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Effects of Novel Vibro-Acupuncture on Healthy Subjects and Those with Experimental and Clinical Pain as Assessed by Quantitative Sensory Testing

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Background: To investigate the analgesic effects of vibro-acupuncture (VA), a novel acupunctuator was developed.

Objectives: To compare the analgesic effects of VA with those of manual acupuncture (MA) and placebo acupuncture (PA) on subjects with normal sensory perception (Study I), experimentally induced acute pain (Study II), and clinical chronic pain (Study III).

Methods: Thirty healthy volunteers (21 males, age: 20-30 years) participated in Study I. Fourteen healthy volunteers (8 males, age: 20-32 years) participated in Study II in which experimental pain was induced by injection of hypertonic saline. Fourteen patients suffering from unilateral epicondylalgia (9 males, age: 30-61 years) participated in Study III. All participants received VA, MA, and PA at LI4 and LI10 points in a randomized, crossover, and double-blinded manner. Quantitative sensory testing (QST) was performed on the ipsilateral forearm before and after each treatment. Data were analyzed using repeated-measures (RM) ANOVA.

Results: A significantly higher vibration detection threshold (VDT) was observed after treatment of VA than after MA and PA ($p < 0.001$). No significant treatment effect on experimental pain intensity was detected ($p > 0.086$). Significantly lower pain intensity ($p = 0.005$) and a smaller drawing area ($p = 0.011$) of unilateral epicondylalgia were found after VA treatment than after PA.

Conclusion: A specific effect on the VDT beyond that of MA and PA was evoked by VA. Patients with epicondylitis showed significantly lower pain intensity during VA than during PA. This study indicated that VA may be beneficial in individuals with clinical chronic musculoskeletal pain; however, further studies are needed.

Keywords: Acupuncture, Vibro-acupuncture, Quantitative sensory testing, Experimental pain, Lateral epicondylalgia

INTRODUCTION

The primary mechanism of acupuncture has been suggested to be the activation of afferent nerve fibers innervating the skin and muscles by needle stimulation [1,2]. The somatic afferent information from nerve fibers excited by acupuncture stimulation has various effects on body functions, including an inhibitory effect on the synaptic

transmission of nociceptive inputs in the central nervous system [3]. Vibrational stimuli are known to attenuate pain intensity in animal models [4,5], human experimental models [6,7], and patients with chronic pain [6,8,9]. Nonetheless, the exact underlying neurobiology of this inhibition is still largely unknown. Previous animal studies have suggested the dorsal horn as an important relay site for this phenomenon [10]. Another assumed mechanism is that vibrational analgesia

relies on high-frequency stimulation of low threshold A β -mechanoreceptors and segmental spinal mechanisms [4] and a possible interaction within the somatosensory cortex [11]. Vibrational stimulation of A β primary afferents produces potent inhibition of dorsal horn nociceptive neurons and somatosensory cortical neurons [12]. Therefore, it seems reasonable to hypothesize that vibration combined with acupuncture may be beneficial for pain relief. However, to the best of the authors' knowledge, no study has previously investigated the analgesic effects of a combination of acupuncture and vibration. We have recently developed a new technique to combine these two modalities, called vibro-acupuncture (VA), and designed an 'acu-vibrator' to facilitate its application [13].

Quantitative sensory testing (QST) has been significantly developed and widely applied in human experimental and clinical studies [14]. Its application permits quantitative assessment of the neurophysiological effects of different acupuncture modalities as the immediate effects can be evaluated; furthermore, the pressure pain threshold has been shown to be increased from a baseline of 25% to 52% after acupuncture [15].

Injection of hypertonic saline into the forearm muscles (brachioradialis) can evoke acute muscle pain that mimics the clinical pain of early-stage lateral elbow pain or tennis elbow [16,17]. This experimental model of muscle pain can be implemented as a standardized experimental pain model in healthy subjects to test the analgesic effects of acupuncture.

Finally, lateral epicondylalgia (LE) is one of the most common chronic pain problems of the upper extremity seen in clinical practice. Acupuncture is well-accepted as an alternative treatment for pain relief of musculoskeletal pain including LE [18,19]. However, whether this is a treatment effect of acupuncture versus placebo is still controversial [20]. Therefore, further well-designed studies with placebo control performed in a double-blinded manner are necessary.

The aims of this series of randomized, placebo-controlled, double-blinded, crossover studies were to compare the possible analgesic effects of acupuncture plus vibration (VA) with conventional manual acupuncture (MA) and placebo acupuncture (PA) using QST to assess 1) somatosensory perception, 2) acute experimental muscle pain, and 3) chronic LE pain.

MATERIALS AND METHODS

1. Subjects

A total of 60 participants were recruited in the three studies. Thirty asymptomatic healthy subjects (21 men and 9 women, aged 20-30 years) were recruited in the Study I. Fifteen asymptomatic healthy subjects (8 men and 7 women,

aged 20-32 years) were recruited in Study II. Fifteen (10 men and 5 women, aged 30-60 years) patients suffering from chronic unilateral LE were recruited in Study III. The inclusion criteria for Studies I and II were healthy subjects aged 20-60 years who were free from any ongoing pain in the previous year. The inclusion criteria for Study III were people diagnosed with unilateral LE lasting more than six months with an ongoing pain intensity rated above 2 on a 10-point Numerical Pain Rating Scale (NPRS) during the previous week. The exclusion criteria for all subjects included pregnancy, use of analgesics, recent acupuncture treatment (within the previous 4 weeks) and lack of the ability to co-operate.

The study was approved by the local ethics committee (reference no. N-20140005). Written informed consent was obtained from all participants prior to the experiment.

2. Experimental protocol

A randomized, placebo-controlled, double-blinded crossover study was designed in which each subject participated in three experimental sessions.

In Study I, a standard QST protocol was performed on the ipsilateral forearm (lateral epicondyle of the humerus) of the acupuncture treatment (testing the local effect) before (baseline) and after treatment by one investigator (Tester-1). The pressure pain threshold (PPT) and suprathreshold pain stimulation (SPS) were assessed in the contralateral forearm (testing the segmental effect) and on the anterior aspect of the contralateral leg (tibialis anterior muscle) (testing the general effect). After baseline QST, a single application of either MA, VA, or PA was randomly performed for 25 minutes by another investigator (Tester-2). The randomization code was opened after the data analysis. All QST parameters were assessed again immediately after each treatment session.

In Study II, experimental muscle pain was evoked by a single bolus injection of hypertonic saline into the belly of the right brachioradialis muscle 5 minutes after the start of the MA/VA/PA treatment. The evoked pain intensity was continuously scored by the subject on an electronic visual analog scale (VAS). The pain profile and all QST parameters were assessed at the end of each treatment session.

In Study III, the MA, VA, or PA treatment was randomly performed for 25 minutes with intervals of one week. The subjects were asked to rate their ongoing pain intensity on a 0-10 numerical rating scale (NRS) and draw the pain area before and after MA/VA/PA treatment. The standard QST protocol was performed before and after each treatment session.

3. Treatment

All subjects received a randomized single session of MA,

VA, or PA for 25 minutes on their right forearm during three separate sessions. A pillow was placed on the chest of each subject to prevent visual recognition of the intervention applied. Sterile single-use acupuncture needles (0.25 × 40 mm or 0.20 × 25 mm, Hwato, Jiangsu, PR China) were used for all treatments. The acupuncture points LI4 (*Hegu*) and LI10 (*Shousanli*) were chosen since Study III evaluated the effects of the same treatment on patients with LE. The acupuncturist (Tester-2) had over 30 years clinical experience of providing acupuncture treatments. After cleaning the overlying skin with alcohol, acupuncture needles were inserted at the selected points to a depth of 10–20 mm. During manual stimulation, the *de qi* sensation, a subjective feeling of numbness, tingling, and/or deep muscle discomfort, was achieved by twisting the needle [21].

In the VA treatment session, the procedure was initially the same as for MA, i.e., the acupuncture needles were inserted at the same points to the same depth and twisted to achieve *de qi*. In addition, an acu-vibrator was connected by a metal clip to the acupuncture needles, through which high-frequency vibrational stimulation was delivered at a frequency of 100 Hz and an amplitude of 140 μ m.

In the PA treatment session, half-cut needles (custom-made) with a blunted tip were used to provoke a pricking sensation without actually puncturing the skin [13,22]. The needle was inserted through cube-shaped elastic foam and just touched the skin surface at the same points [13].

In all three sessions, the acupuncture needles were connected with a vibrator that was activated as indicated with a green flash, but no power output was used in the MA or PA session.

4. Vibrator

The newly developed acu-vibrator consists of a micro-controller and microvibration motor components. The small vibration motor is connected to the acupuncture needles with a metal clip and is capable of producing vibrations with different frequencies (0–125 Hz) and amplitudes (0–200 μ m) according to demand. The microcontroller displays the amplitude and frequency values on a liquid crystal display. A 0–10 arbitrary scale is shown on the display to allow calibration of the amplitude output [13].

5. Experimental muscle pain

To evoke forearm muscle pain, a single bolus of hypertonic saline (5.8%, 0.5 ml) was injected into the belly of the right brachioradialis muscle with a 27-G needle over 15 s at a point 2 cm away from the testing point. The pain intensity was continuously scored by the subjects using an electronic device on a 0–10 VAS (Aalborg University, Denmark); the lower extreme of the VAS scale indicated ‘no pain’, and the upper extreme indicated ‘the most pain imaginable’. The

pain intensity was sampled every 2 seconds and recorded for a period of 600 seconds. The area of the pain was drawn by the subject at the end of the session. The area under the VAS curve (VASAUC) was used to obtain a measure of the overall pain intensity extracted from the VAS data.

6. Quantitative sensory testing

Thermal detection and pain thresholds were tested using a computerized thermal stimulator (TSA 2001-II, MEDOC, Israel). The cold detection threshold (CDT), warm detection threshold (WDT), cold pain threshold (CPT), and heat pain threshold (HPT) were measured in this specific order. Three consecutive measurements were averaged as the final value for each parameter.

The mechanical detection threshold (MDT) was assessed using a standardized set of Semmes Weinstein Von-Frey aesthesiometers with 20 different diameters (Touch-Test® Sensory Evaluators, North Coast Medical, US). The mechanical pain threshold (MPT) was assessed using a set of 7 custom-made weighted pinprick stimulators (Aalborg University, Aalborg Denmark). The mechanical pain sensitivity (MPS) was assessed using the same device that was used for the MPT. Dynamic mechanical allodynia (ALL) was measured using a set of three light tactile stimulators (a cotton wisp exerting a force of about 3 mN, a cotton tip exerting a force about 100 mN, and a standardized brush (Somedic) exerting a force of approximately 200 to 400 mN). The windup ratio (WUR) was determined using a single pinprick (128 mN). The NRS score on a 0 to 100 scale (0 indicating “no pain” and 100 indicating the “most intense pain imaginable”) of a single stimulus and that of a series of 10 repetitive stimuli were recorded. The WUR was calculated as the ratio of the mean rating of the 5 series of 10 stimuli divided by the mean rating of the single stimulus.

The vibration detection threshold (VDT) was obtained with a Vibrometer (100 Hz/ 0–400 μ m, Somedic, Sweden). The vibrator of the Vibrometer (weight: 650 g) was put to the testing site and gradually increased the vibration amplitude. The subjects were instructed to say “yes” as soon as they felt the vibration. Three measurements were averaged for each site.

The PPT was obtained using a pressure algometer (Somedic, Sweden). The algometer probe (contact area: 1 cm²) was pressed against the testing site with a constant advancing rate of 30 kPa/second. The subjects pushed a button to stop the stimulation as soon as they felt the pressure turn to pain on the testing site. Three trials were averaged for each site. Once the PPT was determined, mechanical SPS was tested by delivering stimuli at 130% of the PPT to the three sites as previously described, and subjects were asked to rate the pain intensity using a 0–10 NRS.

7. Statistical analysis

The sample size was calculated a priori. Assuming intra-individual variation of 30% (a conservative estimate), it was calculated that 24 or 15 subjects would be required to detect a minimum clinically relevant difference of 25% or 30%, respectively, at an alpha level of 0.05 and 80% power (i.e., the risk of a type I and type II error was 5% and 20%, respectively). We recruited a total of 30 subjects in Study I and 15 subjects in Studies II and III.

All data were analyzed using repeated measures analysis of

variance (RM ANOVA) with treatment type (MA, VA, and PA) and time (before and after treatment as within-subject factors). Necessary logarithmic transformation was performed [14]. All results are reported as the mean \pm standard error of the mean (SEM) unless otherwise indicated. The statistical calculations were performed using the Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, IL, USA). A p -value < 0.05 (with Bonferroni correction applied where appropriate; $p = 0.017$) was considered statistically significant.

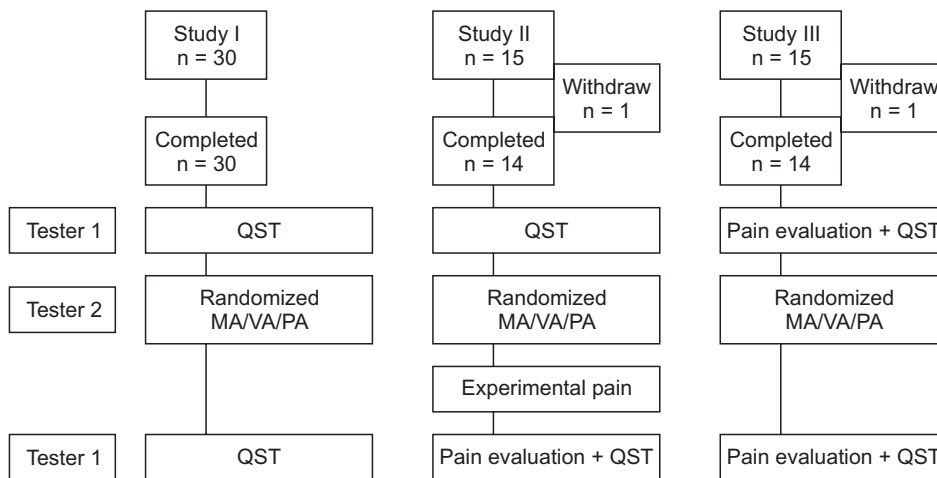


Fig. 1. Flow diagram of the study. An intervention of manual acupuncture (MA), vibro-acupuncture (VA), or placebo acupuncture (PA) was performed in a randomized, double-blinded, crossover study.

Table 1. Time and treatment effects on the QST parameters of healthy subjects

	Time effect			Treatment effect			Treatment \times time effect		
	F	p	+/-	F	p	+/-	F	p	+/-
CDT	20.67	< 0.001	+	0.46	0.64		1.16	0.32	
WDT	5.26	0.029	+	0.18	0.84		0.74	0.48	
CPT	5.1	0.032	-	0.59	0.56		0.94	0.39	
HPT	29.48	< 0.001	-	1.1	0.35		2.41	0.9	
MDT	2.15	0.15		1.35	0.27		2.98	0.059	
MPT	34.32	0.001	-	0.18	0.84		1.68	0.19	
MPS	0.11	0.75		0.07	0.93		0.07	0.93	
ALL	0.41	0.53		0.51	0.6		2.02	0.14	
WUR	0.05	0.83		0.67	0.52		0.23	0.79	
VDT	4.48	0.043	+	4.57	0.014	+	5.35	< 0.001	+
PPT	0.06	0.82		0.32	0.73		0.0003	1	
PPT-contr	6.22	0.019	+	0.19	0.82		1.32	0.27	
PPT-leg	6.48	0.016	+	3.36	0.052		0.41	0.63	
SPS	12.69	0.001	-	0.5	0.61		0.41	0.66	
SPS-contr	5.57	0.025	-	0.2	0.82		0.44	0.64	
SPS-leg	2.16	0.15		0.03	0.98		0.93	0.4	

Main results from the two-way RM ANOVA on QST parameters in healthy subjects after the treatments. Significant changes are shown in bold. "+" indicates an increase in the detection threshold (desensitization); "-" indicates a decrease in the detection threshold (sensitization).

CDT = cold detection threshold; WDT = warm detection threshold; CPT = cold pain threshold; HPT = heat pain threshold; MDT = mechanical detection threshold; MPS = mechanical pain threshold; ALL = dynamic mechanical allodynia; WUR = windup ratio; VDT = vibration detection threshold; PPT = pressure pain threshold; PPT-contr = pressure pain threshold on the contralateral side; PPT-leg = pressure pain threshold on the leg; SPS = suprathreshold pain stimulation; SPS-contr = suprathreshold pain stimulation on the contralateral side; SPS-leg = suprathreshold pain stimulation on the leg.

RESULTS

Thirty subjects fulfilled the inclusion criteria and completed all three experimental sessions in Study I. In addition, 14 asymptomatic healthy subjects (8 men, 6 women) and 14 patients (9 men, 5 women) with unilateral chronic LE completed all three experimental sessions in Study II and Study III (Fig. 1).

1. Effects on quantitative sensory testing in healthy participants (Study I)

The two-way RM ANOVA for the QST data revealed a significant main effect of time with increased CDT ($p < 0.001$)

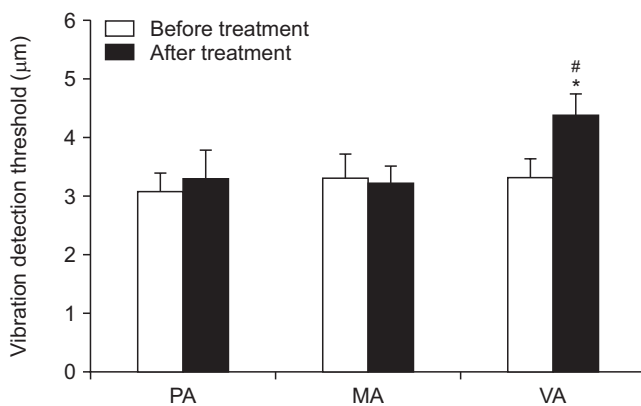


Fig. 2. The two-way repeated-measures analysis of variance (RM ANOVA) for the quantitative sensory testing (QST) data revealed a significant treatment \times time interaction for the vibration detection threshold (VDT) measurements. The *post hoc* analysis showed a significantly higher VDT after treatment with vibro-acupuncture (VA) than after manual acupuncture (MA) and placebo acupuncture (PA). Data are reported as the mean \pm standard error of the mean (SEM). # indicates a significant difference ($p < 0.05$) before and after treatment. * indicates a significant difference ($p < 0.05$) between treatments.

and WDT ($p = 0.029$) and decreased CPT ($p = 0.032$), HPT ($p < 0.001$) and MPT ($p < 0.001$) after treatment (Table 1). Significantly higher PPT values for the left arm ($p = 0.019$) and tibialis ($p = 0.016$) were also found (Table 1). However, no significant treatment \times time interactions were found for the abovementioned QST parameters (all $p > 0.05$). A significant treatment \times time interaction was found only for the VDT ($p = 0.004$). The *post hoc* analysis showed a significantly higher VDT after VA than after MA and PA ($p < 0.001$) (Fig. 2). One-way RM ANOVA showed no significant differences in the relative percentage changes in the PPT and SPS among the three treatments on any tested site ($p > 0.27$) (Table 1).

2. Effects on experimental muscle pain (Study II)

For Study II, pain intensity evoked by hypertonic saline injection was analyzed with one-way ANOVA. No significant interaction or main effect of treatment on the VAS intensity or area under curve (AUC) among the three different treatments was detected ($p > 0.086$) (Fig. 3). No significant treatment \times time interaction was detected for any QST parameter ($p > 0.179$).

3. Effects on clinical elbow pain (Study III)

The two-way RM ANOVA for the NPRS and pain area revealed a significant treatment \times time interaction ($p < 0.015$). The NPRS and pain area values after treatment were normalized to baseline values, and an additional one-way ANOVA was performed on the normalized data to test for a possible treatment effect on the relative changes. The normalized data showed significant decreases in the NPRS and pain area after VA (46%, 33%), MA (67%, 65%), and PA (85%, 94% respectively) compared with before treatment (100%). The *post hoc* analysis showed a significantly lower NPRS ($p = 0.005$) and smaller pain area ($p = 0.011$) during VA

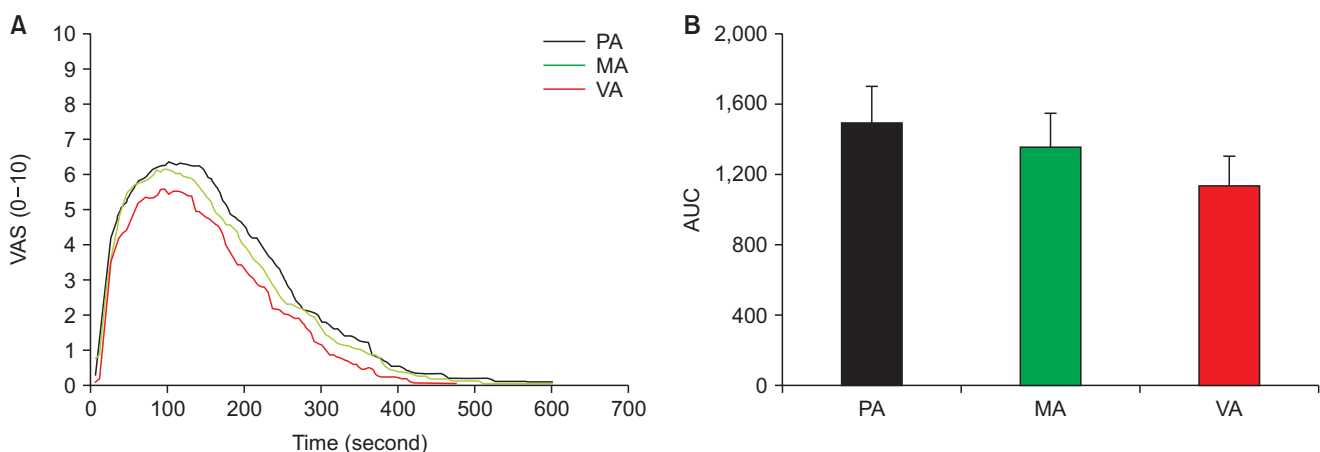


Fig. 3. Pain profile of hypertonic saline injection during treatment. No significant interaction or main effect of manual acupuncture (MA), vibro-acupuncture (VA), or placebo acupuncture (PA) was detected on the pain intensity as assessed by the visual analog scale (VAS) intensity (A) and area under curve (AUC) (B).

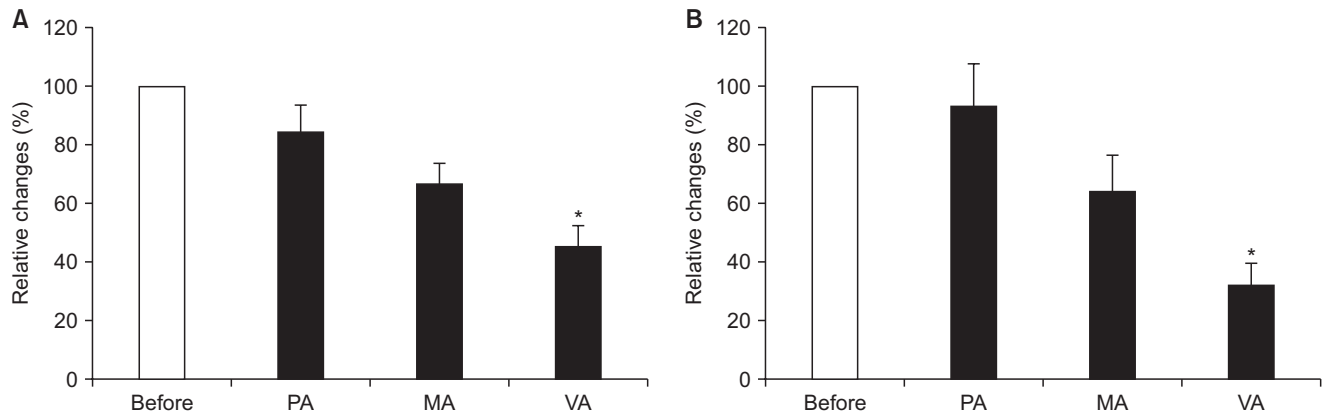


Fig. 4. The two-way repeated-measures analysis of variance (RM ANOVA) for the pain intensity (A) and drawing area (B) revealed a significant treatment \times time interaction. The normalized data showed significant decreases in the pain intensity and pain drawing area after compared with before treatment (100%). The post hoc analysis showed a significantly lower intensity or smaller area ($p < 0.05$) after vibro-acupuncture (VA) treatment than after placebo acupuncture (PA). *indicates a significant difference compared with PA.

treatment than during PA (Fig. 4). No significant difference was detected between MA and PA ($p = 0.072$) or between MA and VA ($p = 0.321$). No significant treatment \times time interaction was detected for any QST parameter ($p > 0.310$).

DISCUSSION

A novel VA device was developed and for the first time employed to investigate its effects in human volunteers and patients with chronic pain. The application of VA increased the VDT in healthy volunteers, suggesting a specific modulation of A β fibers. No significant effect was found on experimentally induced muscle pain, and significantly lower pain intensity and smaller area were found during VA treatment than during PA treatment in patients with LE.

1. Effects on QST parameters in healthy volunteers

Study I tested the immediate effects of different acupuncture interventions on somatosensory perception in healthy adults. The acupuncture stimulus influences specific types of activated receptors, and increasing evidence has revealed that the types of afferent nerve fibers activated by acupuncture are diverse. An early study demonstrated that different afferent fiber types are implicated in conveying the sensory response for *de qi*, with soreness mainly carried by C-fibers, numbness by A β -fibers, and aching and fullness (heaviness and distention) by A δ -fibers [23]. MA stimulation induces the *de qi* feeling by exciting mainly A δ -fibers [24,25]. However, when the needles penetrate through the skin and are twisted down into deeper tissues, the tissues are locally injured, proinflammatory mediators are released, and nociceptors are directly or indirectly excited [24]. It is therefore conceivable that C-fibers are also involved during MA [2]. The noninvasive PA treatment does not evoke the

traditional *de qi* sensation, but the short pinprick sensation may evoke activity in nociceptors due to a blunt needle penetrating the skin around the acupuncture points, and C-fibers as well as A δ -fibers could also be stimulated. Our previous study showed nonsignificant differences in the Massachusetts acupuncture sensation scale for PA compared with MA and VA across the different *de qi* descriptors, indicating that the placebo needle, at least in part, induced sensations comparable to those of real penetrating needles [13]. Our results suggest that VA, different from MA and PA, stimulated mainly A β -fibers by mechanical vibrostimulation [13,14].

Significant time-dependent differences were found for the thermal QST parameters, with significant increases for the CDT and WDT (hypoesthesia) and decreases for the CPT and HPT (hyperalgesia), suggesting the involvement of A δ - and C-fibers. A similar local effect was found also for the MPT post-treatment compared with baseline, indicating sensitization of A δ -fibers. The hyperalgesic effect for the CPT, HPT, and MPT was unexpected. Several previous studies have shown that acupuncture stimulation potentially increases thresholds, i.e., has a desensitization effect on QST parameters [15,26-28]. However, in contrast to these findings, other studies did not detect significant changes in QST parameters after acupuncture [29,30]. Moreover, another study tested the CPT in the lower extremity following electroacupuncture with different stimulation time periods and demonstrated a significantly reduced CPT (hyperalgesia) at 30 minutes [31]. Importantly, the different modalities, stimulated points, and experimental timeframes used in the previous studies may have yielded heterogeneous results. Considering the controversial results of previous studies, it is difficult to make any firm conclusion about the effect of acupuncture treatment on QST parameters.

The time-dependent differences found in our study could be an inherent impact of repeated measurements of the QST protocol over a short time period. The significant changes in the CDT, WDT, and HPT were reported when running several cycles of thermal testing (i.e., repeated testing) within one hour using the same DFNS protocol [32]. Nonetheless, QST parameters have exhibited good test-retest reliability in one-day intervals [33,34]. The timeframe of testing in the current study was close to one hour, and as such, the local skin could have been irritated by the noxious stimuli due to the repeated ascending and descending of thermal and mechanical stimuli during the QST, which could explain why several parameters were sensitized over time, rather than due to desensitization effects after acupuncture treatment. The timeframe of the QST should be considered in future studies to avoid evoking local sensitization of the testing point.

In the present study, a significant treatment-dependent difference was found only for the VDT, which was significantly increased after VA compared with after PA and MA, suggesting that VA produced significant desensitization of A β -fibers compared with PA and MA in healthy subjects. Vibration is primarily conveyed by large myelinated A β -fibers [35]. A β -fiber sensitization is a common problem in neuropathic pain conditions in which nociceptor function may be selectively impaired within the allodynic skin and light moving mechanical stimuli can often produce severe pain, i.e., dynamic mechanical allodynia [36]. If VA is capable of providing desensitization of A β -fibers, patients with such painful conditions could perhaps be a population who could benefit from VA. Numerous results support that excitation of A β -type fibers are involved in electroacupuncture or transcutaneous electrical nerve stimulation analgesia, which have different analgesic effects by modulating the stimulation modalities [37]. A specific vibrational sensation evoked by VA could potentially exhibit more effects in chronic pain. However, further research is needed to elucidate the specific effects of VA.

2. Effects on experimental muscle pain in healthy volunteers

Acupuncture stimulation is thought to exert its effects through activation of A δ -fibers or A β -fibers, which, based on the modified gate-control theory, could act to inhibit nociceptive transmission [2]. The activation of these afferent fibers might alter pain transmission at the spinal cord, midbrain, and hypothalamus [38]. Hypothalamic activation can then lead to systemic, pain-modifying effects by releasing substances such as endorphins [39]. The diffuse noxious inhibitory control mechanism [40] should also be considered as a possible explanation for the analgesic effect of acupuncture. However, the efficacy of acupuncture on human

experimental pain models is still controversial. A previous study used a dental pain model for acupuncture research and found that experimentally induced dental pain can be influenced by acupuncture [41]. In contrast, another study using cold-pressor and capsaicin injection models concluded that acupuncture on predefined points has a minor effect on experimentally induced pain [42].

The results of the current study showed that the pain intensity was lower and that the pain duration was shorter after MA and VA than after PA; however, these differences were not statistically significant (Fig. 4). In our experimentally induced pain model, hypertonic saline was injected 5 minutes after starting each acupuncture intervention. The evoked pain lasted approximately 6-7 minutes when the treatment effect was evaluated. A previous animal study demonstrated that the analgesic effect of acupuncture reached its maximum within 15 to 30 minutes and decreased towards baseline 50 minutes after the treatment [43]. Considering the results from this animal model, it is possible that the optimal time to evaluate the pain intensity was missed in the participants due to the timeframe of the experiment. Another possible reason could be the relatively small sample size. Thus, the current results regarding the efficacy of VA for acute experimentally induced pain should be interpreted cautiously.

3. Effects on musculoskeletal chronic pain

Increasing clinical evidence supports that MA or electroacupuncture has convincing therapeutic effects in various painful conditions, and these effects can last for a long period of time even hours after the application [31,39,44,45]. A randomized, controlled, multicenter clinical trial with a large sample size was conducted to confirm the efficacy of acupuncture for chronic pain including low-back pain, tension-type headache, migraine, neck pain, and knee osteoarthritis [46]. Overall, the results demonstrated that acupuncture is effective for these chronic conditions [46].

Our results indicate that pain intensity was decreased in patients with chronic LE immediately after acupuncture treatment, with VA showing better effects than the other two treatment modalities. The distinct vibrational sensation evoked by VA may have analgesic properties in chronic pain conditions such as LE. Vibration has long been considered a therapeutic modality capable of alleviating pain. The underlying mechanisms, however, are unclear. Earlier evidence in animal studies has suggested that the analgesic effect could be achieved through high-frequency stimulation of the low-threshold A β -fibers, which in turn alters and inhibits nociceptive transduction through dorsal horn neurons [4]. Moreover, high-frequency vibration has been shown to suppress activity in areas related to pain processing in the somatosensory cortex, which further supports the

notion that vibrational stimuli induce central changes in pain perception [19]. Previous studies have shown that high-frequency (100-200 Hz) vibration stimulation can increase pain thresholds in healthy humans [47,48] and decrease pain in patients suffering from chronic myofascial pain including LE [49]. Moreover, the possible application of vibration therapy in dental pain conditions has also been reported [50]. To date, it is not clear if the analgesic effects of the vibration involve other underlying mechanisms, e.g., descending inhibitory pathways, neuropeptides, or neuroplasticity, that were not assessed in our experiments. The many different pain scenarios in which vibration therapy has been applied and found effective support the original idea that the gate control theory is involved, i.e. innocuous vibration stimulation carried predominantly by A β -fibers may alter nociceptive transmission on a segmental and central level [10]. Current and previous findings therefore support that vibration combined with acupuncture may be a beneficial combination for providing improved pain relief in different pain conditions.

Han [37] demonstrated that different electrical parameters, such as stimulation frequencies, have different therapeutic benefits in terms of pain relief provided by electroacupuncture. Low-frequency stimulation (2 Hz) can facilitate the release of β -endorphins, enkephalins, and endomorphins, which have better analgesic effects on neuropathic pain [37]. In contrast, high-frequency stimulation (100 Hz) can selectively increase the release of central dynorphins, which have better analgesic effects on inflammatory pain and muscle spasms [37]. The frequency-dependent effect could also exist in vibration stimulation. However, only high-frequency vibration (100 Hz) was employed in our studies; thus, the possible frequency-dependent effect of VA should be investigated in future studies.

4. Methodological considerations and limitations

The present novel method of VA can provide an extra mechanical stimulation in addition to conventional acupuncture. Similar to what is performed with the well-known electroacupuncture, stimulation parameters, such as the stimulation frequency and intensity and interval of stimulations, can be adjusted to maximize the therapeutic benefit. However, the possible differences in the mechanism and treatment effect between mechanical stimulation and electrical stimulation need to be investigated in future studies.

Some of the results obtained by QST should be interpreted with caution since QST is a psychophysical test and therefore depends on patient cooperation. The test-retest in a short time might have had an effect on the results. The local sensitization of the skin evoked by the test-retest procedure

over a short time may have offset the desensitizing effect of the acupuncture treatments, which might have result in absent changes in the QST parameters. An interval of 24 hours between sessions is suggested for the test-retest procedure for QST in future studies [33,34]. Furthermore, for practical reasons, the age and gender of the participants were not matched in the groups. Therefore, the data could not be directly compared between groups. Finally, the present study investigated only immediate effects after a single treatment with two needles; this does not represent clinical practice in which multiple sessions and multiple needle stimulations are usually applied.

CONCLUSIONS

A specific desensitization of A β -fibers was observed following the VA treatment in healthy volunteers. No significant effect was found on experimentally induced muscle pain. The pain intensity and pain area decreased more in patients with chronic LE immediately after VA than after PA. Further larger cohort, long-term follow-up clinical studies are needed to elucidate the specific effects and mechanisms of VA.

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AUTHORS' CONTRIBUTIONS

All the authors contributed to the study. The study was designed by KW and LAN, and performed by DBL, SAQ, YZ and KW. The data were analyzed by DBL, YZ, and HL. The manuscript was drafted by KW, DBL, CFP and LAN. All the authors helped to revise the manuscript and approved the final version for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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