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Targeting intestinal epithelial cell–programmed necrosis alleviates tissue injury after intestinal ischemia/reperfusion in rats

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ABSTRACT

Background: Intestinal dysfunction, especially acute pathologies linked to intestinal ischemia/reperfusion (I/R) injury, is profoundly affected by inflammation and improper execution of cell death. Few studies have examined the efficacy of combined strategies in regulated intestinal epithelial necrosis after intestinal I/R. Here, we evaluated the functional interaction between poly (adenosine diphosphate-ribose) polymerase 1 (PARP-1)-induced parthanatos and receptor-interacting protein 1/3 (RIP1/3) kinase-induced necroptosis in the pathophysiological course of acute ischemic intestinal injury.

Methods: Anesthetized adult male Sprague–Dawley rats were subjected to superior mesenteric artery occlusion consisting of 1.5 h of ischemia and 6 h of reperfusion. The PARP-1-specific inhibitor PJ34 (10 mg/kg) and the RIP1-specific inhibitor Necrostatin-1 (1 mg/kg) were intraperitoneally administered 30 min before the induction of ischemia.

Results: Intestinal I/R was found to result in PARP-1 activation and RIP1/3-mediated necrosome formation. PJ34 or Necrostatin-1 treatment significantly improved the mucosal injury, while the combined inhibition of PARP-1 and RIP1/3 conferred optimal protection of the intestine. Meanwhile, results from terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling assay showed a decrease in intestinal epithelial cell death. Interestingly, we further showed that PARP-1 might act as a downstream signaling molecule of RIP1 in the process of I/R-induced intestinal injury and that the RIP1/PARP-1-dependent cell death signaling pathway functioned independently of caspase 3 inhibition.

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Conclusions: The results of our study provide a molecular basis for combination therapy that targets both pathways of regulated necrosis (parthanatos and necroptosis), to treat acute intestinal I/R-induced intestinal epithelial barrier disruption.

Introduction

Intestinal ischemia/reperfusion (I/R) injury is a critical condition that results from acute mesenteric ischemia or is secondary to a variety of pathological conditions and surgical procedures, including sepsis, strangulated bowel, vascular surgery, and hemorrhagic shock.[1] In its severest form, intestinal I/R injury may clinically result in multiple organ dysfunction syndrome or death, which contributes to an overall mortality rate of up to 60%-80%.[2,3] However, the exact mechanisms of intestinal I/R-induced intestinal injury remain obscure, and effective preventive strategies remain unavailable.

Because the small intestine is particularly susceptible to hypoperfusion and the following reperfusion is caused by various pathological stimuli, the functions of the intestinal epithelial barrier (IEB) are often disrupted.[4] Studies in intestinal I/R patients and animals have demonstrated that a key aspect of intestinal I/R injury is the increased occurrence of apoptotic and necrotic cells in the IEB.[5-7] The presence of apoptotic cells or cellular damage in reperfused ischemic organs has serious implications in the pathogenesis of I/R-induced tissue injury.[8] We previously demonstrated that receptor-interacting protein 1/3 (RIP1/3) kinase and mixed lineage kinase domain-like protein recruitment mediate the regulated necrosis pathway (necroptosis), which acts as an important mediator of enterocyte loss in intestinal I/R injury.[9] Given that regulated necrosis is a form of genetically programmed necrotic cell death and encompasses a range of pathways, its complex molecular interactions after intestinal ischemic insult have not been sufficiently explored.

Although we previously demonstrated that targeting necroptosis and apoptosis appears to be an ideal therapeutic strategy,[9,10] the combined targeting of these and other cell death pathways with pathway-specific inhibitors is likely to bring a superior benefit and possibly a cure for acute intestinal ischemic insult. Recently, poly (adenosine diphosphate-ribose) polymerase 1 (PARP-1)-mediated regulated necrosis, referred to as parthanatos, has also been shown to be a distinct pathway in programmed necrosis[11] although there may be shared molecular components with necroptosis.[12] As previously demonstrated, PARP activation has already been implicated in the I/R injury of the retina and cochlea, as well as in both hemorrhagic and endotoxin shock, and has been associated with multiorgan failure.[13] Indeed, much remains to be understood regarding the role of intestinal epithelial parthanatos in the disruption of the IEB after intestinal I/R.

In this study, we investigated the role of different pathways in intestinal I/R-dependent–regulated necrosis and further identified the functional interaction between PARP-1-induced parthanatos and RIP1/3-induced necroptosis in the pathophysiological course of acute ischemic intestinal injury.

Materials and methods

Animals and intestinal I/R model

All animal experiments were approved by the Animal Care Committee of Sun Yat-sen University (Guangzhou, China) and were performed in accordance with the National Institutes of Health guidelines. Adult male Sprague–Dawley rats (weighing 220-250g) were purchased from Guangzhou University of Chinese Medicine (Guangzhou, China) and were housed in individual cages with controlled temperature and alternating 12-h light/dark cycles. All rats were fasted overnight but had free access to water before the experiment. After the rats were anesthetized with pentobarbital (30 mg/kg, intraperitoneal), the rat intestinal I/R injury model was established as previously reported.[14] Briefly, a 3-cm midline abdominal laparotomy was performed, and the small intestine was then exteriorized. The superior mesenteric artery (SMA) was occluded with a noncrushing microvascular clamp for 1.5 h followed by 6 h reperfusion. A heating blanket was used to keep the body temperature stable at 37°C. At the onset of reperfusion, resuscitation was performed by intraperitoneally administering 0.5 mL/100 g of normal saline, and the wound was then closed by sterile suture. The rats were sacrificed 6 h after the SMA was reperfused.

Experimental groups and drug treatments

One cohort of rats were randomly divided into the following groups ($n = 8$ per group): 1) a sham group, in which the animals just underwent isolation of the SMA without occlusion; and 2) an I/R group, in which the rats received 1.5 h of ischemia, followed by 6 h reperfusion. Another cohort of rats was randomly categorized into four groups ($n = 8$ per group): 1) the I/R group, which served as the vehicle control; 2) the PJ34 (acetamide, N-(5,6-dihydro-6-oxo-2-phenanthridinyl)-2-(dimethylamino) hydrochloride, PARP-1 inhibitor, Catalog No. S7300, Selleck) treatment group, in which rats received 10 mg/kg[15] of PJ34 dissolved in normal saline; 3) the Necrostatin-1 (Nec-1, necroptosis inhibitor, BML-AP309-0100, Enzo Life Science, New York, NY) treatment group, in which rats received 1.0 mg/kg[9] of Nec-1 dissolved in normal saline; and 4) the PJ34+Nec-1 combined treatment group. The aforementioned drugs or vehicle (normal saline) were intraperitoneally administered 30 min before the induction of ischemia, and the animals underwent 1.5 h of ischemia followed by 6 h of reperfusion.

Preparation of specimens

After euthanizing the rats, a 10-cm segment of the intestine was further divided into two segments. The segments were