Production of Consistent Pain by Intermittent Infusion of Sterile 5% Hypertonic Saline, Followed by Decrease of Pain With Cryotherapy

Blaine C. Long, Kenneth L. Knight, Ty Hopkins, Allen C. Parcell, and J. Brent Feland

Context: It is suggested that postinjury pain is difficult to examine; thus, investigators have developed experimental pain models. To minimize pain, cryotherapy (cryo) is applied, but reports on its effectiveness are limited. Objective: To investigate a pain model for the anterior knee and examine cryo in reducing the pain. Design: Controlled laboratory study. Setting: Therapeutic modality laboratory. Participants: 30 physically active healthy male subjects who were free from any lower extremity orthopedic, neurological, cardiovascular, or endocrine pathologies. Main Outcome Measures: Perceived pain was measured every minute. Surface temperature was also assessed in the center of the patella and the popliteal fossa. Results: There was a significant interaction between group and time ($F_{68,864} = 3.0$, $P = .0001$). At the first minute, there was no difference in pain between the 3 groups (saline/cryo = 4.80 ± 4.87 mm, saline/sham = 2.80 ± 3.55 mm, no saline/cryo = 4.00 ± 3.33 mm). During the first 5 min, pain increased from 4.80 ± 4.87 to 45.90 ± 21.17 mm in the saline/cryo group and from 2.80 ± 3.55 to 31.10 ± 20.25 mm in the saline/sham group. Pain did not change within the no-saline/cryo group, 4.00 ± 3.33 to 1.70 ± 1.70 mm. Pain for the saline/sham group remained constant for 17 min. Cryo decreased pain for 16 min in the saline/cryo group. There was no difference in preapplication surface temperature between or within each group. No change in temperature occurred within the saline/sham. Cooling and rewarming were similar in both cryo groups. Ambient temperature fluctuated less than 1°C during data collection. Conclusion: Intermittent infusion of sterile 5% hypertonic saline may be a useful experimental pain model in establishing a constant level of pain in a controlled laboratory setting. Cryotherapy decreased the induced anterior knee pain for 16 min.

Keywords: therapeutic modality, pain perception, experimentally induced pain

Investigating pain after an injury is difficult due to injury differences and patient subjectivity. Injured patients often have pain intensities that fluctuate in severity, thus making pain difficult to measure. To resolve this dilemma, experimental pain models have been developed to induce pain in uninjured people. Current experimental pain models involve a single injection or constant infusion of sterile hypertonic saline. Single injections of hypertonic saline produce mild to severe pain with a gradual onset and rapid decline when injected into the anterior knee. Constant infusion has been delivered into skeletal muscle with various infusion rates and volumes. Some include short infusions lasting 20 to 40 seconds, while others involve large volumes infused for approximately 15 minutes. These saline-infusion models either do not cause constant pain or cause too much pain within the first 5 minutes of the infusion, resulting in the subject’s decision to discontinue their participation in the study.

Since single injections and constant infusion do not create a constant level of pain for at least 20 minutes, they would be inadequate when examining a cryotherapy treatment. Cryotherapy is often applied for at least 20 minutes to decrease pain. To determine its effectiveness on decreasing pain, however, a constant level of pain must be established. Establishing a constant level of pain before a cryotherapy treatment would show that any change or fluctuation in pain perception is truly the result of the experimentally induced pain.

Through pilot work, we developed an experimental pain model of inducing constant anterior knee pain with intermittent infusion of sterile hypertonic saline. Such a model needs to be evaluated in a larger subject population so that investigators may examine the effectiveness of various therapeutic interventions. The purpose of this investigation was 2-fold: to investigate intermittent infusion of sterile 5% hypertonic saline solution in producing an experimental pain model for the anterior knee and to investigate the effectiveness of 20-minute cryotherapy treatment in reducing the experimentally induced pain.
Methods

Study Design

We used a controlled laboratory study to determine if group (saline infusion/cryotherapy [saline/cryo], no saline infusion/cryotherapy [no-saline/cryo], and saline infusion/sham [saline/sham]) influenced perceived pain with a visual analog scale (VAS) across time (every minute during a preapplication [5], application [20], and postapplication [10]). In addition, we measured surface temperature to assess the effects of cryotherapy and to determine if surface temperature changed in the saline/sham group.

Subjects

Thirty physically active, healthy male subjects (saline/cryo, n = 10: age 23.2 ± 4.1 y, height 180.3 ± 6.2 cm, mass 78.4 ± 9.0 kg; no saline/cryo, n = 10: age 23.7 ± 2.9 y, height 180.3 ± 6.3 cm, mass 78.9 ± 20.1 kg; saline/sham, n = 10: age 22.1 ± 2.7 y, height 182.1 ± 12.9 cm, mass 88.8 ± 15.4 kg) volunteered to participate in this investigation. We only included male subjects because of the reported differences in experimental pain observed between genders. Each subject went through a short orientation session before participation, which consisted of an explanation of the risks and benefits of the study and instructions on how to report perceived pain on a VAS. Subjects then filled out a preparticipation health-history questionnaire to ensure that they were free from any lower extremity orthopedic, neurological, cardiovascular, or endocrine pathologies. The study was approved by the institutional review board, and subjects gave written informed consent before being randomly assigned to 1 of the 3 groups by selecting a number 1, 2, or 3 from a container.

Instruments

Pain was induced with an implanted 24-gauge × 0.75-in. (0.7 × 19 mm) Teflon catheter (Alliance Medical, Russellville, MO) or the same Teflon catheter model with simultaneous infusion of 5% hypertonic saline (B. Braun Medical, Inc, Irvine, CA). To infuse hypertonic saline, a 30-cm extension set (B. Braun Medical Inc, Bethlehem, PA) was interfaced between the Teflon catheter and a 5-mL syringe filled with sterile 5% hypertonic saline. The syringe was positioned in a constant infusion pump (Harvard Apparatus, Millis, MA, model # 975) set to intermittently deliver the hypertonic saline over 25 minutes. Infusion rate was set on 0.54 mL/min during 5 consecutive on and off cycles. Each on/off cycle was 3 minutes long. Total volume of hypertonic saline infused was 7.02 mL. Once the first 5-mL syringe was emptied, we exchanged the syringe with a second 5-mL syringe.

Pain perception was measured with a new 100-mm VAS. The VAS was labeled no pain on the left polar end and unbearable pain on the right. When measures are taken every minute, the VAS is reported to be a reliable tool in assessing acute pain (r = .87). Temperature was measured with 3 PT-6 Kapton insulated surface thermocouples (Physitemp Instruments, Inc, Clifton, NJ) interfaced to a 16-channel Iso-Thermex electrothermometer (Columbus Instruments, Columbus, OH). We used one thermocouple to measure ambient air temperature. The other 2 thermocouples were secured to the center of the patella and popliteal fossa. To minimize the insulating effect from the pillow on the popliteal fossa temperature, we positioned a 20-oz plastic bottle with holes on each end and in the center between the pillow and the patient’s leg (Figure 1). Temperature data were recorded every minute throughout data collection and stored on a desktop computer.

To assess uncertainty (ie, reliability and validity) of the 3 thermocouples, we used a National Institute of Standards and Technology–calibrated mercury thermometer (Fisher Scientific International Inc, Hampton, NH) graded at 0.1°C immersed in a constant water bath. To establish the constant water bath we circulated the water with a stirrer (model 103, Corning PC, Corning, NY) and magnetic stir bar.

Testing Procedures

Subjects reported to the laboratory dressed in shorts and a T-shirt. On arrival, they were positioned supine on a treatment table with their dominant leg (ie, leg with which they kick a ball) slightly bent. A baseline pain-perception measure was then reported with a vertical mark placed on the 100-mm VAS. Pain measures on the VAS were recorded before their random assignment, because know-

Figure 1 — Teflon catheter inserted into the infrapatellar fat pad from the lateral side of the knee. The catheter was inserted perpendicular to the patellar tendon. To minimize interface temperature beneath the popliteal fossa, we customized a 20-oz plastic bottle so that air could pass freely over the thermocouple sensing tip.
ing what group they had been randomly assigned to may have influenced the pain score.

Two 2.5 × 2.5-cm areas, one over the patella and the other in the center of the popliteal fossa, were shaved and cleansed with isopropyl alcohol for surface thermocouple application. One thermocouple was secured to the center of the patella and the other in the center of the popliteal fossa. A small area inferolateral to the patella was shaved and cleansed with 70% isopropyl alcohol and povidone-iodine solution for catheter-needle insertion. To find the infrapatellar fat pad, the lateral border of the patellar tendon was palpated. The catheter needle was then inserted on the lateral side, posterior to the patellar tendon to a depth of 19 mm. Once inserted, the needle was extracted leaving the flexible Teflon catheter implanted in the infrapatellar fat pad (Figure 1). To ensure consistency the same investigator performed all procedures.

For the 2 saline-infusion groups, saline was intermittently infused for 25 minutes. At minute 5 during infusion, the pillow and plastic bottle were removed from the posterior side of the knee joint and 2 1-kg bags of crushed ice or cat litter (sham bags) were secured to the knee joint with a double 6-in. elastic wrap (6 in. × 10 yd) for 20 minutes (Figure 2). One bag was applied to the anterior surface of the knee joint and the other bag was applied to the posterior surface of the knee joint. The no-saline/cryo group also received ice bags at the same time interval as the saline/cryo group.

After the 20-minute application, the ice bags or sham bags were removed and subjects remained on the table for an additional 10 minutes. During the 10-minute postapplication period, subjects continued to report their perceived pain on new VAS every minute. Immediately after the postapplication period, the 30-cm connection tube was disconnected from the Teflon catheter, the catheter was extracted from the infrapatellar fat pad, and the insertion site was treated with antibiotic ointment and a protective bandage.

Immediately after the wound treatment and 24 hours after data collection, we contacted each subject to see if he was experiencing any lingering pain from the infused hypertonic saline or inserted Teflon catheter.

**Statistical Analysis**

We computed means and standard deviations for the perceived pain (VAS) at each minute during data collection. We also computed means and standard deviations for surface and ambient temperature of the patella and popliteal fossa during each application phase.

To determine if there were differences in perceived pain between and within each group across time, we used two 3 × 35 (group × time) mixed-model ANOVAs with random effects for subjects and within-subject order-1 autoregressive correlations.

Difference in patella, popliteal, and ambient temperatures were determined with three 3 × 3 (group × application phase) mixed-model ANOVAs with random effects for subjects and within-subject order-1 autoregressive correlations.

Modified Tukey–Kramer post hoc multiple-comparison tests were used to examine individual differences for each measurement variable. For all differences, the level of significance was set at $P < .05$. Data were analyzed with the MIXED procedures of Statistical Analytical Software (SAS; 9.1 Carey, NC).

Uncertainty consists of both random and systematic error. Random error is reported to contribute to reliability and systematic error is reported to contribute to validity. To calculate the uncertainty (reliability and validity) of our thermocouples we immersed the thermocouple sensing tip in the constant-room-temperature water bath. Water-bath temperatures were then recorded every 10 seconds for 10 minutes and compared with the National Institute of Standards and Technology–certified mercury.

**Results**

**Pain Perception**

Pain-perception data (mean ± SD) between and within each group across time are summarized in Figure 3. There was a significant interaction between group and time for perceived pain ($F_{68,864} = 3.0, P = .0001$). At the first minute, there was no difference in pain between the 3 groups (saline/cryo = 4.80 ± 4.87 mm, saline/sham = 2.80 ± 3.55 mm, no saline/cryo = 4.00 ± 3.33 mm; $P = 1.0$). During the first 5 minutes, pain increased from 4.80 ± 4.87 to 45.90 ± 21.17 mm in the saline/cryo group and from 2.80 ± 3.55 to 31.10 ± 20.25 mm in the saline/sham group ($P < .02$). Pain, however, did not change in the no-saline/cryo group, 4.00 ± 3.33 to 1.70 ± 1.70 mm ($P > .69$). At minutes 3, 4, and 5, pain in the saline/cryo and
saline/sham groups increased more than in the no-saline/cryo group (Figure 3; \( P < .0001 \)). At minute 5, pain in the saline/cryo group was greater than in the saline/sham (saline/cryo = 45.90 ± 21.17 mm and saline/sham = 31.10 ± 20.25 mm, \( F_{1,864} = 6.0, P = .03 \)).

From minute 10 to minute 27, pain did not change in the saline/sham group (43.2 ± 22.5 and 44.5 ± 26.9 mm, respectively; \( P > .05 \); 17 minutes of constant pain). Pain in the saline/cryo group decreased from minute 11 during application (40.50 ± 22.46 mm) to minute 7 after application (6.30 ± 8.47 mm; \( P < .05 \)), a 16-minute decrease in pain. For the no-saline/cryo group, pain did not change from minute 2 during application to minute 2 after application (20 minutes, \( P = .08 \)).

No subject experienced a level of pain that caused him to discontinue the study. In addition, no subject reported pain 24 hours after the investigation.

### Temperature and Thermocouple Uncertainty

Surface-temperature data between and within each group across application phase are summarized in Table 1. There was a significant interaction between group and application phase for patella (\( F_{4,99} = 456.52, P < .0001 \)) and popliteal surface temperatures (\( F_{4,99} = 523.36, P < .0001 \)). Both patella and popliteal preapplication temperatures did not differ between groups (patella \( P = .32 \) and popliteal \( P < .05 \)).

![Figure 3](image-url) — Perceived pain as measured on a new VAS each minute during each group across time (preapplication = 5, application = 0, postapplication = 10; mean ± SD). \(^a\)Saline/sham > saline/cryo (cryo; \( P < .05 \)). \(^b\)Saline/cryo > saline/sham (\( P < .05 \)). \(^c\)Saline/cryo and saline/sham > no-saline/cryo (\( P < .05 \)). \(^d\)Constant level of pain in the saline/sham and no-saline/cryo groups.

### Table 1 Average Patella and Popliteal Surface Temperature During Each Application Phase for Each Group (n = 10 Subjects/Group; Mean ± SD)

<table>
<thead>
<tr>
<th>Location/Phase</th>
<th>Saline/cryotherapy(^a)</th>
<th>No saline/cryotherapy(^a)</th>
<th>Saline/sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patella</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>preapplication</td>
<td>28.3 ± 1.1</td>
<td>27.7 ± 1.6</td>
<td>28.0 ± 1.2</td>
</tr>
<tr>
<td>application(^b)</td>
<td>7.2 ± 4.5</td>
<td>6.7 ± 4.2</td>
<td>28.2 ± 1.1</td>
</tr>
<tr>
<td>postapplication(^b)</td>
<td>10.9 ± 2.3</td>
<td>11.5 ± 2.4</td>
<td>28.3 ± 1.5</td>
</tr>
<tr>
<td>Popliteal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>preapplication</td>
<td>32.8 ± 0.7</td>
<td>31.7 ± 1.9</td>
<td>32.6 ± 1.2</td>
</tr>
<tr>
<td>application(^b)</td>
<td>8.4 ± 4.6</td>
<td>7.4 ± 4.2</td>
<td>32.4 ± 1.3</td>
</tr>
<tr>
<td>postapplication(^b)</td>
<td>17.7 ± 3.9</td>
<td>18.5 ± 4.3</td>
<td>33.0 ± 1.3</td>
</tr>
</tbody>
</table>

\(^a\)Application < postapplication < preapplication (\( P < .05 \)).

\(^b\)Saline/cryotherapy and no-saline/cryotherapy < saline/sham (\( P < .05 \)).
For the saline/cryo and no-saline/cryo groups, there was no difference in temperature between any group’s application and postapplication phases (patella $P = .29$, popliteal $P = .1$). However, temperature for the application and postapplication phases for the 2 cryo groups were less than for the saline/sham group ($P < .0001$). Patella and popliteal surface temperature remained the same within and between all phases for the saline/sham group ($P = .6$). Ambient temperature fluctuated less than $1^\circ C$ during data collection.

The calculated thermocouple uncertainty for both thermocouples used to measure patellar and popliteal fossa temperature was the same before and after data collection ($\pm 0.05^\circ C$). This was also true for the thermocouple used to measure ambient air temperature ($\pm 0.04^\circ C$). Calculations were done within 24 hours before and after data collection.

**Discussion**

Intermittent infusion of sterile 5% hypertonic saline into the anterior knee resulted in a constant level of pain that lasted for approximately 20 minutes. The constant level of pain produced by our intermittent infusion technique may be due to changes in extracellular osmolarity. It is hypothesized that increased extracellular osmolarity decreases cell size, and this decrease is linked to increased perceived pain. Intermittent infusion of sterile hypertonic saline may therefore alter extracellular osmolarity levels, thus constantly depolarizing the nociceptors, resulting in the observed pain.

Another interesting observation was the increased pain perception in the saline/cryo group at minute 5 of infusion. Uncontrollable variables such as anticipation, attitude toward the pain stimulus, stress, anxiety, or conscious awareness of knowing ice bags were going to be applied may have added variability in the pain measures.

The observed pain reduction during cold application is likely due to either a reduction in nerve conduction velocity or to changes in cerebral blood flow. Reductions in nerve conduction velocity occur as a result of increased refractory periods. As tissue temperature decreases, sensory-nerve impulses increase in duration, resulting in longer depolarizations. Longer depolarizations lengthen the time for impulses to reach the spinal cord, resulting in a fewer number being conveyed at a given time. This decrease in the number of impulses is responsible for the reduction in nerve conduction velocity. Any reduction in nerve conduction velocity may increase the amount of time it takes supraspinal regions to integrate nociceptive information, resulting in less information being processed. This increased amount of time may be responsible for the decreased pain perception.

Increased cerebral blood-flow changes during cold application may also be responsible for the decrease in pain perception. Casey et al. reported that a 90-second hand immersion in “painfully” cold water ($1^\circ C$) increased cerebral blood flow in the contralateral secondary somatosensory cortex, insular cortex, thalamus, and cerebellum—regions that contain endogenous opioid receptors that, when activated, are known to decrease pain. Therefore, applying cold modalities via ice-bag application in addition to the experimentally induced pain may increase cerebral blood flow and endogenous opioid-receptor activation, resulting in the decreased pain. More data are needed to determine the exact mechanism.

The different pain response to cold in the saline/cryo and no-saline/cryo groups is interesting. The saline/cryo group experienced an immediate decrease in pain after 1 minute of cold application, and the no-saline/cryo group experienced an increase in pain (Figure 3). These data support previous reports that cold application decreases pain in patients who have experienced a soft-tissue injury and increases pain in subjects not experiencing pain (ie, cold-induced pain). Theories to explain cold-induced pain have been reviewed elsewhere; see Knight.

As expected, there was no difference in cooling between the no-saline/cryo and saline/cryo group. Cryo-therapy decreased surface temperature at the center of the patella and popliteal fossa during application and increased during postapplication. For the saline/sham group, our data contradicted a previous investigation. Cat litter secured with a double-length 6-in. ACE wrap to the thigh muscles withdraws heat from the body and thus may not be a good control variable. We, however, observed no difference in surface temperature when the cat litter was secured to the anterior and posterior knee joint (Table 1). Therefore, the location of the cat-litter bag or the amount of compression may be responsible for these differences.

**Conclusion**

Pain caused by intermittent infusion of sterile 5% hypertonic saline is effective in producing a constant level of anterior knee pain for approximately 20 minutes. Cryotherapy immediately decreases experimentally induced pain within the first minute of application, with a gradual decline during application. This experimental pain model may be useful in examining the effectiveness of various interventions on pain or the effects of pain on skeletal-muscle activation.

**Acknowledgments**

This study was partially funded through an internal grant from the Mary Lou Chair for Health and Human Performance.

**References**

2. Bennell K, Hodges P, Mellor R, Bexander C, Souvlis T. The nature of anterior knee pain following injection of


