Immediate effects of acupuncture and cryotherapy on quadriceps motoneuron pool excitability: randomised trial using anterior knee infusion model

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ABSTRACT

Objective The authors asked the following research questions: will an anterior knee infusion model induce constant pain? will perceived pain alter motoneuron pool (MNP) excitability? and will treatments alter perceived pain and/or MNP excitability?

Methods Thirty-six neurologically healthy volunteers participated in this randomised controlled laboratory study. To induce anterior knee pain (AKP), 5% hypertonic saline (0.12 ml/min with a total volume of 8.5 ml over 70 min) was injected into the infrapatellar fat pad of the dominant leg. One of four 30-min treatments was randomly assigned to each subject after pain was induced (acupuncture, cryotherapy, sham cryotherapy and no treatment). Five acupuncture needles (SP9, SP10, ST36, GB34 and an ah shi point) were inserted to a depth of 1 cm. Vastus medialis (VM) maximum Hoffmann reflexes normalised by maximum motor response were recorded from each subject at baseline, 20 min post-injection, 50 min post-injection and 70 min post-injection. To record pain perception, a visual analogue scale was used every 5 min after injection.

Results An anterior knee infusion pain model increased perceived pain (p<0.0001). No change was found in VM MNP excitability among the four treatments (p<0.19) or at any of the time intervals (p<0.52). Cryotherapy reduced perceived pain compared with acupuncture (p=0.0003) and sham treatment (p=0.0002).

Conclusions A pain model may be used in other neurophysiological intervention studies related to AKP. AKP alone may not directly alter quadriceps activation. None of the treatments altered VM MNP excitability. Cryotherapy reduced pain while a single session of acupuncture and sham treatments did not.

Quadriceps inhibition associated with anterior knee pain (AKP) has been suggested in individuals with clinical pain. AKP is believed to cause abnormal sensory input to the central nervous system, which may reduce the excitability of the α-motoneuron in the surrounding musculature. However, the direct relationship between pain and muscle activation is inconclusive because swelling, inflammation and structural damage may confound the results. To remove the other factors associated with clinical knee pain, researchers have used an induced pain model. An injection of 5% hypertonic saline into the infrapatellar fat pad causes chemical irritation, resulting in an increase in nociceptor activity. This has been considered to be a safe and effective injury model for examining neuromuscular activity changes under the condition of isolated pain.

Although the artificial pain model quickly induces moderate dull and achy pain, the induced pain only lasts approximately 10 min. A single injection pain model is limited to studies in which the measurements or interventions take less than 10 min. A continuous infusion of 5% hypertonic saline has previously been used in muscle to elicit longer-lasting pain. We hypothesised that a continuous infusion of 5% hypertonic saline into the infrapatellar fat pad would also induce a longer constant AKP allowing longer measurements to be taken or interventions to be used.

Multiple sessions of acupuncture treatments have been shown to reduce pain in patients with AKP and knee osteoarthritis. Activation of somatic afferents in peripheral nerves by acupuncture needle stimulation has been considered to be a primary neurophysiological mechanism behind the effects on pain reduction. Increased activity in A-δ and C-fibres due to noxious stimulation may release β-endorphins and enkephalins, resulting in analgesic effects. Acupuncture may also produce psychological (placebo) effects to evoke an increase in the release of endogenous opioids, resulting in central pain inhibition. In addition, a single session of acupuncture treatment immediately increased maximal voluntary quadriceps torque in healthy individuals. However, potential changes in motoneuron pool (MNP) excitability due to acupuncture treatments in patients experiencing isolated AKP have never been examined.

Cold application has been widely used to decrease pain and facilitate active exercise. Knee joint cooling has increased quadriceps activation in individuals with tibiofemoral osteoarthritis, artificial effusion and in healthy knees.

response following a cold application may include a reduction in nerve conduction velocity, discharge rate of receptors and receptor sensitivity. Cryotherapy also stimulates cutaneous mechanoreceptors that excite Ia interneurons, resulting in excitatory potentials at the MNP. However, little is known about how cryotherapy effectively reverses quadriceps inhibition in isolated AKP individuals.

The purpose of this study was to examine the immediate effects of cryotherapy and acupuncture on vastus medialis (VM) MNP excitability in subjects with experimentally induced AKP. We asked the following research questions: will an anterior knee infusion model induce constant AKP? If so, will perceived AKP alter quadriceps MNP excitability? And will treatments (acupuncture, cryotherapy, sham and control-no treatment and no injection) alter perceived AKP and/or MNP excitability? We hypothesised that an anterior knee infusion model would produce constant AKP, an increased knee pain would decrease quadriceps MNP excitability and acupuncture and cryotherapy would reverse quadriceps inhibition and decrease perceived knee pain.

**Figure 1** CONSORT flowchart. VM, vastus medialis.
METHODS

Experimental design
A randomised controlled laboratory study with repeated measures on time was used. Dependent variables were the VM H-reflex normalised to the M-response (H:M ratio) and pain perception. The independent variables were treatment (acupuncture, cryotherapy, sham treatment and control) and time (baseline: preinjection; pretreatment: 20 min post-injection; post-treatment-0: 50 min post-injection; and post-treatment-20: 70 min post-injection).

Subjects
Thirty-six neurologically healthy volunteers (age 22.7±1.79 years, height 1.82±0.08 m, mass 76.72±10.15 kg) with no history of lower extremity conditions resulting in surgery, no lower extremity injury in the past 6 months, and a measurable VM H-reflex participated in this study. One hundred and twelve subjects were initially recruited in the study. After screening, 72 subjects were excluded due to an unmeasurable VM peak H-reflex (n=72). After data analyses, an additional four subjects were excluded due to unstable M-responses (n=3) and missing H-reflex data at the pretreatment time interval (n=1). The CONSORT flow chart is presented in figure 1. Before participation, all subjects read and signed a written informed consent form approved by the university’s institutional review board.

Instrumentation

Anterior knee infusion model
A 20 gauge catheter (Becton Dickinson Medical Systems, Sandy, Utah, USA) was inserted into the lateral aspect of the infrapatellar fat pad on the subject’s dominant limb (figure 2). The catheter was inserted directly under the patellar tendon at an angle of 45°, in an inferior-medial direction, with a depth of 1 cm. A 100 cm extension tube (B.Braun Medical, Bethlehem, Pennsylvania, USA) was interfaced between the catheter and a 30 ml syringe (Becton Dickinson Medical Systems, Franklin Lakes, New Jersey, USA) filled with 5% hypertonic saline (B. Braun, Irvine, California, USA). This syringe was placed on an infusion pump (Graseby Medical Ltd., Hertfordshire, UK) to deliver hypertonic saline constantly over 70 min. The infusion rate was set to 0.12 ml per min with a total volume of 8.5 ml over 70 min.

H-reflex and M-response (H:M ratio)
Subjects remained supine with their involved knee supported at approximately 15° of flexion and the hip slightly flexed (figure 3). Subjects were instructed to place their hands along their sides with their palms facing the ceiling. Subjects were also instructed to maintain this position while they looked at a spot on the ceiling. Subjects listened to white noise through headphones to avoid any possible sound-induced variability in measurements of the H-reflex.

Two locations on the testing limb (dominant leg) were shaved, debrided (with sandpaper) and cleaned with alcohol prep wipes before the placement of self-adhesive surface electromyography (EMG) electrodes (silver–silver chloride, 10 mm diameter; EL 503-10; Biopac Systems, Goleta, California, USA). EMG electrodes (2 cm apart) were attached to the bulk of the VM (figure 4). The ground electrode was attached to the medial malleolus of the ipsilateral limb.

The stimulating module (Biopac STM 100C), isolation adaptor (Biopac STIMISOC) and a bar stimulating electrode (Biopac EL 300) provided the stimulus. The intensity of the stimulus varied between 100 and 180 V with...
The stimulating electrode was placed over the femoral nerve, just lateral to the femoral artery in the femoral triangle of the ipsilateral limb. The bar electrode was secured with medical tape and compression warp (figure 3).

Surface EMG (MP 150; Biopac Systems, Santa Barbara, California, USA) was used to measure the peak H-reflex and M-response. Gain was set at 500 to amplify the signal (DA 100B; Biopac Systems) from the electrodes. EMG data were sampled at 2000 Hz for 50 ms. Maximum H-reflex and M-response were found by gradually increasing the stimulator in 0.1–1 V increments, allowing for a 15 s rest interval between stimulations. As the H-reflex values are extremely variable between subjects, we obtained the M-response to normalise the H-reflex in each individual. Once the maximum H and M waves were found, five measurements for each were recorded and used to calculate the H:M ratio. The calculated H:M ratios were then used for data analysis.

Pain perception
Subjective pain perception was quantified using a 10 cm visual analogue scale (VAS).

Testing procedures
The University’s institutional review board approved the testing procedures of this study (figure 5). During the first visit, subjects read and signed the informed consent form approved by the university’s institutional review board. Subjects also read and completed a brief health history form. Subjects were screened for a measurable VM H-reflex. Subjects with a measurable VM H-reflex were asked to come back after a week for data collection. Subjects who did not have a measurable H-reflex were excluded.

On the second visit, baseline (preinjection) VM MNP excitability data were measured. The catheter was inserted into the infrapatellar fat pad. The extension tube linked with the 50 ml syringe loaded with 5% hypertonic saline was connected to the catheter. Twenty minutes after the initial infusion, pretreatment VM MNP excitability data were taken. Each subject was then randomly assigned a treatment (acupuncture, cryotherapy, sham and control) for 30 min. These treatments were administered in a counterbalanced order. Upon completion of the treatment (50 min post-injection), the post-treatment-0 VM MNP excitability data were recorded. Twenty minutes after the treatment, the same data at post-treatment-20 were measured. Pain perception on the VAS was measured before and immediately after the catheter insertion, then every 5 min until catheter removal (70 min post-injection). The catheter was detached after the last measurement. The puncture site for catheter insertion was cleaned and covered with sterilised gauze. Subjects were dismissed when self-reported pain was zero on the VAS.

Treatment groups
Acupuncture (n=9)
Acupuncture treatment was performed under direct supervision of a licensed acupuncturist. Acupuncture points (acupuncture needle sites: SP9, SP10, ST36, GB34, and one next to the catheter—ah shi point; table 1 and figure 4) were cleaned with alcohol prep before and after the treatment. Five disposable sterile stainless steel acupuncture needles (0.2×43 mm; Dongbang Acupuncture, Sungnam, Korea) were manually inserted into each acupuncture point, followed by bidirectional rotation to elicit needle sensation (de qi). A guide tube (0.5×40 mm) was used to decrease acupuncture-related discomfort.
Each acupuncture needle was inserted to a depth of 1 cm. The acupuncture needles were left in place for 30 min.

Cryotherapy (n=10)
Two plastic ice bags (1.5 l of crushed ice) were directly placed on the anterior and posterior surfaces of the knee for 30 min. The ice bags were secured with a 4-inch wide compression wrap (figure 6).

Sham (n=9)
The same methods used for the cryotherapy treatment group were applied, but cat litter (1.5 l) was filled in the plastic bag instead of crushed ice.

Control (n=8)
Subjects received no treatment. The catheter was inserted. An extension tube was interfaced between the catheter and a 30 ml syringe filled with isotonic saline (0.9% sodium chloride). This syringe was placed on the constant infusion pump, but the infusion pump was turned off.

Statistical analysis
Our sample size was calculated using an expected change in the H-reflex of 1.8 and a SD of 1.44. Based on these estimations, 11 subjects in each group would be necessary in order to have an 80% chance of detecting a significant difference with p<0.05.

Means were calculated from five trials of each time interval. To test treatment effects over time, a 4×4 mixed model analysis of covariance for the VM MNP excitability (covariate: baseline H:M ratio) was used. A separate 4×16 mixed model analysis of covariance tested the treatment effects for pain perception (covariate: baseline VAS measurement). Tukey–Kramer post-hoc tests were used for comparisons (p<0.05 for all tests).

RESULTS
Mean (SD) perceived AKP as a percentage change from the baseline measurement is depicted in figure 7. Continuous infusion of 5% hypertonic saline increased perceived AKP (interaction F_{4,44}=4.58, p<0.001). Fifteen minutes following the injection, subjects statistically began to feel AKP when the control group to the acupuncture, cryotherapy, or sham treatment group (p<0.0001). Compared with the control group, perceived AKP was maintained until catheter removal (55 min) in the sham treatment group (p<0.0001).

Mean (SD) VM MNP excitability (H:M ratio) for each treatment over time is summarised in table 2. We did not find any change in VM MNP excitability (H:M ratio) among the four treatment groups (F_{3,31}= 1.68, p<0.19) at any of the time intervals (F_{2,64}=0.66, p<0.52).

<table>
<thead>
<tr>
<th>Points</th>
<th>Segmental innervations</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP (stomach) 9</td>
<td>(S1–2)</td>
<td>In a depression in the angle formed by the medial condyle of the tibia, on the level of the lower border of the tibial tuberosity</td>
</tr>
<tr>
<td>SP (spleen) 10</td>
<td>(L2–4)</td>
<td>Two cun(^*) above the mediosuperior border of the patella</td>
</tr>
<tr>
<td>ST (stomach) 36</td>
<td>(L4–5)</td>
<td>One finger breadth below and lateral to the tibial tuberosity</td>
</tr>
<tr>
<td>GB (gall bladder) 34</td>
<td>(L5–S1)</td>
<td>In the tender depression approximately one cun anterior and inferior to the head of the fibula</td>
</tr>
<tr>
<td>Ah shi point†</td>
<td>(L2–4)</td>
<td>Medial aspect of infrapatellar fat pad</td>
</tr>
</tbody>
</table>

\(^*\)Cun, the breadth of the distal phalanx of the thumb.

†Ah shi point, a point that is painful on palpation.
Cryotherapy reduced perceived AKP compared with acupuncture 20 min following application (p=0.0003) and sham treatment 15 min following application (p=0.0006). A reduced pain level in the cryotherapy group was maintained until the last measurement (70 min post-injection, p=0.002). Acupuncture only reduced AKP compared with sham treatment 20 min following application (p=0.02). At other times, there was no difference in reducing perceived AKP between acupuncture and sham treatment (p=0.98).

**DISCUSSION**

Continuous infusion of 5% hypertonic saline at a rate of 0.12 ml per min produced constant AKP over a 55 min period. The pain perception in this study peaked at 15 min post-injection and the pain level was maintained until catheter removal (70 min post-injection, p=0.002). Acupuncture only reduced AKP compared with sham treatment 20 min following application (p=0.02). At other times, there was no difference in reducing perceived AKP between acupuncture and sham treatment (p=0.98).

The infra-patellar fat pad has nociceptive innervations, and an increase of nociceptive activity from this structure may affect spinal-mediated reflexes, resulting in quadriceps inhibition. These alterations could include an increase in the flexion withdrawal response and/or a decrease in flexion reflex thresholds. For this reason, we expected that our pain model might produce similar effects in the involuntary measurement (VM MNP excitability). Our results support the idea that isolation of nociceptive activity in the infra-patellar fat pad may not directly cause quadriceps inhibition.

Compared with pain, it is relatively well established that knee joint effusion causes quadriceps inhibition. An increase of Ruffini ending activity within the joint capsule excites the Ib inhibitory interneurons in the spinal cord. Ib interneurons receive information from afferents (joint receptors, mechanoreceptors, Golgi tendon organs and some Ia fibres) and descending signals originating from supraspinal centres (other neurons in the central nervous system). A negative, net effect change of the Ib interneurons is thought to contribute to quadriceps inhibition. It is unlikely, given our findings, that a pain stimulus (types III and IV) facilitates the Ib inhibitory pathway. We speculate that different types of stimuli may follow different or shared pathways. Based on the available data, spinal-mediated reflexes are more affected by joint effusion and appear to play a bigger role in arthrogenous muscle inhibition than pain. Motor responses from isolated pain and effusion should be compared in future studies.

### Table 2 Quadriceps MNP excitability

<table>
<thead>
<tr>
<th></th>
<th>Acupuncture</th>
<th>Cryotherapy</th>
<th>Sham</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.37 (0.27)</td>
<td>0.33 (0.24)</td>
<td>0.30 (0.12)</td>
<td>0.38 (0.23)</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>0.29 (0.15)</td>
<td>0.30 (0.26)</td>
<td>0.27 (0.17)</td>
<td>0.40 (0.38)</td>
</tr>
<tr>
<td>Post-treatment-0</td>
<td>0.27 (0.19)</td>
<td>0.27 (0.21)</td>
<td>0.26 (0.15)</td>
<td>0.41 (0.4)</td>
</tr>
<tr>
<td>Post-treatment-20</td>
<td>0.29 (0.22)</td>
<td>0.26 (0.23)</td>
<td>0.26 (0.12)</td>
<td>0.40 (0.32)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

MNP, motoneuron pool.
Our acupuncture treatment did not produce a significant effect on VM MNP excitability or pain perception. There have been many studies demonstrating pain reduction; however, multiple sessions of acupuncture treatment may be necessary to generate treatment effects. There is a wide variation in the parameters of acupuncture treatment for patients with knee pain in terms of the number of acupuncture needles (four, six, nine, eight to 10 and seven to —15), the diameter of the acupuncture needle, and the depth of acupuncture needle insertion (1–1.5 cm and 0.5–3.5 cm). In order to minimise the confounding factors between subjects, the acupuncture points were set as those commonly used for knee pain (SP9, SP10, ST36, GB 34) using an ah shi point with a depth of 1 cm. The depth of acupuncture needles is commonly decided by the sensation of de qi. De qi is the sensation felt by a patient as a tension and tenderness with acupuncture needle insertions, which is believed to be necessary to achieve positive outcomes. This may explain why our acupuncture treatment did not show any observable change in the MNP excitability or pain perception, as we used a set protocol with a standard depth.

Application of two ice bags around the knee joint did not change VM MNP excitability. Cryotherapy is thought to be an effective disinhibitory modality due to empirical evidence of cryokinetics and scientific demonstrations. An increase in quadriceps activation by knee joint cooling has been reported in the measurement of voluntary (maximal isometric contraction and central activation ratio) and involuntary activations (H-reflex). In addition, cryotherapy increased quadriceps activation in a healthy population. Therefore, we expected our 30 min cold application would increase the VM MNP excitability under all conditions. H-reflex is a highly variable measurement between subjects (0.93±0.14 in a crossover design study; 0.32±0.2 in a non-crossover design study). To resolve this issue, we normalised the H-reflex to the M-response. Subjects were nested with treatments in our study. This may have increased variability in VM MNP excitability, failing in the demonstration of significant differences. For this issue, we ended the trial even though we did not have 11 subjects in each group.

The H-reflex amplitude represents the measurable portion of excited MNP and the M-response represents the entire MNP. Decreased amplitude of the quadriceps H-reflex indicates quadriceps inhibition. As subjects have to relax completely in the supine position, this measurement allows us to estimate the physiological (involuntary) capacity to recruit available MNP. We were interested to see how an isolated pain stimulus and therapeutic modalities change the physiological capacity of MNP excitability. For this reason, we measured the H-reflex and the M-response. We excluded 72 subjects due to unmeasurable quadriceps H-reflex. The quadriceps H-reflex is more difficult to obtain due to the deeper location of the femoral nerve. Previous reports also mentioned exclusion of the subjects due to unobtainable H-reflex in the quadriceps. Safety issues related to the anterior knee infusion model should be considered. First, as the 5% hypertonic saline is infused via the catheter and the perceived pain is maintained for an extended period of time, subjects may feel vasovagal syncope. Vasovagal syncope is from a sudden drop in blood pressure, resulting in light-headedness (faint) due to lack of oxygen supply in the brain. In this study, we did not observe any vasovagal reaction. We believe that this was due to the subjects’ testing position—subjects were laid on the treatment table during infusion. However, subjects may have vasovagal syncope if the testing procedures require functional movements during infusion. We recommend having subjects lay down and elevate their legs above the heart. Second, subjects’ physical activity should be limited after infusion. We tested the anterior knee infusion model in 41 individuals, including five pilot subjects. We asked the subjects not to participate in any physical activity (more demanding than activities of daily living) for 48 h after the infusion. No subjects reported residual pain or discomfort after the infusion.

CONCLUSION
In conclusion, our anterior knee infusion pain model increased perceived pain in 5 min post-injection, pain peaked at 15 min post-injection, and the pain level maintained over a 55 min period after the peak. However, an increased perceived pain did not change VM MNP excitability. AKP alone may not directly alter quadriceps function. None of the treatments altered VM MNP excitability. Cryotherapy reduced pain while the acupuncture and sham treatments did not.

Contributors Both corresponding authors and coauthors, JH and JTH, have contributed the following criteria: conception and design, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version published.

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Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was granted by the Brigham Young University’s institutional review board.

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Original paper

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