

Dexmedetomidine Administration before, but Not after, Ischemia Attenuates Intestinal Injury Induced by Intestinal Ischemia-Reperfusion in Rats

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ABSTRACT

Background: Intestinal ischemia-reperfusion (I/R) injury is a devastating complication in the perioperative period. Dexmedetomidine is commonly applied in the perioperative period. The authors aimed to determine the effects of different doses of dexmedetomidine (given before or after intestinal ischemia) on intestinal I/R injury and to explore the underlying mechanisms.

Methods: Intestinal I/R injury was produced in rat by clamping the superior mesenteric artery for 1 h followed by 2 h reperfusion. Intravenous infusion of dexmedetomidine was performed at 2.5, 5, and 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before or after ischemic insult. In addition, yohimbine hydrochloride was administered intravenously to investigate the role of α_2 adrenoreceptor in the intestinal protection conferred by dexmedetomidine.

Results: Intestinal I/R increased mortality of rats and caused notable intestinal injury, as evidenced by statistically significant increases in Chiu's scores; serum diamine oxidase and tumor necrosis factor- α concentration, accompanied by increases in the intestinal mucosal malondialdehyde concentration; myeloperoxidase activity; and epithelial cell apoptosis (all $P < 0.05$ vs. Sham). Except malondialdehyde and myeloperoxidase, all changes were improved by the administration of 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine before ischemia

What We Already Know about This Topic

- The mortality caused by intestinal ischemia-reperfusion (I/R)-induced intestinal injury remains high, and few techniques or drugs are effective enough to use clinically.

What This Article Tells Us That Is New

- Dexmedetomidine administration before an ischemic insult dose-dependently reduces intestinal I/R injury. However, the initiation of dexmedetomidine administration after ischemia produced no protective effect.

(all $P < 0.05$ vs. Injury) but not after ischemia. Infusion of 2.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine before or after ischemia produced no beneficial effects, and infusion of 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine led to severe hemodynamic suppression. Yohimbine abolished the intestinal protective effect of the 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine infusion before ischemia and was accompanied by the disappearance of its antiapoptotic and antiinflammatory effect.

Conclusion: Dexmedetomidine administration before, but not after, ischemia dose-dependently protects against I/R-induced intestinal injury, partly by inhibiting inflammatory response and intestinal mucosal epithelial apoptosis *via* α_2 adrenoreceptor activation.

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INTESTINAL ischemia-reperfusion (I/R) injury is a potentially serious consequence of acute mesenteric ischemia; hemorrhagic, traumatic, or septic shock; severe burns; or some surgical procedures, including cardiopulmonary bypass, small bowel transplantation, and abdominal aortic surgery.¹ Intestinal I/R is associated with the exacerbation of intestinal injury and a systemic inflammatory response leading to progressive distal organ impairment, finally resulting in cardiocirculatory, respiratory, hepatic, and renal failure.^{2,3} In the critical care setting, the development of intestinal ischemia contributes to high mortality (67–80%).⁴

Dexmedetomidine, a potent and highly selective α_2 adrenoreceptor agonist, is widely used for sedation in intensive care units (ICU).⁵ Dexmedetomidine also offers good perioperative hemodynamic stability and an intraoperative anesthetic-sparing effect.⁶ Thus, dexmedetomidine is used as an anesthetic adjuvant during surgery.⁷ Either *in vivo* or *in vitro*, dexmedetomidine has demonstrated a protective effect

against the I/R injury of heart, kidney, brain, and testis in animal models.^{8–12} Schaak *et al.* reported that the activation of α_2 adrenoreceptor increased intestinal epithelial cell proliferation,¹³ which indicates that α_2 adrenoreceptor agonist might be able to accelerate the wound healing process in the intestine. However, whether or not dexmedetomidine administration can provide protection against intestinal injury induced by intestinal I/R injury remains unclear. Based on the characteristics of dexmedetomidine, we hypothesized that dexmedetomidine could attenuate intestinal mucosal injury and mortality in rats after intestinal I/R.

Therefore, the current experiment was designed to investigate the effects of different doses of dexmedetomidine (given before or after the ischemic phase) on intestinal injury after I/R injury and explore the potential mechanisms involved in the effects of dexmedetomidine.

Materials and Methods

Animals and Operative Procedure

The current study was approved by the Animal Care Committee of Sun Yat-sen University, Guangzhou, China, and was performed in accordance with National Institutes of Health guidelines for the use of experimental animals. Adult pathogen-free male Sprague-Dawley rats weighing between 220 and 270 g were housed in individual cages in a temperature-controlled room with alternating 12-h light–dark cycles, and acclimated for 1 week before the study. Food was removed 8 h before the study, but all animals had free access to water.

All animals were anesthetized with pentobarbital (30 mg/kg body weight, intraperitoneal). Tracheotomy were performed, and 14-gauge intravenous catheters were inserted as tracheotomy tubes. The tubes were connected to small-animal ventilators (DH-150; Medical Instrument Company of Zhe Jiang University, Hangzhou, China). All rats were mechanically ventilated with a standard tidal volume ventilation protocol (tidal volume 10 ml/kg; respiratory rate 50 breaths/min; inspiratory/expiratory ratio of 1:1; 100% oxygen). A polyethylene catheter was inserted into the left carotid artery and connected to a digital data acquisition system (PowerLab/4SP; AD Instruments, Comerio, Italy) for monitoring the heart rate and mean arterial pressure (MAP), as described previously.¹⁴ The femoral vein was cannulated to draw blood samples and for the drug infusion. The small intestine was exteriorized by midline laparotomy, and the intestinal I/R injury was established by occluding the superior mesenteric artery with a microvessel clip for 1 h, followed by 2 h reperfusion, as described previously.^{15,16} During the study period, the rat rectal temperature was maintained at $\sim 36^\circ\text{--}38^\circ\text{C}$ with the aid of a heating pad.

Groups and Drug Administration

As figure 1A shows, animals were randomly allocated into 10 groups ($n = 12$ per group), as follows. Sham group (Sham):

the rats received continuous intravenous infusion of normal saline and sham surgical preparation, including isolation of the superior mesenteric artery without occlusion. Injury group (Injury): the rats received continuous intravenous infusion of normal saline, and intestinal I/R was induced by clamping the superior mesenteric artery for 1 h followed by declamping (reperfusion) for 2 h. Dexmedetomidine 2.5, 5, and 10 administration before ischemia (ABI) groups: intravenous dexmedetomidine (Orion Pharma, Turku, Finland) was infused continuously at 2.5, 5, and 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia was induced. Dexmedetomidine 2.5, 5, and 10 administration after ischemia (AAI) groups: intravenous dexmedetomidine was infused continuously at 2.5, 5, and 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the beginning of reperfusion. The different doses of dexmedetomidine (2.5, 5 and 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) had been used previously to investigate the dose-related effect of dexmedetomidine on mortality and inflammatory responses in endotoxemic rats.¹⁷ In the current study, all drugs were dissolved in normal saline, and all rats received intravenous fluid at a rate of 0.5 ml/h to ensure the same volume of fluid administration (fig. 1).

Experiment 1: To Investigate the Effects of Different Doses of Dexmedetomidine on Hemodynamics and Blood Gas Analysis

The rats' heart rate and MAP values at various time points were recorded and compared among groups. At 2 h after reperfusion, arterial blood (0.25 ml) was drawn into a heparinized syringe for blood gas analysis (G3+ chips, i-STAT; Abbott Laboratories, East Windsor, NJ).

Experiment 2: To Investigate the Effects of Different Doses of Dexmedetomidine on Intestinal I/R Injury

Based on the results of experiment 1, the appropriate doses of dexmedetomidine were chosen to investigate the effect of dexmedetomidine on intestinal injury induced by intestinal I/R.

An additional 2-ml blood sample was drawn 2 h after reperfusion. The whole blood was centrifuged at 3,500 rpm for 15 min at 4°C and plasma collected and stored at -70°C . The rats were then killed with an overdose of pentobarbital sodium. A segment of intestine, 0.5–1.0 cm, was cut from 5 cm to terminal ileum, fixed in formaldehyde polymerization (4%), and embedded in paraffin for preparation. Another segment of small intestine (9–10 cm) was cut from 10 cm to terminal ileum and washed with cold saline. The intestinal mucosa was scraped off gently, dried with suction paper, and preserved at -70°C .^{15,16}

Histologic Measurement of Intestinal Mucosal Injury. The segment of small intestine was stained with hematoxylin-eosin. Damage of intestinal mucosa was evaluated using the criteria of Chiu's method¹⁸ by two independent experienced pathologists who were blinded to the study groups. A minimum of six randomly chosen fields from each rat were eval-

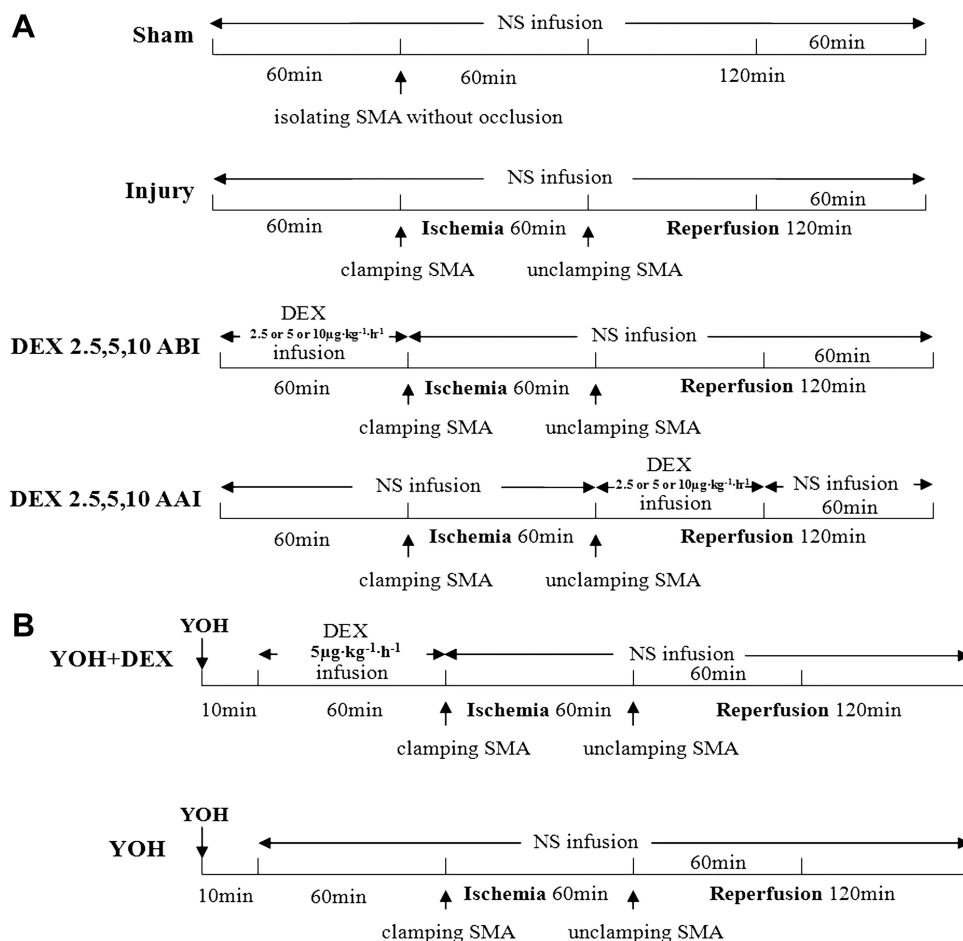


Fig. 1. The protocol of experiments 1, 2, and 3. Sham group: involving isolation of the SMA without occlusion; Injury group: performed by 1 h occlusion of SMA followed by 2 h of reperfusion without any intervention; DEX 2.5 or 5 or 10 ABI group: dexmedetomidine continuous intravenous infusion at 2.5 or 5 or 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for 1 h before ischemia; DEX 2.5 or 5 or 10 AAI group: dexmedetomidine infusion at 2.5 or 5 or 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for 1 h after ischemia (A). The protocol of experiment 4. YOH + DEX group: yohimbine hydrochloride (1 mg/kg) was administered intravenously before dexmedetomidine infusion; YOH group: yohimbine hydrochloride (1 mg/kg) was administered alone as control (B). AAI = administration after ischemia; ABI = administration before ischemia; DEX = dexmedetomidine; NS = normal saline; SMA = superior mesenteric artery; YOH = yohimbine hydrochloride.

uated and averaged to determine mucosal damage, and then the results of two pathologists were averaged.

Detection of Diamine Oxidase Concentration in Serum.

Diamine oxidase is an enzyme synthesized primarily in gastrointestinal mucosal cells. The intestinal tissue and serum concentrations of diamine oxidase have been used as an indicator of the integrity and functional mass of the intestinal mucosa.¹⁹ In this study, serum diamine oxidase was detected using a chemical assay kit (Nanjing Jiancheng Biologic Product, Nanjing, China) with a spectrophotometer according to the provided protocol of the manufacturer. Results were expressed as unit per liter serum.

Survival Analysis. The rats ($n = 15$ per group) receiving the same protocols (fig. 1A) were used to detect survival time. From the onset of reperfusion, animals were monitored *via* video recording for 24 h. Two hours after reperfusion, the survivors were transferred to their individual cages and allowed free access to food and water. Twenty-four hours after

reperfusion, all animals were sacrificed by lethal sodium pentobarbital injection.

Experiment 3: To Investigate the Protective Mechanisms of Dexmedetomidine against Intestinal I/R Injury

Based on the results of experiment 2, the optimal regimen with the most effective dose of dexmedetomidine were chosen to investigate the mechanisms of dexmedetomidine protection against intestinal I/R injury.

Detection of Tumor Necrosis Factor- α (TNF- α) in Serum.

Serum concentrations of TNF- α were determined using commercially available ELISA kits (R&D, Minneapolis, MN) according to the manufacturer's procedure, as described previously.¹⁴ The results were expressed as picograms per liter serum.

Detection of Lactic Acid Concentration in Serum. Serum concentrations of lactic acid were determined using a chemical assay kit (Nanjing Jiancheng Biologic Product) with a

spectrophotometer according to the protocol provided by the manufacturer. Results were expressed as micromoles per liter serum.

Detection of Malondialdehyde Concentration and Myeloperoxidase Activity in Intestinal Mucosa. Intestinal mucosal tissues were homogenized on ice with normal saline, frozen in a refrigerator at -20°C for 5 min and centrifuged for 15 min at 4,000 rpm. Supernatants were transferred into fresh tubes for the evaluation. The lipid peroxidation product malondialdehyde concentration was measured using chemical assay kits (Nanjing Jiancheng Biologic Product). The results were expressed as nanomoles per 100 mg protein.

The myeloperoxidase activity was used as an index to reflect neutrophil migration into the small intestine.²⁰ The intestine assay sample was homogenized and the homogenate was frozen–thawed twice, and then centrifuged at 13,000 rpm for 5 min. The resulting supernatant was assayed spectrophotometrically for myeloperoxidase activity. One unit of myeloperoxidase was defined as that degrading 1 μmol peroxide per minute at 25°C . Results were expressed as unit per gram intestinal tissue.

In Situ Detection of Intestinal Mucosal Epithelial Apoptosis. The ileal fragments were fixed in formaldehyde polymerization (4%) and embedded in paraffin. The apoptosis of intestinal mucosal epithelial cell was detected by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling method, as described previously.¹⁶ Cell death was assessed using an assay kit (Roche, Indianapolis, IN). The cells with clear nuclear labeling were defined as positive cells. The apoptotic index was calculated as: (the number of positive cell nuclei/the number of total cell nuclei) $\times 100$.

Detection of Caspase-3 Expression in Intestinal Mucosa.

To further confirm the existence of apoptosis, caspase-3 was detected *via* western blot. The intestinal mucosal tissues were suspended in RIPA buffer (50 mM Tris, 150 mM NaCl, 1% sodium deoxycholate and 0.05% sodium dodecyl sulfate, pH 7.4), broken into pieces on ice, and subjected to three freeze–thaw cycles. The insoluble cell debris was removed by centrifugation. Supernatants were collected, and the protein concentration was determined using a bicinchoninic acid assay reagent (Pierce Chemical Company, Rockford, IL). Protein samples (30 μg) were subjected to sodium dodecyl sulfate (15%) polyacrylamide gel electrophoresis, then transferred electrophoretically to Hybond ECL Nitrocellulose Membranes (Amersham, Arlington Heights, IL) and incubated with a blocking solution composed of 5% fat dry milk in Tween-containing Tris-buffered saline (pH 8.0, 10 mM Tris, 150 mM NaCl, 0.1% Tween). Membranes were incubated overnight with rabbit monoclonal anticaspase-3 antibody (number 9664; Cell Signaling Technology, Danvers, MA) or rabbit polyclonal antiactin antibody (number 4967; Cell Signaling Technology). After the membranes were washed with Tween-containing Tris-buffered saline three times, they were incubated for 1 h at room temperature with the appropriate horseradish peroxidase-conju-

gated secondary antibody (number 7074; Cell Signaling Technology) directed at the primary antibody. The band density was analyzed densitometrically and normalized with the housekeeping protein β -actin and then presented as percentage of injury.

Experiment 4: To Explore the Role of α_2 Adrenoceptor in the Intestinal Protective Effect of Dexmedetomidine

Based on the results of experiments 2 and 3, the α_2 adrenoceptor antagonist yohimbine hydrochloride (YOH; Sigma Aldrich, St. Louis, MO) was used to examine whether the α_2 -adrenergic receptor is involved in the protective effect of dexmedetomidine. The rats were randomly divided into the YOH + dexmedetomidine and YOH groups ($n = 12$ per

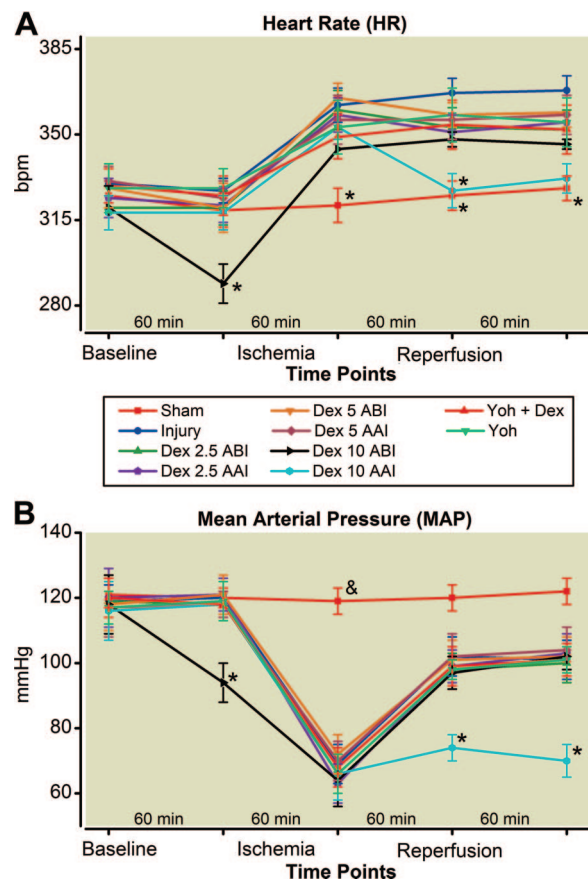


Fig. 2. The effects of dexmedetomidine infusion on heart rate (A) and mean arterial pressure (B) during the study period. Animals underwent various interventions based on the protocol of experiment 1. Data are expressed as mean \pm SE, $n = 4$. Results were compared by two-way ANOVA with repeated-measures followed by Bonferroni posttest. & $P < 0.01$, * $P < 0.05$ versus the Injury group. AAI = administration after ischemia; ABI = administration before ischemia; DEX = dexmedetomidine; DEX 2.5 or 5 or 10 AAI = dexmedetomidine was infused at 2.5, 5, or 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; DEX 2.5 or 5 or 10 ABI = dexmedetomidine was infused at 2.5, 5 or 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; YOH = yohimbine hydrochloride.

Table 1. The Effects of Different Doses of Dexmedetomidine on the Blood Gas Analysis

	pHa	Pao ₂	Paco ₂
Sham	7.34 ± 0.15	522.8 ± 5.6	40.3 ± 1.4
Injury	7.23 ± 0.23	517.5 ± 5.3	38.8 ± 1.1
Dexmedetomidine 2.5 ABI	7.23 ± 0.28	517.8 ± 5.8	39.5 ± 1.5
Dexmedetomidine 2.5 AAI	7.28 ± 0.21	511.8 ± 4.5	40.5 ± 1.5
Dexmedetomidine 5 ABI	7.28 ± 0.18	521.3 ± 5.9	39.8 ± 1.1
Dexmedetomidine 5 AAI	7.24 ± 0.19	512.5 ± 5.3	40.8 ± 1.0
Dexmedetomidine 10 ABI	7.22 ± 0.17	512.3 ± 5.0	40.0 ± 1.2
Dexmedetomidine 10 AAI	7.11 ± 0.19*	516.8 ± 4.9	39.3 ± 0.8
YOH + Dexmedetomidine	7.25 ± 0.08	517.8 ± 3.7	39.8 ± 1.3
YOH	7.25 ± 0.14	520.0 ± 4.5	41.8 ± 1.3

Data are expressed as mean ± SE, n = 4. Results were compared by ANOVA with Tukey posttest.

* $P < 0.01$ vs. the Injury group.

AAI = administration after ischemia; ABI = administration before ischemia; Dexmedetomidine 2.5 or 5 or 10 AAI = dexmedetomidine was infused at 2.5, 5, and 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 hr after the intestinal ischemic insult; Dexmedetomidine 2.5 or 5 or 10 ABI = dexmedetomidine was infused at 2.5, 5, and 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before intestinal ischemia; Paco₂ = arterial carbon dioxide pressure; Pao₂ = arterial oxygen pressure; pHa = arterial pH; YOH = yohimbine hydrochloride.

group). Based on the results of a previous study involving dexmedetomidine and YOH,²¹ YOH (1 mg/kg) was administered intravenously 10 min before dexmedetomidine or normal saline infusion (fig. 1B). Blood samples and intestinal tissues were harvested and preserved according to the methods of experiments 1 and 2.

Statistical Analysis

Statistics were analyzed with SPSS 15.0 software (SPSS Inc, Chicago, IL). Hemodynamic data during the study were expressed as mean ± SE and analyzed using two-way ANOVA with repeated measures, followed by Bonferroni posttest. Survival time from the beginning of reperfusion was expressed as median (range), and results were compared by Kaplan–Meier log-rank test. The mortality rate was analyzed by Fisher exact test. The other data were expressed as mean ± SE, and one-way ANOVA (Tukey posttest) was used for comparisons. Biochemical assays for diamine oxidase, lactic acid, malondialdehyde, and myeloperoxidase were performed in triplicate for each specific sample. Therefore, all data points are means of numbers that themselves are means of triplicate measurements for these parameters. $P < 0.05$ in two-tailed testing was considered statistically significant.

Results

Experiment 1: Effects of Different Doses of Dexmedetomidine on Hemodynamics and Blood Gas Analysis

Changes of Hemodynamic Variables and Blood Gas Parameters. As shown in figure 2, there were no statistical differences in baseline heart rate or MAP among the groups. Dexmedetomidine in dosages of 2.5 and 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the ABI and AAI groups did not affect heart rate and MAP (all $P > 0.05$, *vs.* Injury). Whereas, 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine in the ABI and AAI groups statistically

significantly reduced heart rate and MAP (all $P < 0.05$, *vs.* Injury). As shown in table 1, the arterial Paco₂, and Pao₂ values did not differ among the groups (all $P > 0.05$). The arterial pH value was reduced in the dexmedetomidine 10 AAI group ($P = 0.003$ *vs.* Injury) but was not reduced in other groups (all $P > 0.05$ *vs.* Injury).

Experimental 2: Effects of Different Doses of Dexmedetomidine on Intestinal I/R Injury

Experiment 1 showed that 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine caused severe hemodynamic instability. Thus, the dexmedetomidine 10 regimens were suspended in the following experiments because hypotension and bradycardia induced by this large dose of dexmedetomidine probably interfered with the investigation of the intestinal protective effect of dexmedetomidine.

Intestinal Mucosal Morphologic Changes. Representative mucosal morphologic changes are presented in figure 3, A–H. Detailed descriptions of morphologic changes in different groups are provided in the legend of figure 3. The mucosal injury was quantified as Chiu's score in figure 3I. Chiu's scores in the Injury group were higher than in the Sham group ($P < 0.001$), and were attenuated by 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine in the ABI group ($P < 0.001$ *vs.* Injury) but not by other regimens (all $P > 0.05$ *vs.* Injury).

Changes of Serum Diamine Oxidase Concentration. As shown in figure 4, the diamine oxidase concentration in the Injury group was higher than that in the Sham group ($P < 0.001$), and was reduced in the dexmedetomidine 5 ABI group ($P = 0.005$ *vs.* Injury) but not in the other regimens (all $P > 0.05$ *vs.* Injury).

Survival Analysis. The survival time of the animals in the Injury group was 2.25 h (1.6–4.5 h). Survival time was prolonged in the dexmedetomidine 2.5 and 5 ABI groups, but not in the dexmedetomidine 2.5 and 5 AAI groups [2.5 ABI: 4 h (1.7–24 h), $P = 0.016$; 5 ABI: 4.5 h (2.25–24 h), $P < 0.001$ *versus* Injury]. Mortality rates at 24 h after reper-

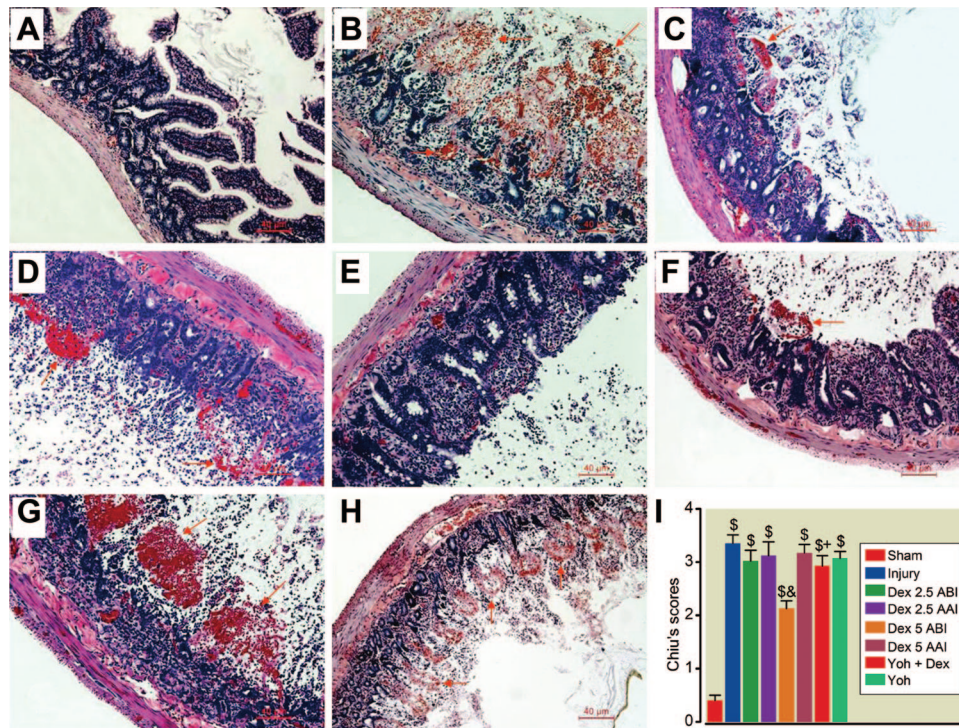


Fig. 3. Morphologic changes of intestinal mucosa and the evaluation of gut injury with Chiu's scores under light microscopy ($\times 200$). Animals underwent various interventions based on the protocol of experiment 2. In the Sham group, the villi and glands were normal (A). By contrast, severe edema of mucosal villi accompanied by severe intestinal gland injury were observed in the Injury group (B). In addition, a large number of intestinal villi disintegrated, the gap of epithelial cells increased, and severe hemorrhage was present, indicative of severe mucosal damage in the Injury group. In the DEX 5 ABI group, only slight mucosal sloughing could be seen at villi tips, and the gap between epithelial cells increased slightly (E). Whereas, in the both DEX 2.5 groups and the DEX 5 AAI group (C, D, F), edema of mucosal villi and denuded villus tips were observed, and a number of capillaries were congested. In addition, hemorrhage could be seen in some specimens (C, D, F), which was comparable with that seen in the Injury group. In the YOH + DEX (G) and YOH (H) groups, the concentration of intestinal mucosal injury was similar to that in the Injury group. The data of Chiu's score are expressed as mean \pm SE, $n = 12$ (I). Results were compared by ANOVA with Tukey posttest. \$ $P < 0.01$ versus the Sham group; & $P < 0.01$ versus the Injury group; + $P < 0.05$ versus the DEX 5 ABI group. AAI = administration after ischemia; ABI = administration before ischemia; DEX = dexmedetomidine; DEX 2.5 or 5 AAI = dexmedetomidine was infused at 2.5 or 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; DEX 2.5 or 5 ABI = dexmedetomidine was infused at 2.5 or 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; YOH = yohimbine hydrochloride.

fusion were 6.7%, 100%, 80%, 100%, 66.7%, and 93.3% for the Sham, Injury, Dexmedetomidine 2.5 ABI and AAI, and Dexmedetomidine 5 ABI and AAI groups, respectively (fig. 5). Only dexmedetomidine 5 in the ABI group statistically significantly reduced mortality ($P = 0.042$ vs. Injury), and mortality rates in other groups were similar to that in the Injury group (all $P > 0.05$).

Experiment 3: The Mechanisms of Dexmedetomidine Conferring Protection against Intestinal I/R Injury

Experiment 2 showed that dexmedetomidine 2.5 in the ABI and AAI groups produced no protective effects on intestinal I/R injury, and dexmedetomidine 5 μg in the ABI, but not the AAI, group exhibited statistical beneficial effect. Thus, in experiment 3, we performed mechanistic investigations to further detect why dexmedetomidine 5 in the ABI group, rather than the AAI group, could confer intestinal protection.

Changes of Intestinal Mucosal Malondialdehyde Concentration. As shown in figure 6, the malondialdehyde concen-

tration in the Injury group was higher than in the Sham group ($P < 0.001$). Dexmedetomidine 5 ABI and AAI did not reduce the malondialdehyde concentration (ABI: $P = 0.511$ and AAI: $P = 0.996$ vs. Injury).

Changes of Intestinal Mucosal Myeloperoxidase Activity.

As shown in figure 7, the activity of myeloperoxidase in the Injury group was higher than in the Sham group ($P < 0.001$). Neither Dexmedetomidine 5 ABI nor AAI reduced myeloperoxidase activity (ABI: $P = 0.839$ and AAI: $P = 0.851$ vs. Injury).

Changes of Serum Lactic Acid Concentration. As shown in figure 8, the concentrations of lactic acid were not statistically significantly different among the groups (all $P > 0.05$).

Changes of Serum TNF- α Concentration. As shown in figure 9, the serum concentration of TNF- α in the Injury group was higher than that in the Sham group ($P < 0.001$), which was statistically significantly reduced by dexmedetomidine 5 ABI ($P < 0.001$ vs. Injury) but not by AAI ($P = 0.269$ vs. Injury).

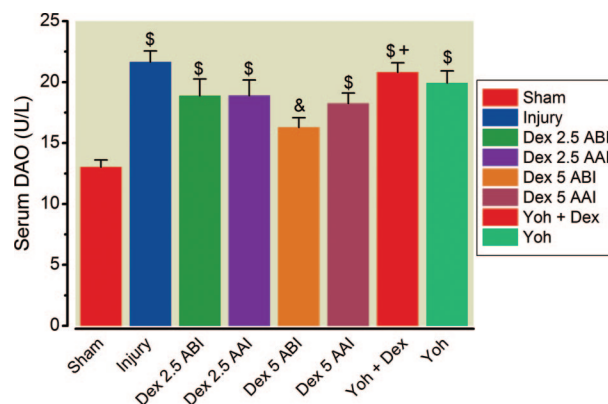


Fig. 4. The changes of diamine oxidase concentration in serum. Animals underwent various interventions based on the protocol of experiment 2. Data are expressed as mean \pm SE, $n = 12$. Results were compared by ANOVA with Tukey posttest. \$ $P < 0.01$ versus the Sham group; & $P < 0.01$ versus the Injury group; + $P < 0.05$ versus the DEX 5 ABI group. DEX 2.5 or 5 ABI = dexmedetomidine was infused at 2.5 or 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; DEX 2.5 or 5 AAI = dexmedetomidine was infused at 2.5 or 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult. DAO = diamine oxidase; DEX = dexmedetomidine; ABI = administration before ischemia; AAI = administration after ischemia; YOH = yohimbine hydrochloride.

Changes of Intestinal Mucosal Epithelial Apoptosis. The terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling positive epithelial cells at the villus surface stained dark brown in the nuclei (fig. 10). Detailed descriptions of mucosal epithelial apoptosis in different groups are shown in the legend of figure 10. The apoptotic index was statistically significantly higher in the Injury group than in the Sham group

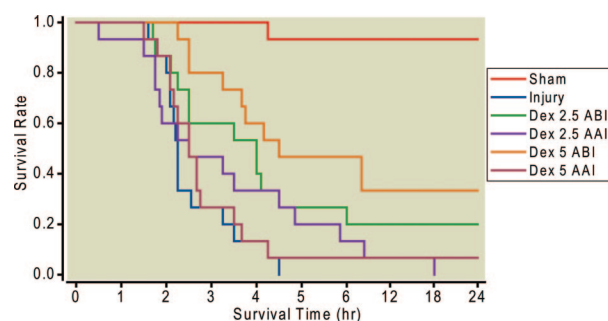


Fig. 5. Effects of dexmedetomidine on survival. Animals underwent various interventions based on the protocol of experiment 2. Survival time is calculated from the beginning of reperfusion. Results were compared by Kaplan-Meier log-rank test and Fisher exact test, $n = 15$. Survival times in the DEX 2.5 and 5 ABI groups were significantly longer than those in the Injury group, whereas only the DEX 5 ABI regimen statistically significantly attenuated the 24-h mortality rate. AAI = administration after ischemia; ABI = administration before ischemia; DEX = dexmedetomidine; DEX 2.5 or 5 AAI = dexmedetomidine was infused at 2.5 or 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; DEX 2.5 or 5 ABI = dexmedetomidine was infused at 2.5 or 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia.

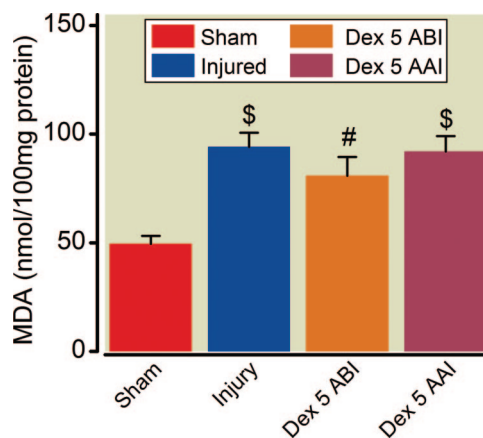


Fig. 6. The changes of malondialdehyde concentration in intestinal mucosa. Animals underwent various interventions based on the protocols of experiments 2 and 3. Data are expressed as mean \pm SE, $n = 12$. Results were compared by ANOVA with Tukey posttest. \$ $P < 0.01$, # $P < 0.05$ versus the Sham group. ABI = administration before ischemia; AAI = administration after ischemia; DEX = dexmedetomidine; DEX 5 AAI = dexmedetomidine was infused at 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; DEX 5 ABI = dexmedetomidine was infused at 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; MDA = malondialdehyde.

($P < 0.001$, fig. 10G). As shown in figure 10G, dexmedetomidine 5 ABI reduced the apoptotic index ($P = 0.018$ vs. Injury). However, dexmedetomidine 5 AAI did not reduce the apoptotic index ($P = 0.900$ vs. Injury).

Changes of Caspase-3 Expression of Intestinal Mucosa.

Representative bands from the western blotting are presented in figure 11A. As shown in figure 11B, cleaved caspase-3

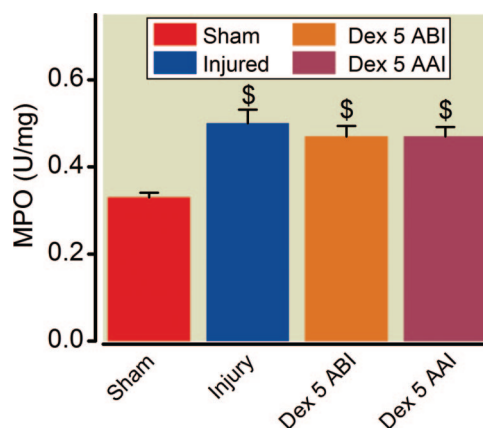


Fig. 7. The changes of myeloperoxidase activity in intestinal mucosa. Animals underwent various interventions based on the experimental protocol 2 and 3. Data are expressed as mean \pm SE, $n = 12$. Results were compared by ANOVA with Tukey posttest. \$ $P < 0.01$ versus the Sham group. DEX 5 ABI = dexmedetomidine was infused at 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; DEX 5 AAI = dexmedetomidine was infused at 5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; MPO = myeloperoxidase; DEX = dexmedetomidine; ABI = administration before ischemia; AAI = administration after ischemia.

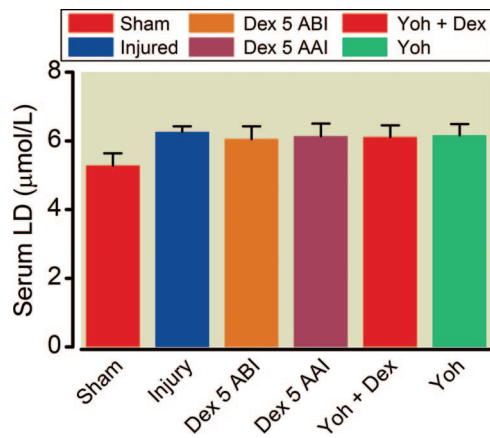


Fig. 8. The changes of lactic acid concentration in serum. Animals underwent various interventions based on the experimental protocol 2 and 3. Data are expressed as mean \pm SE, $n = 12$. Results were compared by ANOVA with Tukey posttest. DEX 5 ABI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; DEX 5 AAI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult. LD = lactic acid; DEX = dexmedetomidine; ABI = administration before ischemia; AAI = administration after ischemia; YOH = yohimbine hydrochloride.

expression of intestinal mucosa in the Injury group was higher than that in the Sham group ($P < 0.001$). Dexmedetomidine 5 ABI, but not AAI, reduced caspase-3 expression (ABI: $P = 0.002$ and AAI: $P = 0.545$ vs. Injury).

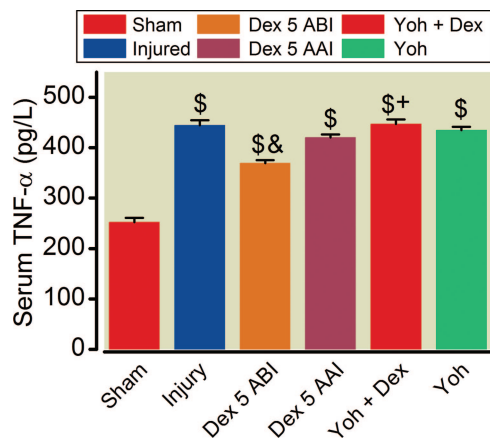


Fig. 9. The changes of tumor necrosis factor- α (TNF- α) concentration in serum. Animals underwent various interventions based on the protocols of experiments 2 and 3. Data are expressed as mean \pm SE, $n = 12$. Results were compared by ANOVA with Tukey posttest. \$ $P < 0.01$ versus the Sham group; & $P < 0.01$ versus the Injury group; + $P < 0.05$ versus the DEX 5 ABI group. AAI = administration after ischemia; ABI = administration before ischemia; DEX = dexmedetomidine; DEX 5 AAI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; DEX 5 ABI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; YOH = yohimbine hydrochloride.

Experiment 4: Effects of α_2 Adrenoceptor Antagonists

As shown in figures 3I, 4, 9, 10G, and 11B, Chiu's score, serum diamine oxidase activity, TNF- α concentrations, and apoptotic index and caspase-3 expression in the YOH + Dexmedetomidine group were statistically significantly higher than that in the dexmedetomidine 5 ABI group (Chiu's score: $P = 0.034$; diamine oxidase activity: $P = 0.033$; TNF- α : $P < 0.001$; apoptotic index: $P = 0.017$; and caspase-3: $P = 0.048$) and similar to that in the Injury group (all $P > 0.05$), suggesting that the YOH pretreatment abolished the protective effects of dexmedetomidine 5 ABI on intestinal I/R injury. On the other hand, YOH alone (YOH group) produced no effects on the above variables in comparison with the Injury group (all $P > 0.05$).

Discussion

Intestinal I/R injury is associated with some critical clinical phenomena, such as severe infection, shock, and some major surgery, including cardiopulmonary bypass, small intestine transplantation, and abdominal aortic artery surgery, which are commonly seen in the operating room and ICU. Dexmedetomidine is often used as a sedative and anesthetic adjuvant for such patients because of its anesthetic-sparing effect and properties of antiinflammation and cardiovascular protection.^{22,23} Thus, we sought to determine whether dexmedetomidine protects against intestinal I/R injury and to find its appropriate dose and administration regimen.

To determine the appropriate dose of dexmedetomidine for intestinal protection, low, median, and large (2.5 , 5 , and $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) doses of dexmedetomidine were used in the current study. Our data showed that a low dose of dexmedetomidine did not confer intestinal protection and a large dose led to severe hemodynamic instability, especially hypotension. The current data about hemodynamics and serum lactic acid concentration indicated that the median dose of dexmedetomidine ($5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) did not contribute to tissue hypoperfusion in the current model. In addition, this dose had been used previously to decrease inflammatory responses and mortality in septic rats.^{24,25} In recent years, the body surface area normalization method was recommended by the Food and Drug Administration for the more appropriate conversion of drug doses from animal studies to human studies.²⁶ Using this method, the dose of $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine used in rats is equal to approximately $0.8 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in humans, which has been demonstrated to be safely applied in cardiovascular surgery and the ICU.^{27–29} The human equivalent dose of $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine far exceeds the clinically licensed dose of $1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, which may cause frequent bradycardia and hypotension in general anesthesia.⁶ This information indicates $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine is the optimal dose in the current study.

We found for the first time that intestinal injury and rat mortality after intestinal I/R were reduced by dexmedetomidine 5 ABI but not by 5 AAI. This finding seems to be similar

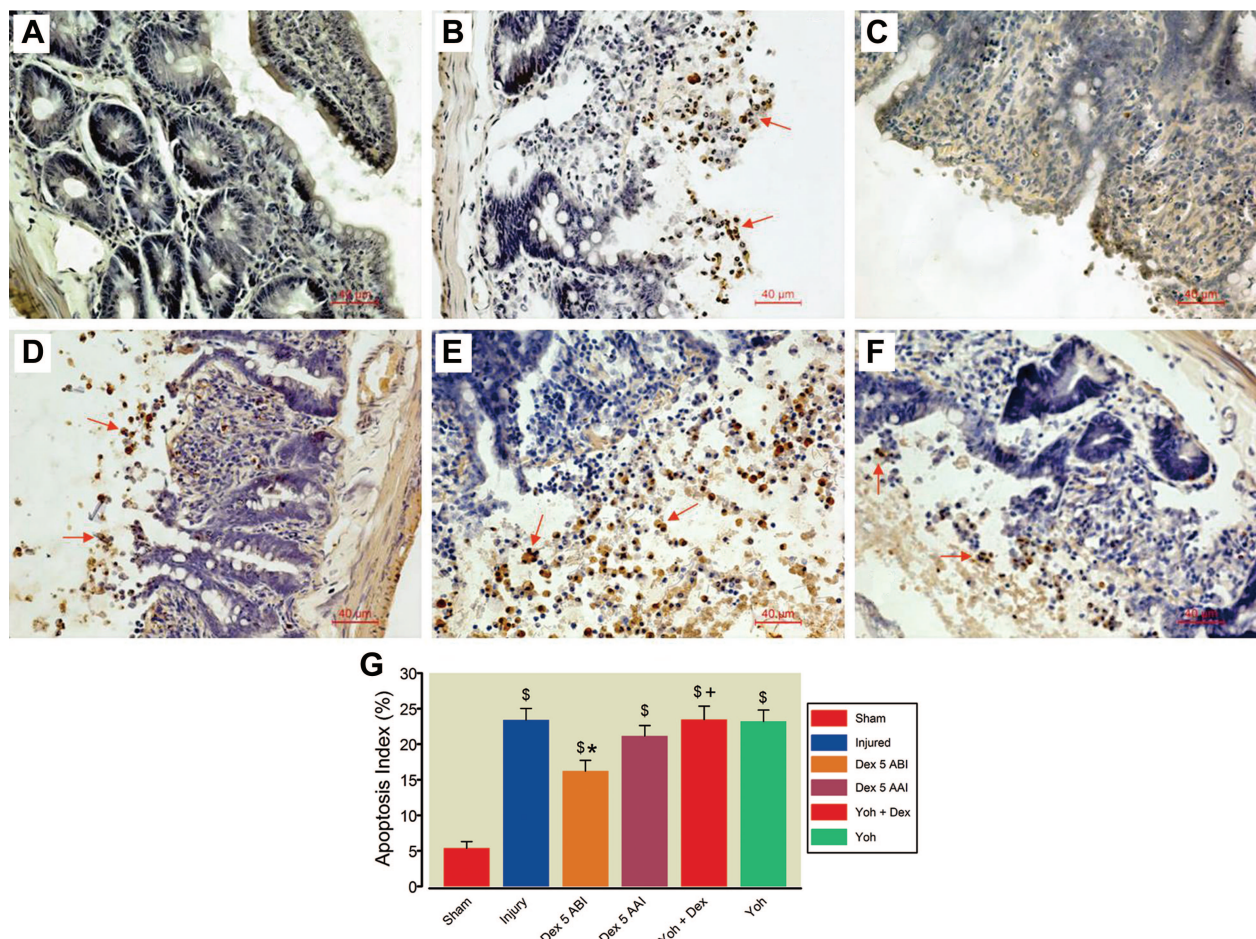


Fig. 10. Effects of dexmedetomidine on intestinal mucosal cell apoptosis by light microscopy of the ileum stained using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling assay ($\times 400$). Animals underwent various interventions based on the protocols of experiments 2 and 3. Apoptotic nuclei are stained dark brown (arrows). In the Sham group, few apoptotic epithelial cells were present at the villous (A). In the Injury group, notable destruction of the villi was evident, and enterocytes with dark brown nuclei were observed in the upper regions of the villi. Many detached apoptotic enterocytes were seen in the enteric cavity (B). In the DEX 5 ABI group, the appearance of villi was normal, and there were fewer apoptotic cells than in the Injury group (C). In the DEX 5 AAI group, the upper regions of the villi were destroyed and a few detached apoptotic epithelial cells were seen (D). In both the YOH + DEX and YOH groups, the numbers of apoptotic epithelial cells were similar to that seen in the Injury group (E, F). The data of the apoptosis index are expressed as mean \pm SE, $n = 12$. Results were compared by ANOVA with Tukey posttest (G). \$ $P < 0.01$ versus the Sham group; * $P < 0.01$ versus the Injury group; + $P < 0.05$ versus the DEX 5 ABI group. AAI = administration after ischemia; ABI = administration before ischemia; DEX = dexmedetomidine; DEX 5 AAI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; DEX 5 ABI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; YOH = yohimbine hydrochloride.

to previous investigations involved in the effects of dexmedetomidine on myocardial I/R injury in which dexmedetomidine administration before ischemia significantly reduced the infarct size in an isolated rat heart model,⁸ whereas dexmedetomidine administration at the initiation of reperfusion with the same dose in the same experimental model increased the myocardial infarct size.³⁰ Our previous studies demonstrated the very early moments of reperfusion (less than 3 min) are critical to intestinal protection in the same model as the current study.^{14,31} These findings all suggested that, although the exact mechanisms are not clear, the timing of performing treatment is of utmost important for conferring

protection against I/R injury. In the current study, the dexmedetomidine 5 AAI regimen did not exhibit intestinal protective effect despite immediate infusion at the beginning of reperfusion. We speculated that this ineffectiveness might be attributed to the slow onset of dexmedetomidine, which reaches its effect approximately 15 min after intravenous administration.³² To our knowledge, the timing of administration of dexmedetomidine is quite different and empirically decided by physicians in clinical settings.²³ Although additional research for investigating the optimal timing of dexmedetomidine administration is mandatory, the current results at least indicate that early intervention of

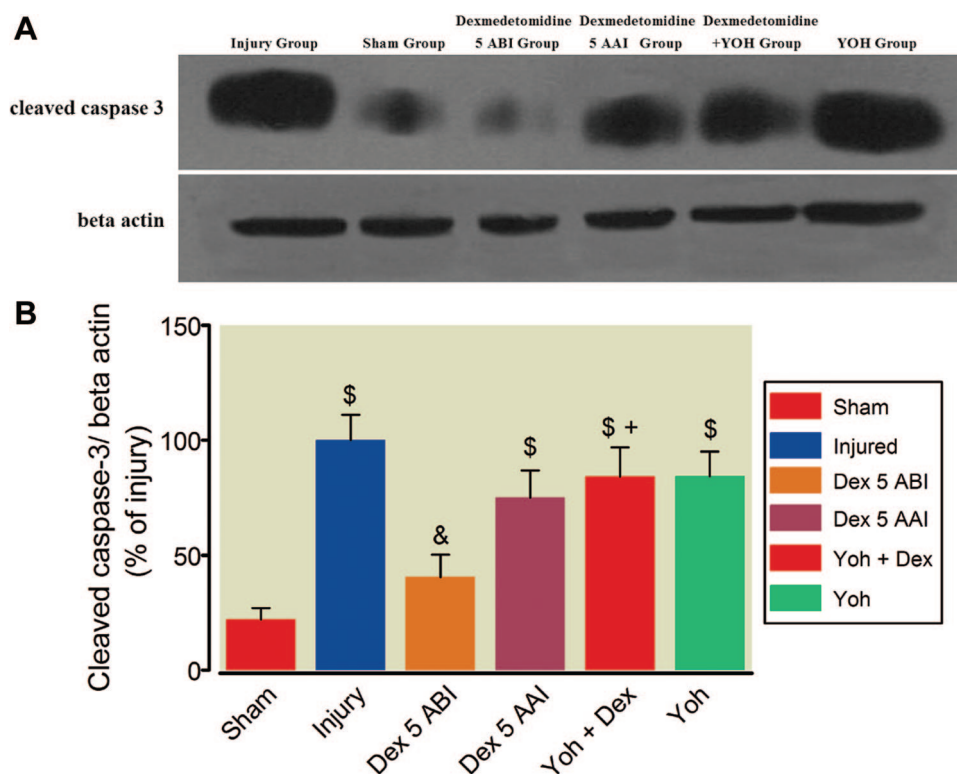


Fig. 11. The changes of caspase-3 expression in intestinal mucosal epithelial cells. Representative bands of each group (A). Densitometry analysis from the western blotting assay showing quantitative changes in caspase-3 concentrations (B). Animals underwent various interventions based on the protocols of experiments 2 and 3. Data were expressed as mean \pm SE, $n = 12$. Results were compared by ANOVA with Tukey posttest. \$ $P < 0.01$ versus the Sham group; & $P < 0.01$ versus the Injury group; + $P < 0.05$ versus the DEX 5 ABI group. AAI = administration after ischemia; ABI = administration before ischemia; DEX = dexmedetomidine; DEX 5 AAI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h after the intestinal ischemic insult; DEX 5 ABI = dexmedetomidine was infused at $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 1 h before the intestinal ischemia; YOH = yohimbine hydrochloride.

dexmedetomidine is critical for its intestinal protection. It may help clinicians to develop a good perioperative administration regimen of dexmedetomidine. In the majority of clinical conditions involving intestinal I/R, the ischemic insult occurs before patient admission to the ICU. Therefore, dexmedetomidine should be used as an anesthetic adjuvant and administered before surgery for patients with potential intestinal ischemia. The initiation of administration of dexmedetomidine after the operation or in the ICU may produce no beneficial effect.

We previously demonstrated that inflammatory response, oxidative injury, and neutrophil infiltration were involved in the pathogenesis of intestinal I/R injury.^{14,16} The current data show that the dexmedetomidine 5 ABI regimen reduced serum TNF- α concentration, suggesting that dexmedetomidine could confer its intestinal protection by inhibiting inflammatory response. Compared with $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine, $2.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ dexmedetomidine failed to attenuate the increases of TNF- α and interleukin-6 in endotoxin-exposed rats,¹⁷ which might be an important reason the low dose of dexmedetomidine did not produce beneficial effects in the current study. However, the current results showed that dexmedetomidine did not reduce intes-

tinal mucosal malondialdehyde concentration or myeloperoxidase activity after intestinal I/R. That finding is not in accordance with previous reports, in which dexmedetomidine showed a superior ability in inhibiting neutrophil infiltration^{25,33} and lipid peroxidation.³⁴ We speculate that two factors could be involved in this finding. First, the median dose of dexmedetomidine was not adequate for suppressing oxidative injury and the neutrophil infiltration induced by severe intestinal I/R in the current model. Second, oxidative stress and neutrophil accumulation could be not involved in the protection of dexmedetomidine against intestinal I/R injury.

Intestinal mucosal epithelial cells are the main component of the intestinal mucosal barrier. Apoptosis is a major mode of cell death in the destruction of rat intestinal epithelial cells induced by ischemia and I/R injury.^{35,36} Several reports have demonstrated that prophylactic antiapoptotic treatment is an effective therapeutic strategy for the prevention of intestinal I/R injury.^{16,37} In the current study, we for the first time showed that dexmedetomidine could attenuate intestinal mucosal epithelial cell apoptosis, as evidenced by decreases in the apoptotic index, caspase-3 protein expression, intestinal injury, and consequently rat mortality. The

antiapoptotic effect of dexmedetomidine might be associated with reducing the production of TNF- α and thereby inhibiting the activation of extrinsic apoptotic cascade.³⁸ It has also been reported that dexmedetomidine could reduce the proapoptotic protein Bax expression and increase the antiapoptotic protein Bcl-2 expression,¹⁰ thereby attenuating apoptosis by inhibiting the activation of intrinsic apoptotic cascade. In addition, previous reports, *in vivo* or *in vitro*, suggest the antiapoptotic effect of dexmedetomidine on axoneuron is related to the α_2 -adrenoceptor.^{39,40} Therefore, in the current study, YOH was applied to further explore whether the α_2 -adrenoceptor is involved in the protective effect of dexmedetomidine. The results show that YOH totally abolished the intestinal protective effect of dexmedetomidine, accompanied by the disappearance of its antiinflammatory and antiapoptotic effects. This is a novel finding, indicating that apoptosis and inflammation mediated by the activation of α_2 -adrenoceptor play an important role in the intestinal protection conferred by dexmedetomidine.

There were several possible limitations in this study. We used only three different doses to investigate the dose-effect relationship of the intestinal protection conferred by dexmedetomidine. The results showed that the large dose of dexmedetomidine severely affected hemodynamics and thereby was removed from the dose-effect relationship study, and the median dose of dexmedetomidine was superior to the low dose in intestinal protection. Nevertheless, more doses should be used to further investigate the dose-effect relationship in the future. In addition, α_2 -adrenoceptor is composed of three subtypes: α_{2A} , α_{2B} , α_{2C} . YOH, a nonspecific α_2 -adrenoceptor antagonist, can not provide an exact profile of which subtype plays the key role in the intestinal protection of dexmedetomidine. Nevertheless, based on the results of Ma *et al.*,⁴⁰ we concluded dexmedetomidine produced its intestinal protective effect mainly *via* the α_{2A} -adrenoceptor subtype.

In summary, this study reports for the first time that dexmedetomidine administration before ischemia, but not after ischemia, dose-dependently attenuates intestinal injury induced by intestinal I/R, in part by suppressing the inflammatory response and intestinal mucosal epithelial apoptosis *via* the activation of the α_2 -adrenoceptor. Large doses of dexmedetomidine may contribute to severe hemodynamic inhibition and are not suitable for clinical application. Our results provide a new insight for the clinical use of dexmedetomidine, showing that an appropriate dose of dexmedetomidine can be initiated before ischemic insult occurs in the critical clinical settings involving intestinal I/R to produce effective intestinal protection.

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