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Postconditioning of the small intestine: which is the most effective algorithm in a rat model?

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ABSTRACT

Background: Mesenteric ischemia is a serious clinical condition requiring immediate surgical intervention. The unavoidable ischemic-reperfusion (IR) injury may be ameliorated using the appropriate postconditioning protocol. The aim of the present study was to investigate the optimal postconditioning algorithm in a rat model of intestinal ischemic–reperfusion injury.

Materials and methods: Male Wistar rats were randomized into five groups ($n = 10$), one sham-operated, one IR, and three postconditioned groups, each with different protocols. The animals were subjected to 60 min of mesenteric ischemia, followed by 60 min of reperfusion. Postconditioning was applied at the onset of reperfusion using three different algorithms. Arterial pressure and mucosal microcirculation were monitored throughout the experiment. Mesenteric pH was determined at the early phase of reperfusion. Blood and tissue samples were taken at the end of reperfusion for histologic evaluation, serum lactate dehydrogenase, serum creatine kinase, serum tumor necrosis factor-α, serum interleukin-6, detailed mucosal antioxidant, and scavenger capacity assays.

Results: The shorter and intermediate length cycles of postconditioning enhanced mucosal microcirculation and redox state and significantly delayed the normalization of mesenteric pH. Furthermore, milder histopathologic lesions and lower concentrations of serum necroenzymes and proinflammatory cytokines were detected compared with the IR group. The protective effect of postconditioning using longer cycles could only be seen in a tendentious manner.

Conclusions: In a rat model of intestinal ischemia–reperfusion, the shorter and intermediate length cycles of postconditioning proved to be more effective than the use of longer cycles.

1. Introduction

Intestinal ischemia may appear as an abdominal emergency. It is also an important cause of mortality in surgical patients. Among the abdominal organs, the intestine is probably the most sensitive organ to ischemic-reperfusion (IR) injury [1]. The cells of the intestine can easily be injured during ischemia. Paradoxically, reperfusion can further damage the

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mucosa, leading to enhanced intestinal permeability, which in turn enables the intestinal gut flora to translocate into the systemic circulation [2–4]. These changes and the formation of reactive oxygen species (ROS) during reperfusion accompanied by release of inflammatory cytokines into the circulation may lead to systemic inflammation or multiple organ failure [5]. Although acute intestinal ischemia accounts for only 1%–2% of all surgical emergencies, in-hospital mortality rates can be as high as 60%–80%, which have remained consistently this high during the last decades [6–8].

Protection of organs from IR injury has always been an immense challenge for surgeons because of the multifactorial etiology. In 1986, Murry *et al.* described a new treatment to minimize the impairment of myocardial ischemic–reperfusion injury [9]. Ischemic preconditioning has become highly useful in increasing the ischemic tolerance of organs, and the beneficial effects of this technique have been demonstrated in several organs. Unfortunately, however—apart from elective intestinal transplantation—the possibility to perform preconditioning before occlusion of the mesenteric artery is only slight (e.g., arterial thromboembolism).

On the basis of a similar concept, a new method, called "postconditioning" was introduced years later, inducing short series of repetitive cycles of brief reperfusion and reocclusion immediately at the very onset of reperfusion. This maneuver was able to reduce the degree of IR injury in a similar extent as preconditioning, in a canine model of myocardial IR injury [10]. In 2008, Santos *et al.* demonstrated that the effect of ischemic postconditioning was comparable with ischemic preconditioning on IR-induced intestinal injury in rats [11]. Although the mechanism of postconditioning remains unclear, the technique has proved to have beneficial effects by means of protecting mitochondrial integrity *via* regulation of mitochondrial permeability transition pores (mPTPs), reduction of sensitivity to the increased intracellular calcium overload, and restoration of nitric oxide-mediated vasorelaxation [12–14]. Other studies [15,16] suggested that prolonged local acidosis, after ischemia during the early period of reperfusion, suppresses mPTP formation, showing a different aspect of postconditioning.

In a model of myocardial infarction, Kin *et al.* emphasized the importance of timing of the first postconditioning cycle [17]. Moreover, it was suggested that the duration of the periods of reocclusion and reperfusion and the interval at the beginning of postconditioning are similarly crucial regarding the beneficial effects of postconditioning [17–20].

Because there is no consensus in the literature about the proper timing and duration of postconditioning cycles [20], the present study focused on examining the effects of postconditioning on postischemic small intestine, using different reperfusion and reocclusion cycles. Three postconditioning protocols were compared: a dynamic algorithm of 6 × 10 s, a moderate algorithm of 3 × 30 s, and a prolonged algorithm of 3 × 1 min.

2. Materials and methods

2.1. Animals

Inbred male Wistar rats (Charles River Hungary Ltd, Budapest, Hungary) weighing 250–280 g were used in this study. The experimental design was approved by License No. 22.1/2408/3/2011 from the Animal Care Committee of the Semmelweis University, and the experiment was performed in accordance with U.S. National Institute of Health guidelines (publication no. 85–23, revised 1996; Bethesda, MD). The animals were kept under specific- and pathogen-free conditions at 22°C–24°C and were fed commercial pellets and water *ad libitum*. Twelve hours before the surgical procedure only water was given. Each experiment was started at the same time of day to avoid the effects of circadian rhythm.

2.2. Surgical procedure

The animals ($n = 50$; 10 in each group, according to the study design) were anaesthetized using an intraperitoneal combined injection of ketamine (75 mg/kg) and xylasin (7.5 mg/kg). They were then placed in the supine position on a heating pad to keep their body temperature between 36.5°C and 37.5°C, monitored by a rectal thermometer (Homeothermic Blanket Control Unit; Harvard Apparatus, Holliston, MA).

A polyethylene catheter was placed into the right jugular vein to maintain anesthesia and to compensate intraoperative fluid loss by the administration of normal saline solution (3 mL/kg/h). Another polyethylene catheter was inserted into the left carotid artery connected to a blood pressure gauge (Kent Scientific Corporation, Torrington, CT) so as to monitor the mean arterial blood pressure and heart rate. Median laparotomy was performed and the superior mesenteric artery (SMA) was identified. For monitoring intestinal microcirculation, the small intestine was mobilized and the antimesenteric side was opened to position the head of the laser Doppler flowmeter 14 cm in oral direction from the ileocecal transition. Warm ischemia was induced to the entire small bowel by clamping the SMA using atraumatic microvascular clips (Harvard Apparatus). The 60 min of mesenteric ischemia was followed by 60 min of reperfusion. During the IR period, the abdomen was covered with a plastic blanket to prevent fluid loss *via* evaporation. Intestinal microcirculation was monitored throughout the ischemia–reperfusion period. After the interval of ischemia, postconditioning was performed in three groups of animals using 60-, 30-, or 10-s algorithm. After 1 h of reperfusion, the animals were sacrificed by exsanguination *via* right ventricular puncture. Blood samples were collected and centrifuged (3000 rpm for 2 × 10 min, at room temperature), serum was snap-frozen in liquid nitrogen, and stored at −80°C until further analysis. Histologic samples were taken from the middle part of the duodenum, jejunum, and ileum: 10-mm long sections were placed in 4% neutral-buffered formalin and further 10-mm large adjacent parts were snap-frozen in liquid nitrogen. The remnant mucosal mass was homogenized, snap-frozen, and stored at −80°C until further analysis.

2.3. Experimental groups

The animals were randomly allocated into five groups ($n = 10$ each) as follows:

(1) Sham-operated: After opening the abdomen the SMA was dissected, but ischemia was not induced. After 2 h of median laparotomy the animals were sacrificed.