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ORIGINAL ARTICLE

Cryotherapy suppresses tendon inflammation in an animal model

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Summary Cryotherapy (or cold treatment) has been a popular treatment to relieve pain caused by injuries to tissues such as tendons. However, the exact mechanisms behind the beneficial effects of cryotherapy in tendons remain largely unclear. As prostaglandin E_2 (PGE_2) is known to be a major mediator of acute inflammation in tissues, which is related to tissue pain, we hypothesized that the beneficial effects of cryotherapy in tendons are mediated by downregulation of PGE_2 levels. To test this hypothesis, we applied cold treatment to mouse patellar and Achilles tendons using two animal models: exhaustive mouse treadmill running and acute mouse tendon injury by needle penetration. We then measured the levels of PGE_2 and protein expression levels of COX-2, an enzyme responsible for PGE_2 production in tissues, under both experimental conditions. We found that treadmill running increased PGE_2 levels in both patellar and Achilles tendons compared to control mice without running. Cold treatment for 30 min after treadmill running was sufficient to reduce PGE_2 levels to near baseline control levels in both tendons. An extension of cold treatment to 60 min resulted only in a marginal decrease in patellar tendons, but a marked decrease in Achilles tendons. Moreover, COX-2 protein levels in both tendons were also lowered by cold treatment, suggesting that the reduction of PGE_2 levels in tendons by cold treatment is at least in part due to the decreased COX-2 expression. Similarly, in the acutely injured tendons, 30 min of cold treatment after needle penetration reduced PGE_2 levels when compared to the controls at room temperature (22°C). This decrease was sustained up to at least 3 h after the administration of cryotherapy. Given

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that PGE_2 is a known pain sensitiser, the results of this study suggest that the ability of cold treatment to reduce pain may be attributable to its ability to decrease PGE_2 production in tendons.

Introduction

Acute injuries to soft tissues such as tendons are some of the most common conditions in orthopaedic/sports medicine. One of the popular modalities used to treat these injuries, which are characterised by swelling, pain, and even haemorrhage, is cryotherapy, or cold treatment. This therapy is known to have a wide range of effects on soft tissues, including changing tissue temperature, blood flow, and metabolism [1]. For example, cold treatment decreases skeletal blood flow (thereby reducing swelling) and metabolism in human knees [2]. In addition, local cold application decreases nerve conduction velocities in the extremities [3], which in turn can reduce muscle spasm and decrease pain sensitivity [3,4]. It is generally thought that cryotherapy offers benefits to patients by lowering tissue temperature, which reduces metabolism and decreases blood flow (microcirculation) and thereby reduce swelling, which collectively result in alleviating the pain associated with tissue injury [5,6].

Given that inflammatory responses dominate shortly after tissue injury, it is possible that cryotherapy may function through an anti-inflammatory mechanism. In fact, a popular therapy in athletic settings is whole-body cryotherapy, in which humans are exposed to extremely cold air (from $-110^{\circ}C$ to $-140^{\circ}C$) in a special cryo-chamber for a very short period (2 min, in general). Such exposure in rugby players after an elite rugby training program effectively relieved pain and inflammatory symptoms by increasing the anti-inflammatory cytokine interleukin (IL)-10, decreasing proinflammatory cytokines (IL-2 and IL-8), and decreasing the levels of prostaglandin E_2 (PGE_2) in blood [7]. Localized cryotherapy has also been shown to reduce inflammation in specific body parts by decreasing the number of leucocytes and granulocytes as well as decreasing macrophage infiltration following soft tissue injury [8]. Except for these and a few other reports that studied the molecular changes associated with the beneficial effects of cryotherapy [7,9,10], the mechanism of cryotherapy-induced pain reduction in tendons remains largely unclear.

It is known that pain is associated with the presence of high levels of inflammatory agents in affected tissues [11,12]. PGE_2 is one such agent; it is produced by inflammatory cells (e.g., macrophages) and tendon fibroblasts in response to tissue injury [13]. PGE_2 is a highly active inflammatory molecule that causes pain and induces vasodilatation [14], hyperalgesia [15], and fever [16].

In general, PGE_2 levels are elevated in tissues subjected to large mechanical loading, such as intense exercise. For example, high levels of PGE_2 were observed in tendon fibroblasts subjected to large repetitive mechanical loading [17]. Recently, we showed a marked increase in PGE_2 levels in tendons and bone marrow in response to a bout of exhaustive treadmill running [18]. A similar increase in PGE_2 levels is also observed when tissues are injured, such as in wounded mouse Achilles tendons [19]. These increases in PGE_2 levels are similar to those observed in tendinopathic tendons [20], for which cryotherapy has often been recommended to reduce associated pain [9]. Therefore, we reasoned that the beneficial effects of cold treatment may be mediated by downregulating PGE_2 levels in tendon tissues. To test this hypothesis, we induced high levels of PGE_2 production in tendons using two animal models: an exhaustive mouse treadmill running model [18] and an acute mouse tendon injury model. We then applied cold treatment to the mice and measured PGE_2 production and COX-2 protein expression in both Achilles and patellar tendons. Our results show that cold treatment effectively decreases PGE_2 levels and COX-2 expression in both tendons. The findings of this study explain, at least in part, the mechanism behind cryotherapy's beneficial effects on tendons and justify cryotherapy's wide use in clinical practice.

Materials and methods

We used two animal models to induce high levels of PGE_2 production in mouse tendons: (1) mouse treadmill running model and (2) acute mouse tendon injury model. The treadmill running protocol, needle penetration procedure, and subsequent cryotreatment of mice were approved by the University of Pittsburgh Institutional Animal Care and Use Committee, Pittsburgh, PA, USA.

Mouse treadmill running model

Twenty-nine C57BL/6J female mice (10 weeks old) were used in the treadmill running experiment. Twenty-five mice were allowed to run on the treadmill, whereas four mice served as controls and were allowed free cage activities. The treadmill running protocol consisted of training for 1 week with mice running at 13 meters/min for 15 min per day, followed by one bout of exhaustive running at the same speed. The longest individual running time was 435 min and the shortest was 193 min. On average, the mice ran for 313 $\pm$ 60 min (mean $\pm$ SD).

Application of cold treatment after treadmill running

After treadmill running, cold treatment was applied by submerging the hind legs and feet of mice in treadmill running groups in a Koolit refrigerant gel (Cold Chain Technologies, Holliston, MA, USA) maintained at $8 \pm 2^{\circ}C$ for 30 min or 60 min. During cold treatment, the mice were restrained using a custom-made device to prevent them from moving around. Control mice remained in cages at room temperature ($22^{\circ}C$).