Microvascular Research 106 (2016) 96–100

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Sustained cutaneous vasoconstriction during and following cyrotherapy treatment: Role of oxidative stress and Rho kinase

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ARTICLE INFO

Article history:
Received 20 January 2016
Revised 25 March 2016
Accepted 13 April 2016
Available online 14 April 2016

Keywords:
Vasoconstriction
Ischemia
Skin-surface cooling
Cryotherapy
Soft tissue injury

ABSTRACT

Cryotherapy is a therapeutic technique using ice or cold water applied to the skin to reduce bleeding, inflammation, pain, and swelling following soft tissue trauma and injury. While beneficial, there are some side effects such as pronounced vasoconstriction and tissue ischemia that are sustained for hours post-treatment. This study tested the hypothesis that this vasoconstriction is mediated by 1) the Rho-kinase pathway and/or 2) elevated oxidative stress. 9 subjects were fitted with a commercially available cryotherapy unit with a water perfused bladder on the lateral portion of the right calf. Participants were instrumented with three microdialysis probes underneath the bladder. One site received lactated ringers (control site), one received the Rho-Kinase inhibitor Fasudil, and one received Ascorbic Acid. Skin temperature (Tskin) and cutaneous vascular conductance (CVC) was measured at each site. Subjects had 1 °C water perfused through the bladder for 30 min, followed by passive rewarming for 90 min. Tskin fell from ~34 °C to ~18.0 °C during active cooling across all sites and this response was similar for all sites ($P > 0.05$ for all comparisons). During passive rewarming Tskin rose to a similar degree in all sites ($P > 0.05$ relative to the end of cooling). %CVC was reduced during active cooling in all sites; however, the magnitude of this response was blunted in the Fasudil site relative to control ($P < 0.001$ for all comparisons) and min 25 and 30 of cooling in the Ascorbic Acid site ($P < 0.05$). During passive rewarming %CVC at the control and Ascorbic Acid sites did not change such that values were similar to the end of cooling ($P > 0.05$ for each comparison). %CVC at the Fasudil site remained elevated during passive rewarming such that values were higher compared to the control and Ascorbic Acid sites throughout the 90 min of passive rewarming ($P < 0.001$ main effect of Fasudil). These findings indicate that the Rho-kinase pathway contributes to pronounced vasoconstriction during cryotherapy as well as the sustained vasoconstriction during the subsequent rewarming period post treatment.

Introduction

Localized cooling accomplished via cryotherapy treatment is commonly used after orthopedic surgery and in sports medicine to reduce bleeding, inflammation, metabolism, muscle spasm, pain, and swelling following soft tissue trauma and injury (Swenson et al., 1996). While cryotherapy treatment yields many therapeutic benefits, it is often associated with a number of side effects including, tissue necrosis, and neuropathy (Babwah, 2011; Bassett et al., 1992; Brown and Hahn, 2009; Lee et al., 2007; Moeller et al., 1997). These conditions are likely the result of profound reductions in local tissue temperature and the subsequent pronounced tissue ischemia during the period of cryotherapy. Furthermore, we have recently reported that this pronounced vasoconstriction is sustained for up to 2 h during passive rewarming of the limb despite skin temperature returning back to near baseline (i.e. pre-cooling) values (Khoshnevis et al., 2015a; Khoshnevis et al., 2015b). A temporarily reduced blood flow is beneficial in treating soft tissue injuries due to reducing the inflammation cascade and edema formation. However, when a prolonged state of ischemia is maintained over sufficient time, the consequences of a reduced supply of oxygen and cell nutrients in conjunction with the buildup of toxic metabolic byproducts may lead to tissue necrosis and neuropathies (Santilli and Santilli, 1999). In addition, a prolonged state of ischemia can lead to reperfusion injury when flow is reestablished (Jia and Pollock, 1999). In this regard it is well documented that reduced skin temperatures, even as high as 24 °C, cause a profound local decrease in blood perfusion of tissues, which, may be an agent of nonfreezing cold injury (Francis, 1984; Francis and Golden, 1985).

The mechanisms of cutaneous vasoconstriction during localized cooling have been the topic of many previous research studies and reviews (Hodges et al., 2006; Johnson, 2007; Johnson and Kellogg, 2010; Thompson-Torgerson et al., 2007a; Thompson-Torgerson et al., 2007b;

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http://dx.doi.org/10.1016/j.mvr.2016.04.005
0026-2862/

Yamazaki, 2010). These studies indicate that the pronounced vasoconstriction during local cooling is due to adrenergic and nonadrenergic pathways (Hodges et al., 2006; Johnson, 2007; Johnson and Kellogg, 2010; Thompson-Torgerson et al., 2007a; Thompson-Torgerson et al., 2007b; Yamazaki, 2010). In vivo human studies have reported an attenuated cutaneous vasoconstrictor response during local cooling following intradermal infusion of the antioxidant ascorbic acid (Yamazaki, 2010). Similar findings have been reported following blockade of the Rho kinase pathway and the subsequent translocation of α_{2c}-adrenoreceptors to the smooth muscle plasma membrane using intradermal infusion of Fasudil (Thompson-Torgerson et al., 2007a; Thompson-Torgerson et al., 2007b).

While these studies are eloquently designed and provide valuable insight into mechanisms of cold-induced vasoconstriction, the application differs from conditions of cryotherapy treatment in a few ways. Many of these studies are performed during cooling of the skin surface to about 24 °C which is approximately 10 °C below baseline values (Hodges et al., 2006; Johnson, 2007; Johnson and Kellogg, 2010; Thompson-Torgerson et al., 2007a; Thompson-Torgerson et al., 2007b; Yamazaki, 2010). In addition, the skin surface area being cooled, equal to approximately 6.3 cm^2, is relatively small (Hodges et al., 2006; Johnson, 2007; Johnson and Kellogg, 2010; Thompson-Torgerson et al., 2007a; Thompson-Torgerson et al., 2007b; Yamazaki, 2010). In contrast cryotherapy application, using commercially available units, is most commonly performed by circulating ice water through a bladder that covers a much larger surface area such as the shoulder, knee, thigh, or shin region (Babwah, 2011; Bassett et al., 1992). As a result skin temperatures drop to approximately 17 °C or lower over a much larger surface area relative to the aforementioned cold applications (Khoshnevis et al., 2015a; Khoshnevis et al., 2015b). Whether or not similar mechanistic pathways involved in cold induced vasoconstriction can be extrapolated to the vasoconstriction that occurs during cryotherapy treatment (Khoshnevis et al., 2015a; Khoshnevis et al., 2015b) remains unknown. Furthermore, to our knowledge no studies have investigated mechanisms of sustained vasoconstriction following termination of the cooling stimulus (Khoshnevis et al., 2015a; Khoshnevis et al., 2015b) particularly using commercially available cryotherapy units.

This study utilized the technique of intra-dermal administration of vasoactive substances into the cutaneous circulation directly underneath the cryotherapy cooling pad. We hypothesized that cold-induced vasoconstriction during cryotherapy treatment would be attenuated following: 1) local infusion of the global antioxidant Ascorbic Acid and 2) blockade of the Rho kinase pathway using Fasudil. Furthermore, we hypothesized that the sustained vasoconstriction for up to 90 min following cryotherapy treatment (Khoshnevis et al., 2015a; Khoshnevis et al., 2015b) would be attenuated following local infusion of Ascorbic Acid and Fasudil.

Methods

Ethical approval

The Institutional Review Board at The University of Texas at Austin approved all study procedures and the consent process used in the present study. Subjects were given a verbal description of all procedures and informed of the purpose and risks involved in the study before providing their informed, written consent.

Subjects

9 healthy young subjects (10 males) participated in this study. Average (mean ± SD) subject characteristics were: age, 24 ± 1 years; height, 180 ± 1 cm; and weight, 81 ± 3 kg. Subjects were non-smokers, were not taking medications and were free from cardiovascular, neurological, or metabolic diseases. None of the subjects reported a history of knee injury or cryotherapy or other form of cold exposure in the lower extremities for at least a year prior to the experiment. All studies were conducted in the morning following an overnight fast (>12 h). Subjects refrained from strenuous exercise and alcoholic beverages for 24 h and from consuming caffeine and food for 12 h prior to the experimental trial that was conducted in a temperature controlled laboratory (~24 °C and 40% relative humidity).

Instrumentation and measurements

All data were collected with the subject seated in a semi-recumbent position. Three microdialysis membranes (CMA 31 Linear Microdialysis Probe, 55 KDalton cut-off membrane; Harvard Apparatus, Holliston, MA) with a 10-mm-long semi permeable portion were inserted ~5 cm apart into the dermal layer of the nonglabrous skin on the lateral side of the right calf. Membrane insertion was accomplished by first placing a 25-gauge needle into the dermal layer of the right calf and then threading the membrane through the needle. Once the semi-permeable portion of the membrane was under the skin surface (i.e. in the needle) the needle was completely removed and the membrane was secured to the skin with tape. Following placement each membrane was perfused with lactated Ringer's solution (Baxter, Deerfield, IL) at a rate of 2 μL/min via a perfusion pump (Harvard Apparatus, Holliston, MA) while insertion trauma associated with membrane placement subsided (minimum 90 min). During this period each membrane site was instrumented with an integrating laser Doppler flow probe (VP7a, Moor Instruments, Wilmington, DE) which provided a continuous index of skin blood flow. A thermocouple (Type T Thermocouple Probe, Physitemp Instruments INC, Cliffton, NJ) was placed immediately adjacent to the Doppler flow probe for continuous assessment of local skin temperature. Following placement of the membranes, Doppler flow probes, and thermocouples a commercially available cryotherapy cooling pad (Arctic Ice Universal Pad; Pain Management Technologies, Akron, OH) was applied overlying the instrumented area, and fixed in place using an Ace bandage. The cooling pad was connected to an Arctic Ice cryotherapy unit (Pain Management Technologies, Akron, OH) which allowed for manipulation of the underlying skin (Tskin) and tissue temperature according to the manufacturer's recommendation (see below for more detail). A cuff was placed around the left arm for intermittent blood pressure measurements from the brachial artery using electrosphygmomanometry (Tango, SunTech Medical Instruments, Raliegh, NC).

Study protocol

After the hyperemic response associated with insertion trauma subsided (minimum of 90 min) each site was perfused with its respective vasoactive agent for a 45 min wash in period. One site received lactated Ringer solution (Baxter, Deerfield, IL) which served as the control site, one site received Fasudil (3 mM, ToCris Bioscience, Ellisville, MO) to locally block the Rho kinase pathway (Thompson-Torgerson et al., 2007a), and the last site received Ascorbic Acid (10 mM, Mylan Institutional LLC, Rockford, IL) to supplement antioxidants (Yamazaki, 2010). Fasudil and Ascorbic Acid were dissolved in lactated Ringer solution. Each site was initially perfused at 52 μL/min for a 30 s priming period after which the rate was reduced to 2 μL/min for the remainder of data collection. After the 45 min wash in period, the cryotherapy unit and cooling pad were perfused with 34 °C water for 15 min of baseline data collection. This was followed by 30 min of active skin-surface cooling which was accomplished by circulating 0–2 °C water through the cryotherapy unit and cooling pad. At the end of the cooling phase the cryotherapy unit was turned off for a 90 min period of data collection during passive rewarming.