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Direct peritoneal resuscitation improves survival and decreases inflammation after intestinal ischemia and reperfusion injury

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ABSTRACT

Background: Direct peritoneal resuscitation (DPR) has previously been shown to alter blood flow in the small bowel mesenteric vessels in models of intestinal ischemia. However, a survival advantage or its effects on local tissue inflammation have not been previously demonstrated. We hypothesized that DPR would increase survival and decrease intestinal tissue inflammation after intestinal ischemia and reperfusion (I/R) injury.

Methods: Eight-week-old male C57Bl6J mice were anesthetized and underwent midline laparotomy. I/R and DPR groups were exposed to superior mesenteric artery occlusion for 60 min with a nontraumatic clamp. Immediately after removal of the clamp, 1 mL of phosphate-buffered saline, 1 mL of minimal essential media, or 1 mL of minimal essential media supplemented with fetal bovine serum, penicillin and/or streptomycin, and glutamine were placed into the abdominal cavity of DPR groups. Animals were then closed in two layers and allowed to reperfuse for 6 h (cytokine analysis, $n = 6$ per group) or 7 d (survival analysis, $n = 10$ per group). After 6 h of reperfusion, animals were euthanized. Intestines were harvested and homogenized. Extracts were quantified for total protein content (Bradford assay), myeloperoxidase activity, tissue inflammatory cytokine, and growth factor production. $P < 0.05$ was significant.

Results: I/R caused marked intestinal ischemia, significant mortality, and a significant increase in tissue cytokine and growth factor levels ($P < 0.05$). Seven-day survival was 30% for I/R without treatment and rose to 60% with DPR therapy using phosphate-buffered saline as the dialysate. DPR using plain MEM or MEM with supplements after ischemia increased 7-d survival to 90% ($P < 0.05$). DPR also significantly decreased intestinal tissue levels of myeloperoxidase, as well as intestinal tissue levels of multiple growth factors and inflammatory cytokines.

Conclusions: DPR increases survival and decreases intestinal inflammation after intestinal I/R injury. Translational applications are readily achievable and should be considered for patients with intestinal ischemic pathology.

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1. Introduction

Intestinal ischemia and necrosis affect multiple patient populations of varying ages and comorbidities. Acute mesenteric ischemia (AMI) is prevalent in the elderly population and those who undergo cardiac bypass surgery. It usually involves the sudden occlusion of the intestinal arterial supply by a thrombus or embolus. AMI affects nearly 5000 patients annually, with many requiring open or endovascular surgical intervention to lyse the clot and salvage the ischemic intestine. The mortality rate for AMI can be as high as 40% for those who progress to surgery [1].

Two other forms of intestinal ischemia found predominantly in the neonatal population include necrotizing enterocolitis and volvulus. Necrotizing enterocolitis affects 10%–15% of the very low birth weight premature population and the mortality for the most severe cases of necrotizing enterocolitis can be as high as 50% [2]. Midgut volvulus associated with malrotation occurs much less frequently but carries a mortality of nearly 65% when 75% or more of the bowel is necrotic [3]. In either case, if the newborns survive these ischemic episodes, they are faced with prolonged hospitalization as well as need for long-term central venous access and parenteral nutrition.

Unrecognized or left untreated, patients can be exposed to a dramatic inflammatory cascade, which can result in shock, multisystem-organ failure, and eventual death. Many times, the ischemic area of bowel has to be surgically removed and this has the potential to leave the patient with a less than adequate length of bowel to absorb nutrients. Clinicians, therefore, have a unique opportunity to salvage ischemic intestine through the direct application of novel drugs, devices, or cellular therapies to the injured bowel.

Significant elevations in serum chemokines, including macrophage inflammatory protein 2 (MIP-2), eotaxin, interleukin (IL)-1, IL-2, and IL-9, have been noted after intestinal ischemia and are thought to be responsible for leukocyte growth, regulation, and mobilization to injured tissues [4–7]. These cells are responsible for injury repair but also promote inflammation, which may be detrimental to the host. For example, lymphocyte influx is thought to be detrimental to recovery, as lymphocyte depleted animals had better outcomes after intestinal ischemia [8]. However, other leukocyte classes may actually promote intestinal recovery by digesting dead cells and repairing the extracellular matrix [9].

Likewise, intestinal ischemia has dramatic effects on various growth factors including fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), and angiopoietin. FGF-2 has a broad mitogenic potential for bowel restitution, whereas VEGF and angiopoietin facilitate vasculogenesis. These factors have been implicated in wound healing and have been shown to accelerate the healing of ischemic colonic anastomoses [10]. Additionally, angiopoietin and IL-6 are elevated in infants with necrotizing enterocolitis and inflammatory bowel diseases [11–13]. IL-6 protects enterocytes by prolonging enterocyte lifespan and decreasing apoptosis, whereas angiopoietin helps to prevent plasma leakage and vascular inflammation [13,14]. Other factors, such as IL-10, promote an anti-inflammatory response, whereas hepatocyte growth factor may decrease apoptosis in intestinal crypts after ischemia [15,16].

In this regard, direct peritoneal resuscitation (DPR) may be a novel therapeutic modality to salvage ischemic bowel and to reduce the inflammatory cascade associated with intestinal ischemia. It has been used in certain trauma patients, has facilitated a decrease in time to definitive abdominal closure, and has reduced intra-abdominal complications after damage control surgery [17]. DPR was associated with improved hepatic flow, decreased organ edema, and lower levels of circulating proinflammatory cytokines [18]. In murine models of necrotizing enterocolitis, application of dialysates directly to the bowel has been shown to increase blood flow to the terminal ileum and to promote intestinal recovery [19]. Although the definitive mechanisms have not been elucidated, this protection may be associated with decreased local or systemic inflammation [20]. We, therefore, hypothesized that (1) DPR would increase survival in a murine model of intestinal ischemia and reperfusion (I/R) injury and (2) DPR would decrease intestinal inflammation after injury.

2. Methods

2.1. *Murine I/R model*

The experimental protocol and use of animals were approved by the Indiana University Institutional Animal Care and Use Committee. Adult male C57Bl6J mice (8–10 wk, 20–25 g; Jackson Laboratory, Bar Harbor, Maine) were allowed to acclimate to their environments for at least 48 h before intervention. They had free access to standard chow and were maintained in a 12-h light–dark cycle. Mice were anesthetized with 3% isoflurane and maintained at 1% isoflurane for the duration of the procedure. Abdomens were then shaved and prepped with 70% ethanol and betadine. One milliliter of 0.9% normal saline was injected subcutaneously and a midline laparotomy performed. The intestines were eviscerated, and the small bowel mesenteric root was identified.

In ischemic groups, the mesenteric root was temporarily occluded with an atraumatic microvascular clamp for 60 min. During the period of ischemia, the abdomen was temporarily closed with silk suture to prevent evaporative heat losses. Animals were maintained on a heating blanket to maintain body temperature. After 60 min, abdomens were reopened and the clamp was removed. The abdominal fascia and skin were then closed in two layers. Before final closure of the facial defect, 1 mL of phosphate-buffered saline (PBS), 1 mL of plain minimum essential medium ("pMEM" [Life Technologies, Grand Island, New York], or 1 mL of minimum essential medium supplemented with 16% fetal bovine serum [FBS; Atlanta Biologicals, Flowery Beach, Georgia], 1% penicillin/streptomycin [Sigma–Aldrich], and 1% glutamine [Sigma–Aldrich, St. Louis, Missouri]) was applied directly into the peritoneal cavity ("sMEM"). Triple antibiotic ointment was applied to the incision, and analgesia (1-mg/kg buprenorphine