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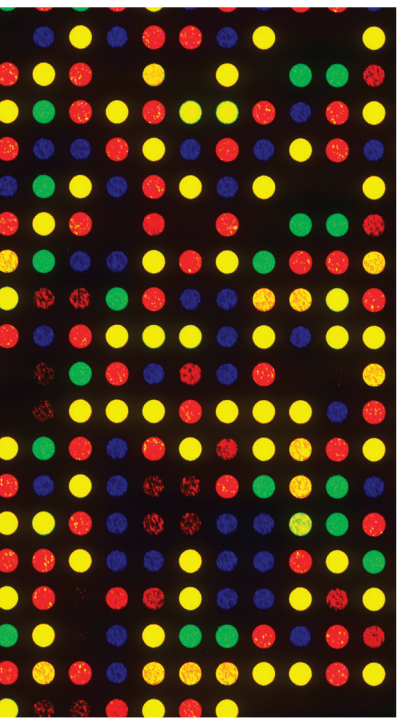
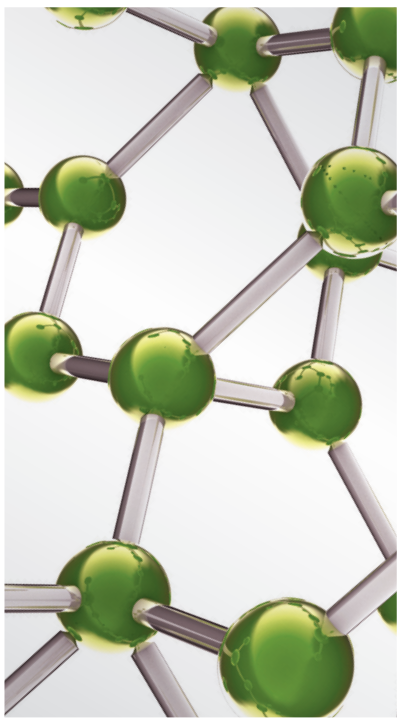
Evidence Based Alternative Medicines in Pain Management

Guest Editors: Haroon Khan, Bruno Eto, Vincenzo De Feo, and Anwar-Ul-Hassan Gilani



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Editorial Board

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Editorial

Evidence Based Alternative Medicines in Pain Management

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Pain is undoubtedly unpleasant sensation that affects the life style of a large population around the world. Several therapeutic approaches are used for the effective management of pain including use of drugs and alternate measures. This special issue describes the various events in traditional/alternative systems treatment for the management of painful condition.

The article entitled "Laser Acupuncture for Postoperative Pain Management in Cats" demonstrated that laser acupuncture significantly reduces postoperative analgesic requirements in cats undergoing ovariohysterectomy. Low back pain (LBP) is one of the most common complaints in the emergency department (ED) but the available therapies are having serious side effects. The clinical study in an article "Efficacy and Safety of Acupuncture for Acute Low Back Pain in Emergency Department: A Pilot Cohort Study" showed that acupuncture offered significant pain reduction in low back pain in patients treated in emergency department without any side effect.

Postoperative pain is very frequent and hard to treat. Dezocine is an opioid μ receptor partial agonist/antagonist widely used because of its potency and safety. The clinical studies in the article "Dezocine Prevents Postoperative Hyperalgesia in Patients Undergoing Open Abdominal Surgery" showed that dezocine offers a significant antihyperalgesic and analgesic effect in patients undergoing elective open gastrectomy for up to 48 h postoperatively.

The review article "Complementary and Alternative Medicine for the Management of Cervical Radiculopathy: An Overview of Systematic Reviews" concluded that current systematic reviews showed potential advantages to CAM for CR in alleviating neck pain or related symptoms. Due to the frequently poor methodological quality of primary studies, the authors suggested that the conclusions should be treated with caution for future clinical practice.

Approximately 75–90% of cancer patients experienced pain while 50% of patients are not satisfied with pain treatment that could affect the cortisol level. The article of this issue "Decreased Cortisol and Pain in Breast Cancer: Biofield Therapy Potential" exhibited marked reduction in cortisol concentration treated with bioenergy, suggesting reduction in stress due to pain.

The review article of Lo et al. "The Effects of Acupuncture on Cerebral and Muscular Microcirculation: A Systematic Review of Near-Infrared Spectroscopy Studies" based on available literature believed that research utilizing Near-Infrared Spectroscopy Studies to investigate the hemodynamics of acupuncture presently lacks in scope and quality. Improved designs, for example, placebo-controlled, randomized trials, and standardized intervention reporting could enhance study quality.

In this research study entitled "Involvement of Cholinergic and Opioid System in γ-Terpinene-Mediated Antinociception" the gamma terpinene (γ-TPN), a monoterpene, when studied for antinociceptive effect showed significant antinociceptive activity in various pain assessment models. The results suggest that the γ-TPN (p.o.) produced antinociceptive effect in models of chemical nociception through the cholinergic and opioid systems involvement.

A research article "Transcutaneous Electrical Acupoint Stimulation Improves the Postoperative Quality of Recovery and Analgesia after Gynecological Laparoscopic Surgery: A

Randomized Controlled Trial" based on prospective, randomized, double-blind, placebo-controlled clinical study displayed that preoperative transcutaneous electric acupoint stimulation enhances quality of recovery, improves postoperative analgesia and patient's satisfaction, alleviates postoperative side effects, and accelerates discharge after general anesthesia for gynecological laparoscopic surgery.

Auricular point acupressure (APA) is a promising treatment for pain management. The pilot prospective randomized clinical trial entitled "The Anti-Inflammatory Actions of Auricular Point Acupressure for Chronic Low Back Pain" demonstrated that APA treatment affects pain perception in CLBP patients through modulation of the immune system, as reflected by APA-induced changes in serum inflammatory cytokine and neuropeptide levels.

The article of this special issue "Study on the Antinociceptive Effects of Herba Epimedium in Mice" when studied in various pain models evoked marked antinociceptive effect possibly mediated through 5-HT receptors (1A and 2A receptors) and adrenaline β1-receptor intervention.

The article "Efficacy of Pulsed Radiofrequency on Cervical 2-3 Posterior Medial Branches in Treating Chronic Migraine: A Randomized, Controlled, and Double-Blind Trial" showed in a randomized, double-blind, and controlled clinical trial that the pulsed radiofrequency on the cervical 2-3 posterior medial branches could provide satisfactory efficacy in the treatment of chronic migraine without obvious adverse effects.

The research article of the issue "Peripheral Neuropathic Facial/Trigeminal Pain and RANTES/CCL5 in Jawbone Cavitation" exhibited regulated on activation, normal T-cell expressed and secreted (RANTES) overexpression in silent inflamed jawbones as a possible cause for atypical facial pain/trigeminal neuralgia (AFP/TRN). Thus, the surgical clearing of fatty degenerated jawbone might diminish RANTES signaling pathways in neurons and contribute to resolving chronic neurological pain in AFP/TRN patients.

The research article entitled "The Nociceptive and Anti-Inflammatory Effects of *Artemisia dracunculus* L. Aqueous Extract on Fructose Fed Male Rats" reported that the extract of *A. dracunculus* elicited significant antinociceptive and anti-inflammatory effects in fructose fed male rats.

The research article entitled "Chinese Herbal Therapy for Chronic Tension-Type Headache" provided the evidence that Chinese herbal therapy can be clinically useful for the treatment of chronic tension-type headache.

In short, the various articles of this special issue explored the importance of each traditional/alternative way of treatment and clinical significance thus signifying that pain is best controlled using coordinated efforts using different traditional/alternate measures.

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Review Article

Complementary and Alternative Medicine for the Management of Cervical Radiculopathy: An Overview of Systematic Reviews

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Background. Complementary and alternative medicine (CAM) is widely applied in the clinical practice of neck pain owing to cervical radiculopathy (CR). While many systematic reviews exist in CAM to improve CR, research is distributed across population, intervention, comparison, and setting. *Objective*. This overview aims to summarize the characteristics and evaluate critically the evidence from systematic reviews. *Methods*. A comprehensive literature search was performed in the six databases without language restrictions on February 24, 2015. We had identified relevant systematic reviews that examined the subjects with neck pain due to cervical radiculopathy undergoing CAM. Two authors independently appraised the methodological quality using the revised assessment of multiple systematic reviews instrument. *Results*. We had included eight systematic reviews. The effectiveness and safety of acupotomy, acupuncture, Jingfukang granule, manual therapies, and cervical spine manipulation were investigated. Based on available evidence, the systematic reviews supported various forms of CAM for CR. Nevertheless, the methodological quality for most of systematic reviews was low or moderate. In addition, adverse reactions of primary studies were infrequent. *Conclusions*. Current systematic reviews showed potential advantages to CAM for CR. Due to the frequently poor methodological quality of primary studies, the conclusions should be treated with caution for clinical practice.

1. Introduction

Cervical radiculopathy (CR) was defined as neck pain in a radicular patter in one or both upper extremities related to compression and/or irritation of one or more cervical nerve roots. A retrospective epidemiology study presented that the annual incidence rate of CR was 83.2 per 100,000 populations. It was reported that up to 80% of CR patients always complained of neck pain, and it would be more and more serious over time [1–3]. For those patients with recurrent condition after initial onset, pain became increasingly frequent. Also, neck pain was a common presenting symptom, which often caused limited cervical range of motion and poor quality of life.

The majority of patients chose conservative, nonoperative treatment course. The main objects of conservative treatments were to relieve pain, improve function, and enhance quality of life [4, 5]. However, a latest systematic review showed that conservative therapies including physiotherapy, collar, and traction were not superior to other interventions on the basis of low-level evidence [6]. Many patients whose symptoms were refractory to conservative treatments and had to undergo surgical therapy probably continued to suffer from neck pain. As an adjunct therapy, complementary and alternative medicine (CAM) approach might help patients improve neck discomfort resulting from CR. At present, the patients usually turn to CAM, which might be considered in rational and individual approach based on the first general

rule in medicine "not to harm," and mainly to treat pain [7, 8]. Meanwhile, many clinicians are reluctant to use these conventional drugs and instead to use CAM approaches, including massage, manipulation, mobilization, exercise, herbal medicines, acupuncture, and cognitive behavioral approach, which are increasingly favored by patients with the hope of alleviating pain-related symptoms with few adverse events [9–14]. However, CAM approaches are not totally without side effects. For instance, patients who receive manipulation treatment or take Chinese herbal medicines may experience dizziness, nausea, vomiting, and other serious risks [15, 16]. Therefore, potential relative benefits or harms of different CAM interventions for CR are worth considering.

Systematic reviews have become a standard method in assessing and summarizing primary studies. With the increasing published systematic reviews of CAM for CR, some interventions are appealing and have been tested in clinical trials. While many systematic reviews exist on CAM to improve CR, research is distributed across population, intervention, comparison, and setting. Therefore, the methodological quality of the reviews is variable and should routinely be appraised. It is necessary to summarize the characteristics and evaluate critically the evidence from systematic reviews in order to give optimal suggestions to future research and clinical practice. The purpose of this overview is to evaluate critically the methodological quality of systematic reviews regarding using CAM to treat CR. To our knowledge, this is the first one which systematically reviewed available systematic reviews of CAM on neck pain due to CR.

2. Methods

2.1. Inclusion Criteria and Exclusion Criteria. All the systematic reviews or meta-analyses had to pertain to the effective or safety of one or multiple CAM modalities, to focus on CR and include evidence from at least one controlled clinical trial. Patients were diagnosed with cervical radiculopathy, regardless of duration of illness. The interventions were CAM or CAM in combination of existing conventional therapies. According to the World Health Organization definition, CAM was described as a comprehensive term used to refer to both traditional medical systems such as traditional Chinese medicine, Indian Ayurveda, Arabic Unani medicine and various forms of indigenous medicine [17]. Various CAM interventions related in the Cochrane library included alternative medical systems (e.g., Chinese herbal drugs, homeopathy), natural product based therapies (e.g., diet therapy, dietary supplements), energy therapies (e.g., acupuncture therapy, electric stimulation therapy), manipulative and body-based methods (e.g., Chiropractic manipulation, massage), and mind-body interventions (e.g., hypnosis, sensory art therapies, Tai Chi, and Yoga) [18]. Among all the CAM treatments, acupuncture, manual therapy, spinal manipulation, mobilization, and soft tissue massage were the most common CAM treatments in the management of neck pain [19, 20]. Systematic reviews with any pain-related outcome measures were included. Narrative reviews, editorials, commentaries, and letters to the editor were excluded.

2.2. Database and Search Strategies. Electronic literature searches were conducted to identify systematic reviews of CAM for CR. The following six electronic databases were searched from their inception through February 24, 2015: PubMed, EMBASE, the Cochrane Central Registry of Controlled Trials (CENTRAL), Chinese Biomedicine (CBM), Allied and Alternative Medicine Database (AMED), and Cumulative Index to Nursing and Allied Health Literature (CINAHL).

The full search strategy of PubMed was presented as follows:

#1 Search ((((cervical radiculopathy [Title/Abstract]) OR cervicobrachial neuralgia [Title/Abstract]) OR cervicobrachial pain [Title/Abstract]) OR neck pain [Title/Abstract]),

#2 Search ((((((((((((((((((((((((((((((((((((((Alternative [Title/Abstract]) OR Acupuncture[Title/Abstract]) OR Alexander technique[Title/Abstract]) OR Aromatherapy[Title/Abstract]) OR Arts therapy [Title/Abstract]) OR Ayurveda[Title/Abstract]) OR traditional Chinese medicine[Title/Abstract]) OR Chiropractic[Title/Abstract]) OR Complementary[Title/Abstract]) OR Dietary supplements[Title/Abstract]) OR Diet therapy[Title/Abstract]) OR Electric stimulation[Title/Abstract]) OR Energy therap* [Title/Abstract]) OR Exercise[Title/Abstract]) OR Herb[Title/Abstract]) OR Homeopathy[Title/Abstract]) OR Hydrotherapy[Title/Abstract]) OR Kampo[Title/Abstract]) OR Magnetic[Title/Abstract]) OR Manual therapy[Title/Abstract]) OR Manipulati* [Title/Abstract]) OR Massage[Title/Abstract]) OR Mind-body intervention[Title/Abstract]) OR Mobilization[Title/Abstract]) OR Non-herbal[Title/Abstract]) OR Physiotherapy[Title/Abstract]) OR phototherapy[Title/Abstract]) OR Reflexology[Title/Abstract]) OR Relax* [Title/Abstract]) OR Reiki therapy[Title/Abstract]) OR Qi gong[Title/Abstract]) OR Spiritual healing[Title/Abstract]) OR Shiatsu [Title/Abstract]) OR Tai Chi[Title/Abstract]) OR traditional Korean medicine[Title/Abstract]) OR Therapeutic touch[Title/Abstract]) OR Tui na[Title/Abstract]) OR Ultrasonic therapy[Title/Abstract]) OR Yoga[Title/Abstract]),

#3 Search ((systematic review [Title/Abstract]) OR meta-analysis [Title/Abstract]),

#4 Search (#1 and #2 and #3).

We also contacted content experts and hand-searched a number of journals published in China. No limits were applied for language and foreign papers were translated. Two authors (X. Wei, S. Q. Wang) independently selected the systematic reviews according to the inclusion criteria; disagreements were resolved by discussion and reached consensus through a third party (L. G. Zhu).

2.3. Data Extraction and Methodological Quality Assessment. First of all, the extracted information summarized essential

characteristics of systematic reviews, including the name of first author, year of publication, sample size of included studies, meta-analysis or not, the intervention and control, clinical outcome, adverse effect, and conclusion for each systematic review.

A measurement tool for the "assessment of multiple systematic reviews" (AMSTAR) was used to evaluate the methodological quality of systematic reviews [21]. The internal and external validity of AMSTAR had been validated. AMSTAR has good agreement, reliability, construct validity, feasibility, and external validity [22, 23]. The tool is also reliable, valid, and easy to use for methodological quality assessment of systematic reviews on Traditional Chinese medicine, as one type of CAM [24]. The eleven items were evaluated: "a priori" design, duplicate study selection and data extraction, comprehensive literature search, the status of publication (i.e., grey literature) used as an inclusion criterion, a list of studies (included and excluded), the characteristics of the included studies, the scientific quality of the included studies, the scientific quality of the included studies used in formulating conclusions, the methods used to combine the findings of studies, the likelihood of publication bias, and the conflict of interests [21]. But AMSTAR failed to produce quantifiable assessments of systematic review quality [17, 25].

On the basis of eleven items of the original instrument, the revised "assessment of multiple systematic reviews" instrument (R-AMSTAR) was developed to quantify the quality of systematic reviews [25]. Each item's score ranges from 1 to 4 (maximum), and the R-AMSTAR total scores has a range of 11 to 44 (maximum). According to the conventional criterion, total score of 22 was considered an acceptable cutoff point [25]. Methodological quality of systematic reviews was classified into three grades in our study: high quality (total score > 33), moderate quality ($22 <$ total score ≤ 33), and low quality ($11 \leq$ total score ≤ 22).

Subsequently, we constructed a data extraction form for this study, in which eleven items of R-AMSATR were adopted directly. Two authors conducted data extraction (J. Yu, M. S. Feng) independently according to the contents. Differences were resolved by discussion and reached consensus through a third reviewer (L. G. Zhu).

3. Results

3.1. Description of Included Systematic Reviews. Our searches generated 792 articles, of which 784 had to be excluded. The reasons for exclusion were duplicates ($n = 147$), not reports of systematic reviews ($n = 575$), not CR ($n = 52$), and not CAM ($n = 8$). Two articles, which were initially included in the review based on information from the abstracts, were later excluded secondary to incorrect literatures enrolled in systematic reviews and therefore had an insufficient R-AMSTAR score [26, 27]. Thus eight systematic reviews met our eligibility criteria. A flow diagram showed the literature search and screening process (Figure 1). They were published between 2007 and 2015. Seven systematic reviews were published in Chinese [28–34]. One recent systematic review was published in English [35].

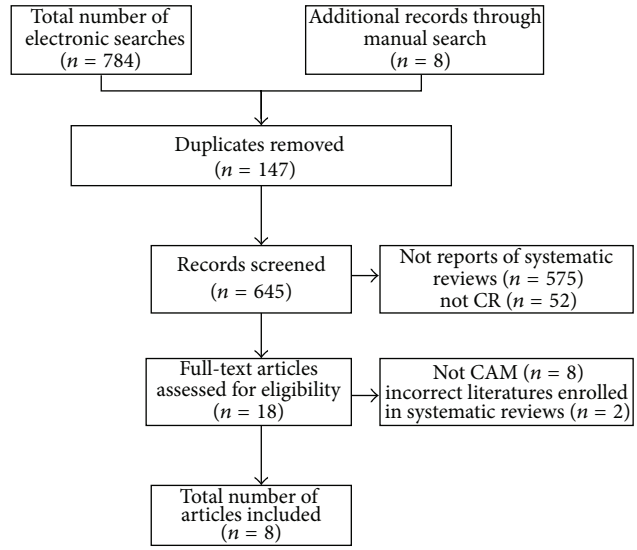

Figure 1: Flow diagram.

3.2. Essential Characteristics of Systematic Reviews. The characteristics of the enrolled systematic reviews were summarized in Table 1. One systematic review was about acupotomy [28], other two systematic reviews focused on all kinds of acupuncture (conventional acupuncture, electropuncture, and abdominal acupuncture) [29, 31], and another one was related to a Chinese patent medicine called Jingfukang granule [33]. Additionally, there were three systematic reviews concerning manual therapies, including manipulation, massage, mobilization, and acupressure [30, 32, 34]. The remaining systematic review was about cervical spine manipulation [35].

3.3. Methodological Aspects of the Included Reviews. Table 2 provides a formal assessment of the quality of all included systematic reviews. Methodological quality scores of the included reviews ranged from 18 to 36 points according to the R-AMSTAR total scores. Of these systematic reviews, two were of low quality [28, 33], four were of moderate quality [29–32, 34], and one was evaluated high quality [35].

Only one study had "a priori" design [35]. All reviews conducted duplicate study selection and data extraction. Two systematic reviews performed a comprehensive literature search [29, 35]. Almost all the reviews did not use the status of publication as an inclusion criterion and provide a list of included and excluded studies. Items 6–8 (the characteristics of the included studies provided, the scientific quality of the included studies assessed and documented, and the scientific quality of the included studies used appropriately in formulating conclusions) satisfied less than half of the total scores for the majority of systematic reviews. Five systematic reviews used appropriate methods to combine the findings of studies [29–31, 34, 35]. Three systematic reviews assessed the likelihood of publication bias [32–34]. In addition, only one systematic review had statement of sources of support and laid emphasis on whether conflict of interests existed [35].

Table 1: Summary of the included studies in the review.

First author (year)	Number of primary studies	Meta-analysis	Intervention	Comparison	Clinical outcome	Adverse effects	Conclusion
Liu, 2007 [28]	3	Yes	Acupotomy	Acupuncture (3 studies)	*Cure, markedly effective, effective, ineffective	All trials did not mention whether adverse events have occurred	There are some defects in clinical study on the treatment for CR by acupotomy; treatment for CR by acupotomy is safe and efficient treatment for CR, but the bad quality of articles and the deficiency of methodological decline the efficacy and the reliability
Sun, 2009 [29]	8	Yes	Electropuncture Acupuncture Abdominal acupuncture Abdominal acupuncture + CCT Acupuncture + CCT + massage Electropuncture + massage + CCT + TDP	CCT (2 studies) CCT CCT CCT CCT + massage (2 studies) massage + CCT + TDP	*Cure, markedly effective, effective, ineffective *MPQ	One trial reported the adverse reaction, including mild pain and bleeding	Acupuncture treatment is effective for CR and is superior to traction in the aspects of effective rate and pain alleviating. The curative effect of traction treatment could be improved when combining with acupuncture. However, the conclusion was uncertain because the quality of enrolled papers was partly low
Guo, 2012 [30]	9	Yes	Long's manipulation Manipulation Rotation manipulation Manipulation Manipulation Massage Massage Manipulation	CCT + instruments CCT + IFTA CCT CCT (2 studies) Buluofen tablets CCT CCT + Buluofen CCT + acupuncture	*Cure, markedly effective, effective, ineffective	One trial reported the mild adverse reaction	Manipulation or massage treatment on CR is safe, effective and both cure rate and the effective rate are much better than other therapies; but due to the limited number of documents included and the quality being not very high, the conclusion is still uncertain
Hu, 2012 [31]	14	Yes	Acupuncture Electropuncture Acupuncture + CCT Electropuncture + CCT	CCT (3 studies) CCT (3 studies) CCT (4 studies) CCT (4 studies)	*Cure, markedly effective, effective, ineffective	Two trials reported the safety, but no adverse reactions were observed	Acupuncture was safe in the treatment of CR. Acupuncture showed better clinical effect than traction therapy. In addition, acupuncture had better analgesic effect and could reduce recurrence. Therefore, acupuncture is probably superior to traction therapy in the treatment of CR, which is not definite due to relatively low level of evidence
Wang, 2013 [32]	28	Yes	Massage Massage + CCT Massage Massage + CCT Manipulation Manipulation Massage Massage Manipulation Massage + TCM capsule Massage + TCM Decoction Manipulation + VCDI Massage Manipulation + acupuncture Manipulation + CCT + TCMI Manipulation + CCT + EH Manipulation + CCT + IFTA	CCT (7 studies) CCT (5 studies) CCT + acupuncture Acupuncture + CCT CCT + TCM Pills CCT + Buluofen Lornoxicam tablets TCM capsule TCM plaster TCM capsule TCM decoction VCDI Acupuncture Acupuncture (2 studies) CCT + TCMI CCT + EH CCT + IFTA	*Cure, markedly effective, effective, ineffective *MPQ	Three studies reported the adverse reactions. Only one study described that skin allergy reaction occurred in seven patients after the plaster therapy	Single-application of manipulation or massage was superior to traction group and medicine group in effective rate, while no significant differences were noted between the manipulation or massage group and other control groups. In the trials of comparison between union-application of manipulation or massage and other treatments, only manipulation plus acupuncture versus acupuncture group was not significantly different. However, the conclusion is uncertain because the quality of enrolled papers is partly low

Table 1: Continued.

First author (year)	Number of primary studies	Meta-analysis	Intervention	Comparison	Clinical outcome	Adverse effects	Conclusion
Zhang, 2013 [33]	3	Yes	Jingfukang granule Jingfukang granule Jingfukang granule	CCT + Buluofen Meloxicam tablets TCM granule	*Cure, markedly effective, effective, ineffective	No significant adverse effects or allergic reactions were reported	Jingfukang granule was superior to the other therapies. To compare Jingfukang granules with western medicine, there was no significant advantage. So Jingfukang granule was effective in the treatment of CR. However, the evidence is insufficient to determine the effect of Jingfukang granules
Yang, 2013 [34]	30	Yes	Manipulation Massage + acupressure Massage Mobilization Manipulation + acupressure Manipulation + massage	CCT (18 studies) CCT CCT (5 studies) CCT (3 studies) CCT CCT (2 studies)	*Cure, markedly effective, effective, ineffective *VAS *TCSSG	Fourteen trials mentioned whether adverse events have occurred. Only one trial showed that ten patients presented red mark left on the cervical skin and the pain getting worse after massage	Manipulation or massage has advantages in treating CR with respect to short-term therapy, pain relief and the signs/symptoms amelioration compared with cervical traction. Manipulation or massage is of higher security. Nevertheless, the wide variety of therapeutic manipulation or massage techniques, diagnosis, and treatment standards is inconsistent
Zhu, 2015 [35]	3	Yes	Cervical spine manipulation	CCT (3 studies)	*VAS	One out of three trials reported the adverse events and none with a small sample size	There was moderate level evidence to support the immediate effect of cervical manipulation in treating CR. However, the safety of cervical manipulation cannot be taken as an exact conclusion

*Definition of "cure," "markedly effective," "effective," and "ineffective," cured: clinical symptoms resolved, the cervical or limb functions restored to normal.
Markedly effective: clinical symptoms significantly alleviated, cervical and limb functions effective.
Effective: clinical symptoms alleviated, but cervical or limb functions remain impaired. Ineffective: clinical symptoms and signs remain unchanged after the treatment.
*MPQ: McGill pain questionnaire; *VAS: visual analogue scale; *TCSSG: total clinical symptoms and signs grading.
CR: cervical radiculopathy; CCT: cervical computer traction; TDP: special electromagnetic therapeutic apparatus; IFTA: intermediate-frequency therapy apparatus.
TCM: traditional Chinese medicine; VCDI: vertebral canal drug injection; TCMI: traditional Chinese medicine injection; EH: electromagnetic heating.

Table 2: Assessment of methodological quality for included systematic reviews.

Study ID	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Total score	Quality
Liu et al., 2007 [28]	BC	ABC	A	0	0	0	0	A	BCE	0	A	18	Low
Sun et al., 2009 [29]	BC	ABC	ABCE	D	0	A	AB	AB	ABCD	0	A	27	Moderate
Guo et al., 2012 [30]	BC	ABC	ABE	0	0	A	AB	AB	ABCE	0	A	25	Moderate
Hu et al., 2012 [31]	C	ABC	AB	0	ABC	A	AB	AB	ABCE	0	A	26	Moderate
Wang et al., 2013 [32]	BC	ABC	AB	0	0	A	AB	AB	A	AB	A	23	Moderate
Zhang et al., 2013 [33]	BC	ABC	AB	0	0	A	A	AB	AB	AB	0	22	Low
Yang et al., 2013 [34]	BC	ABC	ABC	0	0	AB	A	AB	ABCE	AB	A	27	Moderate
Zhu et al., 2015 [35]	ABC	ABC	ABCE	AD	AC	ABC	ABCD	ABC	ABCD	0	AB	36	High

Item 1: was an "a priori" design provided?
Item 2: was there duplicate study selection and data extraction?
Item 3: was a comprehensive literature search performed?
Item 4: was the status of publication (i.e., grey literature) used as an inclusion criterion?
Item 5: was a list of studies (included and excluded) provided?
Item 6: were the characteristics of the included studies provided?
Item 7: was the scientific quality of the included studies assessed and documented?
Item 8: was the scientific quality of the included studies used appropriately in formulating conclusions?
Item 9: were the methods used to combine the findings of studies appropriate?
Item 10: was the likelihood of publication bias assessed?
Item 11: was the conflict of interests stated?

3.4. Acupotomy. Liu et al. aimed to evaluate the quality of clinical study and efficacy of the treatment for CR by acupotomy [28]. Meta-analysis showed that the group of acupotomy was better than that of acupuncture. However, included three studies had some methodological flaws such as inadequate study design, poor reporting of results, and small sample size. In addition, a nonrandomized controlled trial was enrolled in the meta-analysis. Accordingly, there were not enough high grades of evidence recommendation.

3.5. Acupuncture. Sun et al. critically assessed the efficacy of acupuncture versus traction in the treatment of CR [29]. The effective rate and improvements in McGill pain questionnaire scores of acupuncture group (including conventional acupuncture, electropuncture, and abdominal acupuncture) were better than traction group, and significant difference was also noted with acupuncture plus traction group versus traction group. But the quality of included studies was partly low.

Hu et al. assessed and compared the clinical effects and safety of acupuncture and traction therapy for CR [31]. Acupuncture (conventional acupuncture or electropuncture) was safe and showed better clinical effect than traction in the treatment of CR. The authors stressed the limitations of the randomized controlled trials included in the analysis and the low methodological quality of the primary studies. The conclusion was not definite due to the low level of evidence eventually.

3.6. Jingfukang Granule. Zhang et al. aimed to evaluate the efficacy of Jingfukang granule for patients with CR [33]. The effective rate of Jingfukang group was better than the other groups. Nevertheless, due to a high risk of selection bias and detection bias in the included studies, the evidence was insufficient. Few primary studies prevented firm conclusions.

3.7. Manual Therapies. Guo et al. appraised the safety and efficacy of manipulation and massage for treating CR [30]. The results suggested a significant effect of manipulation and massage for the treatment of CR. The authors described that limited primary studies, poor study design, and different treatment methods were the reasons of low quality. In a word, these findings should be treated with caution.

Wang et al. aimed to evaluate the evidence from randomized controlled trials and quasi-randomized controlled trials for the effectiveness of manipulation and massage for CR in detail [32]. The result of meta-analysis showed that both single-application and union-application of manipulation or massage were effective for CR and superior to other treatments. But the heterogeneity of enrolled studies decreased methodological quality and reliability of the conclusion. The authors of the systematic review recommended more rigorous randomized controlled trials.

Yang et al. assessed the efficacy and safety of manual therapies (manipulation, massage, mobilization, and acupressure) for CR [34]. The results of systematic review showed manual therapies had advantages in short-term therapy and performed more efficiently on the long-term treatment, but it was of no statistical significance. In one study, adverse reactions of massage were on record (Table 1). This systematic review reported that the wide variety of therapeutic manual techniques, diagnosis, and treatment standards of CR was inconsistent. The authors were uncertain about the effectiveness of manual therapies and recommended more and better research.

3.8. Cervical Spine Manipulation. Zhu et al. evaluated the effectiveness and safety of cervical spine manipulation for CR [35]. Meta-analysis suggested that cervical spine manipulation improving visual analogue scale for pain showed superior immediate effects compared with cervical computer traction. The overall strength of evidence was judged

to be moderate quality according to GRADE (grades of recommendation, assessment, development, and evaluation) approach. However, there are still selection bias and attrition bias in the primary studies. Moreover, the adverse event of cervical spine manipulation in treating degenerative CR was not clear.

4. Discussion

More recently, CAM has shown high usage in the developed countries, especially for those with chronic diseases, such as neck pain [36–41]. CAM can be the "mainstay" of health care delivery, particularly in remote or rural areas in the developing countries [42]. A multitude of patients suffering from CR used CAM to address their symptoms, including neck pain [8]. Despite significant evidence for the use of CAM on CR into professional clinical practice, it is necessary to use the methods of overview of systematic reviews to summarize available evidence. There was a paucity of reports evaluating the potential for the therapeutic effect and safety of CAM for CR. This paper was aimed at providing an overview of systematic reviews. Eight systematic reviews were included [28–35]. We placed the discussion in the text of existing evidence.

The effectiveness and safety of acupotomy, acupuncture, Jingfukang granule, manual therapies, and cervical spine manipulation were investigated. Based on available evidence, the systematic reviews provided some evidence to support various forms of CAM for CR. All the systematic reviews showed the CAM intervention was superior to the control group, respectively.

In this paper, we used R-AMSTAR to evaluate the quality of systematic reviews. Regrettably, the methodological quality for the majority of the systematic reviews was low or moderate. Those "positive findings" might be unreliable because of the frequently poor quality of previous studies, such as poor study design, small sample size, selection bias, and detection bias. One of the common problems was high heterogeneity across studies, especially in the systematic reviews of acupuncture and manual therapies [29–32, 34]. Wide differing estimates of the treatment effect across individual trials implied true differences in underlying treatment effects [43]. For example, three systematic reviews paid attention to comprehensive effect of manual therapies [30, 32, 34]. But the effect of single manual therapy was not necessarily identical. We suggested that systematic review of single manual therapy which included massage, manipulation, or mobilization for CR should be performed. Another problem was the inappropriate choice of control group in the randomized controlled trials. There was no placebo-controlled study design. Additionally, not all therapies as control group were recommended by the clinical practice guideline [28, 32]. In the future, the randomized controlled trials that compared CAM with placebo or "gold-standard treatment" should be well done. But besides that, primary studies with no randomization, allocation concealment, blinding, outcome not to be measured in a large proportion of patients, patients lost to follow-up, or failure to adhere to the intention-to-treat principle during the analysis were highly susceptible to bias [44–47]. Although the latest systematic review was judged to be high quality, the positive results were presented with limited eligible studies [35]. According to the evidence we collected, we could not recommend any CAM therapeutic option for CR.

Risk assessment was an inherent component of CAM therapy practice as well. Six out of eight systematic reviews mentioned adverse reactions in the overview [29–32, 34]. In the systematic reviews, adverse reactions were infrequent. Two systematic reviews did not report any significant adverse effects or allergic reactions [28, 33]. The total number of adverse reactions after acupuncture was low in two systematic reviews by Sun et al. and Hu et al. [29, 31]. Mild pain and bleeding were observed in a randomized controlled trial [29]. The analysis of the publications indicated that fainting, allergy, and pain were the common adverse reactions. And various causes lead to adverse reactions to acupuncture. So the researchers took the attitude that the safety guidelines for the risk management of acupuncture operation should be established [48, 49]. Meanwhile, as the most commonly used treatment method for CR, published cases of severe adverse events following chiropractic manipulation illustrated the need for the safety evaluation of manual therapies [50, 51]. In this overview we discussed, only one trial reported that neck pain became more serious after massage [30, 34]. Nevertheless, there was no confirmative evidence to identify the safety of other CAM interventions for CR. Further safety testing of CAM therapies, no doubt, was an essential part for future research.

In our opinion, this overview of systematic reviews had some limitations. On the one hand, although comprehensive searches were conducted, there is no guarantee that all relevant systematic reviews were enrolled. Also, we did not include primary randomized controlled trials that evaluated CAM for CR and keep up with the latest research progress. For instance, a new research program about a compound traditional Chinese herbal medicine for neck pain in patients with CR is ongoing [12]. On the other hand, the paucity of primary studies included in systematic reviews influenced conclusions. Only three trials were enrolled in systematic reviews, which was associated with low quality [28, 33, 35]. When studies included relatively few patients and few events occurred, estimates of the effect usually had indeterminate results [43]. To make progress in this area, further high-quality randomized controlled trials are required to prove the role of CAM in the treatment of CR. We also need more effective CAM interventions around the world, better implementation of existing therapies, better quality of reporting, and more reliable systematic reviews.

5. Conclusion

In conclusion, current systematic reviews showed potential advantages to CAM for CR in alleviating neck pain or related symptoms. Acupotomy, acupuncture, Jingfukang granule, manual therapies, and cervical spine manipulation were the CAM interventions. The adverse reactions of primary studies were infrequent. However, the safety of other CAM therapeutic methods could not be adequately judged. Our

overview suggested that the methodological quality for most of systematic reviews (7/8) was low or moderate. Due to the poor study design of previous studies, small sample size, selection bias and detection bias, and high heterogeneity across studies, these conclusions of available systematic reviews should be treated with caution for future clinical practice.

Abbreviations

CAM:	Complementary and alternative medicine
CR:	Cervical radiculopathy
AMSTAR:	Assessment of multiple systematic reviews
R-AMSTAR:	Revised "assessment of multiple systematic reviews" instrument
GRADE:	Grades of recommendation, assessment, development, and evaluation
MPQ:	McGill pain questionnaire
VAS:	Visual analogue scale
TCSSG:	Total clinical symptoms and signs grading
CCT:	Cervical computer traction
TDP:	Special electromagnetic therapeutic apparatus
IFTA:	Intermediate-frequency therapy apparatus
VCDI:	Vertebral canal drug injection
TCMI:	Traditional Chinese medicine injection
EH:	Electromagnetic heating.

Conflict of Interests

All authors declare that they have no conflict of interests. The funders had no role in the paper.

Authors' Contribution

Xu Wei and Shangquan Wang contributed equally to this paper. They are the co-first authors.

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Research Article

Efficacy and Safety of Acupuncture for Acute Low Back Pain in Emergency Department: A Pilot Cohort Study

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Introduction. Low back pain (LBP) is one of the most common complaints in the emergency department (ED). There are several research articles providing evidence for acupuncture for treating chronic LBP but few about treating acute LBP. This study assessed the efficacy and safety of acupuncture for the treatment of acute LBP in the ED. *Materials and methods*. A clinical pilot cohort study was conducted. 60 participants, recruited in the ED, were divided into experimental and control groups with 1 dropout during the study. Life-threatening conditions or severe neurological defects were excluded. The experimental group ($n = 45$) received a series of fixed points of acupuncture. The control group ($n = 14$) received sham acupuncture by pasting seed-patches near acupoints. Back pain was measured using the visual analog scale (VAS) at three time points: baseline and immediately after and 3 days after intervention as the primary outcome. The secondary outcomes were heart rate variability (HRV) and adverse events. *Results*. The VAS demonstrated a significant decrease (P value <0.001) for the experimental group after 15 minutes of acupuncture. The variation in HRV showed no significant difference in either group. No adverse event was reported. *Conclusion*. Acupuncture might provide immediate effect in reducing the pain of acute LBP safely.

1. Introduction

Most adults have the experience of low back pain (LBP) in their lives [1, 2]. Low back pain is one of the most common complaints when patients visit the emergency department (ED) [3, 4]. Most cases of acute LBP are not related to any specific disease [5–7]. After checking the patients and excluding any life-threatening conditions or severe neurological deficits, sometimes the pain has still not been eased. Patients must be kept in the ED for further observation. The prolonged hospital stay due to poor pain control is a potential factor that can cause the overcrowding of the ED [8].

Pain has been regarded as the fifth vital sign (temperature, heart rate, blood pressure, and respiratory rate) recently [9], and every patient has the right to receive adequate pain management. Pain relief is an important work in the ED and there are many medications for LBP with each medication having both benefits and side effects [10–13].

Acupuncture is one of the oldest and most popular complementary alternative medicines in the world and it has been widely utilized for pain, including low back pain, osteoarthritis, headache, and cancer [14–19]. We found that there are many studies assessing the effectiveness of acupuncture for chronic LBP but few for acute LBP [15, 20].

In light of the aforementioned observation, this study focused on evaluating the efficacy and safety of acupuncture in patients with acute LBP through outpatient care in the ED.

2. Materials and Methods

2.1. Population. A clinical pilot cohort study was conducted. Patients were recruited from the emergency department (ED)

of Changhua Christian Hospital (a medical center in Taiwan) with a target sample size of 60 subjects. Participants were divided into either the experimental group or control group based on their willingness to accept acupuncture treatment. All candidates received a standardized interview process. And the purpose, procedures, potential risks, and benefits of the study were explained thoroughly to the candidates. Participants had the right to withdraw from the study at any time without any consequence. All participants' written consents were obtained. The trial was conducted from March to December, 2014. The clinical trial protocol was approved by the Institute Review Board (IRB) of Changhua Christian Hospital (CCH IRB number 140214).

2.2. Inclusion Criteria. Participants meeting all of the following criteria will be included:

(1) age 20 to 90 years, either gender;

(2) visit and stay in emergency department;

(3) the chief complaint being acute low back pain;

(4) diagnosis with International Classification of Diseases 9th revision (ICD-9) code 724.2 Lumbago.

2.3. Exclusion Criteria. Participants meeting one or more of the following criteria were excluded:

(1) serious comorbid conditions (e.g., life-threatening condition or severe neurological defects);

(2) patients who cannot communicate reliably with the investigator or who are not likely to obey the instructions of the trial;

(3) pregnancy status.

2.4. Baseline Assessment

2.4.1. The Oswestry Disability Index (ODI). This questionnaire (also known as Oswestry Low Back Pain Disability Questionnaire) was designed to measure a patient's functional disability resulting from spinal pain [21].

2.5. Interventions. Participants were divided into experimental and control groups based on their willingness to accept acupuncture treatment. The experimental group received a series of fixed points of acupuncture: Bilateral Hegu (LI4), Shousanli (LI10), Zusanli (ST36), Yanlingquan (GB34), and Taichong (LR3) [22]. Needles were correctly inserted and manually stimulated until the "De Qi" sensation was elicited. The needles stayed in place for 15 minutes. The control group received sham acupuncture by pasting seed-patches next to the same location as correct acupoints of experimental group; see Figure 2 [22].

2.6. Evaluations. The primary outcome evaluation was the visual analog scale (VAS) for pain. It is graded from 0 (no pain) to 10 (worst possible pain) and has proven its usefulness and clinical validity for the evaluation of pain [23]. Patients were evaluated at three timepoints in this study: before intervention (VAS-1), after intervention (VAS-2), 3 days after the intervention (VAS-3).

The secondary outcomes were heart rate variability (HRV) and adverse events. HRV was measured 2 times in this study: before the intervention and after the intervention. Many studies have shown a relation between HRV and pain [24, 25]. We tried to further identify the correlation between the intensity of pain and HRV [26, 27]. An additional secondary outcome was participants reporting any adverse events they experience, including discomfort, bruising at the sites of needle insertion, nausea, or feeling faint during or after treatment.

2.7. Data Analysis. First, the experimental group and control group were analyzed for comparability according to the baseline characteristics, including gender, age, body mass index (BMI), blood pressure (systole and diastole), heart rate (HR), and ODI. Chi-square test and Mann-Whitney U test were used to assess categorical variables. Second, in order to analyse the outcome of this study including VAS and HRV, we used Wilcoxon Signed Ranks Test and Mann-Whitney U test because of the sample size. All tests were conducted using SPSS (V.18.0).

3. Results

The flowchart of this study is presented in Figure 1.

Participant Recruitment. All study participants, from the emergency department (ED), were evaluated by emergency medicine specialists to exclude serious comorbid conditions and severe neurological defects, such as infection, cauda equina syndrome, and aneurysm. Sixty participants (21–89 years old, 20 men and 40 women) were recruited into the study and divided into experimental group ($n = 46$) and control group ($n = 14$). The VAS was conducted to evaluate the maintenance of the pain relieving effect by phone interview 3 days after treatment. There was 1 participant lost to follow-up in the experimental group at the 3 days after intervention timepoint.

Baseline Characteristics. Tables 1(a) and 1(b) show baseline participant characteristics, including gender, age, BMI, blood pressure, heart rate, and Oswestry Disability Index. The two groups were homogeneous while no significant difference was shown at baseline assessment.

VAS. Comparison of VAS-1 (before intervention) and VAS-2 (after intervention) indicated that there was significant difference in the experimental group ($P < 0.001$) but not in control group ($P = 0.109$). Comparison of VAS-1 and VAS-3 (3 days after intervention) found significant differences in both experimental group ($P < 0.001$) and control group ($P = 0.011$) (see Table 2).

In addition, comparison of ΔVAS1-VAS2 (changes of VAS-1 and VAS-2) between two groups also showed a significant difference ($P < 0.001$). No significant difference was observed in ΔVAS1-VAS3 (changes of VAS-1 and VAS-3)

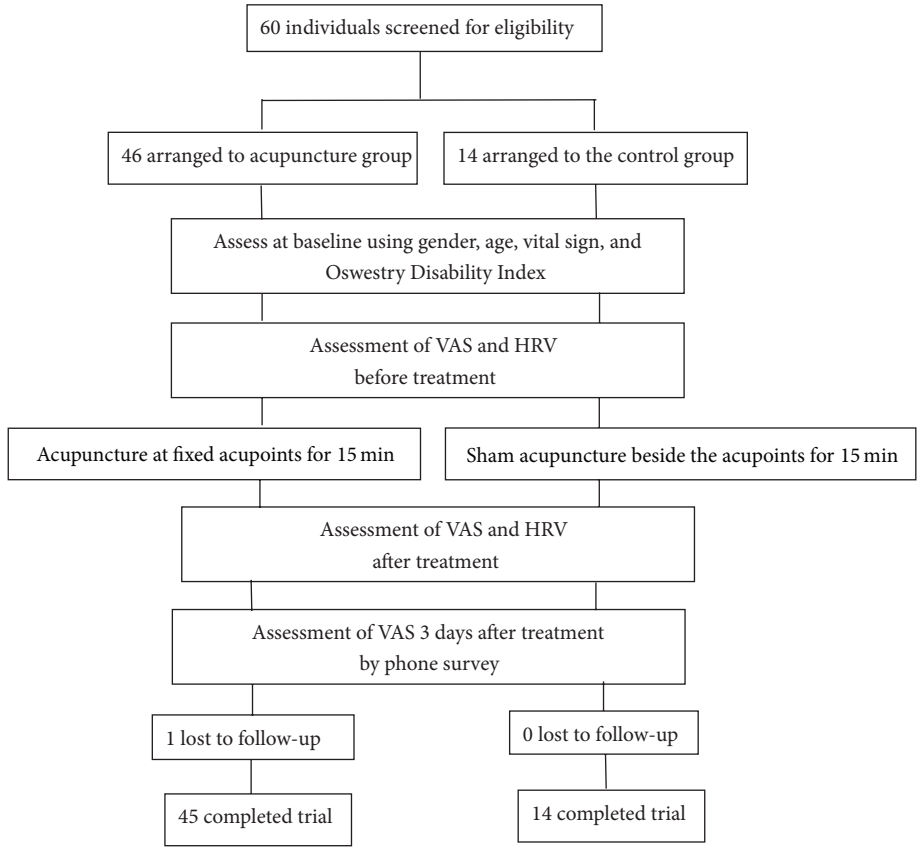
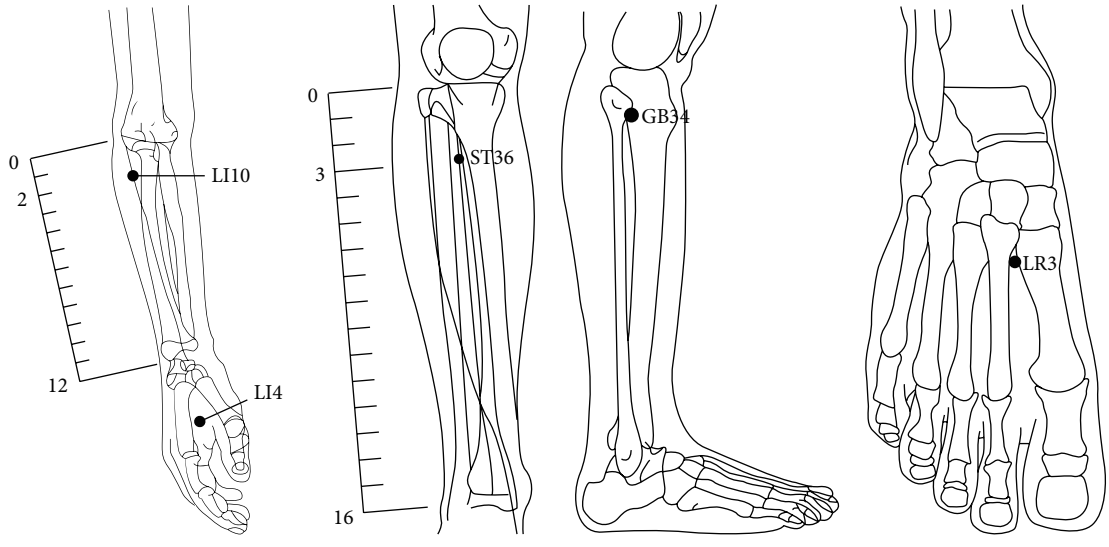


Figure 1: Flowchart of the study.



(a) LI10 and LI4 (b) ST36 (c) GB34 (d) LR3

Figure 2: Acupoint locations (LI4, LI10, ST36, GB34, and LR3).

Table 1: (a) Distribution of participants' gender. (b) Baseline of participant characteristics.

(a)

	Control		Acupuncture		P value
	N	%	N	%	
Gender	14		45		0.942
Female	7	50.0	23	51.1	
Male	7	50.0	22	48.9	

P value by Chi-square test.

(b)

	Control (n = 14)			Acupuncture (n = 45)			P value
	Median	Q_1	Q_3	Median	Q_1	Q_3	
Age	65	52	79	56	46	75	0.423
BMI	26	23	28	24	22	27	0.454
SYS	134	119	137	122	117	138	0.741
DIA	76	73	78	74	71	79	0.533
HR	81	77	91	75	67	88	0.303
Oswestry							
(1) Pain intensity	3	2	4	3	3	4	0.861
(2) Personal care	2	1	5	3	2	4	0.930
(3) Lifting	5	5	5	4	3	5	0.024
(4) Walking	4	3	5	4	3	5	0.767
(5) Sitting	4	2	5	4	3	5	0.745
(6) Standing	4	3	5	4	1	4	0.099
(7) Sleeping	2	1	4	3	1	4	0.648
(8) Sex life	4	4	4	4	2	4	0.448
(9) Social life	4	3	5	4	3	5	0.205
(10) Traveling	5	3	5	5	3	5	0.853

P value by Mann-Whitney U test.
Q_1: Percentile 25.
Q_3: Percentile 75.
BMI, body mass index; SYS, systolic pressure; DIA, diastolic pressure; HR, heart rate.

Table 2: Comparison between groups of VAS before, after, and 3 days after intervention.

	Control (n = 14)				Acupuncture (n = 45)				P value[b]
	Median	Q_1	Q_3	P value[a]	Median	Q_1	Q_3	P value[a]	
VAS-1	5.5	4	7		7.0	5	8		0.059
VAS-2	4.5	4	6	0.109	4.0	2	5	<0.001*	0.161
VAS-3	3.0	0	4	0.011*	3.0	1	6	<0.001*	0.465

P value[a] by Wilcoxon Signed Ranks Test (take VAS1 as reference) (intergroup).
P value[b] by Mann-Whitney U test (between groups).
Q_1: Percentile 25.
Q_3: Percentile 75.
VAS-1, VAS before intervention; VAS-2, VAS after intervention; VAS-3, VAS of 3 days after intervention.
*Statistically significant difference ($P < 0.05$).

($P = 0.370$) and ΔVAS2-VAS3 (changes of VAS-2 and VAS-3) ($P = 0.181$) (see Table 3).

Furthermore, when we do the gamma regression model with GEE method on VAS, the results also indicate a significant change after treatment in the experimental group ($P < 0.001$) but not in the control group. The VAS reduced significantly in all patients after 3 days ($P = 0.031$) (see Table 5).

HRV. Table 4 shows the comparison of all parameters of HRV before and after intervention in experimental group and control group. No significant change was observed in HRV,

Table 3: Changes in VAS between control group and acupuncture group.

	Control ($n = 14$)			Acupuncture ($n = 45$)			P value
	Median	Q_1	Q_3	Median	Q_1	Q_3	
ΔVAS2-VAS1	0.0	0	0	−2.0	−4	−1	<0.001*
ΔVAS3-VAS1	−1.5	−3	0	−4.0	−5	−1	0.370
ΔVAS3-VAS2	−1.5	−3	0	−1.0	−3	2	0.181

P value by Mann-Whitney U test.
Q_1: Percentile 25.
Q_3: Percentile 75.
*Statistically significant difference ($P < 0.05$).
ΔVAS2-VAS1, changes of VAS-2 and VAS-1; ΔVAS3-VAS1, changes of VAS-3 and VAS-1; ΔVAS3-VAS2, changes of VAS-3 and VAS-2.

Table 4: Comparison of parameters of heart rate variability (HRV) before and after intervention in two groups.

Group		Before			After			P value
		Median	Q_1	Q_3	Median	Q_1	Q_3	
Control ($n = 14$)	HRV	39.0	33.0	49.0	31.0	26.0	45.0	0.311
	HF%	50.0	38.0	58.0	53.0	48.0	76.0	0.421
	LF%	50.0	42.0	62.0	47.0	24.0	52.0	0.421
	LF/HF	1.0	0.7	1.6	0.9	0.3	1.1	0.133
	VLF	976.0	567.0	1436.0	628.0	501.0	1098.0	0.463
	Number of irreg. hb.	8.0	0.0	48.0	2.0	0.0	13.0	0.229
	LF	305.0	92.0	509.0	154.0	54.0	199.0	0.552
	HF	258.0	196.0	323.0	224.0	133.0	428.0	0.916
	Total power	1521.0	1089.0	2401.0	961.0	676.0	2025.0	0.311
	Variance	1521.0	1089.0	2401.0	961.0	676.0	2025.0	0.311
	RMSSD	45.0	29.0	52.0	41.0	22.0	54.0	0.674
	PNN50	13.0	8.0	30.0	20.0	1.0	30.0	0.753
Acupuncture ($n = 45$)	HRV	40.0	25.0	83.0	34.0	24.0	58.0	0.273
	HF%	45.0	32.0	61.0	46.0	32.0	60.0	0.694
	LF%	55.0	39.0	68.0	53.0	39.0	68.0	0.905
	LF/HF	1.2	0.6	2.1	1.1	0.7	2.1	0.923
	VLF	891.0	382.0	4272.0	732.0	423.0	2274.0	0.561
	Number of irreg. hb.	11.0	0.0	49.0	6.5	0.0	22.0	0.158
	LF	204.0	77.0	1095.0	185.0	53.0	667.0	0.891
	HF	208.0	68.0	932.0	141.5	71.0	503.0	0.446
	Total power	1600.0	625.0	6889.0	1157.0	576.0	3364.0	0.401
	Variance	1600.0	625.0	6889.0	1157.0	576.0	3364.0	0.401
	RMSSD	34.0	22.0	75.0	32.0	22.0	59.0	0.573
	PNN50	11.0	1.0	45.0	8.5	2.0	31.0	0.353

P value by Wilcoxon Signed Ranks Test.
Q_1: Percentile 25.
Q_3: Percentile 75.
HF, high frequency; LF, low frequency; VLF, very low frequency; Number of irreg. hb., number of irregular heart beats; RMSSD, root mean square successive difference; PNN50, NN50 count divided by the total number of all NN intervals.

Table 5: Results of gamma regression model with GEE method on VAS.

Predictor	Coefficient	SE	Mean ratio	95% C.I.	*P* value
(Intercept)	1.701	0.368	5.478	2.665–11.259	<0.001*
Age	0.001	0.002	1.001	0.997–1.005	0.637
BMI	−0.001	0.012	0.999	0.977–1.022	0.920
Gender					
Male	0.008	0.096	1.008	0.835–1.215	0.936
Female	0.000		1.000		
Group					
Acupuncture	0.156	0.098	1.169	0.964–1.417	0.113
Control	0.000		1.000		
Time					
3	−0.377	0.175	0.686	0.487–0.966	0.031*
2	−0.132	0.077	0.876	0.753–1.019	0.086
1	0.000		1.000		
Interaction					
Acupuncture Time 3	0.021	0.196	1.021	0.695–1.499	0.916
Acupuncture Time 2	−0.380	0.092	0.684	0.571–0.819	<0.001*
Acupuncture Time 1	0.000		1.000		
Control Time 3	0.000		1.000		
Control Time 2	0.000		1.000		
Control Time 1	0.000		1.000		

*Statistically significant difference ($P < 0.05$).

HF%, LF%, LF/HF, VLF, LF RMSSD, and PNN50 in both groups in this study.

Adverse Events. No side effects were reported in this study. No patients reported bleeding, nausea, vomiting, feeling faint, or any other complication during or after intervention.

4. Discussion

This study was designed to demonstrate that acupuncture can benefit patients with acute LBP. Instead of recruiting participants from acupuncture outpatients, we cooperated with emergency medicine specialists in order to make the first contact with patients with acute LBP. Clinically we found that most patients with acute LBP would not be able to maintain the face-down posture during the treatment time. Therefore, all the acupoints we chose in this study were at the limbs and based on traditional Chinese medical meridian system, so patients could keep a relatively comfortable lying down position.

In the results of this study, the significant difference between VAS-1 and VAS-2 in the experimental group might prove the efficacy of acupuncture while no statistical variation was shown in control group. Another significant variation was shown in the change of VAS-1 and VAS-2 (ΔVAS1-VAS2) between two groups. It also indicated that acupuncture intervention might reduce the pain intensity. The other significant variation was between VAS-1 and VAS-3 in both groups. And it was considered as acute LBP could be mitigated through appropriate treatment without immediate recurrence [28].

HRV measures the balance of autonomic nervous system which reflects physiological, hormonal, and emotional balance within our body [29]. Many studies have proved that there are statistical differences of HRV between healthy people and patients in pain [24, 30]. But the correlation between HRV and pain intensity has not been clearly demonstrated [24, 31]. In our study, no significant difference was shown in both experimental and control group after intervention. We assume that patients might feel much less pain after 15 minutes of acupuncture (mean 6.64 ± 1.87 to 3.98 ± 1.74) but have not yet fully recovered to pain-free state.

We used the adverse event record to assess the safety. No complication was reported showing that acupuncture could be a safe treatment in patients with acute LBP. However, our study has several limitations. One limitation concerned the different number of participants between two groups. Acupuncture is a common and popular medical service in the Chinese society. Patients are usually willing to accept it. It resulted in the fact that less participants were recruited into control group when our strategy was to divide participants into two groups based on their willingness to accept acupuncture.

Another limitation was that this study was not designed as randomized blind. Considering that acupuncture is well-known in the Chinese society, it is difficult to do blinded study about acupuncture. In order to minimize the bias from this, we used seed-patches as sham acupuncture. Seed-patches are often used in auricular acupuncture. Auricular acupuncture is another well-known Chinese medical service.

We tried to convince participants in control group that they were also receiving another effective treatment by pasting the seed-patches near the correct acupoints [32, 33]. Still, biases introduced by this unblinded approach cannot be ruled out.

A larger sample size in future studies is indispensable to provide well-defined types of acute low back pain for our evidence-based practice.

5. Conclusion

We conclude that acupuncture could provide immediate effect in reducing pain of acute low back pain significantly. The results from this study provide clinical evidence on the efficacy and safety of acupuncture to treat acute low back pain in the emergency department. Nevertheless, further larger studies are needed to replicate the findings of this study.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Yen-Ting Liu collected the data, did the literature search, and drafted the paper. Yen-Ting Liu, Chih-Wen Chiu, Chin-Fu Chang, Tsung-Chieh Lee, Chia-Yun Chen, Shun-Chang Chang, Chia-Ying Lee, and Lun-Chien Lo participated in the conception and design of the study. Tsung-Chieh Lee, Chia-Yun Chen, Shun-Chang Chang, and Chia-Ying Lee conducted acupuncture treatment and seed-patches. Lun-Chien Lo did the critical revision of the paper and was the corresponding author.

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Research Article

Effects of the Fourth Ventricle Compression in the Regulation of the Autonomic Nervous System: A Randomized Control Trial

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Introduction. Dysfunction of the autonomic nervous system is an important factor in the development of chronic pain. Fourth ventricle compression (CV-4) has been shown to influence autonomic activity. Nevertheless, the physiological mechanisms behind these effects remain unclear. *Objectives*. This study is aimed at evaluating the effects of fourth ventricle compression on the autonomic nervous system. *Methods*. Forty healthy adults were randomly assigned to an intervention group, on whom CV-4 was performed, or to a control group, who received a placebo intervention (nontherapeutic touch on the occipital bone). In both groups, plasmatic catecholamine levels, blood pressure, and heart rate were measured before and immediately after the intervention. *Results*. No effects related to the intervention were found. Although a reduction of norepinephrine, systolic blood pressure, and heart rate was found after the intervention, it was not exclusive to the intervention group. In fact, only the control group showed an increment of dopamine levels after intervention. *Conclusion*. Fourth ventricle compression seems not to have any effect in plasmatic catecholamine levels, blood pressure, or heart rate. Further studies are needed to clarify the CV-4 physiologic mechanisms and clinical efficacy in autonomic regulation and pain treatment.

1. Introduction

Dysfunction of the autonomic nervous system is considered to be an important factor in the development of chronic pain [1]. Studies using muscle blood flow, heart rate, and blood pressure as outcome measures have suggested an excessive sympathetic activation in patients with different pain conditions such as fibromyalgia [2, 3], musculoskeletal and myofascial pain [1, 4], and chronic pelvic pain [5] and patients who had undergone abdominal surgery [6]. The relationship between sympathovagal balance and psychological distress has also been proven in children with abdominal pain, irritable bowel syndrome [7, 8], and complex regional pain syndrome [9, 10].

Catecholamines are important hormones which regulate ANS activity in both central and peripheral sympathetic nerve endings [11]. Studies with animals have suggested that chronic adrenergic stimulation or impaired epinephrine homeostasis may contribute to the pathophysiological mechanisms of pain syndromes [12, 13]. On the other hand, dopamine has proved to have antinociceptive effects both in animals and in humans [14–16] and dopaminergic neurotransmission has been shown to be affected in chronic pain syndromes [17, 18].

(a) (b)

Figure 1: Physiotherapist's hand position during the procedure. (a) CV-4 technique, (b) placebo technique.

Fourth ventricle compression (CV-4) is a cranial manipulation technique aiming at influencing on brain and cranial nerve function [19]. Some authors have suggested that CV-4 may produce changes in the regulation of the autonomic nervous system (ANS). For instance, Cutler et al. [20] reported muscle sympathetic nerve activity after applying the CV-4 technique. The use of CV-4 technique has also been purported to modify heart rate, blood pressure, blood flow velocity [21, 22], and cerebral tissue oxygenation [23]. Moreover, CV-4 has been observed to produce an increment of electroencephalography alpha absolute power [19], reduce sleep latency [20], and decrease anxiety [21] in healthy subjects. In addition, CV-4 has been shown to be effective in pain relief in tension type headaches [24]. Due to the established interactions between the autonomic and nociceptive systems, the potential effects of CV-4 on the autonomic regulation could represent a novel approach to control the development or maintenance of pain. Nevertheless, the physiological mechanisms undergoing CV-4 autonomic regulation remain unclear. The aim of the present study was to assess the immediate effects of the CV-4 technique compared to sham placebo intervention in plasma catecholamine levels, heart rate, and blood pressure on healthy adults.

2. Methods

2.1. Participants. Forty healthy adults (19 males and 21 females; age range = 18–33 years) were recruited in the Faculty of Physiotherapy of UNILUS (Santos, SP, Brazil). A member of the research group did a personal interview with every volunteer informing them of the objectives of the study and the procedure. The established exclusion criteria were (1) any local or systemic pathologic condition (recent fracture in the cranial base, osteitis, tumor, encephalopathy, stroke, traumatic brain injury, depression, or asthma crisis), (2) arterial hypotension, low heart rate, or parasympathetic state, (3) pregnant women or women in menstrual state on the evaluation day, (4) tobacco or drug consumption, and (5) having received a manual therapy procedure in the last month. Subjects participating in the study were instructed not to consume coffee, tea, chocolate, vanilla, fruit or fruit derivates, soft drinks, or alcohol for 4 hours before data collection. Subjects who reported having consumed any of these foods were excluded from the study ($n = 1$).

The study was approved in accordance with the principles of the Declaration of Helsinki by the Ethics Committee of Hospital Guilherme Álvaro de Santos, SP, Brazil.

2.2. Procedure. Intervention and assessments were performed in a university-based physiotherapy research clinic at Lusíada University (UNILUS, Santos, SP, Brazil). The assessments and intervention were performed between 7 and 9.30 a.m., in a room with a temperature of 21–23°C.

Participants were assigned randomly to one of the treatment groups: intervention group and control group. The intervention group received the CV-4 technique for 10 minutes. The CV-4 procedure was administered by an experienced physiotherapist and member of the research group according to the standards established previously in the literature [19]. The participant lay down in a supine position. The physiotherapist, situated behind the participant's head, made a slight approximation of the occipital squama lateral angles towards the posterior occipital convexity, while taking the cranium into extension. The traction was maintained until a motionless state was perceived in the cranial pulse and released when a perception of movement was noted. The procedure was repeated until the 10-minute session time expired (Figure 1(a)).

The control group underwent a bilateral nontherapeutic contact on the occipital bone for 10 minutes. The participant lay down in a supine horizontal position. The physiotherapist, situated behind the participant's head, overlapped their hands touching the occipital bone and provided random slight touch of random duration until the end of the 10-minute session time. Figure 1(b) displays physiotherapist's hands' position in the control procedure.

2.3. Assessments. Both groups (intervention and control group) underwent assessments of blood pressure, heart rate, and blood test to determine plasmatic catecholamine levels before and 5 minutes after the procedure. Blood pressure and heart rate assessments were performed by the same evaluator

Table 1: Mean and SD of catecholamine plasmatic levels, blood pressure, and heart rate for both groups (intervention and control) in the preintervention and postintervention.

	Intervention group		Control group	
	Preintervention	Postintervention	Preintervention	Postintervention
Dopamine	53.73 (20.15)	53.63 (15.62)	36.85 (14.47)	52.35 (19.13)
Norepinephrine	119.68 (39.55)	114.00 (44.17)	113.85 (39.64)	87.65 (37.63)
Epinephrine	43.58 (20.16)	49.26 (16.27)	53.55 (14.94)	52.15 (22.13)
Systolic blood pressure	119.74 (9.50)	116.53 (8.08)	121.55 (13.12)	117.00 (15.52)
Diastolic blood pressure	74.00 (9.18)	74.26 (7.12)	81.48 (10.25)	80.50 (12.48)
Heart rate	72.39 (6.95)	70.40 (6.93)	80.95 (13.39)	77.35 (7.99)

and the blood test was performed by a second evaluator. Both evaluators entered the room only during the assessment time and were not aware which intervention was done on the participant. Before the intervention, the subject was instructed to sit on a chair in a comfortable position for 10 minutes; after this time, evaluators entered the room and started the assessment protocol. Evaluations were made of blood pressure, heart rate, and blood test, following this order. After the intervention, the participant was instructed to relax in a seated position for 5 minutes before postintervention evaluations were performed.

Plasmatic catecholamines levels were obtained by an analysis of every participant's blood sample. Epinephrine, norepinephrine, and adrenaline were measured according to the methods described by Sealey [25].

Blood pressure was determined by the use of a mercury sphygmomanometer (UNITEC). The assessment was performed 3 times in each arm, with 1-minute interval between them. The average of all measurements was made to obtain systolic and diastolic blood pressure scores.

Heart rate was assessed by stethoscope (Littmann 3M) that was placed on the participant's thorax, over the left heart ventricle. Heart rate was assessed three times with an interval of 1 minute between the measurements. An average of the three measurements was computed to obtain the heart rate score for study purposes.

2.4. Statistical Analysis. Analyses of variance (ANOVA) were performed to assess changes in heart rate, blood pressure, and plasmatic catecholamine levels, with GROUP (intervention versus control) as between-subjects factor and TIME (preintervention versus postintervention) as within-subjects factor. Post hoc analyses were performed using post hoc Bonferroni corrected test for multiple comparisons. Analysis was performed with STATA version 7.0. (StataCorp 2001, Stata Statistical Software: Release 7.0, College Station, TX: Stata Corporation, Texas, USA). Significance level was set at $P < .05$.

3. Results

Nineteen healthy adults were included in the intervention group (7 females, mean age = 23.58 ± 4.19 years) and twenty healthy adults were in the control group (14 females, mean age = 20.05 ± 1.96 years). Table 1 displays the values of plasmatic catecholamines, blood pressure, and heart rate for both groups before and after the procedure.

Dopamine plasmatic levels displayed a significant interaction GROUP × TIME ($F(1.37) = 7.16$, $P < .05$). The control group had lower dopamine levels than the intervention group in the preintervention assessment (post hoc pairwise comparison $P < .01$). Only the control group showed a statistically significant increase in dopamine levels when comparing postintervention scores to preintervention values (post hoc pairwise comparison $P < .01$), whereas no significant changes were seen in the intervention group (post hoc pairwise comparison $P > .82$). Moreover, a main effect TIME ($F(1,37) = 6.97$, $P < .05$) was found.

Norepinephrine levels witnessed a significant main TIME effect ($F(1,37) = 4.57$, $P < .05$), showing a decrease of plasmatic norepinephrine levels when comparing the mean score changes from postintervention to preintervention. No significant statistical effects were found for the main effect GROUP ($F(1,37) = 2.34$, $P = .14$) or the interaction GROUP × TIME ($F(1,37) = 1.89$, $P = .177$). Nevertheless, pairwise comparisons found that the control group showed lower norepinephrine plasmatic levels in the postintervention assessment, compared to the preintervention values ($P < .05$), whereas no changes were found in the intervention group ($P = .60$). Even though both groups had similar norepinephrine baseline levels ($P = .65$), pairwise comparisons after intervention showed a nonsignificant tendency for the control group to have lower norepinephrine levels than the intervention group ($P = .052$).

Likewise, no significant main effects were found for epinephrine (TIME ($F(1,37) = .32$, $P = .36$); GROUP ($F(1,37) = 2.51$, $P = .12$)). Although the interaction GROUP × TIME was not statistically significant ($F(1,37) = .87$, $P = .58$), post hoc pairwise comparisons showed lower baseline epinephrine levels for the intervention group than for the control group ($P < .05$), whereas no significant differences were found in the postintervention evaluation ($P = .65$). Pairwise comparisons did not show significant changes between the preintervention and postintervention levels for any of the study groups ($P > .30$ in all cases).

Systolic blood pressure had a significant main TIME effect ($F(1,37) = 10.02$, $P < .01$), showing a reduction of heart rate after the procedure. No statistically significant effects were found for the main factor GROUP ($F(1,37) = .10$, $P = .76$) or the interaction GROUP × TIME ($F(1,37) = .30$, $P = .59$). Although pairwise comparisons did not show significant

differences between the control and the intervention group neither before intervention or after intervention ($P > .63$ in all cases), the control group showed a statistically significant decrease of the systolic blood pressure after the intervention ($P < .05$), whereas the intervention group showed only a nonsignificant tendency to decrease ($P = .075$).

With regard to diastolic blood pressure, the findings achieved a significant main effect GROUP ($F(1,37) = 5.30$, $P < .05$), revealing lower diastolic blood pressure in the intervention group than in the control group. No statistically significant effects were found for the main factor TIME ($F(1,37) = .09$, $P = .76$) or the interaction GROUP × TIME ($F(1,37) = .28$, $P = .60$). Although pairwise comparisons did not show significant changes of the diastolic blood pressure after the intervention in any of the study groups ($P > .56$ in all cases), the control group showed higher diastolic blood pressure levels than the intervention group in the preintervention assessment ($P < .05$), whereas only a nonsignificant tendency was found for the postintervention comparison ($P = .065$).

There were significant main effects GROUP ($F(1,37) = 7.81$, $P < .01$) and TIME ($F(1,37) = 7.09$, $P < .05$) for heart rate. A lower heart rate was observed in the intervention group compared to the control group and the score changes also showed lower heart rate values in the postintervention evaluation compared to baseline scores. Although the interaction GROUP × TIME did not show any statistical significance ($F(1,37) = .58$, $P = .45$), pairwise comparisons showed that only the control group decreased the heart rate after the intervention ($P < .05$). On the contrary, no changes were found in the intervention group ($P = .19$). Nevertheless, the control group showed higher heart rate than the intervention group both before and after the intervention ($P < .05$ in all cases).

4. Discussion

The present study aimed to evaluate the effects of the CV-4 technique in the regulation of the autonomic nervous system (ANS). More specifically, the objective of the study was to evaluate changes in plasma catecholamine levels, blood pressure, and heart rate in healthy adults that were randomly assigned to either an intervention group, who received the CV-4, or a control group, who underwent a placebo intervention. The present findings confirmed a reduction of norepinephrine, systolic blood pressure, and heart rate after the intervention, in both study groups. Likewise, only subjects of the control group showed an increase of dopamine blood levels after intervention.

Our results are in contrast with other studies reporting an influence of the CV-4 technique in autonomic-related parameters, such as heart rate, blood pressure, blood flow velocity, the electroencephalography alpha power, and the muscle sympathetic nerve activity [19–22]. The previously mentioned previous studies have used several tools such as microneurographic recording, skin conductance, skin temperature, or heart rate variability assessments to test the effects of the CV-4 technique in the ANS. However, to the best of authors' knowledge, this is the first study to assess the immediate effects of CV-4 in the plasmatic catecholamine levels. The present study has addressed this issue in a different way and, therefore, the observed findings may be different. Previous research has reported that heart rate variability can be unstable in some situations and must be cautiously interpreted to characterize sympathovagal interaction [26, 27]. Other studies have not found relationships between heart rate variability and plasmatic norepinephrine levels [28]. In this sense, our design could have included catecholamines' analysis to better indicate ANS activity. It could also be argued that the lying-down position and the relaxation provided by the procedure in both groups could have had more influence in the autonomic regulation than the CV-4 technique per se. This would be in accordance with studies reporting that increments in the head-up tilt angle have been shown to increase plasma epinephrine and heart rate [29]. Plasma catecholamine activity has also been concluded to be influenced by stressors [30], and we must also take into account the influence of the procedural setting on the sample's ANS. On the other hand, previous research has also suggested that the duration of manual intervention may have a significant impact on the autonomic response [31]. According to this, higher differences between the groups may have emerged if longer procedure times had been used. This is an important area for further research, especially when no clinical present evidence has established a proper duration for the CV-4 technique. The high standard deviations found in the catecholamine levels in the present study subjects may also explain why more significant differences have not been found. Finally, our study was performed with a healthy population; the application of CV-4 in individuals with chronic pain or autonomic dysfunction or in an experimental condition using pain stimuli could have displayed different results.

In contrast with previous research, in our study, the application of the CV-4 technique was not directly related to variation in heart rate or blood pressure [21]. It has been stated that the heart rate does not quantitatively reflect the degree of autonomic activation and Ng et al. [32] have reported changes in sympathetic nerve activity without changes in heart rate or blood pressure. Mannelli et al. [33] have related changes in catecholamine plasmatic levels to the modification of the low-frequency/high-frequency ratio, which would disregard variations in changes in heart rate. In our study, both groups experienced a reduction of norepinephrine levels after intervention. Although the present findings may indicate a tendency to a higher reduction of diastolic blood pressure and heart rate in the intervention group, the absence of significant interactions does not allow us to maintain the fact that these effects are related to the technique.

Our study has some limitations that must be taken into account for the adequate interpretation of the results. Firstly, previous research has reported significant changes in the ANS after manual techniques and placebo conditions when compared to a control group with no intervention [31]. In our study the absence of a control group with no intervention does not allow us to conclude if the mere hand contact in a lying position is sufficient to elicit a different autonomic response than in a control condition. Hence, we must question whether the proposed placebo intervention is not really a powerful intervention itself and it should not be considered as a sham intervention. Secondly, catecholamine plasmatic

concentration can be influenced by factors such as age, sex, nutritional condition, emotional factors, or heat exposure [34, 35]. Although some factors have been controlled in the present research, trying to homogenize the groups and procedure conditions, some other parameters, such as the nutritional or emotional conditions, have not been controlled. The statistical analyses to observe the influence of sex in the outcome measures displayed only main statistical effects not related to the technique application. Scientific literature concludes contradictory results on the topic, going from studies reporting gender variations in catecholamine levels during rest, exercise, or stressors [35, 36] to studies not reporting gender differences [37, 38]. Our results seem to be in accordance with the latter authors, although further studies with specific designs and bigger samples are needed to control all the possible influencing variables. Moreover, heart rate measurement would have been more accurate with the use of ECG.

5. Conclusions

The CV-4 technique does not seem to have an immediate influence on catecholamine plasmatic levels. This finding is relevant as manual techniques may have concurrent effects on pain and on the ANS activity. In this sense, manual techniques likely to regulate sympathetic activity may be of paramount importance for providing analgesia and reducing pain sensitivity. Further research on the topic is warranted to better understand the physiological mechanisms underlying the CV-4 technique and its potential effects in the autonomic and nociceptive systems.

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

Chinese Herbal Therapy for Chronic Tension-Type Headache

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Objective. To investigate the effects of Chinese herbal therapy on chronic tension-type headache. *Method*. 132 patients with chronic tension-type headache were enrolled in the study. All patients filled in headache questionnaire at baseline phase and 4, 8, and 12 weeks after baseline. As an alternative therapeutic method, the patients were orally administrated Chinese herbal concoction for ten days. Therapeutic effects were evaluated during 12 weeks of followup. *Result*. In the primary outcome analysis, mean headache scores were significantly lower in the group. Scores fell by 25%–40% during 12 weeks of followup. Patients fared significantly well for most secondary outcome measures. From baseline to 4–12 weeks of followup, the number of days with headache decreased by 6.8–9.5 days. Duration of each attack also significantly ($P < 0.05$) shortened from 5.3 hours at 4 weeks to 4.9 hours after 8 weeks of followup. Days with medication per four weeks at followup were lower than those at the baseline. The differences were significant ($P < 0.05, 0.01$) for all end points. Days with medication fell by 56.6% at 12 weeks. *Conclusion*. The study has provided evidence that Chinese herbal therapy can be clinically useful for the treatment of chronic tension-type headache.

1. Introduction

Every year the lives of many people throughout the world are affected by headaches. Tension-type headache is classified as episodic if it occurs on less than 15 days a month and as chronic if it occurs more often [1]. Episodic tension-type headache can be treated with rest and analgesics, while chronic tension-type headache demands a more fundamental treatment [2]. Chronic tension headache represents a considerable social burden in terms of both costs to the health services and also the costs of lost productivity [3–5]. Despite the undoubted benefits of medication, many chronic tension-type patients continue to experience distress and social disruption. This leads to alternative approaches to headache care. One of the approaches seems to be Chinese herbal therapy.

While some researchers recommended that Chinese herb is valuable for various types of headache, including chronic tension-type [6–8], the data was limited. In this study, Chinese herbs were used with the aim of exploring their effect on chronic tension-type headache.

2. Methods

The study was a clinical trial performed at outpatient department in the First Affiliated Hospital to Changchun University of Chinese Medicine, China, from 3rd of March, 2011, to 18th of December, 2012. The study protocol was approved by the Research Ethics Committee of the Hospital (Approval number CC201102).

Patients were selected consecutively by the neurologists of the outpatient department, according to the inclusion and exclusion criteria below. Protocol summaries were reviewed by the participants, and written informed consents were obtained on the day of the study after a detailed explanation of the study purpose and methods. Those who were eligible and willing to participate were assessed by an independent physician. This assessment included a detailed history, physical examination, and collection of baseline data. All patients filled in headache questionnaire at baseline phase and 4, 8, and 12 weeks after baseline. As the main outcome measures, the headache questionnaire included analogue scale of headache score on a scale from zero (no pain) to 10

(most severe pain), duration of each attack (in hours), and the number of days on which headaches occurred per four weeks.

The main inclusion criterion was chronic tension-type headache diagnosed by criteria of International Headache Society [9], for which the subject had not received any treatment in the previous one week, besides symptomatic medication. Patients were excluded for any of the following: onset of headache disorder less than one year before; patients who had papilloedema, or pulsating headaches, or asymmetrical pupillary reflexes, or neurological deficits, or systemic disorders; pregnancy; and patients with creatinine, serum glutamic oxaloacetic transaminase (SGOT), or alkaline phosphatase levels 50% greater than the upper limit of normal for the investigator's laboratory.

At the first visit, all patients underwent initial assessment and completed questionnaires. Following this, as an alternative therapeutic method, the patients were orally administrated Chinese herbal concoction. The prescription was as follows: Tu Fuling (*Smilax glabra* Roxb) 30 g, Jin Yinghua (*Lonicera japonica* Thunb) 20 g, Deng Xincao (*Juncus effusus* L. var. decipiens Buchen) 15 g, Yuan Husuo (*Corydalis yanhusuo* W.T. Wang) 15 g, Man Jingzi (*Vitex trifolia* L.var.simplicifolia Cham.) 15 g, Fang Feng (*Saposhnikovia divaricata* (Turcz.) Schischk) 15 g, Tian Ma (*Gastrodia elata* Bl.) 15 g, Chuan Xiong (*Ligusticum wallichii* Franch.) 20 g, Bai Zhi (*Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. J. ex Franch.ex Sav.) 15 g, and Xin Yi (*Magnolia liliflora* Desr.) 3 g. The concoction was prepared by mixing the crude drugs in 800 mL water, getting 200 mL liquor after the drugs are decocted in 800 mL water (100°C for 30 minutes twice). After cooling, concoction was stored with temperature 18–24°C, humidity 55%–70%. The concoction was orally administrated by 200 mL/day, 100 mL twice per day, for ten days.

Laboratory tests that had been performed at baseline (complete blood cell count; SGOT, serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, and serum creatinine determinations) were repeated after the treatment. Subjective data were collected from daily diaries that were given to patients at each treatment session and collated by one observer.

Therapeutic effects were evaluated during 12 weeks of followup. Assessments were made at baseline and every 4 weeks up to 12 weeks. The primary outcome measures were the headache score at 12-week followup. Secondary outcome measures included the duration of each attack (in hours), the days with headache in 4 weeks [10], and use of medication scored with the medication quantification scale [11].

2.1. Statistical Analysis. Statistical methods used included paired t-tests for comparison of mean values. All analyses were carried out using SPSS Statistics 19.0. $P < 0.05$ was considered statistically significant.

3. Results

A total of 132 patients with chronic tension-type headache, aged 26–55, who met the inclusion criteria, were included (Figure 1). Three patients did not complete therapy. One patient developed scattered red skin rash 3 days after the first oral administration. This patient discontinued the therapy. Two days after the discontinuation, the skin rash disappeared. The rate of adverse events was 0.76% in the group. The other two participants discontinued for no therapy-related reasons. No clinically significant changes in hematologic or biochemical laboratory parameters were identified in laboratory monitoring.

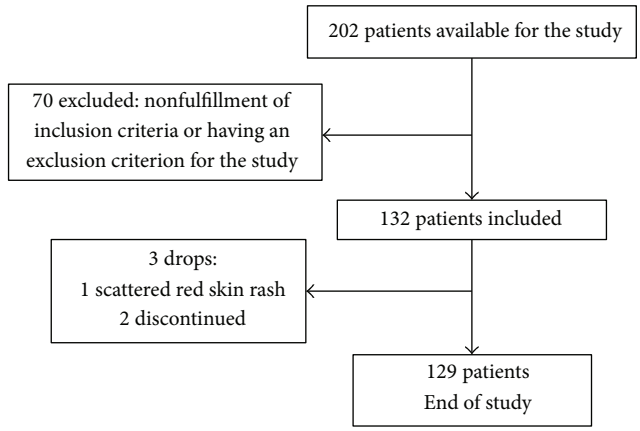

Figure 1: Flow chart of entry and discontinuation by patients during the study.

Table 1: Baseline characteristics.

Characteristics	
All patients (n)	132
Male [n (%)]	35 (26.5)
Age [mean (SD)]	40.5 (13.2)
Body mass index [mean (SD)]	23.3 (3.5)
Duration (years) [mean (SD)]	8.5 (2.8)
Days with headache per four weeks [mean (SD)]	16.0 (0.5)
Duration of each attack (hours) [mean (SD)]	9.8 (3.5)
Headache score [mean (SD)]	6.0 (3.3)
Days with medication per four weeks [mean (SD)]	13.6 (2.2)

Table 2: Outcome measures.

Outcome measures	Baseline	4 weeks	8 weeks	12 weeks
Headache score	6.0 (3.3)	3.6 (2.1)*	3.8 (3.0)*	4.5 (3.5)
Duration of each attack (hours)	9.8 (3.5)	4.5 (3.0)*	4.9 (3.8)*	5.6 (4.1)
Days with headache per four weeks [mean (SD)]	16.0 (5.2)	6.5 (6.0)#	7.8 (4.3)*	9.2 (5.5)
Days with medication per four weeks [mean (SD)]	13.6 (2.2)	4.5 (3.9)#	4.8 (2.8)#	5.9 (2.3)*

Paired t-tests: $^{*}P < 0.05$; $^{\#}P < 0.01$.

The baseline characteristics are shown in Table 1. Table 2 summarizes the results for medical outcomes for patients completing 12 weeks of followup at baseline and 4, 8, and 12 weeks after baseline. There were significant changes over time after therapy. In the primary outcome analysis, mean

headache scores were significantly lower in the group. Scores fell by 25%–40% during 12 weeks of followup compared with baseline. But the effects of Chinese herbs did not seem to be long lasting. Headache scores were slightly higher at 12 weeks than 4 weeks after treatment. Patients fared significantly well for most secondary outcome measures. From baseline to 4–12 weeks of followup, the number of days with headache decreased by 6.8–9.5 days in the group. Duration of each attack also significantly ($P < 0.05$) shortened from 5.3 hours at 4 weeks to 4.9 hours after 8 weeks of followup. Days with medication per four weeks at followup were lower than those at the baseline. The differences were significant ($P < 0.05$, 0.01) for all end points. Days with medication fell by 56.6% at 12 weeks.

4. Discussion

This trial assesses the key variables of headache in patients with chronic tension-type headache given herbal therapy. Herbal therapy results in clinically relevant benefits for patients with chronic headache. We also found decreases in use of medication. There was a significant improvement compared to the baseline for each time point. Symptoms of chronic tension-type headache abated significantly, and the effects were sustained through the followup period of 12 weeks. Methodological strengths of our study include a large sample size and high follow-up rates.

Safety is an important consideration in the management of chronic conditions such as headache. The common pharmacological therapies, such as metoprolol and flunarizine, have associated side effects, including drowsiness, ataxia, and blushing [12, 13]. In this analysis, we found a low incidence of side effects, which were related to mild allergic reaction.

There were some limitations. For lack of similar herbal drugs, our study did not have control group. One hypothesis might be that the effects seen resulted not from the action of herbal therapy but from the "placebo effect." Nonetheless, the effects of Chinese herbs were sustained during the 12 weeks of followup. This implies that our findings perhaps cannot be explained purely in terms of the placebo effect.

Patients recorded the use of analgesics for headache during the course of the study, which were lower after therapy, indicating that the superior results were not due to influence of effective cointerventions.

Chinese herbal medicine is a method of treatment rooted in an ancient Chinese culture that has existed for at least two millennia. Although the exact underlying neurophysiological mechanisms remain unclear, the results suggest that herbal therapy provides neuromodulating effects.

Traditional Chinese Medicine (TCM) is a system of healing that originated thousands of years ago. It has evolved into a well-developed, coherent system of medicine that uses several modalities to treat and prevent illness. The philosophy behind TCM revolves around the balance of the Yin and Yang. The Yang energy tends to flow up. In TCM, a common internal cause of headache is (1) liver yang rising up to the head, as a result of long-term deficiency of liver yin; (2) liver fire, a condition of extreme heat, liver fire going in an upward direction in the body leading to excess in the upper part of the body; and (3) qi stagnation and blood stasis. The Yang meridians intersect in the head. The above incentives lead to a blockage of the Yang meridians and cause headaches. When qi stagnation and blood stasis start to set in, the headache gets worse [14].

Chinese herbs applied are to lead the liver yang to flow downward, regulate the qi, and disperse blood stasis. Herbal therapy works to clear the blockages of the Yang meridians, harmonize the organs, and reestablish a balance of Yin and Yang.

The current study has provided evidence that Chinese herbal therapy can be clinically useful for the treatment of chronic tension-type headache.

Conflict of Interests

It is declared that the authors have no financial and personal relationships with other people or organizations that can inappropriately influence their work; there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the paper entitled.

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Research Article

The Nociceptive and Anti-Inflammatory Effects of *Artemisia dracunculus* L. Aqueous Extract on Fructose Fed Male Rats

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Aim & Objective. *Artemisia dracunculus* L. (*Tarragon*) species have been used as a traditional medicine. The present study was designed to evaluate the nociceptive and anti-inflammatory effects of *A. dracunculus* L. leaf aqueous extract on fructose drinking water (FDW) in male rats. *Materials & Methods*. Forty-eight Wistar-albino male rats weighing 200–250 g were divided into control (C), control extract (CE), FDW, and FDWE groups ($n = 12$). Group C did not receive any agents; Group CE did 100 mg/kg *A. dracunculus* L. aqueous extract on a daily basis for duration of four weeks. FDW Group received fructose drinking water (10%, weight/volume) but did not receive any agents during trial period. FDWE group received 100 mg/kg *A. dracunculus* L. aqueous extract during trial period. At the end of experiment, a biphasic pain response was induced following interplanetary injection of formalin (50 μL, 1%). Obtained data were analyzed using SPSS software version 17 and using ANOVA and Tukey post hoc tests. Results were expressed as mean ± SE. Statistical differences were considered significant at $P < 0.05$. *Results*. Our findings revealed that acute and chronic pain scores in FDW group are significantly higher than other ones and *A. dracunculus* L. aqueous extract causes significant decreasing of this parameter in FDWE group ($P < 0.001$). Moreover, IL6 and TNF values in this group were significantly decreased compared to FDW group ($P < 0.05$). *Conclusion*. Results in the present study show that FDW causes the pain response score to increase and cause proinflammatory cytokines in rat model but *A. dracunculus* L. leaf aqueous extract improves values of these parameters.

1. Introduction

Metabolic syndrome (Met S) is illustrated as clustering several metabolic abnormalities of an individual based on hyperlipidemia, abdominal obesity, and insulin resistance [1]. This disorder is associated with low-grade chronic inflammatory activity state and it increases the cytokines serum concentration [2]. In addition, there are associations among insulin resistance, abdominal obesity, and serum immune markers such as IL6 and TNFα [3]. Moreover, metabolic syndrome related to insulin resistance (IR) is a state in that insulin is higher than normal concentration and it plays an important role in the pathophysiology of most common human diseases such as type 2 diabetes mellitus, hypertension, and coronary heart disease [4]. A mixture of several botanical medicines is used to treat diabetes mellitus and insulin resistance accompanied by hypertension in fructose-fed rats (FFR) [5]. *Artemisia* is a widespread traditional plant and it has varied genus with different species of the family *Asteraceae* as well as great therapeutic and economic importance [6]. A botanical extract obtained from *Artemisia dracunculus (A.D.L)* has been shown to improve insulin sensitivity by increasing cellular insulin signaling in vitro in muscle cell culture [7]. Moreover, investigation confirmed that Artemisinin, a component taken from *Artemisia annual*, can affect as a potential contraceptive agent with antimalarial activity [8]. The essential oil obtained from the aerial parts of *AD* has orally been used as an antiepileptic remedy in traditional medicine [9]. Additionally, Artemisinin is an intrinsic product showing powerful anticancer activity in different types

of human tumors [10]. Furthermore, experiments indicated that Artemisinin induced the generation of regulatory T cells with extraordinarily inhibitory effect on IL-17 production, diminishing the level of IL-6 in mouse model [11]. Based on above investigations, the aim of the present study was to evaluate the nociceptive and anti-inflammatory effects of *Artemisia dracunculus (AD)* leaf aqueous extract on FDW male rats.

2. Materials & Methods

Forty-eight Wistar-albino adult male rats weighing 200–250 g were selected from Medical Sciences of Zahedan University animal house and they were reserved in individual cages (one rat in each cage). The rats had free access to water and food and they were kept in a room at 23 ± 2°C with a fixed 12:12-h artificial light/dark period (timer model: SUL180a, AC220V, China, 6 Am to 6 Pm) and a suitable humidity of 45–60%. After a week of accommodation, the rats were randomly divided into C, CE, FDW, and FDWE groups ($n = 12$ in each group) as follows.

Group C did not receive any agents during experiment period.

Group CE received daily water tap, rodent's diet, and 100 mg/kg *AD* aqueous extract for four weeks by gavages.

Group FDW received fructose-enriched water (10% w/v) and rodent's diets during experimental period but group FDWE did intake fructose-enriched water (10% w/v) daily, rodent's diets, and 100 mg/kg *ADL* aqueous extract at this time by gavages.

2.1. Preparation of the Extracts. Plants of the genus *Artemisia* (family Asteraceae) were collected from local area around Tehran, the capital of Iran, in August 2013 and identified by the Biology Taxonomy Centre in Science Faculty of Sistan and Baluchestan University, Zahedan, Iran. *Artemisia dracunculus* aerial parts were separated, shade-dried in a room temperature, and then converted into powder by hands. Extraction was performed by mixing 20 grams of powder in 200 mL of distilled water for 24 hours in soxhlet extractor [12]. The prepared extract was filtered through a gauze cloth followed by filtration through a normal filter paper Whatman no. 1 [12]. The product was a dark brown aqueous extract dried afterward in incubator for one day at 45° and sustained in appropriate temperature (regenerator).

2.2. Pain Behavioral Response Scoring. Acute pain was assessed using the 1% formalin test [13]. The rats were located in open Plexiglas observation chambers for 30 minutes to permit them to provide accommodation to their condition. Then they were removed for formalin administration. After formalin administration, each rat was placed in a Plexiglas observation box measuring 40 cm × 20 cm × 20 cm. A mirror was placed under chamber floor at a 45° angle to allow a clear view of the rat's paws. Pain behavior responses were recorded beginning from the subcutaneous injection of 1% formalin (50 μL/paw) with a 30-gauge needle into the dorsal surface of the right hind paw. Formalin induced biphasic flinching behavior in rats.

The primary acute phase (0–10 min) was then followed by a quite short period, which was followed by an expanded constant response (15–60 min). Pain behavioral responses were measured every minute and continued at 5-minute intervals for one hour [13]. The scores follow the Dubinson method:

1 = the injected paw which is not superior indicates no pain;

2 = the injected paw with little or no weight on it, with no toe splaying, indicates mild pain;

3 = the injected paw elevated and the heel is not in contact with any surface indicates moderate pain;

4 = the injected paw licked, bitten or taken aback indicates severe pain.

At the end, animals were deeply anesthetized by diethyl ether (Merck Germany) and sacrificed and blood samples were immediately collected from cervical vessels. All blood samples were collected in ordinary vials and centrifuged at 3000 rpm for 10 minutes in order to separate serum. Serum was removed (BH-1200 type Iran) and stored at −70°C for further analyses. Serums IL6 and TNF were measured by sensitive rat kit (Cusabio Biotech Co. Ltd., China), using double antibody enzyme-linked immunosorbent assay (ELISA). These experiments on animals were carried out in accordance with recommendations from the pronouncement of Helsinki and internationally conventional principles for the use of experimental animals, and they received institutional ethical approval from the committee for Animal Research of Zahedan University of Medical Sciences.

The normal distribution of data was approved by Kolmogorov-Smirnov test, and then all data were analyzed by SPSS software version 17, via ANOVA and Tukey post hoc test. The results were expressed as mean ± SE. Statistical differences were considered significant at $P < 0.05$.

3. Results

Results obtained from the present study indicated that insulin-resistance indexes (IRI) in FDW group (0.14) were significantly higher than those of C and CE groups (0.07, 0.12). In addition, IL6 and TNF value in FDW groups increased significantly in comparison with C and CE groups. Moreover, IRI (0.12), IL6, and TNF values in FDWE group decreased noticeably compared to those of FDW group (Figures 3 and 4). On the other hand, acute and chronic pain response scores in FDW extract group fell considerably down comparison with FDW group (Figures 1 and 2).

4. Discussion

Fructose is a monosaccharide found in fruits and used as sweetener in foods and drinks, and its consumption is related to incidence of abdominal obesity, insulin resistance, and

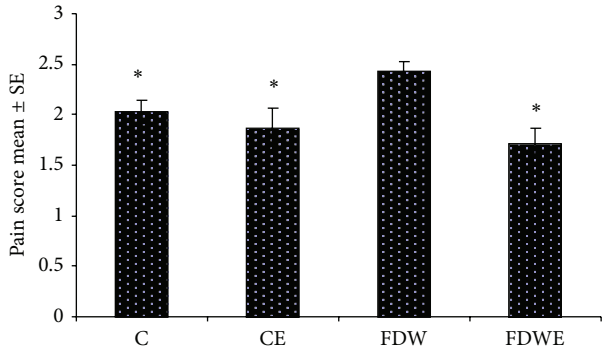

Figure 1: Mean acute pain score in C, CE, FDW, and FDWE. $n = 12$, mean ± SE and $^{*}P < 0.05$. Based on ANOVA and Tukey post hoc, consumption of fructose dirking water enhances the acute pain response in rats but *Artemisia* aqueous extract administration results in decreasing of the acute pain response in FDWE group.

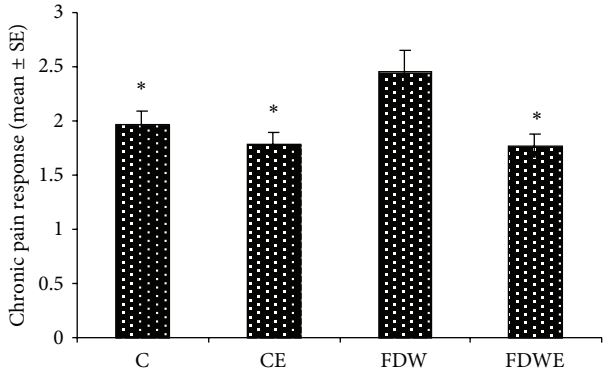

Figure 2: Chronic pain in C, CE, FDW, and FDWE. $n = 12$, $^{*}+P < 0.05$, mean ± SE. Based on statistical tests ANOVA and Tukey post hoc, consumption of fructose drinking water enhanced the chronic pain response in rats but *Artemisia* aqueous extract administration leads to decreasing of this parameter in FDWE group.

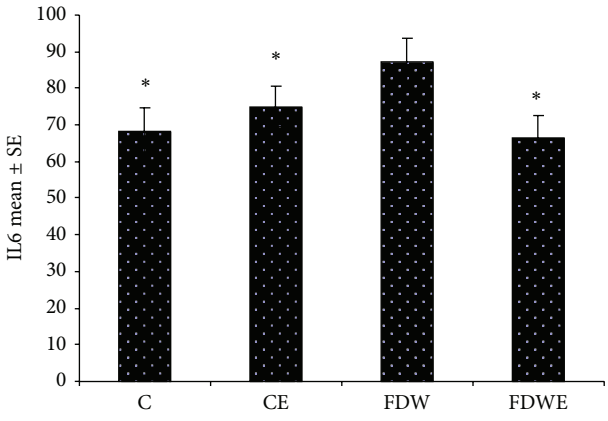

Figure 3: IL6 (ng/L) value in C, CE, FDW, and FDWE groups. $^{*}P < 0.05$, $n = 12$. Based on statistical tests ANOVA and Tukey post hoc, consumption of fructose dirking water enhanced the cytokines value (IL6) in rats but *Artemisia* aqueous extract administration causes this value to decrease for FDWE group.

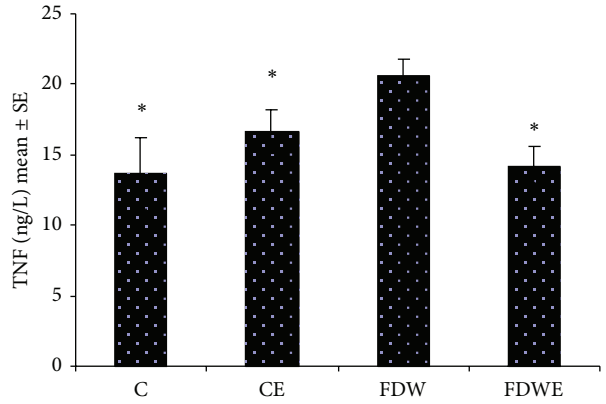

Figure 4: Tumor necrosis factor (ng/L) in C, CE, FDW, and FDWE. $^{*}P < 0.05$, mean ± SE, $n = 12$. Based on statistical tests ANOVA and Tukey post hoc, consumption of fructose drinking water improved the TNF value in male rats. On the other hand, *Artemisia* aqueous extract administration causes this parameter to fall in FDWE group.

type II diabetes mellitus [14]. Insulin and leptin are two important hormones interfering with the regulation of food intake and body weight gain in all vertebrata and human [15]. However, dietary fructose contributes to increased energy input and weight gain and alters blood lipid and carbohydrate homeostasis [15]. Insulin resistance and hyperinsulinemia are common results among patients with MS, insulin resistance, type II diabetes, and essential hypertension, which are high risk factors of cardiovascular diseases and have an important role in the development of coronary artery disease [5]. Our finding in this study revealed that FDW consumption, in a month, induced insulin resistance index (IRI), hyperinsulinemia a serum proinflammatory cytokines values such as IL6 and TNF in male rats. These results were confirmed by previous studies indicating that fructose-fed and FDW cause central obesity, insulin resistance, and hyperinsulinemia to increase in rats [4]. Previous studies indicated that TNF serum concentration elevated and altered insulin signaling in skeletal muscles cell membranes in insulin resistance disorders (type II diabetes and MS), but *Artemisia dracunculus* L. extract improved insulin action by increasing insulin signaling in skeletal muscle [16]. Investigational evidence determined that metabolic pathways associated with glucose transport, glycolysis, and cell signaling is probably influenced by *Artemisia dracunculus* L. aqueous extract and modulates carbohydrate metabolism as well as translocation of GLUT4 to the plasma membrane skeletal cells [17]. This observation pointed out that a molecular mechanism may be influenced by the *Artemisia dracunculus* L. aqueous extract components and it enhances glucose uptake, glucose transport in that sensitive-to-insulin cells, improving whole body insulin sensitivity [17]. Our results in this study showed that consumption of *Artemisia* aqueous extract causes IRI to improve in FDWE group compared to that of FDW group and it is in agreement with those of literatures. Some experimentation has shown that diabetic

and insulin-resistant patients have higher adipose tissues-derivatives TNFα in comparison with normal samples [18]. On the other hand, TNF inhibits insulin-stimulated activation of the tyrosine kinas in rat adipocytes and finally suppresses glucose transporter 4 (GLUT4) and glucose intake in these cells [19]. *Artemisia* crude extract has shown anti-inflammatory effects, but its associated anti-inflammatory mechanisms are not clear [20]. Fructose-drinking water treated by *Artemisia* aqueous extract had significant enhancement to insulin-responsive [21]. Our results expressed that TNF serum concentration in FDW rats is significantly higher than control and it is confirmed by previous studies. Moreover, *Artemisia dracunculus* L. aqueous extract improves these parameter values in FDWE group. Previous studies indicated that *Artemisia* hydromethanolic extract has shown an antinociceptive property in normal mice [22]. In addition, experimental studies revealed that *Artemisia* species has antihyperalgesic and antiallodynic properties through the inhibition of different seditious pains signaling a significant component for the treatment of inflammatory pain [23]. The results in the present study indicated that *Artemisia* aqueous extract decreased the acute and chronic pain responses in FDWE group compared to FDW group. Maham et al. in 2014 confirmed that essential oil of *Artemisia dracunculus* (EOAD) has peripheral and central antinociceptive effects in normal mice and it seems that a variety of mechanisms compared to opioid receptors are involved in the analgesic effect of EOAD [24]. Our results in this study showed that acute and chronic pain score in FDWE group is significantly lower than that of FDW group and it is confirmed by prior studies. Literature investigated that *Artemisia* species have shown a high ferric reducing antioxidant properties against oxidant damages [25]. In addition Saoudi et al. in 2010 reported that *Artemisia campestris* leaves aqueous extract contains a lot of cations such as K(+), Na(+), and Ca(++) and antioxidant such as polyphenols and scavenging activities and superoxide anion which has antioxidant and protective effects against liver oxidant [26]. Our finding in the present study showed that *Artemisia dracunculus* L. leaves aqueous extract have anti-inflammatory and analgesic effects and supported by literatures.

5. Conclusion

Our finding in the current study indicated that *Artemisia dracunculus* leaves aqueous extract has antinociceptive and anti-inflammatory effects in FDW rats.

Conflict of Interests

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

Authors' Contribution

Shahraki Mohammad Reza, Mirshekari Hamideh, and Samadi Zahra developed the original idea and protocol, collected and analyzed data, and wrote the paper.

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Review Article

The Effects of Acupuncture on Cerebral and Muscular Microcirculation: A Systematic Review of Near-Infrared Spectroscopy Studies

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Acupuncture produces physiological effects via stimulating acupoints, proximal or distal to the region of effect. Near-infrared spectroscopy (NIRS) noninvasively measures tissue-level hemodynamics in real time. We review the literature investigating the effect of acupuncture on muscular and/or cerebral microcirculation. As the basis, we queried PubMed in June 2014 for articles mentioning both acupuncture and NIRS in title/abstract. The reviewed papers investigated either cerebral ($n = 11$) or muscular hemodynamics ($n = 5$) and, based on STRICTA for reporting acupuncture methodology, were overall poor in quality. Acupuncture was found to influence regional oxygen saturation in cerebral and muscular tissue. The cortical response in healthy subjects varied across studies. For subjects with stroke or cerebrovascular dementia, findings suggest that acupuncture may modulate dysfunction in cerebral autoregulation. The muscular response to pressure techniques was more intense than that to needling or laser. Probe proximity could impact measurement sensitivity. No one study simultaneously investigated the direct and remote responses. Research utilizing NIRS to investigate the hemodynamics of acupuncture presently lacks in scope and quality. Improved designs, for example, placebo-controlled, randomized trials, and standardized intervention reporting will raise study quality. Exploiting NIRS in clinical settings, such as stroke, migraine, or other pain conditions, is worthwhile.

1. Introduction

Acupuncture is the practice of stimulating specific points of the body (acupoints), most commonly by needling, with roots in traditional Chinese medicine, and aims to treat a wide range of ailments [1]. Physiological responses include analgesic and hemodynamic responses. The analgesic response, a reflection of the influence on the autonomic system, has been documented, although not without controversy [2–4]. The hemodynamic response is also of clinical interest. Reflexive responses include erythema, a local, relatively benign effect around the stimulation site, and syncope, a systemic, serious adverse effect in poorly compromised subjects [5–8]. These are rare but well known to acupuncturists. More commonly, the response is therapeutic and able to modulate autoregulation under pathological status, such as stroke and migraine [9–11].

Modes of acupuncture are several. Modern practice generally applies stainless steel needles. Variations are application of electricity to the needles, the use of laser at the acupuncture points, manual pressure at the points (acupressure), or moxibustion. Auricular acupuncture involves a collection of points/regions on the ear theorized in 1957 by Nogier [12–14]. Point locations may then be categorized by location (and tissue type): body (muscle/tendon), ear (cartilage), and scalp (subcutaneous tissue). Furthermore, the intended effects of acupuncture stimulation are generally proximal or distal. The distal effects depend on the meridian theory, while the proximal effects follow the theory of *A Shi* points, per Chinese traditional medicine [15–18].

The scientific mechanisms behind acupuncture have long been and still are mysterious in large part because the needling locations are often remote from the intended area of effect. Such responses to needling stimuli most likely arise from interactions within the nervous system, particularly the brain. Recent tools have made it easier to study these interactions in both muscular and cerebral tissues, from multiple angles. Near-infrared spectroscopy (NIRS) is one such tool that observes the hemodynamics at the tissue level. The muscular hemodynamics reflects the direct response, while the cerebral hemodynamics reflects the remote response to acupuncture stimulation.

NIRS observes tissue hemodynamics by using near-infrared light to monitor blood oxygenation in real time. It is a safe, noninvasive technique but has limited reading depth, while the breadth of the monitored region depends on the number and placement of probes [19–21]. Nonetheless, its portability, ease of use, and high temporal resolution are significant advantages over the more spatially comprehensive BOLD-fMRI (blood oxygenation level-dependent functional magnetic resonance imaging), while its ease of use and focus on microcirculation make it an attractive alternative to transcranial Doppler ultrasound (TCD), which focuses on blood flow, usually in specific arteries [10, 22–25].

These advantages lend themselves well to monitoring the immediate hemodynamic response in cerebral or muscular tissues to acupuncture stimulation. Our aim is to review the studies reporting the use of NIRS in investigating acupuncture, its effectiveness, and its mechanisms.

2. Materials and Methods

We queried the PubMed database as of June 9, 2014, for all articles mentioning both acupuncture and NIRS in title or abstract, regardless of language. We included all original articles and excluded reviews. Any reviews were combed for relevant citations not found in the database search. For analysis, we focused on articles written in English or Chinese. Analysis of articles written in other languages was limited to abstracts and provided data and figures. All articles marked for analysis were obtained (Figure 1).

Inclusion criteria are as follows:

(i) being in PubMed database, up to June 9, 2014,

(ii) mentioning "acupuncture" in title/abstract or as a MeSH term and "near-infrared spectroscopy (NIRS)" in title/abstract,

(iii) being an original article,

(iv) having no restriction on language,

(v) having, for analysis, language restricted to English or Chinese.

Exclusion criteria are as follows:

(i) it is a review;

(ii) for analysis, languages other than English or Chinese were excluded, aside from abstract, tables, and figures.

Methods of Analysis. To assess study quality, we adapted the checklist for STRICTA (standards for reporting interventions in clinical trials of acupuncture) [26] (Table 1). Information on study designs, population, interventions, hemodynamic measures, and outcomes was organized in the tables (Tables 2–4). A summary table is also provided (Table 5).

3. Results and Discussion

Our query on June 9, 2014, produced ($n = 18$) results. We excluded ($n = 3$) reviews [10, 43, 44]. From a review, an additional three candidates, not covered in the database search, were added for consideration, of which only one was obtained and included [27, 44–46]. The two excluded are an animal study and a study involving two healthy subjects that observed changes in NIRS parameters (unspecified in the review) following acupuncture on ear, hand, and body [45, 46]. The articles ultimately included for review investigated either cerebral hemodynamics (CH) ($n = 11$) or muscular hemodynamics (MH) ($n = 5$) [27–42] (Figure 1).

3.1. Quality of Studies according to STRICTA. By STRICTA, the quality of studies under review may be considered poor in their reporting of acupuncture. We took a broad interpretation of acupuncture to include laser needling, moxibustion, and acupressure (Table 1). Details of needling, particularly number of needle insertions, depth of insertion, clarity between unilateral and bilateral application, and response sought to stimulation, were not reported in 44% of the studies (7 of 16) (Table 1, Item 2). Depth of insertion and response sought may not be applicable to some of these studies, since laser stimulation, electric stimulation, moxibustion, and acupressure were included, yet four (three) investigated manual needling among the seven underreporting depths of insertion (response sought) (Table 1, Items 2c and 2d). The number of needle insertions was often obscured from lack of distinction between unilateral and bilateral application. Regarding treatment regimen, the frequency of sessions, or time between sessions, was not reported in the majority of the studies (63%, 10 of 16) largely because most of these studies involved only one session (Table 1, Item 3). For other components of treatment, most of the studies did not have any additional interventions, as the subjects under investigation were generally healthy (Table 1, Item 4). Practitioner background for participating acupuncturists was fully reported in only 13% (2 of 16) of the studies. Three studies qualified participating acupuncturists as "expert" or "experienced" only. Of the remaining 11 studies, seven administered acupuncture but did not provide any description of the acupuncturists (Table 1, Item 5). Control or comparator interventions were also underreported (50%, 8 of 16), attributable to the majority (76%, 12 of 16) of these being observatory studies (Table 1, Item 6).

3.2. The Cerebral Hemodynamic Response. Five of 11 studies observed a significant increase in regional cerebral blood volume (rCBV) or oxyhemoglobin parameters. One involved a multisession, multipoint (body or body + scalp acupuncture) intervention for 20 stroke patients aged 41–75 and was

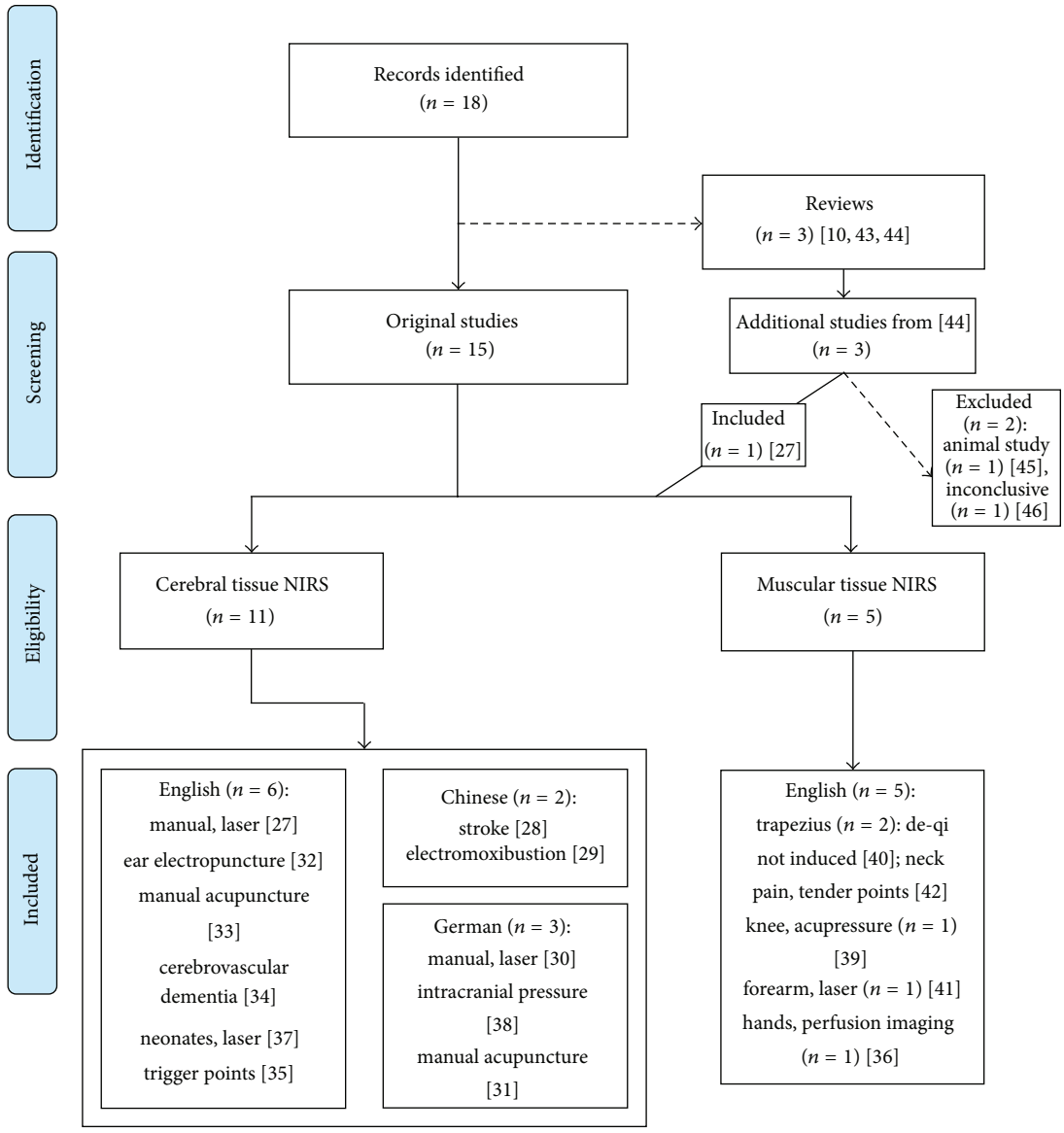

Figure 1: Flow diagram. Articles included for review targeted either cerebral hemodynamics or muscular hemodynamics with NIRS. No study attempted both.

one of two to record rCBV as the principal NIRS parameter [28]. The other investigated single-point electric moxibustion in 20 healthy subjects, aged 25–53, with mean 46 [29]. The principal oxyhemoglobin parameter in the remaining three studies was O_2Hb measured by the NIRO 300 and involved healthy subjects aged 19–38. Two involved brief needle stimulations (20 s), with a retention time of 5 or 10 min [27, 30]. The other used continuous electrical stimulation on auricular acupuncture points, finding steady increase in O_2Hb during each 15-minute stimulation of 100 Hz that persisted on level in the periods between stimulations [32].

It is likely that rCBV is synonymous with, or at least closely related to, total hemoglobin, as defined in Table 4. The interstudy populations assessed by rCBV were not comparable—one suffering stroke, the other, healthy—although both were older (age ranges: 41–75, 25–53) than the participants in the studies mentioned below [28, 29]. Among the studies finding increased oxygenation, one recorded the maximum amplitude of the changes in response to seven types of acupuncture stimulation (164 total) randomly distributed among 88 subjects [27]. The other two involved one or two subjects [30, 32]. All of these volunteers were healthy and aged 19–38. The stimulation times are comparable to the ones used in the studies finding oxygenation decreases. This complicates any attempt to draw a correlation between age and the cerebral hemodynamic response to acupuncture on healthy subjects.

Four of 11 detected significant decrease in oxyhemoglobin parameters. One involved a patient (age 77) with cerebrovascular dementia as a case study and found decreases after each NIRS-recorded session, coupled with increases in cerebral arterial mean blood flow velocity (measured by TCD). These

Table 1: Adherence to STRICTA. Articles organized by category according to a checklist provided by STRICTA.

Item	Detail	Provided		
		Yes	Unclear or incomplete	No or not applicable
(1) Acupuncture rationale	(1a) Style of acupuncture	16		5 [27–31]
	(1b) Reasoning for treatment	11		
	(1c) Extent to which treatment was varied	16		
(2) Details of needling	(2a) Number of needle insertions	9	7 [30–36]	
	(2b) Points used (uni/bilateral)	16; u/b: 9	u/b: 3 [29–31]	u/b: 4 [32–34, 36]
	(2c) Depth of insertion	9	1 [32]	6 [28–31, 34, 37]
	(2d) Response sought	9		7: [27, 29, 31, 32, 37–39]
	(2e) Needle stimulation	14		2 [33, 34]
	(2f) Needle retention time	14	2 [30, 31]	
	(2g-1) Needle (dimensions)	13		3 [29, 32, 39]
	(2g-2) Type (material/mfc)	12	3 [35, 36, 40]	1 [39]
(3) Treatment regimen	(3a) Number of sessions	13	3 [27, 30, 31]	
	(3b-1) Frequency or time between treatments	6 [27, 28, 34–36, 38]	2 [30, 32]	8
	(3b-2) Duration of treatment sessions	14	2 [30, 31]	
(4) Other components of treatment	(4a) Details of other interventions for the acupuncture group	2 [33, 34]		14
	(4b) Setting and context of treatment	16: N = 15 [27–35, 37–42]; T = 4 [32–34, 38]; P = 2 [33, 34]; O = 3: eeg [33], lds [42], icg [36]		
(5) Practitioner background	(5) Description of participating acupuncturists	2 [35, 36]	3 [32, 38, 39]	11
(6) Control or comparator interventions	(6a) Rationale for the control or comparator	2 [35, 42]	7 [27, 28, 30, 32, 33, 38, 40]	7 [29, 31, 34, 36, 37, 39, 41]
	(6b) Precise description of the control or comparator	8	2 [30, 33]	6 [31, 34, 36, 37, 39, 41]

(2b) u/b: uni/bilateral.
(2d) Acupressure [39], laser acupuncture [37], electric moxibustion [29], and P-Stim, a form of auricular electroacupuncture [32].
(2g) mfc: manufacturer; no mfc [35, 36, 40], material not mentioned [40].
(4b) All studies [27–35, 37–42], except [36] are conventional NIRS; N: NIRS, T: TCD, P: Pointselect (a tool to help identify acupuncture points), O: other; eeg: electroencephalogram, lds: laser Doppler spectroscopy, and icg: indocyanine green perfusion imaging (an application of NIRS).

decrements diminished in magnitude over the course of treatment (from a 13% decrease after the first treatment to a 4% decrease after the last) [34]. Another found oxygenation decrease in neonates after a single session of, but not during, laser acupuncture of Hegu LI 4. Peripheral oxygenation saturation (measured by means other than NIRS) was relatively constant, which implies that fractional tissue oxygen extraction increased [37] (see Table 4 for definition). The other two found decreases during brief stimulations (15 or 20 s) in subjects aged 19–30/19–45 (mean 23.5/23.9). The first of these correlated de-qi induction with the decreases in oxygenation in several areas of the brain, namely, the supplementary motor area, presupplementary motor area, and dorsomedial prefrontal cortex; the other observed decrease from manual needling at Hegu LI 4 but without clearly indicating induction of de-qi [31, 35].

Excepting the cerebrovascular dementia case, the populations are young and somewhat comparable in size (20, 20, and 16); also, the number of acupuncture points used is single to a few [31, 35, 37]. Manual needling showed quick response among healthy adults, but response to laser in neonates only emerged after the laser was turned off [31, 35, 37]. Also, needling stimulation was brief, comparable to the multiple-type acupuncture study finding increase in oxygenation, discussed above [27]. In spite of some common points among the studies investigating acupuncture in healthy young adults, the findings appear inconsistent: some found oxygenation increase; others found oxygenation decrease [27, 30–32, 35].

Table 2: Study designs and populations. Summary of objectives, study design types, and populations involved in the reviewed articles.

Purpose	Study design	Population	Type (Pop.)
Compare body (A) versus body & scalp (B) acupuncture for stroke [28]	Comparative	$n = 20$: A/B = 10/10, f : m = 4 : 6/3 : 7, by intervention, age range 41–72/42–75	Stroke
Electric moxibustion at (a) Baihui GV 20 or (b) Shenque CV 8 for healthy subjects [29]	Comparative	$n = 20$ (a) $n = 10$, f/m = 5/5, age range 25–53; (b) $n = 10$, f/m = 5/5, age range 27–51	Healthy
Changes in regional cerebral oxygenation after various methods of acupuncture [27]	Observational	$n = 88$: f/m = 50/38, age range 19–38	Healthy
Effects of manual and laser acupuncture on cerebral oxygenation [30]	Observational	$n = 3$, male, ages 25, 50, and 70	Healthy
P-STIM auricular electroacupuncture [32]	Observational	$n = 2$, female, ages 23 and 27	Healthy
Regional cerebral oxygenation changes during and after acupuncture [33]	Observational	$n = 12$: f/m = 4/8, age range 26–41	Healthy
Cerebral parameters of healthy subjects after stimulating acupuncture points associated with intracranial pressure [38]	Observational	$n = 34$, f/m = 24/10, age range 20–35 Intensive care patient after severe head injury ($n = 1$, age 15)	Healthy
Acupuncture for cerebrovascular dementia [34]	Case study	$n = 1$, age 77, female	Cerebrovascular dementia
Changes in regional cerebral oxygen saturation in neonates undergoing laser acupuncture at Hegu LI 4 [37]	Observational	$n = 20$: f/m = 8/12, age < 1	Neonates
Effects on brain activity of trigger point (TP) versus nontrigger point stimulation and de-qi induction [35]	Comparative	$n = 20$: f/m = 5/15, age range: 19–30 TP first: f/m = 1/9, non-TP first: f/m = 3/7	Healthy
Effects of acupuncture at Hegu LI 4 on central frontal cortex [31]	Observational	$n = 16$, f/m = 9/7, age range 19–45	Healthy
Compare blood oxygenation in stimulation region and distant region in trapezius muscle [40]	Controlled	$n = 19$: AS $n = 9$: f/m = 7/2 age 36 no AS $n = 10$: f/m = 7/3, age 29	Healthy, acupuncture-experienced
Tender dry point needling for neck pain (katakori) Experiment I [41]	Controlled	$n = 9$: f/m = 7/2, age range 22–48; control $n = 4$: f/m = 0/4, age range 25–27	Neck pain
Tender dry point needling for neck pain (katakori) Experiment II [41]	Observational	$n = 13$: f/m = 8/5, age range 24–48	Neck pain
Effect of acupressure at Xiyangguan GB 33 on regional oxygen saturation of deeper knee tissues [39]	Observational	$n = 12$: f/m = 5/7, age: 23.8 ± 1.6 yrs	Healthy
Effect of laser needle stimulation at acupuncture point on blood flow and oxygenation in forearm [42]	Randomized Double-blinded Placebo-controlled	$n = 33$: age 26.6 (3.4) laser/no-laser = 18/15	Healthy
Near-infrared optical imaging to evaluate efficacy of acupuncture on peripheral tissue perfusion [36]	Observational	$n = 2$: f/m = 1/1, age 20/39	Healthy

Table 3: Acupuncture interventions. Summary of the acupuncture interventions, including placebo treatments. No medications, except in one case study, were involved in these studies [34].

Technique	Duration	Session (s)	Retention time	Points	De-qi
MB [28]	37 days	22-23 sessions/37 days: 15/15 + 0/7 + 7-8/15	30 min: every 5 min, apply 1 min stim (6x)	Varies with symptom and timing of treatment (3–15 points)	Yes
MB + S [28]	37 days	15/15 + 0/7 + 7-8/15 sessions/days	30 min: every 5 min, apply 1 min stim (6x)	Varies with symptom and timing of treatment (7–22 points)	Yes
EX [29]	1 day	1	30–40 min: 2 × 15 min stim + 5 min rest btw	One of GV 20 or CV 8	
MB, MA, MH, C, C + L, C + L′, Pt* [27]	1 day	1.86 (avg.), >30 min btw, randomized*	10 s stim + 10 min (retention or laser)	MB: BL 2, Ex-HN 4; MA: "eye" (ear) and "liver" (ear); MH: Yandian, "eye" (E2) Korean hand points; C: all the above; Pt: placebo point*	
Light stimulation, MB, L, Pt [30]	1 day	4: one of each	20 s stim of each	LI 4, St 36, BL 60, BL 65, BL 66, BL 67	Yes
L, Pl [30]	1 day	2: one of each	20 s stim of each	GB 14, PC 6	
L, Pl [30]	1 day	2: one of each	20 s stim of each	GB 14, PC 6, CV 6, St 36, SP 6, LV 3	
EA [32]	1 week	4 sessions of different stim patterns	Several hours: varied (5, 15 min, or 3 hr stim)	Ear points: "eye" and "liver"	x
MB [33]	1 day	1	20 min retention after de-qi	PC 6, CV 6, ST 36, SP 6	Yes
MB, R, L [38]	1 day	3: randomized, one of each, >10 min btw	5 min for each (MB: 20 s stim + 2 min btw)	St 7, SJ 22	
MB + L [34]	13 weeks	11 (10 needle + 1 laser) sessions/13 weeks	20 min	He 5, He 7, Sp 6, BL 10, BL 17, BL 23, St 36	Yes
L [37]	1 day	1	5 min stim + 10 min undisturbed	LI 4	
MB [35]	1 day	2: 5 min btw alternate types	13 min: 3 min after insert, 8 × (15 s stim + 1 min no stim)	2 types**: TPs with de-qi; non-TPs with or without de-qi	Yes
MB [31]	1 day	1	6 min: 2 × 20 s stim + 5 min btw	LI 4	
MB [40]	1 day	1	2 min	GB 21	No
MB [41]	1 day	1	15 min	Tender points of the trapezius (6 needles obliquely inserted)	
MB [41]	2 days	1	15 min	Tender points of the trapezius (6–10 needles perpendicularly inserted)	
R [39]	1 day	1	5 min	GB33	
L [42]	1 day	1	10 min	Pe 6	
Pl [42]	1 day	1	10 min	Pe 6	
MB [36]	7 days	3	10 min	LI 4, SI 3	Yes

C: combination acupuncture; EA: electroauricular acupuncture; EX: electromoxibustion; L: laser acupuncture; L′: laser at 30% greater intensity; MA: manual auricular acupuncture; MB: manual body acupuncture; MH: manual hand acupuncture; Pl: placebo laser (laser off); Pt: placebo point needling; R: manual acupressure; S: scalp acupuncture; btw: between; stim: stimulation; min: minutes; s: seconds; 164 total sessions of 7 possible types of acupuncture randomized among the recipients ($n = 88$); the number of instances of each type (MB, MA, MH, C, C + L, C + L′, and Pt) is 23, 23, 23, 27, 27, 18, and 23, respectively. The placebo point was located 6 cun above the wrist on the radial ledge, off the lung meridian in the forearm.

**Trigger points (TPs) are located in the right extensor muscle of the forearm; non-TPs are 2 cm away from TPs. De-qi was induced from all TPs, but not all non-TP stim's.

Table 4: NIRS results. Summary of hemodynamic outcomes as measured by NIRS.

NIRS	Measure	Anatomy	Time frame	Outcomes[†]
Not available [28]	rCBV	Prefrontal cortex	At the (A) 0th, (B) 10th, (C) 20th, (D) 30th min of 30-minute acupuncture	During MB, rCBV ↑ at growing rate (130% ↑ from A to D); during MB + S, rCBV ↓ at B, then ↑ at C and D (136% ↑ A to D). At A, base MB + S >base MB
Not available [29]	rCBV	Prefrontal cortex	At the 0th, 10th, 20th, 30th min of EM[†]	rCBV ↑ during intervention
NIRO 300 [27]	O_2Hb, HHb[††]	Prefrontal cortex	During 10-minute needle retention/laser and a period 5 min after stim	O_2Hb ↑ and HHb ↓ during MH, MB, C, C + L′; O_2Hb ↑ and HHb ↓ slightly from Pt; O_2Hb ↓ and HHb ↑ slightly from A. Same response at least 5 min after
NIRO 300 [30]	O_2Hb, HHb, t-Hb, CtOx, TOI	Central cortex (crown of head)	During all stim and rest periods between (20 s) stim's	O_2Hb ↑ and TOI ↑ from MB or L. Response to MB >Response to L. O_2Hb • and TOI • from needling or laser of Pt
NIRO 300 [32]	O_2Hb	Frontal areas of brain	Before and during all stimulation periods	O_2Hb ↑ each time during 15-minute EA stim of 100 Hz on "eye" acupuncture points
INVOS 3100 [33]	rSO_2 (NIRS)	Forehead	(A) 10 min before, (B) 2 min into, (C) 10 min after (20 min) needling	rSO_2 ↑ slightly at B and C
INVOS 5100 [38]	$rcSO_2$	Prefrontal cortex	1 min before, 3 min into, and 1 min after	$rcSO_2$•
INVOS 5100 [34]	$rcSO_2$	Prefrontal cortex	Before and 10 min after needling, for the 1st, 2nd, 3rd, and 11th sessions	$rcSO_2$ ↓ (4%–13%) after each needling. Magnitude of change at each session reduced with successive sessions
NIRO 300 [37]	$rcSO_2$ SpO_2 cFTOE	Prefrontal cortex	3 × 5-minute sampling periods: before, during, and after laser stim	$rcSO_2$ ↓, SpO_2•, and cFTOE ↑ in the postintervention period. No changes before or during stimulation
fNIRS: 2 × OMM 3000 [35]	O_2Hb	Whole cortex	11 min: 2 min after needle insertion to end of acupuncture	Over 20 s interval, in SMA, pre-SMA, and mPFC: O_2Hb ↓ during and 5 s after de-qi stim's; O_2Hb • during stim with no de-qi. O_2Hb • in the other cortical regions
NIRO 300 [31]	O_2Hb, HHb, CtOx	Central region of cortex	7 min: 1 min before to end of acupuncture	After each stim, O_2Hb ↓ HHb ↑ CtOx •
HEO-200 [40]	O_2Hb, HHb, t-Hb	Trapezius muscle[**]	From 3 min before to 5 min after non-de-qi stim	O_2Hb ↑ t-Hb ↑ HHb • in stim region during and after stim compared with distant region. Parameters for controls •
OM-200 [41]	t-Hb SdO_2	Trapezius muscle	5 min before to 5 min after needling	t-Hb • SdO_2• after needling
OM-200 [41]	T_R	Trapezius muscle	Before, during, and after 1-minute exercise	T_R ↓ one day after needling in 10/13 patients
INVOS 5100 [39]	rSO_2	Knee	Just before, 2 min into, and immediately after	rSO_2 ↑ during and after stim on the stim side ($P = 0.033$); rSO_2• on opposite side
InSpectra [42]	O_2Hb and t-Hb	Forearm	4 × 2-minute sampling periods over 14 min: before, during, and after	O_2Hb • t-Hb • in the sampling periods
NIR imaging: Vas View [36]	Perfusion rate	Hands	4 × 15 min: 10 min before (A_1, A_3) and after (B_1, B_3) 1st and 3rd MB[†]	B_1: perfusion ↑. A_1 versus A_3 baselines: one case ↑, but the other •. B_3: perfusion •

↑: significant increase; ↓: significant decrease; •: insignificant or no change; stim: stimulation; rCBV: regional cerebral blood volume; rSO_2: regional oxygen saturation; $rcSO_2$: regional cerebral oxygen saturation; cFTOE: cerebral fractional tissue oxygen extraction, calculated by $(SpO_2 - rcSO_2)/SpO_2$, where SpO_2 is peripheral oxygen saturation; O_2Hb: concentration of oxyhemoglobin; HHb: concentration of deoxyhemoglobin; t-Hb: total hemoglobin (Δt-Hb = $\Delta O_2Hb + \Delta HHb$); CtOx: concentration of cytochrome oxidase aa3; TOI: tissue oxygenation index; SdO_2: oxygenation rate (%) calculated by $\Delta O_2Hb/\Delta$t-Hb; T_R: half recovery time of SdO_2 after maximum exertion of trapezius for 1 min; SMA: supplementary motor area; mPFC: dorsomedial prefrontal cortex.

INVOS 3100, 5100: Somanetics, Troy, USA; NIRO 300: Hamamatsu, Japan; OM-200 (number P/N 101-40200), OMM 3000: Shimadzu Co. Ltd, Kyoto, Japan; Model HEO-200: OMRON Ltd. Inc., Japan; Vas View: Vieworks Corp., Seongnam, Gyeonggi-do, South Korea; InSpectra: Hutchinson Technology Inc., Netherlands.

[†]Abbreviations for interventions are in Table 3. [††]Maximum amplitude of the changes in oxyhemoglobin and deoxyhemoglobin.

[*]On midpoint between the C7 spinous process, near neck tender points; [**]near and 50 mm away from Jianjing GB 21 stimulation point.

Table 5: (a) Cerebral hemodynamic response. Comparative view of articles studying the cerebral hemodynamic response with NIRS. Brief details of populations and interventions are provided, with articles arranged by response to acupuncture. (b) Muscular hemodynamic response. Comparative view of articles studying the muscular hemodynamic response with NIRS. Brief details of populations and interventions are provided, with articles arranged by target of measurement.

(a)

Type	Population Size (f : m ratio)	Age (mean)	Stimulation type	Parameter[†]	Response
Stroke [28]	20 (7 : 13)	41–75	Needling: multipoint, multisession, intensive	rCBV	+
Healthy [29]	20 (10 : 10)	25–53 (46)	Electric moxibustion: single point, single session	rCBV	+
Healthy [27]	88 (50 : 38)	19–38 (25.7)	Needling or needling + strong laser: multipoint, 1.86 average sessions/subject[a]	O_2Hb	+
Healthy [30]	1 m	25	Needling: multipoint	O_2Hb	+
Healthy [32]	2 f	23, 27	Electrical ear stimulation[b]	O_2Hb	+
Healthy [33]	12 (4 : 8)	26–41 (35.2)	Needling: single session, multipoint	rSO_2 (INVOS 3100)	0+
Healthy [38]	34 (24 : 10)	20–35 (25.2)	Separate needling, acupressure, laser at two points (ICP)	$rcSO_2$ (INVOS 5100)	0
Dementia[c] [34]	1 f	77	Needling: multisession, multipoint	$rcSO_2$ (INVOS 5100)	−
Neonates [37]	20 (8 : 12)	<1	Laser: single session, single point	O_2Hb	−
Healthy [35]	20 (5 : 15)	19–30 (23.5)	Needling: trigger points and nontrigger	O_2Hb (OMM 3000)	−/0[d]
Healthy [31]	16 (9 : 7)	19–45 (23.9)	Needling: single session, single point	O_2Hb	−

+: significant increase; 0: no significant change; −: significant decrease; 0+: slight increase in parameter.

[†]The parameter measures either tissue-level oxygenation or regional blood volume. See Table 4 for definitions. Except for one case, all measurements for O_2Hb use the NIRO 300.

[a]164 instances of acupuncture chosen from 7 possible schemes (including placebo needling) randomly applied to the pool of 88 subjects.

[b]Electrical ear stimulation at a frequency of 100 Hz.

[c]Cerebrovascular dementia.

[d]Oxygenation response significant only during de-qi-inducing stimulations.

(b)

Type	Population Size (f : m)	Age (mean)	Target	Stimulation	Probe location	Parameter	Response
Healthy [40]	9 (7 : 2)	(36)	Trapezius	Needling: single point	Needling at center of probe	O_2Hb HHb t-Hb	+ + 0
Neck pain [41]	9 (7 : 2)	22–48 (35.1)	Trapezius	Tender point dry needling	Needles angled under probe[a]	t-Hb SdO_2	0
Neck pain [41]	13 (8 : 5)	24–48 (36.5)	Trapezius	Tender point dry needling	During exercise[b]	T_R	−
Healthy [39]	12 (5 : 7)	(23.8)	Knee	Acupressure: single session, single point	Near stim point and away[c]	rSO_2	+
Healthy [42]	33 m	(26.6)	Forearm	Laser: single session, single point	M. flexor carpi ulnaris[d]	O_2Hb t-Hb	0 0
Healthy [36]	2 (1 : 1)	20, 39	Hand	Needling: 3 sessions, two points	Whole hand	Perfusion rate	0+

+: significant increase; 0: no significant change; −: significant decrease; 0+: slight increase in parameter.

[a]Six needles angled obliquely to 20 mm under the center of the probe.

[b]T_R is calculated during maximal exertion of trapezius conducted once before and again one day after needling. Needles angled perpendicularly. See Table 4 for definition.

[c]Two probes: one 2 cm from the stimulation point at Xiyangguan (GB 33) and the other on the opposite side of the patella.

[d]The stimulation site, Neiguan (Pe 6), is located 2 cm proximal to the midpoint of the carpal fold between the tendons of M. flexor carpi radialis and M. palmaris longus.

Two of 11 observed either a slight increase or no significant changes in oxyhemoglobin. Both of these also used transcranial Doppler ultrasound (TCD) to measure blood flow in the middle cerebral artery (MCA). The one finding no significant change in oxygenation generally found increased mean blood flow velocity in the left and right MCA (and, to a lesser degree, reduced pulsatility index) but no change in blood pressure parameters in response to (needle, pressure, or laser) stimulation of acupuncture points known to increase intracranial pressure in 34 healthy subjects, aged 20–35 (mean 25.2) [38]. The other study also found increased mean blood flow in the right MCA in response to an acupuncture scheme designed for "general increase of Qi-energy" in 12 subjects, aged 26–41 (mean 35.2) [33]. Aside from using both TCD and NIRS, too many parameters differ between the two to infer anything substantial.

In summary, the findings above indicate that the cerebral tissue oxygenation response to acupuncture, even in healthy young adults, varies widely, with no clear correlation to any single factor. Further research is required to investigate whether the variation in response carries over to subjects exhibiting dysfunction in cerebral autoregulation, as in stroke or migraine, since acupuncture has been found to have modulating effects [10, 47]. We recommend that future investigations consider the following for control: population age and fitness/health level; acupuncture type and intensity of stimulation (number of sessions, frequency, and duration); and NIRS machine model and recorded parameters and the number and positioning of probe(s).

3.3. The Muscular Hemodynamic Response. The response in the trapezius muscle was mixed between the two relevant studies. One found an increase in regional tissue oxygenation in the site of stimulation starting with needling, which stayed constant at least 5 min after stimulation ended, and identified no changes in a region centered 50 mm away [40]. The implication is that the direct oxygenation response to needling is detectable in the region surrounding the stimulation site, but not so in a region less than 1 cm away. The other found no increase but even a slight decrease in the ratio of oxyhemoglobin to total hemoglobin in the recorded region, which was located amidst six needles angled obliquely under the probe [41]. Some of the key differences between the two studies were population type (healthy versus "neck pain"), number of needles (1 versus 6), needling location (Jianjing GB 21 versus tender points), needling angle (vertical versus oblique), needle retention (2 versus 15 min), and NIRS parameters (oxyhemoglobin versus ratio of oxyhemoglobin to total hemoglobin).

A significant response in tissue oxygenation from acupressure stimulation of Xiyangguan GB33 was detected in the knee tissues on the stimulated side, while no significant response registered on the opposite side of the knee [39]. Acupressure may have a wider range of impact on muscular tissue oxygenation compared to manual or laser needling, simply owing to the nature of the techniques (pressure from the thumb versus needling at a point).

No significant response in tissue oxygenation was detected in the forearm from laser needle stimulation at Neiguan Pe 6, located 2 cm proximal to the middle point of the carpal fold between the tendons of M. flexor carpi radialis and M. palmaris longus [42]. The NIRS probe was located on M. flexor carpi ulnaris. Increased blood flow in a nearby region (5 cm proximal to the middle point of the carpal fold between the tendons of M. flexor carpi radialis and M. palmaris longus) was detected by laser Doppler spectroscopy. It is possible the NIRS probe was too distant from the site of stimulation or that laser needling may not have a strong enough effect for NIRS to detect a significant response.

A significant change in the perfusion rates of the hands was found in response to the first session of manual needling of Hegu LI 4 and Houxi SI 3. However, at the third session five days after, the responses failed to register significance. The baseline perfusion rates from the first to the third trial increased in one subject, but not the other [36]. This may reflect an acclimatization of muscular tissue perfusion to repeated acupuncture (three 10-minute sessions over 5 days). The study only has two subjects of different gender and age. No firm conclusions can be drawn from this study.

In summary of the MH studies, the technique of stimulation and proximity of the probe to the stimulation site appear to have a discernible impact on the detection and intensity of a response in the muscle and connective tissues. The findings suggest that in muscular tissue, acupressure has a greater impact on regional oxygenation than acupuncture, which in turn exceeds that of laser stimulation. Oxygenation has not been found to decrease in response to acupuncture, but the number of studies is few.

4. Conclusion

Research using NIRS to investigate the hemodynamic effects of acupuncture is presently lacking in scope, number, and quality. Further studies may exploit the ease of use and real-time capacity of NIRS to monitor regional, tissue-level blood oxygenation to examine the concurrent response locally in the muscular tissue and remotely in the cerebral tissue. Improved study designs, accounting for the limitations of NIRS, placebo-controlled RCTs, and standardized reporting on interventions, such as adherence to STRICTA, will raise the quality of studies. Although the hemodynamic response to acupuncture varied widely among healthy subjects, it is worthwhile to extend the use of NIRS to clinical settings, such as stroke, neck pain, migraine, or other pain conditions.

Conflict of Interests

There is no conflict of interests to declare for the writing of this paper.

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Research Article

Dezocine Prevents Postoperative Hyperalgesia in Patients Undergoing Open Abdominal Surgery

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Objective. Postoperative hyperalgesia is very frequent and hard to treat. Dezocine is widely used and has a modulatory effect for thermal hyperalgesia in animal models. So, this study was designed to investigate the potential role of dezocine in decreasing postoperative hyperalgesia for patients undergoing open abdominal surgery. *Methods*. This is a randomized, double-blinded, and placebo-controlled trial. 50 patients for elective open gastrectomy were randomly allocated to either a true treatment group (0.15 mg/kg intravenous dezocine at the end of surgery) or a sham treatment group (equivalent volume of saline) in a 1 : 1 ratio. Patients were followed up for 48 hours postoperatively and pain threshold to Von Frey filaments, pain scores, PCIA consumption, rescue analgesics use, sedation score, and occurrence of postoperative nausea and vomiting were recorded. *Results*. Patients in the true treatment group experienced statistically significantly higher pain threshold on forearm and smaller extent of peri-incisional hyperalgesia than the sham treatment group. Rescue analgesic use, cumulative PCIA consumption, and pain scores were statistically significantly decreased in the true treatment group compared to the sham treatment group. *Conclusions*. Dezocine offers a significant antihyperalgesic and analgesic effect in patients undergoing elective open gastrectomy for up to 48 hours postoperatively.

1. Introduction

Hyperalgesia frequently occurs after surgery, especially in the early postoperative period [1]. The occurrence of postoperative hyperalgesia can be either due to nervous system sensitization by surgical nociception or as an adverse effect of perioperative opioid use. Nociception induced hyperalgesia is generally considered as a consequence of surgical tissue and nerve trauma [2] while opioid induced hyperalgesia (OIH) is a paradoxical response whereby a patient receiving opioids during surgery for the treatment of pain may actually become more sensitive to certain painful stimuli after surgery [3]. Postoperative hyperalgesia may occur in a restricted area where the pain is treated or in a more generalized manner [4].

Remifentanil is a potent μ opioid receptor agonist. Its short context sensitive half-life and short elimination half time make it suitable for anesthetic maintenance during surgery. After intravenous administration, remifentanil exhibits a stable plasma concentration [5], organ independent elimination [6, 7], minimal alveolar concentration reducing effect [8], and attenuated autonomic, somatic, and adrenocortical responses to noxious stimuli [9]. Nevertheless, remifentanil-induced hyperalgesia (RIH) is more severe and frequent than other opioids [10, 11]. Postoperative hyperalgesia is always complicated with RIH and other contributing factors including tissue trauma, whereas the precise mechanisms remain unclear [12]. Postoperative hyperalgesia usually results in unsatisfied pain control and increased extra morphine consumption. Current treatments such as opioids, ketamine, and NSAIDs drugs are insufficient to solve this problem. So, novel strategies are highly needed.

Dezocine, first developed in 1970s, is an opioid μ receptor partial agonist/antagonist [13]. Dezocine is largely preferred as an alternative medication for perioperative pain management because of its good tolerance, mild adverse effects, and good potence [14]. There has always been debate for its κ receptor profile. Although initially identified as a κ receptor agonist, recent studies suggest that dezocine might be a κ receptor antagonist [13, 15]. As κ receptor antagonist, it can probably have a modulatory effect on spinal dynorphin, preventing it from binding to κ receptor, which plays a crucial role in the spinal effect of hyperalgesia [3]. Studies have also showed that dezocine produced high levels of thermal

hyperalgesia modulatory function in animal models [16]. However, whether clinically administrated dezocine could modulate postoperative hyperalgesia and provide a more satisfying pain control has never been documented.

Therefore, the present study aimed at investigating the effect of dezocine in preventing postoperative hyperalgesia using a random double-blinded study.

2. Methods

2.1. Study Design and Patient Population. This was a double-blinded, placebo-controlled randomized trial. This study was approved by the Ethic Committee of Shanghai Renji Hospital affiliated to Shanghai Jiaotong University of Medicine (document number 2012032) and was also registered in the Chinese Clinical Trials Registry (ChiCTR-TRC-14004723). Patients scheduled to have open gastrectomy from August 2012 to February 2014 were included in this study.

2.2. Criteria for Inclusion and Exclusion. Patients were included if they were (1) aged 18~64 years and (2) with American Society Anesthesiologists physical statuses I-II.

Patients were excluded when (1) they had chronic use of analgesics or had used opioids within 24 h of surgery; (2) immediate extubation was not planned after surgery; (3) patients had neurological or psychiatric disorders; (4) there was end-stage cancer in preoperative evaluation; (5) there was diabetes or severe hypertension; (6) there were either abnormal findings in laboratory tests including complete blood count, liver and renal function, electrolytic analysis, and thrombin time; (7) they were unable to understand the usage of the patient-controlled intravenous analgesia (PCIA) device; (8) body mass index (BMI) was > 30 or < 17; (9) there was alcoholic abuse or coffee drinking > 2 cups/day, and (10) there was rejection either by the surgeon or by the patient. Moreover, after initial enrollment, if any of the following activities occurred, the patients were also excluded: (1) ICU hospitalization after surgery; (2) transient perioperative hypothermia; (3) blood loss > 400 mL or hemoglobin decreasing $> 30\%$ in the last perioperative ABG; (4) perioperative acid-alkaline or electrolytes disorders; (5) advanced gastric cancer found on the surgical table. After initial inclusion and exclusion, we had 93 patients enrolled in the study and 43 patients were finally excluded because of perioperative findings as listed above.

2.3. Preoperative Preparations. During the preoperative evaluation on the day before surgery, after informed consent was signed, patients were instructed in the use of the PCIA pump, the quantitative sensory tests with calibrated Von Frey filaments (0.6–180 g/mm^2, North Coast Medical, Inc.), the visual analogue scale (VAS; from 0 to 10; 0, no pain; 10, worst pain imaginable), and a four-point verbal rating scale (VRS) for pain evaluation (0, no pain; 1, slight pain; 2, moderate pain; 3, intense or severe pain). The static hyperalgesia was also assessed proximally to the surgical wound and on the forearm. Tactile pain thresholds were measured 2 to 3 cm away from the potential incision at 3 levels (top, middle, and bottom), separated by about 5 cm on the right side. It is defined as the smallest force that was just perceived as painful [17]. No premedication or fasting was required after midnight on the day before surgery.

2.4. Anesthesia Protocol. In the operating room, standard monitoring was performed and the baseline values were recorded. Anesthesia was induced with midazolam 0.05 mg/kg, propofol 1.5 mg/kg, fentanyl 4 μg/kg, and rocuronium 0.6 mg/kg to facilitate the tracheal intubation. After tracheal intubation, the patients were ventilated to normocapnia. An infusion of a cisatracurium was reached at 2 μg/kg/min and was discontinued about 15 min before the end of the surgery. Residual neuromuscular blockade was antagonised by 15–20 μg/kg atropine and 40–60 μg/kg neostigmine. Anesthesia was maintained with remifentanil at 0.4 μg/kg/min and sevoflurane at a concentration from 1% to 2.5%.

2.5. Sample Size Estimation. In our preliminary of 20 patients, we concluded that an estimated sample size of 22 patients per group would give us a β-risk of 80% at an α-level of 0.05 for detecting a difference of 30% in the PCIA consumption. We have also anticipated a lost prevalence of 10%. Thus, the study size was prospectively set to 50 patients (25 patients/group).

2.6. Assignment and Postoperative Management. These 50 patients were randomly assigned to one of the two groups: dezocine group and control group. Each group had a random-number generated by computer precising the group assignment and envelopes containing the results were prepared. In the morning of surgery, a person not involved in the evaluation procedure opened the envelope and prepared the drugs. Thirty minutes before the end of the surgery, a titration of 0.1 μg/kg sufentanil was given intravenously for postoperative analgesia. After incision closure, sevoflurane and remifentanil were discontinued, a dose of 0.15 mg/kg dezocine (Yangtze River Pharmaceutical Group, Taizhou, Jiangsu, China) was administered to dezocine group, and the same volume of normal saline was administered to group control. The patients and the investigators involved in patient management or data collection were all unaware of the group assignment.

Inspired sevoflurane concentration was increased stepwise by 0.5%–1% when insufficient anesthesia was considered which was defined as an accelerated heart rate by 15% or a systolic arterial blood pressure exceeding baseline values by 20% with or without clinical signs of inadequate anesthesia such as patient movement, coughing, and tearing. Inadequate anesthesia was also treated with propofol. Atropine and ephedrine were prepared to treat bradycardia and hypotension perioperatively. After recovery of adequate spontaneous ventilation and the obeisance to verbal commands (eye opening and limb moving), the tracheal tube was removed and the patients were kept in the postanesthesia care unit (PACU) for an hour, where standard monitoring was recorded every 15 min. Additional analgesics were given to the patients who had VAS > 5. Background infusion of PCIA was started just before leaving PACU and the postoperative pain was controlled by PCIA, which was programmed to deliver demand doses of sufentanil 3 μg/h with a 15 min lockout

and continuous infusion of 3 μg/h. In general ward this PCIA regimen was maintained and rescue analgesics were administered if patient required extra pain control or VAS > 5.

2.7. Measurements. Baseline MAP and HR were defined as the mean of the two lowest measurements recorded during a 5 min interval just before the induction of anesthesia. Values from all routine anesthetic monitors were recorded at a 5 min interval perioperatively. Duration of anesthesia and surgery, the total doses of remifentanil, and the use of atropine or ephedrine were also recorded.

Rescue analgesics, VAS score evaluating the pain intensity, and sedation score monitored by Ramsay scale (1, anxious and agitated; 2, cooperative, tranquil, and oriented; 3, responding only to verbal commands; 4, asleep with brisk response to light stimulation; 5, asleep without response to light stimulation; 6, nonresponsive) were recorded during the PACU stay. The cumulative consumption of sufentanil given by PCIA and pain scores (VAS and VRS) were also recorded at 6, 24, and 48 h after surgery. The primary outcome was the consumption of sufentanil during the first 24 h after surgery. The incidences of postoperative nausea and vomiting were recorded during the visit at 48 h postoperatively. Subjects who experienced severe postoperative nausea and vomiting (PONV) were treated with ondansetron 4 mg intravenously. Other side effects such as respiratory depression, muscle rigidity, pruritus, and dysphoria were also recorded.

The pain threshold for mechanical static stimuli was evaluated both at 2 cm proximal to the surgical wound and on the forearm by calibrated Von Frey filaments (0.6–180 g/mm^2) at 6, 24, and 48 h after surgery. The extent of mechanical static hyperalgesia to punctuate stimulation proximal to the wound was assessed with Von Frey filament number 17 (60 g/mm^2) as previously described [17]. Hyperalgesia was determined by stimulating along three linear paths at right angles to the top, middle, and bottom side of the surgical wound in steps of 0.5 cm at 1 s interval, starting from 10 cm outside the surgical wound. The distance (in cm) from the incision to where sensations changed was measured and a total of the three measurements were calculated and used for statistical comparisons.

2.8. Statistical Analysis. Age, weight, height, BMI, duration of surgery and anesthesia, intraoperative remifentanil use, haemodynamic variables, cumulative sufentanil consumption, and VAS scale were analysed by Student's t-test. The χ^2 test was used to compare the sex, intraoperative atropine or ephedrine use, PONV incidence, and PACU rescue analgesics use. Mann-Whitney test was applied to compare the pain threshold, the extent of mechanical static hyperalgesia, VRS scores, and Ramsay scale during PACU stay. All data analysis was performed using SPSS (version 13.0, IBM). P values less than 0.05 were considered to be statistical significance.

3. Results

A total of 50 patients (25 patients in each group) were recruited in our study. Table 1 shows the patients' morphometric and demographic characteristics as well as the details of surgery. No significant difference was found between the two groups ($P > 0.05$).

During the surgical procedure, hemodynamic status and drug consumption were noted. Table 2 presents the perioperative drug consumption including remifentanil, atropine, and ephedrine, and no significant difference was observed between the two groups ($P > 0.05$). Figure 1 shows perioperative mean arterial pressure (MAP) and heart rate (HR), which were comparable between the two groups ($P > 0.05$).

During postoperative follow-up, patients treated with dezocine showed statistically significantly decreased PCIA consumption at 6 postoperative hours ($P < 0.05$) and lower VAS pain score at the first hour after the surgery compared with the patients in the control group ($P < 0.05$) (Figure 2). Table 3 shows that patients in the treatment group required statistically significantly less rescue analgesic during PACU stay ($P < 0.05$). Figure 4 showed that, at 24 postoperative hours, pain threshold on forearm of the patients in the treatment group was statistically significantly higher than the patients in the control group ($P < 0.05$). The extent of peri-incisional hyperalgesia was statistically significantly smaller in patients of the treatment group than the control group ($P < 0.05$) (Figure 4), whereas no significant difference was observed in postoperative VRS pain score, peri-incisional pain threshold, incidence of postoperative hyperalgesia, Ramsay sedation score (Figure 3), and the incidence of PONV ($P > 0.05$).

4. Discussion

To the best of our knowledge, this study firstly showed that intravenous injection of 0.15 mg/kg dezocine at the end of surgery leads to a better postoperative pain control manifesting as indicated by a fewer PACU rescue analgesics demands, a decreased cumulative postoperative PCIA consumption during the first 6 postoperative hours, and a more satisfactory VAS score at 1 hour postoperatively. Dezocine can also provide antihyperalgesia effects, including a higher pain threshold on the forearm and a smaller extent of hyperalgesia proximal to the surgical wound.

Pain after surgery is a major management challenge in clinical practice and postoperative hyperalgesia is usually complicated in such situations. A considerable number of surgical patients suffer from moderate to severe acute postoperative pain and a large number of patients experience inadequate pain relief with available pain managements. Postoperative hyperalgesia manifesting as an exaggerated original underlying painful condition or a more generalized state mainly involves both surgical nociception and opioid administrations and it is considered as a very important contributive cause of inadequate postoperative pain control [3]. Postoperative hyperalgesia usually results in higher postoperative pain scores and earlier morphine consumption requirements, thus leading to an increased occurrence of adverse effects in patients [17, 18], making it a clinical management concern. Although being studied for several years, the exact molecular mechanism seems to be multifactorial and is not clear yet. But the possible mechanisms of postoperative hyperalgesia are thought to share similar underlying mechanisms such

Table 1: Morphometric and demographic data and details of surgery.

	Dezocine	Controls	*P* value
Sex (F/M)	9/16	7/18	0.77
Age (yr)	54.64 ± 10.71	54.44 ± 7.27	0.94
Weight (kg)	63.96 ± 9.21	69.48 ± 10.94	0.06
Height (m)	1.68 ± 0.07	1.70 ± 0.08	0.39
BMI (kg/m^2)	22.62 ± 2.64	23.99 ± 2.85	0.08
Duration of anesthesia (h)	3.75 ± 0.91	4.08 ± 0.80	0.20
Duration of surgery (h)	3.43 ± 0.65	3.29 ± 0.78	0.49
Gastric cancer/gastric ulcer	22/3	24/1	0.30
Partial gastrectomy/total gastrectomy	10/15	8/17	0.56

All values, except sex, diagnosis, and surgical type, are expressed as mean ± SD.

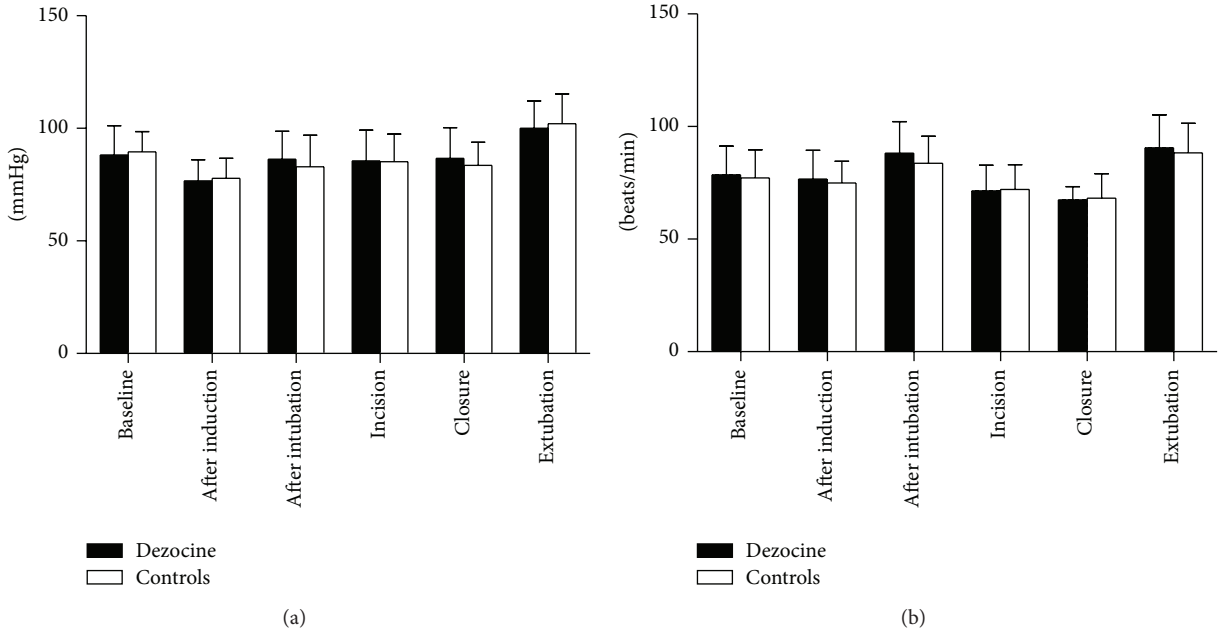

Figure 1: Mean arterial pressure (MAP) (a) and heart rate (HR) (b) (mean ± SD) values at various times points. No statistical significant difference was observed.

Table 2: Perioperative drug consumption.

	Dezocine	Controls	*P* value
Remifentanil (mg)	3.00 ± 0.63	2.85 ± 0.70	0.40
Atropine	8/17	10/15	0.56
Ephedrine	8/17	7/18	0.76

Remifentanil consumption is expressed as mean ± SD; atropine and ephedrine consumption were showed as number of patients using drugs versus number of patients without administration of those drugs.

as the involvement of the sensitization of primary neurons, neuroplastic changes of central pain transmission pathway [3], and excitatory amino acids via the N-methyl-D-aspartate (NMDA) receptor [1]. Our study applying Von Frey filaments has showed decreased pain threshold in dezocine and control groups, proving the evidence of the existence of postoperative hyperalgesia. We did an infusion of remifentanil at an initial dose of 0.4 μg/kg/min and then adjusted to the hemodynamic changes or the anesthetic depth for the maintenance of anesthesia, which is widely considered dose-dependently [17] to generate remifentanil-induced hyperalgesia [19]. Surgical nociception and opioid induced hyperalgesia share the same molecular mechanism including sensitization of primary afferent neurons, enhanced production and release of excitatory neurotransmitters, sensitization of second-order neurons to excitatory neurotransmitters, and neuroplastic changes in the spinal level which lead to upregulation of spinal dynorphin. It should be pointed out that postoperative hyperalgesia can be a more generalized state. We considered the hyperalgesic state on the forearm as a generalized state and our results showed that high incidence of generalized postoperative hyperalgesia is observed in our cohort, which was accordant with other reports [20]. Here based on a random double-blinded study, dezocine is found to be effective in preventing postoperative hyperalgesia in patients undergoing open abdominal surgery.

Table 3: Extra analgesic consumption, PONV, and incidence of hyperalgesia.

	Dezocine	Controls	*P* value
Analgesic use in PACU	10/25 (40%)	18/25 (72%)	0.02
PONV	15/25 (60%)	11/25 (44%)	0.26
Peri-incisional hyperalgesia	22/25 (88%)	20/25 (80%)	—
Generalized hyperalgesia	14/25 (56%)	20/25 (80%)	0.07

All data are expressed as number of patients.

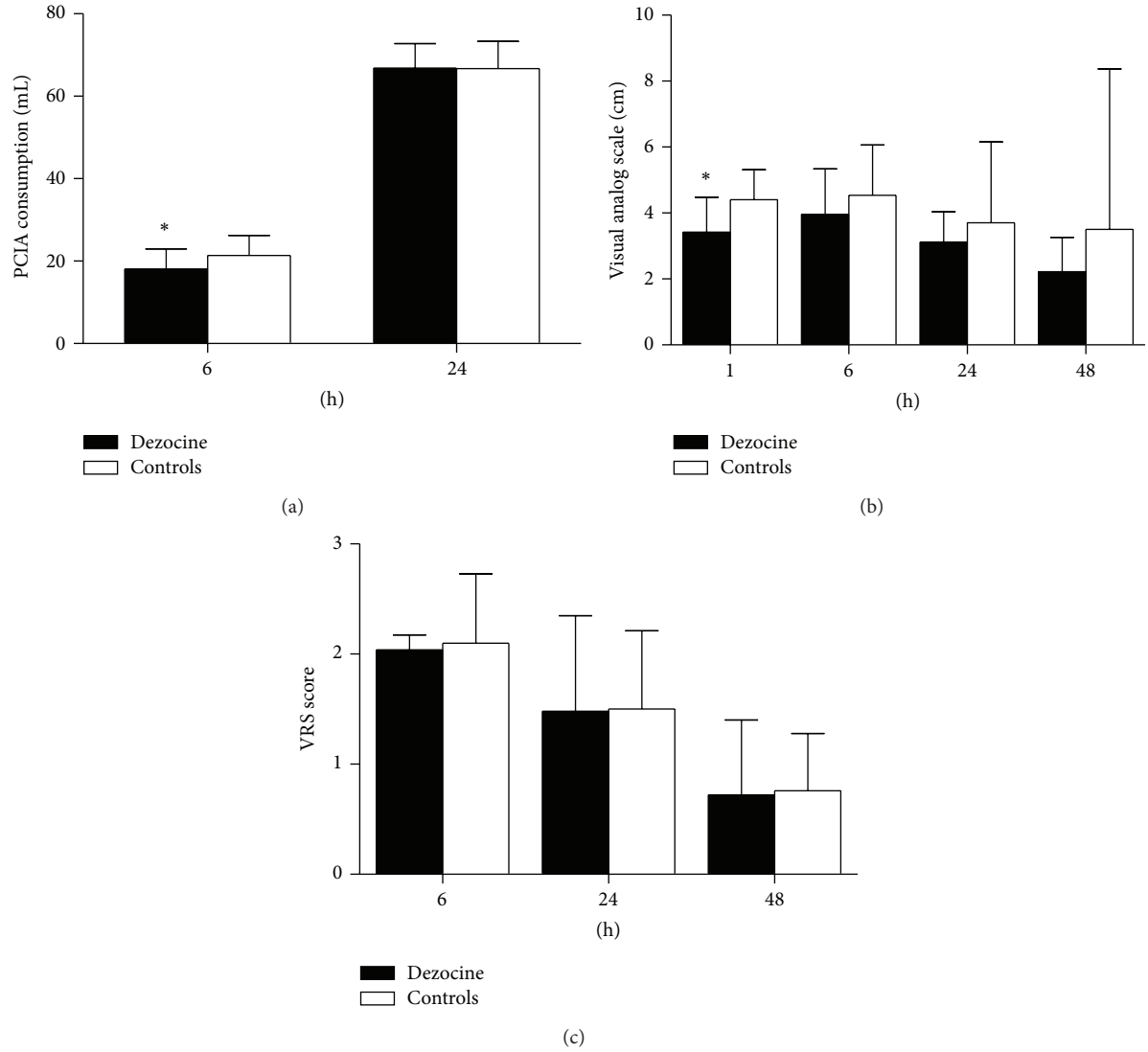

Figure 2: Postoperative PCIA consumption (a), VAS score (b), and VRS score (c) (mean ± SD). $^{*}P < 0.05$.

Dezocine, as a combined opioid agonist/antagonist, is of great interest because of its ability to produce analgesia, decreased liability to addiction, and limited depression of respiration compared with the classical opioid [21]. Clinical observations have suggested that analgesic efficacy of dezocine was similar to that of morphine [22] and a combined use of dezocine with morphine can greatly increase the analgesic effects, which indicates that dezocine may have an additional mechanism of analgesic effect [21]. This is because dezocine should diminish the analgesia effect significantly as a partial μ receptor antagonist. A recently published study identified that dezocine is a κ opioid antagonist and also inhibits norepinephrine and serotonin reuptake in vitro [13], which may have potential interactions with antinociceptive pathways since antagonistic effect of dezocine against spinal dynorphin might play a nonnegligible role in the central pathway of nociceptive transmission. Here we reported that dezocine could greatly increase the generalized pain

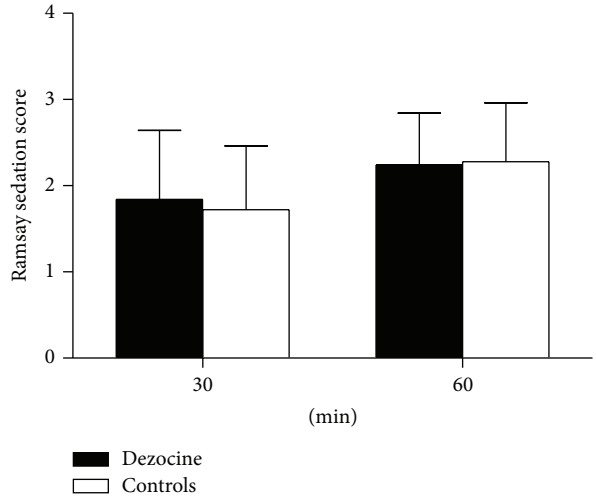

Figure 3: Ramsay sedation score during PACU stay (mean ± SD).

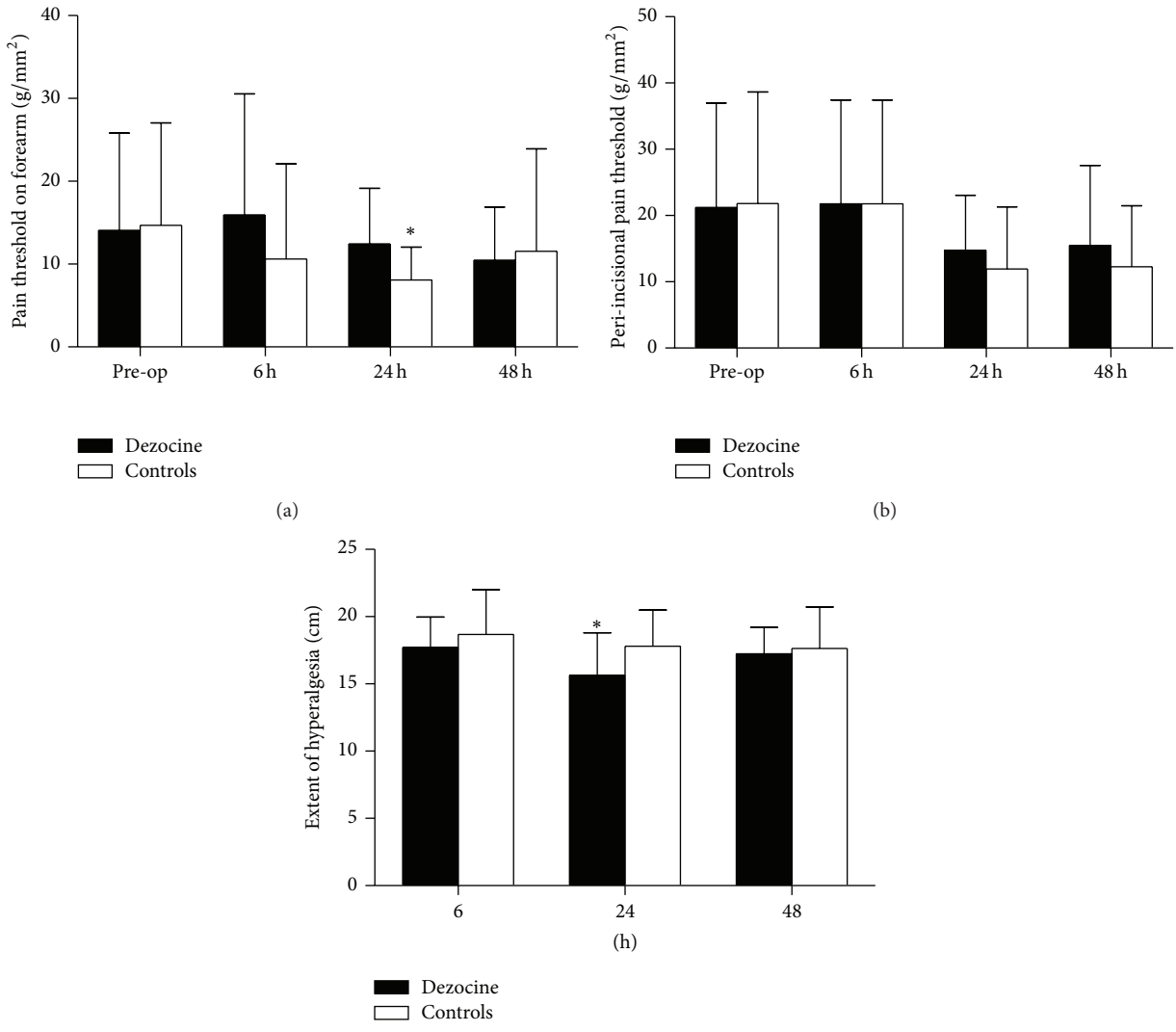

Figure 4: Pain threshold on forearm (a) and proximal to the surgical wound (b) and the extent of peri-incisional hyperalgesia (mean ± SD).
$^*P < 0.05$.

threshold instead of relieving the peri-incisional hyperalgesia, which might explain the central modulatory effect and further studies should be carried out to explain the exact molecular mechanism.

Several limitations of the present study should be highlighted. Firstly, the dosage of remifentanil used in our study was less than 0.4 μg/kg/min and 0.4 μg/kg/min is the most widely reported concentration inducing significant postoperative RIH in the literature. In the clinic, we found that patients with high perioperative dose of remifentanil (0.4 μg/kg/min) had frequently unstable haemodynamics and usually required repeated administration of ephedrine and atropine. Thus, in terms of patients' safety and as required by both of the anesthesiologists and the surgeons, we decreased the dose and hyperalgesia was still induced as proven by the quantitative pain test, calibrated Von Frey filaments. Secondly, we did not note the delay of the first demand of PACU rescue analgesics and the dosage of the analgesics used during PACU stay. The length of PACU stay was not assumed, which should be dependent on the patient status. But in our institute it might be more dependent on the department policy which usually requires one-hour PACU stay. Thirdly, our study was powered on the postoperative PCIA consumption. A larger study powered on the pain threshold change for about several weeks warrants being conducted to see the long term hyperalgesia relief effects of dezocine since evidences have showed that there might be a link between postoperative hyperalgesia and the risk of chronic pain development.

In conclusion, our study shows that, for patients undergoing open gastrectomy, intravenous injection of dezocine at the end of surgery decreases PCIA consumption and helps achieve a better postoperative pain control as well as a decreased generalized and peri-incisional extent of hyperalgesia level. Dezocine may be a potentially useful adjunctive agent for the management of postoperative hyperalgesia and further studies are needed to investigate the optimal dose and to explain the mechanisms.

5. Conclusions

This prospective, double-blinded, and randomised study has confirmed that dezocine provides analgesic and antihyperalgesic effects for patients undergoing elective open gastrectomy. No adverse effects were observed for up to 48 postoperative hours.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Research Article

Decreased Cortisol and Pain in Breast Cancer: Biofield Therapy Potential

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Breast cancer is one of the leading causes of cancer death among women of all races. Pain is a common symptom associated with cancer; 75–90% of cancer patients experience pain during their illness and up to 50% of that pain is undertreated. Unrelieved pain leads to increased levels of the stress hormone cortisol. The purpose of this study was to examine the impact of bioenergy on fecal cortisol levels for mice injected with murine mammary carcinoma 4T1 in two separate pilot studies. Using a multiple experimental group design, six to eight week old female BALB/c mice were injected with tumor and randomly assigned, in groups of 10, to daily treatment, every other day treatment, and no treatment groups. Five days after tumor cell injection, bioenergy interventions were begun for a period of ten consecutive days. Fecal samples were collected for each study and ELISA analysis was conducted at the end of both studies. For both studies, cortisol levels were decreased in the every other day treatment groups but remained high in the no treatment groups. Future studies utilizing bioenergy therapies on cortisol levels in a murine breast cancer model can begin to describe pain outcomes and therapeutic dose.

1. Introduction

Breast cancer is the most common cancer diagnosis for women in the United States and is one of the leading causes of cancer death among women of all races. According to the Center for Disease Control and Prevention, 211,831 women in the United States were diagnosed with breast cancer in 2009 and 40,676 women in the United States died from that diagnosis [1]. Pain is a common symptom associated with cancer; 75–90% of cancer patients experience pain during their illness and up to 50% of that pain is undertreated [2]. Pain that continues or is unrelieved (up to 50%) significantly impacts the patient and his/her family, making the diagnosis of cancer and progression of the disease even more difficult [2]. Unrelieved pain has been linked to increased levels of stress, as measured by the stress hormone cortisol [3], and increased levels of anxiety. Opioids are recognized by the WHO as the first line of treatment for cancer pain. Opioid therapies are effective and are relied upon heavily for management of cancer pain, but these therapies are not without side effects such as constipation, urinary retention, nausea, sedation, respiratory depression, myoclonus, delirium, sexual dysfunction, and hyperalgesia [3].

Complementary and Alternative Medicine (CAM) therapies are increasingly used by practitioners and patients alike to manage pain and are without the side effects known to be associated with opioids. In 1998, surveys on the use of CAM therapies by cancer patients were reported as high as 64% and as low as 7% [4]. According to the 2014 report by the National Cancer Institute at the National Institutes of Health, more than 50% of cancer patients use some form of CAM therapy [5]. CAM therapies that are found to be effective for pain related symptoms without the opioid side effects include acupuncture, biofield (Reiki, healing touch), massage, cranial stimulation, music therapy, and foot baths [6]. Acupuncture, biofield therapies, massage, and cranial stimulation remove or lessen blockages in pathways or channels that can lead to disruptions or disturbances in the flow of energy throughout the body. Once those blockages are removed, balance is restored to enable the body's innate tendency for healing to occur. More recently, psychoeducational interventions, Chinese herbal medicine, compound kushen injection, reflexology,

lycopene, TENS, qigong, cupping, cannabis, homeopathy (Traumeel), and creative arts have also been found to have some positive impact on cancer pain [7–11]. These therapies impact the flow of energy as well and promote homeostasis, balance, and relaxation which is believed to impact pain and stress in a significant way. An area of special interest to the National Center for Complementary and Integrative Medicine (NCCIM) is alleviating pain and inflammation processes which makes continued research in this area of particular importance [7].

Women fear breast cancer more than any other disease and their levels of breast cancer-specific intrusions are related to their increased stress and perceived risk of breast cancer [12]. Cortisol is viewed as a physiological marker of stress [12, 13]. Chronic stress and cortisol deregulation can influence inflammation and immune function in ways that promote fatigue, depression, and risk of cancer recurrence [14, 15]. The stress of advancing cancer and management of it is associated with endocrine and immune dysfunction that has significant, negative consequences for host resistance to cancer progression [16].

At least a third of all patients who undergo treatment for cancer develop psychological morbidity, persisting throughout the disease continuum from suspicion to diagnosis, treatment, and beyond [17]. Others would say that psychological symptoms of distress are reported in as many as 41% of patients with a new diagnosis of breast cancer [18]. In an earlier study, 49.6% of women with early breast cancer were clinically anxious and 37.2% were clinically depressed in the first three months following surgery [19]. Later studies would show that 48% of women diagnosed with early breast cancer were clinically anxious and/or depressed in the first year [19].

The impact of anxiety for breast cancer patients has the potential to impact treatment response, decision making, and overall quality of life [17, 20, 21]. High levels of stress reactivity have recently been related to poor compliance with medical care and low quality of life scores for breast cancer survivors [22].

Stress-induced immunosuppression associated with diagnosis and treatment of breast cancer is well established [23]. High levels of stress in cancer patients have also been shown to negatively impact the immune system leading to elevations in proinflammatory cytokines and stress hormones. Studies have also demonstrated a relationship between stress and decreased immune measurements, even describing a relationship between the types of stress (acute versus chronic) involved and decreased function of immune systems [23–31]. Complementary alternative modalities, in particular bioenergy therapies, are showing promise in their abilities to mediate the impact of these dysfunctions.

Biofield modalities are putative in nature, meaning they are yet to be measured using Western empirical measures. Practices based on putative energy fields (also called biofields) generally reflect the concept that human beings are infused with subtle forms of energy. Practitioners are thought to modulate human biofields by identifying and then removing energy movement blockages. Some would argue that because this energy has never been demonstrated beyond a reasonable doubt, it lacks biological plausibility [32]. However patients continue to seek out these modalities and more sophisticated research methods are leading to a body of evidence that would support continued inquiry into the practice and results of bioenergy.

As reviewed above, stress and increased cortisol can negatively impact pain, immune function, and influence the formation and growth of cancers. Because stress hormones (like cortisol) are known to enhance tumor growth, angiogenesis, and invasion as well as impair cellular immune responses, a variety of cancer growth processes may be blunted and immunity supported by biofield therapies [17, 28, 32–37].

Published reviews regarding the utility of CAM therapies, to include bioenergy studies, have concluded that more research is needed to demonstrate, more specifically, the efficacy, meaning, and underlying mechanisms influenced by energy therapies and invited a more expansive view of what constitutes evidence. These reviews have recommended continued examination of specific biomarkers associated with stress and relaxation response systems to assist in determining the impact of biofield therapies on physiology and concluded that because of small sample size, high heterogeneity across studies, and high risk of bias for primary studies they could not recommend any CAM interventions for adult cancer pain. Murine models may be one mechanism that can begin to address some of these issues.

The current study explores the effects of biofield therapies on cortisol levels in mice injected with breast cancer cells. Aims of the study are as follows.

(1) To determine whether mice injected with breast cancer cells and then treated with bioenergy demonstrate decreased levels of cortisol.

(2) To determine if the results of the first study could be replicated with a different bioenergy practitioner.

2. Materials and Methods

2.1. Design. This study used a randomized, two-group repeated measures design. In two separate studies, two groups of interventional mice (daily healing touch and every other day healing touch) were compared to each other and to a group of untreated mice. Data were collected at two points: day 3 (before treatment) and day 12 (after treatment) (Figure 1).

2.2. Samples. For each study, thirty-six- to eight-week-old BALB/c mice (15–25 g) were obtained from Charles River Laboratories. The mice were housed in a ventilated barrier rack in a temperature controlled facility on a 12 h photoperiod. The mice were given food and water ad libitum. This research was conducted under a protocol approved by the Montana State University, Bozeman Institutional Animal Care and Use Committee (IACUC).

The 6-thioguanine-resistant 4T1 mouse mammary carcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD) and grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and

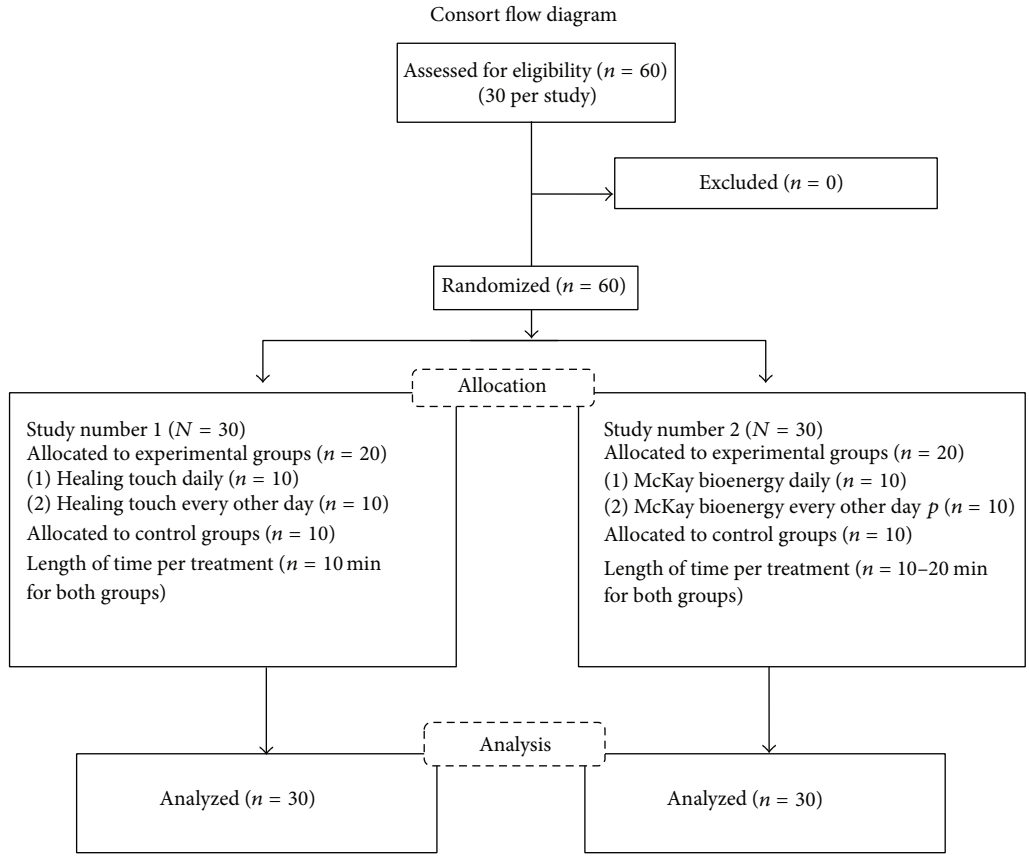

Figure 1: Consort Flow Diagram.

1% each of essential amino acids, L-glutamine, penicillin/streptomycin, and 10 mM HEPES. Cells were harvested for injection with 1X Trypsin/EDTA (Corning) to detach cells from the flask. Cells were then washed three times with Dulbecco's PBS and resuspended in DPBS for injection.

2.3. Procedure. Mice were randomly assigned to cages (five mice per cage, two cages per group, as per power analysis and literature review) when they arrived to the Animal Resource Center (ARC) and remained in those groups for the duration of the study. Five days after arrival, allowing time for acclamation to the ARC and their group, each mouse was injected with 100,000 cells of 4T1 murine mammary breast cancer tumor subcutaneously in 0.1 mL in the lower right mammary gland (day 1). Five days after injection (day 5), allowing time for tumor establishment, the intervention for each group began and continued for ten consecutive days.

Study one employed a certified healing touch practitioner. Group 1 received bioenergy treatment daily, group 2 received bioenergy treatment every other day, while group 3 received no treatment. The literature is not clear on duration of therapeutic treatment time. After consulting with an animal healing touch practitioner and reviewing the literature, it was decided to set a treatment time of 10 minutes per group.

Study two employed a bioenergy practitioner trained in The McKay method. Following the same procedure, group 1 received bioenergy treatment daily and group 2 received bioenergy treatment every other day, while group 3 received no treatment. For this study, treatment time lasted 10–20 minutes depending on the practitioner's daily assessment.

For each study, at the same time every day, the researcher would gown, enter the room where the mice were kept, glove, turn on the air exchange fan in the biosafety hood, move the appropriate cages from the rack, and place them under the hood. For each intervention group, the two cages (five mice in each cage) were placed side by side under the air exchange hood. Once the cages were placed under the hood, the plastic cover of the cage, along with the water which is kept inside the cage, was moved to the side of the hood leaving the metal slotted cover over each cage intact providing access to the mice for the practitioner. The researcher would then leave the room and the bioenergy practitioner would gown, enter the room, and prepare for the session (Figure 2).

Each healer prepared for the session by centering and aligning themselves, attuning to the mice and assessing their energy fields. The bioenergy practitioners then used a "hand scan" over the cages to determine levels of energy or auric field for each group. The practitioner would then "hold the field" to intensify energy to the mice and use "pain drain" to drain away irregularity from a specific or general area of the mouse bodies. "Hands in motion" would then be used to soothe and calm the field, and "hands still" was used to energize the spleen and adrenals. At no time did the practitioner come in physical contact with any of the experimental mice.

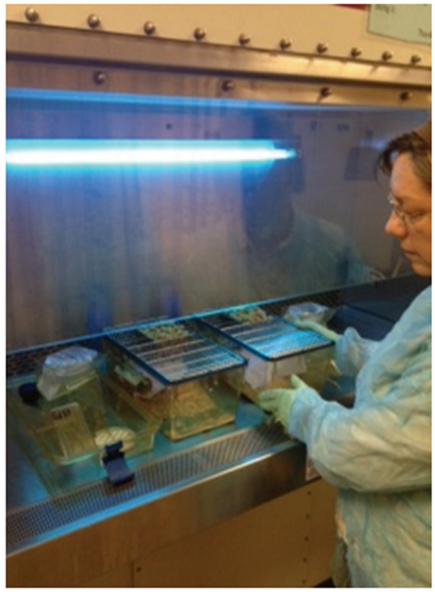

Figure 2: Practitioner setup for bioenergy intervention.

At the end of each treatment the practitioner would acknowledge the contribution of the mice, remove themselves from the mouse energy field, and leave the room. The researcher would return to the room, replace the water and plastic cover over each of the mice cages, and place the mice back in the same spot on the rack.

On day 3 and day 12 (three hours after receiving the bioenergy treatment) fecal samples were collected from each of the intervention and control mice. Stool samples were stored at −80 until analyzed. On day 15 of each study all of the mice were euthanized following IACUC protocol.

2.4. Extraction and Analysis of Fecal Cortisol. Fecal samples were weighed and then homogenized in 1 mL of 80% methanol. Samples were then shaken for 30 minutes on a multivortex. After shaking, samples were centrifuged for 10 min at 2500 g. An aliquot of each supernatant was then diluted (1 : 5) in assay buffer and analyzed by ELISA (Cortisol EIA kit, Enzo Life Sciences, Farmingdale, NY) as recommended by manufacturer.

2.5. Statistical Analyses. Statistical analyses were performed using Prism 4 (GraphPad Software, San Diego, CA). Fecal cortisol levels were compared by two-way ANOVA.

3. Results and Discussion

The goal of the first study was to determine if cortisol levels in mice with cancer could be influenced by healing touch. Fecal cortisol levels of pooled fecal matter from each cage of mice (2 cages/group) were examined at two time points. The first was two days after tumor injection and two days before the first treatment (day 3) and the second was near the end of the experiment, seven days after treatment began (day 12). At day 3, the fecal cortisol levels (measured as pg/mL/g) for the untreated ($\overline{x}$ = 167958.2 and SD = 87684.2), every other day treatment ($\overline{x}$ = 169258.9 and SD = 80096.4), and daily treatment ($\overline{x}$ = 134221.5 and SD = 96297.9) groups were similar (Figure 3(a)). However, at day 12, fecal cortisol levels appeared to be lower in the every other day ($\overline{x}$ = 55784.7 and SD = 13396.8) and daily ($\overline{x}$ = 70997.9 and SD = 31523.8) treatment groups compared to the untreated group ($\overline{x}$ = 154480.9 and SD = 9562.4). In addition, fecal cortisol levels appeared to be reduced between day 3 and day 12 in the every other day and daily treatment groups, but not the untreated group. The data was not statistically significant, likely due to the small number of samples per group, but these data provided preliminary evidence that cortisol levels in mice with cancer could be influenced by healing touch.

In study 2, we analyzed the cortisol levels of each mouse and enhanced our sample numbers (9 or 10 mice/group). We also wanted to determine if we could get similar results to the first study with a different practitioner. Before treatment, at day 3, fecal cortisol levels were higher in the every other day ($\overline{x}$ = 246525.3 and SD = 229323.6) and daily treatment ($\overline{x}$ = 151709.3 and SD = 118512.0) groups than the untreated group ($\overline{x}$ = 55104.3 and SD = 61825.2) (Figure 3(b)). This difference was statistically significant between the untreated and every other day treatment group. However, after treatment, on day 12, fecal cortisol levels were actually lower in the every other day ($\overline{x}$ = 134028.0 and SD = 86815.5) and daily treatment ($\overline{x}$ = 196557.1 and SD = 122664.4) groups compared to the untreated group ($\overline{x}$ = 307396.8 and SD = 255547.9). Again, this difference was statistically significant between the untreated and every other day treatment groups. Furthermore, fecal cortisol levels significantly increased in the untreated group between days 3 and 12, but not in the treated groups.

Bioenergy interventions were successful, in two separate pilot studies with two different practitioners, at reducing the levels of cortisol in female mice injected with mammary carcinoma 4T1 compared to untreated mice. These data are in agreement with what has been observed in clinical studies [11, 13–15, 20, 25–30]. While the first study results were not statistically significant, the direction of the relationship was compelling and the results from the second study further supported these preliminary findings.

In both studies, the mice receiving bioenergy treatments every other day had the lowest cortisol levels towards the end of the experiment. This suggests that there may be a dose effect to bioenergy interventions, but further studies would be needed to confirm this. Altogether, these data provide preliminary evidence that murine models may be a useful tool for exploration into the efficacy and mechanisms of action of bioenergy interventions on stress and possibly other physiological responses. Future studies that build on these findings are in line with NCCAMs call for the employment of animal models and methodology in basic and translational research to study the biological effects and mechanisms of action underlying CAM approaches [6].

Sample sizes for the two studies, though small, were typical for murine research and pilot studies. Cortisol levels are known to be variable, and in murine models there are no

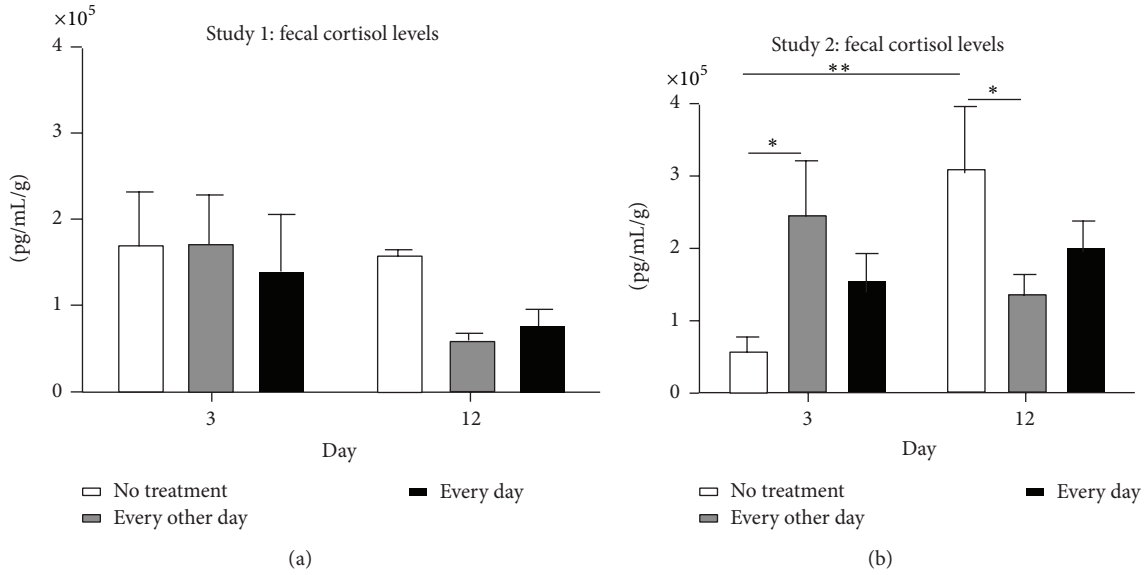

Figure 3: Fecal cortisol levels measured by ELISA. (a) Study 1 levels for untreated group ($n = 2$), every other day treatment group ($n = 2$), and daily treatment group ($n = 2$). (b) Study 2 levels for untreated group ($n = 9$), every other day treatment group ($n = 10$), and daily treatment group ($n = 10$). The data are expressed as mean +/− SEM. Statistical significance was measured by two-way ANOVA with Bonferroni posttest. $^{*}P < 0.05$, $^{**}P < 0.01$.

indicators available for normal cortisol levels or ranges, so normal values are not available for comparison.

4. Conclusions

Both of the aims developed for this study were accomplished. Through this research we were able to prove that mice injected with breast cancer cells and then treated with bioenergy demonstrated decreased levels of cortisol. Though the statistical significance for the first study was not as strong as the second, in both studies the cortisol levels decreased. These results would support earlier studies reporting the effectiveness of bioenergy on cortisol levels, stress, and immune function.

In regard to the second aim, a second bioenergy practitioner was able to achieve more significant results. As described in the methodology section, each practitioner employed the same techniques, but in the second study the practitioner was able to extend the time of the intervention. Because of the need for information on therapeutic dose and outcomes, the results of this study can be used in future protocol development.

The provision of complementary alternative modalities for women with breast cancer must include evidence either supporting or negating its impact. Incorporating a CAM modality such as bioenergy, with evidence from rigorous controlled studies, could have far reaching practice implications. Continued research into dose, length of treatment, and other variables which could impact efficacy as well as mechanisms of action is needed to optimize its use in the relief of pain and the provision of care for cancer patient. These studies can be difficult to perform in clinical settings. The results presented here, while limited in scope, provide evidence that larger scale studies in murine models could be used to address some of these questions and further examine the impact of bioenergy interventions in disease models. This knowledge could then potentially be translated into clinical studies to provide further evidence for practice. Because cancer pain will be experienced by as many as 90% of patients and because there are no known parameters for therapeutic dose for many CAM therapies, scientists and practitioners must work together developing and testing safe interventions to address this statistic. These pilot studies are a first step in that process.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Research Article

Involvement of Cholinergic and Opioid System in γ-Terpinene-Mediated Antinociception

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The literature shows that the monoterpenes are great candidates for the development of new drugs for the treatment of various pathological processes, including painful conditions. The gamma terpinene (γ-TPN) is a monoterpene present in plant species that have multiple pharmacological properties and has structural similarity to antinociceptive monoterpenes, such as limonene and alpha-phellandrene. The γ-TPN molecular mass was evaluated by mass spectrometry and showed a pseudomolecular ion with m/z 137.0 Da. The animals did not present any signs of acute toxicity at 2 g/kg, p.o. γ-TPN (1.562 to 50 mg/kg, p.o.) showed an antinociceptive effect in the formalin, capsaicin, and glutamate tests. γ-TPN has antinociceptive action when administered by others routes in glutamate test. To eliminate a possible sedative effect of γ-TPN, the open field and rota-rod test were conducted and the γ-TPN did not show muscle relaxant activity or central depressant effect. To investigate the mechanisms of action, the animals were pretreated with naloxone, glibenclamide, atropine, mecamylamine, or L-arginine in the glutamate test. γ-TPN antinociception was inhibited in the presence of naloxone, glibenclamide, atropine, and mecamylamine. The results suggest that the γ-TPN (p.o.) produced antinociceptive effect in models of chemical nociception through the cholinergic and opioid systems involvement.

1. Introduction

The essential oils are volatile, natural, complex, and characterized by the presence of a strong odour and are formed by aromatic plants as secondary metabolites. Their pharmacological properties include virucidal, fungicidal, analgesic, anti-inflammatory, and antispasmodic [1, 2]. In turn, the monoterpenes represent a group of naturally occurring organic compounds named "terpenes," whose basic structure consists of two linked isoprene units, which are formed by 5-carbon-base (C5) each. Moreover, monoterpenes are the most representative molecules constituting about 90% of essential oils content and have a great variety of structures [1].

For more than two decades, many researchers have studied the analgesic potential of monoterpenes through of the in vivo and in vitro assays [3]. Studies of proposed analgesic-like activity mechanisms have been conducted with some monoterpenes, acting on several receptors, including opioids, adenosine A1 and A2, or cholinergic M2, producing changes in K+ channels, inhibition of peripheral mediators, and nitric oxide synthesis modulation, among others mechanisms [4–11]. The great diversity of mechanisms that may be associated with the analgesic effect of these monoterpenes is amazing, and several monoterpenes presented more than one mechanism of action that can be related to this effect [3].

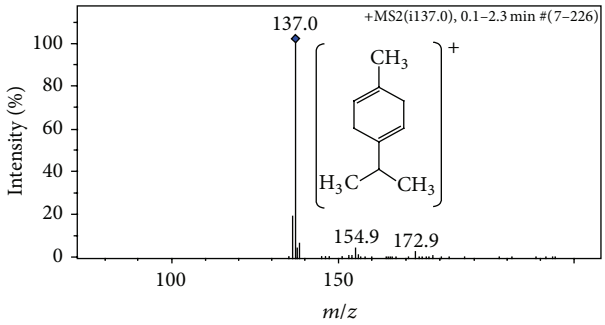

Figure 1: ESI+ MS/MS spectra of γ-terpinene (1-methyl-4-isopropyl cyclohexadiene-1,4) confirm the m/z ratio characteristic, $[M + H]^+$ = 137.01 Da.

The γ-terpinene (γ-TPN) (1-methyl-4-isopropyl cyclohexadiene-1,4) (Figure 1) is a monoterpene present in several plant species pharmacologically active, for example, in the essential oils from *Protium icicariba* (DC.) Marchand [12], *Citrus deliciosa* Tenore [13], and *Origanum onites* L. [14], among others. The presence of unsaturation in its cyclic chain structure confers the olefin characteristic for the γ-TPN, which allows easier absorption through biological membranes, due to the lipossolubility [15]. In acute toxicity tests, γ-TPN showed LD_{50} in rats of 3.65 g/kg and the acute dermal LD_{50} in rabbits exceeded 5 g/kg [16].

Then, considering the structural similarity with other monoterpenes with antinociceptive activity, such as limonene [17] and α-phellandrene [18], as well as no evidences of antinociceptive activity of γ-TPN have been reported, the aim of the present work was to investigate the antinociceptive potential of γ-TPN in experimental nociception models in mice, as well as the possible contribution of cholinergic (i.e., muscarinic and nicotinic acetylcholine receptors) and opioidergic (i.e., opioid receptors) systems, K^+_{ATP} channels, and the L-arginine-nitric oxide pathway to the pharmacological activity.

2. Materials and Methods

2.1. The Monoterpene γ-TPN. The molecular mass was confirmed by mass spectrometry in positive electrospray ionization mode (AmaZon SL, Bruker Daltonics, Bremen, Germany), under the following conditions: capillary voltage of 2,000 V; temperature of 250°C; 12 psi of pressure to nebulizer; and 10 L/min of flux to dry gas. The mass spectra were acquired in mass range of m/z at 70–500 Da. The 137 Da ions were selected within an isolation width of 2 u and scans were accumulated with variable RF signal amplitudes. The m/z scale of the mass spectrum was calibrated using the external calibration standard G2421A electrospray "tuning mix" (Agilent Technologies, Santa Rosa, USA).

2.2. Animals. In the acute toxicity evaluation, female Wistar rats (180–200 g, $n = 6$ per group) were used. The acute pain tests were carried out on male Swiss mice (20–30 g, $n = 6$–9), reared at the Medicinal Plants Research Center of the Federal University of Piauí. The animals were housed at 22 ± 1°C under a 12 h light-dark cycle with free access to food and water. Animals were acclimatized at least 1 h before testing and were used only once throughout the experiments. The protocols were approved by the Institutional Ethics Committee (Ethics Committee on Animal Experimentation/UFPI, n°. 008/12) and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigation of experimental pain in conscious animals [19].

2.3. Drugs and Chemicals. The following substances were used: γ-TPN, glutamic acid, glibenclamide, mecamylamine, nicotine, atropine, pilocarpine, MK 801, capsaicin, Nω-nitro-L-arginine (L-NOARG), L-arginine (all purchased from Sigma-Aldrich, USA), morphine, naloxone hydrochloride, and diazepam (purchased from Cristália, SP, Brazil). Doses of antagonists were based on previous studies from our group. For the pharmacological studies, the γ-TPN was suspended in 2% Tween 80 in 0.9% NaCl (10 mL/kg). The doses were reported as milligrams of γ-TPN per body weight (mg/kg). The doses range was 1.56 to 50 mg/kg. The results of lower or upper doses for some protocols were not shown, due to similarity in pharmacological effects between the previous and next doses. Capsaicin was prepared in 2% Tween 80 solution in 2% ethanol. The γ-TPN was administered orally (p.o.), intrathecally (i.t.), intracerebroventricularly (i.c.v.), and intraplantarly (i.pl.) at different doses in order to construct the dose-response curves.

2.4. Determination of Acute Toxicity in Rats. The toxicological evaluation of γ-TPN was performed by the fixed dose procedure, recommended by the Organization for Economic Cooperation and Development (OECD) number 420 [20]. The animals were divided into 2 groups of 5 animals each; one test group was orally treated with γ-TPN at a dose of 2 g/kg, and a control group orally treated with 2% Tween 80 in 0.9% NaCl solution. Immediately after administration, the animals were evaluated clinically and behaviorally with greater attention during the first 4 hours after administration, as recommended by the protocol of recognition and evaluation of clinical signs of OECD [21]. Then, the evaluations were performed daily, and the weights of the animals were obtained during 14 days. After toxicological investigation, the animals were euthanized, the relative weights of internal organs were determined, and the serum biochemical and hematological parameters were performed, as well as the quantification of reduced glutathione and catalase activity.

2.5. Antinociceptive Effect of γ-TPN

2.5.1. Effect of γ-TPN in Formalin Test. Mice were orally treated with γ-TPN (6.25, 12.5 and 25 mg/kg) or vehicle 1 h before the test. Morphine (5 mg/kg) was subcutaneously administered 30 min before the test as a positive control. The right hind paw was injected with formalin (20 μL, 2%) in the intraplantar region. Nociception was evaluated by quantifying paw licking time during the first 5 min (first phase) and at 15–30 min (second phase) [22].

2.5.2. Effect of γ-TPN in Capsaicin Test. Mice were treated with γ-TPN (12.5, 25 and 50 mg/kg, p.o.), vehicle, or morphine

Table 1: Antinociceptive effect of the γ-terpinene in the formalin-induced nociceptive response in mice.

Treatment	Dosage (mg/kg)	Licking time (s)			
		0–5 min	Inhibition (%)	15–30 min	Inhibition (%)
Vehicle	—	76.35 ± 10.21	—	70.68 ± 8.18	—
γ-TPN	6.25	50.48 ± 2.58	33.89	84.79 ± 12.78	–19.96
	12.5	5.96 ± 3.46***	92.20	24.13 ± 5.60**	65.87
	25	13.74 ± 4.08***	82.01	21.18 ± 6.72***	70.04
Morphine	5	18.19 ± 3.10***	76.18	17.32 ± 5.46***	75.50

Mice were treated with γ-terpinene (γ-TPN) 60 min (p.o.) before formalin test. Data represent the mean ± SEM of 6–9 mice. Statistical analysis was determined by one-way ANOVA followed by Tukey's test. $^{**}P < 0.01$; $^{***}P < 0.001$ compared with vehicle.

(5 mg/kg, s.c.). One hour (p.o.) and 30 min (s.c.) after these treatments, the right hind paw was injected with capsaicin (2 μg/20 μL/paw). Nociception was assessed immediately after injection and quantified by paw licking time during a 5-min period [23].

2.5.3. Effect of γ-TPN in Glutamate Test. The procedure used was similar to a previously described method [24]. Mice received an injection of glutamate by intraplantar route (20 μmol/paw) after a previous treatment with γ-TPN by oral (1.56, 3.125, or 6.25 mg/kg, 60 min beforehand), intrathecal (i.t., 5–20 μg/site, 15 min beforehand), intracerebroventricular (i.c.v., 5–20 μg/site, 15 min beforehand), or intraplantar (10 and 20 μg/paw, coadministered with glutamate) routes. Control animals were treated with vehicle by oral, spinal (i.t., 5 μL/site), supraspinal (i.c.v., 1 μL/site), or intraplantar (20 μg/paw, coadministered with glutamate) routes before glutamate injection. MK801 (0.03 mg/kg, i.p.) was used as positive control and administered 30 min before glutamate.

2.6. Investigation of Mechanisms of γ-TPN-Induced Antinociceptive Action. In order to elucidate the mechanisms underlying γ-TPN-induced antinociception, mice were pretreated intraperitoneally in the glutamate model ($n = 6$–9) with naloxone (2 mg/kg), a nonselective antagonist of opioid receptor; glibenclamide (3 mg/kg), an antagonist of K^+_{ATP} channels; atropine (1 mg/kg), an antagonist of muscarinic receptors; mecamylamine (2 mg/kg), an antagonist of nicotinic receptors; and L-arginine (600 mg/kg), a substrate for NO biosynthesis. The doses of these drugs were selected in according to literature data and previous results from our laboratory [25–27].

2.7. Measurement of Motor Performance

2.7.1. Open Field Test. The apparatus for the open field test consists of an acrylic box with transparent walls (30 cm × 30 cm × 15 cm) and a black floor divided into nine squares of equal area. One day before the experiment, mice were placed in the box for adaptation. The mice were treated with γ-TPN (12.5 and 25 mg/kg, p.o.), vehicle (p.o.), or diazepam (4 mg/kg, i.p.) 30 min and 1 h before individual observation, and the number of crossings (crossed squares with all paws) was counted during a 5 min session [28].

2.7.2. Rota Rod Test. The rota-rod (Model RR-2002, Insight equipment) consisted of a 2.5 cm diameter bar, subdivided into four compartments by 25 cm diameter disks, rotating at 14 revolutions per minute. Mice were submitted to a trial 24 h before experiment, in order to eliminate those animals that did not remain on the bar for three consecutive periods of 60 s. The mice were treated with γ-TPN (12.5 and 25 mg/kg, p.o.), vehicle (p.o.), or diazepam (4 mg/kg, i.p.) 0.5 and 1 h before the experiment. Results are expressed as the time (s) that mice remained on the rota-rod, and the cut-off time used was 60 s [29].

2.8. Statistical Analysis. The results were expressed as the mean ± SEM and analyzed by one-way analysis of variance, followed by Tukey's or Bonferroni's post hoc tests. Significative differences among groups were considered when $P < 0.05$ (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com/).

3. Results

3.1. Mass Spectrometry. The MS analysis of γ-TPN revealed a pseudomolecular ion signal with m/z 137.0 Da $[M + H]^+$ (Figure 1), in acordance to its molecular weight of 136.234 Da, confirming the identity and purity of γ-TPN.

3.2. Evaluation of Acute Toxicity in Rats. The γ-TPN at 2 g/kg (p.o.) did not demonstrate any sign of evident toxicity during the 14 days of observation as well as any death was observed. Therefore, the LD_{50} of γ-TPN was not possible to be determined. Furthermore, no behavioral and clinical alterations after administration of γ-TPN were observed. The γ-TPN did not alter the internal organs relative weights, the biochemical parameters, and the catalase and reduced glutathione, when compared to vehicle (data not shown).

3.3. Antinociceptive Effect of γ-TPN

3.3.1. Effect of γ-TPN in Formalin Test. As shown in Table 1, γ-TPN at 12.5 and 25 mg/kg (p.o.) significantly reduced the licking time of the stimulated paw in both phases of the test when compared with vehicle ($^{**}P < 0.01$; $^{***}P < 0.001$), while the dose of 6.25 mg/kg (p.o.) did not do this.

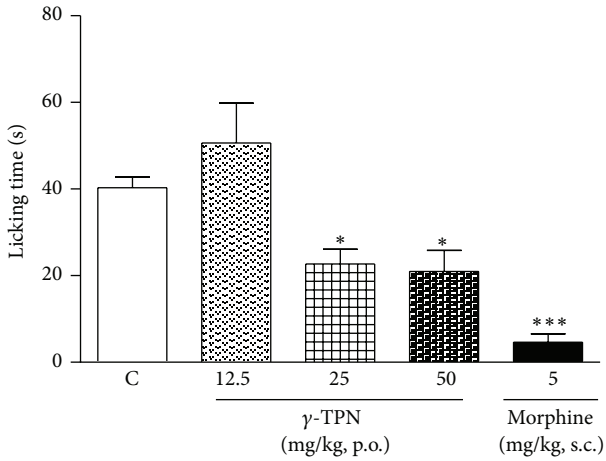

Figure 2: Effect of the γ-TPN on capsaicin-induced nociception in mice. Animals were treated with γ-TPN 60 min (p.o.) before capsaicin test. Data represent the mean ± SEM of 6–9 mice. $^{***}P < 0.001$, $^{*}P < 0.05$ compared with vehicle.

3.3.2. Effect of γ-TPN in Capsaicin Test. The effect of γ-TPN against capsaicin-induced nociception in mice is shown in Figure 2. A significant reduction in time length spent on licking the paw was observed in mice administered with γ-TPN (25 and 50 mg/kg p.o.) compared with vehicle ($^{*}P < 0.05$), indicating an antinociceptive effect in neurogenic pain. Morphine (5 mg/kg s.c.) was used as positive control and showed a decrease of the response when compared with vehicle ($^{***}P < 0.001$).

3.3.3. Effect of γ-TPN in Glutamate Test. The results in Figures 3(a), 3(b), 3(c), and 3(d) show that γ-TPN given either systemically (p.o.) (1.56, 3.125, and 6.25 mg/kg) ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$) or centrally (i.t or i.c.v) (10 and 20 μg/site) ($^{**}P < 0.01$, $^{*}P < 0.05$) caused significant inhibition of glutamate-induced nociception when compared to vehicle, while intraplantar treatment with γ-TPN (20 μg/site) partially inhibited glutamate-induced nociception ($^{*}P < 0.05$).

3.4. Analysis of Possible Antinociceptive Mechanisms of γ-TPN. As shown in Figure 4(a), naloxone significantly inhibited the antinociceptive effect of γ-TPN, as well as glibenclamide (Figure 4(b)). Figures 5(a) and 5(b) demonstrate the involvement of the cholinergic system. In Figure 5(a), atropine significantly inhibited the antinociceptive effect of γ-TPN, as well as mecamylamine (Figure 5(b)). However, pretreatment with L-arginine did not alter this effect (data not shown).

3.5. Measurement of Motor Performance

3.5.1. Open Field and Rota Rod Test. In order to evaluate any nonspecific muscle-relaxant or sedative effects of γ-TPN, mice were submitted to the open-field and rota-rod test. In the test of open field, the γ-TPN (12.5 and 25 mg/kg, p.o.) did not alter the frequency of animals movement or the length of time the animals stayed in the bar in rota-rod test for 1 min when compared with vehicle (data not shown).

4. Discussion

Several studies have reported monoterpenes with a wide sort of biological properties, such as anti-inflammatory, anxiolytic, anticonvulsant, and antinociceptive [30–33]. Interestingly, this study demonstrates for the first time that the monoterpene γ-TPN promotes antinociceptive activity at low-range doses in different models of chemical nociception after oral administration, as well as spinal, supraspinal, or intraplantar in the glutamate-induced nociception model in mice.

For accuracy of pharmacological tests, the identification of the molecular mass of investigated compounds is strongly important due to surely confirming their chemical identities and the purity of the analyzed samples. Therefore, mass spectrometry analysis was performed by direct infusion in ion trap ESI positive mode. A pseudomolecular ion with m/z 137.0 Da $[M + H]^{+}$ then confirms the identity and purity of γ-TPN samples [34].

The γ-TPN did not show any sign of acute oral toxicity in rats at the dose of 2 g/kg. Therefore, the 50% lethal dose (LD_{50}) could not be determined. Accordingly, a previous work reported for γ-TPN low oral toxicity in rats, with LD_{50} of 3.65 g/kg [16]. The monitoring of the body weight is an important indicator for assessing the toxicity of a substance [35]. In turn, no significant changes in body weights were observed during 14 days after acute oral administration of γ-TPN. Furthermore, no changes in the relative weight of internal organs, as well as in serum biochemical parameters (ALT, AST, creatinine, and serum urea) and hepatic oxidative damage, were observed after treatment with γ-TPN, compared with vehicle. ALT, specific to hepatocytes, and AST, found in liver, cardiac muscle, and kidney, both are well-known as markers of cell damage, especially hepatocyte necrosis [36, 37]. Moreover, serum urea and creatinine are the most commonly used clinical markers of renal injury in routine toxicity studies [38]. Therefore, these data allowed a safe choice of the used doses for acute experimental protocols.

The γ-TPN was orally effective in inhibiting both phases of the formalin-induced nociception, which indicates the likely involvement of different mediators. The formalin-induced nociception test is commonly employed as a model of acute tonic pain, characterized by the presence of a distinct biphasic nociceptive response. The first phase corresponds to neurogenic pain by direct activation of the transient receptor, potentially A1 cation channels located at the sensory C-fibers, thus reflecting a centrally mediated pain [39]. The second phase of nociceptive response, also known as inflammatory pain, is mediated by a combination of peripheral input from inflammatory mediators released from injured tissues, causing the sensitization of central nociceptive neurones [40]. Previous reports point out the substance P and bradykinin participate in the appearance of the first-phase responses, while histamine, serotonin, prostagladin, and bradykinin are involved in the second-phase responses [40, 41]. Moreover, it is well established that drugs that act primarily on

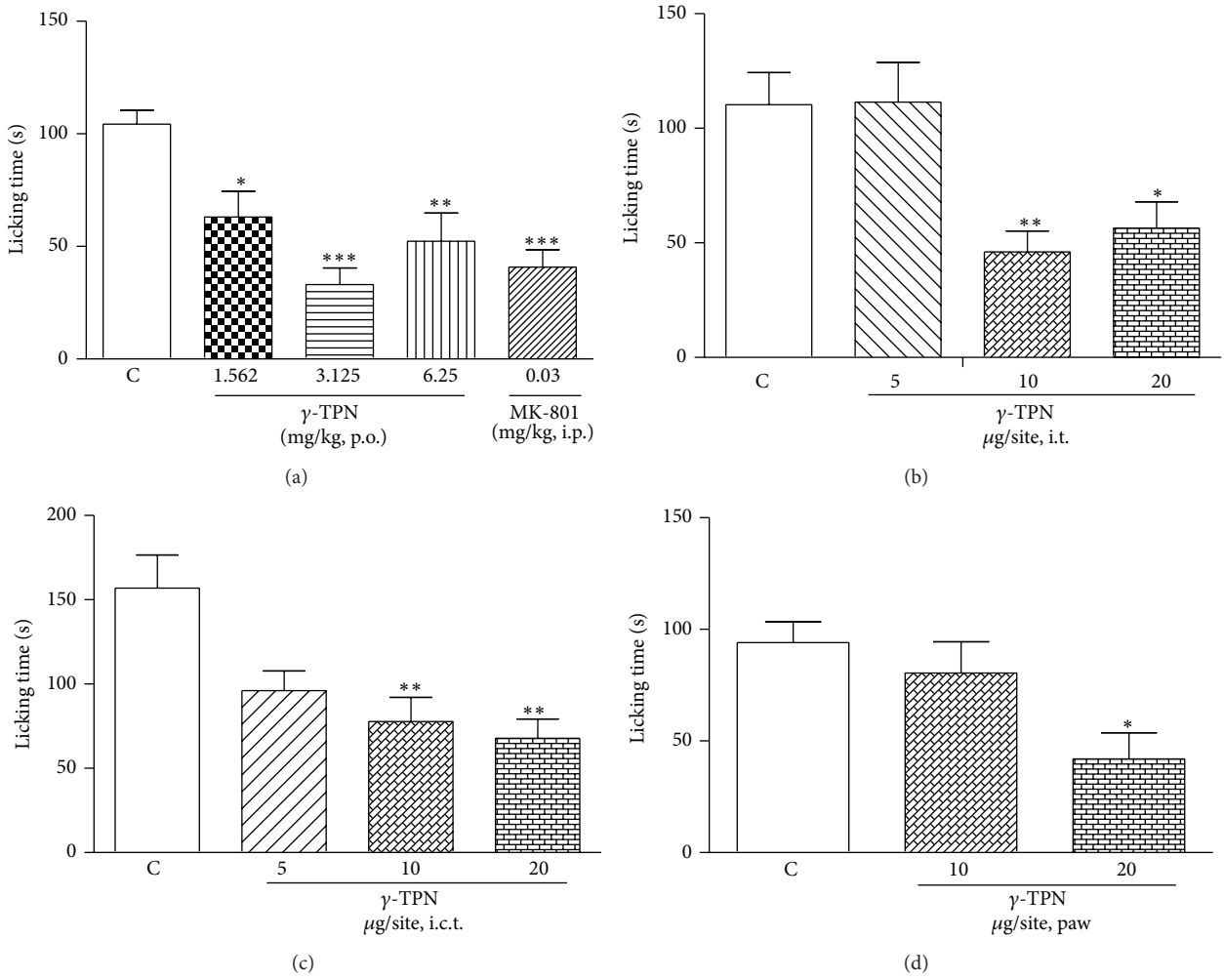

Figure 3: Effect of the γ-TPN administered orally (a), intrathecally (b), intracerebroventricularly (c), or intraplantarly (d) against licking induced by intraplantar injection of glutamate (20 μmol/paw) in mice. Each column represents the mean of 6–8 animals and the error bars indicate the S.E.M. Control values (c) indicate the animals injected with saline and the asterisks denote the significance levels $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ when compared with control group values (one-way ANOVA followed by Tukey's test).

the central nervous system inhibit both phases equally, while peripherally acting drugs inhibit the second phase [40, 42]. Thus, γ-TPN could be acting by inhibition of direct and indirect acting inflammatory mediators and possibly affecting also the neurotransmission pathways at the SNC level (such as substance P or CGRP). Other plant essential oil-derived substances, such as bisabolol [43] and carvacrol [44], also had similar antinociceptive effects.

The present study also revealed that γ-TPN was effective in capsaicin-induced nociception model. Capsaicin is an alkaloid extracted from red pepper capsicum, which stimulates nociceptive and thermal nerve endings causing intense pain. Capsaicin acts specifically in unmyelinated type C fibers and poorly in myelinated and thin Aδ fibers, at the vanilloid receptor (TRPV-1) in the peripheral nervous system, by opening a nonselective cation channel, mainly Ca^{2+} and Na^{+}, and causing depolarization and initiation of action potentials. Capsaicin determines the release of neuropeptides, especially tachykinis (substance P, neurokinin B), which operate on transmission of pain sensation in nociceptive pathways and inflammatory processes [45]. Accordingly, the effect of the γ-TPN on the neurogenic phase of the formalin-evoked response was supported by the data obtained in the capsaicin test. Monoterpenes, such as carvacrol and citronellal, promote antinociceptive activity in the capsaicin test [10, 46].

Another interesting finding of this study is that γ-TPN, when administered by oral, intrathecal, intracerebroventricular, or intraplantar routes, protects the mice from glutamate-induced nociception in a significant manner, promoting not only peripheral, but also central action in this model. Glutamate is a major excitatory neurotransmitter which transmits nociceptive signals by promoting the direct activation of receptors in nociceptive fibers. Once activated, these neurons release several inflammatory mediators and neuropeptides that are involved in nociceptive transmission in the central and peripheral nervous system [47, 48]. Glutamate exerts its effects through both ionotropic and metabotropic glutamate receptors [49, 50]. Studies with the monoterpene linalool

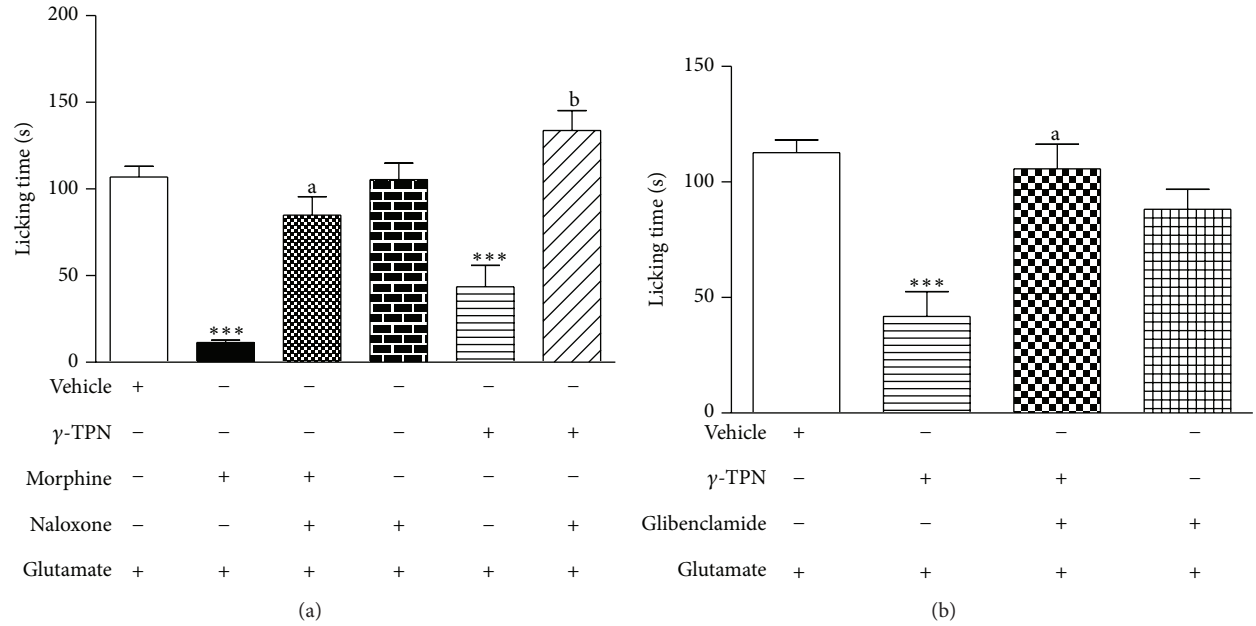

Figure 4: Effect of the γ-TPN (3.125 mg/kg, p.o.) against the action of naloxone (2 mg/kg, i.p.) (a) and glibenclamide (3 mg/kg, i.p.) (b) on glutamate-induced nociception (20 μL, 20 μmol/paw) in mice. Data represent mean ± SEM of 6–9 mice. Figure 4(a), the symbols indicate the level of significance: $^{**}P < 0.001$ compared with vehicle, $^{a}P < 0.001$ compared with the morphine group, $^{b}P < 0.001$ compared with γ-TPN group. Figure 4(b), $^{***}P < 0.001$ compared with vehicle, $^{a}P < 0.01$ compared with γ-TPN group; + present treatment; − missing treatment (one-way analysis of variance, Bonferroni's test).

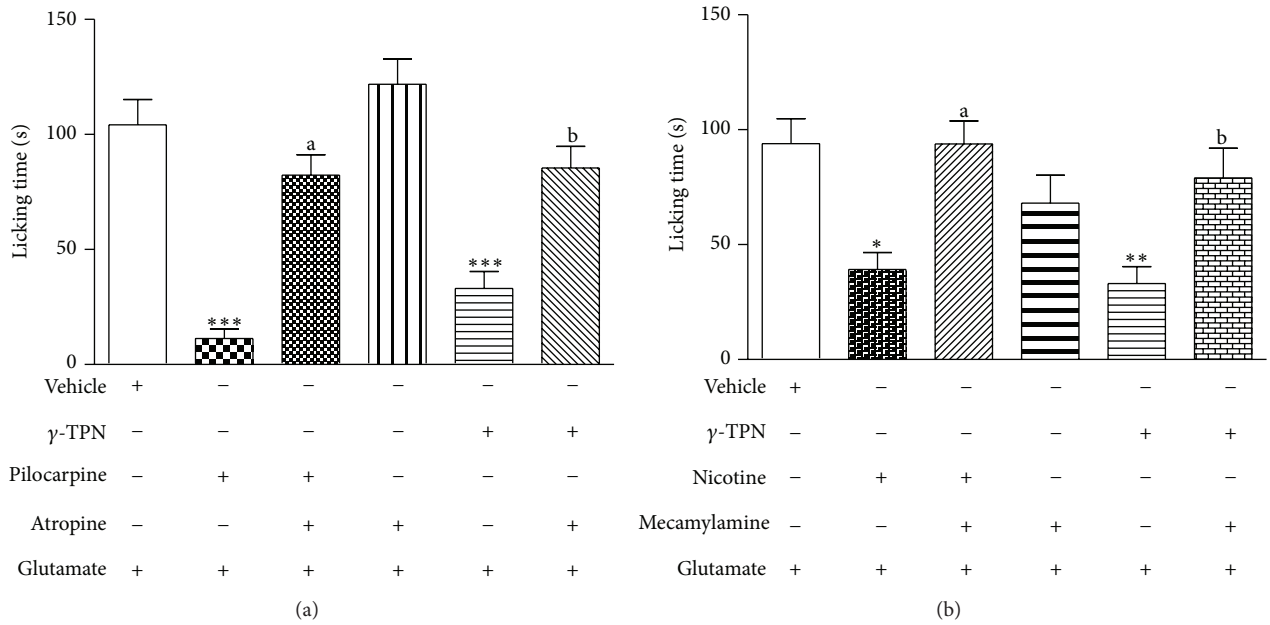

Figure 5: Effect of the γ-TPN (3.125 mg/kg, p.o.) against the action of atropine (1 mg/kg, i.p.) (a) and mecamylamine (2 mg/kg, i.p.) (b) on glutamate-induced nociception (20 μL, 20 μmol/paw) in mice. Data represent mean ± SEM of 6–9 mice. In Figure 5(a), the symbols indicate the level of significance: $^{***}P < 0.001$ compared with vehicle, $^{a}P < 0.001$ compared with the pilocarpine group, and $^{b}P < 0.001$ compared with γ-TPN group. In Figure 5(b), $^{***}P < 0.001$ compared with vehicle, $^{a}P < 0.001$ compared with the nicotine group, $^{b}P < 0.01$ compared with γ-TPN group; + presents treatment; − presents missing treatment (one-way analysis of variance, Bonferroni's test).

showed antinociceptive activity by the same routes [33]. Considering the structural similarity of these two monoterpenes, other studies corroborate our results showing that the linalool can also acting on the glutamatergic neurotransmission, since the NMDA receptor antagonism can cause supraspinal analgesia mediated by central opioid receptors stimulation [7, 51].

Therefore, in order to elucidate the possible antinociceptive mechanisms of γ-TPN, animals were pretreated with several drugs that interfere with different systems and evaluated on glutamate-induced nociception model.

The mechanism of action for γ-TPN, at least in part, seems to be from a direct action on the opioid receptors, since pretreatment of animals with naloxone inhibited the antinociceptive activity. The γ-TPN antinociceptive effect was also antagonized by pretreatment with glibenclamide, suggesting the involving of the opioid system via K^+_{ATP} channels in γ-TPN antinociception. The opioid system is an important inhibitory system in nociception, which acts through two main pathways, central and peripheral. In central pathways opioid agonists act on the periaqueductal gray matter, rostroventromedial bulb, and dorsal horn of the spinal cord, activating the pain control descending pathways, in part, by activating potassium channels and inhibit voltage-dependent calcium channels [49].

Our results clearly indicate the involvement of cholinergic receptors (muscarinic and nicotinic) in this process, since atropine (nonselective muscarinic antagonist) and mecamylamine (a $\alpha 2\beta 3$ selective preferential nicotinic receptor antagonist) inhibited the antinociceptive effect of γ-TPN. A major site of action for cholinomimetics in analgesia is the spinal cord. Painful stimuli are known to increase acetylcholine in the spinal cord. The activation of muscarinic receptors in the spinal cord results in the increased release of inhibitory transmitters and decreased release of excitatory transmitters, and this activation, in part, mediates the antinociceptive effect [52]. In addition, the results of this study provide strong evidence supporting the involvement of nicotinic receptors in the γ-TPN antinociception, since mecamylamine at a dose similar to that known to prevent antinociception induced by the selective agonist of the $\alpha 2\beta 3$ nicotinic receptor, consistently attenuated both nicotine- (nicotinic receptor nonselective agonist) and the γ-TPN-induced antinociception in the glutamate test [53, 54].

Finally, another worthy finding of the present study was the demonstration that γ-TPN was largely devoid of significant effects on the motor performance of mice in the open field and rota-rod tests, thereby eliminating a nonspecific muscle relaxation and sedative effects in γ-TPN-induced antinociception.

5. Conclusions

The results presented here provided, for the first time, convincing evidence that oral administration of the monoterpene γ-TPN exerted pronounced antinociception when assessed in chemically induced nociception models in mice. In addition, γ-TPN has antinociceptive effect when administered into central (intracerebroventricular and intrathecal) and peripheral pathways, and this action occurs possibly with the involvement of the opioid system via K^+_{ATP} channels and cholinergic system. So, this monoterpene seems to be a promising molecule as a future analgesic drug, taking into consideration the combination of efficacy and safety.

Conflict of Interests

The authors declare that they have no conflict of interests to disclose.

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Research Article

Laser Acupuncture for Postoperative Pain Management in Cats

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The aim of this study was to evaluate laser acupuncture as an adjuvant for postoperative pain management in cats. Twenty cats, undergoing ovariohysterectomy, were sedated with intramuscular (IM) ketamine (5 mg kg^{-1}), midazolam (0.5 mg kg^{-1}), and tramadol (2 mg kg^{-1}). Prior to induction of anaesthesia, the subjects were randomly distributed into two groups of 10 cats: Laser: bilateral stomach 36 and spleen 6 acupoints were stimulated with infrared laser; Control: no acupuncture was applied. Anaesthesia was induced using intravenous propofol (4 mg kg^{-1}) and maintained with isoflurane. Postoperative analgesia was evaluated by a blinded assessor for 24 h following extubation using the Dynamic Interactive Visual Analogue Scale and Multidimensional Composite Pain Scale. Rescue analgesia was provided with IM tramadol (2 mg kg^{-1}), and the pain scores were reassessed 30 min after the rescue intervention. If the analgesia remained insufficient, meloxicam (0.2 mg kg^{-1} IM, single dose) was administered. Data were analyzed using *t*-tests, the Mann-Whitney test, and the Friedman test ($P < 0.05$). The pain scores did not differ between groups. However, postoperative supplemental analgesia was required by significantly more cats in the Control (5/10) compared with the Laser group (1/10) ($P = 0.038$). Laser acupuncture reduced postoperative analgesic requirements in cats undergoing ovariohysterectomy.

1. Introduction

In recent decades, several studies have investigated the use of acupuncture for analgesic purposes and reported its effectiveness for the relief of both acute and chronic pain [1–5].

Traditionally, acupuncture is based on the philosophy of energy balance, so that any alteration, block, or stagnation in the flow of energy circulating through the body can promote the development of disease or pain [6]. From the point of view of traditional oriental medicine, pain is due to the stagnation of energy (Qi) and/or blood (Xue) flow along the meridians. Thus, the energy can be renewed and the body rebalanced by inserting needles at specific points, called acupoints [7, 8].

In addition to needles, stimulation of the acupuncture points can also be triggered through electrical stimulation [1–3], radiation (infrared laser) [5], and heat (moxibustion) [7].

Low intensity lasers can be used directly on the acupuncture points for treatment of pain [5, 9]. The main advantages of this technique over traditional acupuncture are shorter session length and the absence of discomfort and risk of infection, due to the noninvasive technique [10]. In rats subjected to experimental models of inflammation and pain, manual stimulation (needling) was as effective as laser stimulation at the same acupoint, resulting in an increased nociceptive threshold and reduced inflammation [11].

In human medicine, this therapeutic approach is common in paediatrics and in patients with needle phobias [10, 12]. In veterinary medicine, this technique may represent a viable alternative for those patients where introduction of a needle may be hindered by the behavior of the animal. In this context, cats represent a suitable population for the use of this mode of acupuncture, due to their quick temper and greater vulnerability to stress and irritability which can be triggered by needling acupoints [13].

Thus, the objective of this study was to evaluate the application of an infrared laser to acupuncture points as

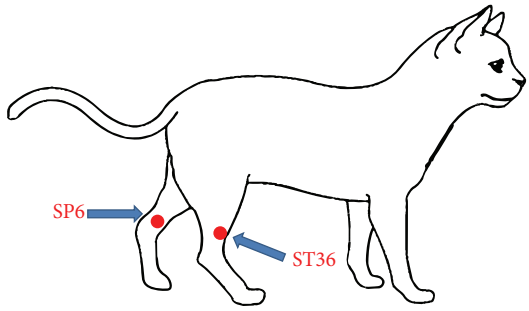

Figure 1: Location of ST36 and SP6 acupoints.

an adjuvant for postoperative pain management in cats undergoing ovariohysterectomy (OH).

2. Methods

This study was performed following the guidelines of the Brazilian College of Animal Experimentation, and the experimental procedure was approved by the Institutional Animal Care Committee (protocol 1975, CEEA). Informed consent was obtained from all of the cats' owners.

2.1. Animals. Twenty crossbreed cats, aged 6 to 36 months (27.3 ± 0.6 months) and weighing between 2.0 and 3.6 kg (median, 2.7 kg) undergoing OH, were enrolled. The cats were selected for this study after a physical examination and laboratory tests (i.e., complete blood cell count and measurements of urea, creatinine, alanine aminotransferase, and aspartate aminotransferase levels). The exclusion criteria were animals with alterations in blood count and/or liver and kidney functions.

2.2. Experimental Groups. Prior to induction of general anaesthesia, the subjects were randomly distributed into two groups of 10 cats: Control ($n = 10$): no stimulation with acupuncture was performed; Laser ($n = 10$): there was application with an infrared laser (Gallium arsenate, AsGA, Laserpulse, Ibramed, Brazil) to the Zusanli (ST36) and Sanyinjiao (SP6) acupoints, bilaterally according to the following specifications: 904 nm wave length, 124 Hz, and 3 J/cm^2 applied for 9 seconds to each acupoint [11]. The stimulation sequence was ST36 right, ST36 left, SP6 right, and SP6 left. The Zusanli (ST36) acupoint is located 3 cun (1 cun = width of the last rib) distal to the lateral head of the fibula. The acupoint Sanyinjiao (SP6) is located 3 cun proximal to the medial malleolus, at the caudal border of the tibia, close to the medial saphenous vein [15] (Figure 1). Cats in both groups had the skin over the acupoints shaved.

2.3. Surgical and Anesthetic Procedures. After fasting periods of 12 and 3 hours for solids and water, respectively, all animals were sedated intramuscularly with 5 mg kg^{-1} ketamine (Cetamin, Syntec, Brazil), associated with 0.5 mg kg^{-1} of midazolam (Dormonid, Cristália, Brazil) and 2 mg kg^{-1} of tramadol (Tramadon, Cristália, Brazil), in the same syringe. Fifteen minutes later the animals were placed in a secluded, quiet room, with no traffic of people and dim lighting, where the catheterization was performed (Insyte, Becton Dickinson, Brazil) in the cephalic vein, followed by the administration of Ringer's lactate solution (5 mL kg^{-1} h^{-1}) by peristaltic infusion pump (Santronic, Brazil), which was maintained until the end of the surgical procedure. During this period, in addition to fluid treatment, acupuncture was performed in the Laser group. Anaesthesia was induced intravenously with propofol (Propovan, Cristália, Brazil), in dose-dependent effect. Immediately afterwards, orotracheal intubation was performed, and anaesthesia was maintained with isoflurane (Isoforine, Cristália, Brazil) in 100% oxygen at a rate of 400 mL kg^{-1} min^{-1} using a small animal circuit without reinhalation of gases (Baraka, Takaoka, Brazil). During the anaesthetic procedure, the end-tidal carbon dioxide concentration ($ETCO_2$), end-tidal isoflurane concentration (ET_{ISO}), oxygen saturation of hemoglobin (SpO_2%), heart rate (HR), respiratory rate (RR), and esophageal temperature (T) were continuously measured using a multiparametric monitor (VAMOS plus; Dräger). Systolic arterial blood pressure (SBP) was measured using a noninvasive method with a Doppler ultrasonic system (Doppler 841-A; Parks Medical Electronics), with the width cuff approximately 40% of the antebrachium circumference. The end-tidal concentration of isoflurane (ET_{ISO}) was adjusted based on arterial pressure and HH changes and using the conventional signs of anaesthesia [16].

The surgical procedures were performed by the same surgeon, who used the same surgical technique and suture material for all the animals.

2.4. Evaluation of the Degree of Analgesia. The animal pain score was evaluated preoperatively (24 h prior to surgery) and postoperatively (0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h after tracheal extubation) using the Dynamic Interactive Visual Analogue Scale (DIVAS) [17, 18] and Multidimensional Composite Pain Scale (MCPS) [19].

For evaluation by DIVAS a 100 mm line was used, with the far left (=0) representing the animal with no signs of pain and the extreme right (=100), maximum pain. The DIVAS pain scoring involved three different phases. Initially, the cat was individually evaluated for 1 minute in its cage. Following this, the animal was stimulated to move around, in order for reactions and behaviour to be observed. Finally, the incision and surrounding area of the abdomen were palpated using 2-3 digits [18].

The MCPS was obtained on the basis of posture, comfort, activity, attitude, vocalization, and interactive behaviour, including palpation of the surgical wound, and abdomen/flank (see Appendix section). Each of the above-mentioned categories contained four descriptive behaviours (0 = normal unaffected behaviour and posture and 4 = severe changes). The observer chose a description in each category that best fits the cat's condition, so that the maximum score obtained was 24 points.

The administration of postoperative analgesic drugs was supervised by one anesthesiologist, and the pain assessment was performed by a blinded assessor. If the pain scores

Table 1: Mean ± standard deviations of the body weight, age, and surgical and anaesthetic recovery times of cats undergoing ovariohysterectomy treated with laser acupuncture (Laser, $n = 10$) or no acupuncture treatment (Control, $n = 10$).

	Body weight (kg)	Age (months)	Surgery time (min)	Extubation time (min)	Recovery time (min)
Laser	2.6 ± 0.6	26 ± 16	11 ± 4	7 ± 5	39 ± 7
Control	2.8 ± 0.4	28 ± 18	12 ± 3	6 ± 2	36 ± 9

exceeded 33% of DIVAS and/or MCPS, 2 mg kg^{-1} tramadol (Tramadon, Cristália, Brazil) was administered intramuscularly as a rescue analgesic. Thirty minutes after the first supplemental analgesia, if the DIVAS and/or MCPS score remained above 33%, meloxicam was administered at a dose of 0.2 mg kg^{-1} (IM). The number of additional administrations of tramadol and/or meloxicam and the interval between them were recorded.

2.5. Statistical Analysis. The statistics were performed using analysis of variance (ANOVA) followed by Tukey's test to compare differences between the means of the different groups, for the parametric variables (HR, f, SBP, SpO_2, $ETCO_2$, ET_{ISO}, surgical time, and time of extubation). The scores obtained from the evaluation of the degree of analgesia were evaluated using the Kruskal-Wallis test to compare the differences between the groups over time, while the Friedman test was used to compare the differences over time within each group. Dunn's posttest was used when significant differences were detected. The analyses were carried out on a standard PC microcomputer using the GraphPad Instat5 program with a significance level of 5%.

3. Results

There was no significant difference between treatments in relation to body weight, age, duration of surgery, and anaesthetic recovery time (Table 1).

The intraoperative HR (Control: 157 ± 19, 120–204 beats min^{-1}; Laser: 165 ± 20, 119–222 beats min^{-1}), SBP (Control: 145 ± 22, 117–150 mmHg; Laser: 134 ± 25, 100–170 mmHg), RR (Control: 24 ± 10, 10–30 breaths min^{-1}; Laser: 22 ± 20, 10–28 breaths min^{-1}), T (Control: 36.6 ± 1.2, 36–37.5°C; Laser: 36.6 ± 1.1, 36–38.1°C), $ETCO_2$ (Control: 37 ± 0.5, 32–45 mmHg; Laser: 38 ± 2, 30–43 mmHg), SpO_2 (Control: 99 ± 1, 97–100%; Laser: 98 ± 0.6, 97–99%), and ET_{ISO} (Control: 1.07 ± 0.25, 0.9–1.7%; Laser: 1.10 ± 0.10, 1.0–1.6%) concentrations were not significantly different between treatment groups at any time point ($P > 0.05$).

The median pain scores (DIVAS and MCPS) did not significantly differ between the treatment groups at any time point ($P > 0.05$). The pain scores were higher than the corresponding baseline values in the first 4 h after extubation in both treatment groups.

The need for supplemental postoperative analgesia was significantly lower ($P = 0.038$) in the Laser treatment group (one animal, one dose tramadol) compared to the Control group (5 animals, with a total of 5 doses of tramadol and 3 doses of meloxicam) (Table 2).

Table 2: Number of rescue doses administered over time in cats undergoing ovariohysterectomy treated with laser acupuncture (Laser, $n = 10$) or no acupuncture treatment (Control, $n = 10$).

	Groups	Postoperative time (h)								Total
		0.5	1	2	4	8	12	18	24	
Rescue doses (number)	Control	1	3	2	2					8[#]
	Laser	1								1

[#]Significantly different from Control group (Mann-Whitney U test, $P = 0.038$).

4. Discussion

This study showed that cats which received laser acupuncture had a significantly lower incidence of rescue medication than the Control group, suggesting a superior level of analgesia when acupuncture was administered preoperatively.

The analgesic effect mediated by acupuncture is closely related to the points stimulated. In the current study, the acupoints stimulated were selected based on previous reports confirming the analgesic potential of points ST36 and SP6 for the control of acute postoperative pain [1, 14, 20]. The combined stimulus of acupoints ST36 and SP6 can promote increased blood circulation and energy, in addition to triggering an anti-inflammatory and analgesic effect [21].

In addition to the choice of acupoints, other factors that can interfere with the analgesic response are the characteristics of the applied stimuli. In treatment with laser acupuncture, the use of an infrared laser with a wavelength between 650 and 1000 nm has a penetration depth of 2 to 3 mm [22], being capable of triggering a feeling of DeQi, which represents a determining factor for the effective stimulation of the acupoint [16, 23]. Additionally, the intensity of the applied radiation may interfere with the analgesic effect. Clinical studies have reported satisfactory analgesia after infrared laser treatment, using radiation intensities ranging from 3 to 10 J/cm^2 [24, 25]. Thus, the radiation characteristics (wavelength 904 nm; intensity of 3 J/cm^2) used in the present study may have contributed to the analgesic effect.

There is evidence that stimulation of acupuncture points is able to activate the descending inhibitory system in the spinal cord, brainstem, and other areas of the central nervous system, such as the thalamus, diencephalon, hypothalamus, and hypophysis [26–28]. Additionally, the release of endogenous opioids such as endorphins, enkephalins, and dynorphins also contributes to the analgesic effect mediated by acupuncture [28]. In addition to the opioid peptides, other factors are involved in the neurochemical mechanisms of acupuncture analgesia such as serotonin, norepinephrine,

Table 3: Multidimensional Composite Pain Scale (Brondani et al., 2011 [31]).

Posture	The cat is in a natural posture with relaxed muscles (it moves normally)	0
	The cat is in a natural posture but is tense (it moves little or is reluctant to move)	1
	The cat is sitting or is in sternal recumbency with its back arched and head down; or the cat is in dorsolateral recumbency with its pelvic limbs extended or contracted	2
	The cat frequently alters its body position in an attempt to find a comfortable posture	3
Comfort	The cat is comfortable, awake, or asleep and interacts when stimulated (it interacts with the observer and/or is interested in its surroundings)	0
	The cat is quiet and slightly receptive when stimulated (it interacts little with the observer and/or is not very interested in its surroundings)	1
	The cat is quiet and is "dissociated from the environment" (even when stimulated it does not interact with the observer and/or has no interest in its surroundings); the cat may be facing the back of the cage	2
	The cat is uncomfortable, restless (frequently changes its body position), and slightly receptive when stimulated or "dissociated from the environment"; the cat may be facing the back of the cage	3
Activity	The cat moves normally (it immediately moves when the cage is opened; outside the cage it moves spontaneously when stimulated or handled)	0
	The cat moves more than normal (inside the cage it moves continuously from side to side)	1
	The cat is quieter than normal (it may hesitate to leave the cage and if removed from the cage tends to return; outside the cage it moves a little after stimulation or handling)	2
	The cat is reluctant to move (it may hesitate to leave the cage and if removed from the cage tends to return; outside the cage it does not move even when stimulated or handled)	3
Attitude	A: satisfied, the cat is alert, is interested in its surroundings (explores its surroundings), and is friendly and interactive with the observer (plays and/or responds to stimuli); the cat may initially interact with the observer through games to distract it from the pain; carefully observe to distinguish between distraction and satisfaction games; B: uninterested, the cat does not interact with the observer (is not interested in toys or plays a little and does not respond to calls or strokes from the observer); in cats which do not like to play, evaluate interaction with the observer by its response to calls and strokes; C: indifferent, the cat is not interested in its surroundings (it is not curious; it does not explore its surroundings); the cat can initially be afraid to explore its surroundings; the observer needs to handle the cat and encourage it to move itself (take it out of the cage and/or change its body position); D: anxious, the cat is frightened (it tries to hide or escape) or nervous (demonstrating impatience and growling, howling, or hissing when stroked and/or handled); E: aggressive, the cat is aggressive (tries to bite or scratch when stroked or handled)	
	Presence of the mental state A	0
	Presence of one of the mental states B, C, D, or E	1
	Presence of two of the mental states B, C, D, or E	2
	Presence of three or all of the mental states B, C, D, or E	3
Miscellaneous behaviors	A: the cat is lying down and is quiet but is moving its tail. B: the cat contracts and extends its pelvic limbs and/or contracts its abdominal muscles (flank); C: the cats eyes are partially closed (eyes half closed); D: the cat licks and/or bites the surgical wound	
	All of the above behaviors are absent	0
	Presence of one of the above behaviors	1
	Presence of two of the above behaviors	2
	Presence of three or all of the above behaviors	3
Reaction to palpation of the surgical wound	The cat does not react when the surgical wound is touched or pressed; or there is no change from presurgical response (if basal evaluation was made)	0
	The cat does not react when the surgical wound is touched but does react when it is pressed; it may vocalize and/or try to bite	1
	The cat reacts when the surgical wound is touched and when it is pressed; it may vocalize and/or try to bite	2
	The cat reacts when the observer approaches the surgical wound; it may vocalize and/or try to bite; the cat does not allow palpation of the surgical wound	3

Table 3: Continued.

Reaction to palpation of the abdomen/flank	The cat does not react when the abdomen/flank is touched or pressed; or there is no change from presurgical response (if basal evaluation was made); the abdomen/flank is not tense	0
	The cat does not react when the abdomen/flank is touched but does react when it is pressed; the abdomen/flank is tense	1
	The cat reacts when the abdomen/flank is touched and when it is pressed; the abdomen/flank is tense	2
	The cat reacts when the observer approaches the abdomen/flank; it may vocalize and/or try to bite; the cat does not allow palpation of the abdomen/flank	3
Vocalization	The cat is quiet, is purring when stimulated, or miaows interacting with the observer but does not growl, groan, or hiss	0
	The cat purrs spontaneously (without being stimulated or handled by the observer)	1
	The cat growls, howls, or hisses when handled by the observer (when its body position is changed by the observer)	2
	The cat growls, howls, or hisses spontaneously (without being stimulated or handled by the observer)	3

dopamine, acetylcholine, gamma-aminobutyric acid, substance P, glutamate, cyclic AMP, calcium ions, and endogenous cannabinoids [6, 29]. Erthal et al. [11] demonstrated the involvement of the opioidergic and serotonergic systems in the antinociceptive effect mediated by stimulation with laser of acupoint ST36 in rats. Therefore, it is suggested that treatment with laser acupuncture potentiated the analgesia mediated by tramadol, whose analgesic properties are attributed to opioid mechanisms and inhibiting the reuptake of serotonin and noradrenalin [30].

However, despite the fact that treatment with laser acupuncture have promoted a reduction in the consumption of analgesics in the postoperative period, pain scores did not differ between treatments. This result is probably associated with increased consumption of analgesics in the Control group, which allowed the reduction in pain scores and hence masked the differences between the groups. The need for supplemental analgesia was observed during the first 4 hours after surgery, being implemented in 10% and 50% of animals in the Laser and Control groups, respectively. In a similar study the need for postoperative analgesic supplementation was reported in 50% of the cats submitted to OH, treated with tramadol in the preoperative period [17].

The assessment of pain in cats is difficult due to the impossibility of verbal communication between the evaluator and the patient. The DIVAS is an interval scale, regularly used by the scientific community to assess pain in cats [17, 18]. Additionally, the MCPS is a valid, reliable, and responsive scale for the assessment of acute pain in cats [19, 31]. Therefore, we sought to employ the most appropriate pain evaluation scales in order to minimize potential interference with the recognition of discomfort in the animal. In addition, all animals were treated by the same surgeon and pain measurement was performed by the same observer, who did not know to which treatment the animal belonged.

One of the limiting factors of the current study was the lack of a control group without treatment with analgesics in the preoperative period. It is possible that the inclusion of this group would have enabled the detection of statistically significant differences in pain scores, promoting greater consistency in the results obtained. However, because the cats in the study originated from a hospital routine and for ethical reasons, we chose not to include this group, since previous studies have shown that postoperative pain was detected in 100% of cats submitted to OSH without preventive analgesic treatment, requiring supplemental analgesia [19, 31].

In summary, our results demonstrate that laser acupuncture reduces postoperative analgesic requirements in cats undergoing ovariohysterectomy.

Appendix

See Table 3.

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

Peripheral Neuropathic Facial/Trigeminal Pain and RANTES/CCL5 in Jawbone Cavitation

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Introduction. In this study, we elucidate the possible causative role of chronic subclinical inflammation in jawbone of patients with atypical facial pain (AFP) and trigeminal neuralgia (TRN) in the local overexpression of the chemokine regulated on activation and normal T-cell expressed and secreted (RANTES/C-C motif ligand 5 CCL5). Neurons contain opioid receptors that transmit antipain reactions in the peripheral and central nervous system. Proinflammatory chemokines like RANTES/CCL5 desensitize μ-opioid receptors in the periphery sensory neurons and it has been suggested that RANTES modifies the nociceptive reaction. *Materials and Methods*. In 15 patients with AFP/TRN, we examined fatty degenerated jawbone (FDOJ) samples for the expression of seven cytokines by multiplex analysis and compared these results with healthy jawbones. *Results*. Each of these medullary jawbone samples exhibited RANTES as the only highly overexpressed cytokine. The FDOJ cohort with AFP/TRN showed a mean 30-fold overexpression of RANTES compared to healthy jawbones. *Conclusions*. To the best of our knowledge, no other research has identified RANTES overexpression in silent inflamed jawbones as a possible cause for AFP/TRN. Thus, we hypothesize that the surgical clearing of FDOJ might diminish RANTES signaling pathways in neurons and contribute to resolving chronic neurological pain in AFP/TRN patients.

1. Introduction

The etiology of chronic facial pain is challenging to diagnose and difficult or frustrating to treat. Many different concepts have been presented and discussed, for example, the presence of a neuroma, implying that the nerve has been damaged in the periphery, and intracranial vascular compression of the trigeminal nerve root at the base of the skull. In 1997, Jannetta published a long-term follow-up study of the surgical approach to move the superior cerebellar artery away from the nerve root, maintaining the artery in its new position with a suture [1]. Various complementary various medical treatments for this problem, such as use of carbamazepine, have been reported [2]. Chronic facial pain can also be related to the temporomandibular joint (TMJ) and can be due to involvement of the cervical plexus [3]. Different terms have been used to describe atypical facial pain (AFP) such as atypical odontalgia (AO, also known phantom tooth pain), psychogenic toothache, and persistent dentoalveolar pain disorder [4]. International associations for the study of pain have adopted the term "persistent idiopathic facial pain" (PIFP) to replace AFP [5]. Pain is also one of the hallmarks of inflammation. Acute trigeminal pain is unavoidable given our interaction with dental decay, but it is just the tip of a disease iceberg. Below the surface of acute bacterial or viral infections lie chronic inflammations, the products of an immune system that is being constantly triggered by overexpressed cytokines. These triggers lead to the stimulation of different signaling pathways, which are instrumental in the development of chronic or "silent" inflammation. The signal messengers, such as the cytokines, carry instructions that are received by cells with specific receptors, which are able to detect them. Most dental procedures consist in eliminating acute inflammation in situations that do not feature typical signs of inflammation like pain and tissue swelling. This is the case with root fillings and surgical procedures like wisdom tooth surgery. The use of antibiotics helps the dentist and the patient overcome inflammation after dental procedures and during

acute infections in daily practice. In daily dental practice, the effects of chronic inflammation on overall health are normally not of interest because local problems seem to be resolved after the symptoms of acute inflammation are gone. Consequently the individually targeted diagnosis of chronic pain in the peripheral facial nerves is a mostly neglected item in normal dental praxis even though this sensory disturbance in particular has a strong negative impact on the quality of life of those who are affected. Peripheral nerves are the source of almost all forms of neuropathic pain. Neuropathic pain is a complex syndrome resulting from many different forms of peripheral nerve damage, such as traumatic nerve damage, diabetes, and infections, as well as immune system and metabolic diseases [6]. For decades, a neuron-centered argument has been frequently used to explain the pathophysiology of chronic pain; however, recent studies have shifted attention towards a neuroimmune interaction. The concept of perineural jawbone inflammation producing or inducing facial neuralgias is an old one, and many oral surgical procedures have been recommended for "*tic douloureux*" [7]. This line of reasoning shifted when, in 1992, *Bouquot* examined 224 tissue samples from the mandibular alveolar bone of 135 patients with AFP or trigeminal neuralgia (TRN). All samples showed the clear presence of fatty-degenerative osteonecrosis spreading up to several centimeters in the form of retromolar cavities in the cancellous bone. This brought *Bouquot* to propose the term "*Neuralgia-Inducing Cavitational Osteonecrosis (NICO)*" to describe the clinical phenomenon of neuralgia in conjunction with fatty-degenerative osteolysis and osteonecrosis of the jawbone (FDOJ) [8]. Further reports in the dental literature suggest that curettage of jawbone lesions is an effective treatment for the pain associated with avascular FDOJ [9, 10]. Notwithstanding these reports, the underlying effects of FDOJ on AFP/TRN remain unexplored by modern immunological means. In contrast to former destructive, intracranial, and extracranial ablative approaches to branches of the trigeminal nerve our hypothesis is that the reduction of acute inflammation might serve as the beginning of a possible development of chronic inflammation in jawbone. Persons with certain risk factors might be prone to developing subsequent chronic AFP/TRN. Although a multidisciplinary approach is required to address the many facets of this pain syndrome, no studies of AFP/TRN have established a connection between the direct role that cytokines and chemokines play in the pain-affected area or in pain syndromes of the jawbone. Elucidating the mechanisms, defining successful treatment strategies and a critical attitude to operation sites with insufficient wound healing in jawbone and treatments tailored to AFP/TRN is a crucial part of the here-presented therapeutic concept.

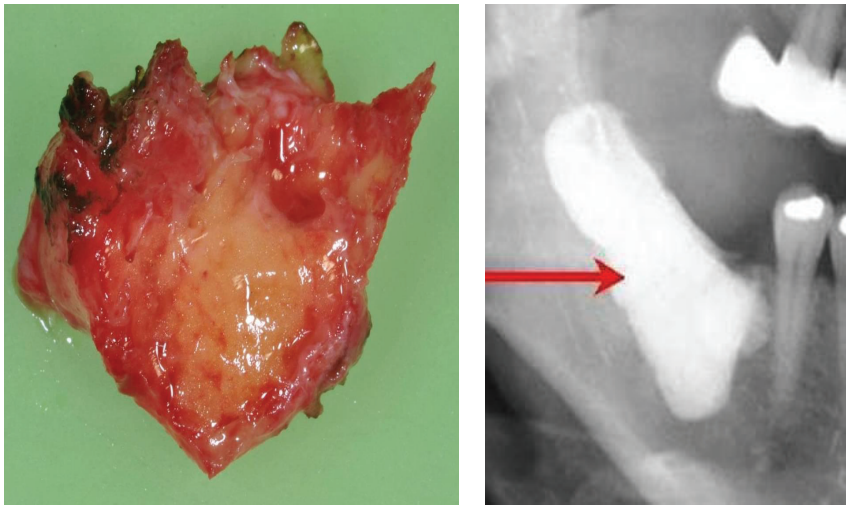
2. Materials and Methods

2.1. Patient Cohort. This study was performed as a randomized controlled trial. We collected FDOJ tissue samples from 15 patients with AFP/TRN. A diagnosis of AFP/TRN was made by neurologists, pain specialists, and physicians. Inclusion criteria were (1) therapy-resistant pain that was clinically similar to AFP/TNR and (2) the local diagnosis of FDOJ in the painful jaw site. Mandatory inclusion criteria were (3) the availability of two-dimensional orthopantomograms (2D-OPG) and (4) cone beam three-dimensional (digital volume tomograms DVT) images. A further inclusion criterion for the group with surgery in the AFP/TRN areas was the measurement of bone density of the jawbone with transalveolar ultrasound technology (TAU). Besides 2D-OPG and 3D-DVT, the definite indication for FDOJ surgery was the additional measurement of bone density by TAU. TAU is a useful tool for establishing FDOJ [11–13]. Patients taking any medications due to neuropathic complaints were not excluded from the study. Demographic data from the AFP/TRN cohort showed an average age of 60 years (standard deviation (SD) = 13.2 years) and a gender ratio of 14 : 1 (female : male). The age range of the control group of 19 patients without FDOJ extended from 38 to 71 with an average age of 54 years and a gender split (female : male) of 11/8. This research was based on data retrieved from patients during normal dental surgery. All patients provided written informed consent.

2.2. Clinical Features of FDOJ Samples. The softening in FDOJ bone marrow is so distinct that the marrow space can be sucked and spooned out. Hollow cavitations with fatty degenerated adipocytes have undergone dystrophic changes accompanied by demyelination of the bony sheath of the infra-alveolar nerve. All 15 FDOJ samples presented clinically and macroscopically as fatty lumps. FDOJ is similar to silent inflammation or subclinical inflammation without the typical signs of acute inflammation. Figure 1 shows a specimen with predominantly fatty transformation of the jawbone (a). The often-impressive extent of FDOJ lesions is illustrated in the right-hand panel by an X-ray with contrast medium.

2.3. Sampling of FDOJ Tissue. The current treatment of FDOJ lesions consists of curettage of the bony cavity, which relieves symptoms of pain with varying rates of success [8, 10, 14, 15]. To elucidate a possible causative link between FDOJ and AFP/TRN at the Munich Clinic for Integrative Dentistry, Germany, 15 patients with AFP/TRN and who were diagnosed with FDOJ had surgery on the affected area of the jaw. After local anesthesia and folding of a mucoperiosteal flap, the cortical layer was removed. All 15 patients exhibited FDOJ inside the bone marrow, which was quite similar to the samples described in the literature [8, 10]. In all 15 cases, surgery was performed on edentulous jaw areas in the sites of former wisdom teeth and the adjacent retro molar areas. The FDOJ samples, with a volume of up to 0.5 cm^3, were stored in dry, sterile, 2 mL collecting vials (Sarstedt AG and Co, Nümbrecht, Germany), which were airtight, and frozen at −20°C. In addition to the cytokine analysis, we checked the FDOJ samples for pathohistological findings.

2.4. Processing of Necrotic Tissue Samples and Cytokine Measurements. In the examining Institute for Medical Diagnostics, Nicolaistrasse 22, 12247 Berlin inspected by DAKKS (Deutsche Akkreditierungsstelle GmbH, accredited to DIN EN ISO/IEC 17025:2005 and DIN EN ISO 15189:2007), the samples were homogenized by mechanical force in 200 μL



(a) (b)

Figure 1: FDOJ sample of fatty and osteolytic degenerated bone marrow (a) and contrast medium X-ray of the FDOJ cavity after curettage (b).

of cold protease inhibitor buffer (Complete Mini Protease Inhibitor Cocktail; Roche Diagnostics GmbH, Penzberg, Germany). The homogenate was then centrifuged for 15 minutes at 13,400 rpm. Next, the supernatant was collected and centrifuged for further 25 minutes at 13,400 rpm. In the 15 supernatants of tissue homogenate, we measured, regulated on activation, normal T-cell expressed and secreted (RANTES), also known as chemokine C-C motif ligand 5 (CCL5), FGF-2, interleukin- (IL-) 1 receptor antagonist (ra), IL-6, IL-8, monocyte chemotactic protein-1 (MCP1), and tumor necrosis factor-alpha (TNF-α). Measurement was performed using the Human Cytokine/Chemokine Panel I (MPXHCYTO-60K; Merck KGaA, Darmstadt, Germany) according to the manufacturer's instructions and analyzed using Luminex 200 with xPonent software (Luminex Co, Austin, TX, USA).

2.5. Pathohistological Examination. Parallel to the cytokine analysis, each FDOJ sample was examined histopathologically (Institute for Pathology & Cytology; Drs. Zwicknagel/Assmus, 85635 Freising, Germany).

3. Results

As we showed in earlier publications [15, 16], the defining feature of the FDOJ areas is overexpression of the proinflammatory messenger RANTES, also known as CCL5. The results of the multiplex analysis of the seven cytokines in the AFP/TRN cohort (n = 15) are shown in Table 1: AFP/TRN patients show elevated inflammatory signals in the FDOJ samples, deriving from painful jawbone areas with an average RANTES/CCL5 value of 4.274,7 pg/mL (SD = 2.778 pg/mL), compared to the randomized controlled sample of 149.9 (pg/mL) in healthy jawbone (Figure 2). All other cytokines were not derailed; only FGF-2 (fibroblast growth factor 2) and IL-1ra (interleukin 1 receptor antagonist) were additionally slightly upregulated in FJOD samples.

Table 1: Pathohistological findings from FDOJ samples in 15 patients with AFP/TRN.

AFP/TRN	15	100%
Ischemia	13	87%
Necrotic adipocytes	10	67%
Myxoid degeneration	12	80%
Increased fat cells	12	80%
Inflammatory cells	1	7%

In the pathohistological findings the amount of fat cells was consistently and strikingly increased in FDOJ samples. Typical signs of inflammation, especially of an inflammatory cell response, were absent. The fatty-degenerative and osteolytic aspects occurred due to insufficient metabolic supply in an ischemic state. The histologic examination of the curetted tissue demonstrated ischemia (n = 13), necrotic adipocytes (n = 10), myxoid degeneration (n = 12), and increased fat cells (n = 12); inflammatory cells were only found in one FDOJ sample. Table 1 shows the pathohistological findings in FDOJ samples from 15 patients with AFP/TRN.

4. Discussion

4.1. Histology in Neuropathic Facial Pain. The presence of inflammatory cells in only one FDOJ sample confirms inflammation-free progression and the absence of inflammatory granulation in FDOJ [17, 18]. This raises an important question: Are typical infections the underlying cause of chronic AFP/TRN? In summary, the pathohistological findings clearly show that AFP/TRN is not caused by an osteitic process that might produce typical symptoms like swelling and local inflammation; this is the likely reason why former attempts to diminish AFP/TRN by serial extraction of apical inflamed teeth exhibited poor success. In these cases,

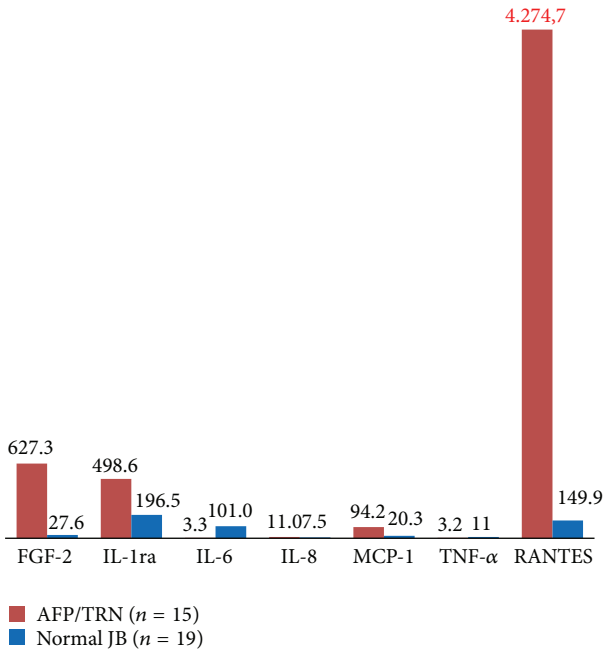


Figure 2: Analysis of seven cytokines in the FDOJ AFP/TRN cohort ($n = 15$) compared to healthy jawbones.

the alveolar jawbone remained untouched and the "silent inflammation" in the affected area continued unabated. FDOJ must not be lumped together with other forms of osteomyelitis, which are defined by a dramatic increase in inflammatory cells.

4.2. Hyperactivated Chemokine RANTES/CCL5 in FDOJ. The absence of acute inflammation in FDOJ indicates that these chronic immunological processes are under the guidance of RANTES/CCL5, a proinflammatory chemokine. The hypothesis that FDOJ is an insidious, subtle process is supported by the fact that typical acute inflammatory cytokines, such as TNF-α and IL-6, were not increased in our samples. Proinflammatory cytokines have been repeatedly associated with demyelination and degeneration of the peripheral nerves, increased excitability of sensory afferents, and the induction of neuropathic pain [19]. The significance of RANTES to the development of disease appears to be substantial: RANTES interferes with immune responses on a number of levels and therefore plays a crucial role in pathological states. The chemotactic properties of RANTES send T-cells, dendritic cells, eosinophils, natural killer (NK) cells, mast cells, and basophils to the sites of inflammation and infection [20]. RANTES is also an effective activator of leukocytes, which play a key role in a wide range of inflammatory disorders [21], including in rheumatoid arthritis [22] and diseases of the central nervous system, such as multiple sclerosis [23]. RANTES has also been associated with the induction or promotion of cancer [24]. RANTES levels were markedly elevated in the primary tumor and metastatic lesions of all patients with breast and cervical cancer in a previous study [25].

4.3. Origin of RANTES in FDOJ—Fatty Tissue and Adipocytes. Reduced blood flow and capillary density followed by ischemia may lead to a hypoxic environment [26]. Moreover, adipocytes and necrotic fat cells are considered immunologically effective ingredients. For instance, Huber et al. found increased expression of RANTES in fatty tissue in obese patients [27]. The role of these immune effects in understanding FDOJ, RANTES/CCL5, and facial pain is an evident issue that will be further illuminated later in the discussion.

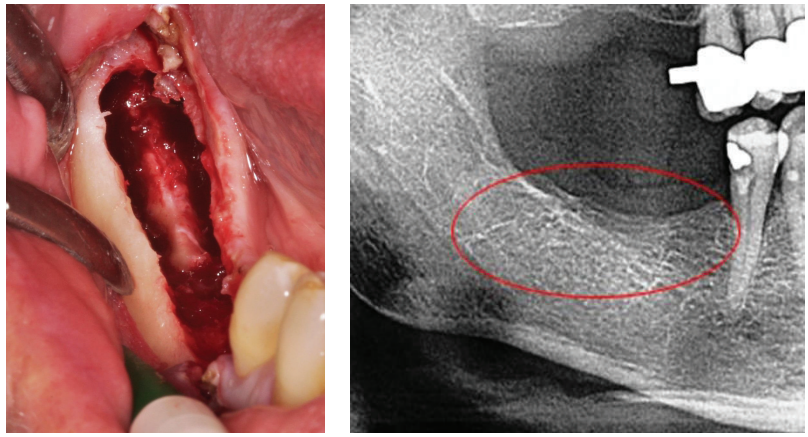
4.4. Immunology in Neuropathic Facial Pain. Recent data suggest that there is a strong link between immune and glial cells and the development of neuropathic pain [19]. The present paper and other researches provide evidence that the nearly 30-fold overexpression of chemokine RANTES/CCL5 that we found in the painful jawbone areas of the AFP/TRN cohort is linked to the disease development. Interactions between the immune and nervous systems occur at multiple levels, at which different types of immunologically active substances are involved in different stages of disease development [28]. Chronic pain is also associated with changes in neuroplasticity or changes in the neural pathways and synapses due to a defective reorganization of both the peripheral and central nervous systems. During tissue destruction, noxious stimuli and inflammation cause an increase in nociceptive input from the periphery to the central nervous system. Extended nociception from the periphery triggers a neuroplastic response at the cortical level and leads to a change in the somatotopic organization in the area of the body affected by pain; this results in central sensitization [19]. Moreover, immune activation near or around the peripheral nerves can cause increased excitability of these peripheral nerves. Both infectious substances and proinflammatory mediators may lead to changes in the blood-brain barrier (BBB) in response to chemotactic molecules that are released to the location of the damaged peripheral nerves which, in turn, leads neutrophils and macrophages to pass from the bloodstream into the nerves. Proinflammatory cytokines take part in this immune activation and shape the early immune response. However, these inflammatory mediators can directly increase nerve excitability, and they can cause damage to myelin and alter the permeability of the BBB. Furthermore, they can simultaneously lead to edema and further infiltration of the immune cells in peripheral nerves. Schwann cells, which ensheath the peripheral nerves, behave in a similar way to macrophages in the sense that they can present "non-self" substances to T-lymphocytes for further activation of immune cells. Schwann cells are also involved in the degradation of damaged myelin and cell debris [29]. Inflammatory mediators from the cells of the dorsal root ganglia (DRG), and those originating in the infiltrating immune cells and activated spinal microglia, are key elements that carry signal transmission of the pain response [10].

4.5. RANTES/CCL5 and Neuropathic Pain Syndromes. Cytokine/chemokine communication between glial cells and neurons is important for the development of neuropathic pain [30]. Studies indicate that prolonged chemokine and

chemokine receptor activation in the sensory ganglia can significantly contribute to neuropathic pain syndromes. Long-term chemokine inflow through RANTES/CCL5 causes neuronal hyper excitability. While proinflammatory cytokines, such as TNF-α, IL-6, and prostaglandins, are already distributed early in the acute stage of an injury or tissue infection, there are many indications that chemokines are activated at a later time, and they can act in the conversion of acute pain into a more chronic phenomenon. Recent data suggest that, in conjunction with tissue damage or infection, ischemia-induced chemokine expression causes an increase in inflammatory cytokines and thus leads to the hyper excitability of sensory neurons [31]. Since some chemokine receptors, such as CCR2, CCR5, CXCR4, and CX3CR1, are located mostly in the primary afferent neurons or secondary neurons of the dorsal spinal horn [32], their chemokine ligands may be able to alter the quality of pain transmission. By means of peripheral administration of the chemokines CCL2, CCL3, CCL5, and CXCL12, it is possible to detect pain patterns that are caused by the activation of chemokine receptors in dorsal root ganglia [33]. A study that examined the effects of CCR5 deficiency on pain responses by employing CCR5 knockout (KO) mice found that the pain responses of CCR5 KO mice to chemical or inflammatory stimuli were milder than those of CCR5 wild-type mice [34]. Another study examined the effects of CCR5 deficiency on pain responses via the use of CCR5 KO mice; it was observed that the pain responses of CCR5 KO mice to chemical or inflammatory stimuli were milder than those of the CCR5 wild-type mice [33].

4.6. Opioid Receptors and Chemokine RANTES/CCL5. Recent studies have suggested that the chemokine RANTES and its receptor CCR5 interact directly with the opioid receptors and modify the nociceptive reaction [29]. Opioid receptors mediate antipain reactions, both in the peripheral and central nervous systems. The analgesic mechanism of morphine occurs when the analgesic opioid (e.g., morphine) excites the opioid receptors located in the brain and spinal cord; the perception of pain is blocked due to an agonistic, opposing effect. Morphine exerts its pain-relieving effect by binding to the nerve cells at the same binding sites as the endorphins; the specific binding sites are the opioid receptors. Fewer nociceptive neurotransmitters are released through morphine-induced opioid receptor excitation, and an incoming pain signal is not propagated. Studies have shown that opioid use suppresses chemokine-mediated chemotactic responses effectively, and this can be seen as a result of heterologous desensitization between opioids and some of the chemokine receptors [34]. The desensitization of opioid receptors through RANTES/CCL5 is part of this mutual "crossover" desensitization [35]. More recently, there have been reports showing that the process of heterologous desensitization is bidirectional, and that chemokine receptor activation leads to an inactivation of the in vitro activity of opioid receptors [36]. An open question that remains is whether some chemokines have the ability to desensitize opioid receptors in vivo. Studies using a rat model found that the analgesic response was blocked in opioids following chemokine application [37, 38]. In these studies, Pizziketti et al. were able to show that proinflammatory chemokines, such as CCL2/MCP-1, CCL5/RANTES, and CXCL8, are able to desensitize μ-opioid receptors on the peripheral sensory neurons [39]. Therefore, these μ-opioid receptors offer novel and potential mechanisms for peripheral inflammation-induced hyperalgesia. Scientists believe that this neural overexcitation materializes during chronic exposure to RANTES/CCL5 through the local overexpression in all trigeminal cases within the FDOJ and thus inhibits RANTES activity on the μ-opioid receptors in the synapses. Moreover, chemokine-induced desensitization is mediated by the chemokine receptors [40]. Animals directly injected with specific doses of RANTES/CCL5 in the periaqueductal gray matter, a brain region that is the first to handle the antinociceptive effects of opioids, experience blocked and altered normal pain response to opioids. Our data indicate that proinflammatory chemokines are capable of desensitizing μ-opioid receptors on peripheral sensory neurons, providing a novel potential mechanism for peripheral inflammation-induced hyperalgesia [40]. When the interval of the chemokine effect was extended to 2 hours, the ability of RANTES/ CCL5 to desensitize opioid receptors was lost. A logical explanation for this is that the desensitization of opioid receptors is a reversible process that occurs via metabolic degradation. In our clinical neuralgia cases, the hypothesis of RANTES/CCL5 as a source of pain has persisted for years, so the experimental time limit for RANTES/CCL5 exposure on the opioid receptors is irrelevant. The above-cited experiments show that the opioid receptors can be desensitized by treatment with chemokines, which suggests that the desensitization of all three opioid receptors is achieved through the activation of RANTES/CCL5 [36]. Although RANTES/CCL5 desensitizes opioid receptors very effectively, desensitization does not work with all chemokines [41]. Recent studies have also shown that the chemokine/RANTES receptor CCR5 interacts with opioid receptors and leads to a change in the nociceptive reaction [42].

4.7. Diagnostic Problems of FDOJ Lesions by X-Ray. The nonvisible nature and lack of radiographic appearance of FDOJ make it difficult to obtain an accurate diagnosis [13]. Therefore, the existence of FDOJ is largely neglected today in mainstream dentistry. The reason for this is that conventional X-ray techniques are limited in their ability to reveal the actual extent and location of FDOJs. To aid the practitioner in diagnosing the debilitating effects of bone marrow softening inside FDOJ lesions, a computer-assisted TAU device was developed [43]. TAU precisely images and identifies cavitational porosity in the jawbone. Studies show that, in 84% of cases, FDOJ lesions on TAU images were more obvious and more readily identified than on radiographs of the same site. TAU imaging proved to be significantly superior to radiology for the detection of microscopically confirmed FDOJ. The efficiency and reliability of TAU in the diagnosis and imaging of FDOJ have been presented in earlier publications [44]. Because of these diagnostic difficulties, FDOJ as a presumably widespread jawbone disease is underdiagnosed by dentists in



(a) (b)

Figure 3: Curettage of FDOJ in the lower jaw with denuded infra-alveolar nerve. Corresponding X-ray without any signs of pathological process in jawbone (b).

general; specifically, in AFP/TRN cases, it may often falsely be referred to as "idiopathic."

The clinical example in Figure 3(a) shows the typical situation during surgical debridement and curettage of the lower jaw. The infra-alveolar nerve is totally denuded from its bony sheath by FDOJ. The ischemic process of FDOJ converts the bony sheath, leaving the nerve tissue intact. As evidenced by what is not shown in the X-ray in the right-hand panel of the figure, this process is inconspicuous and does not show any signs of inflammation or FDOJ. Because of this diagnostic problem of identifying FDOJ on common dental X-rays [13], this patient suffered from AFP for 7 years and received antidepressants during this time as a singular therapy.

4.8. Clinical Relevance of FDOJ Surgery in AFP/TRN Cases. The neurological theories and the data we retrieved from the FDOJ surgery resulted in pain relief in our AFP/TRN cohort. The subjective pain intensity in our AFP/TRN cohort was measured using the Numeric Rating Scale (NRS) [45]. The results of the NRS (ranging from 1 to 10) were changed into a percentage to evaluate pain relief. Figure 4 shows the mean time of AFP/TRN (45 months), the pain-free period after FDOJ curettage (21 months at the time the statistic was measured), and the overall percentage of pain relief (88%) in our 15 patients. Details of pain reduction in each patient are shown in Figure 5, which documents a mean percentage of 88%. Similar results in AFP/TRN pain relief were reported in other papers discussing FDOJ curettage [46].

4.9. A Clinical Case of FDOJ Surgery (Figure 6). To show the extent to which curettage of FDOJ in patients affected by AFP/TRN can contribute to alleviating facial pain and to give an example of the clinical relevance of FDOJ, our patient Mrs. N. T. reported the following: "*Since spring 2009 I had been getting recurring stabbing pain on the left-hand side of my face and earache, tinnitus and pain in my shoulder/arm. During the night I suffered palpitations and panic attacks. My physical*

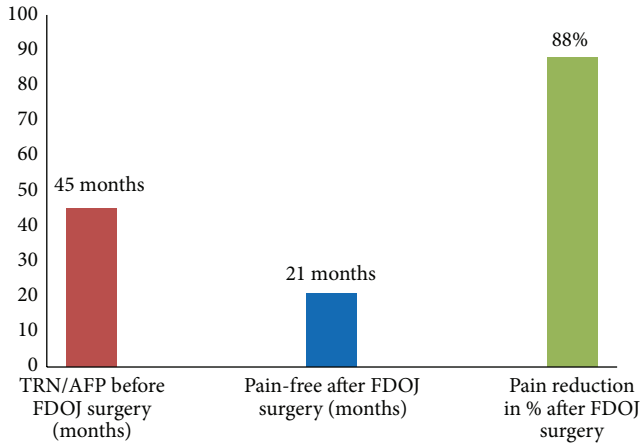


Figure 4: Mean time of AFP/TRN (45 months), the pain-free period after FDOJ curettage (21 months), and the overall percentage of pain relief (88%).

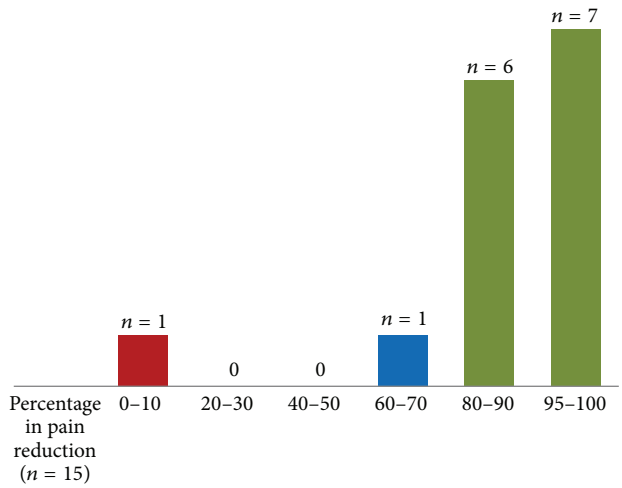


Figure 5: Percentage of pain reduction in the AFP/TRN cohort (n = 15).

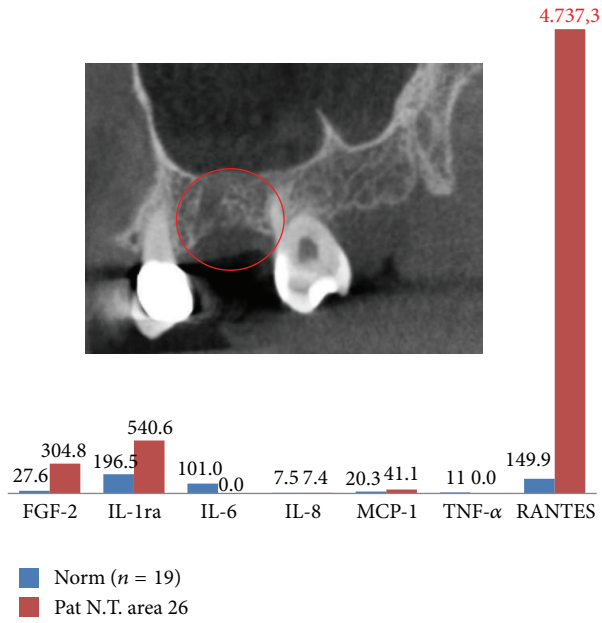


Figure 6: A patient with AFP in the left upper jaw with overexpression of RANTES/CCL5 in the painful area. The corresponding X-ray marked in red is inconspicuous; pain relief after FDOJ surgery was 90%.

energy levels also dropped. I consulted a further two dentists to no avail. One recommended that I went to see a neurologist, who prescribed me strong painkillers and psychotropic drugs. A trip to an osteopath was also unfortunately fruitless. In summer 2011 I was in a horrendous amount of pain, particularly at night. I could barely sleep through the night. I was taking strong painkillers every day just to get me to work. Then came the day when everything was solved. On 15 February 2012 I had an operation on the left side of my upper jaw and bone was excavated. After about 4 weeks I was almost pain-free without medication."

5. Conclusions

Although the role of proinflammatory cytokines and chemokines has been identified in neuropathic pain [38], the exact relationship between the chemokine–cytokine network and neuropathic pain is not fully understood. Jawbone cavitations are hollow dead spaces in the jaw bone, where the bone marrow is dying or dead. The research suggests that this jawbone disease, known popularly as "cavitations" and in some technical publications as "NICO," might serve as a fundamental cause of neuropathic pain, through the inflammatory cytokines that it produces. Opioid receptors mediate antipain responses in both the peripheral and central nervous systems, and RANTES/CCL5 is able to enhance the pain response. As RANTES/CCL5 is overexpressed in jawbone areas defined by FDOJ, this process close to the trigeminal nerve might contribute to the development of AFP/TRN. Data from our research points to the local overexpression of RANTES/CCL5 in jawbones as a possible additional cause of AFP/TRN. Treatment for more advanced stages of FDOJ requires surgery. Surgical debridement of FDOJ areas can diminish RANTES/CCL5 overexpression and thus reduce chronic facial pain. The success of such surgery is by no means guaranteed and it depends on the technique and the skill of the dentist doing the surgery. FDOJ, as a contributing factor to AFP/TRN, is a widely neglected form of "silent inflammation" characterized by the overexpressed chemokine RANTES/CCL5. When doctors or dentists are presented with AFP/TRN of undetermined origin or that is "idiopathic," a complete differential diagnosis should include FDOJ lesions. The presence of FDOJ is often not entirely obvious from examination of a panorex or other X-rays. Many case histories in our clinic show that removing the diseased FDOJ from the jawbone may be the key to reversing the course of different forms of AFP/TRN. Further studies are needed to fully understand the neuropathic regulatory mechanisms that underlie neuroinflammation following nerve damage by cytokines deriving from FDOJ.

Disclosure

Dr. Volker von Baehr is the coauthor.

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

Transcutaneous Electrical Acupoint Stimulation Improves the Postoperative Quality of Recovery and Analgesia after Gynecological Laparoscopic Surgery: A Randomized Controlled Trial

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Background. We conducted this prospective, randomized, double-blind, placebo-controlled study to evaluate the effects of transcutaneous electric acupoint stimulation (TEAS) on the quality of recovery (QoR) and postoperative analgesia after gynecological laparoscopic surgery. *Methods*. 74 American Society of Anesthesiologists physical status (ASA) I or II patients undergoing gynecological laparoscopic surgery were randomly allocated to TEAS or control groups. The primary outcome was the quality of recovery, which was assessed on the day before surgery and 24 h after surgery using a 40-item questionnaire. Secondary outcomes included postoperative pain scores, the incidence of postoperative nausea and vomiting (PONV), duration of postanesthesia care unit (PACU) stay, and patient's satisfaction. *Results*. The TEAS group had higher QoR scores than control group upon 24 h after surgery (177 versus 165; $P < 0.001$). Compared with the control group, postoperative pain scores and the cumulative number of opioids administered were lower in the TEAS group patients ($P = 0.04$). TEAS reduced the incidence of PONV and dizziness, as well as duration of PACU stay. Simultaneously, the patient's satisfaction scores were higher in the TEAS group ($P = 0.002$). *Conclusion*. Preoperative TEAS enhances QoR, improves postoperative analgesia and patient's satisfaction, alleviates postoperative side effects, and accelerates discharge after general anesthesia for gynecological laparoscopic surgery.

1. Introduction

Gynecological laparoscopy is considered to be a minimally invasive procedure. However, as common complications of anesthesia and surgery, postoperative pain, nausea, and vomiting remain problematic despite use of analgesics and antiemetics. These complications delay the patient's recovery from anesthesia, extend their hospital stay, and increase overall healthcare costs [1, 2]. Due to side effects of drug therapy, an integrated approach combining pharmacological methods and various complementary analgesic techniques has been recommended in clinical practice [3]. These nonpharmacological therapies include acupuncture, transcutaneous electrical nerve stimulation (TENS), and acupressure.

Acupuncture is widely accepted in China, Japan, and Korea, which is considered as a complementary intervention for acute and chronic pain of various origins [4, 5]. In addition, acupuncture is commonly used and recommended as part of a balanced anesthetic technique in the above countries [6, 7]. But, acupuncture is invasive, and its application requires a physician experienced in this technique. TEAS is a form of noninvasive electrical stimulation that produces a perceptible sensation via electrodes attached to the skin. It has no risk of infections and can potentially be applied by medical personnel with minimal training. Clinical trials have demonstrated that TEAS reduces the consumption of intraoperative anesthetics and general anesthesia related side-effects [6, 8, 9]. However, the effect of TEAS on the quality of

recovery and postoperative pain in patients undergoing gynecological laparoscopic surgery remains unclear. Therefore, we conducted this prospective, randomized, double-blind study to verify the hypothesis that preoperative TEAS could improve the quality of recovery (QoR) and postoperative analgesia after gynecological laparoscopic surgery.

2. Materials and Methods

2.1. Patients. This study was a single-center, prospective, randomized, double-blind, placebo-controlled trial. The study protocol was approved by the Institutional Review Board of Fujian Provincial Hospital (Ref. K2014-05-008). We enrolled 74 consecutive subjects, aged 18 to 60 years and American Society of Anesthesiologists physical status I-II, who underwent general anesthesia for elective gynecological laparoscopic surgery from May 2014 to November 2014 at Fujian Provincial Hospital. The exclusion criteria were as follows: potentially difficult airway, sore throat, a history of chronic pain, drug or alcohol abuse, mental disorder, obesity (BMI $> 30\,kg/m^2$), intake of any analgesic drug within 48 h before surgery, and previous experience with acupuncture treatment. The study was performed in line with the principles of the Declaration of Helsinki and the CONSORT statement. Written informed consent was obtained from all subjects before randomization.

2.2. Randomization and Blinding. Patients were assigned to either the TEAS group or the control group by a table of computer-generated random numbers. The allocation ratio was 1 : 1 for the two groups. Group assignments were sealed in sequentially numbered opaque envelopes. The patients, attending anesthesiologist, surgeons, recovery ward nurses, data collectors, and the person who performed the final statistical analysis were blinded to group assignment.

2.3. Study Protocol. Patients in the TEAS group received preoperative TEAS for 30 min before the induction of anesthesia in the holding area. TEAS was applied to four pairs of acupoints: bilateral Hegu (LI4), Neiguan (PC6), Zusanli (ST36), and Sanyinjiao (SP6). These acupoints were identified according to the traditional anatomical localisations (Figure 1). TEAS was performed with a dense-disperse frequency of 2/10 Hz and an intensity of 6–9 mA for 30 min using the Hans electronic acupuncture apparatus (HANS-100B, Nanjing Jisheng Medical Technology Company, Nanjing, China). The optimal intensity was adjusted to maintain a slight twitching of the regional muscle according to individual maximum tolerance. In the control group, the patients were connected to the apparatus, but electronic stimulation was not applied.

2.4. Standardized Anesthesia. All patients were fasted for at least 8 h and premedicated with IV midazolam 0.05 mg/kg 30 min before anesthesia induction. Standard monitoring, including electrocardiogram, noninvasive blood pressure, pulse oximetry, and temperature, was used in all patients for the duration of surgery. General anesthesia was induced

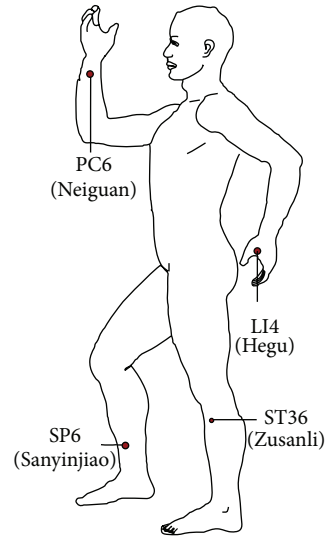

Figure 1: Location of Hegu (LI4), Neiguan (PC6), Zusanli (ST36), and Sanyinjiao (SP6) acupoints.

with IV sufentanil 0.5 μg/kg and propofol 2.0 mg/kg. Tracheal intubation was facilitated with cisatracurium 0.15 mg/kg. After intubation, mechanical ventilation was used to maintain $P_{ET}CO_2$ at 35–45 mmHg. Anesthesia maintenance was achieved with sevoflurane 2%-3% according to both hemodynamic parameters and bispectral index (BIS) of 40–60. Perioperative fluids were administrated in a standardized way, and normothermia (36°C to 37°C) was maintained by a warming device (Bair Hugger; Augustine Medical Inc., Eden Prairie, USA). All patients received IV tropisetron 5 mg 30 min before the end of surgery. Neuromuscular blockade was antagonized using neostigmine 0.02 mg/kg and atropine 0.01 mg/kg.

2.5. Study Outcomes. The primary outcome was the quality of recovery, which was assessed on the day before surgery and 24 h after surgery using a 40-item questionnaire (QoR-40) [10]. The global QoR-40 score ranges from 40 to 200, representing extremely poor to excellent quality of recovery, respectively.

Secondary outcomes were postoperative pain scores, the incidence of nausea and vomiting, duration of PACU stay, and patient's satisfaction. Postoperative pain was assessed using Visual Analogue Scale (VAS) in 24 h after surgery. If the VAS score was greater than or equal to 4, the patient received IV sufentanil 0.05 μg/kg as rescue analgesia. Patient's satisfaction was evaluated on postoperative 24 h with a 10-point numerical rating scale: 10 = excellent, 1 = bad. Patient's satisfaction is defined as the scale with a threshold value of greater than or equal to 8.

2.6. Statistical Analysis. Our sample size calculation for the two-tailed testing of the TEAS superiority hypothesis was based on the global QoR-40 score. A 10-point difference represents a clinically relevant improvement in quality of recovery based on data from a previous study [10]. A mean

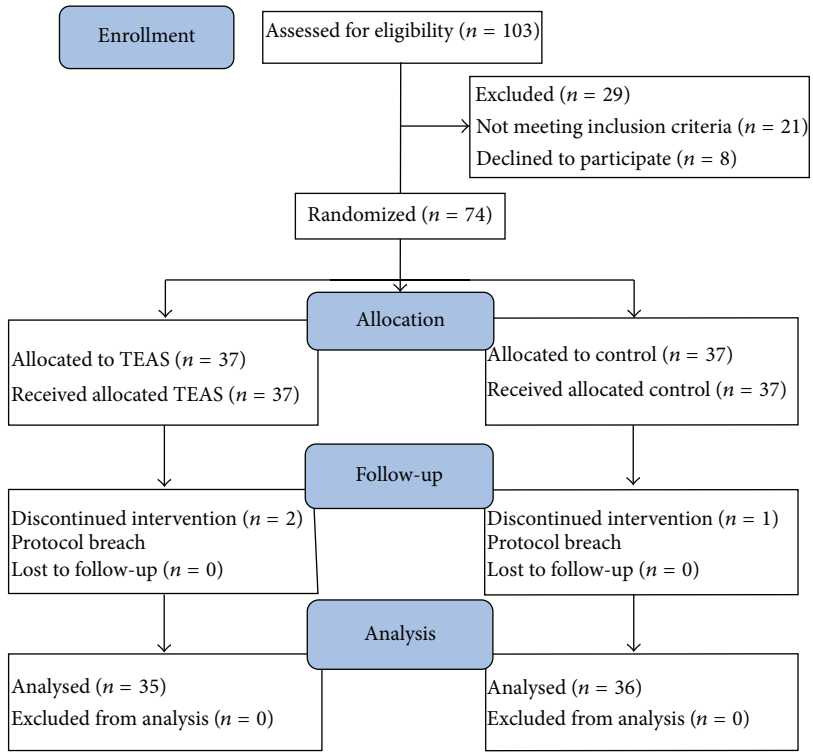

Figure 2: Consolidated Standards of Reporting Trials (CONSORT) flow diagram depicting the progress of subject through the trail. TEAS: transcutaneous electric acupoint stimulation group.

(standard deviation) of the QoR score at 24 h postoperative equivalent to 171 (14.3) was estimated based on our pilot study. A power analysis using a type I error estimate of 5% (alpha = 0.05) and a power (1-Beta) of 80% indicated that a sample of 34 subjects per group would be required. Allowing for approximately 10% incomplete follow-up or dropout, a total of 74 subjects were enrolled in this study.

Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The normality of distribution was assessed with the Kolmogorov-Smirnov test. Parametric data were reported as mean (standard deviation (SD)) and analyzed with the independent t-test, and nonparametric data were reported as median and (interquartile range (IQR)) and analyzed using the Mann-Whitney U test. Categorical variables were reported as the number of patients (%) and evaluated using the x^2 or Fisher's exact test when appropriate. The level of significance was considered at a P value of less than 0.05.

3. Results

We initially assessed 103 patients for eligibility to participate in this study (Figure 2). Of these, 21 patients did not meet the inclusion criteria, 8 declined to participate, and the remaining 74 patients enrolled to the study. Two patients from the TEAS group and one patient from the Control group were later excluded because of protocol breach. A total of 71 patients randomized to treatment allocation completed the study and their data were included in the analysis. Patient demographic characteristics, type, and durations of procedures were similar with no statistically significant differences ($P > 0.05$) between groups (Table 1).

Patients in the TEAS group had significantly higher QoR scores on 24 h after surgery ($P < 0.001$). As shown in Table 2, the QoR-40 scores (mean (SD)) were 176.5 (10.2) and 164.8 (14.7) in the TEAS group and the control group, respectively. The improvement in QoR scores in the TEAS group reflected improvements in the dimensions of emotional status (3 points), physical comfort (4.5 points), psychological support (3 points), physical independence (1 point), and postoperative pain (2.5 points).

Postoperative pain scores were reduced after TEAS at 0.5 h, 1 h, 2 h, 4 h, 8 h, and 24 h after surgery (Figure 3). Compared with the control group, the cumulative number of opioids administered was significantly lower in the TEAS group patients ($P = 0.04$). In addition, the time to first request of rescue analgesia was longer in the TEAS group ($P = 0.039$).

As shown in Table 3, patients in the TEAS group alleviated the incidence of PONV and dizzy, as well as shortened the duration of PACU stay (7.9 min or 22.1%). Simultaneously, the patient's satisfaction scores were significantly higher in the TEAS group than in the control group ($P = 0.002$).

4. Discussion

This study demonstrates that the preoperative TEAS at Hegu (LI4), Neiguan (PC6), Zusanli (ST36), and Sanyinjiao (SP6) enhances the QoR-40 and postoperative analgesia in patients

Table 1: Patient characteristics and operation details.

	Group TEAS ($n = 35$)	Group C ($n = 36$)	P value
Age (year)	34.2 (7.2)	35.6 (8.7)	0.47
ASA (I/II)	34/1	34/2	1.0
Height (cm)	159.1 (6.5)	160.4 (4.9)	0.293
Weight (kg)	53.5 (6.7)	55.8 (8.2)	0.209
Type of surgery			0.562
Ovarian cystectomy	30 (85.7%)	29 (80.6%)	
Myomectomy surgery	5 (14.3%)	7 (19.4%)	
Preoperative QoR-40	186.7 (9.9)	184.6 (8.5)	0.339
Duration of surgery (min)	64.5 (9.2)	63.9 (9.3)	0.776
Duration of anesthesia (min)	72.1 (9.5)	72.8 (9.8)	0.791

Values are mean (SD), or number (%). TEAS: transcutaneous electric acupoint stimulation; C: control.

Table 2: QoR-40 dimensions and global scores.

	Group TEAS ($n = 35$)	Group C ($n = 36$)	P value
QoR-40 dimensions			
Emotional state	40.5 (3.4)	37.9 (5.1)	0.013
Physical comfort	50.8 (4.1)	46.3 (5.7)	<0.001
Psychological support	32.9 (2.3)	31.7 (2.3)	0.029
Physical independence	20.6 (2.4)	19.7 (3.0)	0.152
Pain	31.7 (2.4)	29.2 (3.6)	0.001
Global QoR-40	176.5 (10.2)	164.8 (14.7)	<0.001

Values are mean (SD). TEAS: transcutaneous electric acupoint stimulation; C: control.

undergoing gynecological laparoscopic surgery. In addition, TEAS reduces the incidence of general anesthesia induced side effects, such as dizziness, nausea, and vomiting, shortens the duration of PACU stay, and improves patient's satisfaction. These results suggest that TEAS may be an interesting complementary and alternative analgesic in human subjects.

In this present study, judging from pain intensity and supplemental analgesic requirement, we revealed that preoperative TEAS is an appropriate procedure for acute postoperative analgesia, which was comparable with the previous studies [11–13]. According to the theory of traditional Chinese medicine, surgery as well as anesthesia breaks the balanced state of the human body and disturbs the movement of both qi and blood. Although several literatures supporting that electroacupuncture inhibits sensory and affective dimensions of pain via activation of endogenous pathways, both by exerting a direct inhibitory effect on opioid-sensitive spinal cord interneurones and by promoting enkephalin release [14–16]. The underlying mechanisms of TEAS's analgesic effects have not been clearly clarified.

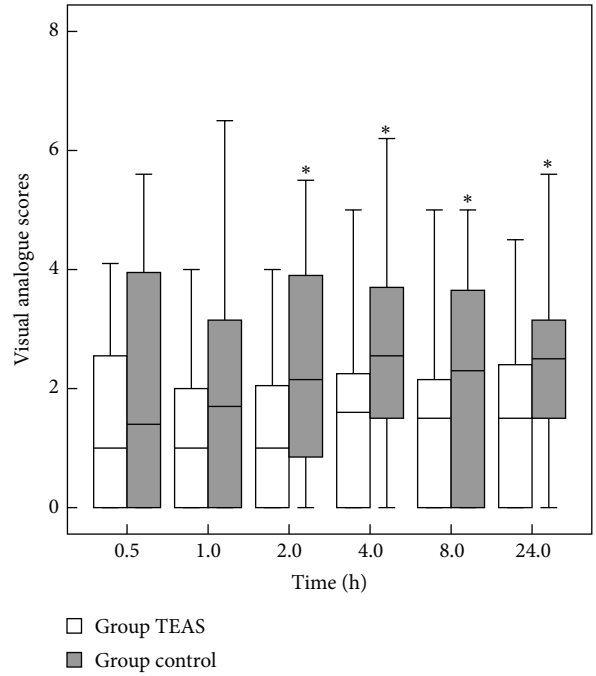

Figure 3: Postoperative visual analogue scores in patients receiving transcutaneous electrical acupoint stimulation (white square) or control treatment (grey square). Error bars are interquartile range. $^{*}P < 0.05$.

Table 3: Patient characteristics in 24 h after surgery.

	Group TEAS ($n = 35$)	Group C ($n = 36$)	P value
Time to first rescue analgesia (min)	59 (31–1440)	47 (13–196)	0.039
Cumulative number of rescue analgesia	1 (1–3)	3.5 (2–7.8)	0.004
Duration of PACU stay (min)	27.8 (8.3)	35.7 (7.2)	<0.001
Dizziness	14 (40.0%)	28 (77.8%)	0.001
Nausea	17 (48.6%)	26 (72.2%)	0.041
Vomiting	7 (20.0%)	19 (52.8%)	0.004
Patient's satisfaction score	8 (6–8)	6 (5–7)	0.002
Satisfaction score ≥8, n (%)	18 (51.4%)	6 (16.7%)	0.002

Values are mean (SD), median (IQR), or number (%). TEAS: transcutaneous electric acupoint stimulation; C: control; PACU: postanesthesia care unit.

The Hegu (LI4) belongs to the Large Intestine Meridian of Hand-Yangming and proved to be associated with analgesic and sedative effect [17]. Stimulation of the Neiguan (PC6), which is one of important acupoints on the Hand-Jueyin pericardium meridian, is suggested to mitigate PONV after surgery [18]. The Zusanli (ST36) is the conjunction point of the stomach channel of the Foot-Yangming. The previous study showed acupuncture at Zusanli (ST36) can improve

upper and lower abdominal symptoms induced by rectal distension [19]. Stimulation of the Sanyinjiao (SP6) is effective in relieving labor pain [20] and dysmenorrhea [21]. From our point of view, applying acupuncture to multiple points achieves greater effects than any single one. Therefore, in our current trial, the acupuncture points Hegu (LI4), Neiguan (PC6), Zusanli (ST36), and Sanyinjiao (SP6) were selected. The dense-disperse frequency of 2/10 Hz was chosen based on previous literatures [22, 23]. The duration of TEAS was 30 minutes in this study, which fits our clinical practice.

In addition, our data confirmed that preoperative TEAS attenuates the incidence of PONV and dizziness. Many studies have supported the efficacy of Neiguan (PC6) acupoint stimulation for preventing PONV [24, 25]. The potential benefits for patients, especially in outpatient gynecological laparoscopic surgery, are faster recovery and rapid discharge after procedure.

There are some limitations to this trial that require consideration when interpreting the results. First, the QoR-40 questionnaire we used was initially tested in the Australian population; cultural differences between countries may limit its generalizability [26]. Second, this is a single center study in a strictly defined patient population. This may potentially limit external validity of the findings [27].

In conclusion, this study is the first verification that preoperative TEAS is effective intervention in improving the quality of recovery, postoperative analgesia, and patient's satisfaction and accelerating discharge after general anesthesia for gynecological laparoscopic surgery. Further studies are required to identify the most beneficial use of TEAS time, frequency, and intensity.

Conflict of Interests

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

Authors' Contribution

Yusheng Yao and Qiuyan Zhao are equal first authors.

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Research Article

Efficacy of Pulsed Radiofrequency on Cervical 2-3 Posterior Medial Branches in Treating Chronic Migraine: A Randomized, Controlled, and Double-Blind Trial

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Objective. The aim of this study was to examine the efficacy and safety of pulsed radiofrequency (PRF) in the treatment of chronic migraine (CM) on cervical 2-3 posterior medial branches. *Methods*. This randomized, double-blind, and controlled clinical trial included 40 subjects with CM, who were randomly divided into two groups: treatment (treated by PRF) and sham (treated by sham treatment). Pain intensity, headache duration (days), the Migraine Disability Assessment Questionnaire (MIDAS), and aspirin dose taken by patients were evaluated at 1, 2, and 6 months after the intervention. Side effects were observed from the time of treatment and throughout the follow-up period. *Results*. During the follow-up, pain intensity, headache duration (days), disability score, and the analgesic dose were significantly improved in the treatment group compared to the sham group ($P < 0.001$) and the baseline ($P < 0.001$) at all measured time points after intervention. No serious complications were reported. *Conclusion*. PRF on the cervical 2-3 posterior medial branches could provide satisfactory efficacy in the treatment of CM without obvious adverse effects.

1. Introduction

CM is diagnosed in patients who suffer from headache at least 15 days per month or who have at least 8 days per month in which the headaches are associated with symptoms that meet the diagnostic criteria for migraine. Migraine affects approximately 2% of patients worldwide [1]. A high frequency of migraines is associated with an increased risk of neck pain and disability [2]. The overall burdens of migraine are higher than the burdens of epilepsy, stroke, or Parkinson disease [3]. Although numerous medications have been available for patients with migraine, still a few patients are insensitive to these therapies [4, 5]. In addition, the overuse of medicine such as opiates and triptans was one of the most important risks of migraine progression [6, 7]. Therefore, effective invasive treatments on CM could not only relieve the pain but also avoid the possible progression of migraine derived from the medicine overuse.

Recently, occipital nerve stimulation (ONS) has become a novel invasive treatment for primary headaches, including cervicogenic headache, occipital neuralgia, cluster, and migraine [8, 9]. ONS could provide benefits to some patients with CM [4, 10, 11]. However, the incidence of complications with ONS was consistently high in published studies [4, 10]. Lead migration, the most common complication of ONS, occurred in 10–100% patients and always required a second surgery [10, 12]. In addition, it was regarded that the incidence of complication is still high even performed by experienced physicians [13]. The possible mechanism of ONS for CM was based on trigeminal vascular reflection and stimulations on upper cervical nerves could enhance the neurons in afferent dural inputs [14]. Therefore, an optimal invasive therapy for CM should not only target upper cervical nerves but also bring fewer complications.

PRF is a non neurodestructive therapy that has been widely used in treating numerous chronic pain conditions such as postherpetic neuralgia and chronic postoperative pain [15–18]. PRF induced very few complications according to previous studies [16, 19]. However, to the best of our knowledge, few studies have emphasized the ability of PRF in treating CM. Anatomically, cervical 2-3 posterior medial

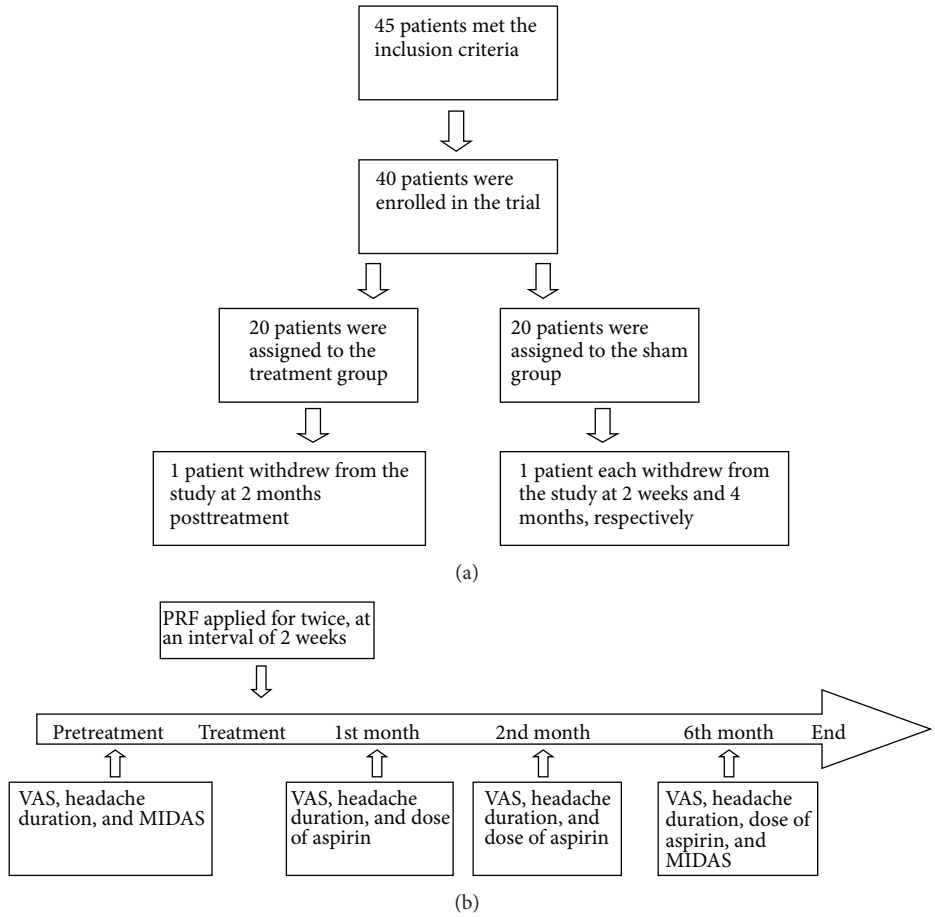


Figure 1: Study flowchart and timeline of the study. (a) Study flowchart. A total of 40 patients were involved in the trial and 37 patients completed the trial. (b) Study timeline described the temporal relationship between the four time points of assessments and the PRF treatments.

branches are the sources of the third occipital nerves (ONs), which could be a possible target for neuromodulation in CM. Therefore, in this study, we designed a randomized, controlled, and double-blind trial to perform PRF on the cervical 2-3 posterior medial branches in the treatment of CM.

2. Methods

2.1. Study Participants. The protocol of this clinical trial was approved by the Human Ethics Committee of Xinhua Hospital. Patients at the Pain Center of Xinhua Hospital from Feb. 2012 to Feb. 2014 were considered for inclusion in this study. All patients had clear understanding of the trial and signed consent forms.

2.2. Inclusion and Exclusion Criteria. Patients were considered eligible for the study if they met the following inclusion criteria: (1) the patient was older than 18 years of age, (2) the patient had suffered for more than 6 months from CM, (3) CM was diagnosed strictly according to the Third Edition of the International Classification of Headache Disorders (ICHD-III) [20], and (4) the patient experienced a greater than 30% reduction in pain after occipital nerve block (ONB) of the cervical 2-3 posterior medial branches before the trial. The exclusion criteria were as follows: (1) obvious psychosis, (2) inability to follow the advice of the physician, (3) involvement in other trials, (4) pregnancy or trying to conceive, and (5) inability to finish the trial for any other reason.

2.3. Grouping, Randomization, and Blinding. Among the 45 patients who met the criteria, 5 patients refused to sign the consent forms. 40 patients were divided into two equal groups by a random number table: a treatment group (treated by PRF) and a sham group (treated with sham treatment). Detailed information on study enrollment and design are shown in the flowchart and timeline (Figure 1). Doctors and patients were blinded to the grouping. Information on grouping was preserved by an investigator who was separated from the operation and follow-up until the end of the trial. There was no communication about the grouping between the investigator who had this information and the investigators related to the clinical trial.

2.4. Intervention Procedure. All procedures were performed within a sterile environment with the patient in a prone

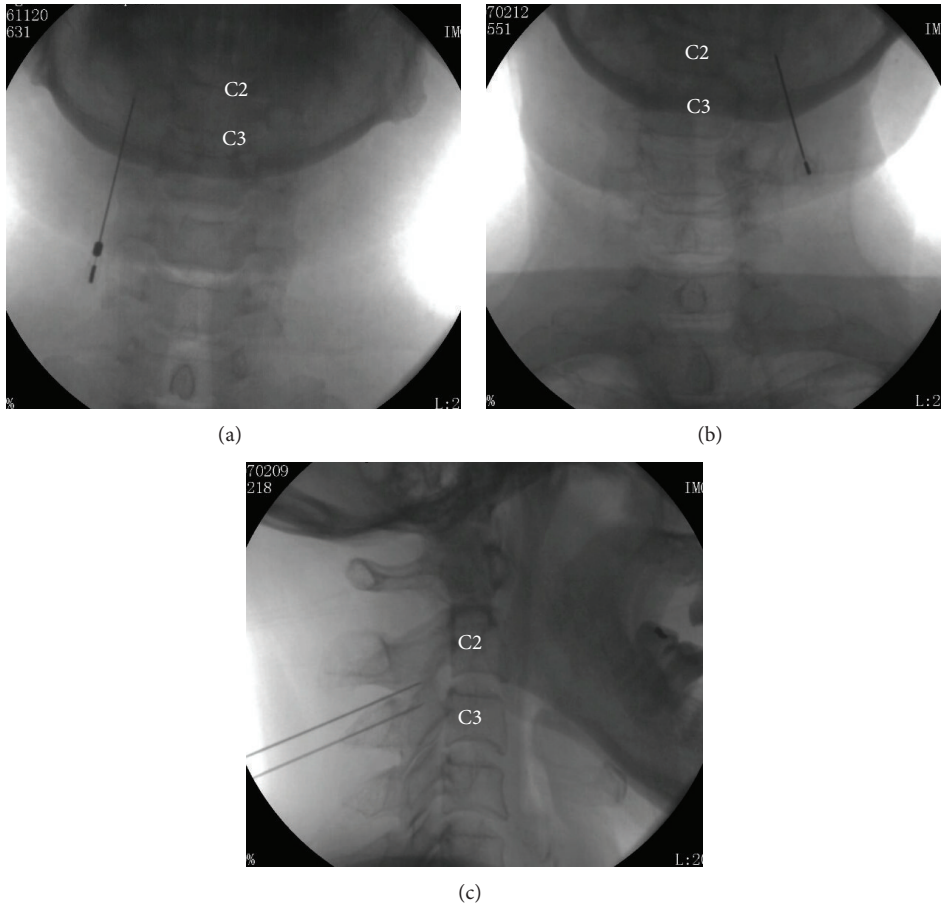


Figure 2: X-ray photos in the PRF treatments. ((a) and (b)) The C-arm machine was placed in the anteroposterior position and the puncture points were at the C2 level. (c) The C-arm machine was placed in the lateral position. The tip of the needles reached the medial branch of C3 and third ONs (C3 and C2, resp.).

position. In the first phase, the C-arm machine was placed in the anteroposterior position. The C2 and C3 levels were confirmed by puncture needles through the C-arm. The first entry point was the intersection of the edge of the C2 vertebral bodies and the midline between the C2 and C3 levels. Lidocaine was injected hypodermically to provide local anesthesia. The C-arm machine was changed to the lateral position. A 21-gauge cannula with a 5 mm exposed tip was punctured vertically at C2, as had been marked by the C-arm previously.

The cannula was inserted slowly until the tip reached the front bottom of the C2 inferior articular process, to align with the third ON under the monitoring of the C-arm (Figure 2). The cannula was connected to the radiofrequency generator and the needle tip was adjusted slightly under the sensation test mode (50 HZ, 0.3 V). An abnormal sensation on the part of the patient indicated that the needle was extremely close to the third ON.

The generator was turned to the PRF mode (42°C, 120 seconds, twice for each level). During PRF, the healthcare provider ensured that the cannula did not move. The second entry point was the intersection of the edge of the C3 vertebral body and C3 level. After local anesthesia, the cannula was inserted slowly until the tip reached level of the zygapophyses, to align with the medial branch of C3. The cannula was connected with the generator and the steps were repeated as for the C2 PRF.

In the sham group, the same procedures were applied except that no energy was used. All treatments in both groups were performed unilaterally. The generator was operated by an investigator who was not involved in the follow-up. Patients left the hospital after 1 day of observation. A second PRF or sham treatment was given after an interval of 2 weeks.

2.5. Outcome Measures. Follow-up procedures were carried out in 1, 2, and 6 months after the intervention. Pain intensity, headache duration (days), analgesic dose, Migraine Disability Assessment Questionnaire (MIDAS) score, and adverse effects were the main outcome measures that were recorded during the follow-up.

Pain intensity was defined as the average pain intensity during the migraine attack, as recorded on the visual analogue scale (VAS). Pain relief of more than 30% at the 6-month follow-up was defined as "effective." The headache duration was defined as the number of days that patients suffered from migraine per month. MIDAS was assessed

Table 1: Patients' demographics and baseline headache data.

Group	Treatment	Sham
Men/women (number of patients)	3/17	4/16
Age (years)	43.5 ± 11.07	43.55 ± 7.82
Headache history (years)	15.25 ± 8.37	18.75 ± 9.98
Baseline VAS	7.75 ± 0.96	7.45 ± 0.88
Baseline headache duration (days/month)	21.05 ± 3.36	19.65 ± 3.66
Baseline MIDAS score	63.05 ± 19.89	63.60 ± 16.59

There was no significant difference between two groups in these data above.

twice: before and 6 months after PRF or sham treatments. Aspirin was used as the routine analgesic, at a dose of 300 mg as needed. The total dose of aspirin used in a month was recorded.

Adverse effects of patients were recorded immediately after the intervention and continued until study completion. In addition to the routine follow-up, patients were able to report the related symptoms to our investigators at the pain clinic. Adverse effects included infection, numbness, increased pain, and paresthesia.

2.6. Sample Size and Statistical Analysis. Sample size was calculated by G-power 3.17. Statistical analysis was performed by SPSS19.0. Continuous data were presented as mean ± standard deviation or as the median (interquartile range) if the data were in a skewed distribution. The difference between two groups was calculated by t-test. The difference at different time points in the same group was calculated by repeated-measures ANOVA. Differences of enumeration data were evaluated by χ^2 test.

A sample size calculation was performed to calculate the sample size needed to detect a statistically significant difference at the 0.05 level with a power of 80%. According to a pilot study, pain of patients in the treatment group was reduced by 30 to 40%, compared to 15% in the sham group. Therefore, the calculated minimum total sample size was 36.

3. Results

In this study, 40 patients were enrolled and 37 patients completed the follow-up. The demographic characteristics of the patients were similar in both groups. There were no significant differences in sex, age, migraine history, or baseline migraine conditions between the groups (Table 1).

The mean VAS decreased by 2.52 points in the treatment group compared to 0.55 points in the sham group at the 6-month follow-up time point. There was a significant interaction between the variables of treatments and follow-up period ($F = 111.7$, $P < 0.001$). The VAS differed significantly between the treatment and the sham groups at the 1-month ($t = 4.08$, $P < 0.001$), 2-month ($t = 4.86$, $P < 0.001$), and 6-month ($t = 3.27$, $P < 0.01$) follow-up periods. When "effective" was defined as a 30% reduction in pain at the 6-month follow-up, there was a significant difference in the numbers of patients with effective outcomes between the treatment and the sham group ($P < 0.05$). No patient in either group achieved a 50% reduction in pain intensity (Figure 3).

Table 2: The mean doses of aspirin taken by patients in the treatment group were significantly lower than those in the sham group.

Group	1st month	2nd month	6th month
Treatment	6.15 ± 2.03* ($n = 20$)	6.16 ± 2.58* ($n = 19$)	6.37 ± 1.83* ($n = 19$)
Sham	14.79 ± 5.10 ($n = 19$)	14.32 ± 5.17 ($n = 18$)	14.00 ± 4.71 ($n = 18$)

*$P < 0.001$ versus the sham group.

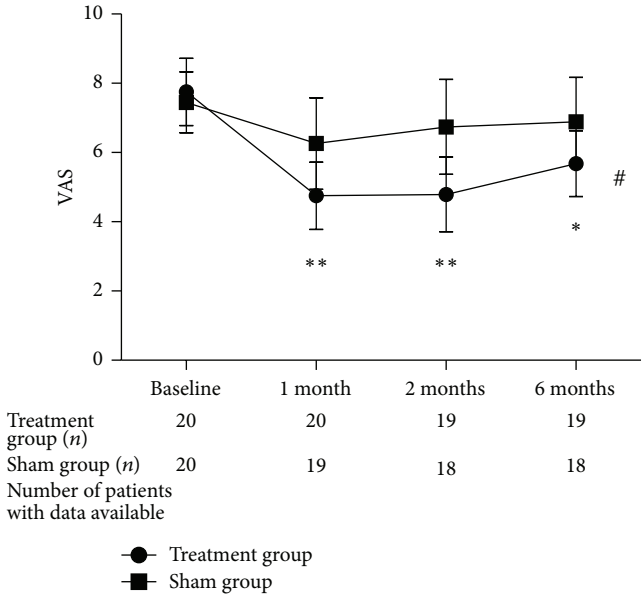
The mean decrease of headache duration in the treatment group was 8.9 days per month at the 6-month follow-up. There was a significant interaction between the variables of treatments and follow-up period ($F = 232.3$, $P < 0.001$). There was a significant difference in the decrease of headache duration between the treatment and the sham groups at the 1-month ($t = 8.14$, $P < 0.001$), 2-month ($t = 7.93$, $P < 0.001$), and 6-month ($t = 7.11$, $P < 0.001$) follow-up time points (Figure 4).

The patients in the treatment group took a significantly lower aspirin dose compared to the patients in the sham group throughout the follow-up period. The aspirin dose differed significantly between these two groups at the 1-month ($t = 7.0$, $P < 0.001$), 2-month ($t = 6.14$, $P < 0.001$), and 6-month ($t = 6.57$, $P < 0.001$) follow-up periods (Table 2). The mean MIDAS score in the treatment group was 21.57 points lower than that in the sham group at the 6-month follow-up time point. The MIDAS scores were significantly decreased after PRF treatment compared to the baseline ($t = 10.25$, $P < 0.001$) and between the two groups ($t = 4.72$, $P < 0.001$, Figure 5).

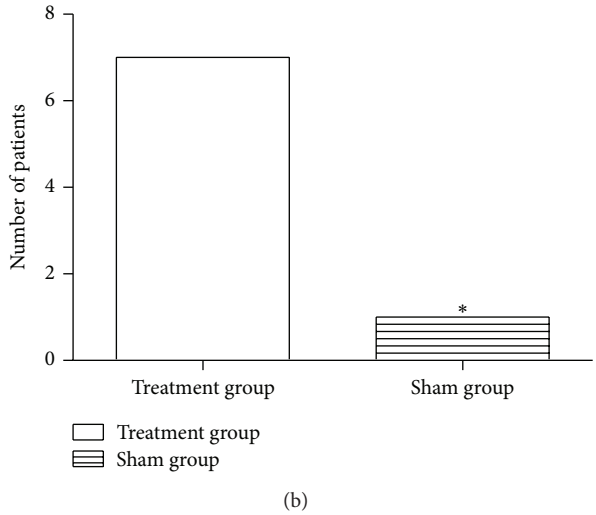
No patient experienced abnormal bleeding, infection, numbness, postoperative paresthesia, increased pain, or any other complication during the perioperative period. One patient in the treatment group reported mild pain at the injection site after the second round of PRF treatments and the pain subsided within 6 hours without any treatment. No complication was recorded at the follow-up.

4. Discussion

In this clinical trial, we have shown that using PRF on the cervical 2-3 posterior medial branches could result in satisfactory efficacy of CM. We chose the cervical 2-3 posterior medial branches as the target in this treatment because of their anatomy. The dorsal ramus of the C2 spinal nerve ultimately becomes the greater ON, which supplies the splenius capitis and semispinalis capitis. The deep branch of the dorsal ramus of the C3 spinal nerve, also known as the third ON, supplies the C2-C3 zygapophyseal joint and the skin over the suboccipital region [9]. The ONs have been regarded as a therapeutic target in migraine. For example, ONB and ONS have been shown to provide benefits in both pain intensity and headache days in migraineurs [1, 21–23].



(a)



(b)

Figure 3: Reduction of pain intensity in the two groups. (a) There was a significant time-related change during the follow-up of the treatment group compared to the sham group. The P value of the independent-sample t-test refers to the difference between groups in the pain intensity at different time points. The VAS was improved in the first month and stabilized by the sixth month. $^{*}P < 0.01$ and $^{**}P < 0.001$ versus the sham group, $^{\#}P < 0.001$ change by time interaction in the treatment group. (b) The histogram demonstrates the number of patients achieving pain reduction. There were significant differences between groups in numbers of the patients achieving more than 30% pain reduction. $^{*}P < 0.05$ versus the treatment group.

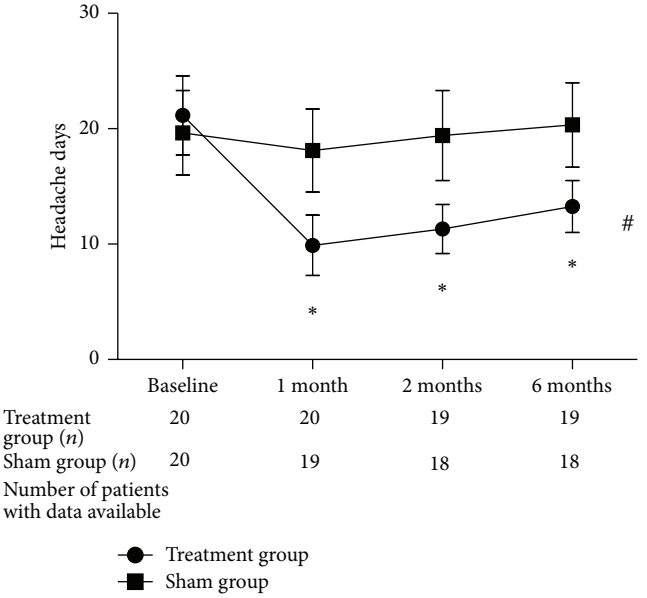


Figure 4: Headache duration in the two groups. Treatments resulted in a significant time-related reduction in the number of days that patients experienced headaches throughout the follow-up period. $^{*}P < 0.001$ versus the sham group. $^{\#}P < 0.001$ change by time interaction in the treatment group.

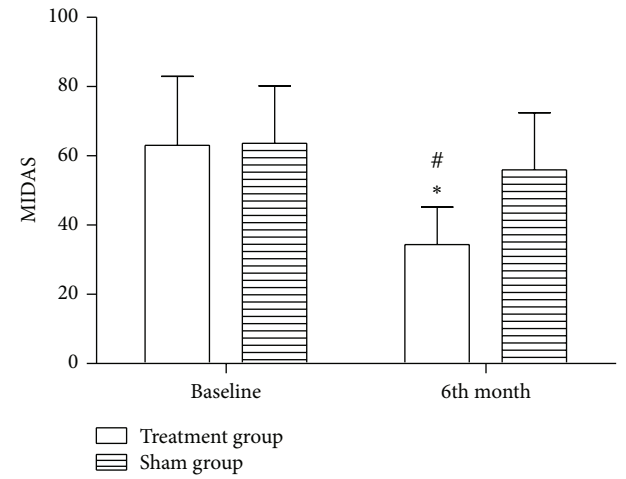


Figure 5: MIDAS scores of the two groups. The MIDAS scores in the treatment group were significantly improved compared to the baseline and to the sham group. $^{*}P < 0.001$ versus the baseline and $^{\#}P < 0.001$ versus the sham group.

For these reasons, the cervical 2-3 posterior medial branches were chosen in this clinical trial.

The mechanism of ON-related treatments is mainly based on the trigeminal vascular system [24]. Pain-sensitive structures, including the intracranial vessels, the meninges, and especially the dura mater, are innervated by the ophthalmic ramus of the trigeminal nerve that arises from pseudounipolar neurons located in the trigeminal ganglion. These neurons project onto second-order sensory neurons in the trigeminal nucleus caudalis in the brain stem [25]. The upper cervical roots and nucleus caudalis of the trigeminal tract converge at the C2 level. This convergence is referred to as the trigeminocervical complex.

During migraine, the stimulation of pain-sensitive structures activates neurons of the trigeminal ganglion, which

projects to the central nervous system and induces peripheral and central sensitization in migraine [25]. Sensitization of meningeal nociceptors arising from the first-order trigeminal neurons, known as peripheral sensitization, could explain the aggravation of intracranial hypersensitivity in physical activities, such as coughing [26]. Central sensitization is based on the concept that the stimulation of pain-sensitive structures also sensitizes the second-order trigeminovascular neurons located in the medullary dorsal horn (MDH). The MDH receives input from the dura and the periorbital skin, which could explain the hypersensitivity in the periorbital skin [26]. In addition, the cutaneous allodynia is regarded as an individual risk factor for the transformation of CM [1]. Direct stimulation on the ONs could excite the second-order trigeminal afferents in rats [14], which may be the potential mechanism of the therapy of stimulating the cervical 2-3 posterior medial branches in this trial.

Before the PRF treatments, an ONB was applied to help predict the potential curative effect of the target nerves. Similar tests have been performed in former PRF studies [27, 28]. In some ONS studies, ONB was administered to help to provide a clear prediction [10, 12]. However, a recent study found that ONB does not sufficiently predict ONS responsiveness [29]. In our study, approximately 50% of patients achieved a pain reduction of less than 30%, even though they had received a 30% pain reduction after ONB. These results indicated that the nerve block could not provide sufficient prediction of PRF efficacy in CM.

PRF is a minimally invasive neuromodulation approach that has been used to treat chronic pain of various origins [15, 17]. A common working temperature of PRF is 42°C, which is below the minimum threshold for irreversible tissue destruction of 45°C. PRF achieves neuromodulation in numerous aspects, including microstructure damage and the endogenous pathway. In a previous study, microscopic damage was found in the internal ultrastructural components of the axons. This damage was more obvious in C-fibers than in the A-delta or A-beta fibers, consistent with the fact that C-fibers and A-delta fibers are the principal sensory nociceptors [30]. In the endogenous pathway, PRF could increase the level of endogenous opioid precursor mRNA and the corresponding opioid peptide [31]. In a recent study, PRF was able to regulate proinflammatory gene expression at the injury site, dorsal root ganglion (DRG), and spinal cord [32]. These changes along the nociceptive pathway could explain the efficacy of PRF in the peripheral and central aspects of neuropathic pain, such as postherpetic neuralgia [16]. Therefore, in this trial, PRF was utilized to provide neuromodulation of the cervical 2-3 posterior medial branches, to reduce the peripheral and central sensitization of CM and, ultimately, to decrease the pain intensity and headache duration.

No obvious side effects were observed in this trial. PRF is an invasive procedure that provides reversible neuromodulation without tissue damage. The puncture was performed under C-arm monitoring to minimize the risks of injury to the carotid artery or spine. PRF on the cervical 2-3 posterior medial branches has not been reported previously. However, PRF on the greater ON has been used in the treatment of cervicogenic headache and no serious complications were reported [28]. This finding indicated that the greater ON (cervical 2-3 posterior medial branches) was a safe therapeutic target for chronic headache. Compared to the numerous complications that were associated with ONS, including lead migration and infection [10, 13], PRF on the cervical 2-3 posterior medial branches was easier to perform and associated with fewer complications. In addition, PRF was a minimally invasive therapy that led to less treatment-related pain compared to ONS.

There were some limitations in this clinical trial. The trial was designed as a single-center study and had a small sample size. The follow-up period was only 6 months. Therefore, the long-term efficacy of this therapy could not be determined. Moreover, it remained unknown whether the efficacy of PRF on the cervical 2-3 posterior medial branches is superior to ONS. These limitations could be addressed with future studies.

5. Conclusion

PRF on the cervical 2-3 posterior medial branches could provide a satisfactory treatment that can reduce pain intensity, headache duration, and disability scores. The procedure was relatively easy to perform and resulted in few side effects.

Conflict of Interests

The authors declared that there is no conflict of interests in their submitted paper.

Authors' Contribution

Yuecheng Yang and Xuehua Huang contributed to the paper equally.

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Research Article

Study on the Antinociceptive Effects of Herba Epimedium in Mice

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The present study was conducted to investigate the antinociceptive action of relationship between Herba Epimedium (HE) and 5-HT_{1A} receptor, between Herba Epimedium (HE) and 5-HT_{2A} receptor. We used the hot-plate method and the writhing assay in mice by the intracerebroventricular (i.c.v.) injection and observed the analgesic effect of HE. Furthermore, through the i.c.v. injection, 5-HT_{1A} receptor partial agonist Buspirone, antagonist Propranolol, the adrenaline β_1-receptor selective blocking agent Metoprolol, and 5-HT_{2A} receptor agonist hydrochloride DOI and antagonist Ketanserin were used, and, 5 min later, HE was used to investigate the impacts of drugs on the analgesic effect in the same way. Results showed that HE had fast and significant antinociception in nervous system, and the effects can persist for a long time. Buspirone and Hydrochloride DOI can remarkably increase the antinociception of HE in nervous system. Ketanserin leads to a significant decrease in its antinociception in nervous system; Metoprolol also has antinociceptive action in nervous system, but it can inhibit the antinociceptive effect of Herba Epimediumin peripheral region. These results suggest that HE has significant antinociception effect and its mechanism is related with 5-HT_{1A} receptor, 5-HT_{2A} receptor, and adrenaline β_1-receptor.

1. Introduction

The 5-hydroxytryptamine (5-HT) plays an important role in the nociceptive transmission. The involvement of central monoaminergic pathways in the antinociceptive mechanisms has been emphasized, and the role of serotonin (5-HT) in the modulation of the nociceptive stimuli has also been demonstrated [1, 2]. The central serotonergic pathways have been claimed to exert antinociceptive effects in defined brain areas through their receptors, notably 5-HT_{1A} and 5-HT_{2A}, as well as through interactions with opioid and γ-aminobutyric acid pathways [3–7].

Many previous studies have shown that activation of 5-HT_{1A} receptor can produce a strong analgesic effect [8–10] in the model of formalin-induced chronic pain, surgery, and postoperative pain of neuropathic pain models.

The distribution of 5-HT_{2A} receptor-labelled neurons in dorsal root ganglia (DRG) neurons is mainly small and medium-sized cells, which are nociceptive stimulation of primary sensory neurons. These cells mostly express both pain-related and calcitonin gene-related peptides [11]. The previous researches demonstrated that the antinociceptive effect of 5-HT_{2A} receptor agonist Hydrochloride DOI in the formalin test is dose-dependent in rats [12].

Herba Epimedium (HE) is a family of plants consisting of Epimedium genera, and also is one of the most frequently used herbs in formulas for the treatment of lots of diseases in China. Modern medical researches have showed that HE contains flavonoid compounds and has the antinociceptive effect [13]. HE has the analgesic effect, but there are few researches about it and the detailed mechanisms underlying the analgesic effects of it are still unclear. In the present study, we investigated the mechanism of its analgesic effect using the hot-plate test and the writhing assay, by i.c.v. injections of 5-HT_{1A} receptor, 5-HT_{2A} receptor agonists, and antagonists,

with the aim to reveal the relationship of analgesic effect between HE and the 5-HT receptors.

2. Materials and Methods

2.1. Animals. Adult female Kunming mice were obtained from Animal Center of Medicine Department in Xi'an Jiaotong University. All mice were housed in standard cages and maintained on a 12:12 h light/dark cycle under conditions of constant 23°C ambient temperature. The mice were given a free access to food and water. After one-week acclimatization, these mice were assigned randomly into a group fed with standard mice chow ($n = 8$, weight: 18–22 g).

2.2. Drugs. Herba Epimedium (Epimedium enshiense B. L. Guo et Hsiao), the 5-HT_{1A} receptor partial agonist Buspirone, the beta-noradrenergic receptors nonselective antagonist propranolol, the β_1-adrenergic selective antagonist metoprolol, and the 5-HT_{2A} receptor preferential antagonist Ketanserin were purchased from Research Biochemicals. The 5-HT_{2A} receptor selective agonist DOI-Hydrochloride was purchased from Sigma.

2.3. Drug Treated Groups. In this study, the mice were divided into the control group, of which each group was treated with i.c.v. injection of saline solution (2 μm/L), the HE + Metoprolol group, of which the mice were treated with Herba Epimedium (1 : 4), the β_1-adrenergic selective antagonist Metoprolol (2 μm/L), and Herba Epimedium, respectively, and four separate groups of mice were treated with the 5-HT_{1A} receptor partical agonist Buspirone (2 μm/L), the beta-noradrenergic receptors nonselective antagonist propranolol (2 μm/L), the 5-HT_{2A} receptor preferential antagonist Ketanserin (1 μm/L), and the 5-HT_{2A} receptor selective agonist DOI-Hydrochloride (1 μm/L), respectively, administered 5 min after Herba Epimedium (1 : 4) administration.

2.4. Preparation of Aqueous Extract of Epimedium. Herba Epimedium (100 g) were soaked in (how much) mL of distilled water for an hour and then boiled three times, every time for 30 min. Then the filtration was combined, and the first extraction was concentrated to 200 mL by simmering and then was extracted with 200 mL of 95% ethanol for 3 times and placed at 4°C or −20°C overnight. The compound was filtered. After filtration, each residual was discarded and the final filtrates were concentrated under vacuum to eliminate solvent and double distilled water was added into the extract to the final volume of 10 mL. 2% NaOH was used to adjust the pH to 7.0. The concentration of the herbal soup is equivalent to crude herb of 1 g/mL, kept in refrigerator.

2.5. Drugs Administration and i.c.v. Injection. The 5-HT receptors agonists and antagonists were dissolved in sterile 0.9% NaCl as vehicle containing 0.1% Evans Blue dye. This saline solution was used as a vehicle and the control treatment. i.c.v. injection was performed according to a method reported previously [14, 15]. The needle was placed vertically on the head, the needle was lightly forward to locate bregma, and the needle position was 1 mm posterior and 1 mm lateral to the bregma. Herbal solutions were infused slowly (over 30 s) into the lateral ventricle and injected i.c.v. in a volume of 10 μL. At the end of each experiment, the mice were sacrificed by injection of an overdose of urethane. Then the brain was removed to confirm the accuracy of injection. The data from brains which confirmed having Evans Blue dye present in the lateral ventricle were used.

2.6. Hot-Plate Test. In this test, animals were individually placed on a hot plate with constant temperature (55 ± 0.5°C) [16]. The latencies to first hind paw withdrawal during thermal stimulation were measured in seconds as indexes of nociceptive threshold with cut-off time of 50-second reaction times were measured with a stopwatch and each of the mice was tested before treatment and 5, 15, and 30 min after treatment. Those mice scores below 10 s or over 60 s in the pretest were rejected, after which the animals were immediately removed from the hot plate.

2.7. Writhing Test. The writhing test described by Hayashi and Takemori [17] was used to assess the analgesia effect. Mice were i.c.v. injected with 2% acetic acid in order to produce the typical writhing reaction, which is characterized by a wave of contraction of the abdominal musculature followed by extension of the hind limbs. The mice were then placed in an individual container and the number of writhes in 35 min was counted, starting from 5 min after acetic acid administration.

2.8. Statistical Analysis. Data from experiments were statistically analyzed with one-way analysis of variance (ANOVA) and t-test. With respect to treatment and time, the Kruskal-Wallis test was used. Data are expressed as means ± SEM. Statistical significance was set at $P < 0.05$ for all experiments.

3. Results

3.1. The Pain Threshold in Hot-Plate Test of HE + Metoprolol Groups. The i.c.v. administration of HE and Metoprolol potently increased the pain threshold 30 min after injection in the mouse hot plate. Antinociception of the Herba Epimedium group reached the maximum after 15 min, and the Metoprolol (2 μmol per mouse) reached the maximum after 5 min. Both of them can persist for up to 30 min. The mice having received i.c.v. HE and Metoprolol exhibited significant antinociception. There was no significant difference in the increase in pain threshold following i.c.v. injection between the HE group and Metoprolol group ($P > 0.05$) (Figure 1).

3.2. The Antinociceptive Effect of HE and Metoprolol on the Abdominal Constriction Test. The antinociceptive effects of Herba Epimedium and Metoprolol on the abdominal constriction test were illustrated in Figure 2. The abdominal constriction test was performed 35 min after i.c.v. administration. The writhing times of mouse were significantly decreased by the HE ($P < 0.01$) and persisted for up to 35 min ($P < 0.05$), but the effect of Metoprolol was different

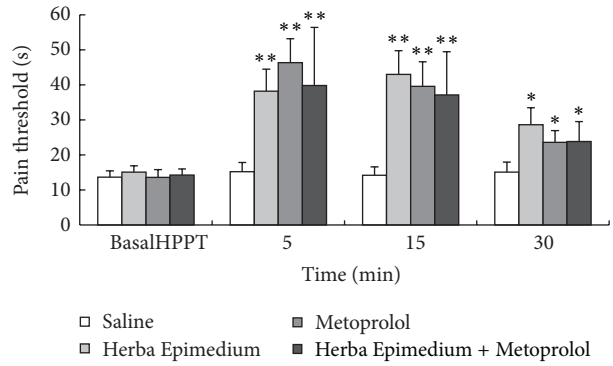

FIGURE 1: The pain threshold in hot-plate test during 30 min after i.c.v. injection of Herba Epimedium and Metoprolol. Values are means ± S.E.M. Significant difference from the control (saline) at each time point represented $^{**}P < 0.01$ and $^{*}P < 0.05$.

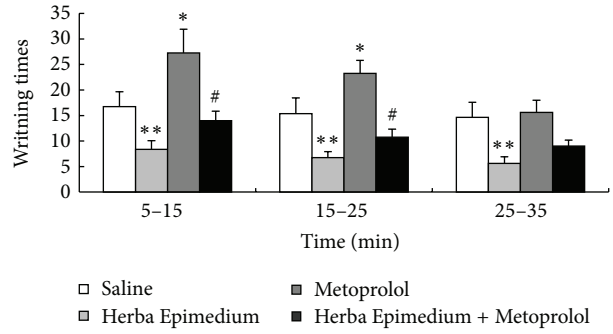

FIGURE 2: The writhing times in the mouse acetic acid abdominal constriction test during 35 min after i.c.v. injection of HE and Metoprolol. Values are means ± S.E.M. Significant difference from the control (saline) at each time point represented $^{**}P < 0.01$ and $^{*}P < 0.05$. $^{\#}P < 0.05$ compared to HE.

from those in the hot-plate test. It had no antinociceptive effect compared to the vehicle group. The writhing times were significantly increased during 25 min after the administration ($^{*}P < 0.05$). Posttreatment after 35 min had no significant difference from the control group. Whereas the writhing time was significantly decreased after injection of HE and Metoprolol compared to the Metoprolol group during 25 min after the administration ($^{\#}P < 0.05$), the magnitude of the three phase did not differ significantly from the Metoprolol group.

3.3. The Effect of i.c.v. Injection of HE, Buspirone, and Propranolol. The effect of i.c.v. injection of HE, Buspirone, and Propranolol on 30 min pain threshold value was shown in Figure 3. In the hot-plate test, HE strongly increased the pain threshold value and persisted for up to 30 min ($^{a}P < 0.01$); 5-HT_{1A} receptor partial agonist Buspirone can increase the antinociceptive effect of HE 15 min after injection ($^{b}P < 0.05$). However, the antagonist Propranolol significantly decreased the antinociception of HE through 30 min after injection ($^{c}P < 0.01$).

3.4. The Effect of 5-HT_{2A} Receptor Agonist (±)-DOI Hydrochloride and Antagonist Ketanserin. The i.c.v. administration of 5-HT_{2A} receptor agonist (±)-DOI Hydrochloride increases the antinociceptive effect of HE at 15 min and 30 min ($^{b}P < 0.05$) in the hot-plate test, but the 5-HT_{2A} receptor antagonist Ketanserin inhibited the antinociception of HE at 30 min after injection ($^{c}P < 0.01$) (Figure 4).

3.5. The Antinociception of HE, Propranolol, and Buspirone in the Acetic Acid Abdominal Constriction Test. This experiment indicated that the 5-HT_{1A} receptor antagonist Propranolol can increase the writhing times in the test compared to the control group and the magnitude of inhibitory effect was similar to that experiment of hot plate. 5-HT_{1A} receptor partial agonist Buspirone can also increase the antinociception of HE in the acetic acid abdominal constriction test. The writhing times significantly decreased compared with the HE group ($^{b}P < 0.05$) (Figure 5).

3.6. The Antinociceptive Effects of Hydrochloride DOI and Ketanserin in the Acetic Acid Abdominal Constriction Test. The abdominal constriction test was performed 35 min after i.c.v. administration. The test indicated that 5-HT_{2A} receptor antagonist Ketanserin could suppress the antinociception of HE; the writhing times of mouse were significantly increased at 35 min after injection, compared to the HE group ($^{a}P < 0.05$). 5-HT_{2A} receptor agonist (±)-DOI Hydrochloride could

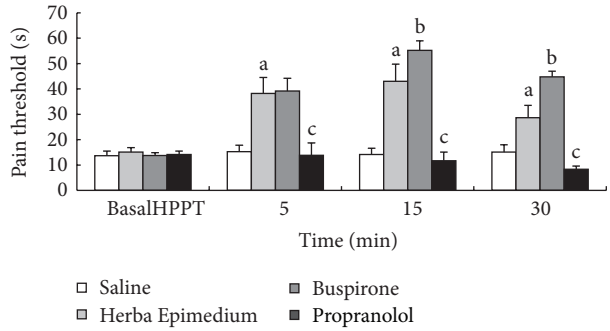

Figure 3: Pain threshold in hot-plate test during 30 min after i.c.v. injection of HE, Buspirone, and Propranolol. Values are means ± S.E.M. $^{a}P < 0.01$ compared to corresponding control group. $^{b}P < 0.05$, $^{c}P < 0.01$ compared to corresponding Herba Epimedium group.

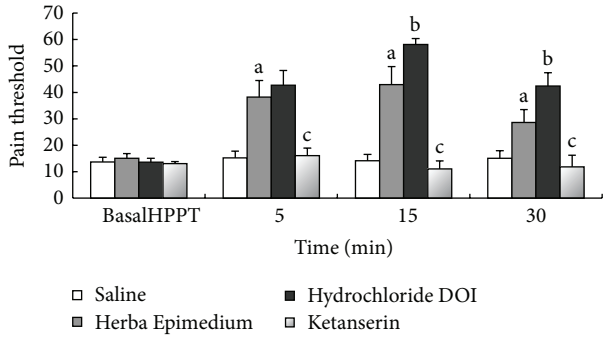

Figure 4: Pain threshold in hot-plate test during 30 min after i.c.v. injection of HE, Hydrochloride DOI, and Ketanserin. Values are means ± S.E.M. $^{a}P < 0.01$ compared to corresponding control group. $^{b}P < 0.05$, $^{c}P < 0.01$ compared to corresponding HE group.

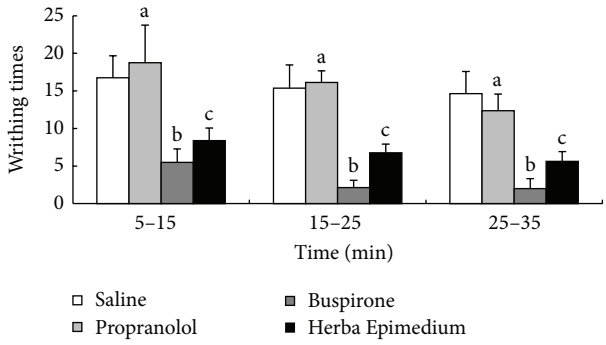

Figure 5: The writhing times in the mouse acetic acid abdominal constriction test during 35 min after i.c.v. injection of HE, Propranolol, and Buspirone. Values are means ± S.E.M. $^{a}P < 0.01$, $^{b}P < 0.05$ compared to corresponding Herba Epimedium group. $^{c}P < 0.01$ compared to corresponding control group.

increase the antinociceptive effect of HE and the writhing times decreased 35 min after injection ($^{b}P < 0.05$) (Figure 6).

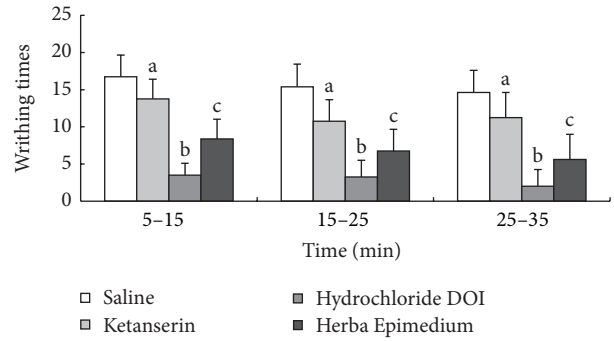

Figure 6: The writhing times in the mouse acetic acid abdominal constriction test during 35 min after i.c.v. injection of HE, Hydrochloride DOI, and Ketanserin. Values are means ± S.E.M. $^{a}P < 0.05$, $^{b}P < 0.05$ compared to corresponding HE group. $^{c}P < 0.01$ compared to corresponding control group.

4. Discussions

Herba Epimedium, which contains several medically active constituents including flavonoids and phytosteroids, has been widely used in China in the treatment of cardiovascular diseases, infertility, impotence, amnesia, lumbago, arthritis, numbness, and weakness of the limbs for thousands of years [18, 19]. Recently, several studies have shown that the crude extract, total flavonoids, and main flavonoid constituents from Herba Epimedium had the antinociception effect [13, 20]. In our study, the Herba epimedium significantly elevated the pain threshold in the mouse hot-plate (Figure 1) and abdominal constriction (Figure 2) tests. Total flavonoids and main flavonoid constituents had the antinociceptive effect [21]. However, the mechanism of antinociception of Herba Epimedium aqueous extract which contained flavonoid was not clear. Adrenaline β_1-receptor selective blocking agent has sedative, analgesic, and anxiolytic effects by blocking the central β receptor [22]. In the present study, Metoprolol showed opposite effects between the hot-plate tests (Figure 1). And in abdominal constriction test (Figure 2), Metoprolol has the significant antinociception in the central nervous system, but has the role of pain in peripheral region. It was possible that Metoprolol only had the antinociceptive effect in central nervous system. The increase of pain threshold was also detected by injection of both Metoprolol and Herba Epimedium, the results indicated that Herba Epimedium had the inhibitory effect to the Metoprolol, especially in the abdominal

constriction test, and the writhing times of mouse were significantly decreased 35 min after injection ($P < 0.01$) in the present study. In vitro and animal experiment had proved that total flavones of Herba Epimedium have the selective block effect on adrenergic β_1 receptor [23].

5-HT is an endogenous bioactive substance released from platelets and mast cells in injured or inflamed tissues. Endogenous or exogenous 5-HT causes noxious and hyperalgesic reactions in peripheral tissues in human and animals. Intracutaneous injection of platelets causes acute pain and hyperalgesia in human [24], it was reported that subtly 5-HT receptors were involved in 5-HT induced pain and hyperalgesia. So many selective drugs have been allowed to develop for treating inflammatory pain and hyperalgesia. Bianchi et al. [25] demonstrated that the full 5-HT_{1A} agonist 8-OH-DPAT was able to increase ACh release from the cerebral cortex of freely moving guinea pigs. 5-HT_{1A} receptor partial agonist Buspirone was able to induce antinociception in mice; the increased pain threshold was detected by using both thermal (hot-plate test) and chemical stimuli (abdominal constriction test) [26]. In the present study, the result indicated that Buspirone can significantly increase the antinociceptive effect of Herba Epimedium; it was possible that analgesic of Herba Epimedium was related to the 5-HT_{1A} receptor. The antinociception of Herba Epimedium was suppressed by the antagonist Propranolol.

It is well known that 5-HT is involved in many complex effects on pain and hyperalgesia through various subtype of receptors located at various levels of the pain transmission system. In a previous experiment, the intraperitoneal administration of acetic acid formalin evoked extremely long-lasting inhibition of somatic inflammatory pain in mice. The study indicated that long-lasting antinociception was completely blocked by the 5-HT_{2A} receptor antagonists, Ketanserin [27]. Peiró et al. reported 5-HT inhibition of the kappa-opioid component of morphine antinociceptive effects and the possibility that this serotonergic inhibition could be reversed through 5-HT2 receptor antagonist Ketanserin [28]. Crisp et al. reported that intrathecal administration of 5-HT agonist, 8-OH-DPAT produced hyperalgesia with the tail-flick test and analgesia with the hot-plate test, and 5-HT_{1B} agonist, TFMPP, 5-HT_{2A} agonist and DOI phenylbiguanide produced analgesia with the hot-plate test in the rat [29]. Therefore, the 5-HT receptor agonists or antagonists may act and change the pain-related behavior. In this study, the i.c.v. administration of 5-HT_{2A} receptor agonist (±)-DOI Hydrochloride can increase the antinociceptive effect of Herba Epimedium at 15 min and 30 min ($P < 0.05$) in the hot-plate test, but the 5-HT_{2A} receptor antagonist Ketanserin inhibited the antinociception of Herba Epimedium through 30 min after injection ($P < 0.01$). As reported previously [30], the Ketanserin has a nonselective action, also exerting some effects on alpha-1-noradrenergic receptors.

In the acetic acid abdominal constriction test, the study indicated that 5-HT_{2A} receptor antagonist Ketanserin could suppress the antinociception of Herba Epimedium. The writhing times of mouse were significantly increased through 35 min after injection, which compared to the Herba Epimedium group ($^aP < 0.05$). 5-HT_{2A} receptor agonist (±)-DOI Hydrochloride could increase the antinociceptive effect of Herba Epimedium and the writhing times decreased during the 35 min ($^bP < 0.05$). Therefore, the hyperalgesia induced by the hot-plate test and acetic acid test was inhibited by Herba Epimedium, and the antinociception of Herba Epimedium was mediated via the 5-HT_{2A} receptor agonist and antagonist.

Several evidences indicated that 5-HT_{1A} and 5-HT_{2A} receptor subtype contributed to antinociception. Moreover, Herba Epimedium has the antinociceptive effect in central nervous system and peripheral region [13, 20]. At the moment, we speculate that the antinociception of Herba Epimedium was mediated by the agonist and antagonist of 5-HT_{1A} receptor and 5-HT_{2A} receptor, but the mediating mechanisms are not clear; further studies are necessary to characterize this pain facilitatory mechanism.

5. Conclusions

From all above experiments, we concluded that HE had significant antinociceptive effect and the mechanism of its antinociception was connected with 5-HT_{1A} receptor, 5-HT_{2A} receptor, and adrenaline β_1-receptor.

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

The Anti-Inflammatory Actions of Auricular Point Acupressure for Chronic Low Back Pain

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Background. Auricular point acupressure (APA) is a promising treatment for pain management. Few studies have investigated the physiological mechanisms of APA analgesics. *Method.* In this pilot randomized clinical trial (RCT), a 4-week APA treatment was used to manage chronic low back pain (CLBP). Sixty-one participants were randomized into a real APA group ($n = 32$) or a sham APA group ($n = 29$). Blood samples, pain intensity, and physical function were collected at baseline and after 4 weeks of treatment. *Results.* Subjects in the real APA group reported a 56% reduction of pain intensity and a 26% improvement in physical function. Serum blood samples showed (1) a decrease in IL-1β, IL-2, IL-6, and calcitonin gene-related peptide [CGRP] and (2) an increase in IL-4. In contrast, subjects in the sham APA group (1) reported a 9% reduction in pain and a 2% improvement in physical function and (2) exhibited minimal changes of inflammatory cytokines and neuropeptides. Statistically significant differences in IL-4 and CGRP expression between the real and sham APA groups were verified. *Conclusion.* These findings suggest that APA treatment affects pain intensity through modulation of the immune system, as reflected by APA-induced changes in serum inflammatory cytokine and neuropeptide levels.

1. Introduction

Chronic low back pain (CLBP) is a major health problem worldwide and is associated with high medical costs, lost productivity, and long-term disability [1–3]. Although various standard pharmacologic and nonpharmacologic treatments have been proposed to alleviate CLBP, their effectiveness is limited [4]. The growing prevalence of CLBP and the limited treatments available underscore an increasing need for complementary therapies, which is reflected by more than one-third of adults with low back pain in the United States who have been treated by an integrative medicine provider over the past decade [5–7].

Auricular point acupressure (APA) is a treatment method similar to acupuncture that may allow improved management of CLBP pain. APA is one form of acupressure in traditional Chinese medicine (TCM) in which specific acupoints on the ear are stimulated without the use of needles [8, 9]. Auricular therapy was modified and updated by Dr. Nogier, the "father of auriculotherapy," in the 1950s [10]. Since then, the World Health Organization has come to recognize auricular therapy as a form of microacupuncture that can affect the entire body [11].

Previous studies using APA for managing pain relief in CLBP have been promising [12–16]. For example, APA provided immediate relief for CLBP (i.e., 40% reduction in

pain intensity after 1 day of APA) [13] and even greater and lasting effects on reducing CLBP (i.e., 75% pain relief and 45% better physical function after a 4-week treatment—both statistically significant compared to a sham APA group) [12]. Additionally, APA is a feasible intervention for older adults [14, 16]. In a study of 37 older adults with CLBP who received 4 weeks of APA, a significant reduction (i.e., 41% for those ($n = 19$) in the real APA group and 5% for the sham APA group ($n = 18$) in worst pain from baseline to the end of the treatment was reported [14]. Improved physical function was also achieved (i.e., the Roland Morris Disability Questionnaire [RMDQ] score decreased in the real APA group by 29% and was unchanged in the sham APA group) [14].

Increasing evidence supports immune activation in the etiology and progression of CLBP [17–19]. Immune biomarkers assessed typically include proinflammatory and anti-inflammatory cytokines and neuropeptides [20]. Changes in proinflammatory cytokines, such as IL-1β, IL-2, IL-6, and TNF-α, have been linked to alteration in pain signaling pathways [21]. Although the relationship between disk degeneration and CLBP remains unclear, evidence suggests that IL-1 and IL-6 may contribute to a local enhancement of pain by promoting matrix degradation [22, 23]. Moreover, IL-1, IL-6, and TNF-α may be associated with the expression of matrix metalloproteinases, which can lead to the herniation of intervertebral disks [24, 25]. These findings suggest a possible role for cytokines, in addition to chronic inflammation, among patients with CLBP.

In contrast, anti-inflammatory cytokines, such as IL-4 and IL-10, may inhibit the proinflammatory cytokine response [26]. Anti-inflammatory cytokines, such as TNF, IL-1, and IL-6, are produced by activated macrophages and monocytes and can act to inhibit the synthesis of proinflammatory cytokines [27]. IL-4 and IL-10 also suppress Th1 cells from releasing proinflammatory cytokines and inducing B-lymphocyte differentiation [27].

The level of calcitonin gene-related peptide (CGRP) has also been linked to pain signaling in CLBP [28]. In humans, CGRP exists in two forms: α-CGRP and β-CGRP [29]. Each binds to a G-protein-coupled receptor, and this activation is thought to produce long-lasting modifications of neurotransmission [30]. CGRP is also a potent vasodilator [31]. Interestingly, IL-1β stimulates the release of CGRP, while IL-6 stimulates the synthesis of CGRP in sensory neurons [32]. Cells of the immune system also synthesize β-endorphin [33]. T-lymphocytes, B-lymphocytes, monocytes, and macrophages have been shown to contain endorphins during inflammatory states [33].

How APA induces changes in cytokines and endorphins in immune and neuronal cell types is still unclear. One explanation is that pain and neuronal excitability impact a neural immune pathway that interconnects the ear microsystem and the somatotopic brain [8]. Neurophysiological connections between ear acupoints and the human CNS have been supported by fMRI studies [34]. Stimulation of acupoints is thought to cause vasodilatation through release of β-endorphin, which elicits either short-term analgesic effects or neuropeptide-induced anti-inflammatory cytokines for long-term effects [35–37].

To examine the underlying biological mechanisms of APA in pain relief, we previously measured serum levels of various cytokines in a prospective 4-week RCT of APA therapy for CLBP [38]. In this pilot study, subjects in the real APA group who reported a 70% reduction in worst pain intensity had changes in serum cytokine levels [38]. In particular, IL-2 and TNF-α decreased, and IL-10 increased, in subjects receiving APA. In contrast, subjects of the sham APA group reported only a 29% reduction in pain, and their levels of cytokines exhibited a different profile: IL-2, IL-4, and TNF-α decreased and IL-1β, IL-6, and IL-10 increased [38]. Among all subjects, levels of IL-1β, IL-2, IL-6, and IL-10 were associated with the worst pain intensity score [38]. This prior pilot study had a small sample size and lacked significance, necessitating further research to determine effect size to use for power calculation. The primary aims of this pilot study were to (1) collect more data for effect size and power calculation, (2) confirm our previous findings of a differential response between groups, which reflected the anti-inflammatory effect of the real APA intervention, and (3) investigate the association between biomarker change and clinical outcome.

2. Methods

Complete details of the study design, sample, and data collection are provided in our previous manuscripts [12, 14], and participant recruitment began after approval by University of Pittsburgh, Institutional Review Board. For this study, we increased the sample size and measured the circulating levels of inflammatory cytokines (i.e., IL-1β, IL-2, IL-4, IL-6, IL-10, and TNF-α) and β-endorphin and CGRP. Levels of these compounds were measured in subjects who received treatment in either a real APA group or a sham APA group. Measures were correlated with clinical outcomes (including worst pain intensity and physical function) for participants who completed the baseline assessment (pre-APA treatment) and the 4-week APA treatment (post-APA treatment). The real APA group had 32 participants and the sham APA group had 29 (including 19 participants for which data had been published) [38]. For these 61 participants, 27% were receiving other treatments (pain medication, $n = 15$; chiropractor, $n = 1$; massage, $n = 1$), 62% ($n = 38$) were not receiving treatment, and 11% ($n = 6$) had never received any treatment related to CLBP. All participants suffered CLBP, which, for the purpose of this study, was defined as low back pain occurring for at least 3 months with an average pain intensity score of 4 or greater on a 0–10-point numerical scale for 1 week prior to enrollment. The majority of the participants' medical diagnoses were osteoarthritis (44%, $n = 27$) and spinal stenosis (39%, $n = 24$). Demographic background information for the participants appears in Table 1. The mean age of the participants was 58.03 years (SD = 17.28) for the real APA group and 62.80 years (SD = 14.75) for the sham APA group.

2.1. Auricular Point Acupressure Treatment Protocol. The APA intervention included one treatment per week for 4 consecutive weeks. Auricular points on the ears of participants were detected with an electrical acupoint finder, which measures auricular cutaneous resistance to identify the potential acupoints for treatment. Using TCM and Chinese ear acupoint maps, the acupoints selected for the real APA group included three for alleviating stress and pain (i.e., *shenmen*, *sympathetic point*, and *nervous subcortex*) and one corresponding to the anatomical site (i.e., *lower back*) [8]. The acupoints selected for the sham APA group were located away from the site where the participant was experiencing pain and included *stomach*, *mouth*, *duodenum*, and *kidney*. In our published APA protocol [12, 14], participants were told to press/stimulate the seeds taped to the acupoints on their ears at least 3 times per day for 3 minutes each time. The seeds and tape were removed at the end of the 5th day each week to insure baseline sensitivity to the site prior to the next treatment. The primary endpoint was the measure of pain intensity and physical function after completion of the 4-week APA. Participants in the sham APA group, who were blinded to this assignment, were provided the opportunity to receive real APA treatment after completing all assessments.

2.2. Data Collection Procedure. Blood (10 mL) was collected from participants in both treatment groups in a red-top vacutainer, using standard phlebotomy procedures. Blood was drawn by a trained nurse before the APA treatment (baseline), once a week for the 4 weeks of APA treatment, after the 4-week APA treatment, and at the 1-month follow-up. Due to budget limitations, data were only obtained for the pre-APA (baseline) and post-APA treatment (after the 4-week APA treatment).

2.3. Blood Extraction. Tubes containing blood samples were labeled with the participant's ID number and time of collection, placed on a level rack at room temperature, and left undisturbed for 1.5 hours. After the tubes were centrifuged at 1,500 rpm for 10 minutes, the serum was transferred into 0.5 mL polypropylene microcentrifuge tubes and stored at −80°C until assayed.

2.4. Inflammatory Biomarker Testing. Luminex cytokine analysis (xMAP, Multiplexed or Multianalyte Platform, Austin, Texas) was used to measure IL-1β, IL-2, IL-6, IL-4, IL-10, and TNF-α. Serum was assayed in the Luminex Core Facility at the University of Pittsburgh Cancer Institute. xMAP technology uses polystyrene microspheres internally dyed with varying ratios of two spectrally distinct fluorophores to create a family of 100 differentially spectrally addressed bead sets. Each bead set was conjugated with a capture antibody specific for a unique target analyte and allowed to react with the serum sample. Beads were washed and secondary (or detection) antibodies were added to a microtiter plate well to perform a capture sandwich immunoassay. The bead suspension was analyzed using a fluorometric array reader with two fluorescence readings obtained for each bead. One reading indicated whether or not a bead was a member of

Table 1: Demographic characteristics of the participants ($n = 61$).

	Mean (SD) or n (%)		P/χ^2
	Real ($n = 30$)	Sham ($n = 31$)	
Age			
Mean (SD)	61 (17.44) (20–82)	66 (16.04) (21–90)	0.91
Gender, n (%)			
Male	10 (33%)	10 (32%)	0.93
Female	20 (67%)	21 (68%)	
Race/ethnicity, n (%)			
White	26 (87%)	25 (81%)	0.73
Black/African American	4 (13%)	6 (19%)	
Marital status, n (%)			
Married or living with partner	14 (47%)	13 (42%)	0.78
Divorced or widowed	10 (33%)	11 (36%)	
Never married	6 (20%)	5 (16%)	
Employment situation			
Working (full time)	6 (20%)	4 (13%)	
Working (part time)	2 (7%)	2 (7%)	
Not employed	4 (13%)	6 (19%)	0.67
Retired	15 (50%)	14 (45%)	
Others	3 (10%)	5 (16%)	
Education level			
≤10th grade	2 (6%)	2 (6%)	0.62
High school	5 (17%)	4 (13%)	
Technical or vocational school	2 (6%)	2 (6%)	
College and/or graduate	21 (71%)	21 (69%)	
Missing		2 (6%)	
Estimated income before taxes			
Less than $10,000	5 (17%)	7 (23%)	
$10,000 to $19,999	2 (6%)	5 (16%)	
$20,000 to $39,999	8 (27%)	2 (6%)	
$40,000 to $59,000	6 (20%)	7 (23%)	0.25
$60,000 to $100,000	3 (10%)	5 (16%)	
More than $100,000	3 (10%)	1 (3%)	
Missing	3 (10%)	4 (13%)	
Medical diagnosis related to back pain			
Osteoporosis	3 (10%)	4 (13%)	
Osteoarthritis	9 (30%)	9 (29%)	
Scoliosis	3 (10%)	5 (16%)	
Kyphosis	1 (3%)	0 (0%)	
Disc herniation	4 (14%)	7 (23%)	
Spinal stenosis	6 (20%)	13 (42%)	
Spondylitis	1 (3%)	0 (0%)	
Spondylosis	3 (10%)	0 (0%)	
Current pain medication use			
Yes	13 (43%)	14 (45%)	
No	17 (57%)	17 (55%)	0.89

one of the 100 possible sets. The other reading corresponded to the amount of fluorescent dye, typically phycoerythrin (PE), bound to the detection antibody in the assay. The amount of PE fluorescence was proportional to the amount of analyte captured in the immunoassay. Bio-Plex Manager software was used to correlate readouts from each bead set with the assay reagent coupled with the bead set. Results were extrapolated to a standard curve that was used to quantify each analyte in the sample. The xMAP assay used in this study measured a maximum of 100 analytes in a 96-well microplate in 1 hour.

Serum levels of β-endorphin and CGRP were determined using commercial ELISA kits (MyBioSource, San Diego, CA) according to the manufacturer's instructions. The level of sensitivity and precision of each assay were as follows: β-endorphin = 2.59 ng/L (intra-assay coefficient of variation [CV] <10%, interassay CV < 12%) and CGRP = 1.12 ng/L (intra-assay CV < 10%, interassay CV < 12%).

2.5. CLBP Clinical Outcomes. CLBP clinical outcomes included assessments of pain intensity and physical functioning. *Worst pain* was an individual item from the Brief Pain Inventory short form (BPI-sf) [39]. From baseline, on a 0–10 numerical scale, the cut-off point of 10–20% was rated as "minimally important," 30% or greater as "moderately important," and 50% or more as "substantial" pain intensity change [40]. *Physical functioning* was measured by the Roland-Morris Disability Questionnaire (RMDQ) [41]. The RMDQ is a 24-item measure to assess the impact of back-related pain on daily functioning. Participants selected "yes" or "no" for statements related to their physical function. The total score ranged from 0 (no disability) to 24 (maximum disability). The RMDQ is a reliable, valid, and sensitive measure that has demonstrated substantial construct validity [41, 42]. A RMDQ reduction of 30% or greater is rated as "minimally clinically important" [43].

2.6. Data Analysis. Descriptive analysis was used to display the outcomes measures (including cytokine, neuropeptide, pain intensity, and physical functions). Because the sample size was approximately 30 per group, independent two-sample t-test was used to compare the mean change from pretreatment to posttreatment between the real and sham APA groups. Pearson product-moment correlation coefficient (r) was used to examine the linear association of the changes from pretreatment to posttreatment in cytokine level and clinical outcomes (i.e., pain intensity and physical functions). Significance was set at a P value < 0.05. All data analyses were performed using SAS software, version 9.2 [44].

3. Results

3.1. Characteristics of Biomarkers and Clinical Outcomes. Table 2 presents the descriptive characteristics of cytokine, CGRP and β-endorphin levels, and clinical outcome at pre- and post-APA treatment. The biomarker data were skewed, and the median should be reported for descriptive characteristics. We also present the mean and standard deviation to understand the trend of change to guide the design of a future study.

Table 3 lists the mean changes from pre- to post-APA treatment for each biomarker. Statistically significant differences between the real APA group and the sham APA group were determined for IL-4 (mean difference = 1.33, SD = 2.49, and $P = 0.05$) and CGRP (mean difference = −8.87, SD = 14.19, and $P = 0.04$) levels. Changes in other biomarker levels did not reach statistical significance between the two groups. For clinical outcomes, pain intensity measures showed a statistically significant improvement among individuals in the real APA group (−3.66, SD = 2.78) compared to participants in the sham APA group (−0.79, SD = 2.46), resulting in a −2.86 (SD = 3.63) difference between the real and sham APA groups (P value < 0.01). However, no statistically significant finding was found for physical function.

Further comparison between groups revealed proinflammatory cytokine and CGRP to display a trend of mean reduction from pre- to post-APA treatment (i.e., −1.33 in IL-1β, −1.24 in IL-2, −2.18 in IL-6, and −6.19 in CGRP) for the real APA group. In the sham APA group, proinflammatory cytokines also displayed a decreasing trend in mean changes yet with smaller magnitudes compared to the real APA group (i.e., −0.39 in IL-1β, −0.39 in IL-2, −1.28 in IL-6, and −5.61 in TNF-α). The anti-inflammatory cytokine IL-4 increased from pre- to post-APA treatment for the participants in the real APA group; however, IL-4 decreased in the sham APA group. IL-10 and β-endorphin decreased for both groups. A statistically significant change from pre- to post-APA treatment for all biomarkers was not observed. For clinical outcomes, the mean pain intensity score and physical function score exhibited a statistically significant change from pre- to post-APA treatment in the real APA group ($P < 0.01$), which indicates that participants in the real APA group experienced a marked reduction in pain intensity (56%) and improved physical function (26%). The effect sizes for each biomarker are presented in Table 3.

3.2. Correlation of Cytokines, Neuropeptides, and Clinical Outcomes. Pearson correlation coefficients for biomarkers and clinical outcomes are presented in Table 4 for pre-APA treatment (upper triangular region) and mean changes from pre- to post-APA treatment (lower triangular region) for the real APA group—data for sham APA group participants is available upon request. In the pre-APA treatment group, proinflammatory cytokines (i.e., IL-1β, IL-2, and IL-6) had strong linear associations ($r > 0.7$) among other cytokines in this category (i.e., IL-1β/IL-2, $r = 0.98$; IL-1β/IL-6, $r = 0.85$; and IL-2/IL-6, $r = 0.82$). Moderate linear associations ($0.3 \leq r \leq 0.7$) were found between IL-4 and IL-10 in the category of anti-inflammatory cytokines ($r = 0.45$). There was also a moderate linear association between cytokines in the proinflammatory and anti-inflammatory categories. Additionally, a negative relationship was found between CGRP and physical function ($r = -0.46$), and a moderate linear association between the mean change in pain scores and physical function ($r = 0.52$) was observed. The mean change score of CGRP was moderate when associated with IL-1β ($r = 0.41$) and IL-2 ($r = 0.42$). The correlations among

Table 2: Descriptive characteristics of participants receiving APA treatment.

Outcomes		Pre-APA					Post-APA				
		Mean	SD	Median	Q1	Q3	Mean	SD	Median	Q1	Q3
Proinflammatory cytokines											
IL-1β	APA	16.17	11.38	12.50	9.00	19.50	14.83	10.00	12.00	8.00	15.00
	Sham	17.70	21.14	11.00	9.00	16.00	17.30	22.25	11.00	9.00	15.00
IL-2	APA	17.73	11.53	13.00	10.00	21.00	16.48	9.99	13.00	10.00	20.00
	Sham	21.29	30.34	11.50	9.50	18.50	20.89	34.39	11.75	8.50	17.00
IL-6	APA	33.28	18.47	26.50	22.00	39.00	31.10	18.09	24.50	20.00	31.00
	Sham	36.48	25.82	28.00	22.00	34.00	35.21	31.80	28.50	20.50	36.00
TNF-α	APA	111.68	44.13	101.50	86.50	129.50	114.23	46.60	107.00	86.00	127.00
	Sham	127.41	51.58	122.00	105.75	161.75	121.80	46.43	121.50	93.00	153.50
Anti-inflammatory cytokines											
IL-4	APA	19.78	17.86	14.00	12.00	16.00	20.18	19.35	14.00	12.00	17.50
	Sham	16.61	8.96	13.75	11.75	19.25	15.68	8.20	13.00	11.50	17.00
IL-10	APA	29.83	12.73	26.00	23.00	33.00	27.55	9.82	26.00	22.00	29.00
	Sham	32.56	14.77	29.00	24.00	34.00	31.19	13.44	28.00	22.50	32.00
Neuropeptides											
CGRP	APA	42.04	53.99	14.34	3.24	59.57	35.85	44.32	9.36	3.77	61.64
	Sham	36.74	31.59	31.10	6.40	65.05	39.43	35.85	30.43	4.34	67.58
β-endorphin	APA	138.21	47.95	131.28	107.55	146.74	129.54	42.57	117.40	103.52	135.99
	Sham	121.81	35.29	121.77	104.88	135.86	113.92	23.88	113.52	96.08	135.23
Clinical outcomes											
Pain intensity	APA	6.31	1.93	6.00	5.00	7.00	2.66	2.01	2.00	1.00	4.00
	Sham	6.07	1.71	6.00	5.00	7.00	5.28	2.37	5.00	4.00	7.00
Function	APA	7.43	5.61	6.00	3.00	11.00	4.77	5.20	4.00	1.00	5.50
	Sham	9.43	5.02	10.00	6.50	13.00	9.00	5.76	10.00	3.00	14.00

Note. SD: standard deviation; Q: quantile; APA: auricular point acupressure; CGRP: calcitonin gene-related peptide.

the mean score changes of biomarkers and clinical outcomes were weak.

4. Discussion

The present study not only expands on data presented in a previous pilot study [38], but also further examines whether or not serum cytokine and/or neuromodulator levels change in response to APA treatment for CLBP. Participants in the real APA group reported a mean 56% reduction in pain intensity and a mean 26% improvement in physical function at the completion of the 4-week APA regimen. We also observed decreases in serum proinflammatory cytokines (i.e., IL-1β, IL-2, and IL-6) and CGRP and increases in IL-4, an anti-inflammatory cytokine. The sham APA group exhibited a 9% pain reduction, 2% improved physical function, and decreased levels of IL-1β, IL-2, IL-6, and IL-4; CGRP and β-endorphin levels also increased. Additionally, the level of IL-4 was significantly higher and CGRP was lower in the real APA groups compared to levels in the sham APA group. These results indicate preliminary associations among CGRP, IL-4, IL-1β, IL-2, IL-6, and the pain intensity score, which suggest a pathophysiological mechanism underlies the APA analgesic effect on CLBP.

These outcomes are consistent with previous studies reporting increased levels of proinflammatory cytokines in CLBP [45–47]. For example, a cross-sectional study of 23 patients with CLBP diagnosed with herniated intervertebral disks and 10 healthy controls showed statistically significant increases in concentrations of IL-6 and TNF-α, but not IL-1β, in patients with CLBP [48]. Another study of 94 patients diagnosed with chronic neuropathic, nociceptive, or mixed pain for more than 6 months and six healthy controls reported a positive correlation between increased cytokine concentration, including IL-1β, IL-6, and TNF-α, and increased pain severity [49].

It has been proposed that peripheral immune responses lead to the activation of discrete circuitries within the central nervous system via both hematogenous and neural pathways, facilitating changes known as sickness responses [50]. Cytokines, as sickness inducing agents, are recognized as key mediators of immune-to-brain communication that facilitate pain [50]. The medulla-to-spinal cord limb of this pathway is proposed to modulate release of neurotransmitters that activate spinal cord glia and enhance pain [50]. Blocking proinflammatory cytokines such as IL-1β and IL-6 by administration of specific antagonists can prevent the generation of sickness responses induced by peripheral immune challenges [50]. Moreover, proinflammatory cytokines administered peripherally in the absence of peripheral immune challenge are sufficient to induce sickness responses [51, 52]. Our results

Table 3: Mean changes from pre- to post-APA treatment of biomarkers and clinical outcomes.

Cytokine change after treatment	Real APA group Mean (SD)	Sham APA group Mean (SD)	Difference Mean (SD)	P value	Effect size*
Proinflammatory cytokines					
IL-1β	−1.33 (5.00)	−0.39 (2.09)	−0.94 (3.88)	0.35	−0.24
IL-2	−1.24 (6.51)	−0.39 (5.34)	−0.85 (5.98)	0.58	−0.14
IL-6	−2.18 (9.68)	−1.28 (16.19)	−0.90 (13.58)	0.80	−0.07
TNF−α	2.55 (24.39)	−5.61 (22.96)	8.16 (23.71)	0.20	0.34
Anti-inflammatory cytokines					
IL-4	0.40 (2.46)	−0.93 (2.52)	1.33 (2.49)	0.05	0.53
IL-10	−2.28 (7.22)	−1.38 (7.07)	−0.90 (7.15)	0.63	−0.13
Neuropeptides					
CGRP	−6.19 (17.91)	2.69 (8.41)	−8.87 (14.19)	0.04	−0.63
β-endorphin	−8.67 (21.57)	−7.88 (24.49)	−0.79 (12.07)	0.90	−0.03
Clinical outcomes					
Pain	−3.66 (2.78)	−0.79 (2.46)	−2.86 (2.63)	<0.01	−1.09
Physical function	−2.86 (3.97)	−0.43 (4.52)	−0.43 (3.99)	0.06	−0.11

Note. SD: standard deviation; APA: auricular point acupressure; CGRP: calcitonin gene-related peptide.
*The effect size is calculated from Cohen's d.

Table 4: Pearson correlation coefficients among biomarkers and clinical outcomes.

Biomarkers	Proinflammatory cytokine				Anti-inflammatory cytokine		Neuropeptides		Clinical outcomes	
	IL-1β	IL-2	IL-6	TNF-α	IL-4	IL-10	CGRP	β-endorphin	Pain	Physical function
IL-1β	—	0.98*	0.85*	0.32*	0.37*	0.20*	0.15*	0.02*	0.05*	−0.02*
IL-2	0.84#	—	0.82*	0.39*	0.29*	0.17*	0.12*	−0.04*	0.06*	0.02*
IL-6	0.54#	0.57#	—	0.29*	0.45*	0.26*	0.06*	0.02*	0.06*	0.09*
TNF-α	0.15#	0.31#	0.33#	—	0.04*	0.31*	0.06*	−0.18*	0.14*	0.25*
IL-4	0.44#	0.42#	0.25#	0.28#	—	0.45*	0.06*	−0.16*	−0.10*	−0.04*
IL-10	0.33#	0.40#	0.48#	0.53#	0.30#	—	0.30*	−0.21*	−0.13*	−0.27*
CGRP	0.41#	0.42#	0.05#	0.08#	0.13#	0.04#	—	0.14*	−0.25*	−0.46*
β-endorphin	0.02#	0.00#	0.32#	0.01#	−0.16#	−0.18#	0.076#	—	0.06*	−0.26*
Pain	0.13#	0.10#	−0.09#	−0.25#	0.26#	0.10#	−0.31#	0.02#	—	0.27*
Function	0.03#	0.13#	−0.21#	−0.52#	−0.03#	−0.09#	−0.27#	0.13#	0.52#	—

Note. *Data in the right triangular region reflects pre-APA treatment; #data in the left triangular region reflects the mean score change from pre- to post-APA treatment. CGRP: calcitonin gene-related peptide.

show a decrease in proinflammatory cytokines (including IL-1β, IL-2, and IL-6), an increase in anti-inflammatory cytokine (i.e., IL-4), and a decrease in CGRP between pre- and post-APA treatments. A moderate correlation was observed between pain intensity change and IL-1β and IL-2 changes. Based on these findings, we hypothesize that APA therapy may exhibit anti-inflammatory efficacy in CLBP in two ways: (1) downregulation of proinflammatory cytokines (i.e., IL-1β, IL-2, and IL-6) and upregulation of anti-inflammatory cytokines (i.e., IL-4) and (2) downregulation of proinflammatory neuropeptides (i.e., CGRP).

An unexpected finding in this study was the decrease in β-endorphin. β-endorphin produces analgesia by (1) binding opioid receptors at both presynaptic and postsynaptic nerve terminals in the peripheral nervous system [53] and (2) inhibiting neuronal firing of somatosensory fibers, especially those involved in nociception [54]. Studies to identify mechanisms of acupuncture-mediated analgesia also suggest a role for endogenous opioid peptides, such as β-endorphin [55]. Acupuncture is thought to cause vasodilation by releasing β-endorphin and, in so doing, elicit short-term analgesic effects [35–37]. The relationship of increased β-endorphin and reduced pain is based on primarily animal studies [56]; we lack empirical studies of this relationship in humans. Additionally, various testing procedures are used to measure β-endorphin levels in serum that may induce variability in measurement [57]. In this study, blood was collected only one time to determine the level of β-endorphin. After collection, blood was kept at room temperature for 1.5 hours, which deviates from the optimum conditions for β-endorphin (i.e., blood is placed on ice immediately after collection, serum separation occurs, and then samples are frozen within 1 hour after collection) [58]. Additionally, we did not record possible confounding variables that may impact β-endorphin level, such as smoking, alcohol consumption, medication, and stress. Additional statistical analysis of the cross-reaction

of β-endorphin with other cytokines could reveal novel interactions.

Despite the strengths of the study as an RCT with a sham control group, this study has limitations. First, we were unable to determine the biological actions of the APA intervention due to the small sample size and complexity of pathophysiology, which involves factors that cross-react within biomarkers. However, we did identify changes in biological biomarker patterns. For example, CGRP decreased in the real APA group while it increased in the sham APA group. Likewise, IL-4 increased in the real APA group and decreased in the sham APA group. Second, chronic pain pathophysiology is complicated. Although we attempted to include most related biomarkers, we are unable to define an underlying biological mechanism of APA therapy on CLBP. Lastly, we did not collect confounding variables that may cross-react with biomarkers, including medication use and stress. Nevertheless, (1) expression of proinflammatory cytokines such as IL-1β, IL-2, and IL-6 decreased and (2) the expression of the anti-inflammatory cytokine IL-4 increased in the real APA group after 4 weeks of APA treatment, both suggesting the interaction of APA therapy and neural-immune signaling. Moreover, CGRP decreased after 4 weeks of APA treatment, and this decrease may impact the sickness response and alleviate symptoms of CLBP. These preliminary findings warrant a larger clinical trial that could further elucidate the biological mechanism of auricular therapy for pain relief.

5. Conclusion

The change in cytokine and neuropeptide levels among the participants who received APA treatment indicates that APA could mediate the expression of inflammatory cytokines and neuropeptides and thus decrease pain intensity. We were able to verify statistically significant differences in IL-4 and CGRP expression between real and sham APA groups. However, the biological mechanism for chronic pain is complicated by pathophysiology, involving factors that cross-react with biomarkers, including medication use and stress. Nevertheless, our findings strongly suggest that pain relief and improved physical function in patients with CLBP experienced through APA treatment may be modulated by the level of inflammatory cytokines (i.e., IL-1β, IL-2, IL-4, and IL-6) and neuropeptides (i.e., CGRP). Our findings warrant additional research, which could include larger-scale studies to determine the underlying biological mechanism linking APA, cytokine/neuropeptide levels, and pain relief.

Conflict of Interests

The authors have no conflict of interests regarding the publication of this paper.

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