Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

The effect of hyperbaric oxygen on nitric oxide synthase activity and expression in ischemia-reperfusion injury

Richard C. Baynosa, MD, Anna L. Naig, MD, Patrick S. Murphy, MD, Xin Hua Fang, MT, Linda L. Stephenson, MT (ASCP), Kayvan T. Khiabani, MD, Wei Z. Wang, MD, and William A. Zamboni, MD*

Division of Plastic Surgery, Department of Surgery, University of Nevada School of Medicine, Las Vegas, Nevada

ARTICLE INFO

Article history:
Received 26 September 2012
Received in revised form
20 December 2012
Accepted 3 January 2013
Available online 1 February 2013

Keywords:
Hyperbaric oxygen
Nitric oxide synthase
Nitric oxide
Ischemia-reperfusion
Nitric oxide synthase activity

ABSTRACT

Background: Hyperbaric oxygen (HBO) mitigates ischemia-reperfusion (IR) injury via a nitric oxide mechanism that is nitric oxide synthase (NOS) dependent. The purpose of this study was to investigate this NOS-dependent mechanism by examining isoform-specific, tissue-specific, and time-specific upregulation of NOS mRNA, protein, and enzymatic activity.
Methods: We raised a gracilis flap in Wistar rats that were separated into early and late phases. Treatment groups included nonischemic control, IR, HBO-treated ischemia-reperfusion (IR-HBO), and nonischemic HBO control. We harvested tissue-specific samples from gracilis, rectus femoris, aorta, and pulmonary tissues and processed them by reverse transcription polymerase chain reaction and Western blot to determine upregulation of isoform-specific NOS mRNA and protein. We also harvested tissue for NOS activity to investigate upregulation of enzymatic activity. Data are presented as mean ± standard error of the mean with statistics performed by analysis of variance. $P \leq 0.05$ was considered significant.
Results: There was no increase in NOS mRNA in the early phase. In the late phase, there was a significant increase in endothelial-derived NOS (eNOS) mRNA in IR-HBO compared with IR in gracilis muscle (79.4 ± 22.3 *versus* 36.1 ± 4.5; $P < 0.05$) and pulmonary tissues (91.0 ± 31.2 *versus* 30.2 ± 3.1; $P < 0.01$). There was a significant increase in the late-phase eNOS pulmonary protein IR-HBO group compared with IR (235.5 ± 46.8 *versus* 125.2 ± 14.7; $P < 0.05$). Early-phase NOS activity was significantly increased in IR-HBO compared with IR in pulmonary tissue only (0.049 ± 0.009 *versus* 0.023 ± 0.003; $P < 0.05$).
Conclusions: The NOS-dependent effects of HBO on IR injury may result from a systemic effect involving an early increase in eNOS enzymatic activity followed by a late-phase increase in eNOS protein expression within the pulmonary tissues.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Ischemia-reperfusion (IR) injury is a common pathophysiologic process that can affect multiple tissues after interruption of perfusion, such as in myocardial infarction, cerebral ischemia, crush injuries, acute vascular insufficiencies, transplantation, replantation after traumatic amputation, and free tissue transfer. Hyperbaric oxygen (HBO) is a treatment

Presented at American College of Surgeons, San Francisco, CA, October 16-20, 2005 and Undersea and Hyperbaric Medical Society, Las Vegas, NV, June 16-18, 2005.

* *Corresponding author.* Division of Plastic Surgery, University of Nevada School of Medicine, Hyperbaric Medicine and Advanced Wound Healing Center, University Medical Center of Southern Nevada, 2040 W. Charleston Boulevard, Suite 302, Las Vegas, NV 89102. Tel.: (702) 671-2256; fax: (702) 671-2245.
E-mail address: rbaynosa@medicine.nevada.edu (R.C. Baynosa).

0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.01.004

modality that has proven to be safe and effective for various conditions including decompression sickness, osteoradionecrosis, and hypoxic wounds [1]. More recently, there has been basic science and clinical evidence indicating a beneficial effect of HBO in the treatment of myocardial infarction and cerebral ischemia, as well as renal and skeletal muscle ischemia [2–8]. The lack of a well-defined mechanism of action, however, has prevented widespread acceptance of HBO as a treatment for IR injury. Our research strives to help further elucidate the mechanism of the beneficial effect of HBO therapy on skeletal muscle IR injury.

Previous studies have demonstrated the deleterious effect of IR injury on the microcirculation. In a morphologic analysis of the skeletal muscle microcirculation after IR injury, a significant increase in adherent neutrophils to the endothelium of postcapillary venules was noted compared to the microcirculation of nonischemic controls [9]. This increased neutrophil adherence was reversed with HBO treatment both during and immediately after the ischemic event. This benefit of HBO on skeletal muscle IR injury has been shown in other studies via significant reductions in skeletal muscle edema and necrosis [6,10,11].

Multiple studies from various researchers have implicated nitric oxide (NO) as an important regulator of cell surface adhesion molecules via cytoskeletal alterations [12–15]. We have confirmed the importance of NO and nitric oxide synthase (NOS) in preventing skeletal muscle necrosis after IR injury using an NOS substrate (L-arginine) and NOS inhibitor (L-NAME) [16]. Our recent work further confirmed that the HBO reduction of IR-induced neutrophil polarization of CD18 and adherence to intercellular adhesion molecule-1 is mediated through an NO mechanism that is NOS dependent [17]. The importance of CD18 and intercellular adhesion molecule-1 in mediating the protective effect of HBO on IR injury has been corroborated in separate rat skeletal muscle experiments by other researchers [18].

The purpose of this study was to investigate the NOS-dependent mechanism of HBO in IR injury by examining isoform-specific, tissue-specific, and time-specific upregulation of NOS mRNA, protein, and enzymatic activity. Nitric oxide synthase is able to produce NO via two separate pathways. Increased NO may result from either an increase in NOS protein expression or an increase in NOS enzymatic activity. We proposed to measure NOS expression by evaluating early- and late-phase mRNA transcription via reverse transcription polymerase chain reaction (RT-PCR) as well as subsequent NOS protein expression via Western blot. In addition, we measured NOS activity *in vivo* by means of a radioisotope assay. Because the pathway of protein expression involves numerous steps to raise overall NOS protein concentration, whereas increasing NOS enzymatic activity may occur rapidly, we hypothesized that HBO treatment for IR injury would result in an early increase in NOS activity and a late-phase increase in NOS expression.

2. Methods

The University of Nevada Institutional Animal Care and Use Committee approved all experimental procedures as well as the animal model and associated animal care during the described study.

2.1. *Rat gracilis muscle model*

We anesthetized male Wistar rats weighing 275 ± 30 g with pentobarbital (50 mg/kg, intraperitoneally) with supplementation (10 mg/kg) as required to maintain anesthesia during the surgical period. We raised the gracilis muscle flap as we previously described [9]. Briefly, the right thigh musculature and femoral vasculature were exposed, and the gracilis muscle was dissected free on its vascular pedicle using standard microsurgical technique. Clamping the femoral artery and vein for 4 h induced global muscle ischemia, after which time the clamp was removed to initiate the reperfusion period.

2.2. *Treatment groups*

We randomly assigned the animals to either early-phase or late-phase study groups. The early-phase group was further subdivided into one of four experimental groups: (1) nonischemic control (NIC); (2) IR, consisting of 4 h ischemia and 30 min reperfusion; (3) IR-HBO, of 4 h ischemia and 30 min reperfusion with HBO treatment during the last 90 min of ischemia; or (4) HBO control (NIC-HBO) with HBO treatment during the last 90 min of mock ischemia. We included the last group to ensure that HBO treatment in normal controls did not affect NOS expression or activity in the early phase.

We subdivided the late-phase group into one of three experimental groups: (1) NIC; (2) IR, consisting of 4 h ischemia and 24 h reperfusion; or (3) IR-HBO, consisting of 4 h ischemia and 24 h reperfusion with HBO treatment during the last 90 min of ischemia.

2.3. *Hyperbaric oxygen treatment*

Hyperbaric oxygen treatment consisted of placing the animals in a research-grade HBO chamber (Model 1300; Sechrist Industries, Inc., Anaheim, CA) with 100% oxygen at 2.5 ATA during the last 90 min of ischemia or NIC-ischemia.

2.4. *Determination of early- and late-phase NOS expression by reverse transcriptase polymerase chain reaction*

We divided Wistar rats randomized to the early-phase group into treatment groups, as described above. For those stratified to the early phase, we subjected the animals to their respective treatments. After 30 min reperfusion or mock reperfusion, we harvested samples from the experimental gracilis muscle, contralateral rectus femoris muscle, abdominal aorta, and pulmonary tissue for RT-PCR ($n = 8$). We harvested samples from four separate and unique tissues to examine whether the changes were local or systemic. We examined the gracilis tissue to assess whether mRNA transcription was elevated in the muscle subjected to the IR injury. Conversely, we also sampled from the contralateral nonischemic rectus femoris muscle to examine whether the increase in NOS mRNA could be seen in skeletal muscle not subjected to IR injury. We isolated and processed the abdominal aorta as well, to determine

Download English Version:

https://daneshyari.com/en/article/6254381

Download Persian Version:

https://daneshyari.com/article/6254381

Daneshyari.com