

Dexmedetomidine Protects against Hepatic Ischemia-Reperfusion Injury by inhibiting Endoplasmic Reticulum Stress and Cell Apoptosis in Rats

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Research Article

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Abstract

Background: Hepatic ischemia-reperfusion injury (IRI) remains a major complication of liver surgery, dexmedetomidine (DEX) has a certain protective effect on liver during ischemia-reperfusion, but the underlying mechanisms are not fully understood. This study explored the protective effects of DEX and investigated whether DEX protects against hepatic IRI by inhibiting endoplasmic reticulum stress (ERS) and its downstream apoptotic pathway in a rat model.

Methods: Thirty-six male Sprague-Dawley (SD) rats were divided into six groups: S, IR, DL, DM1, DH and DM2 group. Group S was subjected to laparotomy, and exposure of the portal triad without occlusion. I-R injury model was induced by clamping the portal vessels supplying the middle and left hepatic lobes for 30 min in IR, DL, DM1, DH and DM2 group. Then DL, DM1, DH group received DEX of 25 µg/kg, 50 µg/kg and 100 µg/kg intraperitoneally at 30 min before ischemia, respectively, DM2 group received 50 µg/kg DEX intraperitoneally 30 min after reperfusion, and IR group received normal saline. After 6 h of reperfusion, assessment of liver function, histopathology, oxidative stress was performed. The liver cell microstructure was detected by transmission electron microscopy. Hepatocyte apoptosis was determined by TUNEL assay. Real-time PCR, Western blotting were performed to analyze various ERS molecules.

Results: We observed that DEX protected the liver by alleviating hepatocytes damage, reducing the content of ALT and MDA, increasing the activity of SOD, reducing the number of TUNEL-positive cells, down-regulating the expression of GRP-78, PERK, ATF-6, Caspase-12 mRNA, and p-PERK, p-IRE-1 α, CHOP proteins, up-regulating Bcl-2 protein. The effect of 50 µg/kg DEX is superior to 25 µg/kg DEX, but not significantly different from 100µg/kg DEX. There was no significant difference in the above monitoring indexes between DM1 and DM2 group.

Conclusions: DEX protects the liver from IRI by inhibiting ERS and cell apoptosis. The protective effect of DEX was dose-dependent in a certain dose range, both DEX administered prior to ischemia and following reperfusion markedly reduced liver injury induced by hepatic IRI in mice.

Background

Ischemia reperfusion injury (IRI) takes place when blood provision to an organ is diminished or interrupted, the restoration of blood reperfusion will aggravate the damage of the physiological function, structure, and metabolism of the organs. Hepatic IRI remain a major complication of severe liver trauma, hemorrhagic shock, liver transplantation and partial hepatectomy ^[1,2]. Especially with the improvement of surgical technology and equipment, more and more liver operations are carried out. However, hepatic IRI remain one of the main factors that deteriorate morbidity and mortality of the liver surgery ^[3]. Hepatic IRI has become a major obstacle in the development of liver surgery. Therefore, to find effective measures to prevent and avoid hepatic IRI have become one of major clinical problems.

Apoptosis, a physiological mechanism of organisms, plays an extremely important role in maintaining the stability of internal environments. During hepatic IRI for the first 24 hours after reperfusion, apoptosis

is one of the main hepatocytes' death modes ^[4]. Thus, we speculated that regulation of key apoptosis-related proteins expression is an important protective mechanism for the attenuation of hepatic IRI. So far, the apoptotic pathway is known to include the death receptor-mediated pathway, the mitochondrial pathway, and the endoplasmic reticulum stress (ERS) pathway.

Endoplasmic reticulum (ER) is an important membranous organelle which is responsible for protein synthesis, folding and secretion. Previous studies have demonstrated that ERS is involved in the pathogenesis of many diseases, including liver diseases ^[5, 6]. Hepatic IRI induces ischemia, hypoxia, Ca²⁺ homeostasis, oxidative stress, and elevates protein secretion, which triggers the disruption of ER internal balance ^[7]. The disturbances of ER function cause ERS, which subsequently leads to the unfolded protein response (UPR). The UPR initially activates three major signal transducers, protein kinase R-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). However, excessive or prolonged ERS leads to the expression of apoptotic signaling pathways. Activation of C/EBP homologous protein (CHOP) is the main pathway for apoptosis in ERS downstream ^[8]. During this process, caspase-12 and JNK are also activated and involved in ERS cell apoptosis pathway ^[9]. Literature show that hepatic IRI is closely related to ERS and apoptosis, many studies have confirmed inhibiting the ERS response pathways can effectively alleviate hepatic IRI^[10-12]. Thus, strategies targeting ERS may have potential protective roles in hepatic IR injury.

Among the therapeutic intervention strategies, pharmacologic strategies have been demonstrated to play an important role in protecting livers against IR injury ^[13]. Dexmedetomidine (DEX) is a selective and potent α_2 adrenergic receptor agonist with a good sedative effect and no respiratory depression, and is mainly used in clinical anesthesia and ICU sedation. Recently, it has been reported that DEX has other possible applications ^[14]. At the same time, there is a large literature showing that DEX exerts various pharmacological effects, such as anti-inflammation ^[15], anti-oxidant ^[16], and anti-apoptosis ^[17]. Accumulating evidence suggested that DEX has a protective effect on ischemia-reperfusion injury of the heart, kidney, and other organs ^[8, 18]. However, its specific mechanism has not been clarified. DEX shows different functions in the protective effect of hepatic IRI. Zong et al. have confirmed that DEX pre-treatment can suppress oxidative stress, decrease NLRC5 expression through inactivating NF- κ B pathway ^[16]. It has also been reported that DEX preconditioning inhibited intrahepatic proinflammatory innate immune activation by promoting macrophage M2 activation in a PPAR γ /STAT3 dependent manner ^[19]. In addition, DEX has been shown to protect the liver against IR injury via the suppression of the TLR4/NF- κ B pathway ^[20]. However, the contribution of ERS-induced apoptosis remains unknown.

Therefore, this study is aimed to explore the protective effects of DEX and investigate whether DEX protects against hepatic IRI by inhibiting ERS in a rat model.

Methods

Animals and Hepatic I-R Model

Thirty-six male SD rats were obtained from the Animal Center of Hebei medical University (Shijiazhang, China). Rats weighed 180-220g and were raised under a steady temperature around 20°C with 12 h light–dark cycles for one week to adapt to the environment. The experimental protocol was approved by the Agricultural University of Hebei Ethical Committee, Baoding, China.

70% Hepatic I-R model was performed according to previous studies^[21]. An atraumatic clip was applied to the portal vessels to induce ischemia of the middle and left hepatic lobes under **isoflurane** anesthesia. The ischemia was confirmed by tissue blanching. After 30 min ischemia, the clamp was removed for reperfusion. The reperfusion was confirmed by immediate color change of the ischemic lobes after removal of the clamp.

Grouping and treatment

The rats were randomly divided into six groups (n=6) as follows:

1. Group S: the rats were subjected to laparotomy, and exposure of the portal triad without occlusion.
2. Group IR: the rats were subjected to laparotomy, and exposure of the portal triad with occlusion, and no drug was utilized.
3. Group DL: the rats received 20 µg/kg DEX intraperitoneal injection 30 min before ischemia of the hepatic lobes.
4. Group DM1: the rats received 50 µg/kg DEX intraperitoneal injection 30 min before ischemia of the hepatic lobes.
5. Group DH: the rats received 100 µg/kg DEX intraperitoneal injection 30 min before ischemia of the hepatic lobes.
6. Group DM2: the rats received 50 µg/kg DEX intraperitoneal injection 30 min after reperfusion.

Blood and tissue sample collection

After 6 h reperfusion, all rats were sacrificed to collect blood samples and parts of the IR liver (middle and left lobes). Blood samples were centrifuged at 3000 rpm for 10 min within 1 h after collection and the serum was stored at -80°C until use. The liver tissues were rapidly separated, washed with 0.01M phosphate-buffered saline, and placed in an ice tray. The middle liver lobe was placed in 10% formalin solution and embedded in paraffin to observe pathological changes. The left lobe was stored at -80°C for further analysis.

Biochemical Analysis

The serum sample was measured to determine the levels of alanine aminotransferase (ALT) using a UniCel DxC800 Synchron chemistry system (Beckman, USA). According to the instructions of the corresponding assay kit (Nanjing Jiancheng Bioengineering Institute, China), liver tissue was prepared into a homogenate to detect concentrations of malondialdehyde (MDA) and the activity of the antioxidant enzyme superoxide dismutase (SOD).

Histopathological Analysis

After fixation with 10% formalin solution, liver tissue samples were embedded in paraffin, sectioned to 5 μm thicknesses, and stained with hematoxylin and eosin (H&E). Histological changes were observed under a light microscope. Histopathologists with no prior knowledge of the experiment evaluated the sections at 200x magnification. Liver injury was scored on a scale of 0 to 4 for sinusoidal congestion, vacuolization of hepatocyte cytoplasm, and parenchyma, as described by Suzuki et al.^[22].

Transmission Electron Microscopy

The liver samples of each group were pre-fixed in glutaraldehyde (2.5%), washed with PBS and then fixed with 1% osmic acid for 2 h. After dehydration in gradient ethanol, the fixed samples were embedded in epoxy resin, cut into ultrathin sections (50 nm) and stained with uranyl acetate and lead citrate. The stained sections were observed using a transmission electron microscope (TEM; H-7650, Hitachi, Japan).

TUNEL Assay

Hepatocellular apoptosis was detected with a TUNEL apoptosis assay kit (Roche, Switzerland). All procedures were performed as described in the assay kit. Ten microscopic fields within the view were randomly selected, and the TUNEL-positive cells were counted. Liver apoptosis rate was evaluated using the average number of positive cells division by the average number of total cells.

Real-time PCR analysis

The total RNA isolated from liver samples using TRIzol reagent was reverse transcribed to obtain cDNA. The primers were synthesized by Takara Biomedical Technology Co., Ltd. (Dalian, China). The primer sequences are listed in Table 1. Real-time qPCR was performed by using FastStart Universal SYBR Green Master (Rox) (Roche, USA). In this experiment, the response system of 20 μL was used and GAPDH was used as the internal reference for relative quantitative analysis of gene mRNA expression level. Relative quantification was performed according to $2^{-\Delta\Delta\text{Ct}}$ method.

Western Blot Analysis

The expression of *p-PERK*, *p-IRE-1 α* , *CHOP*, *Bcl-2* proteins were detected via Western blot. The liver tissues were homogenized on ice, diluted with 10 volumes of natural saline, and then centrifuged at 2500 rpm for 10 min. The supernatants were transferred into fresh tubes for biochemical analysis. Nuclear and cytoplasmic proteins were extracted by using nuclear and cytoplasmic extraction reagents according to the manufacturer's procedure (Solarbio, China), and then separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes. Blocking by 10% skimmed milk for 2 h, and then the membranes were incubated at 4°C overnight with rabbit anti-CHOP (1:1000; CST, USA), rabbit anti-Bcl-2 (1:1,000; CST, USA), rabbit anti-p-PERK (1:500; Bioss, China), and rabbit anti-p-IRE-1 α (1:500; Bioss, China), and β -actin (1:500; Bioss, China). After cleaning with TBST buffer, the membranes were incubated with secondary antibodies

labeled by alkaline phosphatase (1:500; Bioss, China) for 2 h. Finally, BCIP/NBT substrate developer (Solarbio, China) was added to examine blots, and analyzed gray level by Image J software.

Statistical analysis

Data were analyzed by SPSS 22.0 (SPSS, IL, USA) statistical analysis software and expressed as mean \pm standard deviation ($X \pm SD$). Differences among groups were determined for statistical significance using oneway ANOVA and were considered statistically significant at $P < 0.05$. Graphpad Prism 5 (San Diego, California) was used to made graphs.

Results

Effects of DEX on IR-induced liver histopathology

Liver histopathological changes were analyzed in all six groups. There were no pathological changes in the liver tissue sections from the sham group, and the hepatocytes showed an intact structure with no degeneration or necrosis, and no inflammatory cell infiltration (Figure 1 aA). However, IR-induced liver histopathological changes were observed in the liver tissue including disrupted hepatic cell cords, extensive hepatic necrosis, hemorrhage, vacuolar degeneration, and inflammatory cell infiltration (Figure 1 aB). Interestingly, DEX administration significantly reduced IR-induced liver histopathological changes with neatly arranged hepatocytes, a small amount of inflammatory cell infiltration and bleeding (Figure a C-F). The pathological score of liver injury is shown in figure 1 b. The liver injury score of IR group was significantly higher than that of other groups ($p < 0.01$). Compared with group DL and DH, the liver injury score in group DM1 was significant lower ($p < 0.01$). There was no significant difference in liver injury score between DM1 group and DM2 group ($p \geq 0.05$).

Effects of DEX on IR-induced hepatocyte morphological changes

The hepatocyte ultrastructural changes were also analyzed in all groups by transmission electron microscopy. As shown in Figure 2 A, the morphology structure of hepatocytes from the sham group was normal with intact cell and nuclear membranes. In the IRI group the hepatocyte mitochondria were swollen and the ridges of mitochondria were uneven, the hepatocytes ER were damaged and expanded (Figure 2 B). DEX alleviated the damage in both organelles, whereas the nuclear membrane structure in the DL and DH group is slightly incomplete.

DEX decreases serum ALT activity and reduces liver oxidative stress induced by IR

We initially investigated specific indicators of liver function and oxidative stress damage, including ALT level, MDA concentration and SOD activity. As expected, serum ALT level and liver MDA concentration in the IR-induced rats were significantly higher compared with that of sham rats (Figure 3 a, b; $p < 0.01$), and IR significantly reduced lipid peroxidation. SOD activity in the liver (Figure 3 c) was significantly decreased in group IR ($p < 0.01$), DEX intervention markedly inhibited the increase in serum ALT activity, liver MDA level and the decrease in liver SOD activity induced by IR, indicating that DEX has a protective

effect in IR-induced liver injury. Among the three pre-administration groups, the concentration of ALT and MDA in DM1 group was the lowest and the activity of SOD was the highest. There was no significant difference in ALT and MDA concentration and SOD activity between DM1 and DM2 group ($p \geq 0.05$).

DEX ameliorates IR-induced hepatocyte apoptosis

TUNEL staining was used to assess apoptosis in the liver. The sham group showed few apoptotic hepatic cells (Figure 4 aA). The number of TUNEL positive cells increased significantly in the IR group compared to that in group S (Figure 4 aB; $p < 0.01$). In addition, the number of TUNEL positive cells in the DM1, DH, DM2 groups decreased significantly compared to that in the IR group (Figure 4 aD-F; $p < 0.01$ or $p < 0.05$). Notably, hepatocyte apoptosis rate in the DM1 group was significantly lower than in the DL group ($p < 0.01$); hepatocyte apoptosis rate in the DH group was not significantly different from that in the DM1 group ($p \geq 0.05$). There was no significant difference in hepatocyte apoptosis rate between DM1 group and DM2 group ($p \geq 0.05$).

Effect of DEX on core protein of ERS

The GRP78 mRNA level is shown in figure 5. The expression of GRP78 mRNA in the IR group was increased significantly compared with that in group S ($P < 0.01$), whereas DEX weakened this increase significantly. There was no statistical difference between group S and group DM1 ($p \geq 0.05$). Compared with group DL and DH, mRNA level of GRP78 in group DM1 was slightly decreased. There was no significant difference in the expression of GRP-78 mRNA between DM1 group and DM2 group ($p \geq 0.05$).

Effect of HRS on expression of UPR signaling protein in liver

We measured the mRNA levels of PERK, ATF-6 and the protein expression levels of p-PERK, p-IRE-1 α , and found them to be significantly higher in the IR group than those in the sham group ($P < 0.01$; Figure 6), whereas DEX treatment reversed these effects significantly. In addition, all the indicators mentioned above in DM1 group were the lowest among the three pre-administration groups. Notably, mRNA levels of PERK, ATF-6 and protein expression levels of p-PERK, p-IRE-1 α in the DH group and DM2 group were not significantly different from that in the DM1 group ($p \geq 0.05$).

Effect of DEX on the expression of ERS apoptosis signaling protein in the liver

Compared with the sham group, the Caspase-12 mRNA level and the expression of the proteins of CHOP, Bcl-2 in the IR group were increased markedly. DEX not only reduced Caspase-12 mRNA level and the expression level of CHOP protein significantly ($P < 0.01$; Figure 7 a, b), but also increased the expression level of Bcl-2 protein ($P < 0.01$; Figure 7 c). Compared with group DL and DH, the Caspase-12 mRNA level and the expression level of CHOP protein in group DM1 was significant lower ($p < 0.01$). There was no significant difference in the above two indicators between DM1 group and DM2 group ($p \geq 0.05$). The expression level of Bcl-2 protein in the DM1 group was significantly higher than in the DL, DH, DM2 groups ($p < 0.01$).

Discussion

Patients undergoing liver surgery remain at high risk of perioperative and postoperative complications despite recent surgical advances in the field. Many of these potential complications are a consequence of hepatic IRI with subsequent free radical formation, organelle failure, and release of pro-inflammatory mediators. Unraveling the underlying processes of hepatic IRI and developing measures to address this devastating condition are therefore of great interest.

In recent years, more and more studies have shown that DEX has a certain protective effect on liver during ischemia-reperfusion, and its mechanism may focus on oxidative stress and apoptosis^[23]. The pathway of apoptosis mediated by endoplasmic reticulum stress has gradually attracted the attention of scholars. It may be a very effective method to reduce hepatic ischemia-reperfusion injury by regulating endoplasmic reticulum stress. Therefore, in this experiment, the rat model of hepatic ischemia-reperfusion injury was established, and different doses of DEX were given at different times to explore the mechanisms underlying these hepatoprotective effects in hepatic IRI with a focus on ERS apoptosis pathways. We found that hepatic IRI could upregulate the expression of ERS marker GRP78, activate the three UPR signaling proteins PERK, ATF6 and IRE1, and induce expression of ERS-related apoptosis proteins CHOP, Caspase-12 and Bcl-2. DEX intervention can ameliorate IR-induced hepatic injuries by suppressing hepatocyte ERS and apoptosis.

In the current study, histological analysis indicated that IR causes changes in the liver cells, such as liver cell necrosis, blood cell destruction and inflammatory cell infiltration, DEX could ameliorate pathological liver damage. Hepatocyte morphological analysis indicated that IR induced mitochondria swelling, endoplasmic reticulum swelling and structure disorder, DEX could protect against liver cell organelles damage. ALT mainly exists in hepatocytes. When hepatocytes are necrotic, ALT is released into the blood, which is one of the important signs of acute hepatocyte injury^[24]. As shown in Fig. 3a, DEX significantly improved liver function in rats undergoing hepatic IR. These results indicate that DEX can significantly reduce hepatic IRI damage.

Oxidative stress balance plays a key role in the process of ischemia and is one of the important ways to lead to hepatocyte injury. MDA is the final product of lipid peroxidation in the process of oxidative stress, which can directly damage the cell membrane structures such as hepatocyte membrane and mitochondrial membrane. The content of MDA is an important index to reflect the degree of hepatocyte injury^[25]. As an antioxidant enzyme, SOD plays an important role in the antioxidant process of cells, so the activity of SOD is also an important index to measure the degree of oxidative stress injury in hepatocytes^[26]. In this study, the increase of MDA level and the decrease of SOD activity induced by IR were reversed by DEX treatment. According to these results, the protective mechanism of IR seems to be related to the antioxidant properties of DEX. There is evidence showing that oxidative stress, ER stress and inflammation are inseparably linked, in particular, the degree of oxidative stress greatly influences UPR signaling pathways. In the present study, we did not demonstrate a causal relationship between oxidative stress and ER stress; this is an area to explore in more details in our future work.

The ER is a dynamic and stable organelle involved in protein translation, lipid biosynthesis and calcium homeostasis [27]. Cells exposure to hypoxia can cause accumulation of unfolded proteins in endoplasmic reticulum lumen, leading to ERS [28]. IRI leads to hypoxia, oxidative stress and calcium overload, all of which can induce ER failure that in turn triggers ERS and cell apoptotic. Li et al. showed that DEX attenuated myocardial ischemia reperfusion injury in diabetes mellitus rats and H/R injury cell, which is associated with the reduction of ERS-induced cardiomyocyte apoptosis [29]. Consistent with this, DEX significantly alleviated the hepatocyte ERS induced by IR.

GRP78 is mainly located in endoplasmic reticulum and plays a key role in promoting protein folding and assembly, protein transport and calcium homeostasis, and is related to the regulation of ER transmembrane transduction [30, 31]. When ERS occurs, GRP78 dissociates from its complex with three sensory proteins, which is then up-regulated and associated with misfolded and unfolded proteins. Free sensory proteins activate three signal transduction pathways of ERS [32]. Therefore, the expression level of GRP78 can be used as one of the markers of ERS. In this study, we found that the level of GRP78 mRNA in liver IR increased, indicating the activation of ERS. In addition, compared with the IR group, the expression of GRP78 mRNA in the liver of other groups decreased significantly. These results suggest that DEX can down-regulate the increase of ERS induced by IR in liver.

ERS is also known as unfolded protein response (UPR). In the process of UPR, GRP78 is separated from ERS receptor proteins PERK, IRE1- α and ATF-6, and binds to unfolded proteins. The isolated three transmembrane proteins begin to induce stress signals and exert their effects, clearing misfolded proteins and leading to apoptosis [33]. In order to further explore the effect of IR on ER signaling pathway and the protective effect of DEX on ERS, we detected the expression level of PERK mRNA, ATF-6 mRNA, p-PERK protein and p-IRE-1 α protein in each group. In our study, IR significantly up-regulated the level of PERK mRNA, ATF-6 mRNA, up-regulated the expression of p-PERK protein and p-IRE-1 α protein, while DEX significantly down-regulated its expression, down-regulated the expression of p-PERK protein and p-IRE-1 α protein. These results suggest that DEX can inhibit the expression of UPR signal proteins to inhibit ERS, and thus participate in the regulation of liver IR.

Excessive or prolonged ERS activates apoptotic pathways and induces cell death. An increasing number studies have shown that apoptosis induced by ERS plays an important role in liver IRI [34, 35]. Under the ERS, Bcl-2 family proteins mediate endoplasmic reticulum Ca^{2+} release and caspase12 activation, which eventually lead to apoptosis. There are three main apoptotic pathways in ERS, CHOP activation pathway, JNK activation pathway, and caspase 12 activation pathways [36]. Caspase-12 is a key protease of ERS-mediated apoptosis attached to the ER. When ER is overstressed, the complete Caspase-12 response is activated, and then a series of proteins of the Caspase family are activated exponentially, resulting in cascade reaction to induce apoptosis [37], so the protein plays an important role in initiating the ER apoptosis pathway. CHOP is a specific ERS transduction factor and an important signal molecule for promoting apoptosis. In ERS, its expression is greatly increased and is considered to be one of the markers of endoplasmic reticulum stress [38]. In this study, we detected the expression of caspase 12

mRNA in each group, and detected the expression of apoptosis-related proteins (CHOP and Bcl-2) after liver IRI by Western blot analysis. The results showed that the expression of caspase 12 mRNA, CHOP and Bcl-2 protein in liver tissue were significantly up-regulated in IRI, while DEX significantly inhibited its expression. The TUNEL assay results further confirmed that IR can cause a significant increase in liver TUNEL-positive cells, and DEX can reduce the number of TUNEL-positive cells. Our results further proved that DEX could inhibit the apoptotic liver of ERS induced by IRI.

In order to determine the dose-effect relationship of DEX's protection against liver IRI and whether the effect is relate with the administration time, 25 µg/kg, 50 µg/kg and 100 µg/kg DEX were administrated by intraperitoneal injection at 30 min before ischemia or 30 min after reperfusion. Our results indicated that 50 µg/kg DEX significantly decreases the expression of ERS proteins and inhibits cell apoptosis, and provides a superior protection compared with 25µg/kg DEX. Our results are consistent with the previous study described by Robert et al ^[39]. that discovered DEX inhibited isofluraneinduced cortical injury in a dose-dependent manner within 50µg/kg. Whereas the effect on liver IR-induced ERS and cell apoptosis did not increase in 100 µg/kg DEX group, the possible reason is that the organ protection effect of DEX is associated with decreased organ vasoconstriction, which does not occur with rapid loading of DEX or an infusion of markedly high doses ^[40]. The specific mechanism remains to be further studied. At the dose of 50µg/kg, DEX injection 30 min before ischemia and 30 min after reperfusion did not present different effect on liver ERS and cell apoptosis. Our results are consistent with the previous study described by Gu et al. that showed both DEX administered intraperitoneally prior to and following ischemia markedly reduced remote lung injury induced by renal ischemia-reperfusion in mice ^[41]. But Schaak et al. discovered that DEX prior to ischemia, but not following it, attenuates intestinal IRinduced intestinal injury ^[42]. This is an area should be evaluated further in our future work.

Conclusions

In conclusion, this study revealed that DEX can protect liver IRI by reducing ERS, thereby inhibiting ERS-related apoptosis. The protective effect of DEX on hepatic IRI was dose-dependent in a certain dose range, and both DEX administered prior to ischemia and following reperfusion markedly reduced liver injury induced by hepatic IRI in mice.

Abbreviations

Dex: dexmedetomidine; IRI: ischemia-reperfusion injury; HE: haematoxylin–eosin; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labelling; ERS: endoplasmic reticulum stress; PERK: protein kinase R-like ER kinase; ATF6: activating transcription factor 6; IRE1: Inositol-requiring enzyme 1; CHOP: C/EBP homologous protein; ALT: alanine aminotransferase; MDA: malondialdehyde; SOD: superoxide dismutase; TEM: transmission electron microscope;

Declarations

Authors' contributions

SZ and JT designed the research; WZ carried out the experiments, analysed the data; JT, HL and LA cooperated on carrying out the research; HL wrote the manuscript; JT co-wrote the manuscript; All authors read and approved the final manuscript.

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Not applicable

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was allowed by the Agricultural University of Hebei Ethical Committee, Baoding, PR China.

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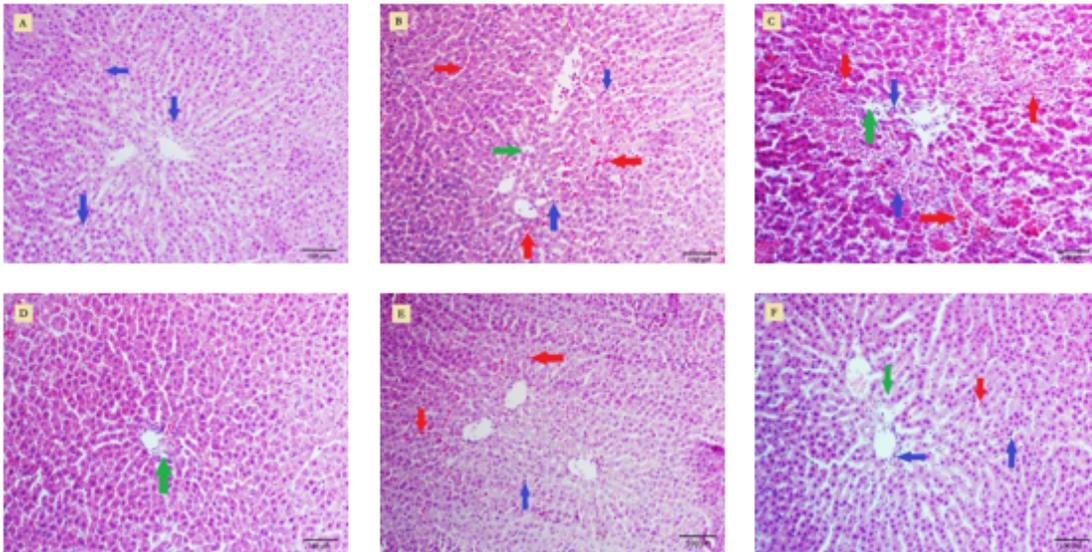
Tables

Table 1 Primer Sequence of the genes were tested in the present study.

Gene	Number	Primer sequences (5'-3')
GaPdh	Nm-017008.4	Forward: GGCACAGTCAAGGCTGAGAATG
		Reverse: ATGGTGGTGAAGACGCCAGTA
PERK	Nm-031599.2	Forward: CCAAGCTGTACATGAGCCCAGA
		Reverse: TTTCTGAGTGAACAGTGGTGGAAAC
ATF-6	Nm-001107196.1	Forward: ATCACCTGCTATTACCAGCTACCAC
		Reverse: TGACCTGACAGTCAATCTGCATC
GRP-78	Nm-013083.2	Forward: TCAGCCCACCGTAACAATCAAG
		Reverse: TCCAGTCAGATCAAATGTACCCAGA
Caspase-12	Nm-130422.1	Forward: AAATGGAGGTAAATGTTGGAGTGG
		Reverse: CTGCCCTTTCTGTCTTCATCGT

Figures

(a)



(b)

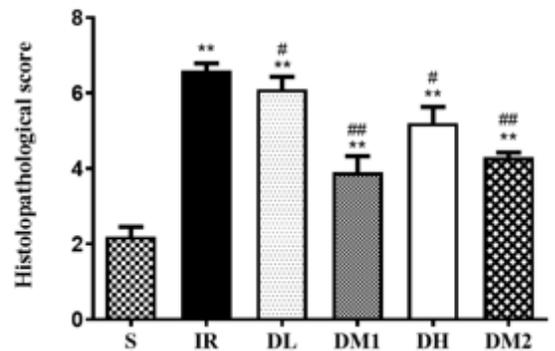


Figure 1

Effects of DEX on IR-induced liver histopathology. Representative H&E-stained sections after 6 h of reperfusion (a) is shown for the livers from group S (A), group IR (B), group DL (C), group DM1 (D), group DH (E), group DM2 (F). (Magnification 200 x, bars = 100 μ m). The red arrows represent congestion; green arrows represent the liver sinus; blue arrows represent liver macrophages. (b) Histopathological mean liver injury scores. Data were presented as mean \pm standard deviation (n = 6). *p < 0.05, **P < 0.01 vs S group. #P < 0.05, ##P < 0.01 vs IR group. + p < 0.05, ++ P < 0.01 DM2 vs DM1 group.

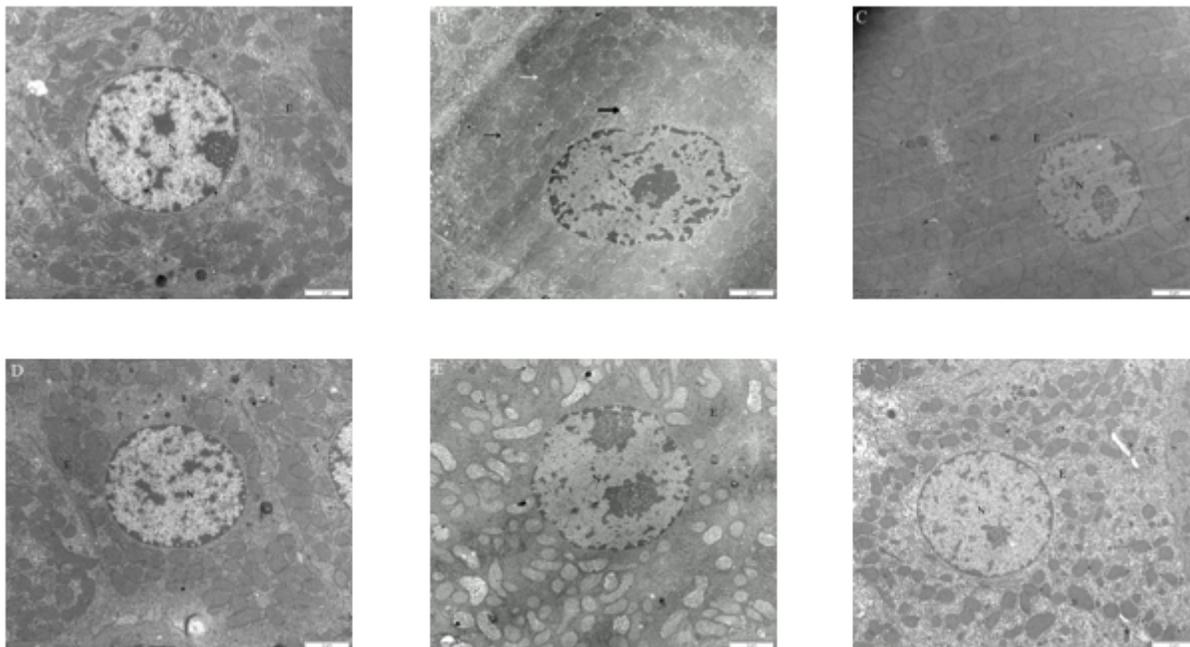
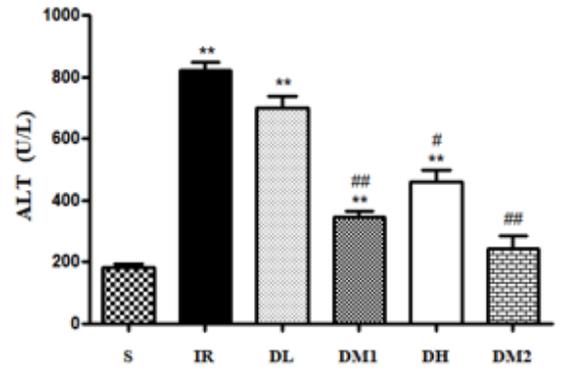


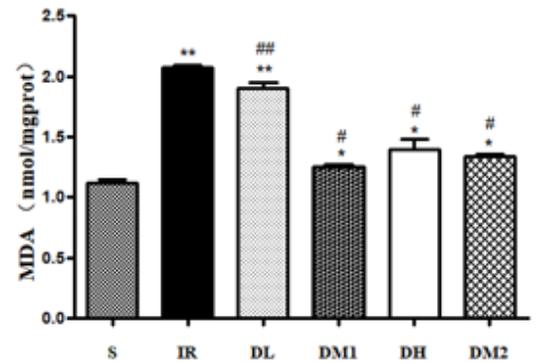
Figure 2

Effects of DEX on IR-induced hepatocyte morphological changes. TEM micrographs is shown for the livers from group S (A), group IR (B), group DL (C), group DM1 (D), group DH (E), group DM2 (F). (Magnification 10000 x, bars = 2 μ m). The thick black arrow indicated ER swelling, the thin black arrow indicated the disordered structure of ER, and the thin white arrow indicated mitochondria swelling.

(a)



(b)



(c)

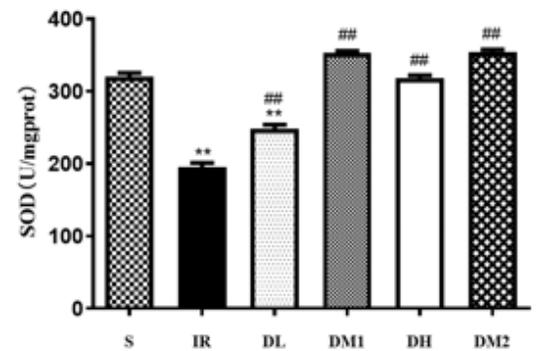
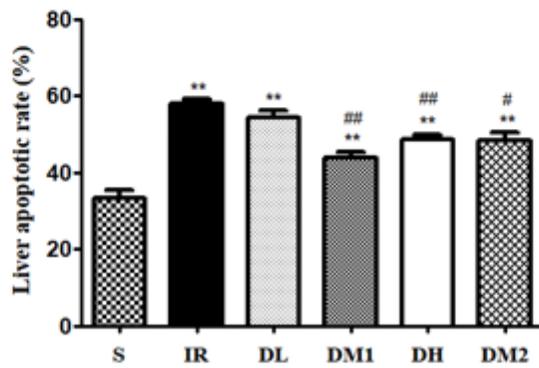
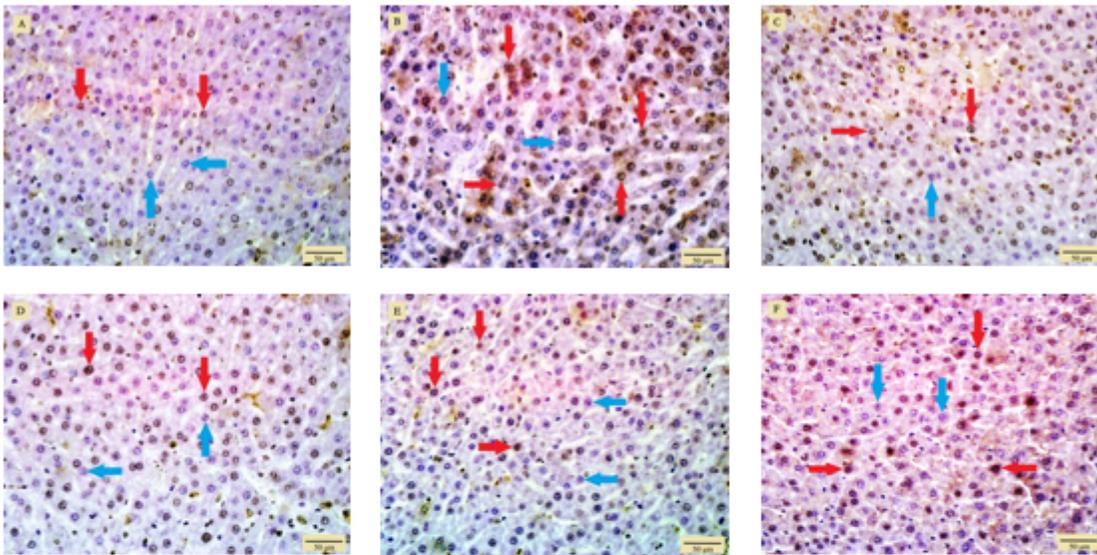


Figure 3

DEX decreases serum ALT activity and reduces liver oxidative stress induced by IR. (a) The content of ALT in serum. (b) The concentration of MDA in liver tissue. (c) The activity of SOD in liver tissue. Data were presented as mean \pm standard deviation (n=6). *p < 0.05, **P < 0.01 vs S group. #P < 0.05, ##P < 0.01 vs IR group. + p < 0.05, ++ P < 0.01 DM2 vs DM1 group

(a)



(b)

Figure 4

DEX ameliorates IR-induced hepatocyte apoptosis. Apoptosis was detected by in TUNEL after 6 h of reperfusion (a) is shown for the livers from group S (A), group IR (B), group DL (C), group DM1 (D), group DH (E), group DM2 (F)(magnification 400 x, bars = 50 μ m). Red arrows indicate TUNEL-positive cells; blue arrows indicate normal cells. * $p < 0.05$, ** $P < 0.01$ vs S group. # $P < 0.05$, ## $P < 0.01$ vs IR group. + $p < 0.05$, ++ $P < 0.01$ DM2 vs DM1 group

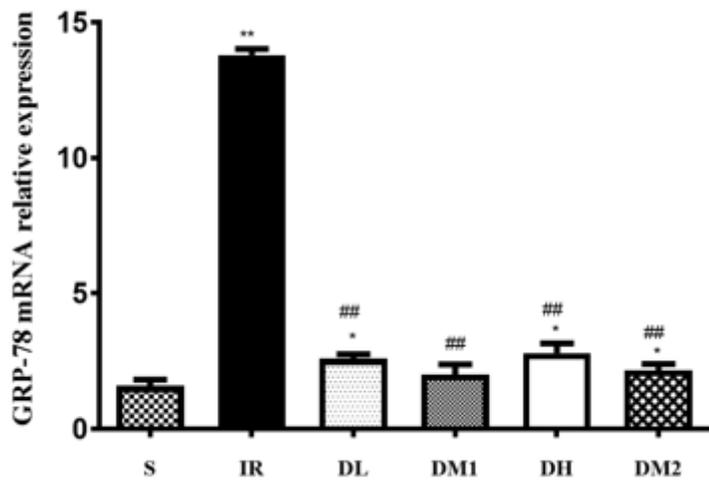
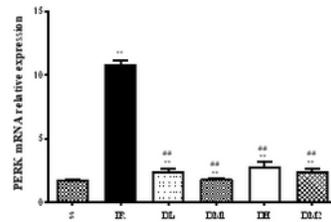


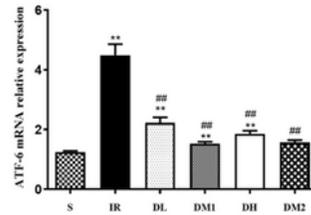
Figure 5

The expression of GRP78 at gene levels. Data were presented as mean \pm standard deviation (n=6). *p < 0.05, **P < 0.01 vs S group. #P < 0.05, ##P < 0.01 vs IR group. + p < 0.05, ++ P < 0.01 DM2 vs DM1 group.

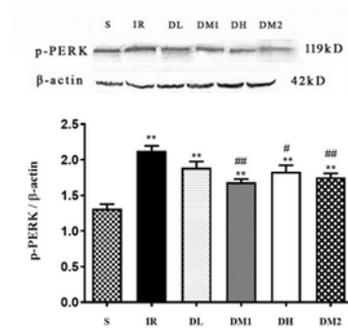
(a)



(b)



(c)



(d)

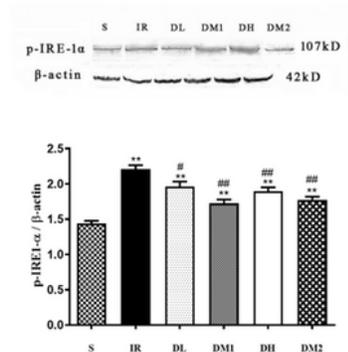
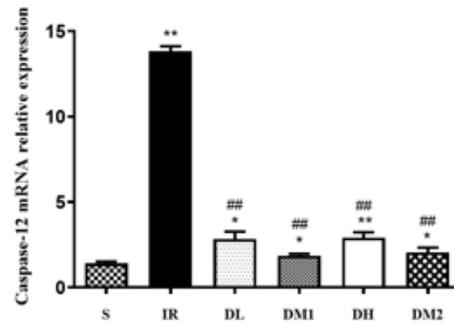


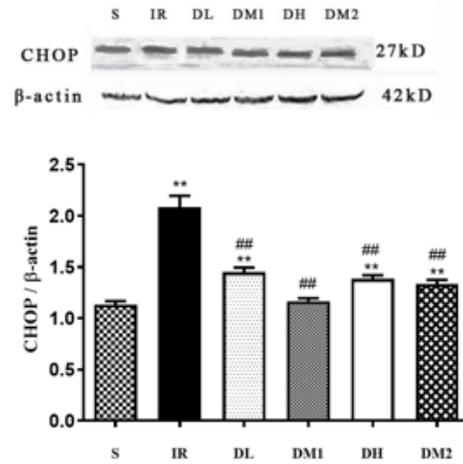
Figure 6

Effect of DEX on expression of UPR signaling protein in liver. The mRNA levels of PERK (a) and ATF-6 (b) were determined by real-time PCR. The protein levels of p-PERK (c) and p-IRE- α (d) were measured by western blotting assay. Data were presented as mean \pm standard deviation (n=6). *p < 0.05, **P < 0.01 vs S group. #P < 0.05, ##P < 0.01 vs IR group. + p < 0.05, ++ P < 0.01 DM2 vs DM1 group

(a)



(b)



(c)

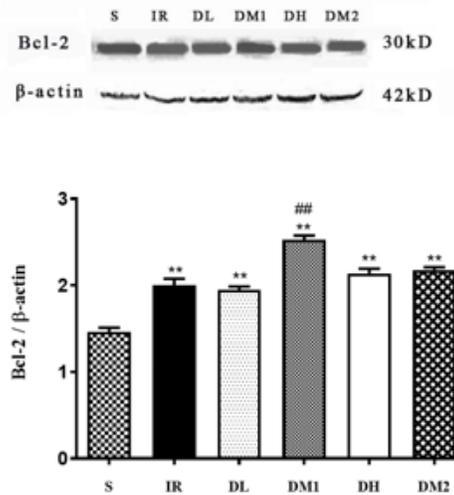


Figure 7

Effect of DEX on the expression of ERS apoptosis signaling protein in the liver. The mRNA levels of caspase-12 (a) were determined by real-time PCR. The protein levels of CHOP (b) and Bcl-2 (c) were measured by western blotting assay. Data were presented as mean \pm standard deviation (n=6). *p < 0.05, **P < 0.01 vs S group. #P < 0.05, ##P < 0.01 vs IR group. + p < 0.05, ++ P < 0.01 DM2 vs DM1 group