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Pretreatment with Bone Morphogenetic Protein-7 (BMP-7) Mimics Ischemia Preconditioning Following Intestinal Ischemia/ Reperfusion Injury in The Intestine and Liver

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Abstract

Intestinal ischemia/reperfusion (I/R) injury has been shown to cause intestinal mucosal injury and adversely affect function. Ischemic preconditioning (IPC) has been shown to protect against intestinal I/R injury by reducing polymorphonuclear leukocyte infiltration, intestinal mucosal injury, and liver injury, and preserve intestinal transit. Bone morphogenetic protein 7 (BMP-7) has been shown to protect against I/R injury in the kidney and brain. Recently, microarray analysis has been used to examine the possible IPC candidate pathways. This work revealed that IPC may work through upregulation of BMP-7. The purpose of this study was to examine if pretreatment with BMP-7 would replicate the effects seen with IPC in the intestine and liver after intestinal I/R. Rats were randomized to six groups: sham, I/R (30 min of superior mesenteric artery occlusion and 6 h of R), IPC+R (three cycles of superior mesenteric artery occlusion for 4 min and R for 10 min), IPC+I/R, BMP-7+R (100 microm/kg recombinant human BMP-7), or BMP-7+I/R. A duodenal catheter was placed, and 30 min before sacrifice, fluorescein isothiocyanate-Dextran was injected. At sacrifice, dye concentrations were measured to determine intestinal transit. Ileal mucosal injury was determined by histology and myeloperoxidase activity was used as a marker of polymorphonuclear leukocyte infiltration. Serum levels of aspartate aminotransferase were measured at sacrifice to determine liver injury. Pretreatment with BMP-7 significantly improved intestinal transit and significantly decreased intestinal mucosal injury and serum aspartate aminotransferase levels, comparable to animals undergoing IPC. In conclusion, BMP-7 protected against intestinal I/R-induced intestinal and liver injury. Bone morphogenetic protein 7 may be a more logical surrogate to IPC in the prevention of injury in the setting of intestinal I/R.

Keywords

BMP-7; ileus; ischemia/reperfusion; intestinal transit

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The authors have no conflicts of interest to declare.

Introduction

Intestinal ischemia/reperfusion (I/R) injury is seen in such clinical scenarios such as hemorrhagic shock and resuscitation, thoracoabdominal aortic aneurysm repair, and small-bowel transplantation. Intestinal I/R injury has been shown to incite ileus formation, intestinal edema, and intestinal barrier dysfunction, and may contribute to multiple organ failure and death (1-5). Various new modalities have been examined to try and protect against this injury, including ischemic preconditioning (IPC) (4).

Ischemic preconditioning refers to short periods of ischemia that provide subsequent protection against a larger sustained ischemic event. This effect has been described in many organ systems, including the heart, kidney, and brain (4, 6-8). Recently, this effect has been studied in the intestine and has been shown to provide protection against I/R injury by preventing intestinal mucosal injury and preserving intestinal function (4). However, although effective, IPC is not readily usable in many clinical settings. As a result, determining the mechanism of IPC may allow for activation of this pathway via more clinically relevant means. As yet, the mechanisms of IPC in the setting of intestinal I/R injury are unknown. Recently, microarray analysis has been used to examine IPC candidate pathways (9). This work revealed that IPC is associated with an upregulation of bone morphogenetic protein 7 (BMP-7), suggesting that IPC may work through the BMP-7 pathway.

Bone morphogenetic proteins are members of the transforming growth factor- β superfamily of proteins and have been shown to regulate growth and differentiation of cartilage, bone, and muscle (10, 11). Disrupted BMP signaling has been associated with chronic intestinal inflammation and malignancy (12, 13). Furthermore, recent studies in the kidney have demonstrated the protective and anti-inflammatory effects of BMP-7 in acute ischemic renal injury (14, 15). In addition, BMP-7 administration has conferred intestinal mucosal protection and reduced systemic IL-6 levels in an inflammatory bowel disease model (16). Given this information, we hypothesized that BMP-7 administration before intestinal I/R injury will protect against intestinal mucosal injury and remote liver injury, preserve intestinal function, and prevent intestinal inflammation in a manner similar to IPC.

Materials and Methods

All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Mesenteric IPC and I/R

The surgical protocol for superior mesenteric artery (SMA) I/R is described in our earlier publications (4, 17-19). Male Sprague-Dawley rats (weighing 270-330g) were fasted 12 to 16 h before surgery and were given free access to water. The rats were placed under general anesthesia with isoflurane and had an external jugular vein catheter placed under aseptic conditions. Next, a midline laparotomy was performed, and a silastic catheter was tunneled from the back of the neck through the subcutaneous tissue and the left abdominal wall musculature, and into the peritoneal cavity. The end of the silastic catheter was placed into the duodenum by needle puncture, advanced 1 cm distally, and secured in place using a 6-0 silk purse-string suture. The external end of the catheter was sealed using a rubber cap. Finally, the SMA was dissected free from its mesenteric attachments. The small bowel was not manipulated.

Rats were randomized into six groups: sham surgery group, I/R group (SMA occlusion [SMAO] for 30 min and 6 h of reperfusion), IPC group (three cycles of SMAO for 4 min and reperfusion for 10 min, followed by 6 h of reperfusion), IPC+I/R group (three cycles of SMAO for 4 min and reperfusion for 10 min, followed by 30 min of SMAO and 6 h of reperfusion), BMP-7 (i.v. administration of 100 µm/kg recombinant human BMP-7 [ProSpec-Tany Technogene Ltd, Rehovot, Israel], followed by 6 h of reperfusion), and BMP-7+I/R (i.v. administration of 100 µm/kg recombinant human BMP-7, followed by 30 min of SMAO and 6 h of reperfusion). This specific IPC protocol (three cycles of SMAO for 4 min and reperfusion for 10 min) was modified from the IPC cardiovascular literature and has been validated in our previous work (4, 20-22).

The BMP-7 concentration used was taken from prior studies on intestine in rats (16). This study has demonstrated efficacy using BMP-7 at 100 µm/kg. Given these data and the prohibitive cost of performing complete dose-response studies, the authors performed this study using BMP-7 dosage from prior successful studies.

Measurement of intestinal transit

After 5½ h, the rats had 0.15 mL of a 5-mM solution of nonabsorbable fluorescein isothiocyanate (FITC)-Dextran (FITC, MW = 9,400, FITC content 0.008 mol/mol glucose, Sigma-Aldrich, St Louis, Mo) injected into the duodenal catheter and flushed with 0.15 mL of isotonic sodium chloride solution.

After 30 min, the rats were placed under general anesthesia with isoflurane and had laparotomy incisions reopened. Next, the entire small intestine was removed and divided into 10 equal segments. The intraluminal contents of each segment were then flushed using 2.5 mL of 5 mM Tris-buffer (pH 10.3) to recover the FITC-Dextran. The FITC-Dextran concentration was then measured using an optical scanner (STORM model 860, Amersham Biosciences, Piscataway, NJ) and expressed as a fraction of the total tracer recovered and presented as the geometric center of distribution as described in the literature (18, 23).

Histology and mucosal injury scoring

Ileal segments harvested from rats in the six experimental groups were snap frozen at -80°C and were stored in 10% formalin until processing. Tissue was embedded in paraffin blocks, sectioned in 5-µm slices, placed on glass microscope slides, and stained with hematoxylin and eosin. Light microscopic evaluation of the tissues was performed in a masked fashion by a pathologist who scored the histology using a system described by Chiu et al. (24): grade 0 = normal mucosa, grade 1 = subepithelial space developing at the tip of the villus, grade 2 = lifting of the epithelial layer from the lamina propria and moderate extension of the subepithelial space, grade 3 = some denuded tips of the villi and massive lifting of the epithelial layer, grade 4 = dilated and exposed capillaries and denuded villi, and grade 5 = hemorrhage, ulceration, and disintegrated lamina propria.

Myeloperoxidase activity

Myeloperoxidase (MPO) enzyme activity was measured in tissue lysates as described previously (4) using the hydrogen peroxide-dependent oxidation of 3,3',5,5'-tetramethylbenzidine and the SureBlue 3,3',5,5'-tetramethylbenzidine 1-Component Microwell Peroxidase (KPL, Gaithersburg, Md). Absorbance changes at 450 nm were measured with a Kinetic Microplate Reader (Molecular Devices, Sunnyvale, Calif) spectrophotometer.

Serum IL-6 determination

Serum samples were isolated at the completion of all experiments and stored at -80°C. Serum IL-6 concentration was determined using a Rat 9-Plex A Panel from Bio-Rad Laboratories (Hercules, Calif).

Serum liver enzyme determination

The release of the hepatocellular enzyme aspartate aminotransferase (AST) into the bloodstream was used as an index of hepatic injury. At the completion of all experiments, blood samples were collected, centrifuged, and the serum was frozen at -80°C until subsequent AST determination. The release of AST was determined by an automated procedure using an autoanalyzer (Boehringer Mannheim, Indianapolis, Ind) and has been described previously (25).

Statistical analysis

All data are expressed as mean $\pm$ SEM. Statistical significance of differences among groups was determined by ANOVA. Post hoc analysis was performed using the Tukey test to identify differences between individual groups. A $P < 0.05$ was considered significant. The number of rats (n) was six for each group.

Results

Transit

Transit (represented by mean geometric center) is significantly depressed in the I/R (3.20 ± 0.33) group versus sham (4.54 ± 0.53), IPC (4.46 ± 0.37), IPC+I/R (4.54 ± 0.37), BMP-7 (4.21 ± 0.49) and BMP-7+I/R (4.36 ± 0.57 ; Fig. 1). There was no significant difference in transit between the sham, IPC, IPC+I/R, BMP-7, or BMP-7+I/R groups.

Chiu score

The Chiu score is significantly increased in the I/R (2.50 ± 0.36) group versus sham (0.92 ± 0.33), IPC (1.00 ± 0.15), IPC+I/R (1.60 ± 0.51), BMP-7 (1.41 ± 0.30), and BMP-7+I/R (1.50 ± 0.62), representing a significant increase in mucosal injury (Fig. 2). There was no significant difference in mucosal injury between the sham, IPC, IPC+I/R, BMP-7, or BMP-7+I/R groups. Representative images of mucosal injury are shown in Figure 3. The image labeled I/R in Figure 3 demonstrates the typical dilated capillaries and denuded villi seen with I/R injury. In contrast, the images labeled sham, IPC+I/R, and BMP-7+I/R demonstrate the intact mucosal and submucosal architecture seen with these treatment modalities.

Intestinal inflammation-MPO and IL-6

The intestinal MPO activity was significantly increased in the I/R (4.39 ± 0.57 ng/mg protein) group versus sham (2.21 ± 0.14 ng/mg protein), IPC (2.36 ± 0.19 ng/mg protein), IPC+I/R (3.10 ± 0.40 ng/mg protein), BMP-7 (2.96 ± 0.25 ng/mg protein), and BMP-7+I/R (2.94 ± 0.22 ng/mg protein), representing a significant increase in neutrophil infiltration and intestinal inflammation (Fig. 4). There was no significant difference in MPO activity between the sham, IPC, IPC+I/R, BMP-7, or BMP-7+I/R groups. Intestinal IL-6 is significantly increased in the I/R (279 ± 47 pg/mL) group compared with sham (170 ± 18 pg/mL), IPC (164 ± 37 pg/mL), IPC+I/R (181 ± 39 pg/mL), BMP-7 (140 ± 42 pg/mL), and BMP-7+I/R (127 ± 62 pg/mL), representing a significant increase in intestinal inflammation (Fig. 5). There was no significant difference in IL-6 levels between the sham, IPC, IPC+I/R, BMP-7, or BMP-7+I/R groups.

Remote liver injury-serum AST concentration

The serum AST concentration was significantly increased in the I/R (308 ± 26 IU/L) group over sham (138 ± 10 IU/L), IPC (120 ± 5 IU/L), IPC+I/R (191 ± 14 IU/L), BMP-7 (127 ± 9 IU/L), and BMP-7+I/R (144 ± 4 IU/L), representing a significant increase in remote liver injury (Fig. 6). There was no significant difference in serum AST level between the sham, IPC, IPC+I/R, BMP-7, or BMP-7+I/R groups.

Discussion

We have shown that ischemic preconditioning before intestinal ischemia reperfusion injury protects against intestinal mucosal injury and remote liver injury, prevents intestinal inflammation, and preserves intestinal function consistent with prior studies (4). In addition, we showed that pretreatment with BMP-7 mimics the effects of ischemic preconditioning in the setting of intestinal ischemia reperfusion.

Ischemia reperfusion injury in the intestine represents a severe clinical problem facing clinicians. Intestinal mucosal injury and dysfunction resulting from I/R injury leads to development of intestinal dysfunction and adversely impacts patient recovery from traumatic injury. In addition, I/R induced ileus delays the initiation of enteral feeding, leading to an increase in septic complications (26). Intestinal I/R injury is associated with a large influx of neutrophils as well as an increase in pro-inflammatory cytokines (27). Ischemic preconditioning seems to protect the intestine and preserve intestinal function by decreasing the inflammatory response seen after I/R injury (4). However, the mechanism of action of IPC is unclear.

Preliminary microarray analysis conducted to elucidate the mechanism of IPC has shown a significant upregulation of endogenous BMP-7 during IPC (9). The BMP-7 has shown anti-inflammatory properties in reducing apoptosis, inflammation, and neutrophil infiltration in I/R injury in the kidney (15). In addition, BMP-7 has been shown to decrease inflammatory mediators in an inflammatory bowel disease model (16). Our data support prior work by showing that BMP-7 decreases inflammation measured by neutrophil infiltration and IL-6 production. In addition, we have shown preserved organ function measured by intestinal transit. These data may provide insight into the mechanism of IPC. However, further studies may be needed to confirm that the effects of IPC are mediated by BMP-7.

Although the mechanism of BMP-7 has not yet been elucidated in intestine, there are many data from other organs that may provide insight. Inhibitory Smad 6 release has been seen with BMP-7 administration in kidney models. The BMP-7, after interaction with BMP receptor 1, leads to phosphorylation of SMAD 1, 5, and 8. This complex interacts with SMAD 4 to enter the nucleus and, along with nuclear binding factors, releases inhibitory SMAD 6 into the cytosol (28). Extensive work in nervous tissue has shown that Smad 6 overexpression leads to downregulation in signal transducer and activator of transcription 3 (STAT3) expression (29). In addition, STAT3 upregulation and activation leads to decreased myosin light chain phosphorylation leading to decreased intestinal contractility and transit (30-33). It is possible that BMP-7 administration may lead to overexpression of inhibitory Smad 6 leading to downregulation of STAT3, with subsequent increased myosin light chain phosphorylation, leading to improved intestinal function. Intestinal I/R injury remains a difficult clinical challenge in such scenarios as trauma, major abdominal vascular repair, or transplantation. At present, there are limited treatment regimens to effectively address this problem. Regardless of whether BMP-7 is the effector pathway of IPC, BMP-7 itself represents a novel therapeutic agent in the treatment of I/R injury. Ischemic preconditioning, although an interesting phenomenon, is not a readily applicable modality in many clinical settings, requiring isolation of mesenteric arteries with subsequent time-consuming

occlusion and release of the vessel. In contrast, i.v. administration of BMP-7 is a more clinically relevant treatment modality that can be used to achieve similar effects to IPC in the intestine. Although more studies will be necessary to identify the therapeutic efficacy and safety of BMP-7 in human subjects, the use of an endogenous mediator to modulate the inflammatory response shows a great deal of promise in future therapeutic strategies.

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Fig. 1.

Comparison of small intestinal transit expressed as the mean $\pm$ SEM ($P < 0.05$, $n=6$). These data show that intestinal transit is adversely affected by I/R injury. IN addition, BMP-7 administration preserves intestinal transit similar to IPC. *Statistical significance compared with other groups ($P.0.05$).

Fig. 2.

Comparison of Chiu score expressed as the mean $\pm$ SEM ($P < 0.05$, $n = 6$). These data show that intestinal mucosa is significantly injured by I/R. Administration of BMP-7 maintains intestinal mucosal architecture similar to IPC. *Statistical significance compared with other groups ($P < 0.05$).

Fig. 3. Representative hematoxylin and eosin-stained cross sections of small intestine (magnification 20 $\times$). The I/R-treated animals demonstrate significant injury to intestinal mucosa as evidenced by denuded villi and dilated capillaries. The IPC and BMP-7 treated animals appear similar to sham.

Fig. 4. Comparison of intestinal MPO levels expressed as the mean $\pm$ SEM ($P < 0.05$, $n = 6$). The I/R injury stimulates a significant increase in MPO levels. Administration of BMP-7 prevents an increase in MPO levels similar to IPC. *Statistical significance compared with other groups ($P < 0.05$).

Fig. 5.

Comparison of IL-6 levels expressed as the mean $\pm$ SEM ($P < 0.05$, $n = 6$). The I/R injury stimulates a significant increase in IL-6 levels. The BMP-7 and IPC treatment maintain IL-6 at sham levels. *Statistical significance compared with other groups ($P < 0.05$).

Fig. 6. Comparison of serum AST levels expressed as the mean $\pm$ SEM ($P < 0.05$, $n = 6$). Intestinal I/R injury causes an increase in serum AST levels. This increase is blunted by BMP-7 treatment similar to IPC.*Statistical significance compared with other groups ($P < 0.05$).