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Original Article

Dexmedetomidine improves DM-induced oxidative stress injury to protect liver function through Nrf2 pathway

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Abstract: Objective: Diabetes mellitus-induced oxidative stress (OS) causes liver injury. Intraoperative pumping of dexmedetomidine (DEX) effectively reduced the postoperative OS response in patients with type 2 diabetes mellitus (T2DM) and had a certain protective effect on liver function. However, the mechanisms of the protective effect on the liver remained unclear. In this study, we investigated the antagonistic effects and the possible mechanism of DEX on T2DM-induced liver injury in the mouse model and Palmitic acid (Pal)-induced injury in hepatocellular carcinoma cells (HepG2). Methods: Seven wt/wt mice served as Control group, and 28 db/db mice were randomly divided into four groups using a random number table method: Model group (n=7), D25 group (n=7), D50 group (n=7) and D75 group (n=7). Different concentrations of DEX were injected intraperitoneally in the D25 group, D50 group and D75 group, while the Control group and the Model group were intraperitoneally injected with the same amount of normal saline for 3 weeks. In the cell intervention experiments, HepG2 cell line was used. The control group (Con group), the palmitic acid group (Pal group) and the DEX treatment group (Pal + Dex group) were set up. The test results were compared among mice groups and cell groups, respectively. Results: DEX alleviated the increase of alanine aminotransferase, triglyceride, total cholesterol and aspartate aminotransferase contents induced by high fat or T2DM. DEX reversed the decrease of nuclear factor E2 related factor 2 (Nrf2) in the nuclear translocation and the lower transcriptional activity of Nrf2 to inhibit the expression of heme oxygenase-1, NADPH quinone oxidoreductase-1 and superoxide dismutase 2 and reduced the activity of superoxide dismutase to increase reactive oxygen species content induced by high fat or T2DM. Conclusion: By attenuating the high-fat or T2DM-induced Nrf2 pathway impairment, DEX can reduce OS injury and inhibit the disorder of lipid anabolism and protect liver function. This study provides a theoretical basis for the protection of liver function by DEX in clinical T2DM patients.

Keywords: Dexmedetomidine, diabetes mellitus, oxidative stress, Nrf2, liver damage

Introduction

Previous studies have shown an estimated 463 million people worldwide with diabetes mellitus (DM) in 2019, and the number may rise to 578 million by 2030 [1]. DM can cause complications including blood vessel abnormalities, heart attack, hepatopathy, nerve disorder, retinopathy and nephropathy [2, 3]. The abnormal state of the liver in DM patients can be defined as diabetic hepatopathy (DH) [4]. The initial symptoms of DH are nonalcoholic fatty liver disease (NAFLD). The prevalence of NAFLD, about 25% worldwide, is as high as 60-75% in patients with type 2 diabetes mellitus (T2DM). Individuals with T2DM also have a doubling risk of nonalcoholic steatohepatitis (NAFLD with inflammation and hepatocyte damage, with or without fibrosis) [6]. Failure to initiate effective intervention will lead the disease to progress to cirrhosis, liver failure as well as hepatocellular carcinoma which significantly increase the risk of cardiovascular disease [7]. Therefore, the mechanisms and treatment options for DH

have been extensively studied for a long time, which modify the disease course progression to some extent, improve the quality of life of T2DM patients and reduce mortality.

The current view is that insulin resistance is only one contributor to the formation of simple fatty liver in patients with T2DM, which is driven by many other factors such as the overproduction and release of inflammatory factors and free fatty acid (FFA), and the accumulation of triglycerides (TG) in liver cells caused by the decline of the ability of the liver to metabolize lipids. Reactive oxygen species (ROS), in the process of NAFLD formation, are potentially vital. The excessive deposition of FFA and TG will lead to liver lipid metabolism disorders, which increase the liver's susceptibility to oxidative stress (OS), resulting in a large amount of ROS, which leads to inflammatory factors release and hepatocyte apoptosis [8-10]. Malondialdehyde (MDA), a product of highly hazardous lipid peroxidation, is positively correlated with hepatic inflammatory response, hepatocellular necrosis and liver fibrosis progression. In addition, the organism is affected by lipotoxicants, genetic polymorphisms and gut flora. Multiple factors contribute to the initiation and progression of T2DM with NAFLD, the so-called "multiple hit" doctrines [11].

As mentioned earlier, playing an important role in the development and progression of T2DM and NAFLD, OS refers to the body's release of excessive ROS and reactive nitrogen species in response to various stimuli, which breaks the balance between the OS response and the antioxidant system and then leads to different degrees of damage to local tissues or organs in the body [12]. The mechanism of action of oxidation versus antioxidation is very complex, combined with various factors that are potentially linked and affect each other. Therefore, we need to further study the mechanism of OS impairment in DH progression to provide a theoretical basis for clinical T2DM patients.

The liver has multiple defense systems against OS injury, among which a very important link is a series of related responses generated by nuclear factor-E2-related factor 2 (Nrf2), which regulates the expression of downstream antioxidant target genes by binding to the antioxidant response element [13]. Nrf2, existing exclusively in the cytoplasm, is constitutively inactive upon mutual binding with kelch-like ECH-associated protein 1 (Keap1). Under OS conditions, Nrf2 will dissociate from Keap1 protein and translocate to the nucleus for activation, where it interacts with the antioxidant response element to regulate the expression of downstream target genes such as heme oxygenase-1 (HO-1), glutathione S-transferase, glutathione peroxidase, NADPH quinone oxidoreductase 1 (NQO-1), superoxide dismutase (SOD) and catalase [14]. Previous studies found that alterations in Nrf2 signaling could affect the development of alcoholic liver injury, viral hepatitis and ischemia-reperfusion liver injury, but its effect on hyperlipidemic diabetes-induced liver injury is still unknown [15, 16].

Dexmedetomidine (DEX) has been widely used in various fields of perioperative anesthesia in recent years. DEX, activated by selective α2 adrenoceptors, exerts pharmacological effects such as sedation, analgesia, anti-sympathetic and opioid actions [17, 18]. DEX is often used as an adjunct to other narcotic analgesics [19]. DEX pumped before induction of anesthesia can reduce the number of other anesthetic drugs [20], attenuate intubation and extubation responses and make hemodynamics more stable [21]. DEX can reduce endoplasmic reticulum stress, protect against sepsis, attenuate histological damage, and reduce OS injury in animal models, which subsides hepatic ischemia-reperfusion injury [22-25]. DEX may also attenuate liver injury by inhibiting inflammation and apoptosis [26]. In addition, clinical studies have confirmed that DEX can improve the repair ability of the liver against ischemia-reperfusion injury through antioxidant and anti-inflammatory effects, thereby exerting a hepatoprotective effect in the perioperative period [27]. Meanwhile, a recent study confirmed that DEX could ameliorate liver injury induced by lower limb ischemia-reperfusion in DM rats via anti-inflammation and anti-oxidation [28]. Intraoperative pump DEX, in our previous clinical study [29], has been found to effectively inhibit OS, restrain inflammatory factor release and improve liver function after elective lower extremity ulcer debridement in patients with T2DM. Here, its mechanism of action is further investigated and discussed.

Materials and methods

Animals and treatments

Both db/db mice (male, 16 weeks old) and wt/wt mice (male, 16 weeks old) were purchased from Jiangsu Jixian Kang Biotechnology Co., Ltd. Seven wt/wt mice served as Control group, and 28 db/db mice were randomly divided into four groups using a random number table method: Model group (n=7), D25 group (n=7), D50 group (n=7) and D75 group (n=7). In the D25 group, DEX (25 μg/kg) was injected intraperitoneally into mice. In the D50 group, DEX (50 μg/kg) was injected intraperitoneally into mice. In the D75 group, DEX (75 μg/kg) was injected intraperitoneally into mice. All Control group and Model group animals received an equal volume of saline intraperitoneally. Mice in each group were dosed daily for 3 weeks with drugs for different groups described above. All animal experiments were approved by the Ethics Committee of Chengdu Medical College and performed following institutional and national guidelines.

Serum and tissue specimens

The animals were anesthetized with 0.3% pentobarbital sodium solution (Huaye Huanyu Chemical, Beijing, China) and euthanized later by cervical dislocation. After taking 1 ml of arterial blood at the left ventricle and kept at 37°C for 1-2 hours, we centrifuged the blood for 10-15 min using a high-speed centrifuge at 3000 rpm. After centrifugation, the supernatant was aliquoted in cryovials, placed at -80°C and stored for further use. Removed from the abdominal cavity intactly, the right lobe of the liver was sheared to approximately 5×2×2 mm pieces and then fixed in 10% formalin for 24 h. The right lobe of the remaining liver was sheared to an appropriate size and then stored in a -80°C freezer.

Cell culture and culture conditions

Currently, the hepatocyte models used in fatty liver research mainly include animal primary hepatocytes [30, 31], human hepatocytes [32, 33] and human hepatocellular carcinoma cells (HepG2) [34-37]. Therefore, HepG2 cells were used in this study. HepG2 cells were purchased from Shanghai Xinyu Biotech Co., Ltd. The cells were incubated in a 37°C sterile incubator with 95% air and 5% carbon dioxide. The high glucose medium containing 10% fetal bovine serum was changed once in 2 days. The cell density of the experiment was 70-80%.

Studies have shown that treatment of HepG2 cells with palmitic acid (Pal) causes hepatocyte lipid damage [38, 39]. Therefore, we used Pal to treat the cells and make the optimal concentration selection. First, six groups were set up: a control group (Con group) and 5 groups of Pal at different concentrations (Pal 0.2 mmol/L, Pal 0.4 mmol/L, Pal 0.6 mmol/L, Pal 0.8 mmol/L and Pal 1.6 mmol/L). After 6 h treatment, cell viability was detected by the methyl thiazolyl tetrazolium method to clarify the optimal concentration. We selected the Pal concentration when cell viability no longer decreased with higher Pal concentration to construct a high-fat hepatocyte injury model, which was then set as Pal group.

DEX has been shown to have antioxidant effects [40]. Following the previous experiments, 7 groups were set up: Con group, Pal group (0.4 mmol/L), and 5 DEX groups treated with DEX at different concentrations (Dex1 μmol/L, Dex5 μmol/L, Dex10 μmol/L, Dex15 μmol/L and Dex20 μmol/L). Having added different concentrations of DEX, the cell viability of hepatocytes under high-fat injury conditions was observed for 6 h. We selected the DEX concentration when cell viability obviously rose as the optimal DEX treatment concentration, which was then set as Pal + Dex group.

Biochemical analysis

SOD, MDA, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), TG, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and FFA were determined with the corresponding biochemical kits (Jian Cheng Biotechnology, Nanjing, China) by enzyme-linked immunosorbent assay.

Measurement of the ROS

Total ROS release from hepatocytes was detected by Dihydroethidium (DHE) staining. After incubation with ROS staining solution for 30 min at 37°C, HepG2 cells were washed three times with PBS. The fluorescence was

detected by a fluorescence microplate reader (Leica instruments Gmbh, Germany) for cells or a microscope for liver sections (Leica instruments Gmbh, Germany).

Histopathological staining

The liver tissue was prepared into paraffin sections at a thickness of 4 μm. Tissue sections were stained with hematoxylin-eosin (HE) to observe liver histomorphology. Cells were also stained with a HE staining kit (keygen Biotech Co., Ltd., Jiangsu, China). Images were obtained using a 40× light microscope (Leica Instrument Co., Ltd., Germany).

Oil Red O staining

The frozen liver sections of 10 μm thick and cells were stained with Oil Red O (Solaibao Technology Co., Ltd., Beijing, China) for 10 min, washed off the dye solution and stained with hematoxylin for 10 s. The slides were fixed with a small drop of glycerol gelatin that was placed around the tissue and observed under the microscope.

Western blot

Liver tissue (20 mg) was added to 150 μL lysate and placed on ice to mix the reaction well. Tissues were lysed thoroughly using an ultrasonic lysing instrument, centrifuged at 4°C in a refrigerated centrifuge (Thermo Fisher, Guangzhou, China) at 15000 r/min, and the supernatant was pipetted into a tube and stored in -20°C freezer for further use. An appropriate amount of tissue samples or HepG2 cells was taken, added to 100 μL lysate, homogenized in an ice bath, and placed for 10 min at 4°C. After 10 min, the cells were harvested by scraping with a cell scraper, coupled with the extracts pipetted by a micropipette into a pre-chilled EP tube. After centrifugation, the supernatant was collected. Protein concentrations were determined by using a BCA protein assay kit (Beyotime Institute of Biotechnology, China). Proteins were subjected to SDS-PAGE. The separated protein samples were transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were then blocked with 5% nonfat milk for 1 h, added with the primary antibodies against Nrf2 (cat. no. ab92946, 1:3000, Abcam, UK), HO-1 (cat. no. sc-390991, 1:3000, Santa Cruz Biotechnology, USA), NQO-1 (cat. no. ab34173, 1:3000, Abcam, UK), SOD2 (cat. no. FNab08104, 1:3000, Fen biotech, China) and GAPDH (cat. no. FNab03343, 1:3000, Fen biotech, China), incubated overnight at 4°C, then added with corresponding secondary antibodies (cat. no. FNab00231 and FNSA-0003, 1:5000, Fen biotech, China) and incubated for 1.5 h at room temperature. With the protein side up on the membrane in uniform contact with the developer, the PVDF membranes were placed into a chemiluminescent gel imager and exposed for imaging.

Statistical analysis

Data analysis and illustration was performed with the use of SPSS 26.0 and Graphpad prism 8.0 software. The data were presented as the mean ± standard deviation (x ± sd). One-way ANOVA was used for comparison among multiple groups, while LSD was used for comparison between each two groups. $P<0.05$ was considered as statistically significant.

Results

DEX attenuated hepatic fat deposition and improved blood lipid metabolism in T2DM mice

The liver appearance of the mice was brown-red and soft in the Control group, while the liver of the Model group was yellow-tinged and greasy. With increasing concentrations of DEX administration, the liver volume of mice gradually shrunk and the color became red (**Figure 1A**). Additionally, the liver weight was increased in the Model group, but DEX treatment reduced the liver weight increase (**Figure 1B**). HE staining showed that, under a 40× light microscope, no inflammatory foci or hepatocyte steatosis was observed in the hepatocytes of mice in the Control group. Hepatic lobule's structure was damaged and the hepatocytes were vacuolized in the Model group. DEX improved liver tissue morphology and reduced liver steatosis in T2DM mice (**Figure 1C** and **1D**). Oil Red O staining found that the hepatocytes of mice in the Control group had normal physiological hepatic lipid content and demonstrated more lipid deposition in the Model group, whereas DEX treatment alleviated this abnormal phenomenon (**Figure 1E** and **1F**). Serum biochemical indexes showed that DEX treatment alleviated the serum TC and TG index in T2DM mice

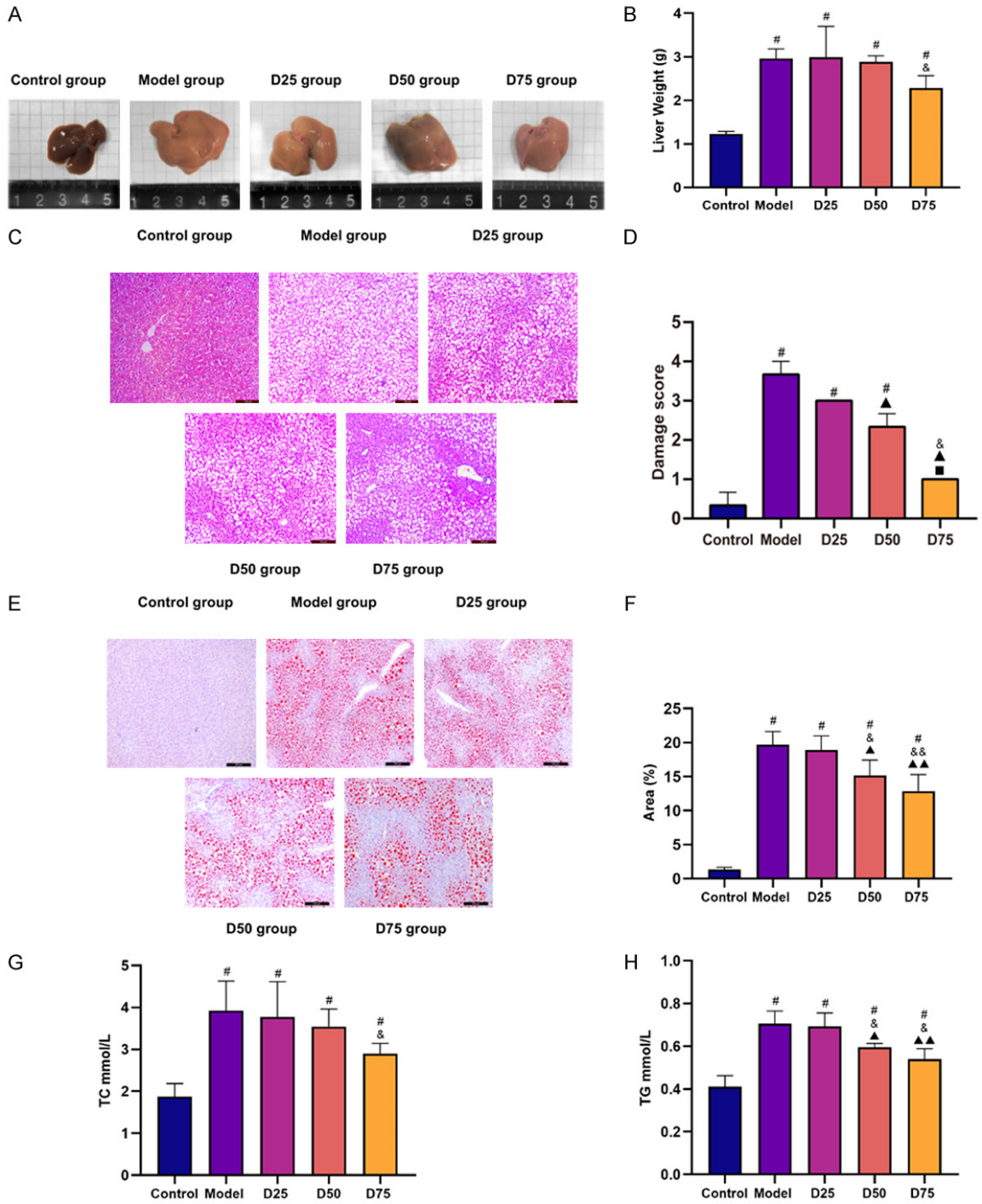

Figure 1. DEX attenuated hepatic fat deposition and improved blood lipid metabolism in T2DM mice. A. The appearance of the livers of each group was examined. B. The weight of livers of each group was examined (n=7/group). C and D. Comparison of HE staining of liver tissue among five groups of mice (×40 times, scale bar, 100 μm). The stained sections were scored using a four-point scale from 0 to 3, with 0, 1, 2, and 3 representing no damage, mild damage, moderate damage and severe damage, respectively. E and F. Comparison of Oil Red O staining of liver tissue in five groups of mice (×40 times, scale bar, 200 μm). Comparison of Oil Red O staining lipid droplet area in liver of mice in five groups. G and H. Changes of lipid metabolism indexes in five groups of mice. $^{\#}P<0.05$, vs. Control group; $^{\&}P<0.05$, vs. Model group; $^{\blacktriangle}P<0.05$, $^{\blacktriangle\blacktriangle}P<0.01$, vs. D25 group. DEX: dexmedetomidine; T2DM: type 2 diabetes mellitus; TC: total cholesterol; TG: triglycerides.

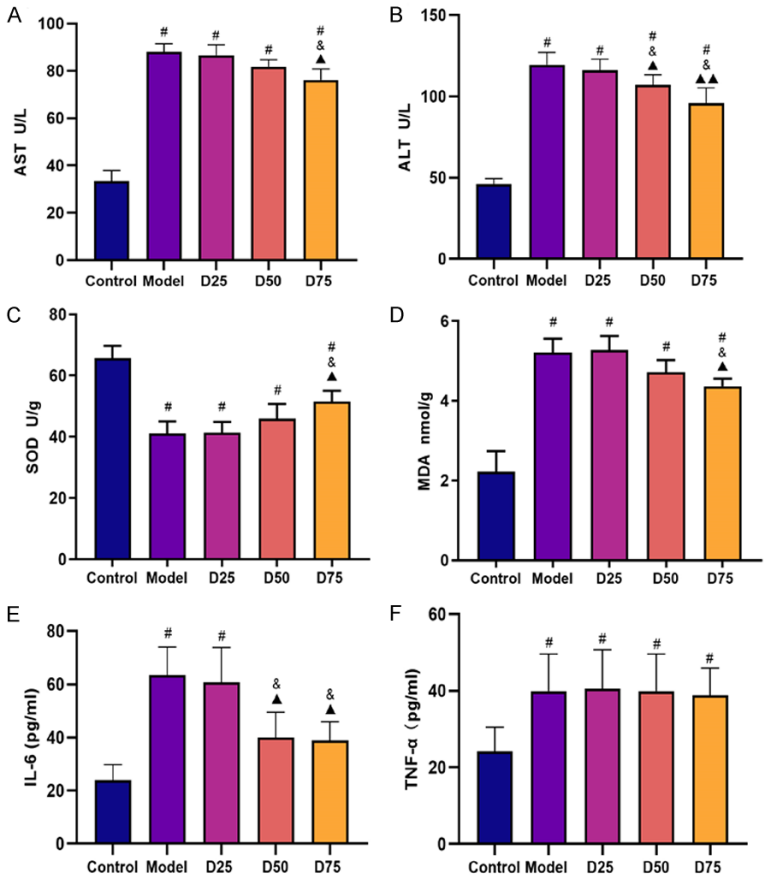

Figure 2. DEX attenuated the inflammatory response, oxidative damage, and improved liver function in T2DM mice. A and B. Changes of liver function indexes in five groups of mice. C and D. Change of oxidative stress indexes in five groups of mice. E and F. Changes of inflammatory factors in five groups of mice. #P<0.05, vs. Control group; &P<0.05, vs. Model group; ▲P<0.05, ▲▲P<0.01, vs. D25 group. DEX: dexmedetomidine; T2DM: type 2 diabetes mellitus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde; IL-6: interleukin 6; TNF-α: tumor necrosis factor-α.

(**Figure 1G** and **1H**). These results demonstrate that DEX can alleviate hepatic fat deposition, reduce fat red-stained areas and improve lipid metabolism in T2DM mice.

DEX treatment reduced the inflammatory response, OS and liver function damage in T2DM mice

Compared with those in the Control group, the AST, ALT, MDA, IL-6 and TNF-α in the Model group were significantly higher, while the SOD was lower. Moreover, DEX treatment significantly reduced AST, ALT, MDA and IL-6, and increased SOD, but no effect was observed on the level of TNF-α (**Figure 2A-F**). These results display that DEX can attenuate inflammation, OS and liver function damage in T2DM mice.

DEX rescued the inhibitory effect of DM on the Nrf2 pathway in vivo

Compared with those in the Control group, the expression levels of Nrf2, HO-1 and SOD2 were significantly decreased in the Model group. However, these levels were significantly increased gradually by the increasing concentrations of DEX. Additionally, HO-1 in D75 group and SOD2 in D50 and D75 groups were higher than those in the Control group (**Figure 3A-D**). Overall, these results indicate that DM can inhibite the activity of Nrf2 to enhance OS, and DEX, with an overactivation effect, can dose-dependently upregulate Nrf2, HO-1 and SOD2 protein expression in the liver of T2DM mice.

DEX elevated the viability of hepatocytes under high-fat injury conditions

The optimal concentration of Pal to induce hepatocyte injury: Compared with that in the Con group, the hepatocyte viability decreased obviously in the Pal groups at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.6 mmol/L, but the hepatocyte viability no longer decreased after the concentration of 0.4 mmol/L, which was judged as the optimal concentration of Pal for hepatocyte injury (**Figure 4A**).

DEX optimal dose of action: Compared with that in the high-fat hepatocyte injury model group (Pal group), the hepatocyte viability was significantly increased in DEX groups at concentrations of 1, 5 and 10 μmol/L, but the viability reached the peak value at 5 μmol/L. In DEX groups at concentrations of 15 and 20 μmol/L, the viability of hepatocytes was significantly lower than that in the Pal group. According to this, we selected the optimal dose of DEX to be 5 μmol/L, which was used to treat the high-fat hepatocyte injury model (**Figure 4B**).

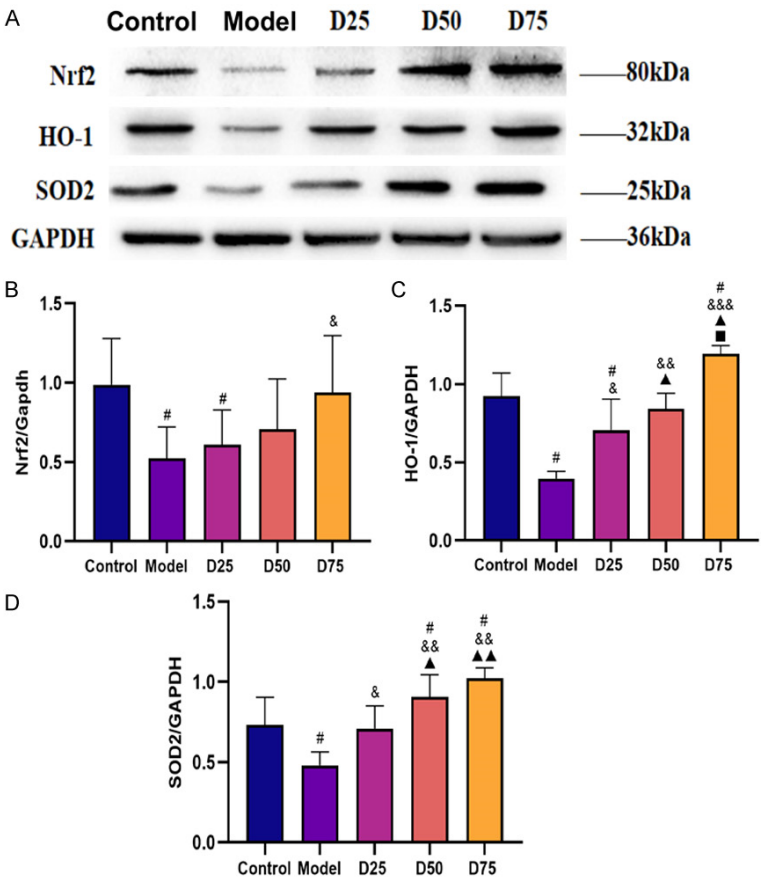

Figure 3. DEX rescued the inhibitory effect of the Nrf2 pathway caused by DM in vivo. A. Western blot assay was used to detect the levels of Nrf2 and the protein expression levels of HO-1 and SOD2 in mouse livers (n=7/group). B-D. Comparison of Nrf2, HO-1 and SOD2 protein expression in five groups of mice. #P<0.05, vs. Control group; &P<0.05, &&P<0.01, vs. Model group; ▲P<0.05, ▲▲P<0.01, vs. D25 group, ▪P<0.05, vs. D50 group. DEX: dexmedetomidine; Nrf2: nuclear factor E2-related factor-2; DM: diabetes mellitus; HO-1: heme oxygenase-1; SOD2: superoxide dismutase 2.

DEX improved hepatocyte function and lipid metabolism under high-lipid injury conditions

The AST and ALT were significantly higher in the Pal group compared with those in the Con group. Moreover, DEX treatment significantly reduced AST and ALT, suggesting that DEX can improve the function of hepatocytes undergoing high-fat injury (**Figure 5A** and **5B**). The oil-red O staining exhibited that, compared with that in the Con group, the cytoplasm of high-fat hepatocytes treated with 0.4 mmol/L Pal displayed significant red lipid droplet formation and fusion and that the nucleus was blue, coupled with the deposition of scattered lipid droplets. However, the red lipid droplets in hepatocytes were significantly reduced in DEX + Pal group. Thus, DEX effectively improved lipid deposition in hepatocytes under high-lipid injury conditions (**Figure 5C** and **5D**). Compared with those in the Con group, the content of TG, T-CHO, LDL and FFA in the Pal group were significantly increased, while the HDL content was significantly decreased. In Dex + Pal group, lipid metabolism indexes improved. These suggest that the abnormal lipid metabolism status of the hepatocytes can be significantly improved after DEX treatment (**Figure 5E-I**).

Activation of Nrf2 signaling by DEX improved OS damage in hepatocytes

As seen by fluorescence microscopy after DHE staining, DEX treatment alleviated the increase in ROS levels induced by Pal (**Figure 6A** and **6B**). In this study, the expressions of Nrf2, NQO-1 and SOD2 protein in hepatocytes were determined by Western blot. Compared with those in the Con group, the expression levels of Nrf2, NQO-1 and SOD2 were significantly decreased in the Pal group, but significantly increased in the Pal + DEX group (**Figure 6C-H**). These results indicate that high-fat injury can enhance OS by inhibiting Nrf2, NQO-1 and SOD2 activity, and DEX can rescue the inhibitory effect of high fat on the Nrf2 pathway in vitro.

Discussion

The development of NAFLD in patients with T2DM begins with simple steatosis and progresses over time to nonalcoholic steatohepatitis, which is characterized by inflammation, fibrosis, apoptosis, OS, lipid peroxidation and mitochondrial dysfunction. NAFLD liver injury is a disease that mainly manifests in the disorder of glucose and lipid metabolism. When body

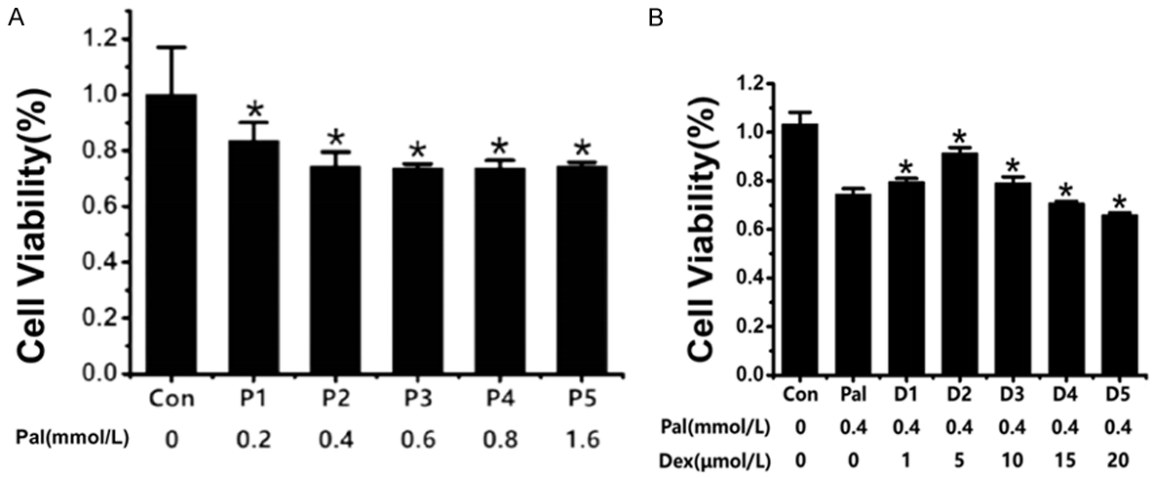

Figure 4. DEX elevated the viability of hepatocytes under high-fat injury conditions. A. The effects of Pal at different concentrations on the activity of HepG2 cells, *P<0.05, P1-P5 vs. Con. B. The effects of DEX at different concentrations on the activity of liver cells under conditions of high lipid injury. *P<0.05, D1-D5 vs. Pal. DEX: dexmedetomidine; Con: control; Pal: palmitic acid; HepG2: hepatocellular carcinoma cells.

ingests sugar and fatty acids that cannot be fully utilized for metabolism and converted into triglyceride storage, it gradually surpasses the compensatory capacity of hepatic lipid metabolism and leads to the development of steatosis in hepatocytes and severe complications such as simple fatty liver, cirrhosis and liver cancer [41]. Our previous studies have demonstrated that DEX inhibits inflammation as well as OS responses after debridement of lower extremity ulcers in patients with T2DM and that DEX has some protective effects on liver function [29]. However, the protective mechanism of DEX on the liver remains unclear and awaits further exploration. The results of the present study proved that DEX could reduce high-fat or diabetes-induced hepatic oxidative damage and watery degeneration, lipid accumulation and vacuolization, which thereby delayed apoptosis and reduced hepatotoxicity. DEX treatment significantly promoted hepatic Nrf2 protein expression, reduced hepatic OS levels and reversed high-fat or diabetes-induced liver pathological changes in vivo and in vitro.

In the clinic, ALT and AST are important indicators for the diagnosis of liver function injury. Normally, AST and ALT are present in small amount in serum and will be released from the intracellular space into the circulation when nociceptive stimuli cause hepatocyte injury with increased membrane permeability and will also be released from the cytosol and mitochondria into the blood when the injury further aggravates the organelle damage [42, 43]. Lipids mainly comprise two major classes, TG and lipoids. TG oxidation generates FFAs. The main function of HDL is to transport excess TC from extrahepatic tissues to hepatic metabolism, thereby preventing excessive TC accumulation in these tissues. LDL, also called “bad cholesterol”, carries TC into peripheral tissue cells [44-46]. Therefore, in the biochemical analysis, we focused on observing AST, ALT, TC and TG, which are indicators of liver function and lipid metabolism. In this study, the serum levels of liver function and lipid metabolism indicators in mice with T2DM and high-fat hepatocytes were significantly higher than those in the corresponding control groups, suggesting that liver damage had occurred and accompanied by a decline in lipid metabolism function. By various histomorphometric staining techniques, severe lipid deposition and a large number of hepatocytes, swelling, necrosis, vacuolization and fibrosis were seen in the livers of the model group. Our study demonstrated many fatty acid deposits in the in vivo and in vitro model groups. After DEX treatment, the liver volume of the mice gradually decreased, the liver mass gradually reduced, and the liver color gradually changed from pale to ruddy. In addition, DEX decreased AST, ALT and lipid metabolism levels. Combined with HE staining and Oil Red O staining, the results of microscopic observation showed that different

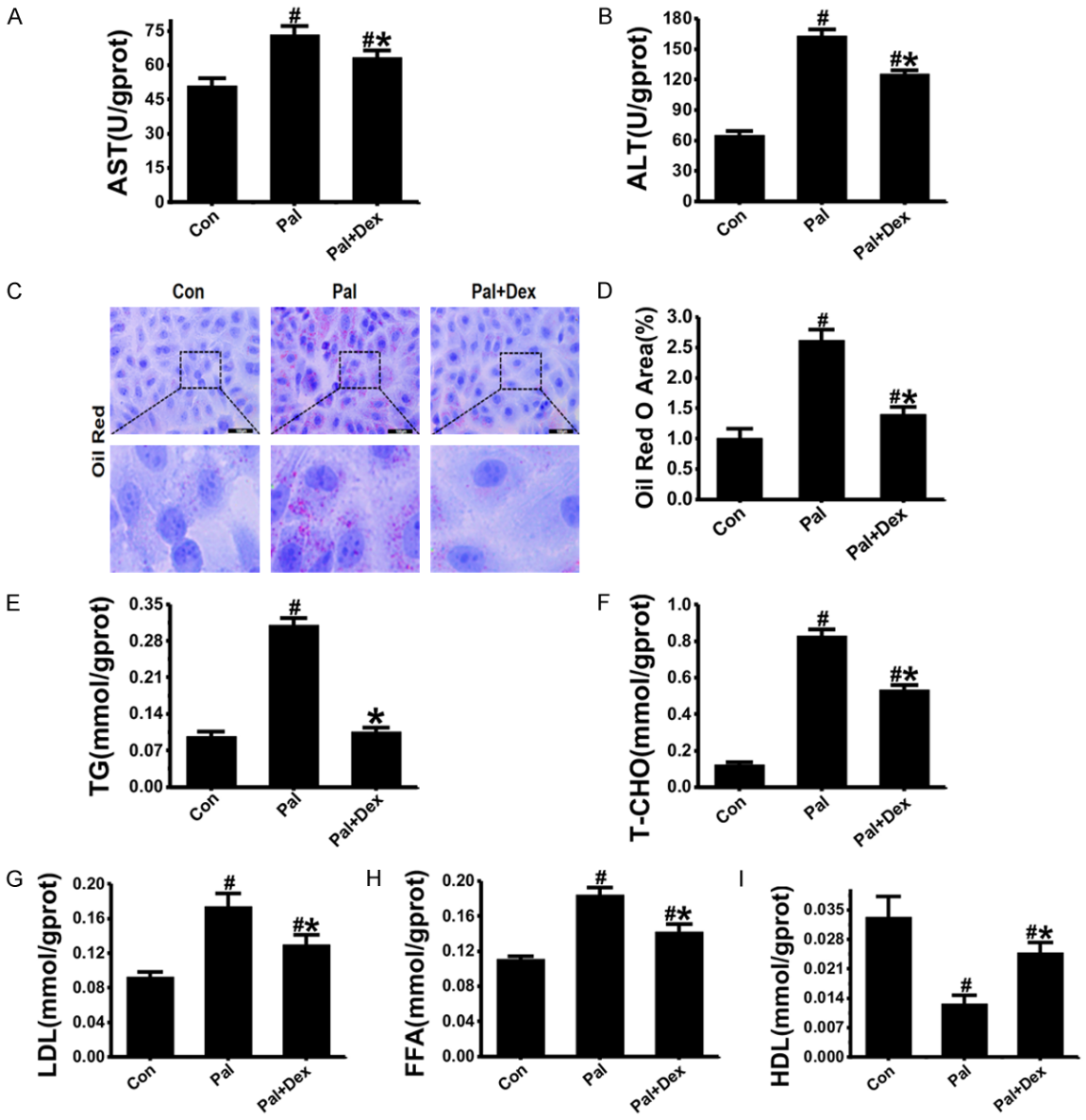

Figure 5. DEX improved hepatocyte function and lipid metabolism under high-lipid injury conditions. A and B. The effects of DEX on the function of liver cells under high lipid injury. C. Oil Red O staining (nuclei were stained purple, and lipids were stained red). The effects of DEX on lipid droplet deposition in liver cells under conditions of high lipid injury (×200 times, scale bar, 100 μm). D. Comparison of Oil Red O staining lipid droplet area of HepG2 cells in three groups. E-I. The effects of DEX on the lipid metabolism of liver cells under high lipid injury. #P<0.05, Pal and Pal + Dex vs. Con; *P<0.05, Pal + Dex vs. Pal. DEX: dexmedetomidine; Con: control; Pal: palmitic acid; HepG2: hepatocellular carcinoma cells.

doses of DEX could ameliorate hepatocyte steatosis and reduce the area of fatty red staining, which indicated that DEX could attenuate hepatocyte injury, reduce blood lipids and improve liver lipid metabolic function in T2DM mice. Unlike this study, our previous clinical study that administered DEX to our patients showed no significant changes in CHOL, LDL, TG or HDL in serum. The reason may be related to the research subjects. This study explored animal and cell models, suggesting that DEX has a relatively obvious improving effect on lipid metabolism, whereas the clinical effect in the perioperative period in patients with T2DM needs further study. In addition, the dose or pump time of DEX adopted by our previous clinical study may not be enough to cause lipid alterations and need further investigation.

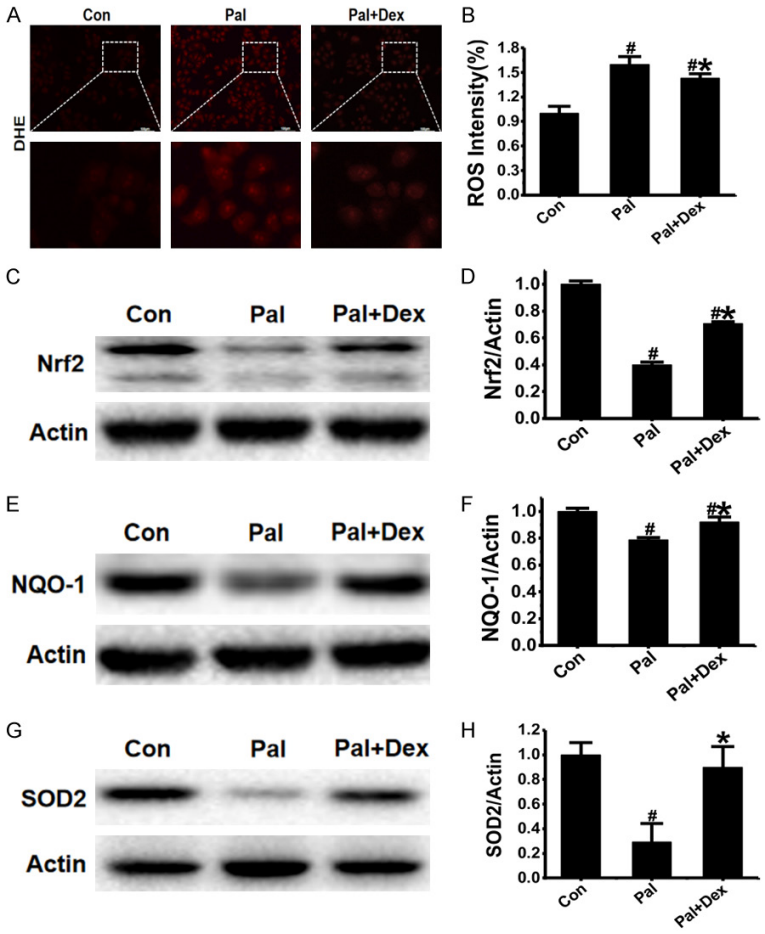

Figure 6. Activation of Nrf2 signaling by DEX improved OS damage in hepatocytes. A and B. Dihydroethidium staining (were passed through the living cell membrane into the cell to emit red fluorescence by ROS oxidation). The effects of DEX on the ROS release of liver cells under high lipid injury (×200 times, scale bar, 100 μm). C-H. Western blot assay was used to detect the levels of Nrf2 and the protein expression levels of NQO-1 and SOD2 in three groups of HepG2. #P<0.05, Pal and Pal + Dex vs. Con; *P<0.05, Pal + Dex vs. Pal. Nrf2: nuclear factor E2-related factor-2; DEX: dexmedetomidine; OS: oxidative stress; ROS: reactive oxygen species; NQO-1: NADPH quinine oxidoreductase-1; SOD2: superoxide dismutase 2; Con: control; Pal: palmitic acid; HepG2: hepatocellular carcinoma cells.

Studies have shown that, in DM patients, the liver is one of the main organs vulnerable to OS, which may be a major cause of liver tissue damage in DM patients [47-49]. Common markers of OS include the lipid peroxide MDA which indicates the level of cellular lipid oxidation [50, 51]. MDA, formed by fatty acid peroxidation, is used to detect ROS. Under normal conditions, ROS levels remain balanced [52, 53]. Excessive ROS levels can cause OS, apoptosis and other disease processes [54, 55] and promote the release of inflammatory factors such as TNF-α, IL-6 and C-reactive protein, which cause further damage to tissues and cells [56]. Furthermore, FFA can cause lipotoxicity by disrupting the balance of the ROS system [57]. Overproduction of ROS causes lipid peroxidation as well as disturbances in antioxidant and peroxidase activities, which leads to cellular dysfunction and apoptosis [58, 59]. SOD is an important antioxidant that scavenges free radicals and effectively alleviates lipid peroxidation injury in tissue ischemia [60, 61]. In this study, the levels of SOD activity in mouse liver and MDA, TNF-α and IL-6 in serum were detected. It was found that compared with those in Control group, SOD activity in the liver decreased, while MDA, IL-6 and TNF-α content in the serum increased in Model group. After the administration of DEX, the activity of hepatic SOD was enhanced and the content of MDA was decreased in the mice. These results suggest that DEX can enhance the anti-OS ability and alleviate hepatic OS injury in mice. Unlike the results of our previous clinical study, there was no significant change in serum TNF-α after the administration of different doses of DEX. The reason for this may be that the study subjects were different and that there were gene polymorphisms [62], leading to differences in the effects of DEX on inflammatory factors in different subjects. In addition, DHE staining results showed that DEX could reduce the release of ROS in hepatocytes under high-fat injury conditions, further confirming that DEX has antioxidant effects, which is consistent with the results of our clinical study.

Nrf2, a key regulator in the cellular OS response [63], is regulated by Keap1 to regulate the expression of antioxidant proteins [64]. Now, it has been found that the Nrf2 signaling pathway

is one of the core pathways of cellular antioxidant response that can significantly induce the body's endogenous antioxidant response [65]. Aberrant Nrf2 signaling aggravates OS injury and disrupts the normal redox balance in cells [66, 67]. Keap1 protein is sensitive to OS. Under normal conditions, Keap1 and Nrf2 exist in the cytoplasm as dimers. Once exposed to OS, Nrf2 will dissociate from Keap1 and be transcribed to regulate the transcriptional activation of a series of cytoprotective genes [68], such as HO-1, SOD2 and NQO-1 [69]. HO-1 has anti-apoptotic, anti-inflammatory and antioxidant functions [70, 71]. As a phase 2 detoxification enzyme, NQO-1 can catalyze the two-electron reduction of quinones and prevent them from participating in redox reactions and ROS production [72]. Some studies have shown that the activation of Nrf2 signaling can upregulate the protein expression of antioxidant genes such as HO-1 and NQO-1 [73]. With a special physiological activity, SOD2 is the first substance to scavenge free radicals in living organisms. These free radicals are present in various tissues of animals, especially the liver. When the liver is damaged by OS, SOD2 expression is significantly reduced [74-76]. To confirm whether DEX could activate the Nrf2 signaling pathway, we examined the expression of Nrf2 pathway proteins and their downstream antioxidant genes HO-1, NQO-1, and SOD2 in mouse liver and HepG2 cells. The results showed that antioxidant proteins Nrf2, HO-1 and SOD2 were significantly decreased in diabetic mice and that Nrf2, NQO-1 and SOD2 were significantly decreased in hepatocytes with high-fat content. Nrf2, HO-1, NQO-1 and SOD2 protein expressions were increased after DEX treatment, and the effects were dose-dependent. The results suggest that DEX can exert protective effects on the livers of T2DM mice and high-fat hepatocytes, the main mechanism of which is upregulating the expression levels of the Nrf2 signaling pathway to increase the antioxidant stress capacity. It should be noted that a large dose of DEX in the D75 group had an over-activation effect on the related proteins of the Nrf2 signaling pathway. This over-activation had not been adversely affected according to the results of this experiment on the SOD and MDA indicators of OS, but the final effects need to be determined in further studies.

The literature reports on fatty liver disease therapeutics mostly point out that drugs can achieve therapeutic effects through their antioxidant effects [77]. T2DM patients with high-fat liver injury need various types of anesthetic drugs during the perioperative period, of which DEX has been proven to have antioxidant effects [78]. This study investigated the protective mechanism of DEX on the liver of T2DM mice and high-fat injured hepatocytes. In the present study, DEX exerted a concentration-dependent protective effect on hepatocytes under high-fat injury conditions and on the liver of diabetic mice. Therefore, the perioperative use of DEX in T2DM patients with high-fat liver injury should also find the appropriate concentration and time to achieve an ideal hepatoprotective effect.

There were certain limitations in the implementation of the experiment due to insufficient time and funding. First, in this study, we only observed the protein expression levels, and we will further explore the mRNA expression in future experiments. Second, HepG2 cells were selected in this study. Many previous studies have confirmed that HepG2 cells can be used for liver steatosis models, but they are not as representative as primary liver cells. In the follow-up study, we will take primary cells as research subjects to deeply explore the mechanism of DEX on hepatocyte protection.

In conclusion, this study demonstrates that DEX can protect the liver from structural and functional damage caused by high fat and diabetes. DEX can reverse the nuclear translocation disorder of Nrf2 caused by high fat and diabetes, leading to increased expression of the antioxidant protein to inhibit OS, reduce lipid deposition, and protect the liver function in vivo and in vitro. The present basic trial validated our previous clinical findings that DEX exerted hepatoprotective effects in perioperative diabetic patients and further elucidated its mechanism of action.

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Disclosure of conflict of interest

None.

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