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Remote ischemic conditioning temporarily improves antioxidant defense

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ABSTRACT

Background: Remote ischemic conditioning (RIC) is the most promising surgical approach to mitigate ischemia and reperfusion (IR) injury. It consists in performing brief cycles of IR in tissues other than those exposed to ischemia. The underlying mechanisms of the induced protection are barely understood, so we evaluated if RIC works enhancing the antioxidant defense of the liver and kidney before IR injury.

Materials and methods: Twenty-one Wistar rats were assigned into three groups as follows: sham, same surgical procedure as in the remaining groups was performed, but no RIC was carried out. RIC 10, RIC was performed, and no abdominal organ ischemia was induced. After 10 min of the end of the RIC protocol, the liver and kidney were harvested. RIC 60, similar procedure as performed in RIC 10, but the liver and the kidney were harvested 60 min. RIC consisted of three cycles of 5-min left hind limb ischemia followed by 5-min left hind limb perfusion, lasting 30 min in total. Samples were used to measure tissue total antioxidant capacity.

Results: RIC protocol increased both liver (1.064 ± 0.26 mM/L) and kidney (1.310 ± 0.17 mM/L) antioxidant capacity after 10 min when compared with sham (liver, 0.759 ± 0.10 mM/L and kidney, 1.08 ± 0.15 mM/L). Sixty minutes after the RIC protocol, no enhancement on liver (0.687 ± 0.13 mM/L) or kidney (1.09 ± 0.15 mM/L) antioxidant capacity was detected.

Conclusions: RIC works through temporary and short-term enhancement of liver and kidney cells antioxidant defenses to avoid the deleterious consequences of a future IR injury.

1. Introduction

The syndrome of ischemia and reperfusion (IR) contributes to the morbidity and mortality in several clinical situations, such as liver and kidney transplantation. Mechanisms for induced tissue injury mainly occur because of reactive oxygen species (ROS) formation when tissue perfusion is restored, which can lead to lethal cell injury. Currently, there is not a drug regimen that can satisfactorily avoid the deleterious consequences of IR [1,2].

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The only action that can stop the ischemic cascade is reestablishment of oxygen inflow as early as possible [3]. In addition to early reperfusion, "tissue conditioning" by a series of alternating intervals of brief episodes of IR is currently the most promising surgical approach to limit cell damage caused by prolonged ischemia [4–7].

Tissue conditioning techniques can be applied locally, before (preconditioning) or after (postconditioning) a major ischemic period. These techniques have been demonstrated to significantly minimize the injury secondary to IR syndrome in several organs [8–12]. However, a shortcoming of both local preconditioning and postconditioning is the prolongation in operative time and the necessity of direct access to the occluded artery, being hard or even impossible to perform in a setting of endovascular or pharmacologic revascularization [13].

Tissue conditioning by brief cycles of IR was discovered to be feasible in tissues other than those exposed to ischemia. This concept has been called remote ischemic conditioning (RIC) and was first used by McClanahan *et al.* [14] (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 ischemic preconditioning [14] and remote ischemic postconditioning [15] were developed. Those techniques are minimally invasive and could be applied in several clinical settings, such as liver and kidney transplantation; however, these techniques also have the shortcoming of increase in the operative time [13].

Schmidt *et al.* demonstrated an even more practical technique. They applied a tourniquet to a porcine limb to produce alternating periods of occlusion and reperfusion while the myocardium was under ischemia. This technique was called remote ischemic perconditioning [16], and it has been demonstrated to be protective against the IR syndrome in various animal models [17–19].

Obviously, the best clinical approach to treatment is the use of noninvasive or minimally invasive techniques because the most important goal is to decrease both frequency of complications and the technical risks [13]. Oxman *et al.* [20] reported the first noninvasive technique, carrying out conditioning cycles on the lower limb by the use of an elastic tourniquet. Such noninvasive model was proved to be feasible in liver and kidney RIC protocols [18,19].

There is scarce evidence as to how brief cycles of IR on a distant organ are able to provide organ protection against injury. It is proposed that the short IR cycles in the remote tissue would release humoral factors [13], such as adenosine [21], bradykinin [22], opioids [23], and endocannabinoids [24]. Those humoral factors and the direct stimuli are sensed by the remote organ innervation [25], and there would be a systemic response modulated through the parasympathetic nervous system [26] leading to the propagation of an effector signal to the target organ that is under ischemia [27].

The effector signal would activate specific receptors in cell membranes, which trigger the well-known participant in the mechanism of the different conditioning strategies, the so-called survivor activating factor enhancement (SAFE) pathway, in which signal transducer and activator of transcription proteins would lead to protection against IR injury. It has been also demonstrated that the activation of another pathways, such Rho-kinases and other certain prosurvival kinase elements of the reperfusion injury salvage kinase (RISK) cascade [28].

Mitochondrial protection seems to represent the final common path elements for both the RISK and SAFE pathways. However, the intracellular mechanism underlying such protection remains unknown [13].

All the previous studies applied the RIC in a setting of IR of a target organ. There are no studies that evaluated the effects of RIC in a target organ that has not been under ischemia. Thus, we analyzed the variations in liver and kidney antioxidant capacities promoted by RIC applied in the hind limb.

2. Materials and methods

2.1. Animals

Twenty-one (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 environment; water and the food were provided *ad libitum*. The research followed the rules of the Brazilian Law for Animal Care (Law: 11.794/08) that is based on NIH guidelines and followed the rules of the 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 three groups ($n = 7$ for each):

1. The sham group (sham): In this group, the same surgical procedure as in the remaining groups was performed, but no remote ischemic conditioning was carried out.
2. The RIC group—10 min (RIC 10): In this group, the RIC was performed, and no other organ ischemia was induced. After 10 min of the end of the RIC protocol, the liver and kidney were harvested for biochemical analysis.
3. The RIC group—60 min (RIC 60): In this group, the RIC was performed, and no other organ ischemia was induced. After 60 min of the end of the RIC protocol, the liver and kidney were harvested for biochemical analysis.

2.3. Surgical procedures

All surgical procedures were performed in anesthesia (ketamine hydrochloride and xylazine hydrochloride 70 and 10 mg/kg, respectively, intraperitoneal). The RIC consisted of three cycles of 5-min left hind limb ischemia followed by 5-min left hind limb perfusion, lasting 30 min in total. Hind limb ischemia was achieved using an elastic rubber band tied around the thigh of the left leg.

Ten or 60 min after the RIC protocols, the animals were subjected to a median laparotomy, and the liver and kidney were exposed and harvested for biochemical analysis.