Effects of Cold Water Immersion on Edema Formation After Blunt Injury to the Hind Limbs of Rats

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Objective: Despite the long history of using cryotherapy to control edema, we found no randomized, controlled studies providing evidence to substantiate this common clinical practice. The purpose of this study was to determine whether cold water immersion affects edema formation following blunt injuries in rats.

Design and Setting: The feet of 16 rats were traumatized after hind limb volumes were determined. Four 30-minute treatments of cold water immersion (12.8°C to 15.6°C, 55°F to 60°F), interspersed with four 30-minute rest periods, began immediately after trauma to one randomly selected hind limb of each rat. The limb remained in a dependent position during all treatments, rest periods, and volumetric measurements.

Subjects: Sixteen anesthetized Zucker Lean rats were used in the study.

Measurements: Limb volumes were measured after each treatment and rest period for a total of 4 hours.

Results: The volume of treated limbs was significantly smaller ($p < .05$) than the volume of untreated limbs after the first treatment and remained smaller throughout the experiment.

Conclusions: Immersing rat limbs in 12.8°C to 15.6°C (55°F to 60°F) water immediately after blunt injury was effective in curbing edema formation.

Key Words: cryotherapy, swelling, animal model

Edema is a natural part of the inflammatory process, which is initiated by virtually any trauma, including athletic injuries. Uncontrolled edema can cause increased pain, prolonged immobilization, and reduced range of joint motion, all of which may extend time to recovery.[1,2] A primary goal in the initial management of orthopaedic injuries is prevention of excessive edema.[2,3] Cryotherapy, in conjunction with compression and elevation, is commonly accepted as an effective method of controlling edema after acute athletic trauma.[4,5,6,7] Cryotherapy is thought to reduce acute edema formation by decreasing blood flow,[8,9] metabolic activity,[8,10] and permeability of post-capillary venules.[11] In addition to cold, elevation, and compression, clinicians often use other modalities such as immobilization,[7] foam rubber horseshoes,[12,13] electrical stimulation,[14] and intermittent compression units[15] to control edema formation. The value of combining modalities is unknown, but the use of multiple modalities and treatments has made it impossible to determine whether cold alone is effective.

Because clinical trials are difficult to control, we have designed mock clinical trials using rats as subjects. Using rats, rather than humans, allowed us to control age, gender, size, and degree and location of injury. In addition, the effects of drugs or placebo were eliminated. Despite the long history of using cold to control edema, we found no positive evidence from randomized, well-controlled studies that cold curbs the formation of edema or reduces pre-existing edema. Indeed, several studies on acute edema formation in nonhuman animals have shown that cold increases edema formation.[16,17] However, in these studies cold was applied for up to 24 continuous hours, not as it is applied in modern therapeutic settings.[17] The purpose of our study was to determine if cold water immersion applied in a manner consistent with current clinical practice is effective in curbing edema formation following traumatic injury in rat hind limbs.

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METHODS

Subjects

Sixteen Zucker Lean rats (Harlan Sprague Dawley, Inc., Indianapolis, IN), weighing 244 g to 332 g (mean, 283 g), were used in this study. The animals were provided food and water ad libitum. The methods of anesthesia and handling procedures, including mode of traumatizing hind limbs and sacrifice, were approved by the Institutional Laboratory Animal Care Committee of the State University of New York at Buffalo.

Instrumentation and Procedures

Impact injury was induced by a procedure similar to that described by Mendel et al.[18] This consisted of dropping a steel rod weighing 85.5 g through a vertical tube from a height of 30 cm onto the plantar aspect of each foot just distal to the malleoli. A rectangular piece of plastic (2 × 2 × 0.5 cm) was

interposed between foot and tube to distribute the force of impact. This method of inducing trauma resulted in changes in limb volume that were attributable to edema formation and not frank bleeding; ie, it caused tissue damage without rupturing major vessels.[14,19,20,21] In previous studies, the height of the weight dropped was adjusted so that bleeding and skin rupture were avoided.[14,19,20,21] Furthermore, the skin on these rats is translucent, and we observed no change in color throughout the procedures.

Limb Volume Measurement

Limb volumes were determined by immersing a hind limb and measuring the amount of water displaced in a manner similar to that reported by Mendel et al.[18] The immersion vessel was 2 cm in diameter and 6 cm long (Fig 1). The inferior end of the vessel tapered and was an additional 6 cm long. A three-way stopcock was attached so that the vessel could be rapidly filled through the tapered end of the vessel. A 23-gauge stainless steel tube, 3 cm long and bent 90° in the middle, was fixed with epoxy to the inside wall so that 1.5 cm of the tube extended into the immersion vessel. The end of the tube outside the vessel was attached via polyethylene tubing and a 23-gauge needle to a 5-cc syringe. Using this tubing complex as a siphon, the water level in the immersion vessel could be brought to the same level repeatedly. A 3-cm piece of 2-0 thread was fixed with white plastic tape to the outside surface at the exact level of the tip of the stainless steel tube in the vessel. Animals, suspended in cloth slings, were prepared for volume measurement by painting lines at the level of their malleoli. Animals were then lowered by camera booms until the lines painted on the hind limbs aligned with the threads fixed to the immersion vessels (at the level of the tips of the stainless steel tubes). Displaced water was collected in 5-cc syringes by siphoning and was weighed on an S-300D micro balance (Fisher Scientific, Pittsburgh, PA). The weight of the fluid collected was equivalent to the animal's limb volume (1 g = 1 mL).

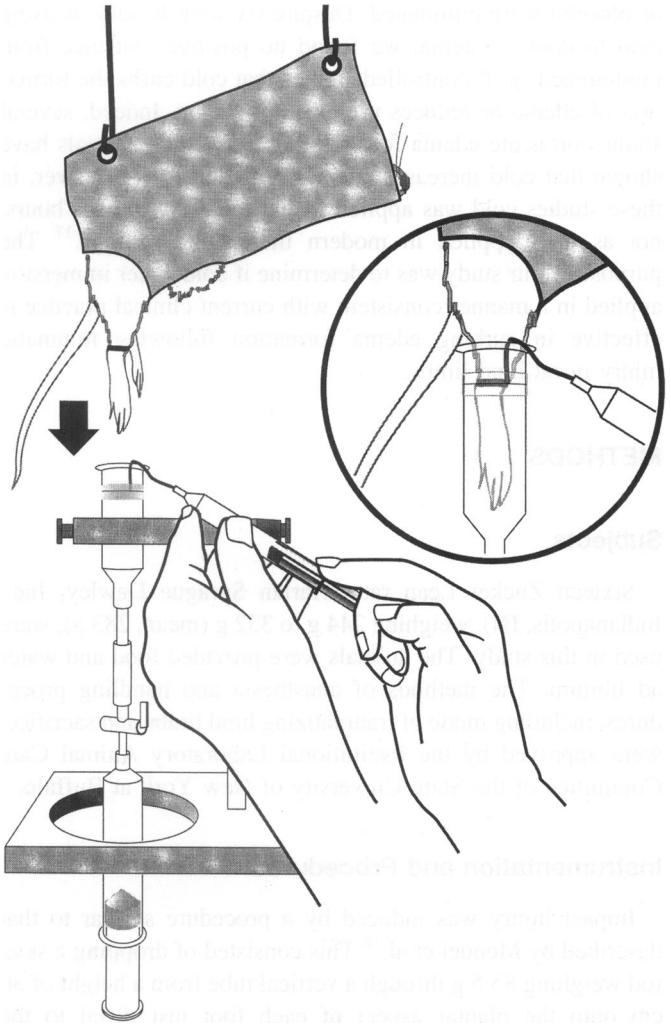

Fig 1. System for measuring limb volumes.

Reliability and Validity of Measurement System

Before initiating experiments, the reliability and validity of the volume measurement system were established by determining the volume displacement of a small aluminum cylinder (1.270-cm diameter by 3.160-cm length). The use of an inanimate object to determine reliability and validity was deemed more appropriate than using volume measurements from animals because it has been shown that, in the dependent position, the volumes of nontraumatized limbs change over time.[18] Mean cylinder volume as determined from 13 consecutive measurements was 3.949 mL (standard error, ± 0.007, range, 3.919 mL to 3.982 mL). When the cylinder volume was measured 13 more times, mean cylinder volume was 3.940 mL (standard error, ± 0.001, range, 3.934 mL to 3.947 mL). The true volume of the cylinder calculated from physical dimensions (see above) was 4.003 mL. This is 0.054 mL and 0.063 mL, respectively, greater than the volume of the cylinder as measured with our method, which represents an underestimation of cylinder volume by our measuring method of 1.57% or less. Thus, our method of determining volume displacement is reliable and valid.

Immersion of traumatized limbs was accomplished by lowering an animal, via a camera boom, until its hind limbs were immersed to the level of their painted lines in 100-mL beakers of water. Water in the treatment beakers was maintained at 12.8°C to 15.6°C (55°F to 60°F). This range was selected after a review of the literature.[16,17,22] Water in the control beakers was at room temperature (22°C to 25.5°C, 72°F to 78°F). This temperature range was selected because Matsen et al[17] reported that it had no therapeutic effect. Both beakers rested on an electric stirring plate that kept the water in constant motion.

Body Temperature

Because anesthesia can cause body temperature to fall,[23] body temperature was regulated throughout these experiments. A rectal probe was inserted 6 cm to 8 cm and connected to a YSI Tele-thermometer (Yellow Springs Instruments Co, Yellow Springs, OH), and body temperature was recorded throughout the experiment. Body temperature was regulated at 37°C ± 1°C by directing a 60W lamp at or away from an

animal. The temperature of the cold water beaker was also monitored continuously with a YSI Tele-thermometer and probe. As the temperature of the cold bath increased, shaved ice chips were added to the beaker to maintain temperature between 12.8°C and 15.6°C.

Experimental Protocol

Each rat was anesthetized by an intraperitoneal injection of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) (60 mg/kg of body weight), which was supplemented over the course of the 4-hour experiment as needed with 0.05-mL doses. After being anesthetized, limbs of animals were shaved and the animals were placed in cloth slings and suspended at 45° (caudal end down) with both hind limbs fully exposed and in dependent position. Lines were painted at the level of malleoli, and rectal probes were inserted.

After being suspended for approximately 20 minutes, the pre-trauma volume of each hind limb was determined. Both hind limbs of each rat were then injured by dropping a steel rod onto the plantar aspect of each foot just distal to the malleolus. Hind limb volumes were measured, and within 5 minutes of injury, the hind limbs were immersed in separate 100-mL beakers. One randomly selected limb was immersed in cold water and the other in room temperature water (22°C to 25.5°C). Treatment continued for 30 minutes, followed immediately by determination of each limb's volume. This was followed by a 30-minute rest period during which animals hung in their slings with their limbs in the dependent position and exposed to room temperature air. After removal from water beakers and before volume measurement, the limbs were dabbed with tissue paper to remove adherent water and to minimize evaporative cooling. At no time were the limbs rubbed or squeezed during drying. The volume of both limbs was again determined after the rest period. This sequence of events continued for three more cycles, so that at the conclusion of an experiment the animal had received four treatments interspersed with four rest periods. At the conclusion of the experiment, animals were sacrificed by overdose of sodium pentobarbital administered by intraperitoneal injection.

Data Analysis

Treated and untreated (control) limbs differed only in the temperature of the water to which they were exposed. Again, assignment of limb to treatment or control was random. Data were expressed as changes from pretrauma hind limb volumes per kilogram of body weight to minimize the effects of size on amount of swelling. Analyses of variance for repeated measures were used to test the null hypothesis that cold would not influence post-traumatic limb volumes. A 0.05 level of significance was selected. Tukey post hoc tests were applied to the limb volumes of treated and untreated limbs at each time interval.

RESULTS

The volume of treated limbs was significantly smaller than that of control limbs ($F(1,9) = 4.86$, $p = .03$); (Fig 2). Tukey post hoc tests showed that the volume of treated limbs was significantly less than that of untreated limbs at all times after the first treatment.

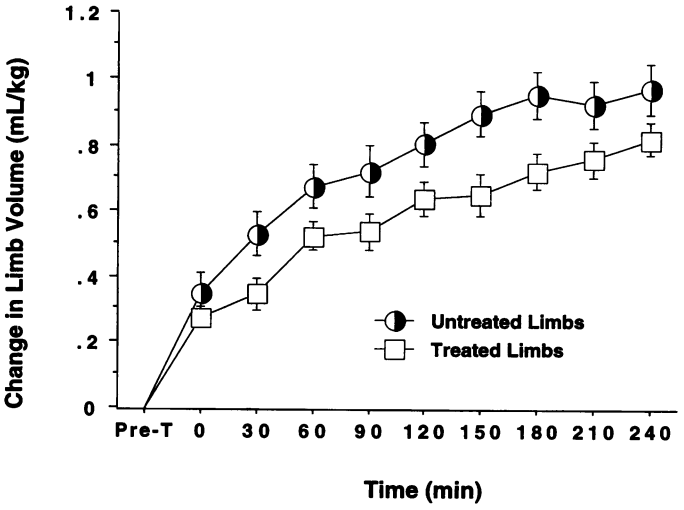

Fig 2. Mean changes in volumes of treated and untreated hind limbs over time. Vertical lines depict standard errors. Pre-T is pretrauma limb volume and Time = 0 is 3 to 4 minutes post-trauma. Tukey post hoc tests showed that volumes of treated limbs were significantly less than those of untreated limbs at all times after the first treatment.

DISCUSSION

In our study, exposure to 12.8°C to 15.6°C water curbed edema formation after blunt trauma in rats. It did so despite limbs' being in the dependent position throughout treatments, rest periods, and volumetric measurements. We maintained limbs in the dependent position throughout data collection because placing them in virtually any other position could be construed as therapeutic. Further, we chose to keep rats anesthetized throughout data collection because we wanted to eliminate as variables pain, stress, and sympathetic activity. Anesthesia also eliminated the exercise effect produced by muscle activity (ie, muscle pump) or behavior such as rubbing or licking that might in some way have influenced limb volumes.

This experiment did not elucidate the physiologic mechanisms cold invokes to curb edema formation. Knight,[10] however, speculated that cold reduces blood flow by increasing viscosity and causes vasoconstriction, which further reduces blood flow in an injured area. Reduced blood flow should also result in less fluid volume at the injury site. Knight[10] further speculated that reducing metabolic activity in cells that survive the original trauma renders them less susceptible to hypoxia (secondary trauma), and, hence, the total number of cells traumatized is reduced, as is the resulting edema. That cold can reduce metabolic demand and therefore reduce blood flow was recently demonstrated in a study by Ho et al.[8] They used triple-phase technetium bone scans to examine the effects of an ice wrap applied to a nontraumatized knee. Cooling with ice resulted in a 38.4% reduction in arterial blood flow to the knee. They interpreted the 19.3% decrease seen in the bone uptake of

technetium as an indication that cold reduced bone metabolism.

Previous Literature

A review of nonhuman animal studies designed to determine the effects of cold on post-traumatic edema reveals that exposure to temperatures lower than 20°C tends to increase swelling. Indeed, when limb volumes are measured 1 or more days after injury, the least swelling occurs when limbs are cooled little if at all. Matsen et al[17] used New Zealand white rabbits as subjects in an experiment to determine the effects of cold on mechanically induced midshaft tibial fractures. Prefracture volumes were determined using a water displacement system. Limbs were treated for 6 hours or 24 hours, with water ranging in temperature from 5°C to 25°C. Limbs were measured immediately after treatment, 6 hours after treatment, and then daily for 4 consecutive days. Limbs that were cooled with water between 5°C and 15°C for 24 hours were significantly more swollen than control limbs (maintained at 32°C) 1 and 2 days after treatment. The volumes of limbs cooled with water at 20°C to 25°C were not different from those of control limbs at any time, and neither were the volumes of limbs cooled with water at 10°C for only 6 hours. Farry et al[22] concluded that no regimen of cooling lessens edema and that there is a detrimental effect with temperatures below 15°C. Farry et al[22] corroborated a detrimental effect when they reported that traumatized radiocarpal ligaments of a small sample of domestic pigs, cooled by ice, showed less histologic evidence of inflammation, but increased swelling relative to controls. They also reported that 48 hours after two 20-minute applications of crushed ice, the volumes of nontraumatized and traumatized limbs were greater than those of controls. Jezdinsky et al[16] also reported that cooling rat limbs at 12.3°C for 2 hours to 10 hours failed to influence edema formation, but caused significant swelling soon after the cold was removed. McMaster and Liddle[24] examined effects of 20°C and 30°C water baths on edema in a small number of rabbit limbs following crush injury. Relative to controls, a single 1-hour exposure to 20°C water seemed to exacerbate swelling 4 hours to 6 hours after injury, whereas exposure to 30°C water did not. Unexpectedly, three 1-hour exposures to either 20°C or 30°C water, interspersed with 1-hour rests, more than doubled swelling relative to control limbs 4 hours and 6 hours after similar injury in another group of rabbits. Rabbits were laid on platforms with their injured limbs in the dependent position, at least during treatments, whereas injured but untreated limbs of control rabbits were not stated to be in this position. Thus, it is possible that prolonged dependent positioning of treated limbs may have enhanced swelling relative to control limbs, which may have been in some other position, perhaps even horizontal. Speculations aside, however, the single 1-hour exposure to 30°C water might be interpreted to curb edema relative to controls, but only in the first hour after injury.

Our results are seemingly the first from a controlled, randomized study to confirm the long-held belief that cold is effective in curbing the formation of acute edema. Cold, as we applied it, curbed edema formation even with limbs in dependent position. We observed, seemingly for the first time, a treatment effect during application of cold that others[16,17,22] observed to exacerbate edema. Further, we did not witness an increase in edema after the removal of such cold temperatures, although we did not track limb volumes for a day or more as other observers did. We speculate that we observed a different outcome because we exposed limbs for much less time than did previous investigators.

Clinical Trials

A review of human studies designed to determine the efficacy of cryotherapy suggests that cold is interpreted to be effective in controlling edema. However, these studies suffer from poor experimental design or lack of proper controls. Basur et al[25] treated patients who had ankle injuries with crepe bandaging or cryotherapy for 48 hours, followed by crepe bandaging. The number of patients who were pain free and without restricted ankle movement was greater at 2 days and 7 days postinjury in the group treated with cryotherapy compared with the group that had not received cryotherapy. However, it was not clear whether, because of the cold gel pack, the cryotherapy group was more restricted in movement than the group that was only bandaged. It is possible that the difference between the two groups was more a reflection of mobility than effectiveness of cold treatment. Sloan et al[26] treated patients within 24 hours of injury with a single 30-minute application of cold and elevation and a sustained course of anti-inflammatory medication. Seven days later, the patients were examined and compared with a group of patients who had not received cold or elevation, but who had been treated with anti-inflammatory drugs. Not surprisingly, the authors concluded that a single cold treatment had little effect.

Two other studies often cited in support of the efficacy of cryotherapy lack proper control groups. Hocutt et al[27] compared the efficacy of cryotherapy with that of heat therapy in patients with ankle sprains. Those receiving cold treatment and adhesive bandages within 36 hours of injury recovered more quickly than those receiving heat and elastic bandages. However, it is unclear whether the patients treated with cold recovered more quickly or if the recovery of those treated with heat was inhibited. Additionally, it is impossible to know if those treated with cold recovered more quickly than if they had not been treated with cold because a control group without treatment was not included in this study. Results from a more recent study by Coté et al[28] are equally ambiguous. In their study, the effects on ankle injury (beginning 3 days after injury) of cold, heat, and contrast baths were compared. The authors concluded that all three treatments increased edema, although cold caused less of an increase than the other two. Descriptive statistics provided by the authors, however, indicate that cold had virtually no effect on pre-existing edema because volumes were essentially the same before and after treatments. Conversely, volumes measured following treatment with heat or contrast baths increased. None of the treatments as applied by Coté et al[28] reduced pre-existing edema.

Our study addressed neither the physiologic mechanism(s) by which cold curbs edema formation nor the effects of cold on pre-existing edema. We, like Knight,[3] think it unlikely that cold will have any appreciable effect on existing edema other than perhaps by reducing pain, which is known[29] to affect the inflammatory process, including, presumably, edema formation. We need to confirm in further experiments that cold is indeed effective in curbing acute edema, and, if so, attempt to optimize its application. Once that task is complete, we can compare outcomes from other modalities or combinations of modalities applied to experimental limbs with a cold standard applied to control limbs. These experiments should allow us to assess the relative efficacy of various current and future protocols. This study represents the first step in this process.

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