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DEXMEDETOMIDINE PREVENTS PDIA3 DECREASE BY ACTIVATING α2-ADRENERGIC RECEPTOR TO ALLEVIATE INTESTINAL I/R IN MICE

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ABSTRACT—**Background:** Dexmedetomidine (DEX) attenuates intestinal I/R injury, but its mechanism of action remains to be further elucidated. Protein disulfide isomerase A3 (PDIA3) has been reported as a therapeutic protein for the prevention and treatment of intestinal I/R injury. This study was to investigate whether PDIA3 is involved in intestinal protection of DEX and explore the underlying mechanisms. **Methods:** The potential involvement of PDIA3 in DEX attenuation of intestinal I/R injury was tested in PDIA3 Flox/Flox mice and PDIA3 conditional knockout (cKO) in intestinal epithelium mice subjected to 45 min of superior mesenteric artery occlusion followed by 4 h of reperfusion. Furthermore, the α2-adrenergic receptor (α2-AR) antagonist, yohimbine, was administered in wild-type C57BL/6N mice intestinal I/R model to investigate the role of α2-AR in the intestinal protection conferred by DEX. **Results:** In the present study, we identified intestinal I/R-induced obvious inflammation, endoplasmic reticulum (ER) stress-dependent apoptosis, and oxidative stress, and all the aforementioned changes were improved by the administration of DEX. PDIA3 cKO in the intestinal epithelium have reversed the protective effects of DEX. Moreover, yohimbine also reversed the intestinal protection of DEX and downregulated the messenger RNA and protein levels of PDIA3. **Conclusion:** DEX prevents PDIA3 decrease by activating α2-AR to inhibit intestinal I/R-induced inflammation, ER stress-dependent apoptosis, and oxidative stress in mice.

KEYWORDS—Dexmedetomidine, I/R, PDIA3, inflammation, endoplasmic reticulum stress, oxidative stress

INTRODUCTION

Intestinal I/R injury is a common life-threatening complication seen in critically ill patients after major surgery, shock, multiple trauma, or sepsis (1). The mortality associated with intestinal I/R injury remains unchanged, exceedingly high up to greater than 90% (2,3). Although great efforts have been made, intestinal I/R injury is still need effective treatment and prevention.

Dexmedetomidine (DEX), an α2-adrenergic receptor (α2-AR) agonist, has sedative, anxiolytic, sympatholytic, and opioid-sparing effects and is applied commonly in a clinical setting (4). It is known to influence inflammation, oxidative damage, endoplasmic reticulum (ER) stress, apoptosis, autophagy, and so on, in several diseases (5–8). The role of DEX in the treatment of intestinal I/R injury has been highlighted in recent studies (5–8). Moreover, it has been reported that activation of α2-AR by DEX could protect the intestine against intestinal I/R injury (9,10). Nevertheless, the exact mechanism remains unclear.

In our previous study (11), protein disulfide isomerase A3 (PDIA3), a classic member of the PDI family, was significantly reduced after intestinal I/R injury while upregulated by ischemic preconditioning with intestinal protection. These results suggest that PDIA3 may be a key protein against intestinal I/R injury. Our another further study revealed that PDIA3 attenuated intestinal I/R injury via inhibiting ER stress-dependent apoptosis and oxidative stress (12). In addition, Tat-PDIA3 is a PDIA3 fusion protein that is used to deliver the protein without any cytotoxicity. It was determined to protect ischemic damage in the spinal cord by reducing immunoreactive microglia and myeloperoxidase (MPO) activity in serum (13), suggesting that PDIA3 played a role in anti-inflammation. However, whether PDIA3 is involved in DEX against intestinal I/R injury remains to be unmasked.

Previously, a study of Prokosch et al. (14) provided evidence that brimonidine, another α2-AR agonist, activates the α2-AR and subsequently regulates the protein PDIA3 expression in the retina, suggesting that ligands binding to α2-AR may modulate the PDIA3 expression. However, it is needed to be confirmed in further research.

As mentioned previously, we hypothesized that DEX exerted its intestinal protective effect through preventing PDIA3 decrease by α2-AR activation to inhibit inflammation, ER stress-dependent apoptosis, and oxidative stress.

MATERIALS AND METHODS

Animals and operative procedures

It has been reported that estrogen has protective effects on intestinal I/R injury in animal studies (15). Therefore, to avoid the effect of hormones on the results, all animal experiments were implemented using 8 to 12-week-old C57BL/6N male mice weighing 20 to 30 g provided by the Experimental Animal Center of Sun Yat-sen University (China). This experimentation was approved by the Animal Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and conformed to the Declaration of Helsinki. After 12-h fasting with free access to water before the experiment, all mice were anesthetized by 5% sodium pentobarbital intraperitoneal injection (30 mg/kg) to perform subsequent operations on a 37°C constant temperature plate until righting reflex disappeared. Using the mice model of intestinal I/R injury, which gives satisfactory anesthesia and abdominal skin

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preparation, a midline laparotomy incision was made aseptically and protected by covering with a sterile saline-moistened gauze. The gut was moved away from the abdominal cavity on the gauze gently with a sterile cotton swab and then exposed the superior mesenteric artery (SMA) close to the right renal hilum. Further dissected carefully from the mesentery, the SMA was clamped using noninvasive microvascular clips. As a result of SMA clipping, ischemia was confirmed by immediate pulsation disappearance and gradual discoloration of the small intestine. After 45 min of intestinal ischemia, the microvascular clips were removed to restore the intestinal blood supply. The renewed pulsation and the natural color recovery of the intestine verified the successful reperfusion. Finally, the gut was placed back within the abdominal cavity gently, and the abdominal incision was sutured layer by layer. Then, the mice had free access to water and chow. After 45 min of ischemia followed by 4 h of reperfusion periods, the mice were euthanized.

Drug administration and groups

DEX, 2.5 μg/mL, was dissolved in normal saline (NS) and yohimbine (Yoh), 1 μg/μL, an α2-AR antagonist, was dissolved in dimethyl sulfoxide (DMSO). All drugs mentioned previously were injected via intraperitoneal injections (i.p.).

Experiment A

Experiment A was conducted to determine the effect of PDIA3 on the intestinal protective effects of DEX. PDIA3 Flox/Flox mice were assigned to F-Sham, F-IR, and F-DEX groups randomly (n = 5 per group), and PDIA3 intestinal conditional knockout (cKO) mice were randomly assigned to cKO-Sham, cKO-IR, and cKO-DEX groups (n = 5 per group) as shown hereinafter (Fig. 1A):

1. F-Sham: 10 mL/kg NS was intraperitoneally injected separately 30 min before sham operation.
2. F-IR: 10 mL/kg NS was intraperitoneally injected separately 30 min before I/R surgery;
3. F-DEX: 25 μg/kg (16) DEX was intraperitoneally injected separately 30 min before I/R surgery;
4. cKO-Sham: 10 mL/kg NS was intraperitoneally injected separately 30 min before sham operation;
5. cKO-IR: 10 mL/kg NS was intraperitoneally injected separately 30 min before I/R surgery; and
6. cKO-DEX: 25 μg/kg DEX was intraperitoneally administered separately 30 min before I/R surgery.

Experiment B

Experiment B was conducted to determine the role of α2-AR in the intestinal protective effects of DEX.

Wild mice were divided into five groups randomly (n = 8 per group) as shown hereinafter (Fig. 1B):

1. Sham: DMSO (1 mL/kg, i.p., 1 h before sham operation) + NS (10 mL/kg, i.p., 30 min before sham operation) + sham operation;
2. IR: DMSO (1 mL/kg, i.p., 1 h before I/R surgery) + NS (10 mL/kg, i.p., 30 min before I/R surgery) + I/R surgery;
3. DEX + IR: DMSO (0.5 mL/kg, i.p., 1 h before I/R surgery) + DEX (25 μg/kg, i.p., 30 min before I/R surgery) + I/R surgery;
4. Yoh + IR: Yoh (1 mg/kg (17), i.p., 1 h before I/R surgery) + NS (10 mL/kg, i.p., 30 min before I/R surgery) + I/R surgery; and
5. Yoh + DEX + IR: Yoh (1 mg/kg, i.p., 1 h before I/R surgery) + DEX (25 μg/kg, i.p., 30 min before I/R surgery) + I/R surgery.

Animal sample collection

Blood was collected by eyeball enucleation at the end of reperfusion period. After centrifugation at 3,500 rpm for 15 min in 4°C, serum was harvested for subsequent biochemical detection. Meanwhile, a distal 10-cm ileum from the ileal-cecal junction was obtained and divided into two segments. The first 1-cm part of the intestine close to the ileal-cecal junction areas was fixed with 4% paraformaldehyde and paraffin-embedded for histological analysis and apoptosis assay of intestinal epithelial cells (IECs). The remaining 9-cm part of the intestine was rinsed with cold NS and saved at −80°C after drying with filter paper and scraping off the mucus layer gently.

Histological assessments of intestinal injury

The intestinal tissue was acquired and fixed in 4% paraformaldehyde. Then, the fixed samples were dehydrated by alcohol, paraffin-embedded, and sectioned successively. Nuclei and cytoplasm were stained with hematoxylin and eosin (H&E), respectively. Finally, the pathological alterations were observed and calculated by two researchers without group information under a microscope (Olympus, Tokyo, Japan).

Detection of malondialdehyde, superoxidase dismutase, and MPO levels in the intestinal mucosa

Intestinal mucosa was homogenized and lysed using the cell lysate (P0013; Biotechnology Co., Ltd., Shanghai, China). The proportion of tissue weight to homogenate or lysate was 10%. The lysate was centrifuged at 10,000 rpm for 10 min, and the supernatant was collected for subsequent determination.

Malondialdehyde (MDA) enzyme-linked immunosorbent assay (ELISA) kits (Enzyme-Linked Biotechnology Co., Ltd., Shanghai, China) was used to detect MDA, a product of lipid peroxidation. A commercial activity superoxidase

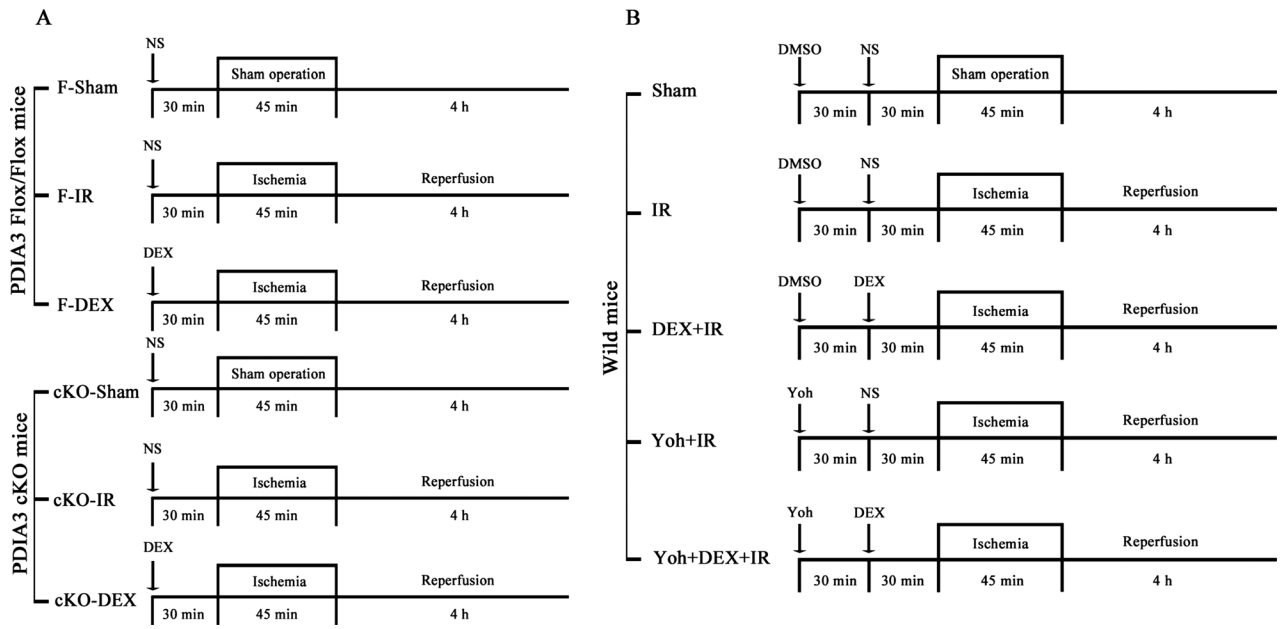

FIG. 1. **The schematic diagram of protocols of *in vivo* experiment.** (A) Experiment 1. (B) Experiment 2. DEX indicates dexmedetomidine; DMSO, Dimethyl sulfoxide; NS, normal saline; Yoh, yohimbine.

dismutase (SOD) kits (Enzyme-Linked Biotechnology Co., Ltd.) was used to measure SOD through suppressing nitroblue tetrazolium reduction by inhibiting xanthine/xanthine oxidase system to produce superoxide anions. MPO ELISA kits (Enzyme-Linked Biotechnology Co., Ltd.) was used to detect MPO, released mainly by activated neutrophils. All ELISA kits were used in accordance with the manufacturer's instructions.

Intestinal fatty acid–binding protein and detection of diamine oxidase in the serum

To evaluate intestinal injury, the markers of circulating intestinal fatty acid–binding protein (i-FABP) and diamine oxidase (DAO) were detected by ELISA kits (Enzyme-Linked Biotechnology Co., Ltd.) according to the instructions of the manufacturer.

Terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling staining

Terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling (TUNEL) staining was carried out with *In Situ* Cell Death Detection Kit (No. 12156792910; Roche, Basel, Switzerland) according to the manufacturer's instruction. Zeiss Axioscan.Z1 digital slide scanner (Carl Zeiss, Oberkochen, Germany) was used to capture images of slides. The numbers of positive cells were measured using ImageJ software (National Institutes of Health, Bethesda, MD).

Quantitative reverse transcription-polymerase chain reaction analysis

RNA was extracted from intestinal tissues with total RNA extraction kit (LS1040; Promega, Madison, WI) according to the manufacturer's instruction. Quantitative reverse transcription-polymerase chain reaction analysis was performed with SYBR qPCR master mix (Q711-02; Vazyme, Nanjing, China) and CFX96 Touch real-time system (Bio-Rad, Hercules, CA). Relative quantification of genes was analyzed using $2^{-\Delta\Delta CT}$ method. The primer sequences are listed in Table 1. β-Actin was set as the endogenous standard that displayed gene expression as the fold change increased.

Western blot analysis

Proteins were extracted from intestinal mucosal tissues using cell lysis buffer (P0013; Beyotime, Shanghai, China), and the protein concentration was assessed with BCA Protein Assay Kit (P0011; Beyotime). Then proteins were run on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and transferred to Polyvinylidene Fluoride membranes (IPVH00010; Merck Millipore, Billerica, MA). Membranes were blocked with 5% milk at room temperature for 1 h. Then membranes were incubated with primary antibodies at 4°C overnight. Primary antibodies included anti-PDIA3 antibody (1:1,000, ab13506; Abcam, Cambridge, MA), anti-Bip antibody (1:1,000, 3177S; CST, Cambridge, MA), anti–caspase 12 antibody (1:1,000, sc-21,747; Santa Cruz, Dallas, TX), anti-Chop antibody (1:1,000, 2895S; CST), anticleaved caspase 3 antibody (1:1,000, 9664S, CST), and anti–β-actin antibody (1:5000, 66,009-1-Ig; Proteintech, Rosemont, IL). After incubating membranes with secondary antibodies at room temperature for 1 h, membranes were incubated with Immobilon Western chemiluminescent horseradish peroxidase substrate (WBKLS0500; Merck Millipore). Blot signals were detected with ImageQuant LAS 500 imager (General Electric, Boston, MA) and analyzed with ImageJ software (National Institutes of Health, Bethesda, MD).

Immunohistochemistry

After fixing with 4% paraformaldehyde and paraffin embedding, the tissue was cut into 5-μm-thick sections and was used for immunohistochemistry (IHC). The sections were heated at 60°C for 60 min; washed in xylene two to three times, 15 min each; and then hydrated in 100%, 95%, 70%, and 50% ethanol for 5 min, respectively. Next, phosphate-buffered saline (PBS) was used to wash the sections three times, and sodium citrate buffer was used for section heat-mediated antigen retrieval. Once incubating in 3% H_2O_2 for 10 min finished, the sections were then washed three times in PBS. After blocking with 10% serum for 20 min at room temperature, the sections were washed with PBS for 2 min. Subsequently, the sections were incubated with anti-PDIA3 antibody (ab13506; Abcam) or anti-Ly6G antibody (ab238132; Abcam) at 4°C for 12 to 16 h. Then, the second antibody (AlexaFluor; Invitrogen, Carlsbad, CA) was used for treating the sections according to the first incubated antibodies. Finally, the sections were observed under a microscope (Olympus), and the integrated optical density value, representing the staining intensity, was calculated by Image Pro Plus 6.0 (Media Cybernetics, Silver Springs, MD).

Statistical analysis

GraphPad Prism 9.0 software (La Jolla, CA) was used to analyze the experimental data, which were showed as mean ± standard deviation. One-way ANOVA was used to determine differences among multiple groups. Tukey post hoc test were used for multiple comparisons of groups. $P < 0.05$ was considered to be statistically significant.

RESULTS

Protective effects of DEX against intestinal I/R injury regulated by PDIA3 in IECs in mice

The H&E staining results of intestinal tissues (Fig. 2A) showed that normal intestinal villi and glands were revealed in the F-Sham group and cKO-Sham group. In contrast, the F-IR group, cKO-IR group, and cKO-DEX group have seen severe intestinal tissue damage accompanied by some visible bleeding and several inflammatory cell infiltrations. In the F-DEX group, intestinal mucosa was partially damaged, inflammatory cells were mild infiltrated, and villi were slightly damaged.

In Figure 2B, Chiu's score was used to quantify the mucosal injury. DAO (Fig. 2C) and i-FABP (Fig. 2D) abundance in serum were measured to further assess small intestinal damage. Chiu's scores and DAO and i-FABP levels in serum were found notably higher in the F-IR group and cKO-IR group than those in the F-Sham group and cKO-Sham group (F-IR vs. F-Sham and cKO-IR vs. cKO-Sham, $P < 0.05$). DEX ameliorated the aforementioned changes, and intestinal PDIA3 cKO reversed the intestinal protective roles of DEX, implying that DEX plays a crucial role in protection of intestinal I/R injury via preventing PDIA3 decrease.

DEX inhibited intestinal I/R-induced inflammation, ER stress-dependent apoptosis, and oxidative stress by preventing PDIA3 decrease in IECs

The results of IHC staining of Ly6G (Fig. 3A) and changes of MPO levels in the intestine (Fig. 3B) showed that I/R in mice significantly recruited neutrophils in the intestine. Moreover, the

TABLE 1. **Primers used for RT-QPCR**

Gene	Forward primer sequence	Reverse primer sequence
β-actin	AGCCATGTACGTAGCCATCC	GCTGTGGTGGTGAAGCTGTA
IL-1β	TGCCACCTTTTGACAGTGATGAG	GCTCTTGTTGATGTGCTGCT
TNF-α	CCCTCACACTCACAAACCAC	ATAGCAAATCGGCTGACGGT
PDIA3	AGAGGCTTGCCCCTGAGTAT	GCTGACAATTCCATCAGCAGT
Bip	TGTCGCCCTCAGACCAGAA	GAACACACCGACGCAGGAATA
Caspase 12	GACATGCTGGATGGGGTTTT	CCTCTACTTTTCTCTTGGATTCTGA
Chop	GCAGCGACAGAGCCAGAATA	ATGTGCGTGTGACCTCTGTT

RT-QPCR, quantitative reverse transcription-polymerase chain reaction.

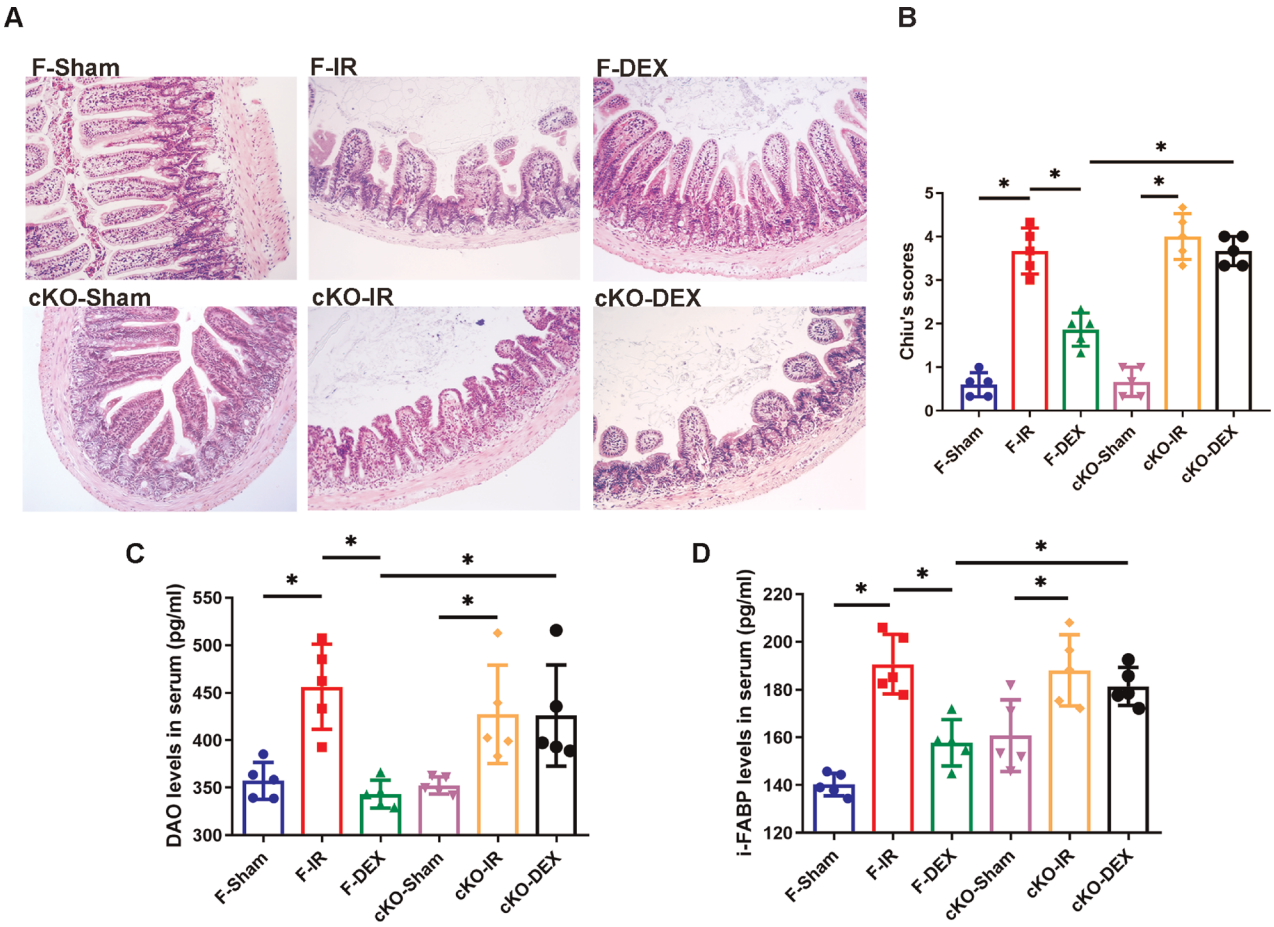

FIG. 2. **Protective effects of DEX on intestinal I/R injury regulated by PDIA3 in IECs in mice.** (A) Histological analysis (H&E staining, original magnification ×200) and (B) Chiu's scores of the intestine (n = 5). (C) Levels of DAO activity (n = 5), and (D) i-FABP in serum (n = 5). Data are expressed as mean ± SD. *P < 0.05. DAO indicates diamine oxidase; DEX, dexmedetomidine; IECs, intestinal epithelial cells; H&E, hematoxylin and eosin; i-FABP, intestinal fatty acid–binding protein; PDIA3, protein disulfide isomerase A3; SD, standard deviation.

expressions of Bip, caspase 12, Chop, and cleaved caspase 3 of the intestinal samples (Fig. 3, C–H), reflecting ER stress-dependent apoptosis, were examined. Bip, caspase 12, Chop, and cleaved caspase 3 levels were significantly higher in the F-IR group than that in the F-Sham group. These increases were significantly attenuated in the F-DEX group. These results suggested that DEX inhibited ER stress-dependent apoptosis induced by intestinal I/R. To further explore whether the effects of DEX were related to PDIA3 of the intestine, PDIA3 cKO mice were applied. Compared with the cKO-Sham group, the protein expressions of Bip, caspase 12, Chop, and cleaved caspase 3 were significantly higher in the cKO-IR group. In addition, these protein expressions were significantly higher in the cKO-DEX group than those in the F-DEX group. These results suggested that PDIA3 cKO in the intestine abolished the inhibition effect of DEX on ER stress-dependent apoptosis. TUNEL staining (Fig. 3I) was performed and apoptosis index (Fig. 3J) was calculated to further evaluate apoptosis in the intestine. Intestinal I/R exposure significantly increased the aforementioned apoptosis index. In addition, intestinal I/R injury led to an increase in MDA (Fig. 3K) and a decrease in SOD (Fig. 3L) in the intestinal mucosa. Pretreatment with DEX improved the aforementioned changes. The effect of DEX was eliminated completely by intestinal PDIA3 cKO. Overall, those findings revealed that DEX inhibited intestinal I/R-induced inflammation, ER stress-dependent apoptosis, and oxidative stress by attenuating PDIA3 decrease in IECs.

DEX prevented PDIA3 decrease via activating α2-AR to alleviate intestinal I/R injury

To further determine the potential protective mechanism of DEX on α2-AR, we next blocked the α2-AR by Yoh, an α2-AR antagonist. First, remarkable intestinal histological damages (Fig. 4, A and B) and messenger RNA (mRNA) expression upregulation of inflammatory cytokines including IL-1β (Fig. 4C) and TNF-α (Fig. 4D) were found after intestine I/R injury, which were alleviated by DEX. In addition, Yoh abolished the aforementioned effects of DEX, whereas Yoh alone did not alter intestinal I/R-induced morphological change or inflammation. These results implied that pretreatment of DEX played a protective role on intestinal injury and inflammation through activating α2-AR.

Furthermore, pretreatment with DEX prevented intestinal I/R-induced ER stress, as evidence by reducing the mRNA and protein expressions of Bip, Chop, and caspase 12 (Fig. 4, F, G, I, and K–M). DEX also attenuated intestinal I/R-induced increase in cleaved caspase 3 (Fig. 4N) and apoptosis index (Fig. 4P), reflecting the cell apoptosis. The administration of Yoh reversed these effects of DEX, whereas Yoh alone did not influence the expression of these proteins and mRNA and subsequent ER stress-dependent apoptosis.

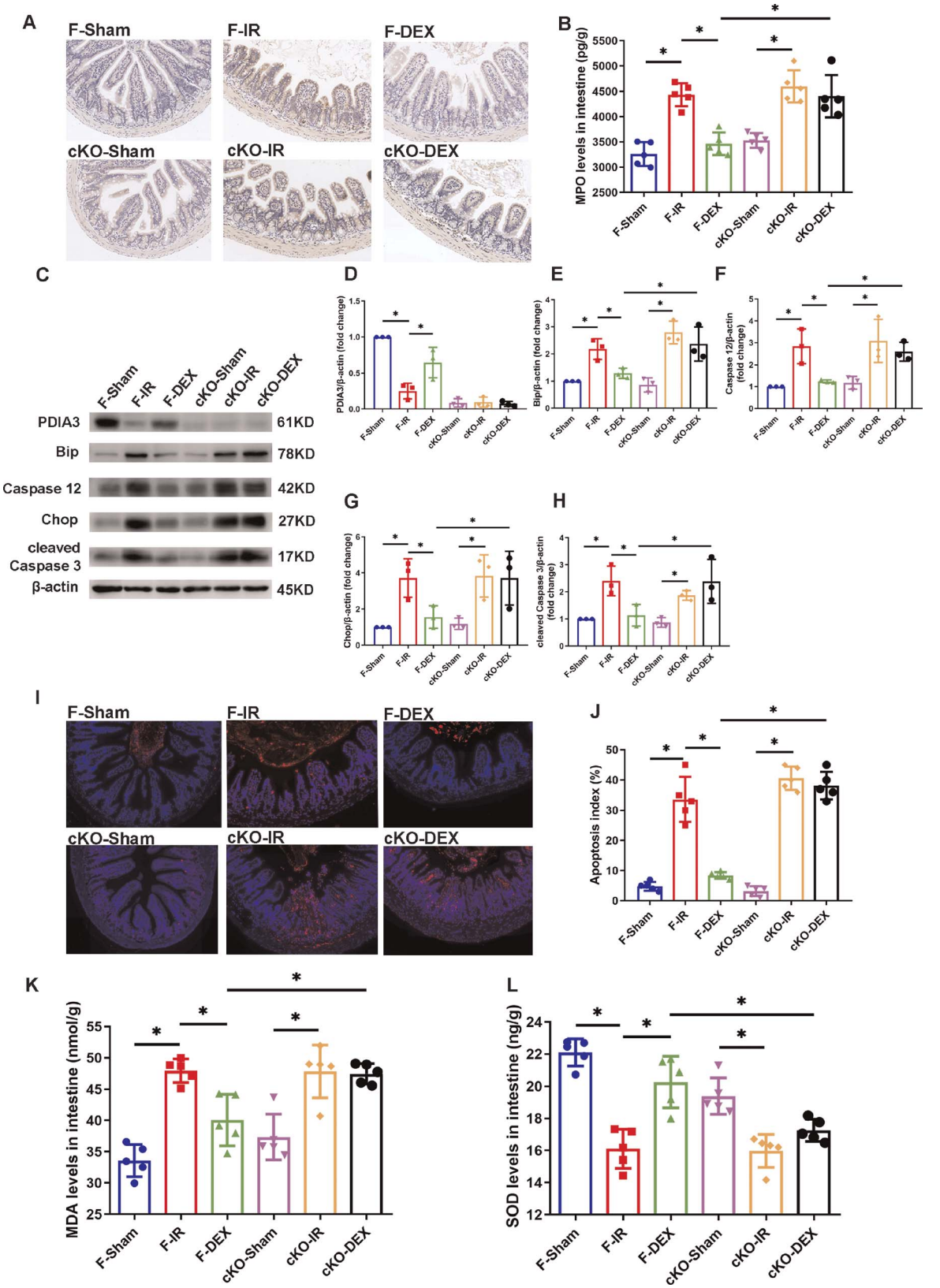

FIG. 3. **DEX preconditioning inhibited intestinal I/R-induced inflammation, ER stress-dependent apoptosis, and oxidative stress by preventing PDIA3 decrease in IECs.** (A) Immunohistochemical staining showing Ly6G localization in the intestine. (B) Levels of MPO in the intestine (n = 5). (C) Representative Western blot bands showing PDIA3, Bip, caspase 12, Chop, cleaved caspase 3, and β-actin expression in the intestinal mucosa. (D–H) Quantitative changes in aforementioned protein concentrations were shown by densitometry analysis from the Western blotting assay (n = 3). Levels of intestinal mucosa (K) MDA (n = 5) and SOD (L) (n = 5). (I and J) Number of TUNEL-positive cells (red) and DAPI (blue) in intestinal tissue (original magnification ×200; n = 5). Data are expressed as mean ± SD. **P* < 0.05. DEX indicates dexmedetomidine; ER, endoplasmic reticulum; IECs, intestinal epithelial cells; MDA, malondialdehyde; MPO, myeloperoxidase; PDIA3, protein disulfide isomerase A3; SD, standard deviation; SOD, superoxide dismutase; TUNEL, Terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling.

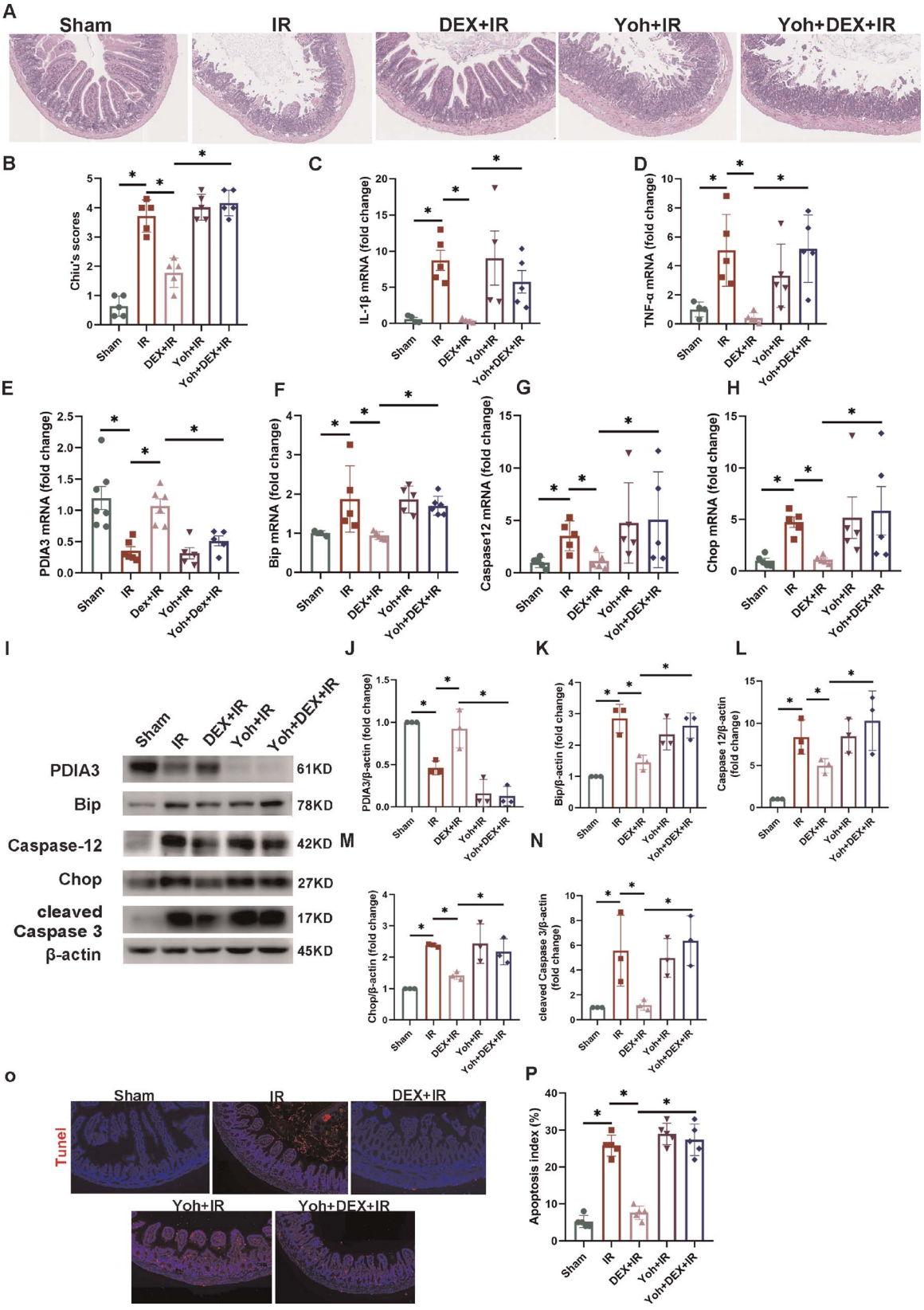

FIG. 4. **DEX preconditioning prevented PDIA3 decrease via activating α2-AR to alleviate intestinal I/R-induced inflammation and ER stress-dependent apoptosis.** (A) Histological analysis (H&E staining, original magnification ×200) and (B) Chiu's scores of the intestine (n = 5). (C and D) mRNA levels of IL-1β and TNF-α in the intestine were measured to reveal intestinal inflammation (n = 5). (E–H) mRNA levels of PDIA3, Bip, caspase 12, and Chop in the intestine were measured (n = 5). (I) Western blot bands showing PDIA3, Bip, caspase 12, Chop, cleaved caspase 3, and β-actin expression in the intestinal mucosa. (J–N) Quantitative changes in aforementioned protein concentrations were shown by densitometry analysis from the Western blotting assay (n = 3). (O and P) Number of TUNEL-positive cells in (red) and DAPI (blue) in intestinal tissue (original magnification ×200; n = 5). Data are expressed as mean ± SD. *$P < 0.05$. α2-AR indicates α2-adrenergic receptor; DEX, dexmedetomidine; ER, endoplasmic reticulum; H&E, hematoxylin and eosin; mRNA, messenger RNA; PDIA3, protein disulfide isomerase A3; SD, standard deviation; TUNEL, Terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling.

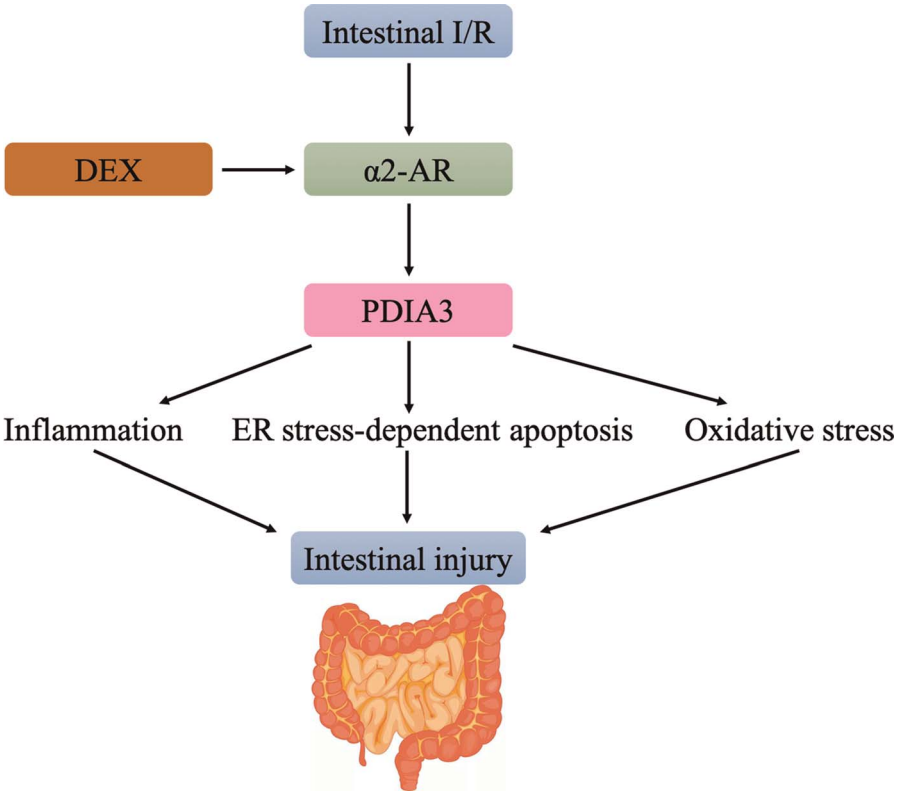

FIG. 5. **Proposed mechanisms of protective effect of DEX via preventing PDIA3 decrease in intestinal I/R injury**. α2-AR indicates α2-adrenergic receptor; DEX, dexmedetomidine; PDIA3, protein disulfide isomerase A3.

As showed in Figure 4, E, I, and J, the mRNA and protein levels of PDIA3 were markedly reduced after intestinal I/R injury, and after administration of DEX, PDIA3 decrease was prevented. Moreover, the pretreatment of Yoh abolished the effect of DEX on PDIA3, indicating that DEX prevented PDIA3 decrease via activating α2-AR.

DISCUSSION

The current study showed that intestinal I/R induced distinct intestinal injury, as supported by an increase in serum DAO and i-FABP levels and intestinal histological structure damages. Pretreatment with DEX (25 μg/kg) effectively improved the aforementioned changes via preventing PDIA3 decrease, which induced an inhibition of inflammation, ER stress-dependent apoptosis, and oxidative stress. Moreover, the α2-AR inhibitor Yoh abolished the intestinal protective effect of DEX (25 μg/kg) and was accompanied by the disappearance of its effect of preventing PDIA3 decrease (Fig. 5).

DEX has been reported to be a potential drug for the prevention and treatment of intestinal I/R injury (7,9,16,18,19). Our results elaborated that the protection from DEX in mice with intestinal I/R injury evidenced with an inhibition of inflammation, ER stress-dependent apoptosis, and oxidative stress in the intestinal mucosa. Consistently, Zhang et al. (9,16) proved that DEX could protect against intestinal I/R-induced intestinal injury by suppressing apoptosis and inflammatory response of intestinal mucosal epithelial, preserving gut-vascular barrier impairment and subsequent liver damage. The other study indicated that pretreatment with DEX markedly attenuate the intestinal I/R injury, probably by enhancing mitophagy and reducing apoptosis of enteric glial cells (7). Moreover, DEX could inhibit hypoxia/reoxygenation-induced IEC ER stress, apoptosis, and pyroptosis via activating SIRT1 expression (20).

DEX is a highly selective α2-AR agonist with rapid onset and short duration of action (21). Recent studies (22,23) demonstrated that the α2-AR played a critical role in organ I/R injury. Moreover, suverys found that DEX alleviated intestinal injury (9) and even remote organ injury (10,24) after intestinal I/R via activating α2-AR, but how it does so remains unclear. In the current study, Yoh, the α2-AR inhibitor, eliminated the intestinal protective effect of DEX, suggesting that DEX protected the intestine against intestinal I/R injury though activating the α2-AR.

PDIA3, which contributes to cells avoiding proteasome dysfunction or prolonged unfolded protein response, could result in apoptosis if the large number of abnormal proteins persists (25). PDIA3 has been identified as a possible new pharmacological target and also suggested to be a biomarker for a large amount of conditions (26–28). Several studies have reported that PDIA3 is involved in I/R-associated diseases. One study showed that PDIA3-dependent activation of NADPH oxidase attributed to its protection of hepatic I/R injury (29). Moreover, PDIA3 has also been reported to be correlated with I/R injury of the hippocampus (30) and spinal cord (13). However, the role of PDIA3 in intestinal I/R injury is still unclear. In the present study, PDIA3 was notably declined after intestinal I/R injury but ascended by DEX. Consistently, several studies have elucidated that PDIA3 is a therapeutic protein for the protective effect and treatment of I/R injury. It was reported that PDIA3 acted as a neuroprotective

agent against ischemia injury by impairing oxidative damage and ER stress-dependent apoptosis (29,30). Another study provided evidence that exogenous PDIA3 protein could modulate the oxidative/antioxidative balance and protect spinal cord neurons from ischemic damage (13). In our previous study, remifentanil was found to promote PDIA3 expression by means of an activation of p38MAPK-associated signal pathway to reduce intestinal I/R-induced ER stress and oxidative stress (12). Nevertheless, the role of PDIA3 in different diseases remains controversial. A traumatic brain injury (TBI) animal model showed that PDIA3 deficiency reduced inflammation of brain tissues after the TBI model. Moreover, in oxygen glucose deprivation and reoxygenation-treated astrocytes, PDIA3 knockout notably attenuated inflammation, oxidative stress, and apoptosis of astrocytes (31). The other study found that siRNA-mediated knockdown of PDIA3 in HL7702 cells and rats could inhibit reactive oxygen species production and NADPH oxidase activity and alleviate transplanted liver injury (29).

Interestingly, in the study by Prokosch et al. (14), brimonidine tartrate, an α2-AR agonist, was used to treat retinal explants from rats and found to significantly decrease the retinal protein expression of PDIA3. In contrast, our results showed that activation α2-AR by DEX prevented PDIA3 decrease in IECs, as evidenced by downregulation of PDIA3 after α2-AR inhibitor, Yoh, administration. We speculate that the relationship between α2-AR and PDIA3 is different, even opposite in different tissues, organs, or diseases.

Several limitations of our study should be noted. First, the current data suggested that PDIA3 in the IECs played a crucial role in intestinal I/R progression and intestinal protective effect by DEX, and activation of α2-AR by DEX was involved in preventing PDIA3 decrease. Further studies are requisite to determine the exact mechanism of DEX-attenuating PDIA3 decrease. Second, PDIA3 cKO mice were applied in the current study, whereas protein overexpression technique is still needed to confirm the intestinal protective effect of PDIA3. Moreover, *in vitro* experiments still need to be performed so as to confirm the exact mechanism of DEX against intestinal I/R injury. Finally, whether intestinal protection of DEX is dose dependent or not remains to be further explored.

In summary, we describe that DEX prevents PDIA3 decrease in IECs by activating α2-AR to attenuate intestinal I/R injury through suppressing inflammation, ER stress-dependent apoptosis, and oxidative stress.

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