Microvascular Research 118 (2018) 12–19

Contents lists available at ScienceDirect

Microvascular Research

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

Ischemia-reperfusion injury alters skin microvascular responses to local heating of the index finger

Gregory W. McGarr, Gary J. Hodges, Matthew M. Mallette, Stephen S. Cheung*

Environmental Ergonomics Laboratory, Department of Kinesiology, Brock University, St. Catharines, ON L2S 3A1, Canada

ARTICLE INFO

Keywords:
Endothelial function
Ischemia-reperfusion
Laser-Doppler
Local thermal hyperemia
Nitric oxide
Spectral analysis

ABSTRACT

Background: Ischemia-reperfusion (IR) injury impairs microcirculatory function by reducing nitric oxide (NO) bioavailability and increasing sympathetic tone. This study non-invasively examined the effects of acute upper limb IR injury on local thermal hyperemia (LTH) in glabrous and non-glabrous finger skin.

Materials and methods: In ten healthy males, LTH was examined twice (~7–10 d apart) for each skin type on the index finger using laser-Doppler flowmetry in a counterbalanced design with either 1) 20 min ischemia, followed by reperfusion (ISCH) or 2) time-matched control (SHAM). LTH tests were performed using a standard heating protocol (33–42 °C at 1 °C·20 s^{-1} + 20 min at 44 °C) and baseline, initial peak, nadir, delayed plateau and maximal heating phases were identified as well as vasodilatory onset time and time to initial peak. Cutaneous vasomotion was evaluated using spectral analysis and comparing absolute and normalized wavelet amplitudes between conditions for both skin types at baseline and during LTH.

Results: In non-glabrous skin, IR injury delayed the vasodilatory onset of local heating by 27.4 [11.3, 43.4] s ($p = 0.004$) and attenuated cutaneous vasodilation during the initial peak and sustained heating by −44.5 [−73.0, −15.9] PU ($p = 0.003$) and −34.4 [−62.9, −5.8] PU ($p = 0.020$), respectively. Analysis of normalized wavelet amplitudes in non-glabrous skin identified impaired microvascular function at baseline via NO-dependent mechanisms (−3.64 [−7.22, −0.05] %, $p = 0.047$), and during LTH via respiratory influences (−2.83 [−5.39, −0.21] %, $p = 0.031$). In glabrous skin, IR injury delayed vasodilatory onset time by 24.9 [1.1, 67.6] s ($p = 0.042$). The vasodilatory response to sustained local skin heating in glabrous skin was increased following IR injury (+56.3 [15.1, 116.5], $p = 0.012$), however, this was not evident when accounting for differences in blood pressure between conditions. Additionally, no other differences in vasodilatory or vasomotor functions were observed in this skin type between conditions (all, $p > 0.05$).

Conclusions: The current IR model elicits impaired cutaneous vasodilatory responses to local heating in young males, primarily in non-glabrous skin, and may be useful for exploring mechanisms of IR-injury and for testing potential countermeasures in otherwise healthy humans.

1. Introduction

Impaired endothelium-dependent vasodilation is a hallmark of ischemia-reperfusion (IR) injury (Carden and Granger, 2000). Alterations in microcirculatory blood flow are initiated by the rapid production of reactive oxygen species (ROS) from enzymatic sources, reducing nitric oxide (NO) bioavailability and promoting a pro-vasoconstrictive state (Carden and Granger, 2000; Granger, 1999; Harrison, 1997). In otherwise healthy humans, acute (20 min) upper limb ischemia is associated with impaired endothelium-dependent vasodilation in conduit and resistance arteries of the affected limb during reperfusion (Gori et al., 2006; Kharbanda et al., 2001). Despite the fact that the microcirculation is more susceptible to the deleterious effects of IR than larger blood vessels (Carden and Granger, 2000; Granger, 1999), little is known about the effects of this reperfusion injury model on microvascular function in humans.

The cutaneous circulation provides an easily accessible microvascular network that can be continuously monitored using laser-Doppler flowmetry (LDF) (Braverman, 1997). Beyond its utility as a representative microcirculatory network, the skin is also directly

Abbreviations: AU, arbitrary units; AVA, arteriovenous anastomoses; CVC, cutaneous vascular conductance; CI, confidence interval; IR, ischemia-reperfusion; ISCH, ischemia-reperfusion condition; LDF, laser-Doppler flowmetry; LTH, local thermal hyperemia; MAP, mean arterial pressure; NO, nitric oxide; PU, perfusion units; ROS, reactive oxygen species; SD, standard deviation; S_pO_2, arterial oxygen saturation; RBC flux, red blood cell flux; SHAM, time-matched control condition; T_{amb}, ambient air temperature; $\overline{T_{sk}}$, mean skin temperature; T_{loc}, local laser-Doppler probe temperature

* Corresponding author at: Department of Kinesiology, Brock University, 1812 Sir Isaac Brock Way, St. Catharines, ON L2S 3A1, Canada.
E-mail address: scheung@brocku.ca (S.S. Cheung).

https://doi.org/10.1016/j.mvr.2018.02.003
Received 25 October 2017; Received in revised form 6 February 2018; Accepted 9 February 2018
Available online 10 February 2018
0026-2862/

impacted by IR injury during limb surgeries requiring a bloodless field, microvascular free tissue transfers, replantation procedures, and by peripheral cold injuries (Schmidt et al., 2012; Wang et al., 2011). Cutaneous blood flow can be examined during early reperfusion and in response to functional microvascular reactivity tests (Roustit and Cracowski, 2013). Rapid, non-painful local skin heating in particular has previously been established as a highly sensitive test of neurovascular and endothelium-dependent vasodilator function in a variety of clinical conditions (Boignard et al., 2005; Khan et al., 2000; Kruger et al., 2006; Medow et al., 2005; Roustit et al., 2008). These examinations can also be combined with spectral analysis of the blood flow signal to provide mechanistic insights into the observed flow changes (Bracic and Stefanovska, 1998; Stefanovska et al., 1999).

In the limbs, reperfusion injury influences both glabrous and non-glabrous skin, which are present on opposite sides of the hands and feet. In non-glabrous skin, an initial vasodilatory peak occurs in response to local heating that is influenced by adrenergic fibers (Hodges et al., 2008; Hodges et al., 2009; Wilson et al., 2005) and NO (Minson et al., 2001), but is primarily mediated by sensory nerves (Minson et al., 2001). During sustained local heating, endothelium-dependent vasodilation produces a delayed plateau that is controlled mainly by NO (Kellogg et al., 1999). In glabrous skin, sensory nerve function also mediates the initial vasodilatory peak (Roustit et al., 2008). However, a delayed plateau does not always develop during sustained heating (Metzler-Wilson et al., 2012; Roustit et al., 2010), which may reflect an increase in efferent sympathetic nerve activity acting on arteriovenous anastomoses (AVA) in order to counteract the heating-induced vasodilation, or perhaps less of a reliance on NO-mediated vasodilation in this skin type (Hales et al., 1985; Metzler-Wilson et al., 2012; Taylor et al., 2014). Spectral analysis of the laser-Doppler signal allows for the non-invasive examination of the effects of IR injury on both skin types in the finger and provides insight into both NO-dependent and NO-independent endothelial function in the skin, as well as local neural and myogenic control mechanisms, in addition to systemic cardiac and respiratory influences on the microcirculation of both skin types (Bracic and Stefanovska, 1998; Stefanovska et al., 1999).

The primary purpose of the current experiment was to evaluate the effects of acute upper limb IR injury on the cutaneous microcirculatory responses to local heating in both glabrous and non-glabrous skin of the index finger. A secondary purpose was to analyze the spectral content of the cutaneous blood flow signals in order to non-invasively evaluate the underlying mechanisms of altered blood flow with IR injury in each skin type. It was hypothesized that 1) acute IR injury would delay the onset and attenuate the magnitude of vasodilation in response to local heating in both skin types and, 2) that spectral analysis of the blood flow signal would reveal reduced cutaneous NO-dependent function following IR injury at normothermic skin temperature and during local heating.

2. Methods

2.1. Ethical approval

Prior to taking part, all participants were fully informed of the experimental procedures and associated risks, and each provided verbal and written informed consent. The study was approved by Brock University's Bioscience Research Ethics Board (File#: 15-077) and all experimental protocols conformed to the latest revision of the Declaration of Helsinki.

2.2. Participants

Ten healthy, recreationally active males were recruited for this study. The participants' age, height, and body mass [mean (SD)] were 26.2 (5.4) y, 178.2 (7.4) cm, and 81.8 (13.2) kg, respectively. Participants were screened for cardiorespiratory, dermatological, and neurological diseases, and all were non-smokers. None were taking prescription drugs and all participants abstained from over-the-counter medications (e.g. non-steroidal anti-inflammatory drugs, vitamins, supplements) for at least two weeks prior to and throughout testing.

2.3. Experimental design

All participants completed one familiarization and two experimental sessions, each separated by ~7 days and were instructed to refrain from caffeine (12 h), alcohol and strenuous exercise (24 h), and exposure to extreme temperatures (48 h), prior to each session. All experimental sessions took place in the morning and participants were instructed to have a light breakfast, no later than 2 h prior to the start of testing. They were encouraged to drink water ad libitum the night before and morning of the trial.

During the familiarization session, height (cm) and body mass (kg) were measured, followed by 20 min of cuff occlusion and reperfusion (see details below). This was done to give participants the opportunity to decide if they were unwilling or unable to continue with the study and to minimize anxiety during experimental sessions to avoid the effects of psychological strain on physiological measurements. Following familiarization, participants underwent two counterbalanced experimental sessions involving 1) IR injury (ISCH) of the upper limb and 2) a time-matched sham session (SHAM).

Upon arrival for the two experimental sessions, hydration was assessed by a mid-stream urine sample and was determined by measuring urine specific gravity with a refractometer (PAL-10S, Atago Co. Ltd., Tokyo, Japan); the euhydration threshold was set to ≤ 1.020 (Armstrong et al., 2010). Participants then changed into a standard clothing ensemble consisting of a cotton t-shirt, underwear, shorts and cotton socks, and body mass (kg) was then recorded.

Following the pre-experimental procedures (above), participants were acclimated for 30 min by resting supine on a padded table in an environmentally controlled room while being instrumented with skin temperature sensors, 3-lead electrocardiogram, and finger pulse oximetry. Throughout the experiment, ambient air temperature (T_{amb}) and relative humidity (%) were maintained at ~25.0 °C and 40–50%, respectively, and overhead lighting was turned off. Skin blood flow assessments were performed on the non-dominant arm, which was supported on a padded side table at heart level with the limb placed in a neutral position and extended ~45° to the long axis of the body.

To assess glabrous skin, one LDF probe was centered on the volar aspect of the distal phalanx (finger pad) of the index finger. To assess non-glabrous skin on the same finger, a second LDF probe was centered on the dorsal aspect of the middle phalanx. At each site, laser-Doppler probes were secured to the skin with double-sided adhesive tape (PF105-3, Perimed, Järfälla, Sweden), in addition to surgical tape (Transpore™, 3M, St. Paul, USA) that was gently placed over the top of the probes to avoid movement throughout testing. Care was taken not to apply added pressure during this taping procedure to avoid restricting blood flow and LDF recordings were examined pre- and post-taping for confirmation.

For each experimental session, following instrumentation the local LDF probe temperature (T_{loc}) was set to 33 °C and measurements were recorded for ~20 min in order to ensure stable temperature and hemodynamic measurements. Following establishment of a steady baseline, a standard blood pressure cuff was manually inflated to 220 mm Hg as fast as possible and maintained for 20 min. The cuff was then rapidly deflated and the arm was reperfused for 20 min prior to the start of the local skin heating protocol (Gori et al., 2006; Kharbanda et al., 2001). The SHAM trial was identical to that described above, except the cuff was not inflated during the protocol. During local heating, T_{loc} was increased from 33 °C–42 °C at $1\,^{\circ}C \cdot 20\,s^{-1}$ and held for 30–35 min, until a stable plateau had been reached. T_{loc} was then increased from 42–44 °C at $1\,^{\circ}C \cdot min^{-1}$ and held for 20 min to induce a maximal heating response. T_{loc} was increased at a slower rate from