Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Combined remote ischemic perconditioning and local postconditioning on liver ischemia–reperfusion injury

Felipe Lobato da Silva Costa, MS,[a,*] *Vitor Nagai Yamaki, MS,*[a] *Thiago Barbosa Gonçalves, MS,*[a] *João Vitor Baia Coelho, MS,*[a] *Sandro Percário, PhD,*[b] *and Marcus Vinicius Henriques Brito, PhD*[a]

[a] *Experimental Surgery Laboratory, Department of Operatory Technique, Para State University, Brazil*
[b] *Oxidative Stress Laboratory, Department of Pharmacology, University of Pará, Brazil*

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form
24 April 2014
Accepted 16 May 2014
Available online 22 May 2014

Keywords:
Liver failure
Transplantation
Transplantation conditioning
Ischemic postconditioning
Ischemia
Reperfusion

ABSTRACT

Background: Remote ischemic perconditioning (rPER) is the newest technique described to mitigate ischemia and reperfusion (IR) injury. Local postconditioning (POS) is also an effective technique for this purpose. It is uncertain if adding local POS to rPER provides superior liver protection, so we tested this hypothesis.

Materials and methods: Twenty five Wistar rats were assigned into five groups: sham, IR, POS, rPER, and rPER + POS. Animals were subjected to liver ischemia for 60 min. POS consisted of four cycles of 5-min liver perfusion followed by 5-min liver ischemia (40 min total) after the major ischemic period. rPER consisted of four cycles of 5-min hindlimb ischemia followed by 5 min hindlimb perfusion contemporaneously to major liver ischemic period, during its last 40 min. After 2 h, median and left lobes were harvested for malondialdehyde and Trolox equivalent antioxidant capacity (TEAC) measurement, and blood for the measurement of serum transaminases.

Results: All tissue conditioning techniques were able to reduce transaminases serum levels, having no differences among them. All tissue conditioning techniques were able to reduce hepatic tissue MDA level; however, only rPER + POS had higher values than SHAM. All tissue conditioning techniques also enhanced TEAC; however, only POS had lower TEAC than SHAM.

Conclusions: rPER appears as the most promising technique to avoid IR injury. This technique reduced oxidative stress of cell membranes and lowered transaminases serum level. There was no additive protection when POS and rPER were held together.

1. Introduction

Reperfusion following temporary tissue ischemia has been identified as an important mechanism contributing to tissue injury [1]. Furthermore, the clinical syndrome of ischemia and reperfusion (IR) is associated with deleterious consequences for several organs. This syndrome contributes to morbidity and mortality in a wide range of pathologies such as myocardial infarction, ischemic stroke, sleep apnea, and circulatory arrest, being a major problem in a setting of liver transplantation [2–4].

Mechanisms for IR-induced tissue injury include intracellular processes such as failure of the Na^+/K^+ ion pump,

* *Corresponding author*. Experimental Surgery Laboratory, Department of Operatory Technique, Para State University, Brazil, Rua dos Pariquis 1880, apt 1401, 66033-590 Belém-PA, Brazil. Tel.: +55 91 84277530; fax: +55 91 32691717.
E-mail address: felipe.lobato@live.com (F.L.daS. Costa).
0022-4804/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2014.05.046

increase in intracellular Ca^{2+} concentration, reactive oxygen species (ROS) formation, and associated inflammatory response. ROS formation can cause lipid peroxidation of cell membranes, leading to malondialdehyde (MDA) formation and depleted tissue antioxidant capacity. ROS can also cause proteins and DNA damage, and eventually, cell death [4,5].

The most critical factor that determines the severity of tissue damage caused by IR appears to be the duration of the ischemia [5]. In addition to early reperfusion, "tissue conditioning" by a series of alternating intervals of brief episodes of IR is currently the most promising approach to limit tissue damage caused by prolonged ischemia [6–9].

Tissue conditioning was first used in 1986 by Murry *et al.* [10], who demonstrated the concept of ischemic preconditioning (PRE) in the heart of a dog by performing short cycles of IR before a major period of cardiac ischemia. Since then, this technique has been applied successfully in many clinical situations such as kidney and liver transplantation [6,11].

In 2003, Zhao *et al.* [12] developed the concept of ischemic postconditioning (POS), which is very similar to PRE, but consists of short cycles of reperfusion and ischemia before the free reperfusion of a tissue that has been under ischemia. The efficiency of POS has been demonstrated in several tissues and has been found to be similarly effective as PRE [13,14].

Tissue conditioning by cycles of IR can also be applied to tissues other than those exposed to ischemia. This concept has been called remote ischemic conditioning, and was first used by McClanahan *et al.* [15] (1993) who showed that a short period of renal ischemia provides protection to the myocardium from IR injury. Based on this study, the concepts of remote PRE [16] and remote ischemic postconditioning [17] were developed.

Schmidt *et al.* [18] (2007) also applied remote ischemic conditioning in the context of myocardial ischemia. They applied a tourniquet to a porcine limb to produce alternating periods of occlusion and reperfusion while the myocardium was under ischemia. This technique is called remote ischemic perconditioning (rPER) and it has been demonstrated to protect the brain, kidney, myocardium, and liver from the IR syndrome in various animal models [19–22].

Mechanisms underlying rPER protective effects are barely understood [23]. Moreover, underlying mechanisms involved in the protective effects of remote and local tissue conditioning techniques might be linked, working through the activation of reperfusion injury salvage kinase pathway. On the other hand, studies demonstrated the importance of an alternative pathway in remote tissue conditioning techniques, where parasympathetic response plays a critical role, and there is activation of the survivor activating factor enhancement [24].

Recent studies found that POS associated with PRE provides synergistic protection against hepatic IR-induced injury [6]; however, there is limited clinical applicability for PRE in a context of unexpected ischemia. Moreover, little data exist on the efficacy of rPER, which could be easily applied in the context of unexpected temporary hepatic ischemia or liver transplantation, and it is uncertain if adding POS to rPER provides superior hepatic protection compared with rPER alone. Thus, we tested the hypothesis that the combination of rPER and POS provides superior hepatic protection compared with rPER alone in a well-established rat model of hepatic reperfusion injury.

2. Materials and methods

2.1. Animals

Twenty-five (12–15 wk) male Wistar rats, weighing 270–300 g, were used in this study. The animals were kept in a vivarium of the Experimental Surgery Laboratory at the Pará State University (Brazil) with a controlled temperature, light, humidity and noise; water and the food were provided *ad libitum*. The research followed the rules of Brazilian National Law for Animal Care (Law: 11.794/08) that is based on National Institutes for Health guidelines, and followed the rules of Council for International Organization of Medical Sciences ethical code for animal experimentation. The project was previously approved by the Animal use and care committee at the Para State University.

2.2. Experimental protocol

The animals were randomly assigned into the following five groups ($n = 5$ for each group):

1. The SHAM group: In this group, the same surgical procedure as in the remaining groups was performed, but no liver ischemia was induced.
2. The IR group: In this group, liver ischemia was induced for 60 min followed by reperfusion without any form of conditioning.
3. The local ischemic POS group: Here, 60 min of hepatic ischemia was followed by 40 min of autologous POS (four cycles of 5-min hepatic perfusion were followed by 5 min of hepatic ischemia).
4. The rPER group: In this group, liver ischemia was simultaneously accompanied by remote ischemic conditioning. rPER consisted of four cycles of 5-min hindlimb ischemia followed by 5-min hindlimb perfusion, starting 20 min after the beginning of the ischemia and lasting 40 min until the end of the ischemic phase. Hindlimb ischemia was achieved using an elastic rubber band tied around the thigh of the left leg, following a model successfully adopted by Yamaki *et al.* [25].
5. The rPER group + local POS group (rPER + POS): Here, liver ischemia was simultaneously accompanied by rPER in the left hindlimb followed by autologous ischemic POS (four cycles of 5-min hepatic perfusion followed by 5-min hepatic ischemia).

2.3. Surgical procedures

All surgical procedures were performed in anesthesia (ketamine hydrochloride and xylazine hydrochloride 60 mg/kg and 6 mg/kg, respectively, injected intraperitoneally). Through a transverse laparotomy, hepatic lobes were exposed and the left hepatic artery was occluded by microsurgical clamp application, leading to left and median lobe liver ischemia.