverse reactions related to treatment were reported. This study had a clear and practical research question with an appropriate trial design, namely, a pragmatic randomized controlled trial, which modelled closely what would happen if patient refers to moxibustion. Compared with available studies of moxibustion for LDH, which included a maximum amount of 120 patients [17–19], our study has a much larger sample size. Other advantages included adherence to current guidelines for acupuncture trials, strictly concealed central randomization, blinded evaluation of statistics and measurement, interventions based on expert consensus provided by qualified and experienced medical acupuncturists, and high follow-up rates. Trial physicians could not be blinded. It was not possible to blind the conventional drug plus acupuncture patients. Therefore, the large and significant difference between HSM and conventional moxibustion and between HSM and conventional drug plus acupuncture could be due to performance bias and detection bias. The results of this study proved the superiority of heat-sensitive moxibustion in patients suffering from LDH. That is, selecting the heat-sensitive acupuncture point obtained therapeutic effect far better than moxibustion at acupuncture point of routine resting states. These heat-sensitive acupuncture points are not fixed, but may, during the progression of disease, dynamically change within

Table 2: Comparison of M-JOA scores.

Variable	Week 2		Month 6	
	Mean (SD)	95% CI	Mean (SD)	95% CI
Group A	3.8 (2.6)	3.4~4.2	3.7 (2.2)	3.3~4.1
Group B	7.9 (3.0)	7.4~8.4	8.9 (3.1)	8.4~9.4
Group C	8.5 (2.9)	8.0~9.0	10.1 (2.9)	9.5~10.6
Comparison between the three groups				
F value	3.8		5.2	
P value	0.016		0.008	
Group A versus Group B				
q value	4.1		5.9	
P value	0.022		0.013	
Group A versus Group C				
q value	5.1		6.7	
P value	0.017		0.002	
Group C versus Group B				
q value	2.0		3.2	
P value	0.146		0.041	

Comparison between the three groups by ANOVA test. Pairwise comparison for the two groups by Student-Newman-Keuls (*q*-test). All data are intended to treat. Each group $n = 152$. SD: standard deviation; M-JOA: Improvement Japanese Orthopedic Association (M-JOA) Lumbago Score Scale; SD: standard deviation; LDH: lumbar disc herniation; Group A: Heat-sensitive moxibustion group; Group B: Conventional moxibustion group; Group C: Conventional drug plus acupuncture group.

a certain range centered on acupuncture points. Several types of heat-sensitization responses might appear alone or in combination. Patients become thermally sensitized to moxibustion stimulation at certain locations on the body, indicated by sensations of strong warmth or heat penetrating into the body (heat penetration), warmth spreading around the stimulation site (heat expansion), warmth conducting in certain directions and reaching some body regions or even internal organs remote from stimulation sites (heat transmission), or other nonthermal sensations [20]. These responses gradually disappear with disease recovery.

In summary, we have provided high-quality evidence that heat-sensitive moxibustion showed significant reduction in symptoms of LDH in the short and long term compared with other two treatments (conventional moxibustion, conventional acupuncture plus medicine). The importance of the therapeutic relationship providing heat-sensitive acupuncture point should not be underestimated in the moxibustion therapy. Therefore, the success of this project is more than providing the efficacy of heat-sensitive moxibustion as a treatment modality in patients with LDH. The findings will be helpful to provide better therapeutic options to enhance the efficacy of moxibustion and to perfect acupuncture point heat-sensitive theory.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Mingren Chen and Rixi Chen obtained fund of the research project. Jun Xiong wrote the final paper. Rixin Chen, Tongsheng Su, Jianhua Sun, Meiqi Zhou, Zhenhai Chi, Dingyi Xie, and Bo Zhang contributed to the trial implement. All authors read and approved the final paper. Rixin Chen and Mingren Chen contributed equally to this work.

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Research Article

Investigation of Hepatic Blood Perfusion by Laser Speckle Imaging and Changes of Hepatic Vasoactive Substances in Mice after Electroacupuncture

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The study was conducted to observe the effect of electroacupuncture (EA) on hepatic blood perfusion (HBP) and vascular regulation. We investigated 60 male anesthetized mice under the following 3 conditions: without EA stimulation (control group); EA stimulation at Zusanli (ST36 group); EA stimulation at nonacupoint (NA group) during 30 min. The HBP was measured using the laser speckle perfusion imaging (LSPI). The level of nitric oxide (NO), endothelin-1 (ET-1), and noradrenaline (NE) in liver tissue was detected by biochemical methods. Results were as follows. At each time point, HBP increase in ST36 group was higher than that in the NA group in anesthetized mice. HBP gradually decreased during 30 min in control group. The level of NO in ST36 group was higher than that in NA group. The level of both ET-1 and NE was the highest in control group, followed by NA group and ST36 group. It is concluded that EA at ST36 could increase HBP possibly by increasing the blood flow velocity (BFV), changing vascular activity, increasing the level of NO, and inhibiting the level of ET-1 in liver tissue.

1. Introduction

Clinically, acupuncture has remarkable effects on various liver diseases. Acupuncture and moxibustion treatment can effectively improve immunity of patients with hepatitis and lower blood lipids of patients with fatty liver and improve their clinical symptoms. It also can relieve pain and reduce the side effects of radiotherapy and chemotherapy in patients with liver cancer, thus enhancing the quality of life and prolonging the life [1–3]. In addition, it is proved that acupuncture and moxibustion have favorable regulative actions on blood circulation in a multiway, multilevel, multilink, and multisubstance manner [4]. It is reported that acupuncture treatment can improve hemorheology and vasoactive substances, such as thromboxane A_2(TXA_2), prostaglandin I_2(PGI_2), endothelin (ET), and atrial natriuretic factor (ANF) [5]. Zhang et al. [6] reported that blood supply in the stomach of ischemia-reperfusion is improved and promoted by EA stimulation. Therefore, the increase of visceral blood perfusion (BP) is an important effect of acupuncture and a basis in the acupuncture treatment for visceral diseases [7, 8].

The hepatic circulation (HC) is very rich. Reports suggest that HBP have a significant association with visceral disorders. Leveson et al. [9] reported that gastrointestinal cancer patients with simultaneous liver metastasis exhibited a high hepatic arterial blood flow (BF). Leggett et al. [10] reported that colorectal cancer patients with simultaneous liver metastases revealed that the hepatic arterial BF was significantly increased and the portal BF was decreased. HBP can be a potential biomarker for predicting clinical progression or outcomes of cancer patients [11, 12]. In addition, computed tomography perfusion (CTP) had been successfully applied in a variety of clinical conditions of the liver. It was detected that HBP was decreasing in hepatocirrhosis condition [13, 14]. Therefore, we got the idea that HBP would be an important biomarker for understanding the mechanism of the effects of acupuncture on HBP for the use of acupuncture therapy in liver diseases. In this study, LSPI technique was used to

display the HBP in mice before and after EA and analyze the time-effect relationships between EA and HBP. At the same time, the relationships between neurotransmitter, vasoactive substances, and the HBP changes were investigated in order to explore the mechanism of the EA effects on HC.

2. Materials and Methods

2.1. Animals and Groups. This study utilized 60 healthy adult male Kunming mice, weighing 21 ± 5 g, average age of 3 months, provided by the Animal Experiment Center, Academy of Military Medical Sciences (China). The 60 mice were randomly allocated to three groups: ST36 group, NA group, and control group, 20 mice in each group. All experimental procedures were approved by the Ethical Committee of Academy of Medical Sciences and were conducted in accordance with the internationally accepted principles for laboratory animal use and care.

2.2. Electroacupuncture. For the ST36 group, bilateral ST36 which was located at the posterolateral knee of hind limbs, about 2 mm below the fibular head, were stimulated with $32^{\#}$ needle (0.18 × 13 mm) to 3 mm deep. Then, the needles were connected to the EA device (Hanshi Pain Healing Device, Hanshi-100A; Nanjing Jisheng Medical Technology Company, Nanjing, China). The stimulation time was 30 min, the current intensity was 5 V, and the pulse frequency was 50 Hz. For the NA group, the bilateral nonacupoints which were located at medial to ST36 and close to the margin of tibia were stimulated. And the method of EA was the same as that for ST36 group. For the control group, acupuncture was not done.

2.3. Preparation of In Vivo Mouse Hepatic Model. The mouse was anesthetized by intraperitoneal injection of 2% pentobarbital sodium (2.5 mg/kg, Sigma-Aldrich, St. Louis, USA) 30 min before preparation of in vivo mouse hepatic model. Under anesthesia, the mouse was fixed on a plank in supine position. Approximately 1 cm of linear incision below the xiphoid process and along the ventral median line was made. Then the liver tissue was exposed. The underneath liver lobe was separated and plainly placed on the top of a bracket above the abdomen.

2.4. Measurement of LSPI. The mouse model was placed in the experimental constant temperature box whose temperature was kept at 30–32°C and relative humidity was 80–90%. The liver lobe was placed about 28–30 cm below the laser scanner. The box was placed in a shielding chamber without direct sunlight, infrared radiation, and ventilation.

Moor-FLPI laser speckle perfusion (LSP) imager (Moor instruments Ltd, Axminster, UK) was used in this study. The scanning mode was the low density and 25 fps, the time interval was 1 s, exposure time was 20 ms, and 10 frames were continually scanned at each time point (10 frames were averagely processed into single frame to obtain the mean HBP at each time point). The instrument system can simultaneously record the LSP image and the digital coded image of the liver (actual positional image of the examined part). The two images were used to analyze the relationships between the BF distribution and the hepatic surface position.

For ST36 group and NA group, the HBP image was recorded before EA and every 5 min in 5 to 30 min during EA. For control group, the HBP image was recorded every 5 min in 0 to 30 min (Figure 5). Then the HBP images were saved and analyzed by the Image Review program of Moor-FLPI-V2.0 software. The location, range, and degree of the HBP of three groups were compared at each time point. The round region of interest (ROI) with the same area in each LSP image was selected for measuring the HBP.

2.5. Determination of Hepatic Vascular Regulators. After the HBP image was recorded, the scanned lobe of liver (about 0.5 g) was taken and homogenized with adequate 0.89% cold saline and centrifuged. Then, the supernatant was taken to detect the level of NO with nitric acid reductase and the level of ET-1 with radioimmunoassay (The NO & ET-1 Assay Kits, Beijing Sinouk Institute of Biological Technology, China; r-911 Radio-immune Counter, Corporation of Industry and Commerce, University of Science and Technology of China, China) and the level of NE with enzyme-linked immunoabsorbent assay (NE Assay Kit, R&D Systems, USA; STET FEX 2100 Enzyme Labeling Meter, Awareness Technology Inc, USA).

2.6. Statistical Analysis. The mean HBP was, respectively, calculated with perfusion unit (PU for short) as the unit. The value of HBP at each time point was expressed as mean ± standard deviation. SPSS13.0 software was used for statistical analysis. Different values of HBP between the time points for the t-test were generated for each group, and the time-effect relationships of BF changes were analyzed.

The level of ET-1, NO, NE in the liver tissue in each group was expressed as mean ± standard deviation. SPSS 13.0 statistical software was used for paired t-test of the same index between groups. $P < 0.05$ was regarded as significantly different.

3. Results

3.1. Analyses of LSP Images of the Liver in ST36 Group. In ST36 group, before EA stimulation (Figure 1(a)), more light green and yellow areas were displayed on LSP images of the liver, indicating that HBP was lower. After puncturing 5 min, red areas increased. The red area gradually added along with EA, it increased more rapidly around the hepatic portal area and much more slowly in the area close to the hepatic edge area. After 25 min (Figure 1(f)), the crimson area was found in the image; the BP reached the maximum. Afterwards, the red was lighter with an even distribution in the liver (Figure 1).

3.2. Analyses of LSP Images of the Liver in NA Group. Before and after EA, in NA group, the changes of LSP images were similar to that of ST36 group, but the range and degree of the red increase after EA were smaller than that of ST36 group (Figure 2).

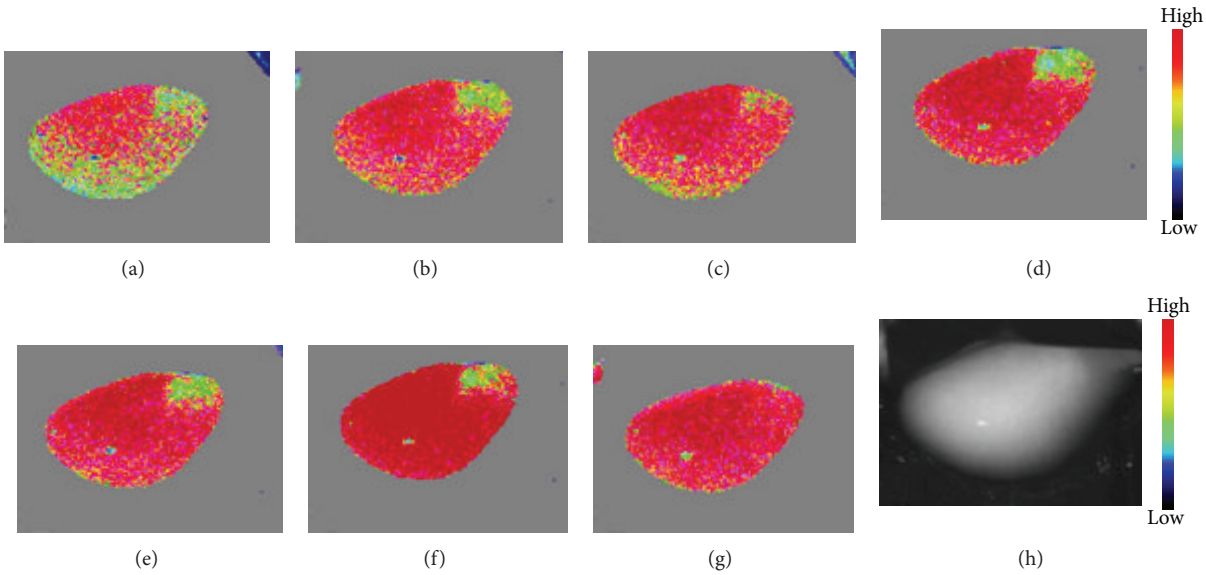

Figure 1: Hepatic LSP images before and after EA at ST36. (a) Before acupuncture; (b) EA for 5 min; (c) EA for 10 min; (d) EA for 15 min; (e) EA for 20 min; (f) EA for 25 min; (g) EA for 30 min; (h) digital coded brightness image of mouse liver. Before EA, LSP on the hepatic surface was displayed as light green, yellow, and red on the image. After EA for 5 min, the red areas were increased on the image immediately. When EA time was prolonged, the red areas were further extended while the light green and yellow regions gradually decreased.

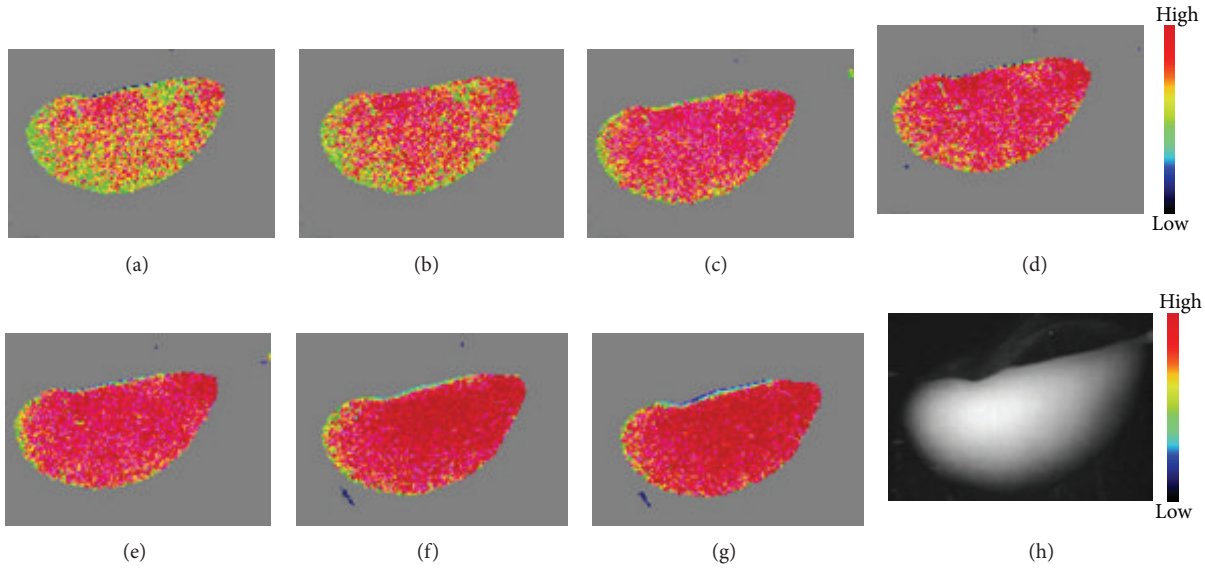

Figure 2: Hepatic LSP images before and after EA at nonacupoint. (a) Before acupuncture; (b) EA for 5 min; (c) EA for 10 min; (d) EA for 15 min; (e) EA for 20 min; (f) EA for 25 min; (g) EA for 30 min; (h) digital coded brightness image of mouse liver. Before EA, LSP on the hepatic surface was displayed as light green, yellow, and red on the image. After EA for 5 min, the red areas were increased on the image immediately. When EA time was prolonged, the red areas were further increased while the light green and yellow areas gradually reduced and almost disappeared.

3.3. Analyses of LSP Images of the Liver in Control Group. From the LSP image at the starting of investigation (Figure 3(a)), it could be seen that the HBP was rich. The HBP was high in the fan-shaped area close to hepatic portal was displayed dark red on the images, and the HBP was decreased from fan-shaped area to the liver anterior edge region were displayed yellow and light green. As investigation time passed by, the light green and yellow regions were extended, and the red areas were decreased gradually, while HBP reduced slowly (Figure 3).

3.4. Quantitative Analyses of HBP Changes. HBP in each EA group was significantly increased at every time point as

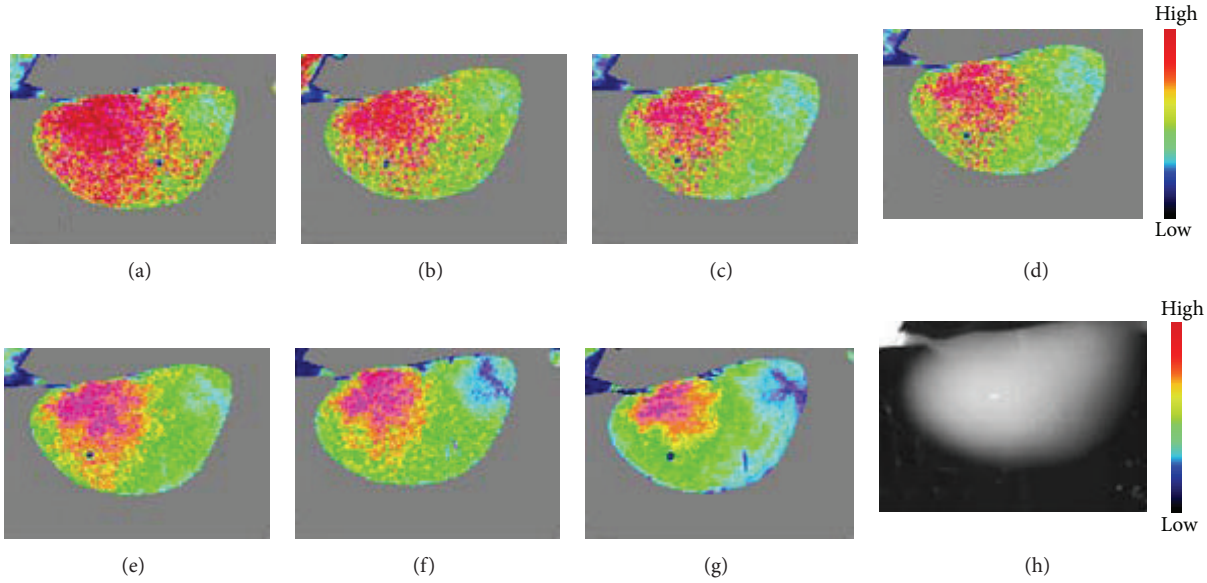

Figure 3: Hepatic LSP images in control group without EA. (a) At 0 min; (b) at 5 min; (c) at 10 min; (d) at 15 min; (e) at 20 min; (f) at 25 min; (g) at 30 min; (h) digital coded brightness image of mouse liver. At beginning of observation, LSP image was displayed as light green, yellow, and red on the image. Moreover the red areas gradually reduced and the yellow and greenish distribution gradually increased along with the observation time extended.

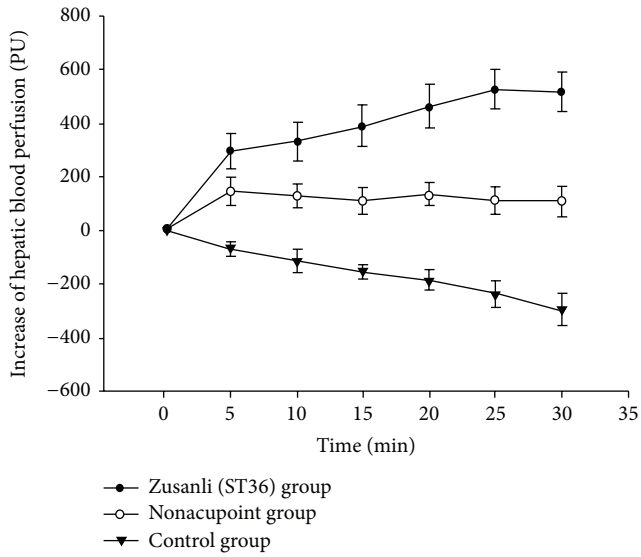

Figure 4: Change of the mean differences of HBP in the three groups. In ST36 or nonpoint group, 0 min was the time before EA; 5–30 min was EA. In control group, 0–30 min was monitoring continually without EA. Data are presented as mean ± SD, $n = 20$ animals for each group.

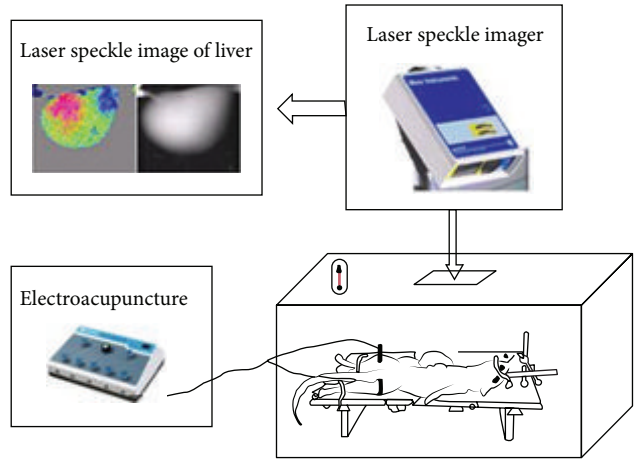

Figure 5: The testing method of HBP using laser speckle imaging.

compared with 0 min. After 5 min, the increase of HBP was 295.54 ± 62.77 (PU), increased by 7.65 ± 1.89%. The highest increase was found at 25 min, which was 527.52 ± 75.96 PU, increased by 14.87 ± 2.35% in ST36 group. After 5 min, the increase of HBP was 88.07 ± 33.36 PU, increased by 2.26 ± 0.92%. The highest increase was found at 25 min, which was 154.04 ± 42.33 PU, increased by 4.14 ± 1.1% in NA group. In control group, HBP was decreased slowly during 30 min (Figure 4). It could be seen from Table 1 that at all time points of EA the increasing rate of HBP in NA group and control group was significantly lower than that of ST36 group ($P < 0.05$).

3.5. Comparison of the Levels in NO, ET-1, and NE. Data describing the levels of NO, ET-1, and NE from three groups were summarized in Table 2. The level of NO was the highest in ST36 group and the lowest in control group. Statistical analysis showed that the level of NO in ST36 group was significantly higher than control group; NA group was significantly higher than control group ($P < 0.05$). The trend was consistent with the HBP.

Table 1: Comparison of the increasing rate of HBP in three groups (mean ± SD, %).

Group	n	$0'$	5 min	10 min	15 min	20 min	25 min	30 min
ST36 group	20	0	7.65 ± 1.89	8.53 ± 2.08	10.67 ± 2.25	12.68 ± 2.49	14.87 ± 2.35	14.54 ± 2.19
NA group	20	0	2.27 ± 0.92†	2.16 ± 0.91†	2.96 ± 1.10†	3.23 ± 1.10†	4.14 ± 1.10†	4.00 ± 1.20†
Control group	20	0	−1.87 ± 0.60†	−3.15 ± 1.10†	−4.25 ± 0.70†	−5.10 ± 1.00†	−6.7 ± 1.50†	−8.16 ± 1.60†

The increasing rate of HBP in the each group was counted. $^{\dagger}P < 0.05$, compared with ST36 group at the same time point.

Table 2: Comparison of levels of NO, ET-1, and NE in three groups (mean ± SD).

Group	n	NO (μmol/gprot)	ET-1 (pg/mL)	NE (ng/mL)
ST36 group	10	70.76 ± 21.33	54.41 ± 7.13	5.30 ± 1.30
NA group	10	39.09 ± 17.83*	121.09 ± 31.07†	8.36 ± 1.06
Control group	10	37.01 ± 14.23*	137.81 ± 24.30†	19.27 ± 7.57

The data of NO, ET-1, and NE contents in liver tissue in the three groups were in Table 2. $^{*}P < 0.05$, compared with ST36 group; $^{\dagger}P < 0.05$, compared with ST36 group.

The level of ET-1 was the highest in control group and the lowest in ST36 group. Statistical analysis showed that the level of ET-1 in NA group and control group was significantly higher than that of ST36 group (both $P < 0.05$)

The level of NE was the highest in control group and the lowest in ST36 group; the trend was similar to the level of ET-1 change but opposite to level of NO change in each group. There were no significant differences in the level of NE among three groups.

4. Discussion

Liver has dual blood supply and is a huge blood bank. In it, many branches of the hepatic artery and portal veins carry the mixed blood to the hepatic sinusoids [15]. When liver functions are damaged, degeneration and necrosis of hepatocytes can cause abnormality of hepatic hemorheology and microcirculation, even in the whole body. On the other hand, abnormality of hepatic hemorheology and microcirculation can induce hepatocyte injury [16, 17]. Traditional Chinese Medicine (TCM) theory holds that "the liver stores the blood," and the liver functions are closely related to the BF. Therefore, it is of a great significance for treatment of liver diseases to improve hepatic microcirculation. Some researches show that EA stimulation can effectively enhance the blood perfusion (BP) of pathologic tissues and organs and improve the physiological function [18–20]. It is reported that the HBP increased after acupuncture treatment detected by the Color Doppler Ultrasonography (CDU) imaging [21]. But the CDU has been applied to detect vascular distribution and BF changes of large vessels and mainly evaluate the vasculopathy and BF state of tissues or organs [22, 23]. Compared with CDU, Moor-FLPI has the following advantages: a use in investigation of microcirculation, a wide range of detection, and noncontact detection [24]. Therefore, it is very suitable to monitor BP of tissues and organs with adequate blood circulation [25, 26]. In this study, Moor-FLPI was used to display HBP in order to accurately record dynamic HBP changes after EA. The LSP images showed that HBP was stable at normal state. After EA stimulation for 30 min, the HBP continuously increased, and the effect on ST36 group was more significant than NA group. Otherwise, the vessels of hepatic portal were dense, BP increased much more in the area, and lower in the region away from the hepatic portal during EA stimulation [27].

NO is a vasodilator with stronger activity in the living body. It mediates relaxation of vascular smooth muscles and vasodilation as a second messenger. Meanwhile NO is involved in maintenance of the physiological functions of the circulatory system such as platelet adhesion and inhibition, matching between BF and ventilation of alveoli, microcirculation of glomerulus and bone marrow, regulation of cardiocerebrovascular tension, protection of myocardial cells, and so on [28–32]. ET-1 is a polypeptide with the strongest vasoconstriction activity. And it is synthesized and released by vascular endothelial cells, myocardium, smooth muscle, and so forth. It can induce vasoconstriction and decrease the blood flow by promoting Ca^{2+} influx in vascular smooth muscle cells and change the form and function of vessel walls by stimulating multiplication of vascular smooth muscle cells, extracellular matrix accumulation, and collagen synthesis [33–35]. Additionally, the functions of NO vasodilation and ET-1 vasoconstriction are antagonistic. The increase of NO would inhibit the synthesis and release of ET-1 in liver tissue [36]. NE is an important sympathetic vasoconstrictive neurotransmitter [37]. EA stimulation can activate the nervous system to adjust the synthesis and release of transmitters, just as NE dose [38, 39].

It could be seen from the results that not only was the HBP significantly increased, but also the vasoactive substances ET-1, NO, and neurotransmitter NE in liver tissue were changed after EA. Then, how did the changes of vasoactive substances in the liver relate to the increase of HBP after EA? According to the results, we think that the more the HBP after EA, the faster the hepatic BFV. Hepatic circulation was promoted by EA. As a result, the friction force between BF and vessels wall was increased. Then, the activities of vascular endothelium cells, vascular smooth muscle, and nerves were motivated by the increasing friction. Therefore, the synthesis and release of vasoactive substances and neurotransmitters must adapt to those changes. At last, in hepatic tissues, vasoconstrictive substance ET-1 and neurotransmitter NE decreased, vasodilative

substance NO increased, blood vessels dilated, and the HBP improved.

ST36 was used primarily for the treatment of digestive system diseases. Clinically, ST36 was an important point in the treatment of liver disease [40–42]. In this study, at the same time point, the increasing rate of HBP and the level of NO in ST36 group were significantly higher than that of NA group and control group. And the level of the ET-1 in ST36 group was significantly lower than that of other groups ($P < 0.05$). It was suggested that the effects of EA on HC in ST36 were superior to nonacupoint. Moreover, the effect of EA on increasing HBP mainly was produced by increasing NO content and inhibiting ET-1 in liver tissue. Additionally, the mechanisms of HBP would be studied from part of vasoactive substances in this study. What would happen in the other side after EA also waits for further study.

Many clinical practices proved that acupuncture had biphasic modulation effects. It can induce abnormal physiological functions to normal levels. There are obvious differences between the effects of acupuncture on pathological state and health state. In this paper, increasing of HBP after EA was detected in health mice. It suggested that EA had an effect on HBP. Based on the result, we will study the effect of EA on HBP of liver injury model mice in the next step.

5. Conclusion

The effect of EA on HC was displayed using LSPI for the first time in this study. The results showed that EA at ST36 could modulate vascular activity of liver tissue and hence obviously increase HBP, providing the new animal experimental evidence for clinical effect of acupuncture on HC and mechanisms of vasoregulation. Meanwhile, it confirmed the TCM theory that acupuncture could promote Qi and blood circulation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

A Unique Electrical Thermal Stimulation System Comparable to Moxibustion of Subcutaneous Tissue

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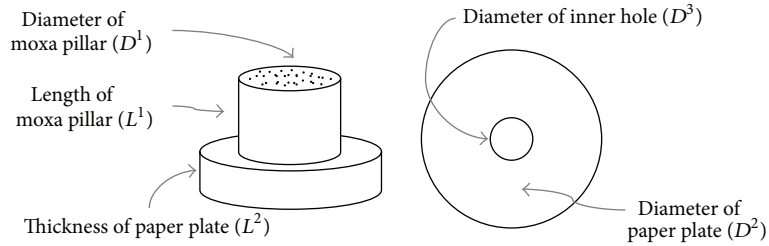
Moxibustion strengthens immunity and it is an effective treatment modality, but, depending on the material quantity, shape, and composition, the thermal strength and intensity can be difficult to control, which may cause pain or epidermal burns. To overcome these limitations, a heat stimulating system which is able to control the thermal intensity was developed. The temperature distributions on epidermis, at 5 mm and 10 mm of depth, in rabbit femoral tissue were compared between moxibustion and the electric thermal stimulation system. The stimulation system consists of a high radio frequency dielectric heating equipment (2 MHz frequency, maximum power 200 W), isolation probe, isolation plate, negative pressure generator, and a temperature assessment system. The temperature was modulated by controlling the stimulation pulse duty ratio, repetition number, and output. There were 95% and 91% temperature distribution correlations between moxibustion and the thermal stimulus at 5 mm and 10 mm of depth in tissue, respectively. Moreover, the epidermal temperature in thermal stimulation was lower than that in moxibustion. These results showed that heat loss by the electric thermal stimulation system is less than that by the traditional moxibustion method. Furthermore, the proposed electric thermal stimulation did not cause adverse effects, such as suppuration or blisters, and also provided subcutaneous stimulation comparable to moxibustion.

1. Introduction

Complementary and alternative medicine has newly emerged among healthy individuals as well as patients in recent years. This treatment includes adjuvant, subcultural, and unorthodox therapies, along with prevention, diagnosis, and treatment outside of traditional western medicine [1]. Among the available alternative therapies, acupuncture and other eastern medicines are important components of disease prevention and treatment. Numerous clinical and pathologic studies have been performed in Korea as well as other countries and have been applied in the treatment of many diseases. However, acupuncture knowledge has progressed primarily owing to clinical experience rather than objective data. Clinical acupuncture studies are lacking; notably, the term acupuncture is virtually unrecognized in eastern medicine. Acupuncture research has focused on experimental studies, and analysis of its clinical effects on the body is lacking. Moxibustion is a traditional eastern treatment that places burning wormwood directly onto the skin or a buffer layer. The heat reportedly stimulates the acupuncture point, accelerates circulation, and enhances immunity.

Current reports show that moxibustion effectively treats poor circulation, chronic urticaria, and chronic cough [2–4], but other studies report unintended adverse effects from the direct stimulation including pain, blistering, and suppuration [5, 6]. These adverse effects are caused by the difficulty in modulating the heat stimulus, which varies according to the material quantity, shape, and composition. Therefore, an easily controlled system minimizing the adverse effects associated with moxibustion is needed.

Previous moxibustion studies only evaluate the specific heat characteristics or placebo effect. Manufactured systems based on currently available data cannot replicate moxibustion but instead provide simple thermal stimulation. For example, high-frequency thermal stimulation provides topical anesthesia during skin treatment and thermal-based tumor removal [7, 8]. However, the currently available



Moxa	D^1 (mm)	D^2 (mm)	D^3 (mm)	L^1 (mm)	L^2 (mm)	Weight (g)
Type 1	7.33 ± 0.17	11.04 ± 0.11	3.08 ± 0.25	7.94 ± 0.22	3.61 ± 0.18	0.38 ± 0.04

Figure 1: Schematic illustration of the commercial indirect moxibustion apparatus used in the present study. Heat is transferred through the cylindrical moxa pillar (top left). The paper plate (top right) serves as a buffer between the pillar and skin and is similarly cylindrical with a central hole. Moxa pillar and paper buffer dimensions are shown (bottom).

method generates intense heat on the skin surface, and the electrode may cause burns; thus, the modality cannot provide heat comparable to that during moxibustion. In a similar study evaluating thermal stimulation as an alternative to moxibustion, the thermal distribution was examined in pig tissue specimens and phantoms. Yet, the study failed to account for vasculature effects and species-specific characteristics and thus its performance in humans is unknown [9].

The present study describes a novel system and protocol with the goal of minimizing the limitations associated with traditional moxibustion, including epidermal burns and poor controllability. An electrical thermal stimulation system targeting the subcutis in anesthetized rabbits was developed and a protocol minimizing heat stimulus was designed to obtain a temperature distribution similar to moxibustion.

2. Materials and Methods

2.1. Moxibustion. Moxibustion can be performed in several ways; among these are the direct method, which directly contacts the skin, and the indirect method, which places a buffer layer of ginger, garlic, and salt between the skin and apparatus. The direct method must be performed by an expert, but the indirect method can be performed by anyone, and thus is the focus of the present study. The commercial moxibustion apparatus includes an air or paper buffer layer. Figure 1 illustrates the apparatus shape and size used in this present study. A paper buffer layer was used.

2.2. Animal Subjects. Five male New Zealand white rabbits aged 18 months and weighing 3.6 ± 0.2 kg were evaluated. The experiment was performed on the left and right femoral skin five times per side. Subjects were anesthetized using combined tiletamine/zolazepam (Zoletil 0.1 mL/kg) and xylazine (Rompun 0.03 mL/kg) administered intraperitoneally; the fur was removed from the target site, and heat stimulation was applied. All animal use and protocols were approved by the Institutional Animal Care and Use Committee of Yonsei University (IACUC).

2.3. Electrical Thermal Stimulation System. The electrical thermal stimulation system was constructed from a clinical high-frequency dielectric heating equipment commercialized for pain relief (Hardville, South Korea). The electrical thermal stimulation generates heat by emitting high-frequency pulses that focus heat energy onto the target tissue. The system comprises a high-frequency dielectric heating equipment, isolation probe, isolation plate, thermometer, and a system control unit; a schematic diagram is shown in Figure 2. The system generated a high dielectric 2 MHz frequency and a maximum 200 W power; an insulating coating minimizes the risk of electric shock. The high frequency was assessed by a medical optical temperature analysis unit (m3300, LumaSense, USA), which determined the subcutaneous temperature distribution. A noncontact infrared sensor (MLX90614, Melexis, Belgium) measured the subcutis and probe temperatures during thermal stimulation. Temperature distribution data were compiled and analyzed using LabVIEW (Ver.8.6, National Instrument, USA) for Windows.

The subject was placed between the positive probe (+) and negative plate (−), and a stimulation protocol mimicking the moxibustion temperature distribution was applied. A 2 MHz frequency and maximum 200 W power output were applied. To minimize the edge current, the probe was constructed as a cylinder, and the 1.5 cm probe diameter simulated a surface area similar to that in moxibustion. Electric shock was minimized by applying a 0.28 mm polyurethane coating onto the probe and using an isolated plate. During conventional moxibustion, there was a 4 min flame period followed by a 14 min thermal effect; electrical stimulation was applied for 18 min to the target site. During thermal stimulation, the skin surface and probe temperatures were monitored and electric shock was minimized using a noncontact infrared

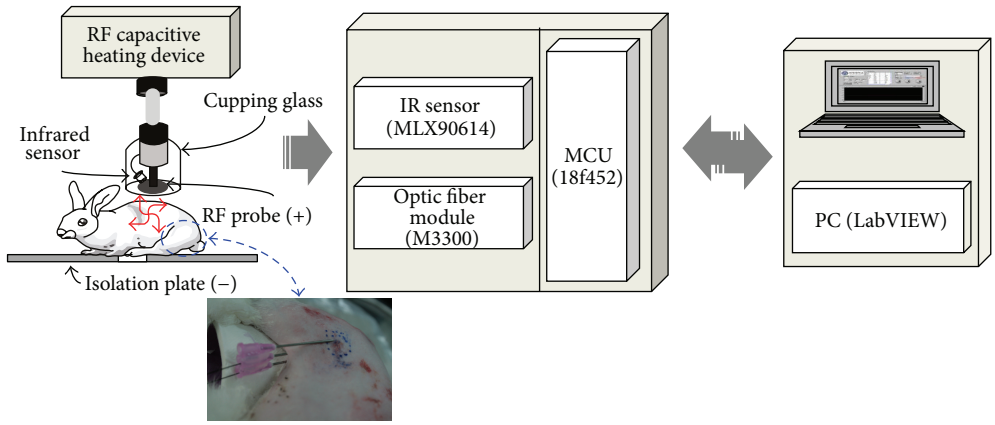
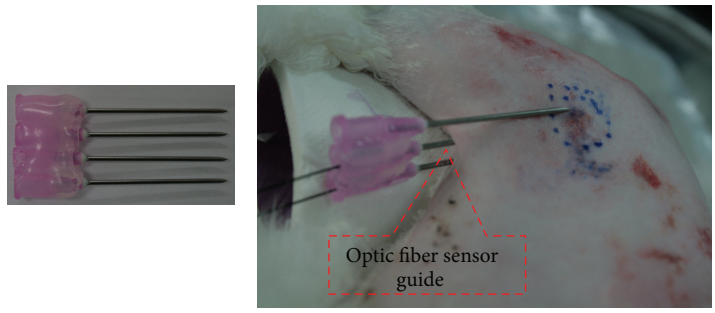
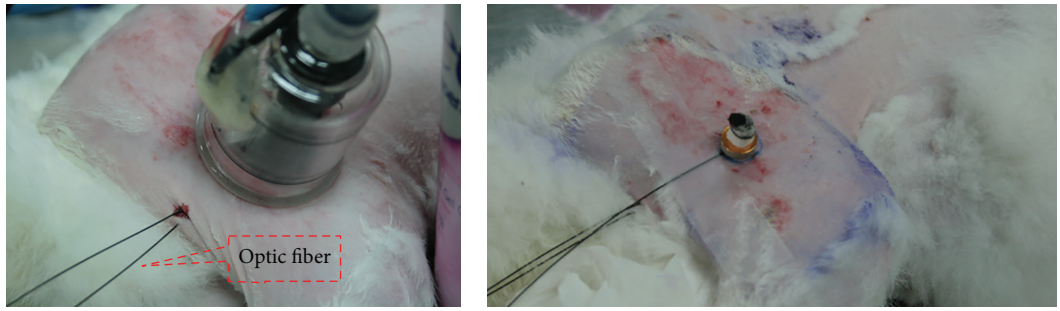


FIGURE 2: Schematic diagram of the electric high-frequency stimulation system.



(a) Inserted the optic fiber



(b) RF stimulate (c) Moxibustion

FIGURE 3: Constructed thermal stimulation and temperature assessment during electric thermal stimulation and moxibustion. (a) Insertion of the optic fiber sensor guide, (b) temperature assessment during electrical thermal stimulation, and (c) temperature assessment during moxibustion.

temperature sensor (Figure 2). Thermal skin damage reportedly occurs at 44°C; therefore, to minimize tissue destruction, the electric unit temperature did not exceed 42°C [10, 11].

2.4. Temperature Assessment. During electrical thermal stimulation, conventional transducers and contact sensors can malfunction due to ambient electrical field and loud noises; thus, assessing temperature in real time is difficult. Therefore, an optical temperature analyzer and noncontact infrared sensor were used to measure temperature distribution during thermal stimulation, summarized in Figure 3. The optical temperature analyzer (Luxtron) is not affected by strong electrical fields and has high repeatability and accuracy. After anesthetic induction, a needle was inserted 5 mm subcutaneously over the femoral region, and the optical temperature probe fiber was inserted into the needle; a fiber sensor guide was then inserted 30 mm subcutaneously (Figure 3(a)). The temperature was measured at the optic fiber endpoint and transmitted through the fiber without outside interference. The thermal probe was attached to the skin using negative pressure applied to the cup using a negative pressure motor (KPV36E, Koge Electronics, Korea) and a solenoid valve maintaining a consistent 10–12 kPa pressure.

As Figure 3 illustrates, the optic fiber sensor guide cannot be inserted at the same anatomic location with each temperature measurement. Therefore, to minimize temperature

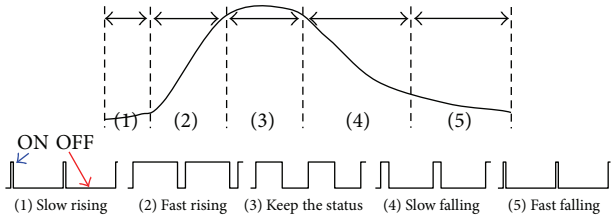


Figure 4: Schematic electric thermal stimulation template protocol. The protocol included ON periods, when high-frequency thermal stimulation was applied, and OFF periods without thermal stimulation; each phase initiates immediately after the previous concludes. The protocol comprised (1) steady temperature increase by shortening the ON period relative to the OFF period, (2) rapid temperature increase by lengthening the ON period, (3) consistent temperature by maintaining equal ON/OFF periods, (4) slow temperature decrease by shortening the ON period relative to the OFF period, and (5) rapid temperature decrease by lengthening the OFF period.

Table 1: Electrical thermal stimulation protocol mimicking the moxibustion temperature distribution.

Phase	ON (ms)	OFF (ms)	REP
1	5	495	80
2	80	420	100
3	499	1	390
4	450	550	45
5	20	980	125
6	10	990	185
7	5	995	150
8	2	998	157
9	1	999	145

ON, application of high-frequency thermal stimulation; OFF, absent stimulation; REP, pulse repetition number. Table 1 shows a protocol for obtaining temperature distribution similar to that of moxibustion at the depth of 5 mm in subcutaneous tissue.

errors with variation of location, the moxibustion and thermal stimulation temperature distributions were measured consecutively.

3. Results

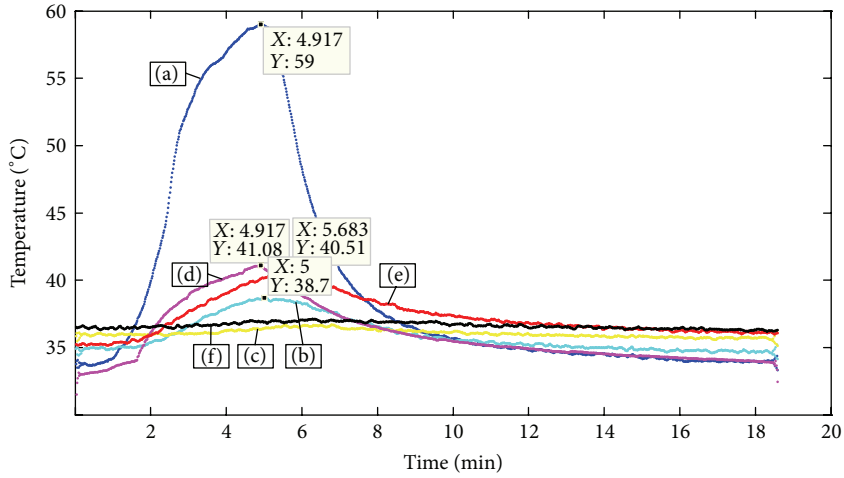
3.1. Stimulation Protocol Design. Prior studies showed that, under repetitive electrical stimulations, the temperature increases and decreases rapidly. However, as shown in Figure 4, the temperature during moxibustion peaked rapidly and then decreased slowly. Therefore, a protocol allowing fine temperature control during electrical thermal stimulation was constructed [12]. A temperature distribution mimicking moxibustion was obtained by repetitive electrical stimulations, controlling the ON/OFF ratio and modulating the ON/OFF repetition, summarized in Figure 4.

An electrical thermal protocol was devised from the template illustrated in Figure 4 to stimulate temperature distributions comparable to that of moxibustion, summarized in Table 1. Phases 1 and 2 correspond to the flame initiation during moxibustion, which slowly applies heat and steadily increases the temperature. Phase 3 corresponds to the phase of rapid temperature rise 5 mm subcutaneously during moxibustion. And phase 4 corresponds to the phase of keeping the temperature 5 mm subcutaneously during moxibustion. During phases 1, 2, 3, and 4, the temperature increases over approximately 5 minutes during moxibustion; the electrical thermal stimulation resulted in a temperature distribution comparable to that with moxibustion. Phases 5, 6, 7, 8, and 9 correspond to the moxibustion phase during which the flame dissipates, and the temperature steadily decreases.

3.2. Temperature Distributions during Moxibustion and High-Frequency Stimulation. Figure 5 compares the temperature distributions in epidermis and subcutis after applying electrical thermal stimulation and moxibustion. Three points (epidermis, 5 mm, and 10 mm in depth) were selected experimentally since the change of temperature rarely occurred at over 10 mm deep during moxibustion in previous study. During moxibustion, the epidermal temperature peaked at 58.41 ± 0.28°C, but the temperature at 5 mm subcutaneously was 39.25 ± 0.55°C, indicating a large heat loss. During electrical thermal stimulation, the maximum epidermal temperature was comparably lower at 39.46 ± 0.29°C and the temperature at 5 mm subcutis was 39.24 ± 0.33°C, indicating minimal heat loss. Similar temperature distributions at 5 mm and 10 mm subcutaneously were observed between moxibustion and electric thermal stimulation, and then the correlation coefficients of $R = 0.95$ and $R = 0.91$ were shown, respectively.

4. Discussion and Conclusion

This study sought to address the limitations of moxibustion due to burns and poorly controlled intensity by designing an electrical thermal stimulation system and protocol that can provide an effective thermal subcutaneous stimulus on an anesthetized rabbit. The subcutaneous temperature distributions between the two techniques were comparable, confirmed by the data correlation coefficients ($R = 0.95$ at 5 mm subcutis and $R = 0.91$ at 10 mm subcutis). The infrared hot plate is the only currently available heat stimulation method comparable to moxibustion. The technique focuses heat epidermally, but significant heat is lost subcutaneously [13]. The moxibustion temperature distribution changed significantly between the epidermis and 5 mm subcutis, with a peak temperature change of up to 19.16°C. Comparatively, the electrical stimulation temperature distribution using the protocol summarized in Table 1 showed minimal heat loss of only 0.22°C between the epidermis and 5 mm subcutis. Despite the electrode mechanism targeting high heat epidermally, the unit effectively transmitted heat subcutaneously. Furthermore, the technique avoided the adverse effects such as suppurative blisters typically associated with moxibustion; yet, it also achieved a similar subcutaneous temperature distribution. Unlike the previous studies evaluating thermal heat on pig tissue and phantom specimens, the new system



(a) Temperature at rabbit skin surface under moxibustion
(b) Temperature at rabbit 5 mm subcutis under moxibustion
(c) Temperature at rabbit 10 mm subcutis under moxibustion
(d) Temperature at rabbit skin surface under electrical thermal stimulation
(e) Temperature at rabbit 5 mm subcutis under electrical thermal stimulation
(f) Temperature at rabbit 10 mm subcutis under electrical thermal stimulation

Figure 5: Epidermal and subcutaneous temperature distributions during moxibustion and electric thermal stimulation. The temperature was measured during moxibustion at skin surface (blue), 5 mm subcutis (light blue), and 10 mm subcutis (yellow) overlying the femoral region and during electric thermal stimulation at skin surface (pink), 5 mm subcutis (red), and 10 mm subcutis (black).

was applied to rabbits, incorporating the vasculature and subcutaneous characteristics; these findings confirm that electric thermal stimulation comparable to traditional moxibustion can be achieved *in vivo*. Also, we can stimulate subcutis at 20 mm thermally by modifying the protocol, which is ensured in our previous study. The presently described clinical protocol has limitations not being applicable to all moxibustion types. However, this study illustrates the potential of electric thermal stimulation to minimize adverse effects and effectively transmit heat subcutaneously. In further research, this electrical stimulation system should be compared with moxibustion having other types and conditions. Also, we will evaluate the therapeutic effect and the application in human.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Mild Moxibustion Decreases the Expression of Prokineticin 2 and Prokineticin Receptor 2 in the Colon and Spinal Cord of Rats with Irritable Bowel Syndrome

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It has been proven that prokineticin 2 (PK2) and its receptor PKR2 play an important role in hyperalgesia, while mild moxibustion can relieve visceral hypersensitivity in a rat model of irritable bowel syndrome (IBS). The goal of the present study was to determine the effects of mild moxibustion on the expression of PK2 and PKR2 in colon and spinal cord in IBS rat model, which was induced by colorectal distension using inflatable balloons. After mild moxibustion treatment, abdominal withdrawal reflex (AWR) scores were assessed by colorectal distension; protein and mRNA expression of PK2 and PKR2 in rat colon and spinal cord was determined by immunohistochemistry and fluorescence quantitative PCR. Compared with normal rats, the AWR scores of rats and the expressions of PK2/PKR2 proteins and mRNAs in colon and spinal cord tissue were significantly increased in the model group; compared with the model group, the AWR scores of rats and the expressions of PK2/PKR2 proteins and mRNAs in colon and spinal cord tissue were significantly decreased in the mild moxibustion group. These findings suggest that the analgesia effect of mild moxibustion may be associated with the reduction of the abnormally increased expression of the PK2/PKR2 proteins and mRNAs in the colon and spinal cord.

1. Introduction

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by abdominal pain or discomfort accompanied by changes in bowel habits [1–3]. The incidence of IBS in China is 5–20.72% [4, 5]. Clinical symptoms such as abdominal pain and diarrhea severely affect the quality of life for IBS patients. Studies have shown that visceral hypersensitivity and abnormal gastrointestinal motility are the main pathogenic factors that cause clinical symptoms such as abdominal pain and diarrhea in IBS patients [6–8]. Reducing visceral hypersensitivity and improving gastrointestinal mobility are the major strategies to improve quality of life for IBS patients.

Prokineticins (PKs) are newly identified peptide family members in mammals. The PK family mainly includes PK1 and PK2. PK1 is the human homolog of a nontoxic protein named venom protein A (VPRA) or mamba intestinal toxin 1 (MIT1) isolated from black mamba venom. PK2 is the human homolog of the protein *Bombina variegate* (Bv8) isolated from skin secretions of the toad. Through G protein-coupled receptors termed prokineticin receptors (PKRs), PKs participate in a variety of biological processes in the body, including regulation of gastrointestinal mobility and transmission of pain signals [9–14]. PKs were initially discovered as regulators of bowel function and are a new type of endogenous regulator of gastrointestinal motility that specifically lead to gastrointestinal smooth muscle contraction, as evidenced by

the observation that they specifically induce the contraction of the ileum longitudinal muscle, the basal muscle, and the proximal colon in guinea pigs [15, 16]. Subsequent studies showed that PKs mediate pain signal transmission and sensitize nociceptors. Through spinal cord and primary sensitive neurons, PKs strongly sensitize peripheral nociceptors to thermal, chemical, and mechanical stimuli and are directly involved in nociceptive threshold changes and nociceptive responses caused by noxious stimuli, thus mediating primary and central pain sensitization. Therefore, inhibition or antagonism of PKs/PKRs can relieve pain [17–21].

Currently, the mechanism of pathogenesis of IBS is not fully understood. Investigation into the association between PK/PKR expression and IBS could further elucidate the mechanism of IBS and provide new targets for IBS treatment. Our group established a rat IBS model based on colorectal distension in neonatal rats stimulated by inflatable balloons according to Al-Chaer et al. [22]. Our previous studies showed that (1), compared with normal rats, pain threshold dropped and visceral sensitivity increased as well as PK1 and PKR1 expression significantly enhanced in the colon and spinal cord in IBS model rats; (2) mild moxibustion was effective at treating IBS as it increased the pain threshold, improved visceral hypersensitivity, and decreased PK1 and PKR1 expression in the colon and spinal cord in IBS model rats [23, 24]. These indicated that PKs and PKRs are closely associated with IBS; PK1 and PKR1 are involved in pathophysiology of IBS and mild moxibustion analgesia in IBS.

PKs and PKRs mainly include PK1, PKR1, PK2, and PKR2. Thus far, reports on the association between PK2/PKR2 and IBS are scarce. Based on previous studies, this study further investigated the mechanism of IBS and mild moxibustion in treating IBS from the perspective of PK2/PKR2.

2. Materials and Methods

2.1. Experimental Animals. A total of 42 male neonatal rats (5-day-old) were provided by the Department of Laboratory Animal Science at Shanghai Medical College of Fudan University. The animals were maintained under light-dark conditions (12 h light : 12 h dark) at a room temperature of 20 ± 2°C with a relative humidity of 50–70%. This study was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

After three days of habituation, the neonatal rats were randomly divided into 3 groups: the normal group, the model group (only modeling), and mild moxibustion group (modeling followed by mild moxibustion).

2.2. Establishment of the IBS Model. The chronic visceral hypersensitivity rat model was established according to Al-Chaer et al. [22].

An inflatable balloon was slowly inserted to a depth of 2 cm through the anus along the colorectal physiological curvature. The balloon was distended with 0.2 mL of air for 1 min and was then deflated and withdrawn slowly. The same stimulation procedure was repeated after 30 min. The balloon stimulation was performed twice a day, for 14 days.

2.3. Mild Moxibustion Treatment. After the model was established, rats in the mild moxibustion group began to receive mild moxibustion treatment at 7th week. The diameter of moxa sticks (Nanyang Hanyi Moxa Co., Ltd., Nanyang, Henan province, China) was 0.5 cm. When the moxa sticks were ignited, moxibustion was performed at 2 cm above bilateral Tianshu points (ST 25, 5 cun above the pubic symphysis and 2 cun away from the midline) simultaneously for 10 min. Moxibustion was conducted once a day for a total of 7 days.

2.4. Abdominal Withdrawal Reflex (AWR) Scoring. According to the method of Al-Chaer et al. [22], AWR scoring was conducted on rats within 90 min after 7 moxibustion treatments. Balloons were slowly inserted into the descending colons through the anus, and four intensities, 20, 40, 60, and 80 mmHg, of colorectal distention (CRD) were applied. Each CRD lasted 20 s every 4 minutes and repeated 5 times. AWR score was assessed by two researchers. The data for each animal were averaged for analysis.

The criteria for AWR scoring [22] were as follows: 0, no behavioral response to CRD; 1, brief head movement followed by immobility during CRD; 2, mild contraction of the abdominal muscles but no lifting; 3, strong contraction of the abdominal muscles and lifting of the abdomen without lifting the pelvic structure and scrotum; and 4, body arching and lifting of the pelvic structure and scrotum.

2.5. Preparation of Colon and Spinal Cord Tissue Samples. After AWR scoring, rats were weighed and anesthetized using 3% sodium pentobarbital (0.1 mL/100 g). Six rats in each group were sacrificed, and their colon (3 cm in length, 5 cm above the anus) and spinal cord tissue (at the lumbar enlargement) were collected immediately. The samples were stored in a −80°C freezer for subsequent fluorescence-based quantitative PCR (FQ-PCR) detection. The remaining rats in each group received a rapid left-ventricular perfusion with 250 mL saline until the liver became white, followed by perfusion and fixation with 500 mL 4% paraformaldehyde. After perfusion, 3 cm of colon tissue located 5 cm above the anus and spinal cord tissue at the lumbar enlargement was removed and fixed in 4% paraformaldehyde (for less than 24 h) for subsequent immunohistochemical detection.

2.6. Immunohistochemical Assay for Expression of PK2 and PKR2. Paraffin sections of colon and spinal cord tissue were deparaffinized to water. After incubation with 3% H_2O_2 at room temperature for 20 min, sections were immersed in 0.01 M citrate buffer (pH 6.0) and heated twice in a microwave oven with medium heat until boiling. Then sections were blocked using 5% bovine serum albumin (BSA) at room temperature for 20 min, and the primary antibodies were added dropwise (PK2: rabbit anti-rat polyclonal antibody, 1 : 400, Novus Co., Littleton, USA; PKR2: rabbit anti-rat polyclonal antibody, 1 : 100, Wuhan USCN Life Co., Wuhan, China) in a moisture chamber at 4°C overnight followed by incubation in a 37°C incubator for 2 h. Corresponding secondary antibody (biotinylated goat anti-rabbit IgG, 1 : 100, Wuhan Boster Bio-Engineering Co., Ltd., Wuhan, China)

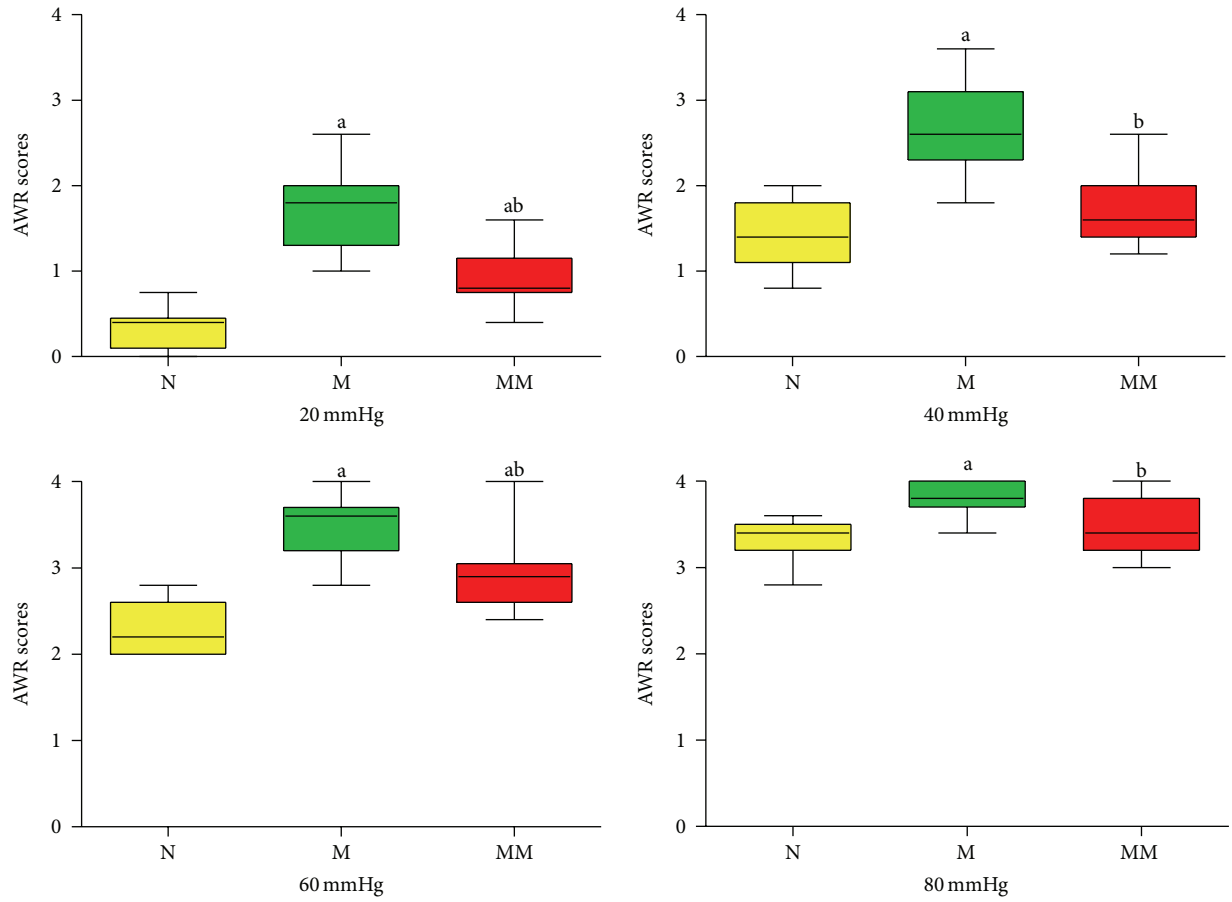


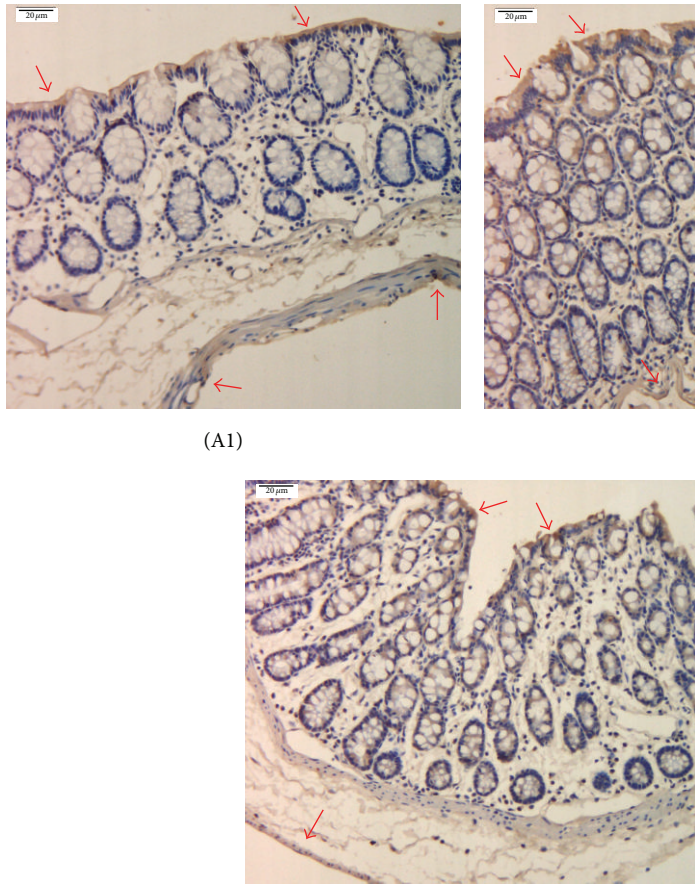
Figure 1: AWR scores of rats in each group. Data are presented as M, min-max. Under the same intensity of CRD stimulation, compared with the normal group: $^{a}P < 0.01$; compared with the model group: $^{b}P < 0.01$. N: normal group; M: model group; MM: mild moxibustion group.

was then added dropwise, and sections were incubated at 37°C for 20 min. After incubation with streptavidin-biotin complex (SABC) (Wuhan Boster Bio-Engineering Co., Ltd., Wuhan, China) at 37°C for 20 min, sections were developed using 3,3′-diaminobenzidine (DAB) (Wuhan Boster Bio-Engineering Co., Ltd., Wuhan, China). After counterstaining with hematoxylin for 1 min, sections were dehydrated, cleared, mounted, and observed under a light microscope (BH2, Olympus, Tokyo, Japan).

Positive signals were represented by a brown-yellow color. Image analysis was performed using the Motic Med 6.0 digital medical image analysis system (Motic Group Co., Ltd., Xiamen, China). Three nonoverlapping fields of each sample were randomly selected, and the data of the integral optical densities of positive signals for each sample were average for analysis. During image analysis process, the intensity of the light source was the same for all samples.

2.7. FQ-PCR Assay for Expression of PK2 mRNA and PKR2 mRNA. Total RNA in colon and spinal cord tissue was extracted using Trizol and reversely transcribed into cDNA according to the manufacturer's instruction manual. The reaction system consisted of 4 μL of 5x reverse transcription buffer, 0.5 μL of oligo (dT), 0.5 μL of dNTPs, 1 μL of MMLV reverse transcriptase, 10 μL of DEPC-treated water, and 4 μL of RNA template; the total volume was 20 μL. The reaction conditions were 37°C for 1 h followed by 95°C for 5 min to inactivate the MMLV reverse transcriptase. The prepared cDNA was used for PCR amplification. The amplification system consisted of 10 μL of 5x PCR buffer, 0.5 μL of upstream primer (F), 0.5 μL of downstream primer (R), 0.5 μL of dNTPs, 0.5 μL of TaqMan fluorescent probe, 1 μL of Taq polymerase, 32 μL of ddH_2O, and 5 μL of cDNA template; the total volume was 50 μL. The amplification process included 40 cycles of 50°C for 2 min, 95°C for 5 min, 95°C for 15 s, and 60°C for 45 s.

The synthesis and purification of probes and primers were provided by Da'an Bio-Technology Co,. Ltd., Qingdao, China. GADPH was used as an internal control. The sequence of the GADPH probe was 5′-CATCTGGGCTACACTAGGACCA-3′; the sequence of the upstream primer for GADPH was 5′-GCTGTTGAGTCACAGGAGCAA-3′, and the downstream primer was 5′-CCGAGGGCCCACTAAAGG-3′. The sequence of the PK2 probe was 5′-TTGCTGCTACCGCTG-CTGCTCACAC-3′; the sequence of the upstream primer for



(A1) (B1)

(C1)

Figure 2: PK2 expression in the colon of chronic visceral hypersensitivity model rats (immunohistochemical detection). Yellow stains denote PK2-positive expression as shown by the arrows. PK2 was mainly expressed in the mucosal epithelium, the proximal glands of the mucosal epithelium, the interstitium, and the muscle layer of the colon. (A1) normal group, (B1) model group, and (C1) mild moxibustion group. Scale bar: 20 μm.

PK2 was 5′-CCTCCACACTGAGAGTCCTTG-3′, and the downstream primer was 5′-GTGCCCCGCTACTGCTAC-3′. The sequence of the PKR2 probe was 5′-TGTGCCTCC-GTCAACTACCTTCGT-3′, the sequence of the upstream primer for PKR2 was 5′-GAGGCGGTCTGGTAATTCATC-C-3′, and the downstream primer was 5′-CTTTCCTGG-GAGCATGGTCAC-3′.

The mRNA expressions of the target genes were analyzed using the ABI Prism 7300 SDS software. The relative mRNA expression of the target gene = $2^{-\Delta CT} \times 100\%$, where ΔCT = CT value of the target gene-CT value of the internal control (GADPH).

2.8. Statistical Analysis. Statistical analysis was performed using the SPSS18.0 software. Statistical description: data are presented as mean ± SD for normal distribution and as M, min-max, for nonnormal distribution. Statistical inference: one-way analysis of variance (one-way ANOVA) was performed if the data followed a normal distribution; the nonparametric test was performed if the data did not follow a normal distribution. If variances were homogeneous, the difference between groups was also compared using the least significant difference (LSD) test; if variances were not homogeneous, the difference between groups was compared using the Games-Howell test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Quantitative Analysis of Experimental Animals. In total, 40 rats were included in the statistical analysis. One rat in the normal group was lost during perfusion and fixation, and one rat of the model group died after anesthesia.

3.2. The Analgesic Effects of Mild Moxibustion on Chronic Visceral Hypersensitivity in IBS Model Rats. The AWR scores of rats in each group after different intensities (20 mmHg, 40 mmHg, 60 mmHg, and 80 mmHg) of CRD stimuli are shown in Figure 1. Compared with the normal group, the AWR scores at all intensities (20 mmHg, 40 mmHg, 60 mmHg, and 80 mmHg) of rats in the model group significantly increased, with $P < 0.01$. Compared with

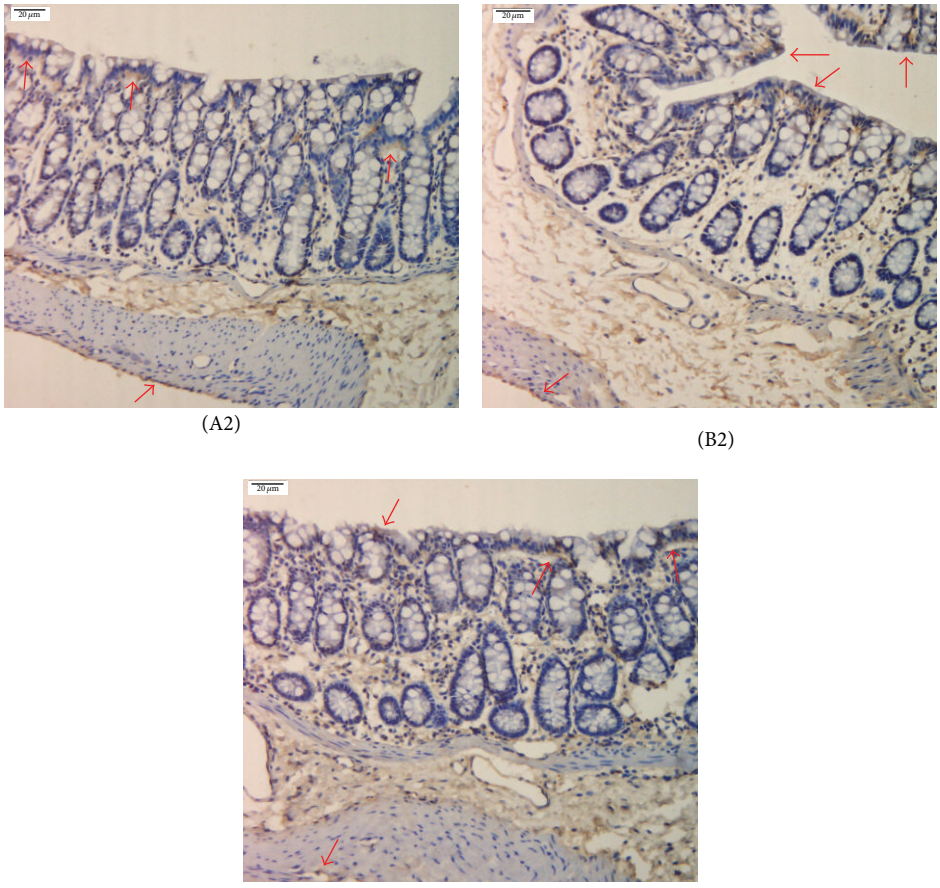


Figure 3: PKR2 expression in the colon of chronic visceral hypersensitivity model rats (immunohistochemical detection). Yellow stains denote PKR2-positive expression as shown by the arrows. PKR2 was mainly expressed in the mucosal epithelium, the proximal glands of the mucosal epithelium, the interstitium, and the muscle layer of the colon. (A2) normal group, (B2) model group, and (C2) mild moxibustion group. Scale bar: 20 μm.

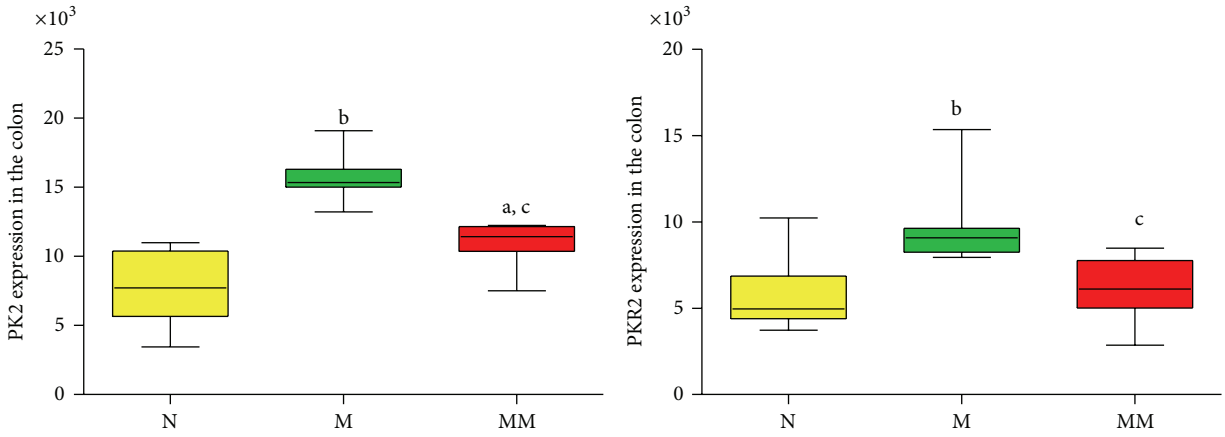
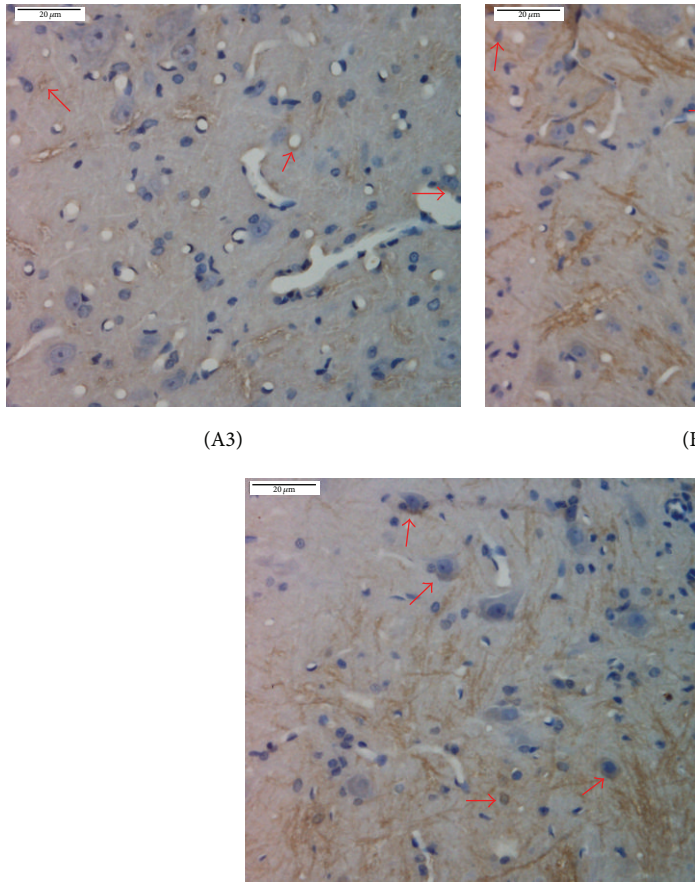


Figure 4: The integral optic density of the expression of PK2 and PKR2 in the colon of each group. Data are presented as M, min-max. Compared with the normal group: $^{a}P < 0.05$, $^{b}P < 0.01$; compared with the model group: $^{c}P < 0.01$. N: normal group; M: model group; MM: mild moxibustion group.



(A3) (B3)

(C3)

Figure 5: PK2 expression in the spinal cord of chronic visceral hypersensitivity model rats (immunohistochemical detection). Yellow stains denote PK2-positive expression as shown by the arrows. PK2 was mainly expressed in the large, medium, and small neurons, vascular endothelial cells, and the interstitium of the spinal cord. (A3) normal group, (B3) model group, and (C3) mild moxibustion group. Scale bar: 20 μm.

the model group, the AWR scores of rats at all intensities (20 mmHg, 40 mmHg, 60 mmHg, and 80 mmHg) in the mild moxibustion group significantly decreased, with $P < 0.01$.

3.3. The Effects of Mild Moxibustion on the Expression of PK2 and PKR2 in the Colons of Chronic Visceral Hypersensitivity Model Rats. Immunohistochemistry showed that compared with the normal group, the expression of PK2 and PKR2 in the colons of the model group significantly increased, with $P < 0.01$. After mild moxibustion treatment, compared with the model group, the expression of PK2 and PKR2 in the colon significantly decreased, with $P < 0.01$ (Figures 2, 3, and 4).

3.4. The Effects of Mild Moxibustion on the Expression of PK2 and PKR2 in the Spinal Cord of Chronic Visceral Hypersensitivity Model Rats. Immunohistochemistry showed that, compared with the normal group, the expression of PK2 and PKR2 in rat spinal cord in the model group significantly increased, with $P < 0.01$. After mild moxibustion treatment, compared with the model group, the expression of PK2 and PKR2 significantly decreased, with $P < 0.01$ (Figures 5, 6, and 7).

3.5. The Effects of Mild Moxibustion on the Expression of PK2 and PKR2 mRNA in the Colon of Chronic Visceral Hypersensitivity Model Rats. The amplification kinetics curves of PK2 mRNA, PKR2 mRNA, and GAPDH mRNA (internal control) in the colon in each group are shown in Figure 8.The results of FQ-PCR demonstrated that, compared with the normal group, the expression of PK2 and PKR2 mRNA in the colon in the model group was significantly increased ($P < 0.05$, $P < 0.01$). After mild moxibustion treatment, the expression of PK2 and PKR2 mRNA in the colon significantly decreased compared with the model group ($P < 0.05$, $P < 0.01$) (Figure 9).

3.6. The Effects of Mild Moxibustion on the Expression of PK2 and PKR2 mRNA in the Spinal Cord of Chronic Visceral Hypersensitivity Model Rats. The amplification kinetics curves of PK2 mRNA, PKR2 mRNA, and GAPDH mRNA (internal control) in the spinal cord in each group are shown in

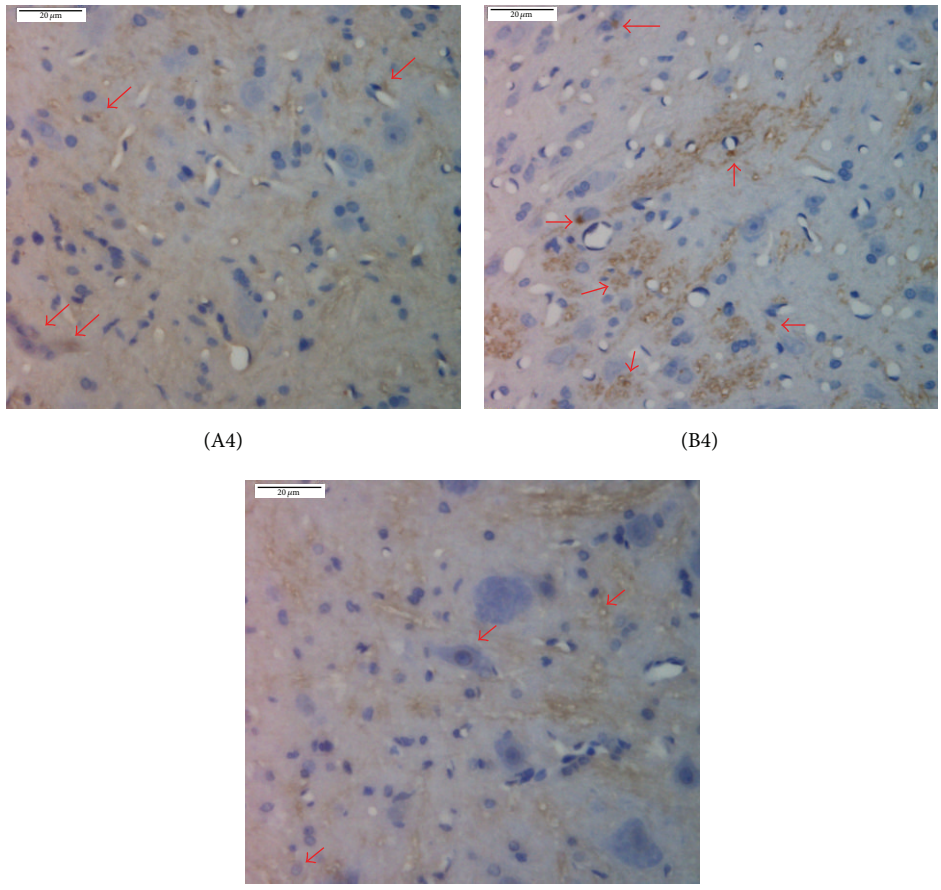


FIGURE 6: PKR2 expression in the spinal cord of chronic visceral hypersensitivity model rats (immunohistochemical detection). Yellow stains indicate PKR2-positive expression as shown by the arrows. PKR2 was mainly expressed in the large, medium, and small neurons, vascular endothelial cells, and the interstitium of the spinal cord. (A4) normal group, (B4) model group, and (C4) mild moxibustion group. Scale bar: 20 μm.

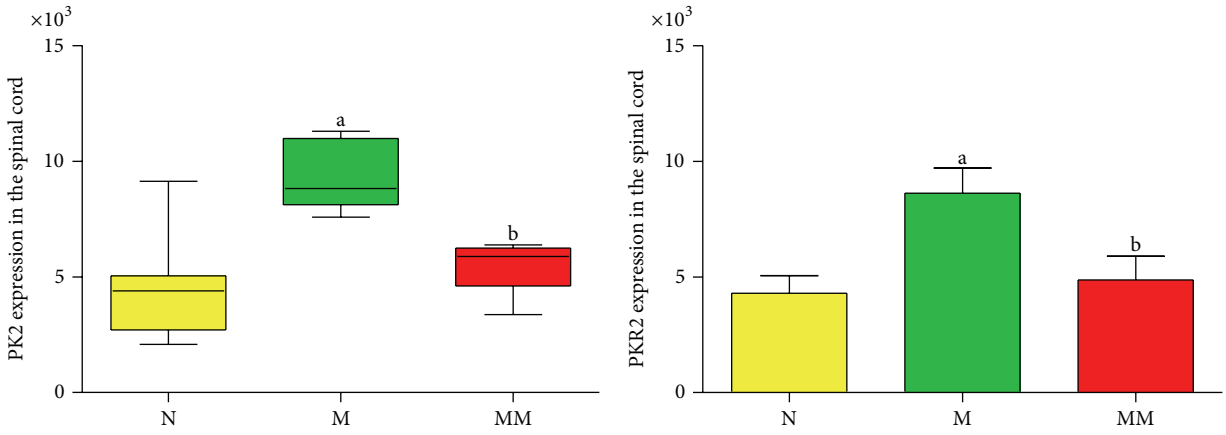


FIGURE 7: The integral optic density of the expression of PK2- and PKR2-positive signal in the spinal cord of each group. Data of PK2 expression are presented as M, min-max, whereas the data of PKR2 expression are presented as mean ± SD. Compared with the normal group: $^{a}P < 0.01$; compared with the model group: $^{b}P < 0.01$. N: normal group; M: model group; MM: mild moxibustion group.

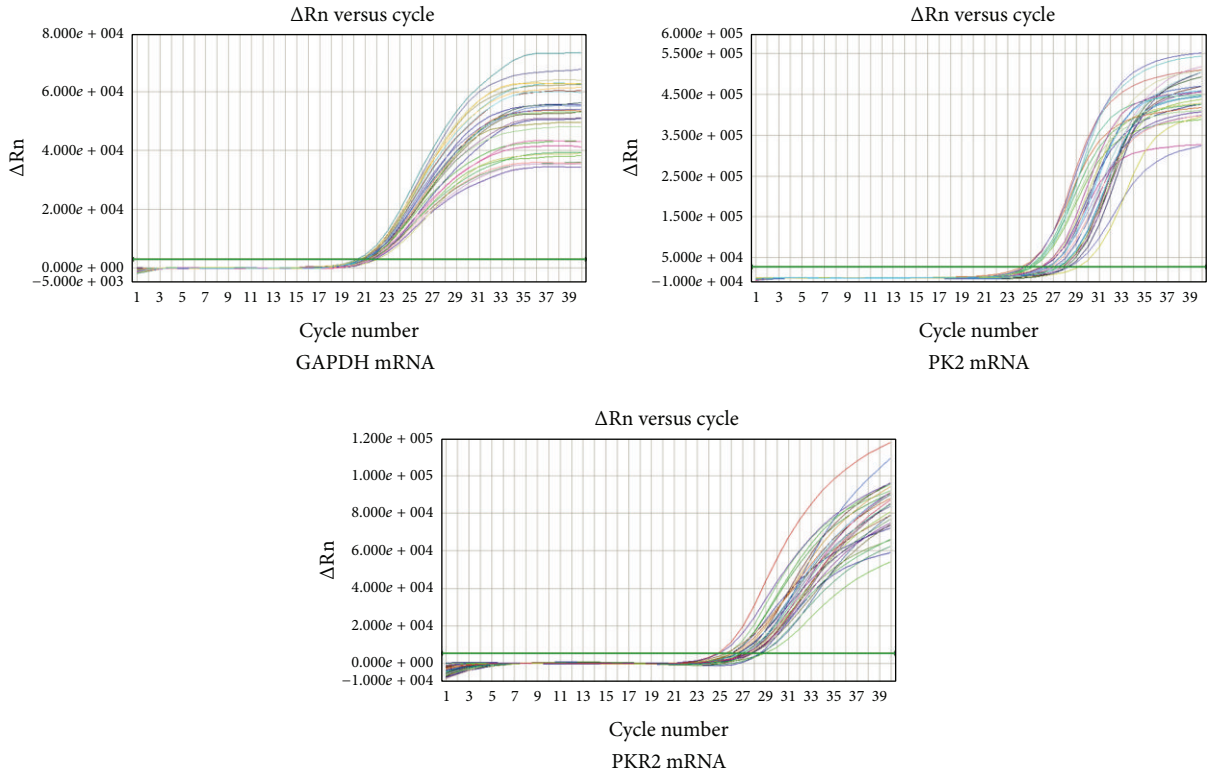


Figure 8: The amplification kinetics curve of PK2 mRNA, PKR2 mRNA, and GAPDH mRNA in the colon in each group.

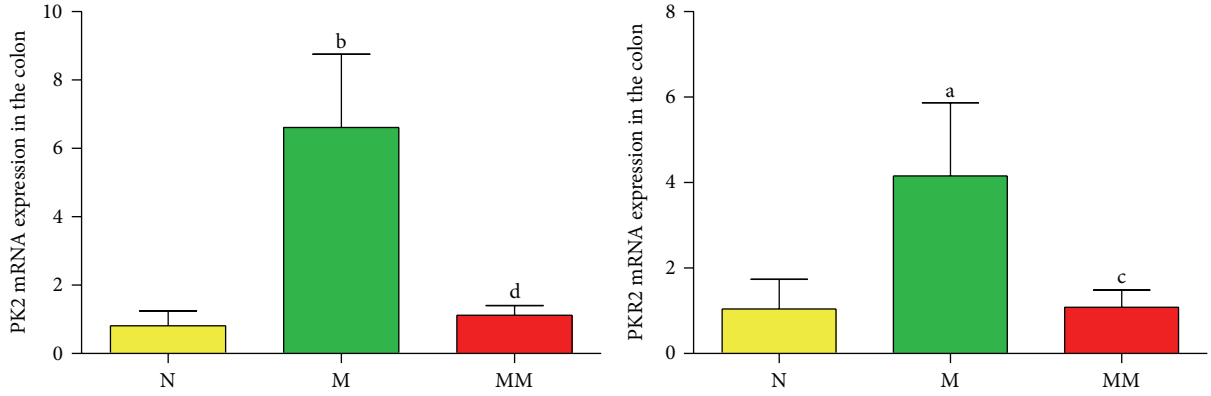


Figure 9: The relative expression levels of PK2 and PKR2 mRNA in the colon in each group (%). Data are presented as mean ± SD. Compared with the normal group: $^{a}P < 0.05$, $^{b}P < 0.01$; compared with the model group: $^{c}P < 0.05$, $^{d}P < 0.01$. N: normal group; M: model group; MM: mild moxibustion group.

Figure 10. The results of FQ-PCR showed that the expression of PK2 and PKR2 mRNA in the spinal cord in the model group significantly increased compared with the normal group ($P < 0.05$, $P < 0.01$). After mild moxibustion treatment, the expression of PK2 and PKR2 mRNA in spinal cord tissue significantly decreased compared with the model group ($P < 0.05$, $P < 0.01$) (Figure 11).

4. Discussion

Previous studies showed that PKs and PKRs participate in the sensitization of nociceptors and hyperalgesia and are closely associated with the transmission of pain signals. Injection of a small amount of PK/Bv8 into the central nervous system [16, 25] and peripheral nervous system [26] of rats produced strong hyperalgesia in a dose-dependent manner [24–27]. A study by Negri et al. [17] showed that intravenous, subcutaneous, and intrathecal injection of Bv8 (the frog homolog of PK2) systemically induced an intense sensitization of nociceptors to mechanical and thermal stimuli in rats. Lacking PKs/PKRs significantly decreased sensitivity to noxious stimuli and reduced pain sensation in rodents [28–30]. Hu et al. [28] reported that mice lacking the PK2 gene displayed a reduction in nociception induced by thermal and

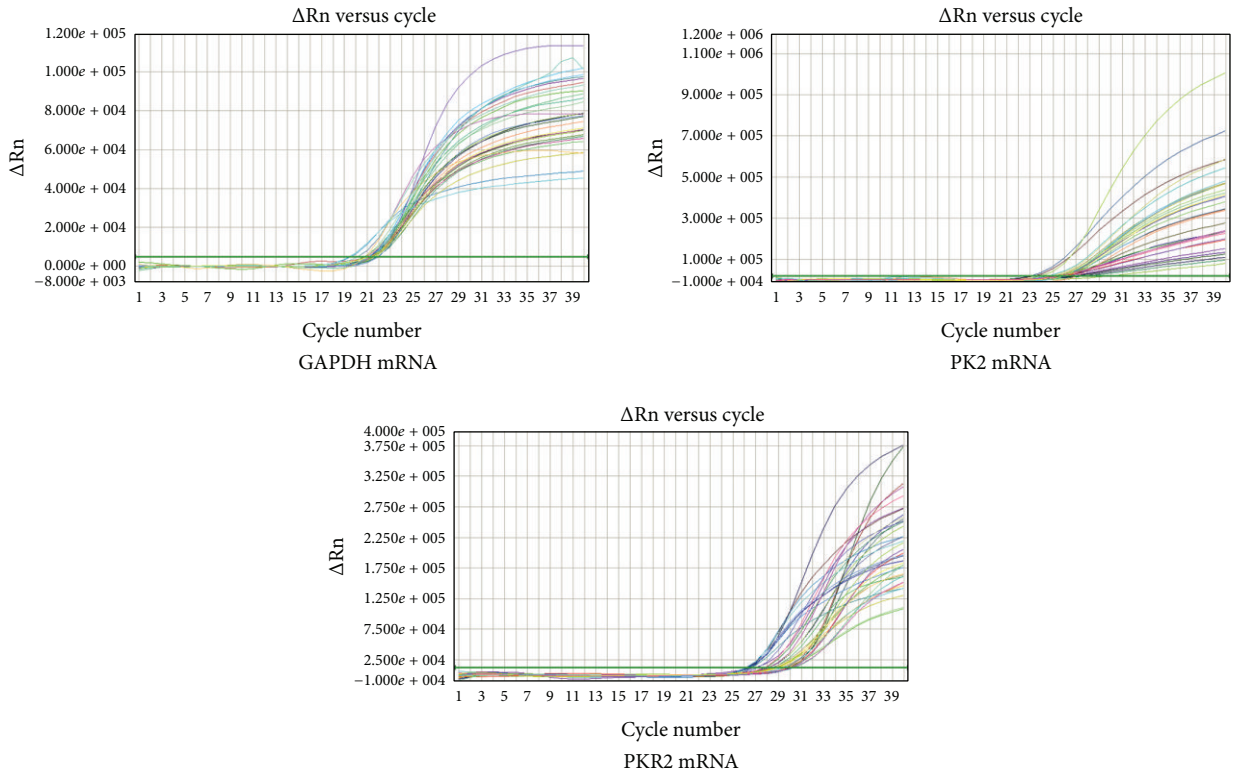


Figure 10: The amplification kinetics curve of PK2 mRNA, PKR2 mRNA, and GAPDH mRNA in the spinal cord in each group.

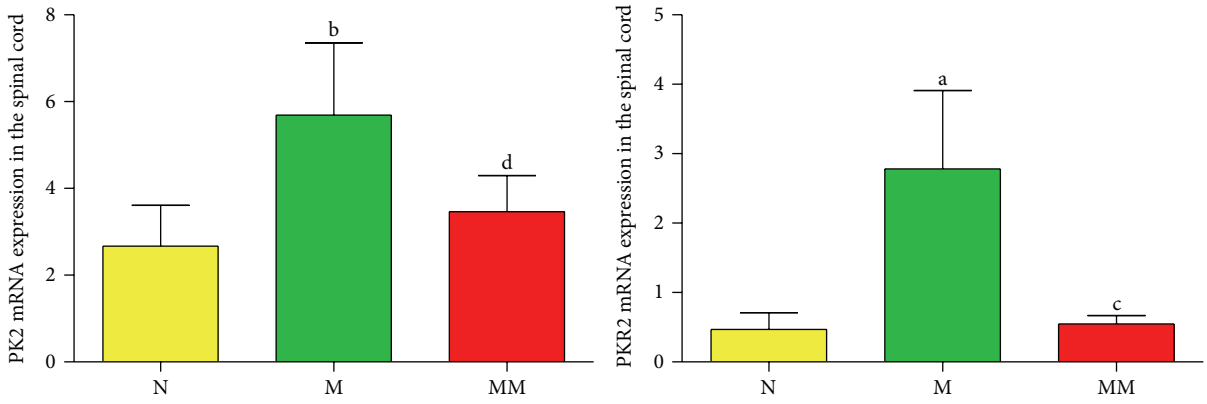


Figure 11: The relative expression levels of PK2 and PKR2 mRNA in the spinal cord in each group (%). Data are presented as mean ± SD. Compared with the normal group: $^{a}P < 0.05$, $^{b}P < 0.01$; compared with the model group: $^{c}P < 0.05$, $^{d}P < 0.01$. N: normal group; M: model group; MM: mild moxibustion group.

chemical stimuli including capsaicin and exhibited significantly reduced late-phase responses to subcutaneous administration of formalin. In acute and chronic inflammation models, mice with PKR-null had reduced pain behavior and significantly decreased inflammatory hyperalgesia [30, 31]. PKR antagonists reduced and eventually abolished PK1 and PK2-induced hypernociception and inflammatory hyperalgesia in a dose-dependent manner [31], indicating that PK2 and PKR2 play important roles in nociceptor sensitization and hyperalgesia, and mediate peripheral and central pain sensitization.

Abdominal pain is one of the major symptoms in IBS patients. Compared with normal individuals, IBS patients have increased pain sensitivity and reduced pain threshold [32–34]. When the balloon pressure in the rectum reached 40 mmHg, 90.7% of IBS patients had abdominal pain, and the specificity was 80% [35]. The severity of IBS symptoms is associated with pain threshold, and there is no significant difference among subtypes [36]. Therefore, increasing the pain threshold and relieving pain are effective methods for treating IBS. In this study, at different intensities of stimulation (20 mmHg, 40 mmHg, 60 mmHg, and 80 mmHg), the AWR

scores of IBS model rats all significantly increased and were significantly different from those of the control group with $P < 0.01$. These results indicated that visceral pain sensitivity intensified and pain threshold decreased in rats after the establishment of the model. A study by Watson et al. [37] showed that PK2 gene expression in the gastrointestinal tract was significantly upregulated in ulcerative colitis patients and rodent models of colitis visceral pain induced by mustard oil, trinitrobenzene sulfonate, water-avoidance stress, and *Citrobacter rodentium* infection. Therefore, it was believed that enhanced PK2 expression induced visceral pain through the PKR2 pathway. In this study, IBS model rats had enhanced visceral pain sensitivity and reduced pain threshold; compared with the normal group, their PK2/PKR2 protein and mRNA expression in colon tissue were significantly increased ($P < 0.05$, $P < 0.01$), indicating that PK2 and PKR2 are associated with enhanced visceral pain sensitivity in IBS model rats.

In this study, immunohistochemistry showed that PK2 and PKR2 were mainly expressed in the mucosal epithelium, the proximal glands in the mucosal epithelium, the interstitium, and the muscular layer of the colon. The colon is innervated by five different types of afferent nerve fibers: serosal, mesenteric, muscular, mucosal, and muscular/mucosal. Different sensory and motor nerve impulses in the colon are transmitted from these afferent nerve fibers to the central nervous system through the dorsal root ganglion (DRG) [38]. The spinal cord is an essential pathway of sensory and motor nerve impulse conduction. Nerve impulses transmitted into the DRG are transmitted into the brain through the spinal cord. Physiological studies on somatic and visceral sensation nerves revealed that hyperexcitability of neurons in the dorsal horn, which can develop either in response to peripheral tissue irritation or in response to descending influences originating in the brainstem, plays a core role in chronic hyperalgesia in the gastrointestinal tract of the human body [39]. Coffin et al. [40] confirmed that IBS patients have spinal cord hyperexcitability through a study on the nociceptive flexion reflex in IBS patients. In this study, the expression of PK2/PKR2 protein and mRNA in the spinal cord of IBS model rats significantly increased compared with that of the normal group ($P < 0.05$, $P < 0.01$), indicating that PK2 and PKR2 also participate in the central mechanisms of visceral pain in IBS model rats.

Previous studies of our project team showed that mild moxibustion was an effective treatment for IBS [23, 24, 41]. In this study, after mild moxibustion treatment, the abnormally increased AWR scores at different intensities of stimuli (20 mmHg, 40 mmHg, 60 mmHg, and 80 mmHg) in IBS model rats all significantly decreased with $P < 0.01$. The results indicated that mild moxibustion effectively improved visceral hypersensitivity and increased pain threshold in rats with IBS, consistent with previous results [23, 24, 41]. Compared with the model group, the expression of PK2/PKR2 protein and mRNA in the colon and spinal cord of IBS model rats significantly decreased ($P < 0.05$, $P < 0.01$), indicating that mild moxibustion suppressed the abnormally increased expression of PK2/PKR2 protein and mRNA in the colon and spinal cord of IBS model rats. These findings suggest that PK2 and PKR2 are involved in mild moxibustion analgesia in rats with chronic visceral hyperalgesia.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Research Article

Preliminary Study on Pain Reduction of Monosodium Iodoacetate-Induced Knee Osteoarthritis in Rats by Carbon Dioxide Laser Moxibustion

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In order to study the effects of CO_2 laser moxibustion on the pain and inflammatory cytokine expression in the spinal dorsal horn of rats with monosodium iodoacetate- (MIA-) induced knee osteoarthritis (KOA), we designed an experiment by randomly assigning 8 SD rats into 3 groups, namely, a CO_2 laser moxibustion group, a sham treatment group, and a blank control group. The treatment group received a laser moxibustion on acupoint Dubi (ST 35; 5 min/treatment, 1 treatment/day) for 8 days, and after treatment, the rats exhibited significantly increased interhindpaw differences compared with their preinduction values. Meanwhile, cytokine microarray analysis showed that one cytokine (TIMP-1) was significantly upregulated and two cytokines (Agrin and MMP-8) were significantly downregulated in treatment group. The present study suggested that CO_2 laser moxibustion created certain pain reduction in the rats with MIA-induced KOA and significantly inhibited the expression of most inflammatory cytokines in the ipsilateral spinal dorsal horn.

1. Introduction

Osteoarthritis (OA) is the most common joint disease in adults [1], prevalently affecting the knee [2]. The major clinical presentation of knee OA is a chronic pain of the affected knee. Current treatment of OA primarily aims at reducing the pain. However, a meta-analysis found that, for reducing short-term pain in OA, commonly used drugs (NSAIDs and nonsteroidal anti-inflammatory drugs) performed only slightly better than placebos [3].

Pain reduction with acupuncture and moxibustion has been a focus in recent years. But these researches mainly concentrated in the field of needling analgesia, whereas little attention has been given to the moxibustion. Although both stimulations target acupoints, needling and moxibustion deliver different modes of actions to acupoints, thereby likely acting via different mechanisms.

Studies on KOA have demonstrated high expression of multiple inflammatory cytokines in synovial fluid, synovium, joint capsular tissue, and cartilage from OA patients with severe pain [4]. These cytokines are believed to further stimulate the function expression of other inflammatory mediators, eventually causing pain [5, 6]. Im et al. [7] proposed that the mechanism of pain in MIA-induced rat knee OA was similar to that in the animal model of neuropathic pain, suggesting a potential overlap in their conduction pathways. A similar situation has been found in a rat model of medial meniscus transection [8].

In the present study, we induced KOA in rat with MIA and treated the acupoint Dubi (ST 35; the point most commonly targeted in needling treatment of knee OA) with carbon dioxide (CO_2) laser simulating the thermal and infrared irradiation effects of moxibustion. Our objective was to investigate whether the CO_2 laser treatment could (1) reduce pain

in this model and (2) affect the expression of inflammatory cytokines in the spinal dorsal horn.

2. Materials and Methods

2.1. Animals. Eight male Sprague Dawley rats weighing 200–220 g (Harlan, USA) were kept under controlled laboratory conditions (22 ± 0.5°C, relative humidity 40%–60%, 12 h alternate light-dark cycles, and food and water ad libitum). Each rat was given a 4-week training for adaptation to the laboratory environment, hindpaw weight-bearing tests, and simulated treatments. The training was conducted daily at regular times. After the training, the rats weighted 250–275 g.

2.2. Induction of Knee OA. The rats were randomly assigned to a CO_2 laser moxibustion group ($n = 3$), sham treatment group ($n = 3$), and blank control group ($n = 2$). For induction of osteoarthritis with MIA, rats from laser treatment group and sham treatment group were anesthetized with isoflurane (Piramal Critical Care, USA) in oxygen and administered a single percutaneous intra-articular injection of 1.0 mg of MIA (Sigma, USA) through the infrapatellar ligament of the left knee. MIA was dissolved in 0.9% saline and administered in a volume of 0.05 mL using a 26 gauge, 0.5 inch needle [9].

2.3. Measurement of Hindpaw Weight-Bearing Distribution. After OA induction, the original balance in weight-bearing capability of hindpaws was disrupted. An incapacitance meter tester (Model-600R, IITC Life Science, USA) was employed for determination of hindpaw weight distribution. Rats were placed in an angled plexiglass chamber positioned so that each hindpaw rested on a separate force plate. The force exerted by each hindlimb (measured in grams) was averaged over a 10 s period. Each data point is the mean of six, 10 s readings. The change in hindpaw weight distribution was calculated by determining the difference in the amount of weight (g) between the left and right limbs.

2.4. Treatment after KOA Induction. From 1 day after KOA induction, the CO_2 laser moxibustion group underwent laser treatment on the depression of the lateral aspect of the infrapatellar ligament (the acupoint is equivalent to ST-35 in human) for 8 days (one treatment per day). The rat was placed on a platform, and the head was covered by the operator's hand. Then the hindlimb was extended to expose the acupoint. After the animal became calm, the acupoint was irradiated with a CO_2 laser beam for 5 min. The wavelength of CO_2 laser is 10.6 μm and the output power density is 63.29 mW/mm^2. The rat of the sham treatment group was similarly immobilized on the platform without undergoing the laser treatment.

2.5. Microarray Analysis of Cytokines. Twenty days after the completion of laser (or sham) treatment, all animals were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg) and killed by decapitation. The Entire spinal cord was ejected, and the dorsal horn was dissected. The dorsal horn was homogenized in protein extraction buffer containing 1% EDTA and 1% Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, USA). After centrifugation (14,000 rpm for 10 min at 4°C), the supernatant containing the proteins was collected. Protein concentration was determined with Bio-Rad protein assay kits (Bio-Rad, USA). Cytokine levels in the dorsal horn were analyzed with cytokine antibody microarrays (Rat Cytokine Array C2, RayBio, USA) following the guideline by manufactory. After development and scan, for each sample, membranes with optimal image qualities (high signal/noise ratio, well-defined background, and absence of signal overlap with adjacent dots) were analyzed using Image J (National Institute of Health, USA) equipped with a protein array analyzer add-on. In image analysis, C1, C2, D1, and D2 served as the background (Table 1) and A1, A2, L7, and L8 as the positive reference. Finally, results recorded from each membrane were normalized.

2.6. Statistical Analysis. Results are expressed as the mean ± SD. Results of repetitive testing of hindpaw weight distribution were analyzed using a repeated-measures general linear model. The mean changes in hindpaw weight distribution over time for the laser treatment group were compared with the mean change in hindpaw weight distribution over time in the sham treatment group via the Bonferroni multiple comparisons procedure. The signal densities of cytokines were analyzed using a multivariate analysis of variance with Bonferroni post hoc test. P values less than 0.05 were considered significant.

3. Results

3.1. Changes in Hindpaw Weight-Bearing Distribution. Before OA induction (Figure 1), the three groups exhibited similar interhindpaw weight-bearing distributions ($P > 0.05$). Throughout the study, the value recorded from the blank control group varied slightly ($P > 0.05$). One day after OA induction, the values recorded from the laser moxibustion group and sham treatment groups increased significantly compared with their baseline values (both $P < 0.001$). And the laser moxibustion group and sham treatment group were similar at this time point. Values measured from the laser moxibustion group and sham treatment group both decreased with time (both $P < 0.05$), but at five time points the laser moxibustion group showed better improvement than the sham treatment group: 1st day ($P = 0.044$), 2nd day (0.029), 12th day (0.031), 15th day (0.007), and 19th day (0.016) after the completion of all the treatment.

3.2. Cytokine Levels. The expression of inflammatory cytokines in the dorsal horn samples was analyzed with RayBio Rat Cytokine Array C2 microarrays, which allowed simultaneous detection of 34 inflammation-related cytokines. In the laser moxibustion group, signal densities of most cytokines were similar or lower than corresponding values recorded in the blank control group (data not shown). Compared with the control group (Figure 2(a)), the signal densities of Agrin ($P = 0.036$) and MMP-8 ($P = 0.006$)

Table 1: Illustration of cytokines distribution as given by the Rat Cytokine Array C2 microarray (image provided by RayBio).

	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	Activin A	Agrin	B7-2/Cd86	beta-NGF	CINC-1	CINC-2 alpha	CINC-3	CNTF
2	POS	POS	NEG	NEG	Activin A	Agrin	B7-2/Cd87	beta-NGF	CINC-2	CINC-3 alpha	CINC-4	CNTF
3	Fas Ligand	Fractalkine	GM-CSF	ICAM-1	IFN-gamma	IL-1 alpha	IL-1 beta	IL-1 R6	IL-2	IL-4	IL-6	IL-10
4	Fas Ligand	Fractalkine	GM-CSF	ICAM-2	IFN-gamma	IL-2 alpha	IL-2 beta	IL-1 R7	IL-2	IL-4	IL-6	IL-10
5	IL-13	Leptin	LIX	L-Selectin	MCP-1	MIP-3 alpha	MMP-8	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-1	TIMP-1
6	IL-14	Leptin	LIX	L-Selectin	MCP-2	MIP-4 alpha	MMP-9	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-2	TIMP-2
7	TNF-alpha	VEGF	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	POS
8	TNF-alpha	VEGF	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	POS

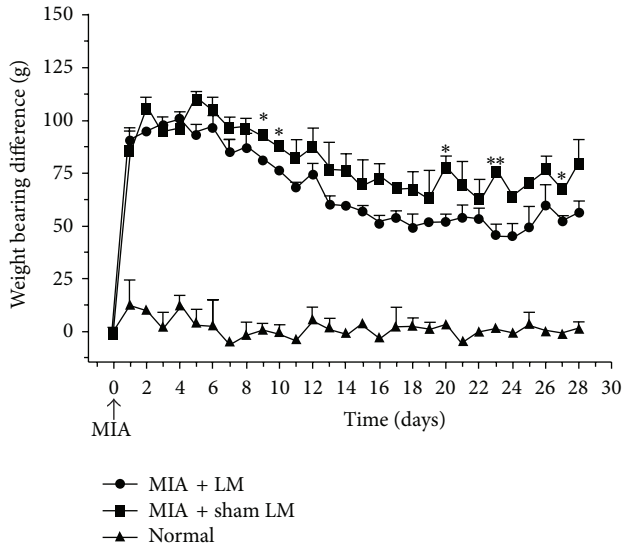

Figure 1: Variation of interhindpaw weight-bearing difference after induction of knee osteoarthritis (MIA + LM: CO_2 laser moxibustion group; MIA + sham LM: sham treatment group; Normal: blank control group; $^{*}P < 0.05$, $^{**}P < 0.01$). Values are the mean and SD.

were significantly downregulated, and only one cytokine (TIMP-1) was significantly upregulated ($P < 0.001$). In the sham treatment group (Figure 2(b)), the signal densities of most inflammatory cytokines were upregulated, including ICAM-1, IL-13 (both $P < 0.05$), Agrin, beta-NGF, Fas Ligand, IL-6, Thymus Chemokine-1, VEGF-A (all $P < 0.01$), CINC-1, CINC-2 alpha, CINC-3, GM-CSF, IL-1 alpha, IL-1 beta, IL-1 R6, IL-2, IL-4, IL-10, Leptin, LIX, L-Selectin, MCP-1, MIP-3 alpha, MMP-8, PDGF-AA, Prolactin R, RAGE, and TNF alpha (all $P < 0.001$). In comparison, only one cytokine (B7-2/CD86) was downregulated ($P < 0.05$).

4. Discussion

4.1. Understanding of Knee OA from the Perspective of Traditional Chinese Medicine (TCM). In the TCM theory, knee OA is categorized as a *Bi* syndrome and frequently diagnosed as cold arthralgia (*Han Bi*) or pain arthralgia (*Tong Bi*). Consequently, in TCM practices, this disorder is commonly treated by moxibustion. In the present study, the lateral depression on the infrapatellar ligament (analogous to acupoint Dubi ST-35 in human) of the affected knee was treated by CO_2 laser moxibustion. According to the acupuncture and moxibustion theory and because of the anatomic proximity of this acupoint to the lesion, moxibustion on this point created a warming effect on the local meridian *Qi and Blood* circulation, thereby reducing pathogenic factors.

4.2. Rational of Laser Treatment. Traditionally, the therapeutic effects of moxibustion have been attributed to the thermal action. Our earlier studies found that, besides the thermal action, infrared (IR) radiation is another potential therapeutic mechanism underlying the moxibustion treatment. We observed that, during indirect moxibustion, the infrared radiation from the burning moxa stick was 20 times higher than the self-IR radiation from human acupoints in energy intensity, but the spectral profiles of the two radiation waves were similar [10]. This finding suggests that infrared radiation may be another important therapeutic mechanism in indirect moxibustion. To test this assumption, here we adopted CO_2 laser moxibustion as a substitute to the traditional indirect moxibustion. The laser treatment may have several advantages. First, with the principal wavelength bands centering around 10.6 μm, the CO_2 laser is easily absorbed by water molecules in the skin and generates heat. By adjusting the laser power, it is possible to control the heating effect and skin temperature. Second, the laser wavelength is close to the peak of self-IR radiation from human acupoints, similar to the traditional indirect moxibustion [11]. Additionally, the use of CO_2 laser moxibustion can eliminate confounding factors in traditional indirect moxibustion, such as smokes.

If the CO_2 laser moxibustion can provide similar effects to traditional indirect moxibustion, it may also offer multiple benefits for better understanding of therapeutic mechanisms of moxibustion. First, the output power of the laser irradiation can be adjusted, thereby allowing determining the effects of different temperatures on acupoints. Second, by controlling the thermal actions, the effects of infrared radiation on acupoints can be directly studied. Finally, even if the laser moxibustion fails to achieve equivalent effects of the traditional moxibustion, it suggests the presence of other unknown mechanisms underlying moxibustion therapies.

4.3. Pain Reduction of Laser Treatment. MIA induces OA via inhibiting glycolysis and, thus, causing chondrocyte death [12]. In the present study, 1 mg of MIA was injected into the left knee to induce OA. One day after OA induction, the rats exhibited clear changes in hindpaw weight-bearing distribution (i.e., preferentially putting more weight load on the right hindpaw). During subsequent 7-day laser (or sham) treatment and 20-day laboratory monitoring, the laser moxibustion group and sham treatment group showed gradual decreases in interhindpaw difference (Figure 1). Although the laser moxibustion group seemed to have a greater decrease than did the sham treatment group, the difference between the groups was not statistically significant. This may have been attributed to the small sample sizes used in this study. A calculation using means and standard deviations recorded from the two groups 1 day after the completion of laser (or sham) treatment suggested that minimally 6 animals are required per group for the recognition of the statistical difference (assuming $\alpha = 0.05$, $1 - \beta = 90\%$, equal group sizes). A calculation using data recorded at the end of this study suggested that 7 animals are required per group (assumptions the same as previous calculation).

4.4. Cytokine Expression in Spinal Dorsal Horn. The pain mechanisms in OA are not fully understood. Inflammatory cytokines in the joint region are categorized into two groups: pro- and anti-inflammatory cytokines [13]. Main proinflammatory cytokines include IL-1 alpha/beta, TNF-alpha, IL-6,

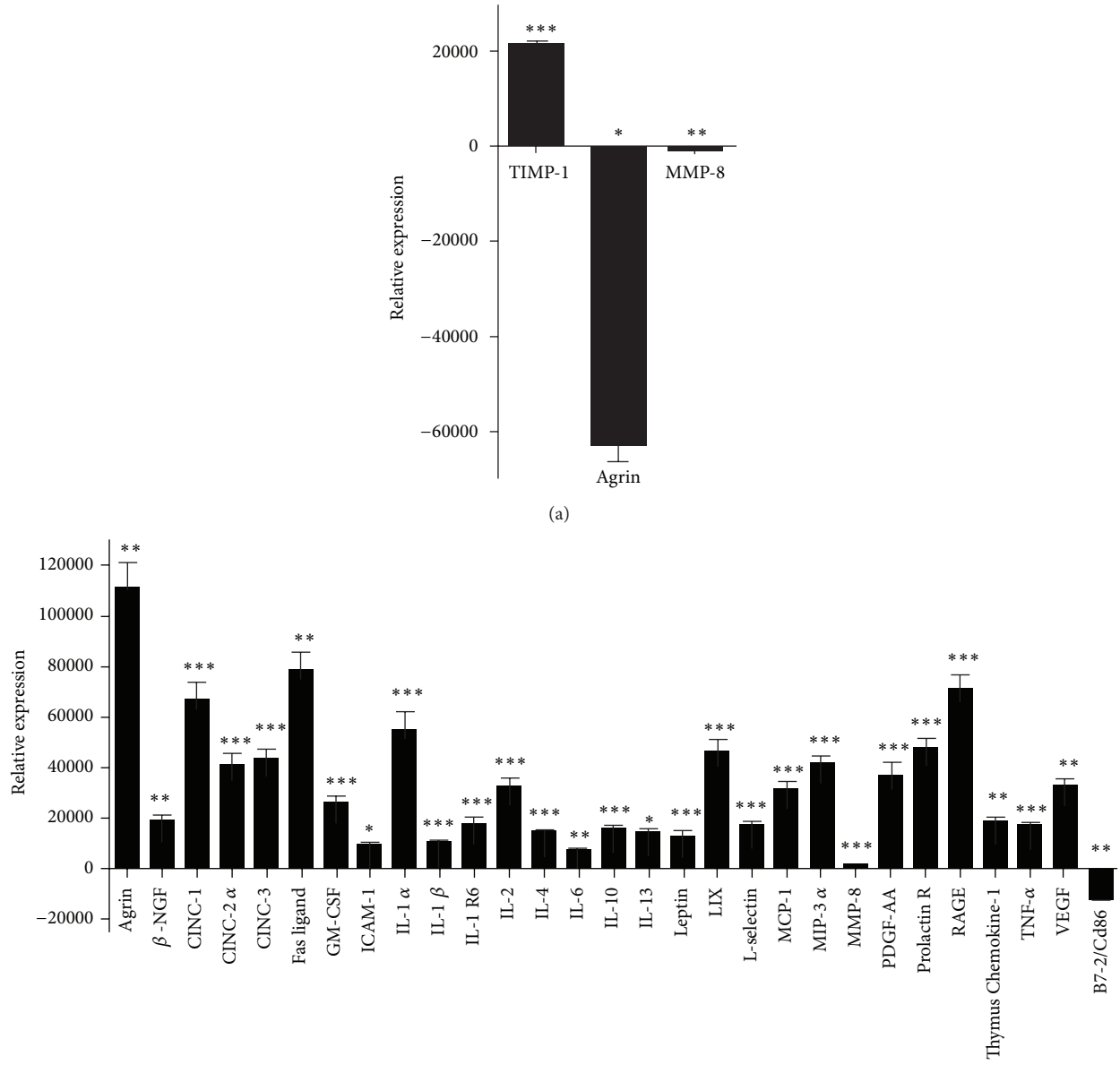

Figure 2: Relative expression levels of cytokine in left dorsal horn tissues measured from the (a) CO_2 laser moxibustion group and (b) sham treatment group 28 d after induction of knee osteoarthritis (results of microarray analyses; upward bars indicate upregulation versus blank control; downward bars indicate downregulation; MIA + LM: CO_2 laser moxibustion group; MIA + sham LM: sham treatment group; Normal: blank control group; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$). Values are the mean and SD. Beta-NGF: beta-nerve growth factor; CINC-1/2 alpha/3: cytokine-induced neutrophil chemoattractant-1/2 alpha/3; GM-CSF: granulocyte-macrophage colony-stimulating factor; ICAM-1: intercellular adhesion molecule 1; IL-1 alpha/1 beta/1 R6/2/4/6/10/13: interleukin-1 alpha/1 beta/1 R6/2/4/6/10/13; LIX: lipopolysaccharide induced CXC chemokine; MCP-1: monocyte chemotactic protein-1; MIP-3 alpha: macrophage inflammatory protein-3 alpha; MMP-8: metalloproteinases 8; PDGF-AA: platelet-derived growth factor-AA; RAGE: receptor for advanced glycosylation end products; TNF-alpha: tumor necrosis factors-alpha; VEGF: vascular endothelial growth factor.

IL-8, IL-17, and IL-18. Anti-inflammatory cytokines mainly include IL-4, IL-10, IL-11, IL-13, IL-1Ra, and IFN-gamma. However, despite the presence of these numerous cytokines, OA is not considered an inflammatory disorder. Because of the absence of nerves in the joint cartilage [14], cartilage degradation cannot directly cause pain. In the knee OA model used in the present study, chondrocytes at the lesion site produced chemokines, cytokines, and proteinases. These molecules sensitized endings of primary afferent fibers in the adjacent tissues [15]. With increasing signal input from nociceptors in the OA site to the spinal neurons, the neurons became more sensitive to signal from the knee [16]. With the

increase of spinal neuron excitability, the sensitive threshold of neurons in the spinal dorsal horn to peripheral injurious stimuli decreased [17].

Current studies on the mechanisms of pain development are primarily focused on spinal glial cells [18]. After the occurrence of peripheral tissue injuries, these cells (including microglial cells and astrocytes) respond by activities such as antigen presentation (primarily microglial cells) and release of inflammatory mediators. These responses may be a cause of the chronic pain in OA [19, 20]. Studies have confirmed that, in animals with MIA-induced knee OA, the proliferation of microglial cells in the ipsilateral spinal dorsal horn is associated with the development of neuropathic pain [21]. In the sham treatment group of the present study, the levels of most analyzed proinflammatory cytokines in the ipsilateral spinal dorsal horn increased significantly compared with the blank control (Figure 2(b)), consistent with earlier findings [22]. This indicated that nociceptive stimulus in the lesion site was transferred into the spinal cord and generated a large number of inflammatory cytokines, probably via pathways involving spinal neurons or glial cells. In comparison, in the laser moxibustion group (Figure 2(a)), only three inflammatory cytokines varied significantly compared with the blank control group. Notably, one cytokine (TIMP-1) significantly upregulated in the laser moxibustion group was not significantly changed in the sham treatment group, and two cytokines (Agrin and MMP-8) significantly downregulated in the laser treatment group were significantly upregulated in the sham treatment group. Of these "oppositely changing" cytokines, MMP-8 and TIMP-1 may deserve particular attention. MMP-8 is a member of the matrix metalloproteinase (MMP) family and believed to be closely related to OA-induced cartilage destruction, as confirmed by studies on humans and animal models [23, 24]. TIMP-1 (tissue inhibitors of metalloproteinases 1) is a MMPs inhibitor and serves to regulate the activities of MMPs. MMPs and TIMP-1 function collaboratively to maintain the homeostasis of the extracellular matrix. Studies have confirmed the roles of the two cytokines in the peripheral and central nervous of model animals with OA. Janusz et al. found that oral administration of TIMP-1 significantly alleviated cartilage destruction in rats with MIA-induced knee OA [25]. Several studies reported that peripheral nerve injuries stimulated upregulation of MMP-9 and MMP-2 in the spinal cord [26] and subsequently activated microglial cells and astrocytes, thereby leading to neuropathic pain [27–29]. Additionally, Kawasaki et al. observed that intrathecal injection of TIMPs significantly reduced the neuropathic pain [26]. The mechanisms of neuropathic pain following peripheral nerve injury-induced glial cell activation in the dorsal horn are not fully understood. However, relevant results suggested that TRPV1 (transient receptor potential cation channel subfamily V member 1) may be related to neuropathic pain involving MMPs [30]. It has been shown that TRPV1 is activated at $\geq 43^{\circ}$C [31]. In the present study, the laser treatment on the acupoint created a local temperature around 45°C. Accordingly, we suggest the following processes as a potential mechanism for explaining the effects of laser treatment on spinal cytokine expression. After the MIA induction of knee OA, inflammatory events occurred in the joint cartilage and synovium. Inflammatory mediators stimulated local nociceptors, leading to sensitization of primary afferent fibers and then central sensitization. Spinal neurons and glial cells in the ipsilateral dorsal horn were activated, thus releasing a variety of inflammatory cytokines. The CO_2 laser treatment on acupoint Dubi activated TRPV1 channels in the irradiated region. TRPV1 probably interacted with various inflammatory cytokines [32] and, thereby, modulated their expression. Of these cytokines, the modulation of MMPs may have produced important roles. Presumably, TRPV1 expression propagated from primary afferent fibers to the dorsal root ganglion and inhibited MMPs expression in the ganglion. Additionally, TRPV1 may have upregulated TIMPs in the dorsal horn and further inhibited their expression there. These functions suppressed the activation of spinal glial cells, thereby providing pain reduction. The acupoint functioned analogous to an amplifier, spreading limited stimuli to a larger space. This hypothetical explanation, however, should be verified by further studies.

As a preliminary work, this study has several limitations. First, the sample size was too small to allow statistical interpretation of behavioral changes. Second, the local affected region (i.e., joint cartilage, subchondral bone, and synovium) was not analyzed by microarray analysis. Consequently, the effects of CO_2 laser moxibustion on the local expression of inflammation-related cytokines could not be determined. Finally, the study only attempted a preliminary screening of inflammatory cytokines in the dorsal horn after CO_2 laser moxibustion. More qualitative and quantitative works are required for the determination of cytokines underlying the effects of CO_2 laser moxibustion and their functional pathways.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Research Article

The Effect of Electroacupuncture on the Extracellular Matrix Synthesis and Degradation in a Rabbit Model of Disc Degeneration

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The present study was aimed at determining if the electroacupuncture (EA) is able to protect degenerated disc in vivo. New Zealand white rabbits ($n = 40$) were used for the study. The rabbits were randomly assigned to four groups. EA intervention was applied to one of the four groups. Magnetic resonance imaging and Pfirrmann's classification were obtained for each group to evaluate EA treatment on the intervertebral disc degeneration. Discs were analyzed using immunofluorescence for the labeling of collagens 1 and 2, bone morphogenetic protein-2 (BMP-2), matrix metalloproteinase-13 (MMP-13), and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1). For protein expression analysis, western blot was used for biglycan and decorin. Outcomes indicated that EA intervention decreased the grades compared with the compressed disc. Immunofluorescence analysis showed a significant increase of collagens 1 and 2, TIMP-1, and BMP-2 positive cells, in contrast to MMP-13 after EA treatment for 28 days. The protein expression showed a sign of regeneration that decorin and biglycan were upregulated. It was concluded that EA contributed to the extracellular matrix (ECM) anabolic processes and increased the ECM components. MMPs and their inhibitors involved in the mechanism of EA intervention on ECM decreased disc. It kept a dynamic balance between ECM synthesis and degradation.

1. Introduction

Low-back pain is a global health problem due to its high prevalence and high socioeconomic burden. It affects 70 to 85% of the population during a lifetime and 15 to 45% in a year [1]. The main cause of low back pain is disc degeneration, of which the etiology is complex and multifactorial, involving age, genetics, and biomechanical and environmental factors such as immobilization, trauma, tobacco use, diabetes, vascular disease, and infection [2–5]. Although low-back pain constitutes a major public health issue, little is known about its precise mechanisms [6]. Nonsurgical treatment modalities currently available for symptomatic disc degeneration include lifestyle modifications, rehabilitation programs, and pain medications. Among the multiple patterns of treatments, acupuncture may have a favourable effect on self-reported pain and functional limitations induced by disc degeneration [7]. Since it originated from China, it has now become worldwide in its practice [8, 9]. Increasing statistics showed that a broader population has granted it acceptance. It was reported [10] that electroacupuncture (EA) inhibits AF cell apoptosis via the mitochondria-dependent pathway and upregulates Crk and ERK2 expression. Neuropeptide, a pain controller, produced by electrical acupuncture stimulation of different frequency [11]. The CB2 receptors also contribute to the analgesic effect of EA in a rat model of inflammatory pain [12, 13]. Although the analgesic effect of acupuncture is well documented, the biological basis is still not fully understood.

Lumbar intervertebral disc is a highly specialized structure composed of a complex system of various connective

(a) (b)

Figure 1: (a) Two K-wires (1.5 mm diameter) inserted into the vertebral body. (b) External dynamic compression device attached to the rabbit lumbar spine.

tissues. The abundant fibrils of intervertebral discs are collagen type 1 and 2. As an important matrix component, the predominant proteoglycan, including decorin, biglycan, ibromodulin, and perlecan were found in the nucleus pulposus [14]. Proteoglycans provide the swelling pressure required to confer a high swelling propensity for load support and collagens resist to the volume increase involved in swelling [15–17]. One of its main functions of intervertebral disc is dampening compressive loads. Depending on the duration and extent of the loading, it leads to significant degeneration [16, 18], thus breaking the balance between extracellular matrix (ECM) synthesis and degradation and resulting in a gradual loss of disc extracellular matrix and, eventually, structural failure [19].

The purpose of the current study was to determine and evaluate the effect of EA on the recovery of disc degeneration. Firstly a custom-made dynamic disc compression device was used to induce a disc degeneration model of rabbits, and then the rabbits received EA administration. For this purpose, Pfirrmann's MRI grade scores were obtained for disc degeneration, and a quantitative molecular and histology analysis was used for (1) extracellular matrix components, including COL-1 and COL-2, biglycan, and decorin; (2) extracellular matrix regulatory factors, catabolic factors, and their inhibitors, including MMP-13, TIMP-1, and BMP-2.

2. Materials and Methods

2.1. Animals. All animal procedures were performed under the approval and guidance of the Animal Care and Use Committee at Wuhan Hospital of Integrated Chinese & Western Medicine, affiliated to Tongji Medical College of Huazhong University of Science & Technology. A total of 40 New Zealand skeletally mature white rabbits (3.5–4 kg) were used for the study. The rabbits were randomly assigned to four groups, and ten for each group were given different interventions at 28-day and 56-day time point [20, 21]. Both the compression group ($n = 10$) and the EA group ($n = 10$) were first loaded for 28 days using a custom-made external compression device to stimulate disc degeneration. After 28-day loading time, in the compression group, five were killed and the tissue was harvested, with the other five using the same device for another 28 days. In EA group, tissue was harvested for five rabbits, and the other five received EA administration for 28 days after removal of the external device. In sham compression group ($n = 10$), the rabbits received surgical preferment, but the lumbar body was only punctured without previous loading for 28 days ($n = 5$) or 56 days ($n = 5$). Ten rabbits, which served as controls, were normally fed without surgical preferment for 28 days ($n = 5$) or 56 days ($n = 5$).

2.2. Surgical Procedure. Rabbits were anesthetized with 10% chloral hydrate administered via the marginal ear vein. Through a dorsal approach to the lumbar spine, the custom-made external device was attached to two K-wires (1.5 mm diameter) inserted into the vertebral bodies L4 and L5 parallel to the adjacent study disc by the use of a variable-speed electric drill [22] (Figure 1(a)). After the wound was closed, in 20 animals, axial compression to the disc was created by a spring within the device to produce a disc compression force of 200 N to induce disc degeneration (Figure 1(b)). The sham compression group was performed the same way, but the external compression device was placed in situ without application of compressive force.

2.3. Magnetic Resonance Imaging. Magnetic resonance imaging (MRI) was obtained for each group at days 28 and 56. Imaging was performed at 30 minutes after removal of the external fixateur to establish a new hydration equilibrium of the disc. A custom-made positioning device consisting of foamed material was used to achieve a standardized supine position of the animal. MRI was performed with a 3.0 T imager (GE, American) with a synergy spine coil receiver. T2-weighted sections in the sagittal plane were obtained in the following settings: fast spin echo sequence and time to repetition (TR) of 2200 milliseconds; time to echo (TE) of 70.7 milliseconds; matrix 336 (h) $*$ 512 (v); field of view at 120 mm; 8 excitations; section thickness of 2 mm; gap of 0.2 mm (T1: TR 375; TE 15; matrix 304 (h) $*$ 512 (v); 18 excitations). Pfirrmann's classification [23] was used for disc degeneration grading from grade 1 to 5 (1 = normal, 2 = mild degeneration, 3 = moderate degeneration, 4 = severe degeneration, and 5 = advanced degeneration).

2.4. EA Treatment. In the EA treatment group, five of the rabbits received EA administration on the Ex-B2 (paravertebral point of L4 and L5 level on both sides) once every day, starting at the second day after the device was removed, and lasted for 28 days. Four acupuncture needles were inserted into 4 acupoints that correspond to Ex-B2 in the rabbits; EA (1 mA and 0.4 or 0.6 ms) was administered at 2 or 15 Hz for 30 minutes. Current was delivered with modified current-constant Han's Acupoint Nerve Stimulator (Beijing, China). Ex-B2 were chosen according to the traditional Chinese medicine meridian theory and the effective use in reducing pain. During EA treatment, each rabbit was placed under an inverted clear wooden box (approximately 40 × 25 × 40 cm) but was neither restrained nor given any anesthetic. The animals remained awake and still during EA treatment and showed no evident signs of distress.

2.5. Tissue Preparation. After 28 or 56 days of different intervention, the lumbar disc was harvested for examination of each group, including complete anulus fibrosus and nucleus pulposus. Using a vertical midline incision, the disc was divided into 2 symmetric parts. One part was immediately quick frozen in liquid nitrogen for protein expression analysis; the second part was used for immunofluorescence.

2.6. Immunofluorescence. Disc samples were fixed in formalin 4%, serially dehydrated in ethanol, and embedded in paraffin. The paraffin blocks were sectioned transversely at a 5 m thickness using a standardized manner to ensure that each slide was obtained from the same disc area. Tissue sections were washed with 5% Tween 20 in PBS for three times, incubated in 1 N HCL for 20 min and in 3% H_2O_2 in distilled water for 15 min, and blocked with 5% goat serum for 30 min at room temperature to block the unspecific staining, respectively. The sections were incubated with a mixture solution of primary antibodies, antihuman COL-1 (Biorbyt, UK), antihuman COL-2 (Biorbyt, UK); antihuman BMP-2 (Bioss, Beijing, China); antihuman MMP-13 (Boster, Wuhan, China); antihuman TIMP-1 (Bioss, Beijing, China) for 48 h at 4°C, washed three times in PBS, and incubated in the secondary antibody, goat antirabbit immunoglobulin G (red fluorescence, diluted 1 : 300; Boster, Wuhan), on a rocking bed (away from light) for 2 h under room temperature, respectively. For control staining, primary antibody was omitted. The tissue sections were mounted on glass slides, washed four more times with running water, dried under room temperature and away from light, and sealed with coverslips at last. The analysis was performed using a light microscope NIKON Eclipse 80i with an objective magnification of 200x and software Analysis Pro 3.1. Visualization was performed with avidin-biotin complex method. The fluorescence intensity was measured by Image-Pro Plus 6.0 (USA).

2.7. Western Blotting Analysis. Total protein was extracted from the tissue in RIPA lysis buffer (containing protease and phosphatase inhibitor mixtures) by using a tissue homogenizer, followed by clearing tissue debris by centrifugation at 13000 rpm at 4°C for 20 min. Fifty micrograms of protein were loaded per lane and separated by 10% SDS-PAGE gel electrophoresis and, then, transferred onto PVDF membranes. Blocking was carried out in 5% nonfat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween 20 for 1 h at room temperature. The membranes were incubated with primary antibody rabbit antidecorin (diluted 1 : 200; Boster, Wuhan, China); anti-biglycan (diluted 1 : 200; Bioss, Beijing, China) over night at 4°C and with secondary antibody (1 : 40000 dilution of goat antirabbit Immunoglobulin G) conjugated to horseradish peroxidase (Boster, Wuhan, China) for 1 h at room temperature on the following day. Immunoblotting signal was detected by ECL (enhanced chemiluminescence) on chemiluminescent films following exposure to an X-ray. For densitometric analyses, the blots were scanned and quantified using BandScan software, and the result was expressed as the ratio of target gene immunoreactivity to GAPDH immunoreactivity.

2.8. Statistical Analysis. The data collected in the present study were expressed as mean ± standard deviation (mean ± SD) and analyzed by one-way repeated measures ANOVA to determine differences between two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. The Effect of EA on MRI Grade Scores in Disc Degeneration. The MRI assessment showed that the healthy and compressed discs are clearly differentiated on the T2-weighted image, and the signal intensity of the nucleus pulposus decreased progressively during the 28-day compression period, with the lowest signal intensity after compression for 56 days. Different images showed that the device was able to induce the IVD model. According to Pfirrmann's MRI grade scores, which indicate the degree of disc degeneration, grade IV degenerative changes were first detected at 28 days after compression, and grade IV or V was detected 56 days after loading. In contrast, the control and sham groups remained relatively constant during the 28- or 56-day period, with grade I on T2-weighted imaging. After EA intervention for 28 days, the degree of degenerated disc was characterized by grade III or IV, compared to the model group in 28 days and 56 days and EA group in 28 days ($P < 0.05$) (Figure 2).

3.2. The Effects of EA on ECM Components in Disc Degeneration. Immunofluorescence labeling was used to detect the immunoreactivity of COL-1 (Figure 3) and COL-2 (Figure 4). In comparison with the control group and the sham compression group, the immunoactivity levels of COL-1 and COL-2 in the compressed disc were found to be decreased significantly ($P < 0.05$). After EA intervention, the immunoreactions positive cells in the 56 days of EA group were found to be obviously higher than that in the model group and the 28 days in the EA group ($P < 0.05$).

Western blot analysis demonstrated that no significant changes were found between control and sham compression groups at any time point. Compared with the two groups, the relative expression levels of biglycan (Figure 5) ($P < 0.01$) and

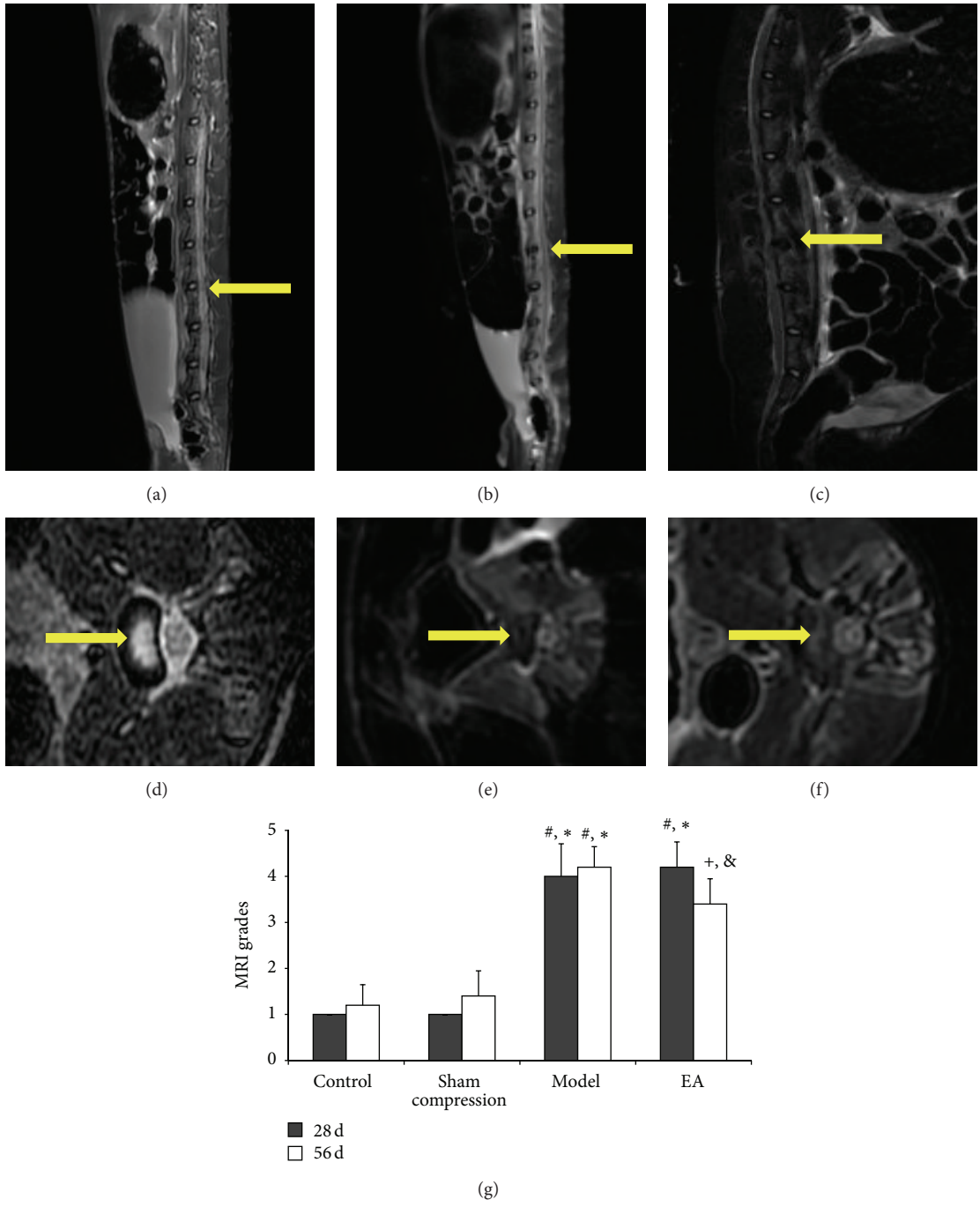


Figure 2: Representative T2-weighted sagittal MRI of the nucleus pulposus at the 56-day time point shows lower signal intensity in model group (b) and EA group (c) than the control group (a). (d), (e), and (f) are the corresponding MRI axial scan, respectively, to (a), (b), and (c). Different signal intensity in the disc is depicted with arrows. Change in disc degeneration of four groups (g). Pfirrmann's classification based on disc height and signal intensity from grade 1 to 5 was used to grade the disc degeneration of the rabbit discs. Data are expressed as the mean ± SD (ANOVA); $^{*}P < 0.05$, compared with the control group; $^{\#}P < 0.05$, compared with sham compressed group; $^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.

decorin (Figure 6) ($P < 0.05$) protein in disc were apparently decreased in the model group. Following EA intervention, the expression in the EA group was considerably higher than those in the model group and the 28 days in the EA group. ($P < 0.05$). A trend to stimulated expression was found in matrix components.

3.3. The Effect of EA on ECM Regulatory Factor in Disc Degeneration. MMPs are a family of inducible, zinc-dependent, secreted, or cell surface based endopeptidases that are centrally involved in the turnover of extracellular matrix (ECM) components. MMP-13, also known as collagenase-3, is the principal interstitial collagenase in this species and has

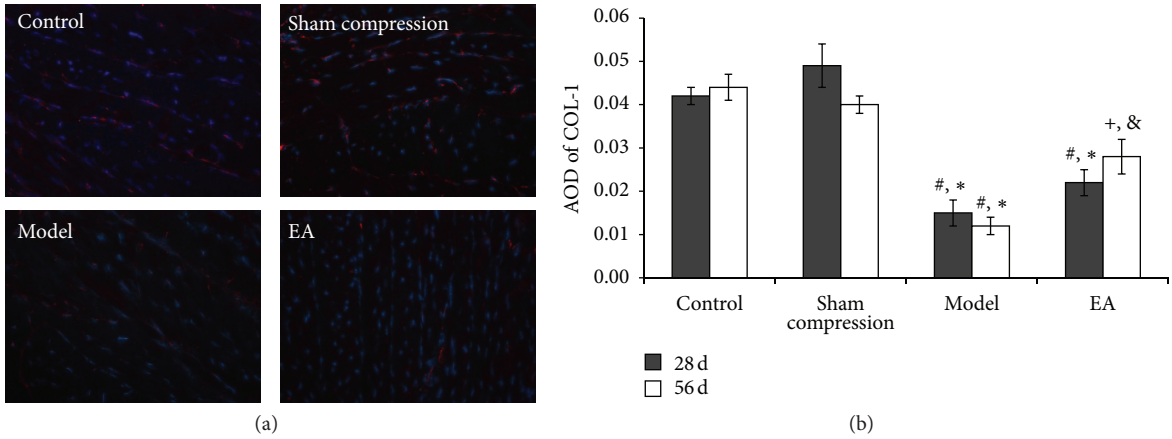


Figure 3: (a) Representative microscopic photos of immunofluorescence staining showing COL-1 immunoreaction (IR) positive products. Nucleus counter stained with DAPI showed blue and red for COL-1 positive products. (b) Average optical density (AOD) was measured and data are expressed as the mean ± SD (ANOVA); $^{*}P < 0.05$, compared with the control group; $^{\#}P < 0.05$, compared with sham compressed group, $^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.

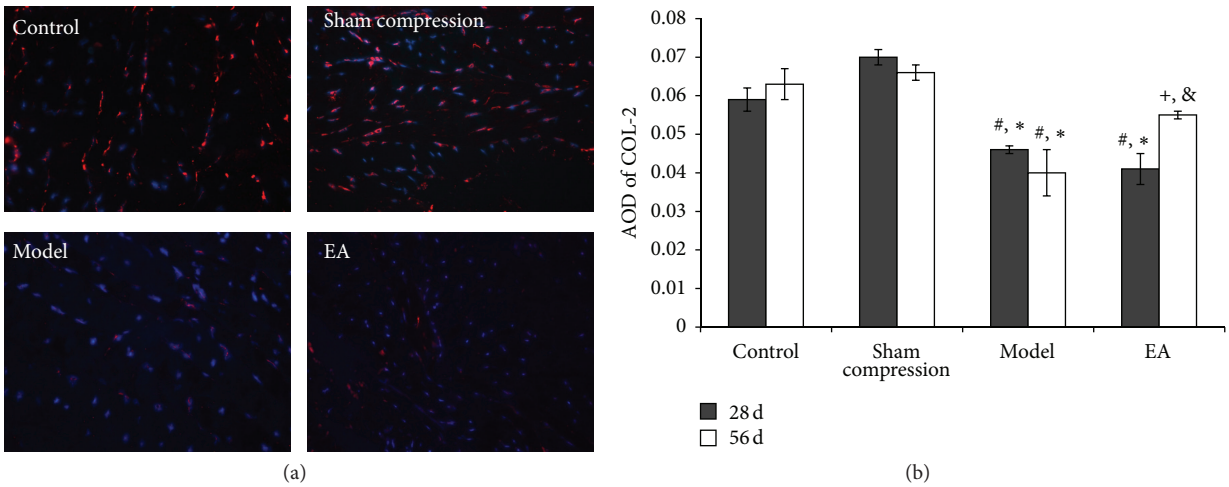


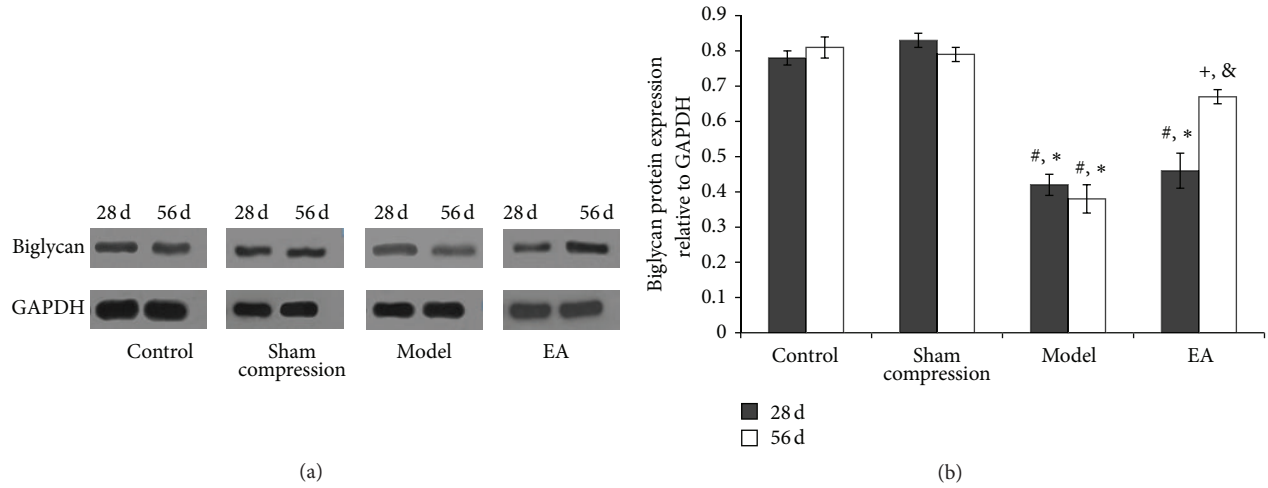
Figure 4: (a) Immunofluorescence staining showing COL-2 immunoreaction (IR) positive products. Nucleus counter stained with DAPI showed blue and red for COL-2 positive products. (b) AOD was measured and data are expressed as the mean ± SD (ANOVA); $^{*}P < 0.05$, compared with the control group; $^{\#}P < 0.05$, compared with sham compressed group, $^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.

a high specificity for degrading insoluble fibrillar collagens, especially types II and I collagens [24, 25]. In contrast, TIMP-1 is an endogenous inhibitor of bone matrix degradation that binds tightly to active MMP-13, thereby downregulating MMP-13 activity. BMP-2, one of the growth factors, has been found to be capable of enhancing cell proliferation and ECM synthesis in vitro and in vivo. We detected MMP-13 by the immunofluorescence method and found that MMP-13 immunoreactivity in the compressed disc was increased compared with the control group, of which the positive cells were not even detectable ($P < 0.05$). Following EA intervention, the immunoreactivity level was downregulated ($P < 0.05$) (Figure 7).

TIMP-1, inhibitor of ECM catabolic factors, and the growth factor BMP-2 were detected by the same method. Compared with the control group and the sham group, the immunoreactivity level of TIMP-1 was lower in the compressed disc ($P < 0.05$), which was in contrast to the MMP-13 (Figure 8). After EA intervention, immunoreactivity level was upregulated in comparison with the model group ($P < 0.05$). The result of BMP-2 was similar to that observed in TIMP-1 (Figure 9).

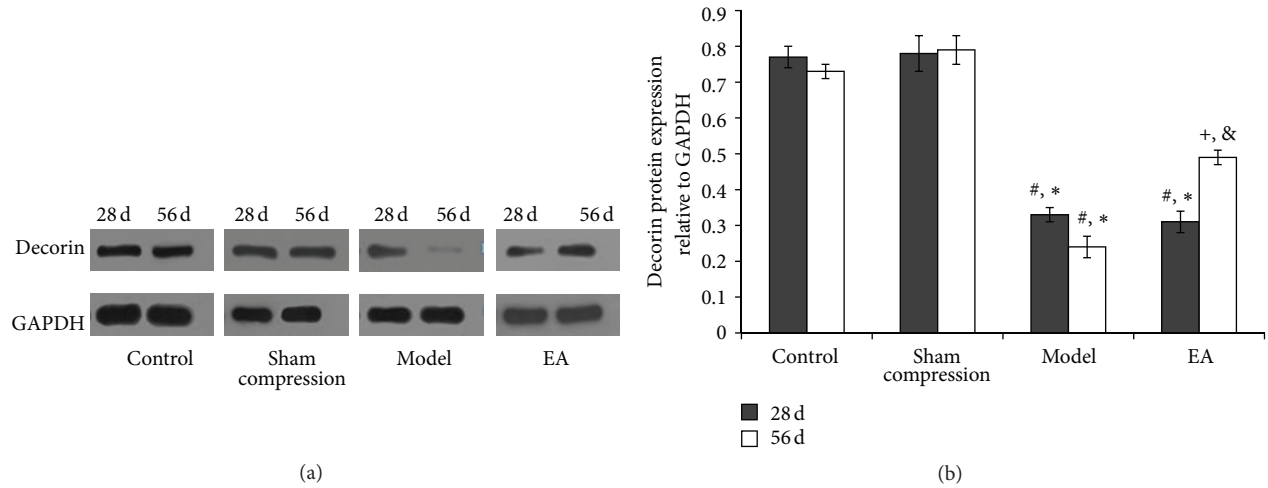
4. Discussion

At present, the methods available to delay degeneration of intervertebral discs include direct injection of cytokines, cell transplantation into intervertebral discs, or tissue engineering. Increasing attention has been paid to the regeneration of functional tissue based on the restoration of the ECM integrity by cell therapy [26, 27]. However, both current nonsurgical treatment modalities and surgical options for severe symptomatic intervertebral disc degeneration have limited and inconsistent clinical results [28]. EA treatments



(a) (b)

Figure 5: (a) Representative western blot analyses showing the biglycan protein levels. GAPDH was analyzed as house-keeping gene. (b) Protein expression of four groups. $^{*}P < 0.01$, compared with the normal control; $^{\#}P < 0.05$, compared with the sham compressed group, $^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.



(a) (b)

Figure 6: (a) Representative western blot analyses showing the decorin protein levels. GAPDH was analyzed as house-keeping gene. (b) The protein expression of four groups. $^{*}P < 0.05$, compared with the normal control; $^{\#}P < 0.05$, compared with the sham compressed group, $^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.

are effective approaches, which offer the potential to halt, retard, or even reverse disc degeneration and restore physiologic disc function. Our study provides new information about the mechanisms underlying the protected effect of EA on disc degeneration. The present study indicated that EA had anabolic and anticatabolic effects on the regulation of extracellular matrix in IVD degeneration model as assessed by MRI, western blot, and immunofluorescence analyses.

First, the current results from the imaging studies support the opinion that EA intervention resulted in a number of slowly progressive and reproducible MRI changes over 28 days. MRI technique allows the definition of IVD based on the tissue hydration shown by the intensity of the T2ws in the NP and various classification systems [29, 30]. It was the gold standard for the clinical investigation of IVD integrity in humans and animal study. The pictures of rabbit lumbar spines in the research showed a significant decrease of nucleus pulposus hydration after 28 days of compression, in contrast to sham compression or controlled discs. Thus indicating a loading-dependent loss and the appearance of a dark transverse band. This data is quite similar to that observed in humans during the course of IVD degeneration. On the other hand, Pfirrmann's classification system was used to assess the effect of EA on the degree of degeneration. It was found that the degeneration grade on MRI was significantly decreased after EA treatment compared with the compressed disc. These findings demonstrate the effectiveness of the EA intervention in a disc degeneration animal model.

Second, we have shown that EA increases extracellular matrix protein expression and the immunoreactivity level, of which biglycan, decorin, COL-1, and COL-2 were significantly stimulated when compared with compressed

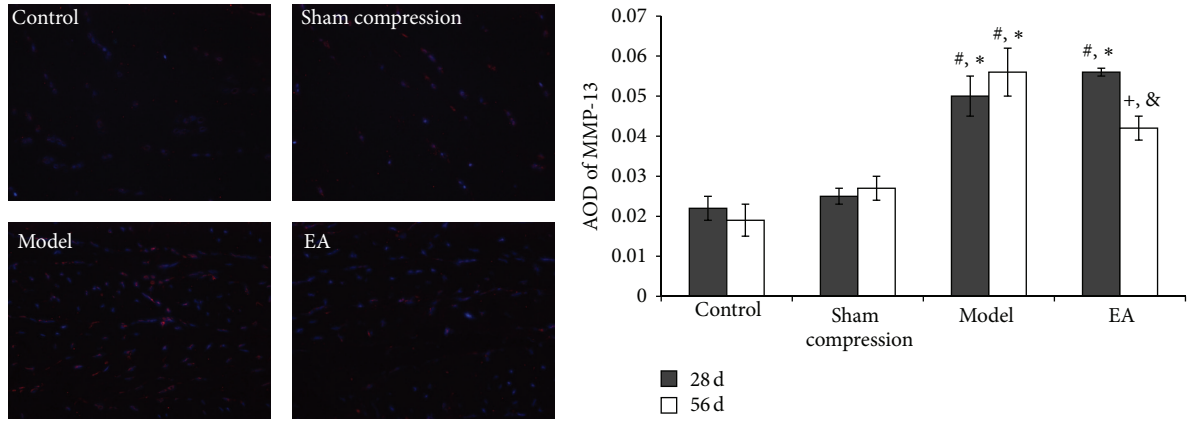


Figure 7: (a) Nucleus counter stained with DAPI showed blue and red for MMP-13 positive products. (b) AOD was measured and data are expressed as the mean ± SD (ANOVA); $^{*}P < 0.01$, compared with the control group; $^{\#}P < 0.05$, compared with the sham compressed group,$^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.

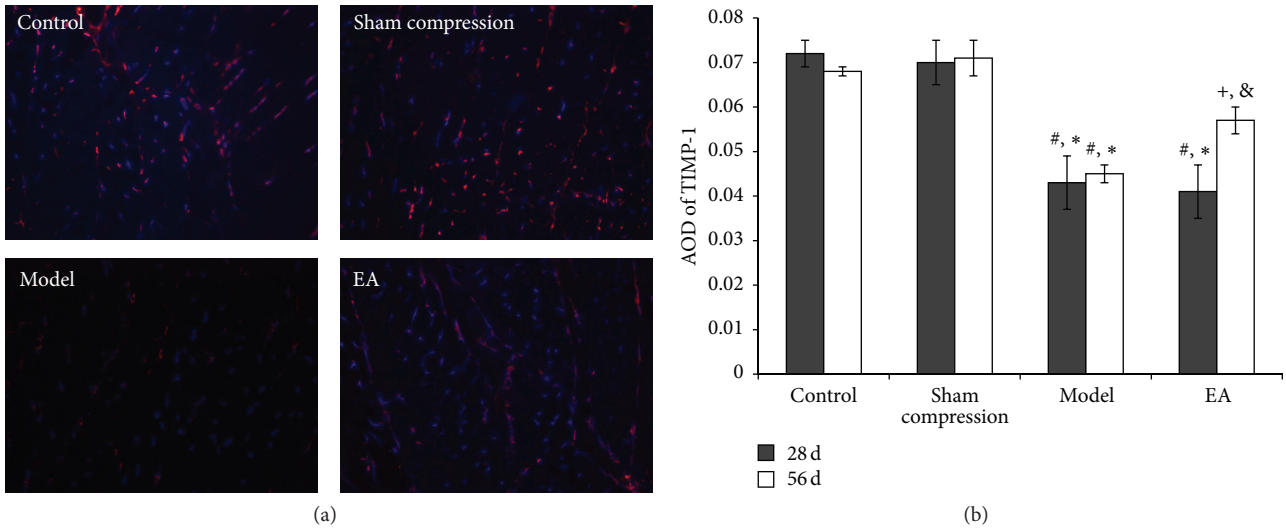


Figure 8: (a) Nucleus counter stained with DAPI showed blue and red for TIMP-1 positive products. (b) AOD was measured and data are expressed as the mean ± SD (ANOVA); $^{*}P < 0.05$, compared with the control group; $^{\#}P < 0.05$, compared with the sham compressed group, $^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.

discs. Histology and protein analysis are consistent with the abovementioned MRI findings. This sequence helps understand the fact that the intervertebral disc degeneration is characterised by ECM decrease, resulting from an imbalance between the anabolic and catabolic processes [31]. It was known that the intervertebral disc consists of COL-1 in the outer anulus fibrosus and the widespread COL-2. Small proteoglycans are represented by decorin in the anulus fibrosus and biglycan in the nucleus pulposus [32]. Those are the important composition of ECM. Its major function is water binding capacity affected by negatively charged glycosaminoglycans [33]. The amount of ECM is dependent on the balance between its production and digestion, and this is a sort of state of dynamic equilibrium [34, 35]. Studies [36] have reported that distraction resulted in stimulated ECM gene expression and increased numbers of protein-expressing cells, showing evidence of regenerative potential. In the current study we achieved the similar result and found that EA method plays the same role as distraction in disc repair. The immunofluorescence findings showed confirmatory characteristics of disc renewal and improved lamellar architecture after EA treatment for 28 days, and the number of COL-1 and COL 2 positive cells was significantly increased. And also the protein expression of biglycan and decorin in the EA group was considerably higher than those in the model group and the 28 days in the EA group. These results suggest that the nutrient supply in the disc was increased by EA treatment, particularly for proteoglycan content.

Third, we studied the role of EA played in the ECM regulatory factors, including MMP-13, TIMP-1, and BMP-2,

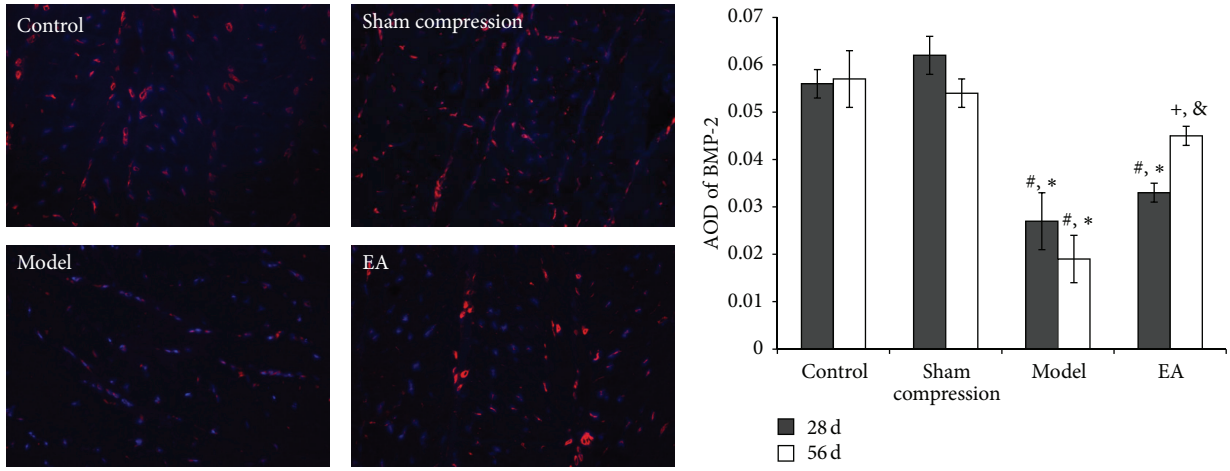


Figure 9: (a) Nucleus counter stained with DAPI showed blue and red for BMP-2 positive products. (b) AOD was measured and data are expressed as the mean ± SD (ANOVA); $^{*}P < 0.01$, compared with the control group; $^{\#}P < 0.01$, compared with sham compressed group,$^{+}P < 0.05$, compared with the model group on 56 days and compared with 28 days in EA group.

in disc degeneration. It is likely that ECM regulatory factors involved in the mechanism of EA intervention on disc ECM decrease.

Matrix metalloproteinases (MMPs) and inhibitors of MMPs, a kind of enzymes which mediate the catabolic process, have been reported to play a major role in the disc degeneration process [37–40], which are stimulated at an early stage of disease and initiate matrix degradation. MMP-13, known as one of the markers for degradation, is probably the most trustworthy among the various MMPs proteins modulated in osteoarthritic chondrocytes. The increase in MMP13 could therefore be a major contributor to IVD degeneration as it has been extensively reported in cartilage degradation during OA [41, 42]. In ECM metabolism, much evidence for TIMP functions has been accumulated [43, 44]. TIMPs inhibit MMPs by 1:1 interaction with zinc-binding site [44]. TIMP-1 is a known endogenous inhibitor of MMP-1 and MMP-3 [45]. BMP-2 is a potent osteoinductive agent [46] and plays a major role as a growth factor during early chondrogenesis [47]. Previous study [36] supports that BMP-2 was involved in disc metabolism and may reflect anabolic behavior in the disc reorganization process. In vitro delivery of the anticatabolic genes TIMP-1 and BMP-2 cause the increase of proteoglycans in cultured degenerated human disc cells. In our study, a positive MMP-13 result was found in compressed disc, whereas, in control or sham compression discs, the immunoreaction positive cells were not even detectable. Following EA intervention for 28 days, the immunoreactivity level decreased compared with the discs loaded for 28 or 56 days. However, the expressions of TIMP-1 and BMP-2 are totally different from MMP-13. Although a causative role has not been proved, our results support the hypothesis that ECM regulatory factors participate in the reorganization process of disc disease treated with the EA method.

In summary, the author performed this in vivo study to determine the effect of EA treatment in a degenerated disc rabbit model. The results in images confirmed that EA reduced Pfirrmann's MRI grade scores. EA increased BMP-2 and TIMP-1 and decreased MMP-13 in the immunoreactivity or protein leve; subsequently it enhances the synthesis of matrix proteoglycan, diminishes the degradation of COL-1 and-2 in disc tissues, and, eventually, showed the anabolic effect on degenerated discs. These results indicate that EA therapy has significant potential for treatment of degenerative disc disease. However, further research is needed on the specific signaling pathways mediated by EA method.

Abbreviations

EA: Electroacupuncture
MRI: Magnetic resonance imaging
BMP: Bone morphogenetic protein
MMPs: Matrix metalloproteinases;
TIMPs: Tissue inhibitor of matrix metalloproteinases
COL: Collagen
ECM: Extracellular matrix
GAPDH: Glyceraldehyde 3-phosphate dehydrogenase
PBS: Phosphate buffered saline
FITC: Fluorescein isothiocyanate
AOD: Average optical density.

Conflict of Interests

The authors declare that there was no conflict of interests.

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Review Article

Safety of Moxibustion: A Systematic Review of Case Reports

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Moxibustion is a traditional medical treatment originating in China. It involves using the heat of burning moxa to stimulate acupoints. It is considered safe and effective and is widely used throughout the world. The increasing use of moxibustion has drawn attention to the procedure's adverse events (AEs). This review covers a total of 64 cases of AEs associated with moxibustion in 24 articles, reported in six countries. Some evidence of the risks of moxibustion has been found in these cases. AEs include allergies, burns, infection, coughing, nausea, vomiting, fetal distress, premature birth, basal cell carcinoma (BCC), ectropion, hyperpigmentation, and even death. The position, duration, distance between moxa and skin, proficiency of the practitioners, conditions of the patients, presence of smoke, and even the environment of treatment can affect the safety of moxibustion. Improving practitioner skill and regulating operations may reduce the incidence of adverse reactions and improve the security of moxibustion.

1. Introduction

Moxibustion is an ancient method of external therapy. It is based on the theories underlying traditional Chinese medicine (TCM). It usually involves stimulating acupoints with burning moxa wool. Moxibustion treatments can be classified as traditional moxibustion, drug moxibustion, and modern moxibustion. Traditional moxibustion is characterized by the use of moxa as a burning material and can be divided into direct moxibustion and indirect moxibustion depending on whether the moxa is in direct contact with the skin during the operation. In direct moxibustion, a moxa cone is placed directly on the skin and ignited. In indirect moxibustion, the moxa cone or stick is kept at a distance from the skin. The insulating materials used in indirect moxibustion can be air, garlic, ginger, aconite, salt, or other substances. In drug moxibustion, also called natural moxibustion, irritant drugs, such as cantharis, garlic, or semen sinapis (mustard seed), are coated on the surface of acupoints. This causes local skin to flush and blister, which is believed to alleviate disease. Modern moxibustion, such as microwave moxibustion, laser moxibustion, and electrothermal moxibustion, involves the simulation of traditional moxibustion stimulation factors through physical and chemical methods, which produces the therapeutic effects of moxibustion. The present review is mainly concerned with traditional moxibustion.

According to TCM theory, moxibustion has a dual effect, tonification and purgation. This involves the actions of the meridian system and the roles of moxa and fire. Studies have shown that the mechanism underlying moxibustion mainly involves the thermal effects, radiation effects, and pharmacological activity of moxa and the products of its combustion [1]. The effectiveness of moxibustion has been tested in the traditional and contemporary moxibustion clinic for more than 2500 years. A bibliometric analysis reported that up to 364 kinds of diseases can be treated with moxibustion. The most common indications of moxibustion therapy are malposition, diarrhea, and colitis. The next common indications are urinary incontinence and dysmenorrhea. The third common indications are knee osteoarthritis, temporomandibular joint disturbance syndrome, soft tissue injury, heel pain, asthma, urinary retention, and herpes zoster [2]. Moxibustion can also be used to treat weakness, fatigue, and problems related to aging [3].

The increasing use of moxibustion has drawn attention to its adverse events (AEs). Some case reports have described

these adverse reactions, side effects, and complications of moxibustion. This review summarizes the published evidence regarding the safety of this medicinal approach.

2. Methods

2.1. Data Sources and Search Strategy. Four Chinese databases (SinoMed, CMCC, CNKI, and VIP) and three English databases (Medline, EMBASE, and Web of Science) were searched for case reports of AEs of moxibustion without any time or language restrictions. Search terms were "moxibustion, moxa, smoke," combined with "safe, safety, adverse event, adverse reaction, side effect, complication, risk, and burn." The literature searches were completed on November 20, 2013.

Review articles on safety of moxibustion were also searched. Two systematic reviews were retrieved, and the case reports cited in these reviews were examined too [4, 5].

2.2. Study Selection. Only firsthand case reports of complications or AEs of moxibustion are included in this review. Comments, letters, and investigations carried out on nonhuman were excluded. Some clinical trials of moxibustion that reported adverse events were excluded for reasons originally given by Lao et al., specifically that they were too small to provide convincing evidence of rare complications [6].

Two authors, Xu and Deng, carried out the screening and selection of articles independently. Disagreements were resolved through discussion.

2.3. Data Extraction. After screening, articles were read in full and extracted by two independent reviewers, Xu and Deng. Information extracted from each case included the author, year of publication, country of occurrence, number of patients affected, disease originally treated, preexisting conditions that might have contributed to the AEs, type of moxibustion, AEs reported and their outcomes, the practitioners' training, and the patients' status at follow-up. We also assessed the likelihood of causality between the events and moxibustion for each case. The causality assessment was based on the following five criteria [4]: time sequence of treatment and AEs, positional relationship between them, single risk factors, dechallenge (symptoms after cessation of moxibustion), and rechallenge (situations emerging during subsequent moxibustion). Each "yes" of the items was noted as 1 point. "No" and "not clear" were 0 points. The likelihood of each given AE was classified as certain (4-5), likely (3), possible (2), and unlikely (0-1) based on total scores. Disagreements in this procedure were resolved by discussion or by consultation with the third author, Shen.

3. Results

In this review, a total of 24 articles reported 64 cases of AEs associated with moxibustion (Table 1). They were from six countries, China, USA, South Korea, Spain, Japan, and Israel (Table 2).

Table 1: Case reports of AEs associated with moxibustion.

Adverse events	Number of reports	Number of cases
Allergies	6	7
Burns	6	43
Infection	6	6
Nausea or vomiting	1	2
Cough	—	1
Fetal harm	2	2
Basal cell carcinoma	1	1
Ectropion	1	1
Death	1	1
Total	24	64

Table 2: Countries in which AEs were reported to be associated with moxibustion.

Country	Number of reports	Number of cases
China	12	51
U.S.	5	6
Korea	4	4
Spain	1	1
Japan	1	1
Israel	1	1

The AEs of moxibustion reported in these articles include allergies, burns, infection, coughing, nausea and vomiting, fetal distress and premature birth, basal cell carcinoma (BCC), ectropion, hyperpigmentation, and other conditions (Table 3).

3.1. Allergy. There were seven cases of allergies associated with moxibustion. Six patients developed local skin flushing, swelling, and itching, and the last one reported a scattered red skin rash covering her whole body. Three patients recovered after 3–7 days of antiallergy treatments, and four recovered immediately after cessation of moxibustion treatment. All allergic reactions occurred in female patients and were reported in China (published in Chinese).

Zhang and Yang reported that a 36-year-old woman experienced mild allergies after moxibustion treatment [7]. This patient also had dysmenorrhea and was treated with acupuncture and indirect moxibustion on the lower abdomen. She underwent more than eight rounds of treatment and experienced redness and itching of the navel skin three times. The skin allergies disappeared the next day and never recurred in subsequent moxibustion treatments.

Feng and An reported a 66-year-old woman who received acupuncture and moxibustion treatments for her shoulder pain [8]. On the third day of moxibustion, she experienced rashes and itching on her neck and shoulders. The symptoms disappeared within 1 day of cessation of moxibustion, but they recurred when her roommates underwent moxibustion therapy in their shared apartment.

Hou reported a patient with a painful puerpera on her right wrist [9]. This was treated with acupuncture combined

Table 3: Adverse events associated with moxibustion.

Author/year/country	Cases age/sex	Disease treated	Moxibustion treatment	Adverse event	Practitioner	Follow-up	Causality
Zhang and Yang 2006, China	36 (F)	Dysmenorrhea	Indirect moxibustion with moxa box	Local allergy	Practitioner	Recovered (1 d)	Possible (1, 1, 0, 0, 0)
Feng and An 2005, China	66 (F)	Shoulder pain	NC[a]	Local allergy	Practitioner	Recovered (1 d)	Certain (1, 1, 0, 1, 1)
Hou 2008, China	1 (F)	Right wrist pain	NC	Local allergy	Practitioner	Recovered	Certain (1, 1, 0, 1, 1)
Li and Liu 2000, China	28 (F)	Facial paralysis	Indirect moxibustion with moxa stick	Local allergy	Practitioner	Recovered	Certain (1, 1, 0, 1, 1)
Li and Liu 2008, China	32 (F)	NC	Stay in moxibustion room	Local allergy	NC	Recovered (3 d)	Possible (1, 0, 1, 0, 0)
	22 (F)	Menstrual abdominal pain	Indirect moxibustion with moxa stick	Local allergy	Practitioner	Recovered (7 d)	Certain (1, 1, 1, 1, 0)
Wang 1998, China	28 (F)	Dyspepsia	Moxibustion in a closed room	Full-body allergy	Self	Recovered (3 d)	Likely (1, 0, 1, 0, 1)
Li 2002, China	Newborns	NC	Spark moxibustion	Burn and infection	NC	NC	Certain[b] (1, 1, 1, 0, 0)
Li 1989, China	18 (F)	Lower limb paralysis	Moxibustion with moxa stick	Burn	Practitioner	Recovered (40 d)	Certain (1, 1, 1, 0, 0)
Condé-Salazar et al. 1991, Spain	40 (M)	Tennis elbow	NC	Burn	Practitioner	NC	Certain (1, 1, 1, 0, 0)
Reinhart and Ruhs 1985, U.S.	2.5 (M)	Hyperthermia and seizures	Folk moxibustion	Numerous burns	Patient's father	Recovered (1 y)	Certain (1, 1, 1, 0, 0)
Fisman 2002, USA	38 (M)	Hepatic cirrhosis	NC	Lesion after burn	NC	NC	Certain (1, 1, 1, 0, 0)
Carron et al. 1974, U.S.	58 (M)	Cervical pain and spasm	Direct moxibustion	Burn	Practitioner	NC	Certain (1, 1, 1, 0, 0)
	62 (M)	Pain	NC	Burn	Self	NC	Certain (1, 1, 1, 0, 0)
Chau 2006, U.S.	53 (F)	Intermittent headaches	Scarring direct moxibustion	Cellulitis	Self	Recovered	Likely (1, 1, 1, 0, 0)
Chong et al. 1982, Korea	48 (F)	Arthritis	Moxa cauterization	*Pasteurella multocida* infection of the calf	NC	NC	Possible (1, 1, 0, 0, 0)
Choi et al. 2013, Korea	69 (M)	Insomnia	Jang-chim moxibustion	Pyogenic liver abscess	Practitioner	Recovered (22 d)	Unlikely (0, 1, 0, 0, 0)
Lee et al. 2008, Korea	78 (F)	Diabetes mellitus	Repeated moxibustion over 4 years	Spinal epidural abscess	Self	Recovered (170 d)	Possible (1, 0, 1, 0, 0)
Bardia et al. 2006, USA	66 (M)	NC	Moxibustion and cutting	Hepatitis C	Practitioner	NC	Unlikely (1, 0, 0, 0, 0)
Sternfeld et al. 1988, Israel	82 (F)	Hypochondrial pain	Indirect moxibustion combined with acupuncture and low-power laser exposure	Infection	Self	Recovered	Possible (1, 1, 0, 0, 0)
Cai 1999, China	26 (F)	Breech presentation	Moxibustion at BL67	Fetal death	Self	Fetal death	Possible (1, 0, 1, 0, 0)
Su 1999, China	1 (F)	Breech presentation	Moxibustion combined with chest-knee position therapy	Premature delivery	Self	Fetal death	Unlikely (1, 0, 0, 0, 0)

Table 3: Continued.

Author/year/country	Cases age/sex	Disease treated	Moxibustion treatment	Adverse event	Practitioner	Follow-up	Causality
Wen et al. 2011, China	47 (F)	Chemotherapy after colorectal cancer surgery	Moxibustion with moxa stick	Nausea and vomiting	Practitioner	Recovered	Likely (1, 0, 1, 0, 1)
	51 (M)	Chemotherapy after lung cancer surgery	Moxibustion with moxa stick	Vomiting and insomnia	Practitioner	Recovered	Possible (1, 0, 1, 0, 0)
	68 (F)	Lung cancer complicated with pulmonary infection	Inhalation of moxa smoke	Cough	NC	Recovered	Likely (1, 0, 1, 1, 0)
Chen 1998, China	41 (F)	Facial paralysis	Direct moxibustion	Lower eyelid loose and ectropion	Practitioner	NC	Likely (1, 1, 1, 0, 0)
Yun et al. 2009, Korea	58 (M)	Abdominal pain	Moxibustion on the same site repeatedly 10 years	Basal cell carcinoma	NC	No recurrence (5 y)	Likely (1, 1, 1, 0, 0)
Ogata et al. 1992, Japan	29 (M)	Asthma	Moxibustion and acupuncture	Death	Practitioner	Death	Unlikely (1, 0, 0, 0, 0)

[a]"NC": not clear.

[b]Although the scores for these burn cases were less than 4, they were classified as "certain" because they had been directly observed to have been caused by moxibustion.

with moxibustion. On the second day of therapy, she experienced redness, itching, and rashes on her face, neck, and chest. The reactions disappeared if only the moxibustion was ceased. It recurred upon subsequent moxibustion.

Li and Liu reported that a 28-year-old woman received moxibustion after cupping and acupuncture for her facial paralysis [10]. Indirect moxibustion was performed with lighted moxa stick on the right Yifeng (SJ17) and the lower front of the earlobe. Two days after the treatment, the patient's right auricle became swollen and red without pain or itching. When the practitioner ceased moxibustion, the patient continued to undergo cupping and acupuncture, and the symptoms disappeared. When the moxibustion was applied nine days later, the patient's right cheek was swelling again.

Li and Liu reported two allergic reaction cases of moxibustion [11]. One patient's mother, 32 years old, accompanied her son to his moxibustion and waited with him in the room during his treatment. Three days later, she was admitted to a hospital for obvious swelling of her head and face. She was discharged after three days of antiallergy treatments. Another 22-year-old girl was cauterized at Shenque (RN8) with a moxa stick to reduce menstrual pain and experienced redness, itching, and papules of the navel. Her allergic symptoms and signs disappeared one week later.

Wang reported an allergic reaction caused by excessive inhalation of moxa smoke [12]. A 28-year-old woman underwent two rounds of moxibustion in an open treatment room without any adverse reactions. However, during the third round, she underwent moxibustion in a closed room in which she later slept. When she woke up, she experienced chest tightness, suffocation, difficulty breathing, throat discomfort, irritability, itching, and scattered papules. The allergies disappeared after three days of antiallergy treatments. Seven days later, similar adverse reactions recurred and again resolved. They never reappeared after the moxibustion had ceased.

3.2. Burns. There were 43 cases involving burns. These were reported in 6 articles. Most of the burns were in newborns, children, and individuals with sensory disabilities. The symptoms of burns caused by moxibustion included pain, local skin blisters, ulcers, and secondary infections. They were usually curable with symptomatic treatments. Another large problem with the more severe burns was permanent and disfiguring scarring. These were associated with direct moxibustion.

Li reported an 18-year-old girl diagnosed with right lower limb paralysis [13]. Treated with indirect moxibustion after acupuncture, the patient's right Yanglingquan (GB34) suffered minor burns and then blistering and ulceration. After 40 days of symptomatic treatment, the wound finally healed. In this case, the patient had sensory disturbance of the right lower limb and could not tell the practitioner administering the moxibustion that she was feeling any pain, which increased the risk of burns.

Li reported 37 cases of newborn burns and infections associated with improper moxibustion from 1990 to 1997 [14]. These newborns had been treated with spark moxibustion, a folk form of moxibustion used to treat neonatal jaundice, most commonly by means of a burning object such as a rush tapped quickly on the skin. All patients showed varying degrees of burns, 34 mild and 3 moderate. Eight patients were diagnosed with secondary systemic infections, including one case of staphylococcal scalded skin syndrome (SSSS), six cases of septicemia, and one case of pyemia. The author inferred that improper and excess moxibustion were the main causes of these burns and infections.

Condé-Salazar et al. reported a 40-year-old male with numerous burns on the wrist and ankle [15]. The patient, who lived in Spain, received acupuncture and moxibustion therapy for his tennis elbow and experienced these burns as a result. However, the moxibustion therapy did cure his complaints effectively.

Reinhart and Ruhs reported an Asian boy, two and a half years old, who had numerous punched-out, circular burns distributed on his face, trunk, and extremities [16]. They were remarkably symmetrical in most areas. The boy had sustained the skin lesions when his father performed folk moxibustion after a visit to an American physician had failed to cure his child's illness. In this case, the patient's father was not an actual practitioner; he was attempting to repeat the practice as recalled from his childhood.

Carron et al. reported two cases of third-degree burns associated with moxibustion [17]. A 58-year-old white man suffered a long history of cervical pain and spasm aggravated by a postural deformity resulting from painful plantar calcaneal spurs. When conventional therapy failed to relieve his symptoms, the patient went to a local acupuncture clinic for acupuncture, scraping, and moxibustion treatments. Examination after more than three rounds of therapy showed two painful, third-degree burns, 3 mm in diameter, 3 mm deep, covered with black eschar and surrounded by a 3×4 cm area of erythema on the left heel. Another 62-year-old man had undergone three lumbar laminectomies and a dorsal rhizotomy for relief of pain of sciatic distribution. He sought acupuncture therapy at a clinic and was instructed to return home to continue his moxibustion therapy. Six months later, when the patient again sought medical attention, permanent scars were present at all moxibustion sites.

Fisman reported a 38-year-old Cambodian man presenting symmetrical hyperpigmented maculae on the abdomen [18]. These unusual skin findings had been caused by burns from the application of small pieces of smoldering cloth.

3.3. Infection. There were six cases of infection involving moxibustion. They were reported in Korea, the U.S., and Israel. Of these cases, three were surface tissues infections, two were deep organ infections, and one was hepatitis C virus (HCV) infection.

Chau reported a 53-year-old Korean woman who was admitted to a hospital with a diagnosis of cellulitis [19]. Before that, the patient, though untrained in Chinese medicine, had attempted to self-administer direct moxibustion for intermittent headaches and experienced cellulitis on her instep as a complication. She was treated with intravenous antibiotics for 24 hours and discharged in a good condition.

Chong et al. reported a *Pasteurella multocida* infection of the calf associated with moxibustion [20]. A 48-year-old woman from South Korea had a history of prednisolone treatment combined with acupuncture and moxibustion for her degenerative arthritis. *P. multocida* infection is rare in humans, but this organism was isolated and identified from fluid aspirated from this woman's calf. It was probable that the organism entered through the burn wound ulcers.

Choi et al. reported a 69-year-old man with pyogenic liver abscess (PLA) following acupuncture and moxibustion treatment [21]. This patient had received acupuncture on his arms and moxibustion on his abdomen three times per week for insomnia. About one month later, he felt nauseous and feverish and lost about 9 kg of body weight. An abdominal computed tomography (CT) scan with contrast revealed multiseptated cystic lesions in the right and left lobes of liver, the largest, which was in the left lobe, measuring about 10.0 cm. Pyogenic abscess was confirmed by ultrasound-guided percutaneous needle aspiration with Gram stain and culture of the aspirate. In this case, authors assumed that the patient had *Streptococcus intermedius* bacteremia after being treated with contaminated acupuncture needles and *Streptococcus intermedius* may have been seeded in the liver. The time sequence of the symptoms and the treatments suggested a possible causal relationship, but there was no direct evidence of that.

Lee et al. reported a spinal epidural abscess originating from cellulitis after moxibustion [22]. A 78-year-old woman was suffering from diabetes mellitus, hypertension, lower back pain, and knee osteoarthritis. She had cauterized her right third finger with moxa repeatedly over 4 years and had experienced recurrent infections with intermittent pus discharge. The authors believed that the patient's spinal epidural abscess originated from osteomyelitis and cellulitis of the finger secondary to burn injuries caused by repeated moxibustion.

Bardia et al. reported a 66-year-old Somalian man diagnosed with hepatocellular carcinoma secondary to cirrhosis due to hepatitis C [23]. The patient did not have the usual risk factors for hepatitis C but had undergone moxibustion and cutting of the wrists and abdomen using sharp objects or needles to release the bad blood. It is possible that he acquired hepatitis C through the sharing of infected knives or the burn wound associated with his moxibustion therapy.

Sternfeld et al. reported one case of *Serratia marcescens* infected silk suture rejected by combined acupuncture, moxibustion, and low-power laser therapy from the abdominal fascia [24]. An 82-year-old Israeli woman was complaining of right hypochondrial pains caused by a forgotten foreign body that had been implanted in her body during a cholecystectomy surgery twelve years earlier. Both acupuncture and moxibustion supplemented by low-power laser beam were performed on the site of infection. Acupuncture and low-power laser beam treatment were carried out by doctors at the clinic; patient was self-treated by moxibustion. After 20 sessions, a silk surgical suture was expelled. From then on recovery was spontaneous and fast.

3.4. Fetal Harm. There were two cases of fetal harm caused by moxibustion reported in China.

Cai reported one case of fetal death following moxibustion [25]. A 26-year-old woman was diagnosed with breech presentation during the eighth month of pregnancy and was told to treat herself using moxibustion at Zhiyin (BL67) to correct it. About ten minutes into her third treatment, the pregnant woman felt chest tightness, palpitations, dizziness, vomiting, and increased fetal movement. She was admitted to hospital with a diagnosis of umbilical cord around the neck of the fetus and fetal distress. There was no improvement after oxygen and drug therapy, and the fetus died three hours after her admission.

Su reported a case of premature delivery caused by moxibustion [26]. A pregnant woman suffered breech presentation at 29-week gestation and was treated with moxibustion combined with chest-knee position therapy. Seven hours after the treatment, the patient felt lower abdominal pain continuously. This pain grew progressively more intense. Three hours later, a large amount of clear liquid was discharged from her vagina, followed by an object which became lodged in the vagina. Emergency examination showed that the fetus had been delivered incompletely in breech presentation and had already died due to suffocation.

3.5. Other Adverse Events. Some rare adverse events related to moxibustion, such as coughing, nausea and vomiting, basal cell carcinoma, ectropion, xerosis, and death, were also recorded.

Wen et al. reported three cases of AEs associated with moxibustion in China [27]. A 47-year-old woman was admitted for chemotherapy after colorectal cancer surgery and sought moxibustion to reduce the discomfort induced by drugs. She was treated with moxa stick at the points of Zhongwan (RN12), Danzhong (RN17), Qihai (RN6), and Guanyuan (RN4). At 15 minutes into the first therapy, the patient felt nausea and vomiting, which recurred during the next day's moxibustion. A 51-year-old man presented similar symptoms. The third patient, a 68-year-old woman, was admitted to the hospital for her lung cancer complicated by pulmonary infection. She complained of the smell of moxa smoke and coughing when her roommates carried out moxibustion.

Yun et al. reported a large superficial basal cell carcinoma (BCC) arising from a burn scar secondary to repeated moxibustion [28]. A 58-year-old Korean man had applied moxa cautery to the same site repeatedly for relief of abdominal pain over ten years. He presented with a 3-year history of a dark reddish plaque on the lower part of the abdomen. Physical examination revealed a well-demarcated, round, 7.2×5.7 cm dark reddish plaque with some brown and black crusts and pigmentation on the lower part of the abdomen. Histopathological examination showed nests of basaloid cells arising from basal layers of the epidermis and extending into the dermis. There was peripheral palisading of the nuclei of the tumor cell nests and peritumoral lacunae between the tumor cells and stroma. Diagnosed as BCC,

the lesion responded well to radiation therapy and there was no evidence of recurrence 5 years later.

Chen reported a case of a loose lower eyelid and ectropion after moxibustion on the craniofacial points [29]. A 41-year-old Chinese woman suffered from the left facial paralysis. She was treated with direct moxibustion at Dicang (ST4), Jiache (ST6), Yangbai (GB14), and Sibai (ST2). After therapy lasting a few days, the patient was better but developed an eversion and sagging of the left lower eyelid. The author believed it to be a consequence of improper moxibustion at Sibai (ST2), where the infraorbital nerve, zygomatic branch of the facial nerve, and infraorbital artery are distributed.

Ogata et al. reported a 29-year-old Japanese man with bronchial asthma who died while undergoing acupuncture and moxibustion treatment [30]. The patient had suffered from periodic asthma attacks for nine years, and the asthmatic attacks became more frequent and persisted over longer periods during the last year before his death. After being treated with three rounds of moxa followed by two needles inserted for acupuncture, he suddenly collapsed and died. The autopsy findings of the lungs in this case were compatible with a diagnosis of severe asthma. The tissue injuries to the patient from the needles and the burns from the moxa treatment were found to be mild. The apparent cause of this death was respiratory dysfunction due to a severe asthmatic attack. It can be speculated that his death from asthma might have been associated with emotional stress and apprehension that he experienced while undergoing acupuncture and moxibustion for the first time.

4. Discussion

4.1. Traditional Understanding of Moxibustion Safety. Ancient literature on Chinese medicine rarely reported AEs of moxibustion, but they did indicate two issues regarding moxibustion safety. First, there are the contraindications of moxibustion, which are based on the TCM theory. For example, *Treatise on Febrile Diseases* advises the reader, "Be cautious and do not apply moxibustion when the patient's pulse is weak and fast." It is also suggested that some points that are located close to important organs, nerves, and blood vessels or where local subcutaneous tissue is thin be avoided during moxibustion to prevent injuries. In the *Zhen Jiu Jia Yi Jing*, moxibustion is forbidden on 28 acupoints. The ancients believed that acupoints on the head, face, and the distal ends of the limbs should not be used or used sparingly to reduce the intensity of moxibustion.

4.2. Experimental Studies on the Temperature and the Products of Moxibustion. Concerns about safety of moxibustion have only been raised in recent decades. There are two approaches to evaluate the safety of moxibustion: experimentation and observation in clinical settings. The therapeutic effects of moxibustion are generally believed to come mainly from thermal effects, radiation effects, and the pharmacological actions of the products of moxa combustion. Studies of the safety of the radiation used in moxibustion are rare, and the focus has been placed mostly on the other two factors, thermal effects and products of combustion. Burning moxa without flame can produce temperatures of about 548–890°C [31, 32]. Some people think that this treatment is essentially a physical thermal effect [33]. Other experiments have confirmed that a single Zhuang (unit of dosage of moxibustion) of moxa cone (2 mg) moxibustion administered to the mouse abdomen can raise the temperature of the mouse's skin to 130°C and that of the area beneath the skin to 56°C [34]. Some have observed the impact of moxibustion at different distances on local skin temperature. The safe distance for indirect moxibustion seems to be 3-4 cm [35, 36]. The temperature of burning moxa can play a therapeutic role, but it offers a risk of burns if administered improperly.

Many of the components of the moxa and the products of their combustion have been identified [37, 38]. They include a variety of biological activities and play important roles in the comprehensive effects of moxibustion [39–41]. Some products of moxa combustion are brown and tar-like. They take effect after entering the human body through burn-damaged skin. Another product of moxa combustion is smoke. Moxa smoke contains many complex components, and its volatile ingredients include ammonia, alcohols (ethylene glycol, pentyl-butanol), aliphatic hydrocarbons, aromatic hydrocarbons, terpene compounds, and their oxides. They may come from products of incomplete combustion, volatile oil, and the products of the oxidation of that oil. There is debate regarding the security of moxa smoke. The mugwort leaf contains terpenes. It may produce polycyclic aromatic carcinogens during combustion. During moxibustion, the concentration of air pollutants, such as nitrogen oxides, carbon monoxide, and particulates, is ten times higher than standard class II as issued by the State Environmental Protection Act. These substances can damage the health of patients and staff [42]. Acute toxicological testing of moxa smoke on SPF degree SD rats showed the median lethal concentration (LC_{50}) to be 11,117 mg/m^3. It was therefore classified as minimally toxic according to WHO standards [43]. An AMES test showed moxa smoke condensation (MSC) with metabolic activation using a liver fraction (S-9) to enhance the mutagenic activity of *Salmonella* strain TA98. This means that MSC may be mutagenic [44]. However, one study gave consideration to short-term and long-term exposure. It showed that the volatile matter and carbon monoxide generated by moxa smoke under normal operating conditions did not exceed safety levels [45].

4.3. Analysis of the AEs in Moxibustion Included in This Review. Many large-sample surveys of the safety of acupuncture have been carried out around the world [46–48]. More than 762 cases (in 219 articles) of AEs related to acupuncture were reported before 2011 [49]. There are far fewer clinical safety survey data for moxibustion and far fewer case reports of AEs. Two previous reviews have been conducted on the safety of moxibustion treatment. Parka reviewed 13 case reports (7 clinical trials and 1 prospective survey) of AEs related to moxibustion [4]. Xu et al. reviewed 4 (294 acupuncture and 10 cupping) [5]. The present review analyzed 64 cases in 24 articles, covering all cases cited in the two articles above.

These cases were mainly found in China (51 cases/12 articles), the U.S. (5/6), and Korea (4/4). This is consistent with the fact that there is far more use of moxibustion in these areas than elsewhere in the world. Adverse reactions do not seem to be related to patient age (from newborn to 82 years old). The most common risk of moxibustion was found to be burns (43 cases/6 articles), which are caused by the high combustion temperature of moxa. Burns can be caused by direct contact with the products of combustion (direct moxibustion) or by radiant heat (indirect moxibustion). However, the issue of whether moxibustion-induced burns are really an adverse event must be addressed. In traditional moxibustion, also known as scarring moxibustion or suppurative moxibustion, local minor burns, purulence, and scarring during treatment were always considered normal. Most ancient Chinese doctors were in favor of using scarring moxibustion. They believed "where there is moxibustion scar, there is cure." Scarring moxibustion is still used in modern clinical practice. Although accidental burns may happen during moxibustion, it was here noted that some patients in reports of burns accepted these skin lesions as the natural results of the therapy. For this reason, to determine whether a burn is a side effect of moxibustion or not, the doctors' expectations and patients' acceptances should be taken into account. The final therapeutic effects may have a nonignorable influence on this assessment.

Allergies (7 cases/6 articles) and infections (6 cases/6 articles) were reported at frequencies similar to those of other adverse reactions to moxibustion. However, the causality between allergies and moxibustion was stronger (average likelihood score: 3.29) than that between the infections and the moxibustion (average likelihood score: 1.83). Most allergic reactions occurred at the local level and may have been caused by physical (e.g., heat) and chemical (e.g., tars, ammonia, and alcohols) stimulations associated with moxa combustion. Only one case of full-body allergy was reported. It was due to excessive inhalation of smoke in a closed room. A survey of moxibustion practitioners in five Chinese medicine hospitals in Guangdong province showed that moxibustion smoke raised the incidence of chronic laryngitis from 3.70% (nonacupuncturists) to 26.67% (acupuncturists) ($P < 0.05$) [50]. Local skin infections were usually secondary to burns. Deep organs infections (e.g., liver abscess, spinal epidural abscess) and viral infection (e.g., hepatitis C) could be secondary to local infections. Very weak causality was found between nonlocal infections and moxibustion.

Moxibustion at Zhiyin (BL67) is often used to correct abnormal presentation in TCM. Recently, a multicenter randomized controlled trial showed moxibustion at acupoint BL67 to be an effective and safe means of correcting nonvertex presentation when used between 33 and 35 weeks of gestation [51]. However, the two cases of fetal distress and premature delivery presented here indicate the potential risks of fetal harm related to moxibustion. Nausea and vomiting after moxibustion were observed solely in cancer patients who were undergoing chemotherapy and were sensitive to the smell, which can easily cause gastrointestinal reactions. The case of loose lower eyelids and ectropion after moxibustion indicated the potential risk of damage to superficial nerves and vessels. In the case of BCC secondary to long-term moxibustion, it is possible that the burn scar secondary to moxa can be repeatedly cauterized. This caused the development of BCC in an area protected from sun exposure. It is rare for reports of death after moxibustion. In the only death evaluated here, the causality between the consequence and moxibustion was assessed as unlikely, but the effects of stress and other consequences of moxibustion should not be ignored, especially considering that this patient was entirely new to moxibustion.

5. Conclusion

Moxibustion is considered a safe and effective traditional therapy, and large numbers of clinical reports have indicated that it is effective and associated with few adverse events. There is no absolutely safe treatment in the world, and moxibustion may be also related with some security risks in certain circumstances. Although the studies examined here included some mistakes, such as nonaccidental injuries as side effects, and some adverse events have little causal relationships with moxibustion, the evidence collected here was consistent with that of previous reports indicating the risks of moxibustion. The position, duration, distance between moxa and skin, proficiency of doctors, patient conditions, stimulations from smoke, and even the environment of treatment can affect the safety of moxibustion. The exact causes of most of these AEs cannot be determined. This issue should be addressed in further experimental studies, clinical trials, and case reports on the safety of moxibustion. Improving practitioner skills, regulating the operations, and controlling for time, distance, dose, and protection can reduce the incidence of adverse reactions and improve the safety of moxibustion.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

The author of Deng contributed to the work equally and should be regarded as co-first author.

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Research Article

The Effect of Different Laser Irradiation on Cyclophosphamide-Induced Leucopenia in Rats

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Objective. To assess the effect of different lasers on cyclophosphamide- (CTX-) induced leucopenia in rats. *Methods*. 11 rats were normal control and 55 rats were injected with a dose of 80 mg/kg CTX for the first time and 40 mg/kg on the 6th and the 11th days to establish a leucopenia model. Rats of the irradiation groups received a 5-minute laser irradiation with either single 10.6 μm or 650 nm laser or alternatively 10.6 μm–650 nm laser irradiation, besides a sham treatment on acupoint Dazhui (DU 14) and acupoint Zusanli (ST 36) of both sides, 8 times for 16 days. Normal and model control group received no treatment. *Results*. On day 16 after the first CTX injection, the WBC counts from all the laser irradiation groups were significantly higher than those from the model control and the sham group ($P < 0.05$), while there were no significant differences compared with the normal control ($P > 0.05$). The TI of 10.6 μm–650 nm laser irradiation group was significantly higher than that of the model control group ($P < 0.05$). *Conclusions*. The single and combined 10.6 μm and 650 nm laser irradiation on ST36 and DU14 accelerated the recovery of the WBC count in the rats with leucopenia.

1. Introduction

Leucopenia, a disease with a white blood cell (WBC) number consistently lower than 4.0×10^9/L in the peripheral blood, can be classified into primary and secondary leucopenia. The main secondary cause of leucopenia is bone marrow suppression that resulted from antitumor drugs. Cancer chemotherapy drugs have long been considered immune suppressive [1]. Bone marrow suppression can severely influence the effect of chemotherapy as well as quality of life after chemotherapy. As a main approach for antitumor therapy, chemotherapy can improve the pathological state of the patients. However, its side effects, such as bone marrow suppression, also bring harm to the patients' physical, mental, and economical states. Clinical studies showed that acupuncture and moxibustion associated with relief of side effects and increase of leukocyte in patients with leucopenia induced by chemotherapy. However, the evidence was of low quality [2–5]. Animal studies found that electroacupuncture and acupoint injection showed benefit on bone marrow suppression after chemotherapy, through protection of hematopoietic function, improvement of WBC counts in the peripheral blood, and enhancement of immune function [6, 7]. However, needling acupuncture might result in some degrees of discomfort, such as pain, while moxibustion produces smog, which could be irritant and harmful to the health of both the practitioners and patients [8–10]. These factors limited the application of traditional acupuncture in the clinical use. Laser acupuncture/moxibustion is a good substitute of traditional acupuncture/moxibustion and has been researched for more than 20 years [11]. Laser acupuncture/moxibustion causes no skin penetration and smoke and is easier to practice and regulate. Our previous research found that the CO_2 laser with the wavelength of 10.6 μm and semiconductor laser with the wavelength of 650 nm have shown some benefit for pituitrin-induced bradycardia and knee osteoarthritis [12, 13]. The purpose of this study was to explore the effect and the mechanism of acupoint irradiation with the single 10.6 μm

CO_2 laser, the single 650 nm semiconductor laser, and the combination of the two lasers in cyclophosphamide- (CTX-) induced leucopenia.

2. Materials

2.1. Animals. A total of 66 healthy adult male SD rats were supplied by Animal Experiment Center of Shanghai Traditional Chinese Medicine University, with body weight of 200 ± 20 g and normal white blood cell (WBC) count. The license number for animal manufacture was SCXK (HU) 2009-0018, bought from Shanghai Xipuer-Bikai Lab Animal Co., Ltd. The license number for the use of experimental facilities in Shanghai University of Traditional Chinese Medicine was SCXK (HU) 2009-0069.

2.2. Reagents. The CTX (0.2 g) used to induce leucopenia was bought from Jiangsu Hengrui Pharmaceutical Incorporated Company. The manufacturing lot number for it was H32020857.

2.3. Apparatus. The CO_2 laser acupuncture apparatus (SX10-C1) and the 650 nm semiconductor laser acupuncture apparatus were produced by Shanghai Wanqi Photoelectric Technology Co., Ltd. The wavelength of the CO_2 laser was 10.6 μm and its output power was 55 ± 5 mw. The wavelength of the semiconductor laser was 650 nm and its output power was 36 mw with a diameter of light spot of 1 mm [12]. ANI full-automatic animal blood cell analyzer (XFA6030) was bought from Nanjing Pulang Medical Equipment Co., Ltd.

3. Methods

3.1. Establishment of Rat Leucopenia Model. All animals were raised and bred in the same standard environment, with the room temperature of (24 ± 1)°C and humidity of 50–70%. An improved method to establish animal model for leucopenia was used [14, 15]. The rats were weighed and CTX was used for intraperitoneal injection in a body weight-dependent dose manner. A total of 80 mg/kg of CTX was injected for the first time and 40 mg/kg on the 6th and the 11th days.

3.2. Selection and Location of Acupoints. Acupoint Dazhui (DU 14) and acupoint Zusanli (ST 36) of both sides were selected on the basis of TCM theory as well as the previous clinical studies [16]. Acupoints were located referring to standards described in "Experimental Acupuncture Science" [17]. We located Zusanli at posterolateral knee joint, about 5 mm underneath the fibulae capitulum, and Dazhui between the spinous process of the 7th cervical vertebra and the 1st thoracic vertebra, in the middle of the back [17].

3.3. Animal Grouping. Rats were numbered according to their body weight. A total of 66 rats were randomly allocated into six groups with a random number produced by SPSS18.0. These groups included normal control group, model control group, sham laser irradiation group, 10.6 μm CO_2 laser irradiation group, 650 nm semiconductor laser irradiation group, and combined 10.6 μm–650 nm laser irradiation group. With the exception of normal control group, the rats in the other five groups were induced by leucopenia with CTX. After inducing leucopenia, the 3 true laser groups were irradiated on the acupoint Dazhui (DU 14) and bilateral acupoint Zusanli (ST 36) with 10.6 μm CO_2 laser, 650 nm semiconductor laser, and combined 10.6 μm–650 nm laser, respectively. Each acupoint received irradiation for 5 minutes (min) every other day, with a total of 8 sessions of irradiation. The sham laser irradiation group was treated the same way as true laser groups, but without laser output.

3.4. Sample Collection and Detection

3.4.1. Detection of WBC Counts in the Peripheral Blood Collected from Tail End of the Rats. Rat tail was cut and blood was collected into tubes with anticoagulant one day before as well as 1, 4, 15, and 16 days after the CTX injection. Animal blood cell analyzer was used to count the blood cell numbers. WBC counts were also recorded.

3.4.2. Calculation of Thymus Index (TI). Thymus gland was excised from each rat on the 16th day after the first injection of CTX, washed with physiological saline, and weighed using a balance with 0.0001 accuracy. The TI was calculated with the formula TI = (index of thymus weight/body weight) × 10.

3.5. Statistical Analysis. The SPSS18.0 software was used for statistical analysis. The outcome data was expressed as mean ± standard deviation (mean ± SD) when the data accords with normal distribution. One-way ANOVA was used for comparison between groups and ANOVA for repeated measurement method was used in comparison within groups. The TI was analyzed with one-way ANOVA and the differences were considered statistically significant when the P was less than 0.05.

4. Results

4.1. Establishment of Rat Leucopenia Model. Before the first CTX injection, WBC counts of the rats in all groups were normal showing no statistically significant differences ($P > 0.05$). After CTX injection, the WBC counts in the other 5 groups significantly decreased compared with the normal control group ($P < 0.05$), while there were no statistically significant differences among the 5 groups ($P > 0.05$). These results indicated that we successfully established the leucopenia model in rat (Table 1). There was one rat that died after CTX injection in each of the model control, sham irradiation, 10.6 μm laser irradiation group, and 10.6 μm–650 nm irradiation group.

4.2. Changes of WBC Counts after Laser Irradiation. Results showed that WBC counts decreased after the first CTX injection and then rose at day 16. On the first and fourth days, WBC counts were significantly lower in all the other 5 groups compared to that of the normal control group ($P < 0.01$). Sixteen days after first CTX injection, the WBC counts

Table 1: WBC counts in rat peripheral blood (mean ± SD, $\times 10^9$).

Group name	Animal number	Before the first CTX injection	After the first CTX injection		
			1 d	4 d	16 d
Normal control	11	11.70 ± 4.36	7.72 ± 2.85	9.47 ± 3.9	11.17 ± 1.48
Model control	10	13.87 ± 3.12[1]	2.50 ± 0.69[2]	2.48 ± 2.01[2]	6.24 ± 3.37[2]
Sham irradiation	10	14.19 ± 2.94[1]	1.26 ± 0.61[2]	1.20 ± 0.54[2]	7.33 ± 4.17[2]
10.6 μm–650 nm	10	11.25 ± 1.77[1]	0.76 ± 0.62[2]	1.35 ± 1.34[2]	10.01 ± 4.17[1,3,4]
650 nm	11	11.83 ± 2.06[1]	1.88 ± 0.61[2]	2.42 ± 0.95[2]	10.11 ± 3.11[1,3,4]
10.6 μm	10	11.66 ± 2.17[1]	1.26 ± 0.47[2]	1.68 ± 1.10[2]	11.89 ± 5.56[1,3,4]

Compared with normal control: [1]$P > 0.05$, [2]$P < 0.05$; compared with model control: [3]$P < 0.05$; compared with sham irradiation: [4]$P < 0.05$.

Table 2: The TI of each group 16 days after model establishment.

Group name	Animal numbers	Weight (g)	TI
Normal control	11	307.36 ± 20.92	1.7637 ± 0.1257
Model control	10	240 ± 12.27	0.6327 ± 0.0610[1]
Sham irradiation	10	232.5 ± 17.46	0.8773 ± 0.0765[1]
10.6 μm–650 nm	10	244.5 ± 19.03	1.1174 ± 0.2950[2]
650 nm	11	243.73 ± 19.92	0.9361 ± 0.0961[1]
10.6 μm	10	242.1 ± 19.90	0.8668 ± 0.0902[1]

Compared with normal control group: [1]$P < 0.05$; compared with model control group: [2]$P < 0.05$.

returned to normal level in the 3 true laser irradiation groups with no statistically significant difference compared to that in the normal control group ($P > 0.05$). WBC counts were still lower in the model control and sham irradiation groups as compared to that in the normal control group ($P < 0.05$) (Table 1).

Sixteen days after the first CTX injection, the WBC counts were statistically significantly higher in each group treated with laser irradiation as compared to that in the model control group ($P < 0.05$) and sham irradiation group ($P < 0.05$). The WBC counts in the sham irradiation and model control groups showed no statistically significant difference at any of the time points ($P > 0.05$).

4.3. Thymus Index. Sixteen days after the first CTX injection, the TI in each group with laser irradiation was significantly lower than that of the normal control group ($P < 0.01$), while the TI in the 10.6 μm–650 nm laser group was statistically significantly higher than that of the model control group ($P < 0.05$) (Table 2).

5. Discussion

Our result indicated that WBC counts in groups with irradiation of 10.6 μm CO_2 laser, 650 nm semiconductor laser, and 10.6 μm–650 nm laser recovered more quickly than those in the sham irradiation and model control groups. Since there was no difference between the WBC counts in the sham irradiation and model control groups, the effect of the factors other than laser acupuncture stimulation on experimental results, such as the fixation of rats, might be excluded. This finding was similar to our previous pilot study, in which we found similar therapeutic effect of 10.6 μm CO_2 laser and 650 nm semiconductor laser for the CTX-induced leucopenia in rat.

Thymus gland reflects the state of the immune function, which might be suppressed by chemotherapy. Results showed that only the combination of 10.6 μm–650 nm laser irradiation improved the TI of the leucopenia model rats, while neither 10.6 μm laser nor 650 nm laser alone improved the TI, indicating that the combination of both lasers is necessary to improve the immune function, which is in accordance with the previous research findings [18].

In the view of traditional Chinese medicine, leucopenia belongs to the category of consumptive disease and blood deficiency. The chemotherapy drugs are normally working well to treat cancerous cell; however, their toxicity is harmful to Qi and blood, as well as the function of Zang and Fu organs. Stomach is a reservoir of food and drink as well as a source of Qi and blood. Zusanli (ST 36), located on the stomach meridian, can regulate and tonify the spleen and stomach Qi, thus regulating and facilitating Qi and blood. Dazhui (Du 14), the crossing point of all Yang meridians on the Du meridian, can invigorate Yang Qi and blood. Both ST 36 and DU 14 are the most commonly used acupoints in treating leucopenia as reported [16].

A general method used for model establishment with the CTX is to inject animals only one time, lasting for 1 to 5 days according to the dose. We have used this method in our previous study and established a model for rat leucopenia, which lasted for 10 days [18]. After iterative trial and error, we finally chose the method of multiple injection of CTX at a low dose in order to establish the model for rat leucopenia. With this method, the established model lasted for about 16 days, which was considered as a relatively steady model that replicated leucopenia caused by clinical chemotherapy and was convenient for observation of curative effects.

The CO_2 laser is a far-infrared light with the wavelength of 10.6 μm, which can be absorbed by epidermis within 0.2 mm and can bring about a rapid, obvious, and abiding heat effect [19]. The 650 nm semiconductor laser can reach the deep epidermis, stimulate several sensors on the nerve ending at the acupoint, and result in effects similar to those of acupuncture. Our previous study on animals indicated that 10.6 μm–650 nm laser irradiation on acupoint Neiguan had effect on pituitrin-induced bradycardia in rabbit, with CO_2 laser playing the main role in this action [12].

In conclusion, irradiation with the 10.6 μm laser, 650 nm laser, and combined 10.6 μm–650 nm laser on certain acupoints boosted the recovery of WBC counts in the peripheral blood and improved the hematopoietic function of the rats with leucopenia. The combination of both lasers might be necessary to improve the immune function. This approach and its mechanism of action need further investigations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Jizhong Zhao, Ke Cheng, and Ling Zhao contributed equally to this study and should be considered as cofirst authors.

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Research Article

Do Changes in Electrical Skin Resistance of Acupuncture Points Reflect Menstrual Pain? A Comparative Study in Healthy Volunteers and Primary Dysmenorrhea Patients

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Electrical skin resistance (ESR) measurements were performed with a four-electrode impedance detector at 10 points bilaterally on the first day of and the third day after menstruation in 48 healthy volunteers and 46 primary dysmenorrhea (PD) patients, to assess whether ESR changes of acupuncture points can reflect menstrual pain or not. The results showed statistical reductions in ESR imbalance ratio between left and right side that were detected at SP8 (Diji) and GB39 (Xuanzhong) ($P < 0.05$), and a statistical increase was detected at SP6 (Sanyinjiao) ($P = 0.05$) on the first day of menstruation compared with those values on the third day after menstruation in dysmenorrhea group. No significant differences were detected at other points within and between two groups ($P > 0.05$). This study showed that the imbalance of ESR at uterine-relevant points in PD patients is not significantly different from those of healthy women on both the 1st day of and the 3rd day after menstruation. The ESR imbalance ratio of certain points can either be lower or higher during menstruation in PD patients. The ESR property of acupuncture points needs to be investigated in further clinical trials with appropriate points, diseases, larger sample sizes, and optimal device.

1. Introduction

In classic Chinese acupuncture theory, acupuncture points are not only the sites receiving needling stimulation for treatment, but also the places reflecting the condition of diseases for diagnosis. Electrical skin resistance (ESR) measurements have been used to identify the electrical properties of points and meridians and particularly used for clinical diagnosis. Since 1950s, many studies asserted that points and meridians possess reduced ESR compared to adjacent areas [1–4]. Based on this understanding, it has been assumed that the differences in ESR at acupuncture points may reflect physiologic processes and pathologic conditions in the human body [5, 6]. Numerous devices have been created and widely used for the purpose of locating acupuncture points, diagnosis, and treatment in the clinical practice and research of acupuncture [7–9].

This widely believed explanation of electrical properties of acupuncture points, however, has been questioned yet. Although many studies conducted recently showed that points and meridians had unique electrical properties [10–13], a systematic review found that those studies were

generally poor in quality and limited by small sample size and multiple confounders; thus the evidence does not conclusively support the claim that points and meridians are electrically distinguishable [14]. Several studies also showed that the majority of measured points or meridian did not show a changed ESR [15–17], or the phenomenon of low skin resistance does not exist to all acupuncture points [18]; therefore, ESR measurement has been equivocal for acupuncture point localization or diagnostic and therapeutic purposes.

Many technical issues in ESR measurement at points including electrode polarizability, stratum corneum impedance, presence of sweat glands, choice of contact medium, and electrode geometry have been considered gradually by researchers [19]. The controversial results of related studies were mainly caused by ESR measurement devices and measuring procedures. Most of devices are based on a two-electrode method, which may cause significant fluctuation of voltage between the two electrodes due to variable contact impedance between electrodes and tissue. Moreover, variation of pressure on electrodes, angle, or duration of the measurement also can influence the results [10, 12, 13, 16, 17]. However, a four-electrode method can minimize error due to electrode-sample contact resistance, which is serious in two-electrode method [15, 20].

Another important issue in relation to ESR of points is the physiologic and pathologic states of the body. Since acupuncture points are used for both treatment and diagnosis, a comparative investigation between healthy people and patients may reflect the exact electrodermal property of points. Some studies showed correlations between changes in ESR at specific acupuncture points and disease states [21–23]. However, the results of those studies might be questioned by using two-electrode devices.

Chinese acupuncture theory believes that specific acupuncture points, particularly *Yuan*-source points and *Xi*-cleft points, have more significant properties to reflect the physiological and pathological conditions of distant corresponding organ systems [24]. Modern research also showed that certain diagnostic zones on the skin in western medicine had relationship with certain specific points [25]. Our previous study showed that different acupuncture points had different therapeutic effects on primary dysmenorrhea (PD) [26]. Therefore, we propose a hypothesis; that is, ESR imbalance ratio at uterine-relevant points when menstrual pain attacks in PD patients may change significantly more than those in healthy women. In order to assess whether ESR changes of acupuncture points can reflect menstrual pain or not, we conducted this study.

2. Materials and Methods

2.1. Setting and Participants. Forty-eight (48) healthy volunteers aged 23.67 ± 2.40 yr and 46 patients with primary dysmenorrhea aged 23.76 ± 2.59 yr were recruited on the campus of Beijing University of Chinese Medicine (BUCM, Beijing, China), where the trial was conducted. All volunteers signed an informed consent form before participation. The Medical Ethics Committee of BUCM approved the trial.

Eligible participants in dysmenorrhea group met the following inclusion criteria: (1) the diagnostic criteria of primary dysmenorrhea in the Primary Dysmenorrhea Consensus Guideline [27]; (2) age 15–30 years without history of delivery; (3) normal menstrual cycle (28 ± 7 days); (4) course of dysmenorrhea varying from 6 months to 15 years; (5) experienced menstrual pain scoring more than 40-mm on a 100-mm VAS in continuous three menstrual periods prior to the trial; (6) no oral administration of any analgesic nor acceptance of other therapies in 24 h before the trial; (7) no common cold in one week before the trial and with normal body temperature.

Women with secondary dysmenorrhea caused by endometriosis, uterine myomas, endometrial polyps, pelvic inflammatory disease, and other gynecological problems were excluded. Women with scars on the skin at measured points were excluded.

Participants in healthy group had no history of chronic diseases and were healthy at the time of enrolment. Inclusion criteria of age and duration of menstrual cycle were the same as those in dysmenorrhea group. Considering that some Chinese people may have a basic knowledge of acupuncture, participants with history of acupuncture treatment and knowledge of acupuncture were excluded in both groups.

2.2. Electrical Skin Resistance Detecting Device. An improved ESR detecting device based on a four-electrode method detector [20] was specially designed and fabricated for this study by School of Physics and School of Electronics Engineering and Computer Science of Peking University (Beijing, China). The new system has been evaluated as a reliable tool for researches on ESR [28]. It consists of two parts, the computer-controlled data obtaining and analyzing system (Figure 1) and 12 metal pipe probes. (Figure 2 shows one probe with three linearly oriented electrodes, which are P_V, P_I, and P_{VR} from left to right, respectively.) The four electrodes consist of three small linearly oriented electrodes (P_V, P_I, and P_{VR}, 5 mm in diameter each, L_1 = 8 mm, L_2 = 12 mm; see Figures 2 and 3) which are sited closely and a reference electrode (P_{IR}) which is much larger (50 mm in diameter) and far from the other three electrodes (Figure 3). A common reference electrode (P_{IR}) is shared by the 12 linearly oriented electrodes. During the measurement, voltage applied is less than or equal to 300 mV. Signal shape is sinusoidal wave which is generated by the signal generator (see Figure 4). An alternating current (with frequency of 5 KHz and amplitude of 20–30 μA) was delivered to two electrodes (P_I and P_{IR}) to eliminate tissue polarization, which only can be induced by direct current. Voltage is measured by a voltmeter located between the other two electrodes (P_V and P_{VR}). The voltage signal was input into a high impedance amplifier and the signal was amplified, filtrated, and sent to a galvanometer and a recorder. The schematic diagram of the device is given in Figure 4; note that for simplicity in the figure only the Nth probe is shown. The specific arrangement

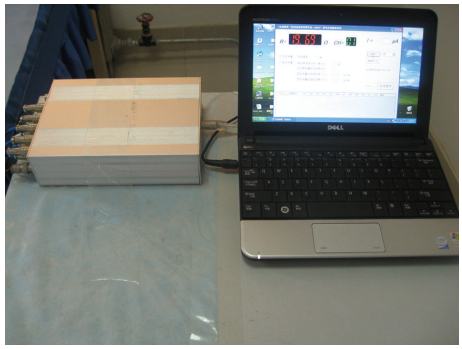

Figure 1: ESR measurement device.

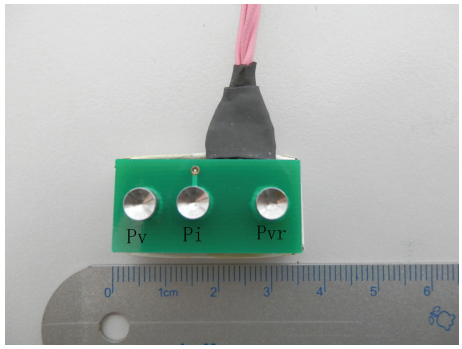

Figure 2: Probe with three electrodes (P_V, P_I, and P_{VR}).

of the electrodes ensures that the device measures only the resistance of the small subcutaneous region lying just below the electrode P_I. An advantage of such a system is that it can be used to monitor, almost continuously, the ESR variation of up to 12 skin points. Therefore, it can be very useful for studying how the ESR varies under same physiological or pathological conditions.

2.3. Detecting Points. According to the theory of Chinese medicine, female reproductive homeostasis is closely related to the organs: "spleen," "liver," and "kidney"; therefore, some specific points on spleen, liver, and kidney meridians including three *Xi*-cleft points (SP8, LR6, and KI5), three *Yuan*-source points (SP3, LR3, and KI3), and a nonspecific point (SP10) were chosen as uterine-relevant points. GB39 and an adjacent nonmeridian point located at the midpoint between stomach meridian and gallbladder meridian on the same level of SP6 and GB39 were chosen as uterine-irrelevant points. All acupuncture points were determined according to standards issued by WHO [29]. To ensure consistency of assessment, the above-mentioned points in all participants were located and marked on the skin by the same senior acupuncturist, who has more than 10-year clinical experience in an academic acupuncture clinic, throughout entire study.

2.4. Blinding. Participants were blinded to investigation, as those who had history of acupuncture treatment and knew the effects of acupuncture points were excluded. The acupuncturist who put the probes on the points maintained neutral communications with all participants. In the process of trial, the acupuncturist was separated from the ESR measurement technician. Both of them were blinded to measurement allocation.

2.5. Outcome Measures. ESR imbalance ratio (absolute differential value of ESR at same points between left and right side/higher ESR at either side) was used as primary outcome measure in this study. The ESR values of each point at left and right side were measured firstly; then we use the following formula to convert the raw data of ESR to imbalance ratios:

$$\text{ESR imbalance ratio} = \frac{\left|\text{ESR}_L - \text{ESR}_R\right|}{\text{Higher ESR (L or R)}}. \quad (1)$$

According to the human body symmetry, significant asymmetry of acupuncture points in terms of ESR, skin temperature, and transcutaneous CO_2 emission may suggest a pathological condition [30–32]. To measure the difference of those indexes of human bilaterally corresponding points are used as an assistant diagnostic method. ESR asymmetry was expressed by the absolute difference of bilateral points, or the ratio of values on left point and right point with the same name. However, the value of the former one will be influenced by large fluctuation of raw ESR values and cannot be used for comparing different indexes. Our pretest on a 74-year-old healthy male volunteer at 12 points showed a significant large difference of ESR (26.5 Ω–32.67 Ω). It suggested that the ESR difference was not suitable for comparison directly. The later one, ratio of left ESR and right ESR, was not a normalized value; thus, it was also not suitable to reflect the point asymmetry. However, one study showed that balance ratio (difference of measured values between left side and right side/higher value at either side) was a satisfied index reflecting the balance condition of acupuncture points [32]. If the value of the ratio is 0, which may indicate well balance of ESR at one point between left and right sides. If the ratio was 1, it indicated a significant imbalance of ESR. Therefore, we used this imbalance ratio as outcome measure in this study.

2.6. Procedure. The procedure of our trial was as follows. (1) Eligible participants were required to contact trial coordinator and arrive at the experimental room on their first day of menstruation (within 24 hr). (2) On arrival, participants acclimatized for 15 min in a standardized room at 22.00 ± 1.42°C and a humidity of 50.48 ± 3.93%. (3) The acupuncturist located all 10 points on both sides and made marks on the surface of skin with ink marker. (4) Glycerine (50%) was used as medium between the electrodes and skin and wiped on 5 points on both sides (LR6-Zhongdu, SP6-Sanyinjiao, GB39-Xuanzhong, KI3-Taixi, and SP3-Taibai, 10 points in total) firstly and then the acupuncturist put 10 probes on those 10 points with current electrodes (P_I) on the center of measured points and attached by elastic straps with proper pressure. The reference electrode (P_{IR}) was bound on the medial side of upper left arm distal to the elbow crease. (5) After 30 min of continuous measurement on first 5 points, 10 probes were moved on another 5 points (SP10-Xuehai, SP8-Diji, nonacupoint, KI5-Shuiquan, and LR3-Taichong)

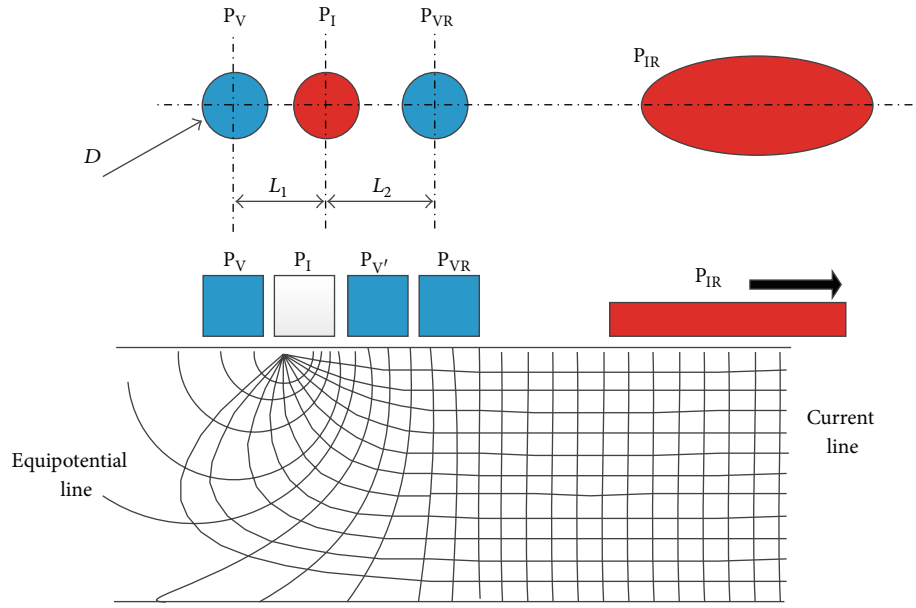

Figure 3: The four electrodes diagram of the ESR detecting device.

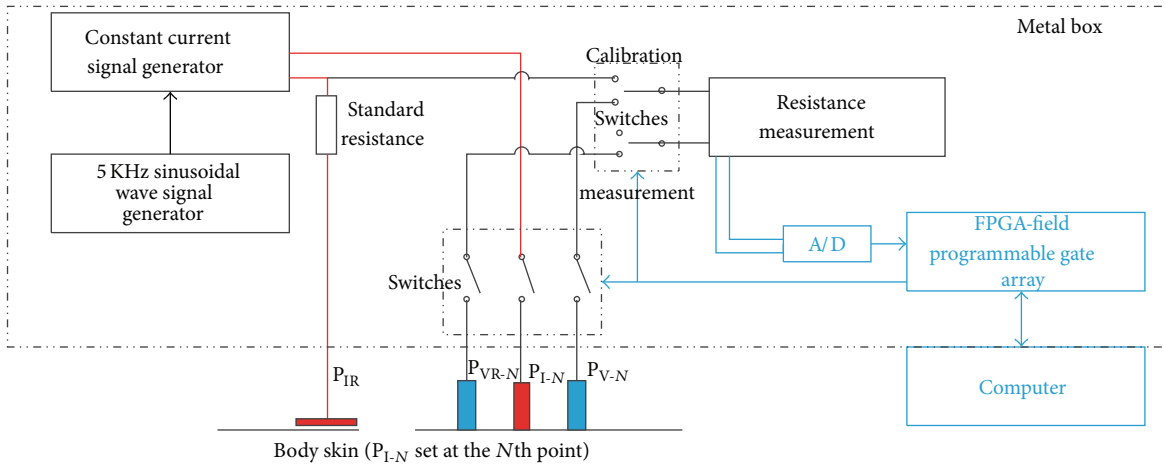

Figure 4: Schematic diagram of the ESR detecting device.

on both sides for another 30 min measurement. (6) On the third day after the period finishes, the same procedure was repeated again at the same time of first measurement. Both the acupuncturist and ESR measurement technician were not allowed to talk with the participant when measurement started. Figure 5 illustrates the procedure of recruitment and measurement.

2.7. Statistical Analysis. Means and standard deviations (SD) and median scores as well as range were determined for ESR difference ratio at the same points between left and right side. The results were analyzed with SAS (version 9.2, SAS Institute, Cary, NC). Median scores were analyzed with the Kruskal-Wallis test to assess differences on the median between two groups on two measurement days and between two days within each group of the study at each point. In this study, we considered $P \leq 0.05$ to be significant.

3. Results

A total of 48 healthy volunteers and 46 patients with PD were recruited into the trial (Figure 5). There were no statistically significant differences when comparing the baseline characteristics between the two groups (Table 1). Thus, the initial condition was comparable in the two groups of the study.

3.1. Comparisons of ESR Imbalance Ratio on Two Days within Groups. Statistically significant reductions in ESR imbalance ratio between left and right side were detected at SP8 (Diji) (0.08 ± 0.08 versus 0.12 ± 0.09, $P < 0.05$) and GB39 (Xuanzhong) (0.09 ± 0.07 versus 0.15 ± 0.12, $P < 0.05$), and a statistically significant increase was detected at SP6 (Sanyinjiao) (0.16 ± 0.10 versus 0.13 ± 0.11, $P = 0.05$) on the first day of menstruation compared with those values on the third day after the period finishes in dysmenorrhea group

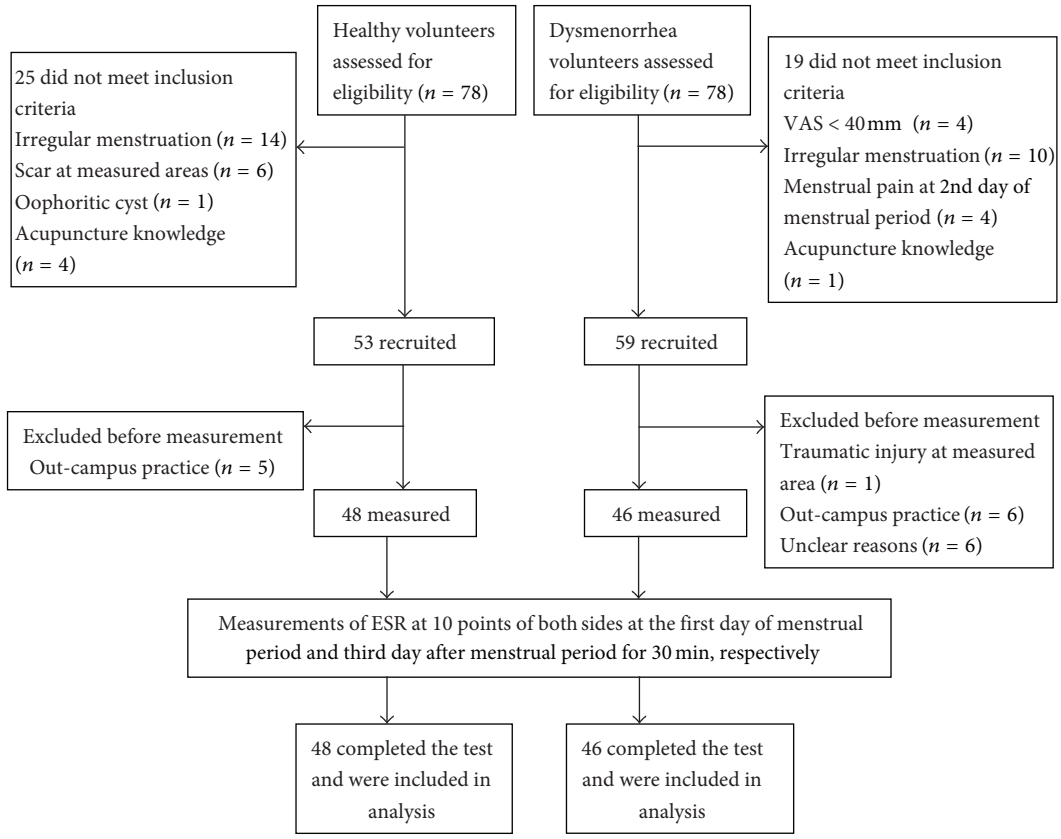

Figure 5: Flowchart of participants through the study.

Table 1: Baseline characteristics of participants in two groups.

	Group A (healthy group) (n = 48)	Group B (dysmenorrhea group) (n = 46)	P value
Age, years	23.67 ± 2.40	23.76 ± 2.59	0.855
Age of menarche, years	13.29 ± 1.20	13.00 ± 1.09	0.223
Duration of menstrual period, days	5.37 ± 1.04	5.26 ± 1.28	0.638
Duration of menstrual cycle, days	29.60 ± 2.11	28.98 ± 1.58	0.109
Body temperature (first day of menstrual period), ˚C	36.44 ± 0.22	36.52 ± 0.30	0.137
Body temperature (third day after menstrual period), ˚C	36.33 ± 0.25	36.46 ± 0.26	0.061

Mean ± SD (standard deviation) is given for each parameter.
P values from between-groups comparisons using t-test.
Note that there is no statistically significant difference when comparing two groups.

(Table 2). No significant differences were detected at other measured points within groups ($P > 0.05$).

3.2. Comparisons of ESR Imbalance Ratio on Two Days between Groups. Between-groups comparisons showed that no significant differences of ESR imbalance ratio were detected at all measured points ($P > 0.05$, Table 2).

3.3. Side Effects. No adverse events were reported in any of the two groups during the trial.

4. Discussion

According to Chinese acupuncture theory as well as some modern research findings, acupuncture points are believed to be distinguishable in bioelectric properties, pathologic responses, therapeutic effects, and anatomical structures compared to adjacent nonmeridian points [33]. The results of this study partly reflected this widely believed claim of electrodermal property of acupuncture points in acupuncture community. Significant changes of ESR imbalance ratio were

Table 2: Comparison of ESR imbalance ratios at same points between left and right side on the first day of menstrual period and third day after menstrual period in two groups.

Points	Group A-1 (healthy group) (n = 48)	Group A-3 (healthy group) (n = 48)	P^a value	Group B-1 (dysmenorrhea group) (n = 46)	Group B-3 (dysmenorrhea group) (n = 46)	P^b value	$P^{ab\text{-}1}$ value	$P^{ab\text{-}3}$ value
SP10 (Xuehai)								
Mean ± SD	0.15 ± 0.10	0.14 ± 0.09		0.17 ± 0.11	0.13 ± 0.10			
Median	0.15	0.13	0.70	0.14	0.13	0.07	0.31	0.39
Range	0.06–0.20	0.08–0.20		0.10–0.26	0.05–0.18			
SP8 (Diji)								
Mean ± SD	0.09 ± 0.06	0.10 ± 0.06		0.08 ± 0.08	0.12 ± 0.09			
Median	0.07	0.10	0.23	0.05	0.10	0.04	0.40	0.67
Range	0.04–0.13	0.05–0.14		0.03–0.13	0.07–0.17			
LR6 (Zhongdu)								
Mean ± SD	0.13 ± 0.11	0.14 ± 0.08		0.13 ± 0.09	0.12 ± 0.09			
Median	0.09	0.14	0.41	0.11	0.10	0.73	0.86	0.19
Range	0.05–0.20	0.07–0.18		0.06–0.18	0.06–0.16			
SP6 (Sanyinjiao)								
Mean ± SD	0.12 ± 0.10	0.12 ± 0.09		0.16 ± 0.10	0.13 ± 0.11			
Median	0.10	0.09	0.97	0.15	0.10	0.05	0.12	1.00
Range	0.04–0.20	0.04–0.19		0.10–0.22	0.04–0.17			
GB39 (Xuanzhong)								
Mean ± SD	0.10 ± 0.08	0.11 ± 0.08		0.09 ± 0.07	0.15 ± 0.12			
Median	0.10	0.10	0.57	0.08	0.10	0.04	0.55	0.29
Range	0.05–0.15	0.05–0.17		0.05–0.12	0.05–0.22			
Nonacupoint								
Mean ± SD	0.12 ± 0.10	0.12 ± 0.10		0.12 ± 0.09	0.11 ± 0.08			
Median	0.09	0.09	0.87	0.11	0.10	0.48	0.54	0.92
Range	0.05–0.16	0.05–0.15		0.05–0.16	0.04–0.16			
KI5 (Shuiquan)								
Mean ± SD	0.12 ± 0.10	0.12 ± 0.08		0.10 ± 0.10	0.12 ± 0.09			
Median	0.08	0.10	0.99	0.08	0.10	0.23	0.58	0.57
Range	0.04–0.16	0.05–0.14		0.04–0.14	0.06–0.16			

Table 2: Continued.

Points	Group A-1 (healthy group) (n = 48)	Group A-3 (healthy group) (n = 48)	P^a value	Group B-1 (dysmenorrhea group) (n = 46)	Group B-3 (dysmenorrhea group) (n = 46)	P^b value	$P^{ab\text{-}1}$ value	$P^{ab\text{-}3}$ value
KI3 (Taixi)								
Mean ± SD	0.12 ± 0.11	0.12 ± 0.08		0.13 ± 0.10	0.11 ± 0.08			
Median	0.09	0.10	0.67	0.10	0.10	0.80	0.53	0.81
Range	0.03–0.17	0.05–0.17		0.05–0.20	0.06–0.16			
SP3 (Taibai)								
Mean ± SD	0.17 ± 0.13	0.19 ± 0.16		0.17 ± 0.15	0.20 ± 0.17			
Median	0.14	0.13	0.84	0.12	0.15	0.43	0.72	0.80
Range	0.08–0.23	0.07–0.30		0.07–0.21	0.09–0.23			
LR3 (Taichong)								
Mean ± SD	0.12 ± 0.09	0.10 ± 0.06		0.10 ± 0.06	0.12 ± 0.08			
Median	0.09	0.09	0.41	0.08	0.09	0.17	0.31	0.23
Range	0.05–0.17	0.04–0.14		0.04–0.13	0.06–0.17			

ESR imbalance ratios at all points on the first day of menstrual period in healthy volunteer group are given in column of Group A-1, while those on the third day after menstrual period are given in column of Group A-3. Corresponding values of dysmenorrhea group on those two days are given in column of Groups B-1 and B-3, respectively.

P^a values are from within-group comparisons of group A on two days using Kruskal-Wallis Test.

P^b values are from within-group comparisons of group B on two days using Kruskal-Wallis Test.

$P^{ab\text{-}1}$ values are from between-group comparisons of groups A and B on the first day of menstrual period using Kruskal-Wallis test.

$P^{ab\text{-}3}$ values are from between-group comparisons of groups A and B on the third day after menstrual period using Kruskal-Wallis test.

only found in three acupuncture points in patients with PD. The ESR imbalance ratios of uterine-relevant points in PD patients did not change significantly more than those in women without PD. We also compared the changes of raw ESR values between PD patients and healthy women; there was still no significant difference. Prior studies also found there were no significant changes of ESR of acupuncture points in healthy subjects [15, 17]. It may suggest that acupuncture points have more sensitive reactions under pathologic states. Recently, acupuncture researchers paid more attention to study the "status" of point, namely, sensitization state and rest state [34, 35]. It is also the core viewpoint of acupuncture point in *Neijing* (the most important classic of TCM). When the human body has disease, acupuncture points on the body surface may be sensitized from a "rest status" with various types of sensitization, such as point heat-sensitization [36–38] and significant plasma extravasation of Evans Blue at points [35]. The result of this study showed another type of acupuncture point sensitization to some extent, namely, electrodermal property. However, since there were no significant different changes in ESR imbalance ratio as well as raw ESR value between PD patients and healthy women, changes in ESR of acupuncture points did not reflect menstrual pain according to this study. Our result was similar to another study, which also showed that there was no significant association between pain intensity and change in ESR of acupuncture meridians [39].

Among 10 measured points, only three specific acupuncture points including crossing point of spleen, liver, and kidney meridians (SP6-Sanyinjiao), influential point of marrow (GB39-Xuanzhong), and *Xi*-cleft point of spleen meridian (SP8-Diji) showed significant changes in ESR imbalance ratio. In acupuncture clinical practice, those three points are commonly used for dysmenorrhea due to their effects of promoting the flowing of qi and blood to relieve pain. The finding in this study also showed that those three points had electrodermal properties in relation to menstruation. To a certain degree, our study has explained why SP6, GB39, and SP8 are more effective for PD in terms of bioelectric properties of points. A study showed that applying moxibustion on sensitized points has achieved better treatment effect [40]. Our previous study focused on the therapeutic effect specificity of SP6 and GB39 and nearby nonmeridian point also showed that the effects of SP6 and GB39 on dysmenorrhea were significantly better than that of nearby nonmeridian point [26]. Therefore, the results of our two studies suggested that needling the acupoints with significant electrodermal property may achieve better treatment effect. Further studies are needed to investigate the changes of ESR at more acupuncture points in relation to different diseases as well as to explore its mechanism, so as to guide acupuncture clinical practice.

Considering the confounding technical factors on ESR measurement, we used a four-electrode method and tried to minimize possible disturbing factors in our measuring procedure. Prior studies that used the old type of this device showed a favorable repeatability [11, 41]. An improved device used in this study with smaller electrodes and computer-control data collection and analysis system enables detecting ESR at 12 points simultaneously at four limbs. The new system's performance is fairly stable even in the presence of various confounding factors such as various pressures on the probe, cleaning the skin with alcohol, and exfoliation [28]. At present, two-electrode method is mainly used in EDSD (electrodermal screening device) in medical community, such as Vegatest made in Germany [2], which is applied to locate acupuncture meridians and assist in diagnosing some diseases [42]. However, the results of diagnosing allergies with the same two-electrode method device were different [42, 43]. Limitations were found in those devices based on two-electrode method in ESR measurement [19]. Compared to a two-electrode method, the four-electrode method has many advantages: (1) it is able to measure electrical impedance approximately 2 mm under the surface of the skin, which is more coincided to the original meaning of acupuncture points [20]; (2) it minimizes error caused by fluctuation in voltage and electrode contact impedance. Therefore, the four-electrode method with some significant modifications has been widely used to measure electrical impedance of biological tissue [28, 44–46]. One prior related study also used a four-electrode method to measure ESR at different meridians [15]. The main differences between the devices used in this study and our study include shape of electrodes and placing order of four electrodes. Four needles were used as electrodes in Ahn's study. Considering that inserting needle into the points may affect the flowing of energy at points resulting in changed ESR, we utilized surface electrodes. Another unique characteristic of our device is the specific arrangement of four skin electrodes (P_V, P_I, and P_{VR} in one straight line and P_{IR} was placed far from the other three electrodes; P_V was moved to the outer side of P_I from its common place; see Figure 3), which ensures that it measures only the resistance of a small subcutaneous region lying just below the current electrode P_I. Unfortunately, this important modification as well as its unique characters has not been realized by others [14, 47].

Since our study measured the resistance of a region 2 mm under the surface of the skin at acupuncture points [20], marker ink on the skin surface of points could not disturb our results. Moreover, resistance of this region is more suitable to reflect the real electrical property of acupuncture points, which are believed located in a stereo-structure under the surface of the skin [33]. Thus only when the needles were inserted into the skin for a certain depth, better treatment effect could be achieved. Some changes in surface acupuncture meridians of dysmenorrhea patients, such as thermal characteristics, should be investigated in further studies.

Finally, we would like to discuss the limitations of this study. Although it serves as a pilot study, no power analysis was performed which lowers the statistical power of the study. Compared with studying points (uterine-relevant points), a few controls including uterine-irrelevant points and nonmeridian points were observed. Since there are numerous complicated factors involved in the studies on the electrical properties of acupuncture points and meridians such as electrode polarizability, electrode geometry, tissue anisotropy, selection of points for measurement, and pathologic states, future studies present challenges. It needs more efforts and

wisdom from both acupuncture clinicians and biomedical engineers.

5. Conclusions

This study showed that the imbalance of ESR at uterine-relevant points in PD patients is not significantly different from those of healthy women on both the 1st day of and the 3rd day after menstruation. However, the ESR imbalance ratio of certain acupuncture points can either be lower or higher during menstruation in PD patients. The ESR property of acupuncture points needs to be investigated in further clinical trials with appropriate investigated acupuncture points and diseases, larger sample sizes, and optimal device.

Conflict of Interests

No competing financial interests exist.

Authors' Contribution

Yan-Fen She and Liang-Xiao Ma are co-first authors of this paper.

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