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The preventive effects of dexmedetomidine against intestinal ischemia-reperfusion injury in Wistar rats

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ABSTRACT

Objective(s): Intestinal ischemia-reperfusion is a major problem, which may lead to multiorgan failure and death. The aim of this study was to evaluate the protective effects of dexmedetomidine on cell proliferation, antioxidant system, cell death, and structural integrity in intestinal injury induced by ischemia-reperfusion in rats.

Materials and Methods: Animals were randomized into three groups: group A, sham-operated or control; group B, intestinal ischemia/reperfusion (IR); and group C, intestinal IR pretreated with 50 µg of dexmedetomidine. Intestine tissue was collected from all rats 30 min after desufflation, and fresh frozen for histological and biochemical evaluation.

Results: The intestinal tissue of group B rats showed a significant decrease in the antioxidant enzyme activities. However, these enzyme activities were improved by the administration of dexmedetomidine. Inhibiting the protein expression of MCP_7, PAR_2, P-JAK, P-$STAT_1$, and P-$STAT_3$ proved the protective effect of dexmedetomidine. The immunohistochemical staining revealed its protective effect by maintaining the normal structural integrity, less caspase-3 immuno reactivity, and increased cell proliferation count in the intestinal tissues.

Conclusions: Intraperitoneal injection of dexmedetomidine significantly protected intestine IR injury in rats by inhibiting the inflammatory response, intestinal epithelial apoptosis, and maintaining structural integrity of intestinal cells.

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Introduction

The condition by which the deprivation of blood flow leads to insufficient oxygen and nutrient supply to the tissue is called ischemia. Reperfusion injury refers to the tissue damage inflicted when blood flow is restored after an ischemic period for a minimum of 10 min. The intestinal ischemia/reperfusion (IR) injury causes damage to the intestinal mucosa, serious impairment of the local microvasculature, increased vascular and mucosal permeability, and multiple organ failure (1). Emerging studies have observed increased cell death or tissue damage during IR of the gut that plays a vital role in the pathogenesis of IR induced intestinal injury. The intestinal IR model has often been practiced as an experimental model to study apoptosis or to investigate reactive oxygen species induced oxidative stress especially during reperfusion in small intestine (2-4). However, a specific treatment for IR is not in practice. Several antioxidants and antibodies against adhesion molecules have shown to be protective against IR injury (3, 5).

Contemporary researches suggest the tissue protective effect of dexmedetomidine by reducing cerebral, cardiac, intestinal, and renal injury (6-8). The highly selective and potent α_2-adrenergic agonist dexmedetomidine is an effective sedative, anxiolytic, and analgesic agent used in postoperative patients as mechanical ventilation (9, 10). Previous studies have suggested that mast cells are critical regulators of physiological function of the intestine and participate in the inflammatory pathogenic process of IR (11, 12). Histamine and tryptase can trigger inflammation and cause tissue injury by increasing the mucosal membrane permeability and promoting inflammatory factor production.

Mast cells protease 7 (MCP_7) is the primary subtype of tryptase synthesized by immature mast cells. Protease-activated receptor 2 (PAR_2) can be activated by tryptase and modulate inflammatory response (13). The Janus kinase/signal transducer and activator of the transcription (JAK/STAT)

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signaling pathway play a vital role in transducing signals for various cytokines and growth factors (14). After a cascade of intracellular phosphorylation events, they dimerize and translocate to the nucleus, modulate the transcription of many target genes, and thereby stimulate cell proliferation, differentiation, cell migration and apoptosis, and other processes (15). Based on the protective effect of dexmedetomidine on various tissues, the present study aimed to investigate the effects of dexmedetomidine on intestinal injury induced by IR in rats.

Materials and Methods

Animals and experimental groups

Male Wistar rats with almost similar body weight (208.62 to 212.87 g) were taken for the experiment. Animals were randomly assigned into 3 groups including group A (sham-operated or control, n=8); group B (intestinal IR, n=8); and group C (pretreated intestinal IR, n=8, and 50 μg of dexmedetomidine was intraperitoneally injected 30 min before intestinal IR); the dosage was determined from previous study (16). The animals were housed in a temperature controlled room with 12 hr light–dark cycles, and were fed with regular rat chow and water *ad libitum,* but were fasted overnight prior to the experiments. All experiments described herein were conducted with the approval of the Institutional Animal Care and Use Committee of First Affiliated Hospital of Nanchang University, Jiangxi, China.

Intestinal ischemia/reperfusion injury

The animals were anesthetized using the combination of sodium pentobarbital (390 mg) and sodium phenytoin (50 mg/ml), and a midline abdominal incision was made. The superior mesenteric artery (SMA) was identified and freed by blunt dissection. A microvascular clamp was placed at the root of SMA to cause complete arrest of blood flow for 60 min, and the clamp was loosened to form reperfusion injury (17). At the end of the experiment, the animals were sacrificed by administering over dose of anesthesia. No analgesics, antibiotics, or euthanasia agents were used. The blood samples and intestinal tissue biopsies were taken. The tissues were fresh frozen for histological and biochemical evaluation. As this is a preliminary study to reveal the protective effect of dexmedetomidine, this study doesn't pursue further with the survival.

Determination of antioxidant enzyme activity

The innate antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (Gpx) were analyzed. To calculate the specific enzyme activity, protein concentration in each sample was estimated by the method of Bradford (18). CAT activity was assayed in hemolysates of erythrocytes by monitoring the consumption of H_2O_2 at 240 nm, as described by Aebi (19). The SOD activity was determined according to the method of Sun *et al* (20). One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the nitroblue tetrazolium reduction rate. The activity of SOD was termed as units per milligram protein. The activity of Gp was determined essentially as described by Rotruck *et al* (21). In the test, the enzyme activity was expressed as units/mg protein (one unit was the amount of enzyme that converted 1 μmol of GSH to the oxidized form of glutathione (GSSH) in the presence of H_2O_2/min).

Western blot analysis

The protein lysates were prepared from frozen tissues in ice-cold RIPA buffer (Sigma-Aldrich). The extracted protein was separated in a 10% sodium dodecyl sulfate (SDS)-PAGE and then electrophoretically transferred to a nitrocellulose membrane (Hybond, Amersham Biosciences, Little Chalfont, UK). Membranes were blocked with 5% nonfat milk powder in TBS for 1 hr at room temperature. These membranes were subjected to immunoblot analysis with antibodies to MCP_7, PAR_2, P-JAK_2, P-$STAT_1$, and P-$STAT_3$. The protein-antibody immune complexes were detected with horseradish peroxidase conjugated secondary antibodies and enhanced chemiluminescence reagents (Pierce Biotechnology, Rockford, IL). β-actin was used as an internal control for protein normalization, and values were compared with those of group A.

Histology and immunohistochemical staining

Paraformaldehyde fixed, paraffin embedded samples of small intestine or jejunum were also cut 5 mm in thickness, deparaffinized in xylene, rehydrated in graded ethanol, and then were stained with haematoxylin-eosin (HE) for histological observation under light microscope (Olympus, Japan) and photographed. Immunohistochemical staining for active caspase-3 was performed using the streptavidin-biotin-peroxidase method. For negative control, phosphate-buffered saline was used instead of antibody. Cytoplasm with dark red staining was assessed as positive. The proliferating cells in intestine sections were detected by proliferating cell nuclear antigen (PCNA) immunohistochemistry.

Statistical analysis

Data were analyzed by statistical software (SPSS Inc, Chicago, IL). All values are given as mean±standard error of the mean. $P<0.05$ was considered as statistical significance.

Results

Dexmedetomidine improved antioxidant enzyme activity

The level of antioxidant enzymes (CAT, SOD, and Gpx) activity was determined using the rat intestinal tissues from all three experimental groups. The activity

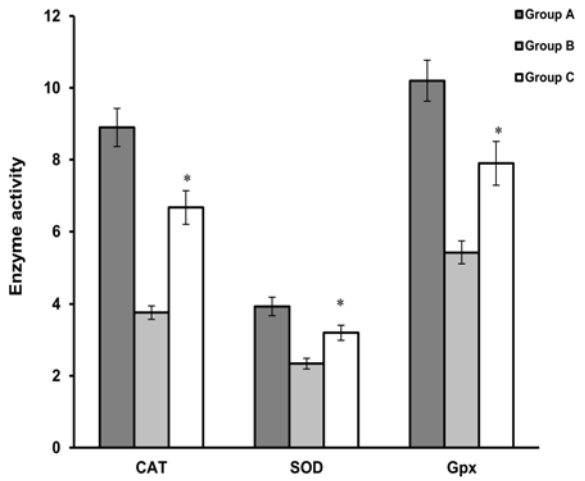

Figure 1. Represents the levels of antioxidant enzyme activity in rat intestinal tissue from experimental group.* -indicates the significant (P<0.05) difference compared with group B

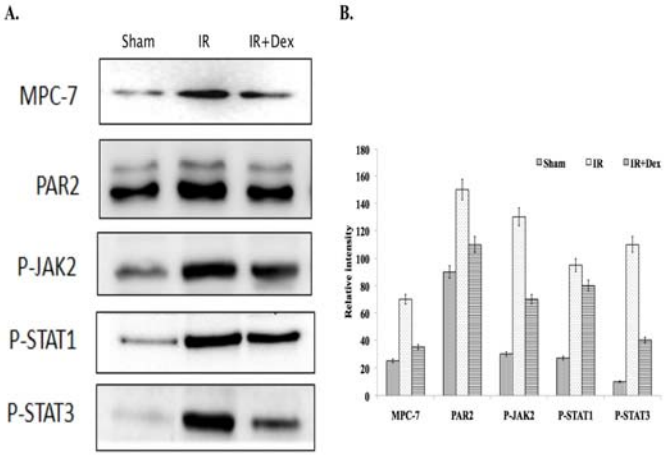

Figure 2. A. Representative Western blot showing the expressions of total MCP_7, PAR_2 and phosphorelation of JAK_2, $STAT_1$, and $STAT_3$ in intestinal tissue. Sham (group A); I/R (group B, intestinal Ischemia/reperfusion rats); I/R^+ Dex (group C, dexmedetomidine administered intestinal ischemia/reperfusion rats). B. Represents the quantification of the band intensity with respect to the Western blot

levels were compared between groups. The enzymes CAT, SOD, and GpPx levels in the intestinal I/R (group B) were lower in comparison with sham (group A) and pretreated intestinal I/R (group C). However, the levels of these enzymes were significantly (P<0.05) lower in I/R^+ dexmedetomidine group compared to the sham group (Figure 1).

Dexmedetomidine inhibited inflammatory protein expressions

The Western blotting analysis was performed to demonstrate the effect of dexmedetomidine on inflammatory proteins. The MCP_7 and PAR_2 protein levels were significantly elevated in intestinal I/R group. However, these elevated protein levels were not seen in dexmedetomidine administered intestinal I/R rats (Figure 2). Similarly, dexmedetomidine administration controlled the JAK/STATs pathway by inhibiting the protein phosphorelation of JAK, $STAT_1$, and $STAT_3$. The protein level of Sham group was used as control. These results confirm that dexmedetomidine inhibits the mast cell proteins and JAK/STAT pathway on intestinal I/R injury (Figure 2).

Dexmedetomidine protects structural integrity from intestinal I/R Injury

To examine the protective effect of dexmedetomidine against I/R injury in small intestinal, we implemented histopathology and immunohistochemical staining methods in the jejunal tissues of experimental groups' rats. The normal histological structure was observed in HE stained sections of sham group rats (Figure 3A). Severe disruption of structural integrity in brush border, including loss of mucus, villi, and wide spread necrotic area was observed in I/R injured rats (Figure 3B). However, these damages were protected in dexmedetomidine-administered I/R rats (Figure 3C). In immunohistochemical staining we observed an

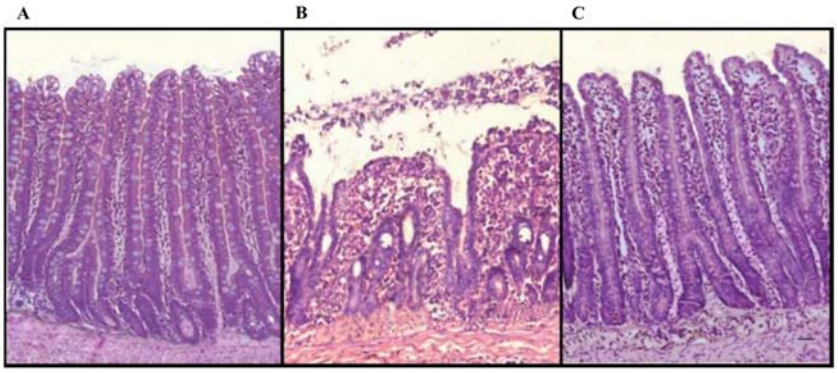

Figure 3. Represents the haematoxylin-eosin staining on histology of rat jejunal tissue in all groups. (A) Normal histological appearance of jejunum in a sham-operated control rats; (B) rats subjected to I/R injury indicates disruption in structural integrity, loss mucus, villi, and wide spread necrotic area; (C) dexmedetomidine administered intestinal I/R rats show the protective effect by maintaining normal structural integrity. Scale bar = 200 µm

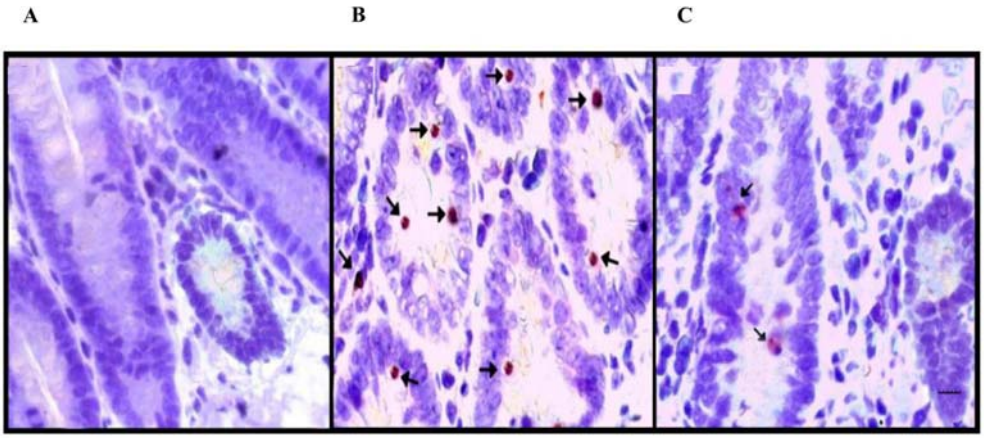

Figure 4. Represents the caspase-3 staining for immunoreactive cells in rat jejunal tissue. (A) No immunoreactive cells appeared in sham-operated control rats; (B) rats subjected to I/R injury exhibited more number of active caspase-3 immunoreactive cells (arrow indicated); (C) dexmedetomidine administered intestinal I/R rats show very less immunoreactive cells. Scale bar= 50 µm

intense staining for active caspase-3 in the cytoplasm of epithelial cells of rats subjected to I/R injury (group B). However very few caspase-3 immuno reactive cells were observed in dexmedetomidine administered I/R group and no caspase-3 stain was observed in sham group (Figure 4). The PCNA immunoreactivity staining clearly showed a significant decrease or no PCNA positive crypt cells in intestinal I/R rats. However, increased cell proliferations intensity and number of PCNA-positive crypt cells similar to that of sham group were observed in dexmedetomidine pretreated I/R rats (Figure 5). These results again proved the tissue protective effect of dexmedetomidine against intestinal I/R injury.

Discussion

In this study, we demonstrated the protective effect of dexmedetomidine against the intestinal injury induced by I/R using rat model. Similar study demonstrated the effect of using testosterone however the mechanistic role was unclear (22). In the present study, the investigation was focused on the role of dexmedetomidine on cell proliferation, differentiation, cell migration, and other processes. The intestinal I/R injury is a complex, multifactorial, and pathophysiological process that involves the dysfunction of absorption, bacterial translocation, production of reactive oxygen species, cytokines, nitric oxide, and initiates multiorgan dysfunction syndrome (23, 24). Intestinal mucosal mast cells are particularly frequent in close proximity to epithelial surfaces, where they are strategically located for optimal interaction with the environment and for their putative functions of host defense (25). The antioxidant enzymes (CAT, SOD, and Gpx) assist cells in repairing damaged membranes against oxidative damage. In the present study, a significant increase in CAT, SOD, and Gpx activities was observed in dexmedetomidine-administered intestinal I/R group. These results indicate that dexmedetomidine has potent free radical scavenging and antioxidant properties during the progress of I/R injury. Our results are consistent to the previous study that dexmedetomidine infusion (10), L-carnitine (26) and resveratrol (27) prevent the production of reactive oxygen species during mesenteric I/R injury in rats.

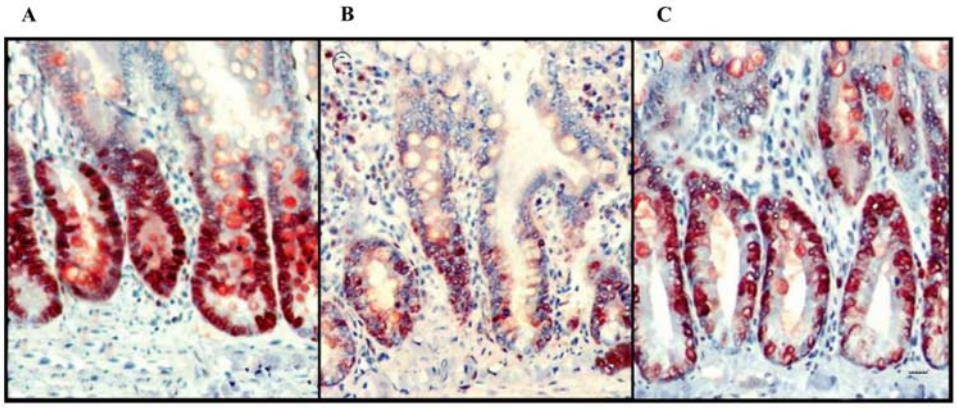

Figure 5. Represents the PCNA staining for immunoreactive cells in crypts of the jejunum. (A) sham-operated control rats; (B) rats subjected to I/R injury; (C) dexmedetomidine administered intestinal I/R rats. Scale bar = 100 µm

Activated mast cells participate in the pathogenesis of I/R related inflammation and intestinal injury (28). To understand the mechanisms underlying the therapeutic effect of dexmedetomidine, we detected the relative levels of MCP_7 in the intestinal tissues from all groups and observed a significantly lower level in treatment group than that of intestinal I/R groups of rat. Furthermore, the relative levels of PAR_2 in the intestinal tissues from the treatment group of rats were significantly lower than those in the intestinal I/R group. Our data were consistent with the report that treatment with anti-PAR_2 mitigates I/R related Inflammation and reduces the levels of TNF-α (29). Next we investigated the relationship between JAK_2 associated signaling cascades and intestinal I/R triggered intestinal injury. Other than sham group, phosphorylations of JAK_2, $STAT_1$, and $STAT_3$, reflecting activation, were significantly potentiated after reperfusion. This shows that JAK/STAT pathway was dramatically activated under intestinal I/R condition. In the present study, we documented that treatment with dexmedetomidine significantly down regulates the phosphorylation of JAK_2 and its downstream molecules STAT1/$STAT_3$, which is consistent with the study of Yang *et al* (30).

The current study evaluated the role of dexmedetomidine on intestinal architecture and apoptosis in small intestinal I/R injury and determined its protective and antiapoptotic effects against intestinal injury. In the present study, the histopathology showed a significant protective effect of dexmedetomidine against intestinal I/R injury. This is consistent with recent report showed dexmedetomidine reduces systemic levels of interleukin-6, tumor necrosis factor-α, and high mobility group box 1 following lipo-polysaccharide infusion or sepsis in animals; indicating its anti-inflammatory effects against renal I/R injury (6). Previous studies have shown that erythropoietin has protective effects against I/R injury in several tissues (31). In addition, dexmedetomidine was known to alter the cardiovascular response during infrarenal aortic crossclamping in sevoflurane anesthetized dogs (32, 33). Similarly, our experiment showed that apoptotic index of caspase-3, as a key caspase involved in the apoptotic pathway in the intestinal mucosa, was markedly reduced in dexmedetomidine pretreated group, which also promotes the cell proliferating index in PCNA staining. Homeostasis of epithelial architecture in the small intestine is regulated by both cell proliferation and cell death or apoptosis (34). Since intestinal epithelial cells have short proliferation cycle and powerful growth ability, regeneration in the intestinal tissue is quite fast. Another factor, which could damage the integrity of the intestinal mucosal barrier, is the inhibition of epithelial cell proliferation (35). Thus dexmedetomidine has been demonstrated as a potent agent for the protection of intestinal I/R injury. The clinical implication of our study represent that "dexmedetomidine" in spite of an effective sedative agent, shows the ability to prevent I/R induced intestinal injury based on its protective effect on cell proliferation, antioxidant system, cell death, and structural integrity.

Conclusion

Our studies showed that dexmedetomidine protects intestine against I/R injury, at least in part, increasing the antioxidant enzyme activity, cell proliferation, and inhibitory effects on injury induced activation of caspase-3, MCP_7, PAR_2 protein level, JAK/STAT signaling pathway. On extrapolating our data to clinical setting, dexmedetomidine may serve as a clinical strategy to prevent intestinal I/R injury.

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