

The effects of Peroxiredoxin VI on the preservation of the small intestine in rats after ischemia / reperfusion damage

**A.E. Gordeeva¹, M.G. Sharapov¹, V.I. Novoselov¹, E.E. Fesenko¹,
A.A. Temnov², M.Sh. Khubutiya²**

¹ *Institute of Cell Biophysics, Russian Academy of Sciences,
Pushchino*

² *N.V. Sklifosovsky Research Institute for Emergency Medicine of
Moscow Healthcare Department, Moscow*

Contact: Andrew A. Temnov, aa-temnov@yandex.ru

Due to its high oxygen requirements, the intestine is extremely susceptible to ischemia/reperfusion (I/R) injury. The aim of this study was to investigate a protective effect of Peroxiredoxin VI (Prx VI) on the small intestine after I/R injury in a rat model of strangulated ileus. An I/R injury in the small intestine strangulation model was induced by the occlusion of the distal ileum loop and mesenterial blood vessels for 60 min followed by a 120-min reperfusion period. In one of 3 experimental groups, the animals received intravenous injections of 10 mg/kg Prx VI at 15 min prior to the intestinal ischemia/reperfusion in the strangulation model. After surgery, specimens of the small intestine tissue were collected for histological examination. The study has shown that the ischemia/reperfusion seriously compromises the integrity of mucosal villi and crypts and is accompanied by a lymphocyte infiltration, an oxidative stress with a serious mucosal loss. A Prx VI administration prior to ischemia/reperfusion protected the rat intestine from the ischemia/reperfusion injury by reducing adverse effects of

the oxidative stress and preserving the intestinal mucosa architecture. The study has demonstrated that Prx VI exerts a favourable antioxidant effect and efficiently attenuates ischemia-reperfusion injury of the strangulated intestine segment.

Key words: small intestine, ischemia/reperfusion, enzymes-antioxidants.

Introduction

The small intestine is the most vulnerable among the visceral organs to an ischemia-reperfusion (I/R) injury. The mucosa of the small intestine consists of cells that are easily damaged in ischemia. The subsequent restoration of blood flow in the ischemic gut, i.e. reperfusion, leads to a further mucosal damage. I/R injury may occur in a variety of situations, such as an intestinal transplantation, neonatal necrotizing enterocolitis, strangulated hernia, hypovolemic/septic shock, and others [1].

Still poorly understood are the changes that take place in the small intestine following the blood flow restoration after the resolution of strangulation ileus. A strangulated small bowel obstruction is especially dangerous since it is associated with blood circulation impairments in the mesenteric and intestinal wall vessels due to their compression. Many investigators have noted that a postoperative blood flow restoration in the ischemic intestine makes the onset of all pathomorphological changes both in the gut and in the whole body [2-5].

The ischemia/reperfusion is mainly considered as a complex two-staged process when the impairments arising from acute ischemia are exacerbated and often become irreversible after the restoration of the blood flow in an organ [6]. During ischemia, the mitochondrial synthesis of

adenosine triphosphate (ATP) discontinues, and a rapid decrease of creatine phosphate, and of ATP later on, occurs in cells. High-energy phosphates degrade to adenosine that is metabolized to inosine and hypoxanthine [7]. A hypoxic stress triggers the conversion of xanthine dehydrogenase to xanthine oxidase that generates oxygen free radicals. The restoration of the blood flow in an ischemic organ initiates a cascade of events leading to an additional damage of the organ mucosa. During the reperfusion, the molecular oxygen penetrates into tissues where it interacts with hypoxanthine and xanthine oxidase. This leads to a "burst" in the production of oxygen free radicals such as superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2) [8].

Thus, the reperfusion in the ischemia-affected intestine may give rise to a powerful oxidative stress and a subsequent destruction of the intestinal epithelium due to a massive cell death. Meanwhile, a pharmacological modulation of the reperfusion may improve the functional integrity of the intestinal tissue and subsequent clinical course.

Currently anti-oxidant enzymes, specifically the family of peroxiredoxins, i.e. thiol-specific antioxidant proteins possessing peroxidase activity, may be among the potential therapeutic agents. Peroxiredoxin VI (Prx VI), one of the agents belonging to this family, is capable of neutralizing organic and inorganic hydroperoxides, including peroxynitrites [9]. Moreover, several studies have already demonstrated a therapeutic effect of Prx VI, specifically its contribution to regeneration processes in the trachea epithelium after thermal [10, 11] and chemical burns [12]. Prx VI has been shown to make a beneficial effect on the healing of cut wounds [14]. Thus, peroxiredoxins may become prototypes of medicinal drugs possessing a potent antioxidant effect [13].

Materials and methods

Animals

The experimental were carried out on white male Wistar rats (body mass of 200-220 g, 6-8 weeks of age) that were maintained in the vivarium of the Institute of Cell Biophysics, Russian Academy of Sciences (ICB RAS), Pushchino. Investigations on laboratory animals were conducted in accordance with the guidelines of the "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" and the legislation of the Russian Federation.

The animals had access to food and water ad libitum. Their feeding was discontinued at 24 hours before surgery.

Recombinant human Prx VI was obtained in the Laboratory of Reception Mechanisms in ICB RAS, Pushchino, Prx VI specific activities being as follows: the peroxidase sp.activity being 80 nmol/mg/min H₂O₂, and 60 nmol/mg/min tert-butyl hydroperoxide.

Ischemia/reperfusion in a rat model of strangulated intestinal obstruction.

According to the experimental study protocol, a prolonged anesthesia was administered to animals intravenously in vena caudalis using a mixture containing 0.5 ml of 3.5% Zoletil® 100 solution (Zoletil® 100, Virbac Sante Animale, France) and 0.1 ml of Rometar solution (Rometar, Bioveta, Czech Republic). Following induction of anesthesia, the intended operative site was prepared by removing fur and decontaminated with 70% alcohol solution. In anesthetized rats, the abdomen was opened via a midline laparotomy incision. The distal ileum loop (4-5 cm long) and adjacent mesentery with blood vessels were pulled out through the laparotomy wound. Strangulation

of the bowel loops with blood supplying vessels was induced by applying an occlusion device consisted of a silk ligature fixed inside the tube of 1 mm in diameter (Fig. 1).

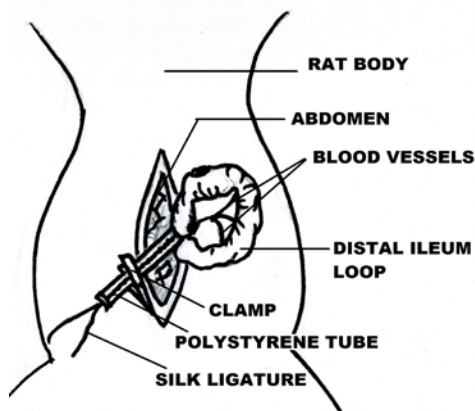


Figure 1. Strangulation of the ileum loops with blood supplying vessels using the occlusion device that consists of a silk ligature fixed inside the tube.

The ischemia of the distal ileal loop and mesenteric vessels was maintained for 60 minutes. After 60 minutes, the occlusion device was removed off the ischemic ileal loop. The restoration of blood flow in the loop was verified by a changing coloration of the strangulated intestinal portion and a visible pulsation in the mesenteric vessels. After the blood flow was restored, the abdominal cavity was closed tightly using a Policon Poliamide sterile surgical suture (Policon, Tonzos 95, Bulgaria). After the strangulation had been resolved, the reperfusion period made 120 minutes.

Experimental study protocol

Animals were randomly allocated into the following groups:

Group I (sham operated animals). The animals in this group (n=5) were subjected to a surgical procedure that comprised opening the abdomen to isolate an intestinal loop for a period of 60 minutes without strangulation.

Group II (I/R). The animals (n=5) were subjected to a surgical procedure that involved opening the abdomen to create an ischemia/reperfusion (I/R) model by inducing a strangulated small bowel obstruction. The ileum loop with mesenteric vessels was occluded for 60 minutes that was followed by a 120-minute reperfusion period.

Group III (Prx VI). The animals (n=5) received 10 mg/kg of human recombinant Prx VI administered intravenously at 15 minutes before intestinal loop ischemia.

Histological assessment of intestinal damage

Strangulated ileum tissue specimens were collected from experimental animals for histological examination. The obtained specimens were fixed with Myrsky's Fixative (Merck, USA) and embedded in paraffin. Serial sections of 2 μ m thick were prepared using a microtome (Thermo Electron Corporation, USA). Sections were stained with hematoxylin-eosin according to Ehrlich (Hematoxylin-Eosin, Fluka, USA). Microscopic examinations of histological sections obtained from different experimental groups were performed using Leica DM 6000 microscope, photomicrographs were taken using Leica DFC 490 digital camera for microscopy.

For each slide, 12 fields of view per section were examined at 200x magnification, and the cumulative damage severity was graded according to the criteria described by Chiu et al. [15]. Sections were evaluated in a blinded manner by two independent pathologists.

Results

Histopathological examination

A histological examination of the ileum segment specimens from the animals of experimental group I demonstrated a structurally intact mucosa; an uneven pattern of mucosal surface typical for a well-developed crypt-villus architecture. A single-layer prismatic limbic epithelium of intestinal villi and crypts had several cell populations: columnar epithelial cells, goblet exocrine cells, exocrine cells with acidophilic granules (Paneth cells) in the crypts (Fig. 2). The mucosal damage in the ileum specimens from Group I was graded as "0 - normal intestinal mucosa" (as defined by Chiu et al. [15]).



Figure 2. Photomicrograph. The ileum wall of the animals from experimental group I: Structurally intact intestinal mucosa (Hematoxylin-eosin stain [H&E stain], x200).

The histological examination of ileum specimens from animals of experimental group II (I/R) demonstrated the following signs of pathological changes: mucosal destruction; a massive disruption and desquamation of

epithelial villi and crypts; submucosa disintegration; lack of clear-cut intercellular junctions in the epithelium. Besides, there were signs of multiple hemorrhages, mucosa inflammation with leukocyte infiltration (Fig.3). An ileum mucosal damage in the specimens from Group II was graded as "5 -disintegration of lamina propria, hemorrhage, and ulceration" (by Chiu et al.).

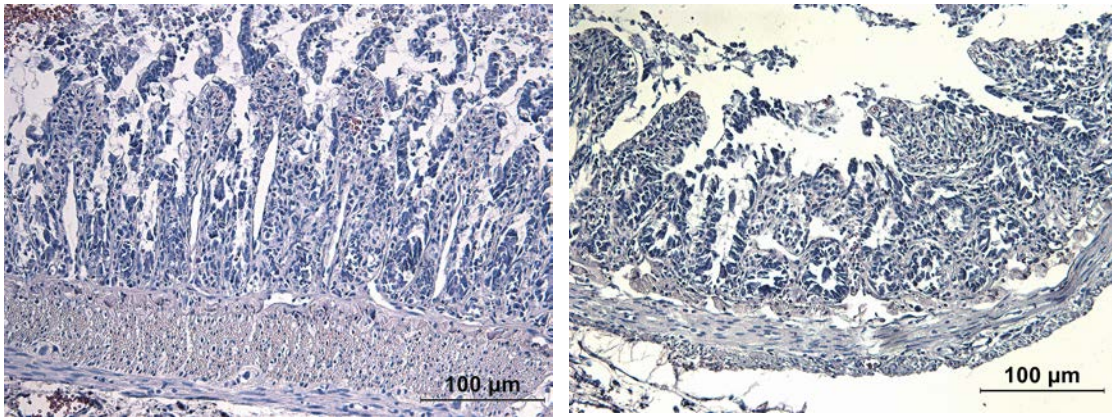


Figure 3. Photomicrograph. The strangulated ileum wall of the animals from experimental group II: Intestinal mucosa structures severely damaged by the I/R injury, (H&E stain, x200).

The histological assessment of the ileum segment from the animals of experimental group III (Prx VI) demonstrated that Prx VI administered intravenously at 15 minutes prior to I/R protected the intestine attenuating the severity of reperfusion injury after the resolution of the strangulated intestinal obstruction. The Prx VI administration posed a protective effect on the intestinal mucosa structure and the integrity of the villus-crypt architecture. However, in some parts of the strangulated ileum we observed discontinuities in the villus-crypt architecture, the leukocyte infiltration, and moderate hyperemia (Fig. 4). The ileum mucosal damage in the specimens from Group III was graded as "3 -significant epithelial lifting along the

length of the villi with a few denuded villous tips " (as defined by Chiu et al.).

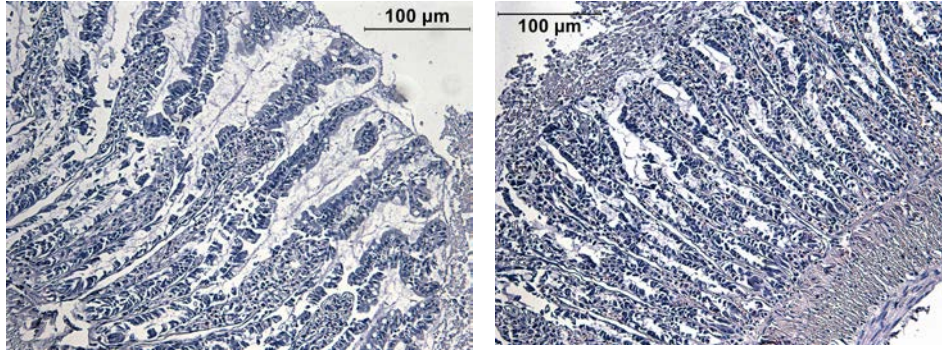


Figure 4. Photomicrograph. The strangulated ileum wall of the animals from experimental group III: Preserved intestinal mucosal structures as a result of intravenous Prx VI administration (H&E stain, x200).

Discussion

An organ I/R injury is a multifactorial pathological process. The reperfusion of the ischemic bowel is associated with dramatic consequences causing the production of reactive oxygen species and a powerful oxidative stress [8, 16]. The oxidative stress developing after a resolved strangulation constitutes an important link in the pathogenesis of subsequent injury to the small intestine [17].

In our study, we investigated the effect of antioxidant enzyme Prx VI on the consequences of reperfusion in the ischemia-affected small intestine.

The study has demonstrated that a 60-minute strangulation of a small intestine loop in a rat followed by the reintroduction of the main blood flow for 120 minutes leads to destructive morphological changes of the intestinal wall, mainly affecting the intestinal mucosa. This is consistent with the literature reports showing that a small intestine reperfusion following the resolution of strangulated ileum is accompanied by a further development of

necrobiotic disorders and necrotic lesions in the intestinal mucosa with progressing disintegration of the simple columnar epithelium [17]. Within two hours of reperfusion period, the pathomorphologic changes in the intestine reach their maximum.

In our study we have found that the administration of Prx VI in a rat may protect the intestine from I/R-injury. In the intestines of the animals who received Prx VI, the intestinal mucosa structure and the villi-crypt integrity were preserved. Thus, exogenous Prx VI prevents a massive loss of ileal cells in a rat protecting the tissue against destructive effects of reactive oxygen species. Worthwhile mentioning, the Prx VI has been found in all mammalian cells and detected in the largest amounts in the epithelial tissues of the lungs, respiratory tract, gastrointestinal tract, and oral cavity [18]. Although mammalian Prx VI gene expression has been shown to be regulated by an oxidative stress [20], probably, a proper endogenous Prx VI activity appears insufficient to neutralize the oxidative stress in I/R. In this case, a significant increase of Prx in the tissue of the small intestine achieved by its administration into the bloodstream might have controlled the oxidative stress at a safe level.

A protective effect of exogenous Prx VI on epithelial tissue has been shown in various organ lesions. Applying Prx VI directly on the tracheal mucosa of a rat after thermal and/or chemical burns of the upper respiratory tract significantly preserves epithelial cells and promotes regenerative processes [10-12], and also markedly accelerates the healing of cut wounds [14]. In this paper we have shown a protective effect of peroxiredoxins on the strangulated small intestine.

Conclusion

The issue of reperfusion injury occurring in the small intestine after its strangulation is crucial. The optimal solution of the problem, apparently, may be a clinical use of medicinal preparations with a potent antioxidant effect. The presented study supports the concept of using peroxiredoxins as a basis for pharmacological agents aimed at recovery of a redox status in a damaged tissue. Furthermore, the obtained results may be applicable in modifying the perfusion media used for preservation of isolated organs prior to their transplantation. A pre-transplantation reperfusion of isolated organs with peroxiredoxin-containing media can significantly improve the antioxidant status of the organ that would result in a reduced severity of the subsequent I/R injury to the organ after transplantation. Further studies of these issues are necessary.

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