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Dexmedetomidine-mediated protection against septic liver injury depends on TLR4/MyD88/NF-κB signaling downregulation partly via cholinergic anti-inflammatory mechanisms

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ABSTRACT

Background: Uncontrolled inflammatory responses exacerbate the pathogenesis of septic acute liver injury (ALI), posing a lethal threat to the host. Dexmedetomidine (DEX) has been reported to possess protective properties in inflammatory conditions. This study aimed to investigate whether DEX pretreatment exhibits hepatoprotection against ALI induced by lipopolysaccharide (LPS) in rats and determine its possible molecular mechanism.

Methods: Septic ALI was induced by intravenous injection of LPS. The rats received DEX intraperitoneally 30 min before LPS administration. α-Bungarotoxin (α-BGT), a specific α7 nicotinic acetylcholine receptor (α7nAChR) antagonist, was administered intraperitoneally 1 h before LPS exposure. The role of the vagus nerve was verified by performing unilateral cervical vagotomy or sham surgery before sepsis.

Results: The expression of α7nAChR, toll-like receptor 4 (TLR4), high mobility group box 1 (HMGB1), and cleaved caspase-3 increased, peaking 24 h during sepsis. DEX enhanced α7nAChR activation and reduced TLR4 expression upon challenge with LPS. DEX significantly prevented LPS-induced ALI, which was associated with increased survival, the mitigation of pathological changes, the attenuation of inflammatory cytokine expression and apoptosis, and the downregulation of TLR4/MyD88/NF-κB pathway. Moreover, the hepatoprotective effect of DEX was abolished by α-BGT. Further investigation established that vagotomy, compared to sham surgery, triggered more severe pathogenic manifestations and higher proinflammatory cytokine levels. The inhibitory effects of DEX were shown in sham-operated rats but not in vagotomized rats.

Conclusions: Our data highlight the pivotal function of α7nAChR and intact vagus nerves in protecting against LPS-induced ALI through inhibiting the TLR4/MyD88/NF-κB signaling pathway upon pretreatment with DEX.

1. Introduction

Sepsis, defined as dysregulated host response to infection, can lead to life-threatening organ dysfunction and is associated with high mortality rates [1,2]. The excessive generation of proinflammatory cytokines is the main contributor to sepsis-initiated organ disorder. The liver acts as a pivotal defense against microorganisms, which explains why 80% to 90% of macrophages are in the liver [3]. These liver-resident macrophages, including Kupffer cells and endothelial cells efficiently phagocytose bacteria and eliminate endotoxins for immunological homeostasis [4]. Alterations in the control of liver function can acutely promote the progression of multiple organ failure in an ill-defined manner. Acute liver injury (ALI) is a complex pathophysiological process involving a series of mediators and signaling pathways, including inflammation, apoptosis, and necrosis that results in multiple organ failure.

Lipopolysaccharide (LPS) is the proinflammatory component of the outer membrane of most Gram-negative bacteria [5]. LPS injection has been widely used as a model of experimental ALI [6]. As the first line of defense against pathogens, the toll-like receptor (TLR) family is one of the molecular mechanisms that mediate the deleterious effects that occur during septic liver injury [7]. It has been reported that Kupffer cells in the liver are among the first cells to initiate the inflammatory response through the sensing of LPS by TLRs [8]. TLR4 is widely

Abbreviations: LPS, lipopolysaccharide; DEX, dexmedetomidine; α-BGT, α-Bungarotoxin; ALI, acute liver injury; CAP, cholinergic anti-inflammatory pathway; α7nAChR, α7 nicotinic acetylcholine receptor; TLR4, toll-like receptor 4; VN, vagus nerve; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor-κB; ACh, acetylcholine; HMGB1, high mobility group box 1; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; VGX, vagotomy; Sham, sham surgery

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expressed on the surface of cells in the liver and participates in promoting inflammatory reactions and organ injury in various pathological disorders [9,10]. The activation of the TLR4 pathway by LPS promotes the binding of myeloid differentiating factor 88 (MyD88) to TLRs and then activates IKK phosphorylation, thereby inducing the phosphorylation and degradation of inhibitory IκB proteins and the subsequent release and nuclear translocation of nuclear factor-κB (NF-κB) [11]. NF-κB activation caused by bacterial endotoxins is required to transcribe and generate cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-6.

Nicotinic acetylcholine receptors (nAChRs) are known for their important roles in modulating the cholinergic anti-inflammatory pathway (CAP), through which the brain modulates inflammation at various locations in the body [12]. In addition, the acetylcholine receptor α7 subunit (α7nAChR) is required for CAP [13]. Acetylcholine (ACh), which is released by CAP, is considered the principal mediator through vagus nerve stimulation; ACh then binds to macrophages and other immunocompetent cells that express α7nAChR, modulating or participating in the inflammatory response to suppress proinflammatory cytokine production [13,14]. A study by Sakata et al. revealed that the highest accumulation of α7nAChR in the human body is in the liver [15]. In a series of in vitro experiments, α7nAChR was detected to be located on a variety of cells such as resident macrophages, including Kupffer cells [16] and endothelial cells [17]. Recently, α7nAChR activation was found to improve spinal cord injury [18], relieve chronic inflammatory pain conditions [19], and protect remote vascular dysfunction [20]. The role of α7nAChR-mediated signaling in the protection against sepsis-induced tissue injury is not well understood. However, this signaling pathway has been demonstrated to have positive effects on the downregulation of TLR4 and NF-κB activation in septic tissue injury [21,22].

The anti-inflammatory and anti-apoptotic effects of dexmedetomidine (DEX), a potent and highly selective α2-adrenoceptor agonist, have been outlined in various pathological settings [23–25]. The clinical application of DEX offers good perioperative hemodynamic stability and reduces intraoperative anesthetic requirements. Furthermore, previous studies have found that the anti-inflammatory mechanism of DEX may be associated with the benefits of the parasympathetic nervous system, which activates the efferent vagus nerve and leads to the suppression of biosynthesis and release of pro-inflammatory cytokines [26]. Limited studies evaluating the effect of DEX in septic ALI and the underlying mechanisms of α7nAChR have been conducted. Therefore, we hypothesized that DEX protects against septic liver injury by activating the α7nAChR subtype and that the mechanism involves the suppression of the TLR4/MyD88/NF-κB pathway.

2. Materials and methods

2.1. Rats

Male Sprague–Dawley rats (200–250 g) were purchased from Tianqin Biotechnology Co. (Hunan, China). The rats were housed at 24 ± 1 °C with a relative humidity of 40%–50% in a clean environment under a 12-h light/dark cycle for 1 week before LPS induction. The rats were allowed access to food and water ad libitum. All the experimental protocols and procedures for this study were approved by the Laboratory Animal Welfare and Ethics Committee of Central South University. The manuscript adheres to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for reporting animal experiments.

2.2. Endotoxemia and drug treatment

Endotoxemia was induced by the tail vein injection of LPS (endotoxin, *Escherichia coli* 0111: B4; Sigma, St. Louis, MO, USA; 6 mg/kg, dissolved in saline). DEX (Hengrui Medicine, Jiangsu, China; 3, 10, and 30 μg/kg) or saline (vehicle) was administered intraperitoneally 30 min before sepsis was established. α-Bungarotoxin (α-BGT; Sigma; 1 μg/kg, dissolved in saline), a specific α7nAChR antagonist, was administered intraperitoneally 1 h prior to LPS induction. The dose and time points of DEX and α-BGT administration were selected based on previous reports [24,27]. Blood samples were obtained 3, 6, 12, and 24 h after LPS from the medial canthus vein. The rats were sacrificed 24 h after LPS or vehicle intraperitoneal injection with sodium pentobarbital (60 mg/kg) anesthesia. Then, the liver tissue was removed and separated into four parts for different assays as follows: the right lobe of the liver was used for Western blotting, the middle lobe was used for quantitative polymerase chain reaction (qPCR), the left middle lobe was used for enzyme-linked immunosorbent assay (ELISA), and the rest of the liver was used for hematoxylin-eosin (H&E) staining.

2.3. Experimental protocol

The rats were randomly assigned to the four separate experiments shown in the timeline of the experimental design (Supplemental File 1). The experimental groups and number of animals used in experiments 1–4 are listed in a table (Supplemental File 2).

2.3.1. Experiment 1

To determine the time course of endogenous α7nAChR, TLR4, and cleaved-caspase 3 expression levels in sepsis-induced ALI, the rats were randomly divided into six groups (n = 6): the control (control) group, the 3 h after LPS (3 h LPS) group, the 6 h after LPS (6 h LPS) group, the 12 h after LPS (12 h LPS) group, the 24 h after LPS (24 h LPS) group, and the 72 h after LPS (72 h LPS) group. H&E staining was employed to assess liver pathological injury after LPS intervention for different durations. Western blot analysis was used to detect α7nAChR, TLR4, and cleaved caspase-3 protein levels in the liver tissues of each group. HMGB1 and ACh levels in the serum samples were measured by enzyme-linked immunosorbent assay (ELISA) analysis. The optimal LPS intervention time was 24 h according to the results above, and this time was used for subsequent experiments.

2.3.2. Experiment 2

To investigate whether DEX preconditioning attenuates lethality or liver injury in septic rats and to determine the optimal concentration of the drug in our experimental conditions, graded doses of DEX (3, 10, and 30 μg/kg) were administered intraperitoneally 30 min before LPS exposures. Ninety-six rats were randomly assigned to six groups (n = 16): the vehicle-treated control (control) group, the 30 μg/kg DEX-treated control (DEX) group, the vehicle plus LPS-treated (LPS) group, the 3 μg/kg DEX plus LPS-treated (LPS + DEX3) group, the 10 μg/kg DEX plus LPS-treated (LPS + DEX10) group, and the 30 μg/kg DEX plus LPS-treated (LPS + DEX30) group. Then, survival rates over 120 h were recorded, and the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the serum were examined 24 h after LPS activation. Based on these results, 30 μg/kg DEX was selected for further biochemical studies.

Next, twenty-four rats were randomly divided into four groups (n = 6): the vehicle-treated control (control) group, the 30 μg/kg DEX-treated control (DEX) group, the vehicle plus LPS-treated (LPS) group, and the 30 μg/kg DEX plus LPS-treated (LPS + DEX) group. Twenty-four hours after infection, the rats were sacrificed. α7nAChR, TLR4, cleaved caspase-3, and Bcl-2 were quantified by Western blotting. Apoptosis was quantified by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining by determining the proportion of TUNEL-positive cells. ELISA assays were performed to examine ACh, TNF-α, and IL-6 levels in the serum 6 h after LPS injection and HMGB1 levels in the serum 24 h after LPS injection.

2.3.3. Experiment 3

The possible mechanisms of DEX-mediated anti-inflammatory

effects in the septic liver were explored. A specific antagonist of α7nAChR, α-BGT was administered by intraperitoneal injection 30 min prior to the administration of 30 μg/kg DEX. LPS was administered intravenously 30 min later. A total of twenty-four rats were randomly divided into four groups ($n = 6$): the vehicle-treated control (control) group, the vehicle plus LPS-treated (LPS) group, the 30 μg/kg DEX plus LPS-treated (LPS + DEX) group, and the 1 μg/kg α-BGT plus 30 μg/kg DEX plus LPS-treated (LPS + DEX + α-BGT) group. TNF-α and IL-6 levels in the serum were detected by ELISA at 6 h. The livers were harvested at 24 h for histological analysis. TLR4, MyD88, NF-κB p65, phosphorylated NF-κB p65, IκB, and phosphorylated IκB were analyzed by Western blot analysis. The mRNA expression of TNF-α, IL-6, TLR4, and MyD88 were further surveyed by qPCR.

2.3.4. Experiment 4

We further investigated the role of intact vagus nerves on the hepatoprotective effect of DEX. Rats were subjected to vagotomy or sham surgery 4 days before LPS intervention. The animals were pretreated with 30 μg/kg DEX 30 min before the establishment of the sepsis model. The subjects were randomly assigned to four groups ($n = 6$): the vehicle plus LPS-treated sham surgery (sham + LPS) group, the vehicle plus LPS-treated vagotomy (VGX + LPS) group, the DEX plus LPS-treated sham surgery (sham + LPS + DEX) group, and the DEX plus LPS-treated vagotomy (VGX + LPS + DEX) group. Six hours after sepsis, cytokine levels were estimated by ELISA. The livers were extracted at 24 h for H&E staining analysis.

2.4. Survival test

Rats ($n = 16$) were subjected to LPS or vehicle injection and administered drugs as previously described. The rats were then monitored, and the time until death was recorded when appropriate. The rats were observed for 120 h after sepsis and sacrificed by the injection of a lethal dose of sodium pentobarbital.

2.5. Assessment of liver damage

Serum samples were obtained from blood collected by cardiac puncture 24 h after LPS to determine ALT and AST levels with an automatic biochemical instrument (Beckman Coulter, Unicel DXC800, USA).

2.6. Histological analysis of liver tissue

Liver tissues were removed and fixed by immersion in 10% neutral-buffered formalin. The samples were then embedded in paraffin, sliced into 4-μm sections, and stained with H&E in preparation for blinded histologic assessment. Histologic changes were evaluated in random, nonconsecutive fields at 400× magnification (Olympus BX51/Olympus DP71; Olympus). Each section was evaluated by three investigators blinded to the experimental information.

2.7. Enzyme-linked immunosorbent assay

Serum samples were obtained from blood collected by cardiac puncture or from the medial canthus vein immediately following euthanasia. The blood was allowed to clot for 1 h and then centrifuged at 3000 rpm for 15 min. The supernatants were subsequently collected to determine TNF-α, IL-6, HMGB1 and ACh levels by using ELISA kits (R& D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.8. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

A TUNEL detection kit (Beyotime Institute of Biotechnology, Jiangsu, China) was adopted to evaluate apoptosis of hepatocyte cells according to the manufacturer's instructions. Sections (4 μm) from the paraffin-embedded blocks were dewaxed before non-DNase protease K was added. The sections were then rinsed three times with phosphate-buffered saline (PBS) for 2 min each, labeled at 37 °C for 60 min with TUNEL reaction mixture, and rinsed again with PBS. The cells were examined and recorded by fluorescence microscopy. TUNEL-positive cells in 10–20 randomly selected fields (200×) from a coverslip were counted with ImageJ software (ImageJ 1.4, NIH, USA).

2.9. Western blot analysis

Western blot analysis was performed as previously described [24]. The right lobe of the liver was homogenized in radioimmunoprecipitation (RIPA) assay lysis buffer mixed with All-in-One and PMSF and centrifuged at 4 °C for 30 min at 14,000 × *g*. The supernatant was collected, and the protein concentration was estimated using BCA reagent. Equal amounts of protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane at 80 V for 20 min. Then, the voltage was increased to 120 V for 60 min. The membrane was blocked with skim milk for 1 h at room temperature and subsequently incubated at 4 °C overnight with the following primary antibodies: mouse anti-TLR4 (2 μg/ml; Novus, NB100-56566SS), rabbit anti-nicotinic acetylcholine receptor alpha 7 (α7nAChR, 1:400; Abcam, ab10096), rabbit anti-cleaved caspase-3 (1:1000; Cell Signaling Technology, #9661), rabbit anti-MyD88 (1:1000; Abcam, ab2064), rabbit anti-NF-κB p65 (1:1000; Cell Signaling Technology, #8242), rabbit anti-phosphorylated-NF-κB p65 (p-NF-κB (Ser536); 1:1000, Cell Signaling Technology, # 3033), rabbit anti-IκB (1:1000; Abcam, ab32518), p-IκB (p-IκB (Ser32); 1:1000, Cell Signaling Technology, #2859), and rabbit anti-β-actin (1:1000; Cell Signaling Technology, #4970). Appropriate secondary antibodies (1:2000; Beyotime) were selected for incubation with the membrane for 1 h at room temperature. The bands were probed with an ECL Plus supersensitive luminescent agent and visualized on film by washing with developing and fixing solution successively. The relative density of the protein immunoblot images was analyzed by ImageJ software (ImageJ 1.4, NIH, USA).

2.10. Quantitative polymerase chain reaction

Total RNA was extracted from the middle lobe of the liver tissue by using Trizol reagent according to the manufacturer's protocol (Roche Diagnostic GmbH, Mannheim, Germany). Total RNA was reverse transcribed to synthesize cDNA with a Roche RNA PCR kit (Roche Diagnostic GmbH, Mannheim, Germany). qPCR was performed with a LightCycler Nano instrument (LightCycler 480, Roche, USA). Rat primers of the following sequences were purchased from Shengon Biotechnology Co., Ltd.: TLR4 forward, AGCCGCTCTGGCATCATCTT; TLR4 reverse, CTCCCACTCGAGGTAGGTGT; MyD88 forward, CCGCA TCGAGGAGGACTG; MyD88 reverse, CTGTGGGACACTGCTCTCCA; TNF-α forward, GCATGATCCGAGATGTGGAACTGG; TNF-α reverse, CGCCACGAGCAGGAATGAGAAG; IL-6 forward, AGGAGTGGCTAAGG ACCAAGACC; IL-6 reverse, TGCCGAGTAGACCTCATAGTGACC; HMGB-1, forward, ACAACACTGCTGCGGATGACAAG; HMGB-1 reverse, CCTC CTCGTCGTCTTCCTCTTCC. The PCR amplification cycling conditions were as follows: initial denaturation at 95 °C for 5 min followed by holding 95 °C for 10 s, 56 °C for 20 s, and 72 °C for 20 s for 40 cycles. We calculated the mean fold change in the expression of TLR4, MyD88, TNF-α, IL-6, and HMGB1 mRNA in the experimental group relative to that in the control group. Glyceraldehyde 3-phosphate dehydrogenase was used as the reference gene. The relative expression levels of these genes in the experimental group were compared with those in the control group using the $2^{-\Delta\Delta Ct}$ method.

2.11. Vagotomy

For this procedure, the rats were anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg) as previously described [28]. The left cervical vagus trunk was exposed, ligated with a 4–0 silk suture, and divided. Subsequently, the skin was closed with three sutures. In the sham-operated rats, the left vagal trunk was exposed and isolated from the surrounding tissue but not transected. Postoperatively, the animals were monitored daily for 4 days before the induction of septic liver injury.

2.12. Statistical analysis

All data are expressed as the mean and standard deviation (mean ± SD). Statistical analysis was performed with GraphPad Prism 7.0 software (Graph Pad Software, San Diego, CA). Survival data were analyzed using a Kaplan–Meier curve and a log-rank test. All other data were analyzed by one-way analysis of variance (ANOVA) followed by multiple comparisons between groups by using Tukey's multiple comparisons test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Animal mortality and exclusion

None of the DEX-treated or vehicle-treated rats died in this study. The mortality rates of the rats from the different experiments and groups in this study were calculated, and extra rats were added. Three rats were excluded from the study because of death after vagotomy or sham operation. (Supplemental File 2: Table 1)

3.2. Time course of liver injury pathology after LPS treatment

Histopathological change is a direct indication of liver injury. Histological assessment was performed 3, 6, 12, 24, and 72 h after LPS. As presented in Fig. 1, the liver lobules were structured clearly, and regular arrangement of hepatocytes with no degeneration, necrosis, or inflammatory cell infiltration was observed in the liver at 0 h. Slight histopathological changes, which involved individual neutrophils in the blood sinus rather than degeneration or necrosis, were found in liver sections obtained 3 h or 6 h after infection. At 12 h, the liver tissues displayed many inflammatory cells into the hepatic sinus, degeneration, and individual hepatocellular necrosis. Typical LPS-induced pathology, including extensive areas of inflammatory cell infiltration in the blood sinus and focal necrosis area of hepatocytes, was observed in liver tissues at 24 h. However, diffuse vacuolar degeneration spread throughout the liver tissue, indicating severe hepatocyte hypoxia 72 h after sepsis. These results suggest that ALI was successfully established in the rats that received LPS injection.

3.3. Hepatic inflammation and cellular apoptosis peaks 24 h after LPS induction

Liver tissues were harvested from rats 0, 3, 6, 12, 24, and 72 h after LPS administration. Dynamic changes in hepatic inflammation were investigated by Western blot analysis and ELISA. Specifically, the expression of α7nAChR, an essential component of the CAP, and its endogenous ligand ACh was detected. Given the important role of TLR4 in the proinflammatory response, the expression of TLR4 and its endogenous ligand HMGB1 was analyzed. In addition, the level of cleaved caspase-3 was examined because it represents apoptosis activation. The results showed that the protein expression of α7nAChR, TLR4, cleaved caspase-3, and HMGB1 increased as early as 3 h, peaked at 24 h, and was decreased 72 h after LPS induction. The ACh level increased starting at 3 h and peaked at 6 h ($p < 0.05$, Fig. 2). Thus, intervention with LPS for 24 h was used for subsequent molecular investigations unless otherwise stated.

3.4. Pretreatment with 30 μg/kg DEX promotes the optimal inhibition of LPS-induced mortality and septic liver injury

Pretreatment with graded doses of DEX (3, 10, and 30 μg/kg) was administered intraperitoneally to determine the optimal dose of treatment for our experimental conditions. The effects of the drug on survival rate and liver function in septic rats were evaluated. DEX at 30 μg/kg alone did not affect mortality or serum ALT and AST levels ($p > 0.05$; Fig. 3). In the survival test, 3, 10, and 30 μg/kg DEX routinely reduced sepsis-induced mortality over 120 h. DEX pretreatment (10 and 30 μg/kg), compared with LPS treatment alone, significantly protected against LPS-induced mortality ($p < 0.05$; Fig. 3A). In

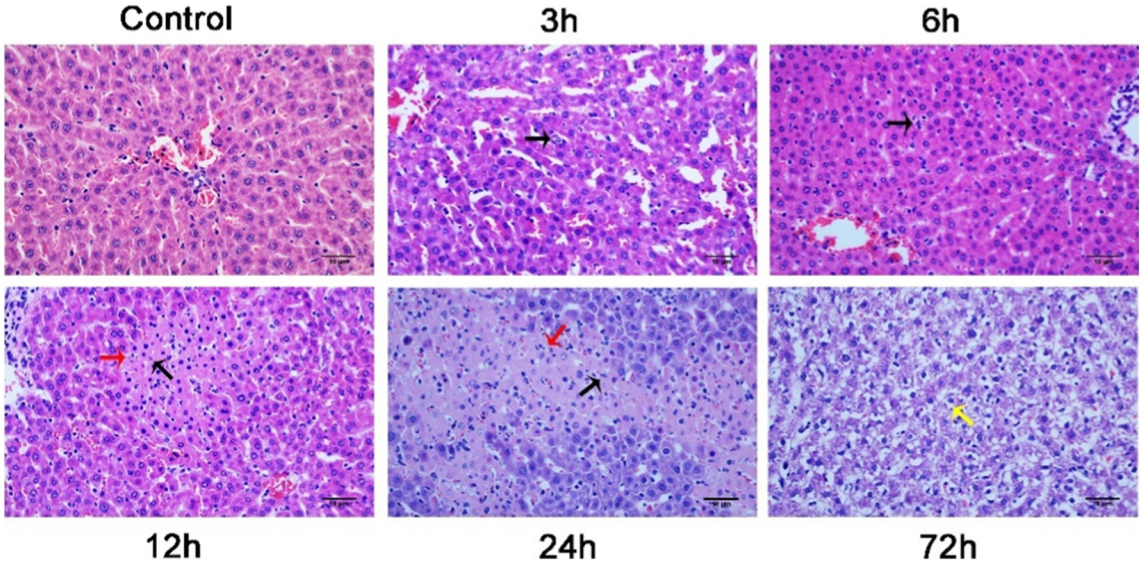

Fig. 1. LPS-induced septic ALI rat model. Rats were intravenously injected with LPS and evaluated after 0, 3, 6, 12, 24 or 72 h. The liver tissues were harvested and stained with H&E (400×) ($n = 3$). The pathological changes in the liver were observed in five nonoverlapping fields using a light microscope. The black arrows indicate inflammatory cells. The red arrows indicate hepatocyte necrosis. The yellow arrow indicates vacuolar degeneration. Scale bar (black) = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

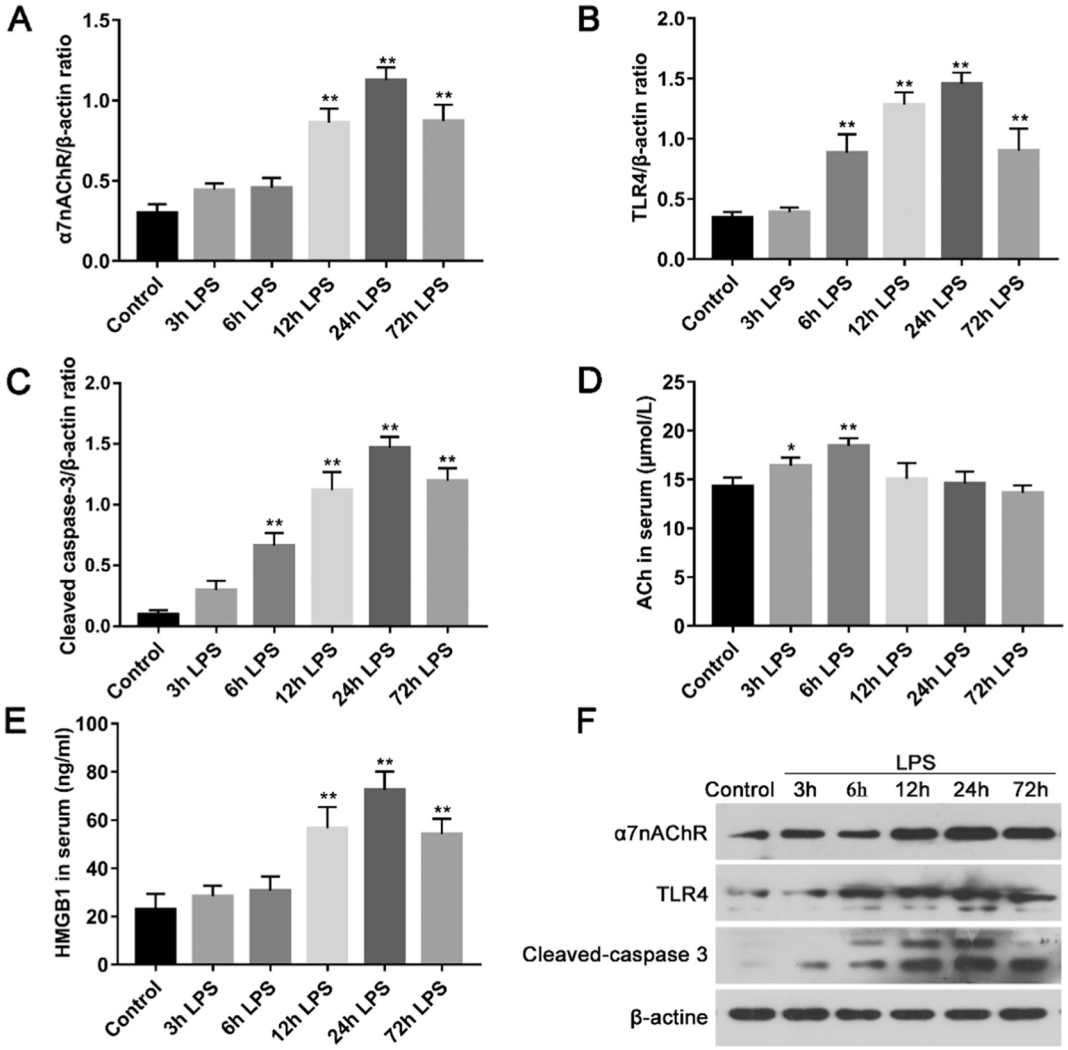

Fig. 2. Proinflammatory and anti-inflammatory proteins are simultaneously and maximally upregulated 24 h after LPS infection in rats. (A) Quantitative results of α7nAChR (n = 3), (B) TLR4 (n = 3), and (C) cleaved caspase-3 (n = 3) bands in (F) at the indicated time after LPS stimulation (n = 3). (D) ACh and (E) HMGB1 expression (n = 6). The data are expressed as the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ versus the control group. α7nAChR = α7 nicotinic acetylcholine receptor; TLR4 = Toll-like receptor 4; ACh = acetylcholine; HMGB1 = high mobility group box 1; LPS = lipopolysaccharide.

particular, DEX preconditioning yielded beneficial hepatic alterations in a dose-dependent manner, as determined by histopathologic evaluation (data not shown). Significant changes in the concentrations of serum ALT and AST were elicited in ALI rats. DEX pretreatment dose-dependently attenuated these increases, which is consistent with its effect on liver pathologic injury (Fig. 3B, C). Based on these results, 30 μg/kg DEX was chosen for biochemical studies in subsequent experiments.

3.5. DEX pretreatment effectively attenuates hepatic inflammation and apoptosis induced by LPS

We then examined the effects of intraperitoneal DEX treatment on the expression of TLR4, α7nAChR, and ACh and the number of apoptotic cells in the septic liver at 24 h. As depicted in Fig. 4, DEX significantly inhibited the overexpression of TLR4 and HMGB1 but further augmented the expression of α7nAChR and ACh in septic rats ($p < 0.05$, Fig. 4A, B, E, F). The levels of the apoptosis-related proteins cleaved caspase-3 and Bcl-2 were measured by Western blotting. Our results showed an increase in cleaved caspase-3 and a reduction in Bcl-2 in the LPS group, whereas these changes were counteracted significantly by DEX pretreatment (Fig. 4C, D). TUNEL staining further revealed that LPS led to a significant increase in the number of TUNEL-positive cells in the liver, but DEX treatment markedly reduced the number of TUNEL-positive cells ($p < 0.05$, Fig. 5A, B). Sepsis is characterized by the dysregulated release of proinflammatory cytokines. In this study, an overwhelming amount of inflammatory mediators, such as TNF-α and IL-6, in the serum of the LPS group was elicited. The administration of DEX 30 min before LPS robustly attenuated the production of these cytokines ($p < 0.05$, Fig. 5C, D). DEX alone showed no influence on the abovementioned proteins ($p > 0.05$; Figs. 4, 5).

3.6. DEX-induced inhibition of septic histopathological injury and inflammation requires α7nAChR signaling

Considering the improvement in liver function and anti-inflammation after DEX treatment, the mechanism that primarily contributes to the hepatoprotective effect of DEX was determined by pre-injecting an α7nAChR antagonist before the administration of DEX. As presented in Fig. 6A, the preemptive administration of DEX substantially blunted the LPS-induced increases in the inflammatory cell infiltration area, hepatocyte degeneration and flake necrosis area, and acidophilic degeneration was the main manifestation. Pretreatment with the selective α7nAChR antagonist α-BGT prevented the protective effect of DEX

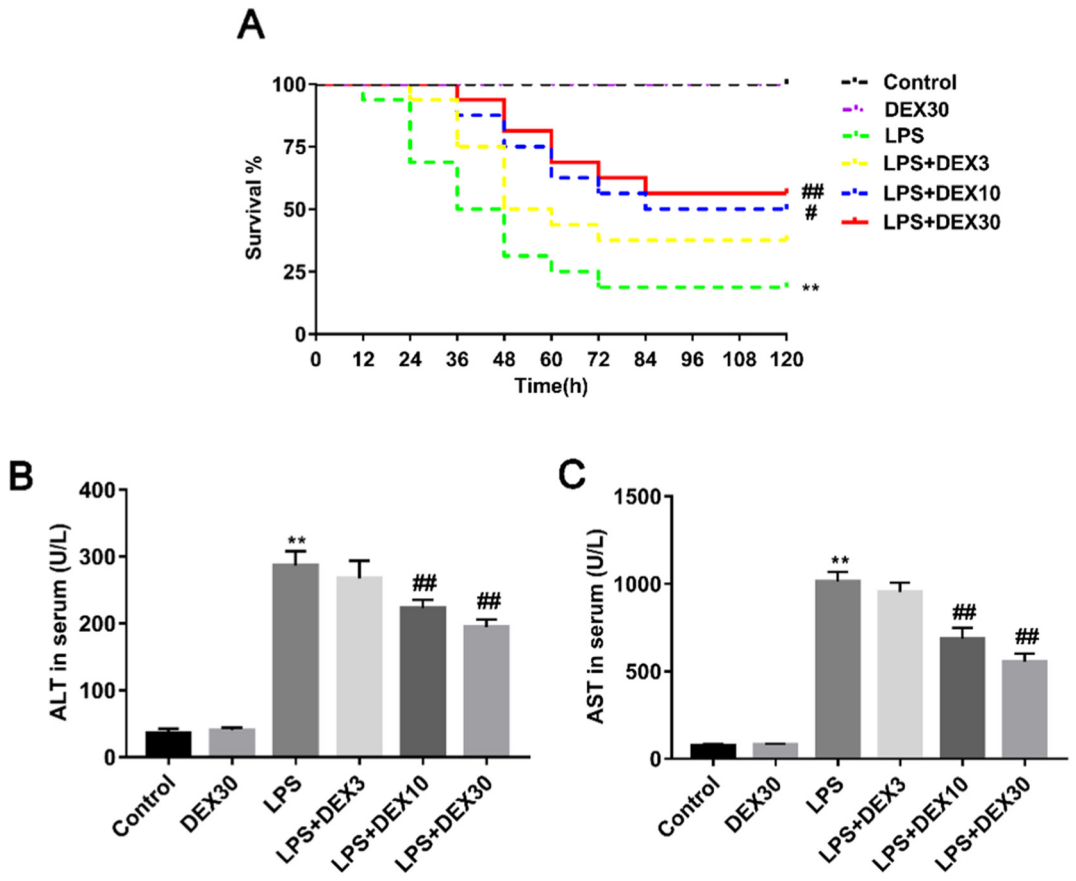

Fig. 3. DEX treatment protects against LPS-induced mortality and restores the liver function. (A) Kaplan–Meier survival curves were generated for rats treated with LPS and normal saline or 3, 10, or 30 μg/kg DEX (n = 16). (B) Serum ALT levels (n = 6) and (C) AST levels (n = 6). The data are expressed as the mean ± SD. $^{**}p < 0.01$ versus the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus the LPS group. LPS = lipopolysaccharide; DEX = dexmedetomidine; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

against liver damage in rats sacrificed 24 h following LPS exposure. H&E staining of liver tissues from the control group demonstrated normal histology, as previously shown. On the other hand, the LPS-triggered severe inflammatory cascade was substantially suppressed by DEX through the inhibition of the massive release of proinflammatory mediators such as TNF-α and IL-6, at both the mRNA and protein levels. However, the anti-inflammatory effect of DEX was inhibited profoundly by α-BGT pretreatment, which is consistent with its effect on liver injury pathology. ($p < 0.05$; Fig. 6B–E). No statistically significant difference was observed in TNF-α or IL-6 expression between the LPS and DEX + LPS + α-BGT groups. The results indicate that DEX pretreatment attenuates the release of factors and histological injury and that inhibition is noticeably abated by α-BGT.

3.7. DEX treatment attenuates hepatic inflammation through the TLR4/MyD88/NF-κB pathway after LPS via an α7nAChR-dependent pathway

We initially determined that DEX has a modulatory role in the expression of the TLR4 and α7nAChR proteins, which are involved in maintaining a regular immune mechanism. The TLR4-mediated MyD88/NF-κB pathway was evaluated by Western blotting and qPCR through the use of α-BGT. The levels of TLR4, MyD88, p-IκB, and p-NF-κB were upregulated in the LPS group. Notably, DEX administration remarkably inhibited the expression of TLR4 as well as downstream proteins. qPCR analysis showed that DEX significantly reduced the overexpression of TLR4 mRNA and MyD88 mRNA caused by LPS. Nevertheless, it is intriguing that the inhibition of DEX on TLR4-mediated signaling was abrogated by α-BGT ($p < 0.05$; Fig. 7).

3.8. Reduction in the inflammatory response after DEX treatment is dependent on, at least partially, the intact vagus nerve

A previous study [28] revealed that histopathological damage to the liver during septic peritonitis as a result of vagotomy is exacerbated. In this report, we assessed the role of the vagus nerve in the initial inflammatory response during septic liver injury and then explored the effect of DEX on both vagotomized and sham-operated septic rats. We established a vagotomy model by left-sided unilateral cervical vagotomy or performed a comparable sham surgical procedure, in which the vagus nerves were isolated but not transected, 4 days before LPS infection. Obvious hepatic tissue injury was described as inflammatory cell infiltration and focal necrosis area in sham-operated septic rats sacrificed at 24 h. Septic liver damage in which the cholinergic system was manipulated by vagotomy, compared to sham operation, was accompanied by more severe histopathological evidence of neutrophil infiltration, flake necrosis, and degeneration. In contrast, DEX pretreatment markedly ameliorated liver damage in sham-operated rats challenged with LPS, in which acidophilic degeneration was the main feature. However, DEX failed to induce any improvement in pathological changes in vagotomized rats upon the administration of LPS (Fig. 8A). In accord with hepatic pathological injury, rats subjected to sham operation showed sharply amplified levels of TNF-a and IL-6 in the serum at 6 h, while more intensive aggravation of the inflammatory response was observed in septic rats subjected to vagotomy. Conversely, DEX treatment in sham-operated septic rats resulted in markedly lower levels of these mediators in the serum compared with those in the vehicle treatment in sham-operated rats ($p < 0.05$; Fig. 8B, C). Similar to its effect on pathological injury in vagotomized rats, DEX pretreatment

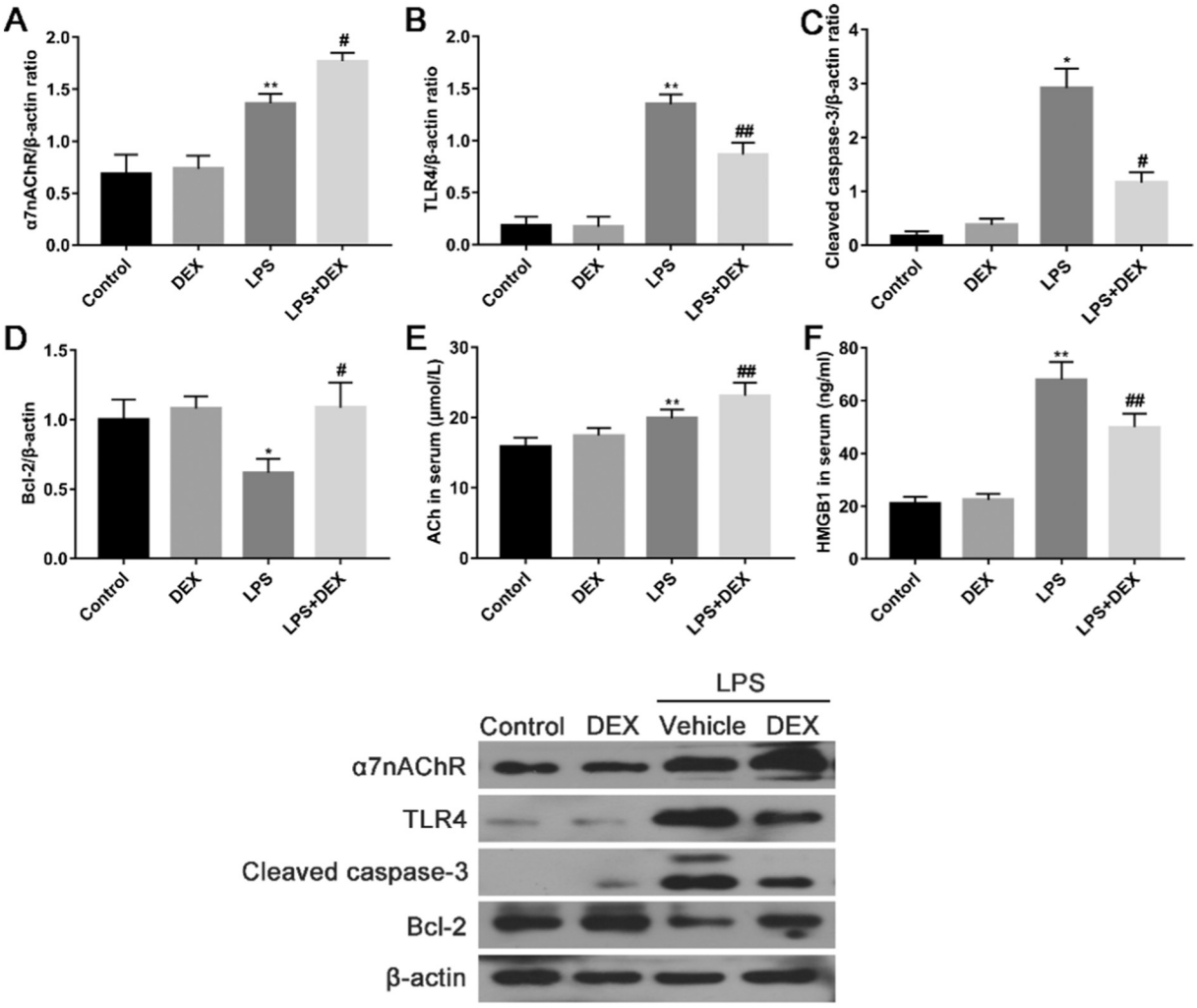

Fig. 4. DEX decreases the expression of the proinflammatory proteins and apoptosis-related proteins but increases the expression of anti-inflammatory proteins in septic ALI rats. Western blot analysis of (A) α7nAChR, (B) TLR4, (C) cleaved caspase-3, and (D) Bcl-2 expression in septic rat livers ($n = 3$). ELISA results for (E) ACh ($n = 6$) and (F) HMGB1 (n = 6). The data are expressed as the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ versus the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus the LPS group. α7nAChR = α7 nicotinic acetylcholine receptor; TLR4 = Toll-like receptor 4; ACh = acetylcholine; HMGB1 = high mobility group box 1; LPS = lipopolysaccharide; DEX = dexmedetomidine.

did not inhibit cytokines in septic vagotomized rats ($p > 0.05$; Fig. 8B, C).

4. Discussion

Liver dysfunction critically impairs the prognosis of sepsis and serves as a powerful independent predictor of mortality in the intensive care unit [3]. The liver plays crucial roles in sepsis by modulating the systemic response to severe infection because the largest mass of hepatic scavenger cells, including Kupffer cells, in the body can clear the bacterial endotoxin that induces the inflammatory response [29]. Pathogen-associated molecular patterns (PAMPs), such as LPS, activate TLRs that exist in innate immune cells, such as Kupffer cells, and then induce cytokine release, which promotes liver inflammation and hepatocyte death, eventually leading to liver failure [8]. DEX, which is widely used for sedation in intensive care units, has been reported to possess anti-inflammatory effects. This study investigated whether DEX pretreatment produces a protective effect against LPS-induced liver injury and explored the underlying mechanisms of cholinergic anti-inflammatory signaling.

In the present study, we found that the preemptive administration of DEX reduced the mortality rate and improved liver dysfunction in LPS-induced ALI. In terms of pathological changes, DEX application also alleviated LPS-induced liver injury. Mechanistically, DEX administration was associated with the upregulation of α7nAChR and ACh, the downregulation of HMGB1, TLR4, MyD88, phosphorylated IκB, and phosphorylated NF-κB, the suppression of cell apoptosis, the modulation of the expression of the apoptosis-related proteins cleaved caspase-3 and Bcl-2, and a reduction in the expression of inflammatory factors such as TNF-α and IL-6 following LPS injection. Importantly, pretreatment with α-BGT to block α7nAChR reversed the beneficial effects of DEX on histopathological changes and inflammatory proteins. The protective effects of DEX were noticeable in rats that received sham surgery but not in vagotomized rats. According to our results, DEX treatment may attenuate septic liver injury through the suppression of the TLR4/MyD88/NF-κB pathway, which is, at least in part, dependent on the α7nAChR-mediated pathway and requires intact vagus nerves.

Histological observations provide further evidence of LPS-induced hepatic injury. In general, mortality in septic ALI is influenced by injury development and liver dysfunction. Serum ALT and AST levels can be used as important indicators of liver function. Our study showed histologic changes in liver tissues, high mortality, and elevated serum ALT and AST levels in sepsis-induced rats (Figs. 1, 3). DEX pretreatment mitigated the histopathological alterations in septic livers harvested at

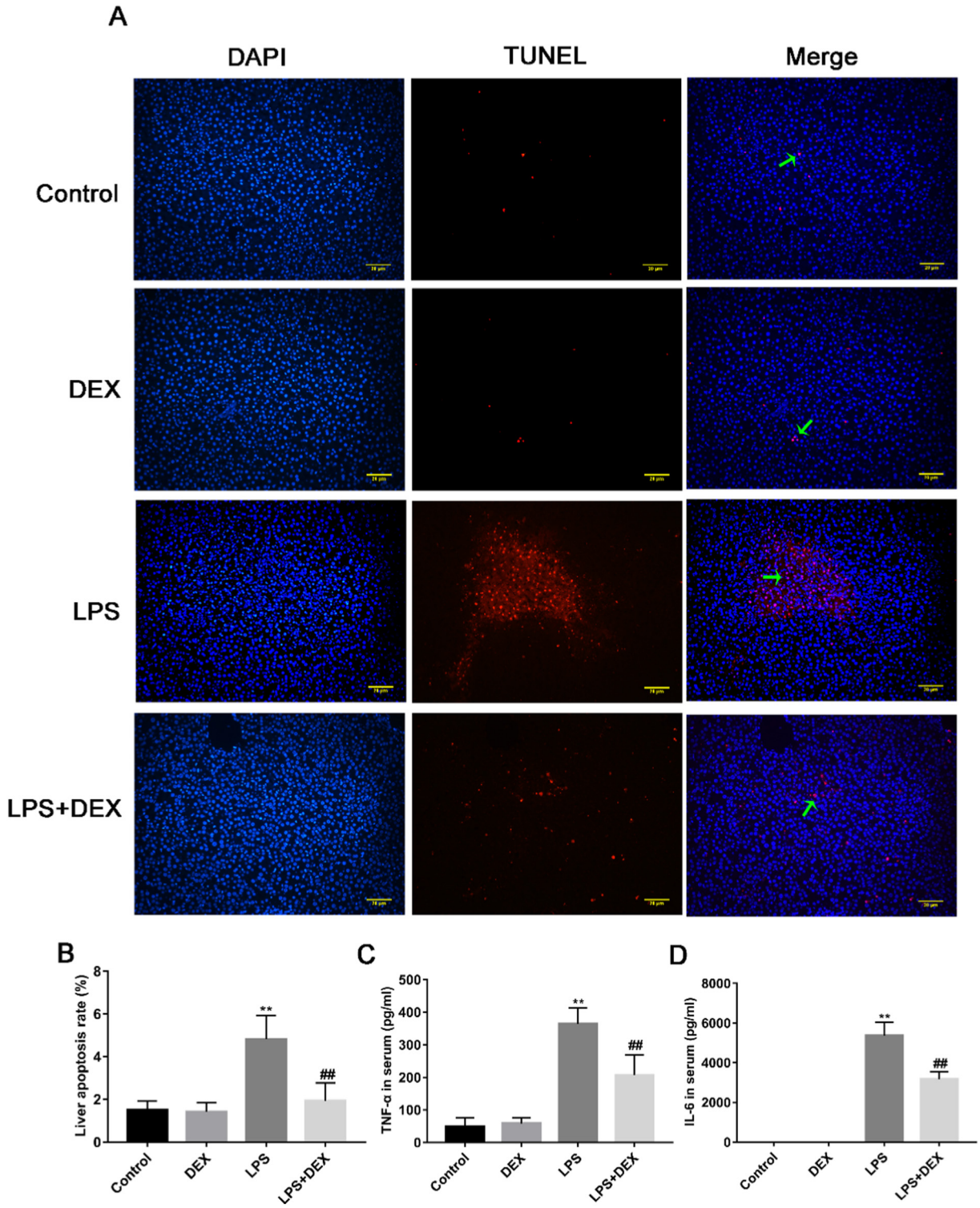

Fig. 5. DEX ameliorates LPS-induced hepatocyte apoptosis and proinflammatory cytokine production. (A) Apoptosis was detected by in situ TUNEL (red; 200×) and DAPI (blue; 200×) staining. The green arrows indicate TUNEL-positive cells. Scale bars = 20 μm. (B) Quantitative analysis of apoptosis based on the proportion of TUNEL-positive cells. (C) Level of TNF-α (n = 6); (D) Level of IL-6 (n = 6). Statistical analysis of apoptotic hepatocytes in the septic liver. The data are expressed as the mean ± SD. $^{**}p < 0.01$ versus the control group; $^{\#\#}p < 0.01$ versus the LPS group. LPS = lipopolysaccharide; DEX = dexmedetomidine; TUNEL = terminal deoxynucleotidyl transferase dUTP nick-end labeling. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

24 h. Log-rank analysis of survival over 120 h demonstrated a significant enhancement of survival in both DEX-treated groups (10 and 30 μg/kg) compared with the LPS-treated group (Fig. 3A), and this effect was accompanied by dose-dependent decreases in the levels of serum markers of liver function after DEX treatment (Fig. 3B, C). Our data are in agreement with the findings of previous studies showing that DEX alleviates hepatocellular congestion and necrosis and reduces inflammatory cell infiltration in the portal tracts in sepsis [23]. A study

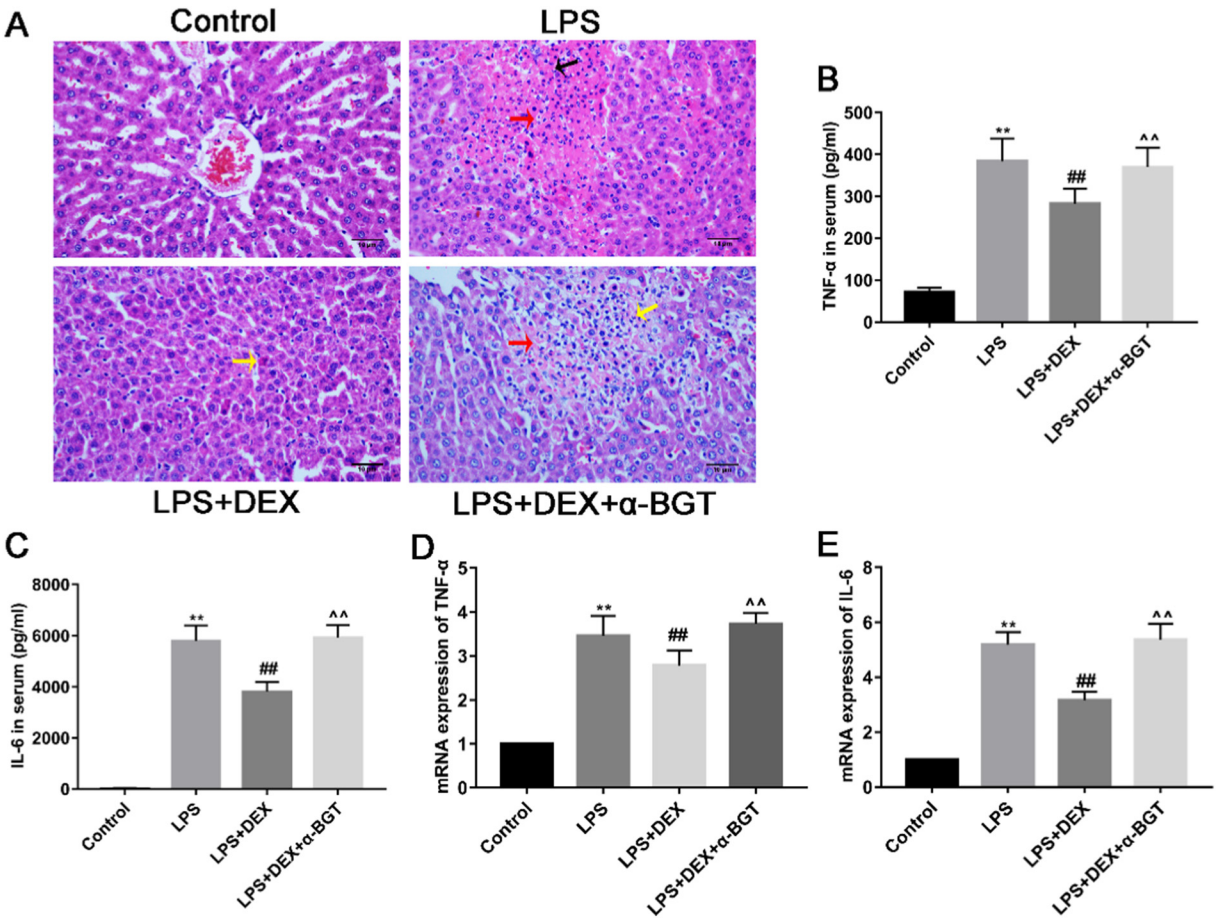

Fig. 6. DEX pretreatment attenuates LPS-induced liver damage and inflammation, which are reversed by the α7nAChR antagonist α-BGT. Liver tissues were extracted for H&E staining (400×) 24 h after LPS injection. Typical images were chosen from each experimental group (A) ($n = 3$). The black arrows indicate inflammatory cells. The red arrows indicate hepatocyte necrosis. The yellow arrow indicates vacuolar degeneration. Scale bar (black) = 10 μm. The levels of (B) TNF-α, (C) IL-6, (D) TNF-α mRNA, and (E) IL-6 mRNA. The data are presented as the mean ± SEM ($n = 6$). $^{**}p < 0.01$ versus the control group; $^{\#\#}p < 0.01$ versus the LPS group; $^{\wedge\wedge}p < 0.01$ versus the LPS + DEX group. LPS = lipopolysaccharide; DEX = dexmedetomidine; α-BGT = α-bungarotoxin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[24] discovered the ability of DEX to improve liver function in the acute stress-induced liver. These results indicate that DEX pretreatment is responsible for protection against septic ALI. Given its ability to improve survival and recovery from hepatic injury, 30 μg/kg DEX shows excellent protective properties.

The study demonstrated that DEX, in addition to having cytoprotective effects in the liver, suppresses the inflammatory cascade and apoptosis, as evidenced by the inhibited release of proinflammatory cytokines, a decrease in the expression of the cleaved caspase-3 and Bcl-2 proteins and fewer TUNEL-positive cells in the LPS-exposed liver. The excessive production of proinflammatory cytokines plays an essential role in ALI pathogenesis and in turn exacerbates damage in liver tissues [4]. The imbalance between the anti-inflammatory system and the proinflammatory system leads to severe liver damage in response to LPS. Numerous in vitro and in vivo investigations have revealed that DEX treatment possesses anti-inflammatory potential in sepsis models [30,31]. In accordance with previous findings, our results revealed that the injection of LPS resulted in elevated mRNA and protein levels of TNF-α and IL-6, which were significantly restrained by DEX treatment, in the liver tissues of septic rats (Figs. 5C, D, 6B–E). Apoptosis is programmed cell death and participates in physiological and pathophysiological processes. Apoptosis is a typical pathological characteristic of liver diseases [32]. Cleaved caspase-3 is involved in the apoptotic pathway and is important for apoptosis induction, and the anti-apoptotic protein Bcl-2 is an essential regulator of mitochondria-mediated apoptosis [33]. The involvement of these apoptosis-related proteins in LPS-induced cell apoptosis is well established [23]. The anti-apoptotic effects of DEX treatment in our experimental settings, demonstrated by reduced cleaved caspase-3 expression and augmented Bcl-2 expression (Figs. 4C, D, 5A, B). The TUNEL assay results further confirmed that sepsis can cause a significant increase in the number of TUNEL-positive cells in the liver and that DEX can dramatically lessen the number of TUNEL-positive cells. Therefore, the results indicate that DEX is capable of depressing the production of proinflammatory cytokines and liver cell apoptosis during sepsis.

In this study, we primarily emphasized the role of α7nAChR, via which DEX exerts hepatoprotection against septic liver damage. The organ protective mechanism of DEX is still not clear but may be a result of anti-inflammatory, anti-apoptosis and other cytoprotective properties via the activation of α7nAChR in sepsis-induced injury to the liver [23] and other remote organs [31]. The pivotal role of CAP as a key anti-inflammatory mechanism is well accepted in infectious diseases [34], in which ACh, the principal neurotransmitter secreted by the vagus nerve, directly inhibits cytokine release via an interaction with α7nAChR [35]. The function of the α7nAChR subtype in the processes of systemic inflammatory cascades and sepsis has been increasingly studied. Emerging animal studies have demonstrated that α7nAChR stimulation by specific agonists, such as nicotine and GTS-21 [36,37], contribute to beneficial outcomes in infectious conditions. Clinical evidence also implies that high α7nAChR expression contributes to

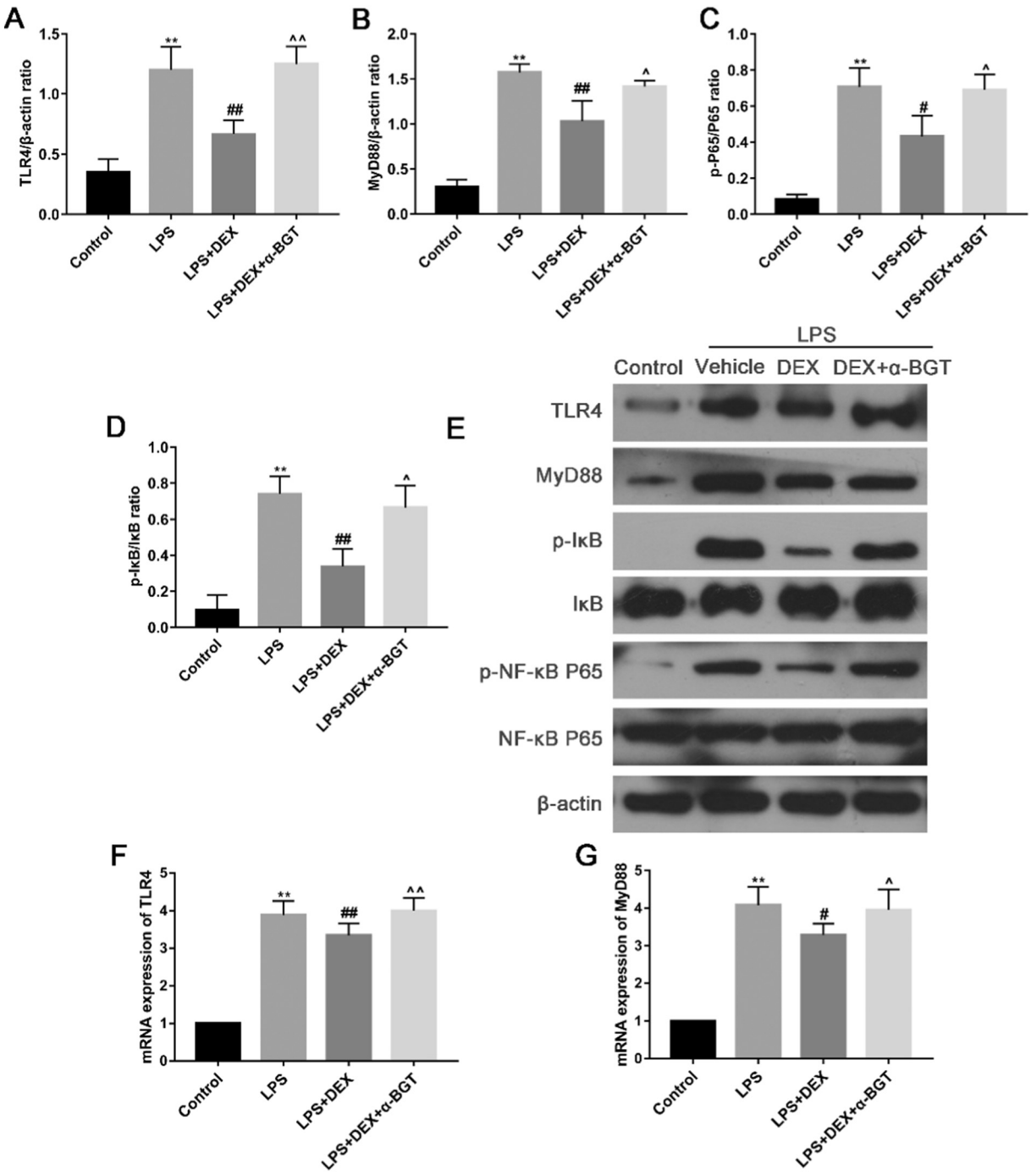

Fig. 7. DEX pretreatment downregulates the expression of TLR4-mediated MyD88/NF-κB pathway-related proteins and mRNA via the α7nAChR-mediated pathway. Quantitative analysis of (A) the TLR4 protein level, (B) the MyD88 protein level, (C) the p-IκB and IκB protein levels, and (D) the p-NF-κB and NF-κB protein levels at 24 h in the liver of different groups from the bands in (E) (n = 3). (F) The TLR4 mRNA level (n = 6). (G) The MyD88 mRNA level (n = 6). The data are presented as the mean ± SEM. $^{**}p < 0.01$ versus the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus the LPS group; $\hat{}p < 0.05$, $\tilde{}p < 0.01$ versus the LPS + DEX group. LPS = lipopolysaccharide; DEX = dexmedetomidine; α-BGT = α-bungarotoxin.

good inflammation control and prognosis [38]. α7nAChR and ACh expression in the septic liver was activated in a time-dependent manner during the infection stage (Fig. 2A, D) and was even more enhanced upon pretreatment with DEX (Fig. 4A, E); to some extent, this implies that DEX probably exerted an anti-inflammatory effect by activating CAP in our study. Besides, it has been determined in in vivo animal models of sepsis that the anti-inflammatory function of DEX may be largely attributable to α7nAChR expression on the surface of immune cells. In vitro experiments have revealed that DEX shows anti-inflammatory activity in various macrophages, such as Kupffer cells upon exposure to LPS [39,40]. In addition, diminished necrotic areas of hepatocytes and few apoptotic cells upon DEX pretreatment were observed in this report. Based on these findings, it is likely that macrophages, especially Kupffer cells and hepatocytes in the liver, are the main targets of the drug for the treatment of ALI.

TLRs trigger the initiation of the inflammatory response via multiple mechanisms and serve as a central target in sepsis treatment [11]. Among them, TLR4 is extensively located in liver tissues, such as in Kupffer cells and hepatocytes [41]. Recent reports have revealed that upregulated TLR4 expression in the liver plays an important role in the

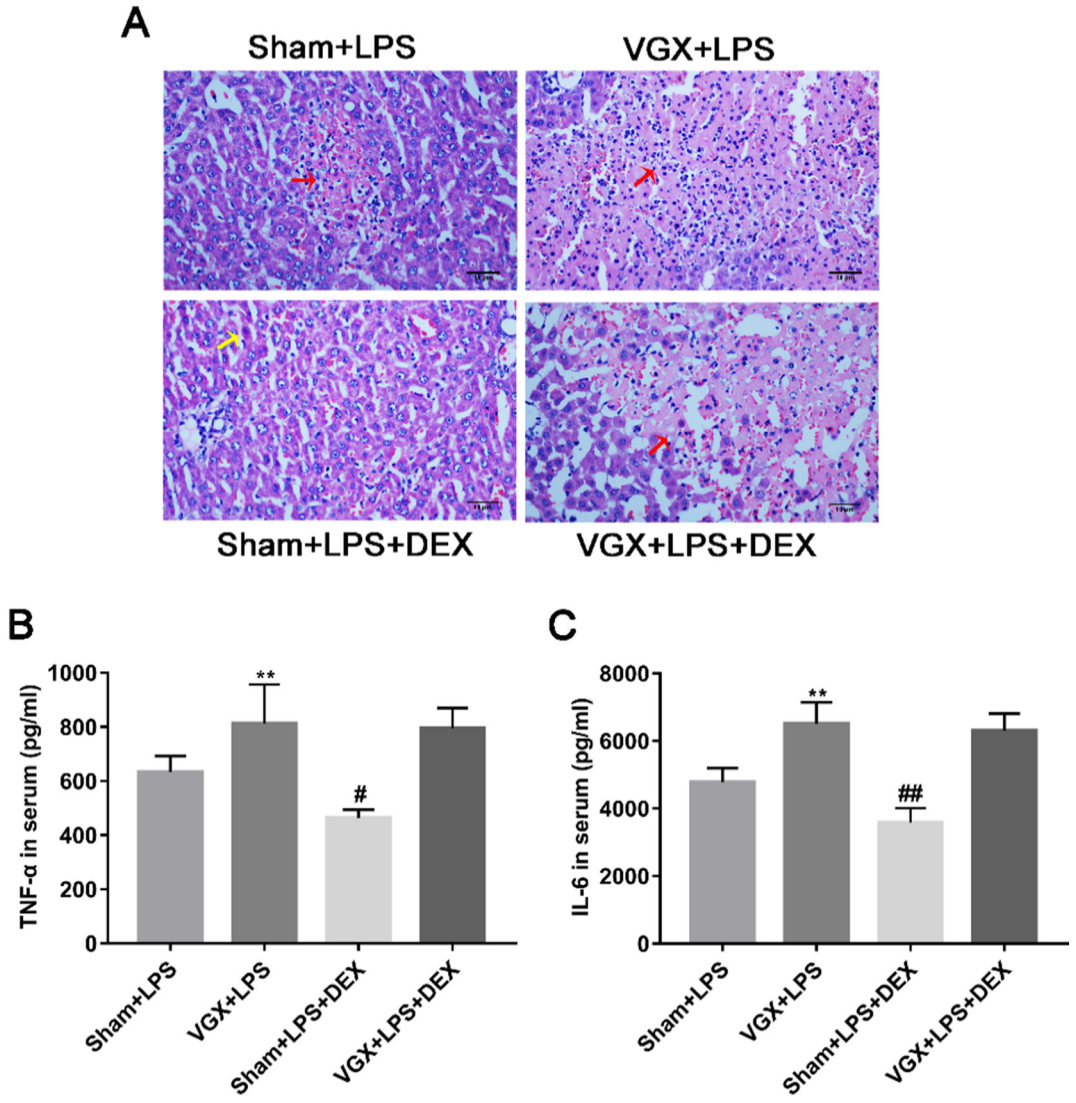

Fig. 8. DEX alleviates liver damage and cytokine release after LPS-induced ALI in sham-operated rats but not in vagotomized rats. (A) Liver tissue sections were stained with H&E (400×) for histological assessment ($n = 3$). The red arrows indicate hepatocyte necrosis. The yellow arrow indicates vacuolar degeneration. Scale bar (black) = 10 μm. The levels of (B) TNF-α and (C) IL-6. The data are presented as the mean ± SEM ($n = 6$). $^{**}p < 0.01$ versus the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus the LPS group. VGX = vagotomy; Sham = sham surgery. LPS = lipopolysaccharide; DEX = dexmedetomidine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pathophysiology and high mortality associated with sepsis [42]. The present data showed that the level of TLR4 in the liver after infection was enhanced by LPS (Fig. 2B). HMGB1, which has been reported to be a potential upstream regulator of TLR4 [43], was increased in septic rats (Fig. 2C). Based on these results, we found that the proinflammatory TLR4 signaling pathway was activated in parallel with the enhancement of the expression of the α7nAChR anti-inflammatory system during sepsis. This phenomenon probably indicates that the host attempted to shift toward the balance between proinflammation and anti-inflammation in the body under infectious conditions. Most importantly, DEX administration diminished TLR4 and HMGB1 expression (Fig. 5B, F), promoted the activation of α7nAChR, and consequently exerted significant beneficial effects on inflammation inhibition and recovery from liver damage.

LPS recognition by TLR4 activates a series of cascades, including MyD88 and further NF-κB dissociation [11]. DEX was discovered to promote inflammation resolution in the kidney by suppressing the TLR4/NF-κB pathway in septic rats [10]. Gao et al. [25] declared that DEX protects against ischemia-reperfusion injury via the suppression of TLR4 signaling. Similarly, TLR4 knockdown partially prevents cell damage in hypoxia/reoxygenation injury, while the overexpression of TLR4 abolishes DEX-mediated cardiomyocyte protection in vitro [25]. In this study, we found that 30 μg/kg DEX significantly increased α7nAChR expression and decreased the expression of TLR4. The inhibition of α7nAChR by α-BGT reversed the inhibition of TLR4 signaling and the anti-inflammatory effect of DEX; the inflammatory cascade was thus amplified (Fig. 7). Thus, the results of this study suggest that the hepatoprotective effect of DEX probably occurs via α7nAChR in associated with TLR4 suppression.

Therefore, we speculate that DEX may activate α7nAChR expressed on Kupffer cells and other sites and subsequently downregulate TLR4-mediating signaling in an α7nAChR-dependent manner, thereby suppressing the inflammatory response and attenuating liver damage during sepsis. It is intriguing that Rong et al. found that α2AR-mediated signaling and α7nAChR-mediated signaling are required for the upregulation of α7nAChR and downregulation of TLR4 expression by DEX pretreatment respectively [18]. Nevertheless, the correlation between the activation of α7nAChR and the inhibition of the TLR4 pathway upon treatment of DEX is unclear. Both α7nAChR and TLR4 are transmembrane receptors, and they may act independently or

synergistically. The specific interaction between them requires further investigation.

Accumulating evidence points to the vagus nerve as an indirect modulator that exerts anti-inflammatory effects on rodents challenged with endotoxins through its major neurotransmitter ACh, which interacts with the α7nAChR [44]. It has been demonstrated that the suppression of inflammation by DEX is involved in a vagal-dependent mechanism in a tibial fracture model [45]. To date, there is little direct evidence indicating that the stimulation of the cervical vagus nerve by DEX is associated with the amelioration of inflammation caused by LPS. In the current study, a profoundly deteriorated inflammatory response and tissue insult were evidenced by detection of inflammatory mediators and H&E staining of liver in rats that underwent vagotomy compared to sham-operated rats; these findings concur with those of previous studies concerning the surgical dissection of the vagus nerve [28,46]. As expected, significant alleviation of cytokine production and improvements in histopathological damage were discovered in septic sham-operated rats pretreated with DEX. However, DEX pretreatment failed to exert anti-inflammatory effects in septic rats that underwent vagotomy (Fig. 8B). Consequently, these results indicate that the intact vagus nerve is required for this hepatoprotective effect.

There are some limitations to this study. First, the distribution and localization of α7nAChR in the liver remain to be verified. We cannot rule out the possibility that other DEX-modulated pathways contribute to the downregulation of inflammatory mediators by activating α7nAChR, or even through other nAChR subtypes in the liver. Second, in addition to Kupffer cells, a number of other cell types exist in the liver, and the cell type that DEX acts on in the septic liver needs to be identified by further research. Third, the relationship between α7nAChR and TLR4 activation upon DEX pretreatment remains to be elucidated. Therefore, more specific antagonists and agonists, as well as transgenic animals with modified receptor genes, are necessary. Finally, unilateral vagotomy was selected because previous experiments [47] showed that bilateral cervical vagotomy is lethal in animals; however, the unilateral cervical vagotomy procedure employed in this study did not eliminate the entire function of the vagus nerve in septic rats. Although we found that DEX regulated the expression of these proteins and genes, the molecular mechanism and related transcriptional regulation remains elusive. Undoubtedly, further experiments are needed to clarify this mechanism.

In conclusion, our study demonstrated that DEX pretreatment offers protection against septic liver damage and the inflammatory response probably through suppressing apoptosis and downregulating TLR4/MyD88/NF-κB signaling through α7nAChR-dependent and vagal-dependent mechanisms in an LPS-induced ALI model. This work provides new insights into DEX-mediated hepatoprotection. Therefore, DEX may be a promising therapeutic strategy for septic ALI management.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105898.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

[1] C. Fleischmann, A. Scherag, N.K. Adhikari, et al., Assessment of global incidence and mortality of hospital-treated sepsis – current estimates and limitations, Am. J. Respir. Crit. Care Med. 193 (3) (2016) Feb 01.
[2] S.M. Fernando, B. Rochwerg, A.J.E. Seely, Clinical implications of the third international consensus definitions for sepsis and septic shock (sepsis-3), CMAJ 190 (2018) E1058–E1059.
[3] P. Strnad, F. Tacke, A. Koch, et al., Liver - guardian, modifier and target of sepsis, Nat. Rev. Gastroenterol. Hepatol. 14 (2017) 55–66.
[4] M. Bauer, A.T. Press, M. Trauner, The liver in sepsis: patterns of response and injury, Curr. Opin. Crit. Care 19 (2013) 123–127.
[5] F. Putker, M.P. Bos, J. Tommassen, Transport of lipopolysaccharide to the Gram-negative bacterial cell surface, FEMS Microbiol. Rev. 39 (2015) 985–1002.
[6] M. Li, S. Wang, X. Li, et al., Diallyl sulfide protects against lipopolysaccharide/d-galactosamine-induced acute liver injury by inhibiting oxidative stress, inflammation and apoptosis in mice, Food Chem. Toxicol. 120 (2018) 500–509.
[7] T.H. Kim, S.J. Kim, S.M. Lee, Stimulation of the alpha7 nicotinic acetylcholine receptor protects against sepsis by inhibiting Toll-like receptor via phosphoinositide 3-kinase activation, J. Infect. Dis. 209 (2014) 1668–1677.
[8] F. Heymann, F. Tacke, Immunology in the liver–from homeostasis to disease, Nat. Rev. Gastroenterol. Hepatol. 13 (2016) 88–110.
[9] L. Meng, L. Li, S. Lu, et al., The protective effect of dexmedetomidine on LPS-induced acute lung injury through the HMGB1-mediated TLR4/NF-kappaB and PI3K/Akt/mTOR pathways, Mol. Immunol. 94 (2018) 7–17.
[10] R. Qiu, W. Yao, H. Ji, et al., Dexmedetomidine restores septic renal function via promoting inflammation resolution in a rat sepsis model, Life Sci. 204 (2018) 1–8.
[11] M. Ruse, U.G. Knaus, New players in TLR-mediated innate immunity, Immunol. Res. 34 (1) (2006) 33–48.
[12] K. Murray, C. Reardon, The cholinergic anti-inflammatory pathway revisited, Neurogastroenterol. Motil. 30 (3) (2018) Mar.
[13] Y.M. Wang H1, M. Ochani, C.A. Amella, et al., Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation, J. Biol. Chem. 293 (36) (2018) 13874–13888 Sep 7.
[14] K.J. Tracey, The inflammatory reflex, Nature 420 (6917) (2002) 853–859 Dec 19-26.
[15] M. Sakata, J. Wu, J. Toyohara, et al., Biodistribution and radiation dosimetry of the alpha7 nicotinic acetylcholine receptor ligand [11C]CHIBA-1001 in humans, Nucl. Med. Biol. 38 (2011) 443–448.
[16] G. Matteoli, P.J. Gomez-Pinilla, A. Nemethova, et al., A distinct vagal anti-inflammatory pathway modulates intestinal muscularis resident macrophages independent of the spleen, Gut 63 (2014) 938–948.
[17] R. Mazloom, G. Eftekhari, M. Rahimi-Balaei, et al., The role of alpha7 nicotinic acetylcholine receptor in modulation of heart rate dynamics in endotoxemic rats, PLoS One 8 (2013) e82251.
[18] H. Rong, Z. Zhao, J. Feng, et al., The effects of dexmedetomidine pretreatment on the pro- and anti-inflammation systems after spinal cord injury in rats, Brain Behav. Immun. 64 (2017) 195–207.
[19] D. Bagdas, M.S. Gurun, P. Flood, et al., New insights on neuronal nicotinic acetylcholine receptors as targets for pain and inflammation: a focus on alpha7 nAChRs, Curr. Neuropharmacol. 16 (2018) 415–425.
[20] M. Zhao, X. He, X.Y. Bi, et al., Vagal stimulation triggers peripheral vascular protection through the cholinergic anti-inflammatory pathway in a rat model of myocardial ischemia/reperfusion, Basic Res. Cardiol. 108 (2013) 345.
[21] Y. He, Z.Q. Ye, X. Li, et al., Alpha7 nicotinic acetylcholine receptor activation attenuated intestine-derived acute lung injury, J. Surg. Res. 201 (2016) 258–265.
[22] Z. Lu, P. Xie, D. Zhang, et al., 3-Dehydroandrographolide protects against lipopolysaccharide-induced inflammation through the cholinergic anti-inflammatory pathway, Biochem. Pharmacol. 158 (2018) 305–317.
[23] J. Sha, H. Zhang, Y. Zhao, et al., Dexmedetomidine attenuates lipopolysaccharide-induced liver oxidative stress and cell apoptosis in rats by increasing GSK-3beta/MKP-1/Nrf2 pathway activity via the alpha2 adrenergic receptor, Toxicol. Appl. Pharmacol. 364 (2019) 144–152.
[24] J. Sha, X. Feng, Y. Chen, et al., Dexmedetomidine improves acute stress-induced liver injury in rats by regulating MKP-1, inhibiting NF-kappaB pathway and cell apoptosis, J. Cell. Physiol. 234 (2019) 14068–14078.
[25] J.M. Gao, X.W. Meng, J. Zhang, et al., Dexmedetomidine protects cardiomyocytes against hypoxia/reoxygenation injury by suppressing TLR4-MyD88-NF-kappaB signaling, Biomed. Res. Int. 2017 (2017) 1674613.
[26] H. Xiang, B. Hu, Z. Li, et al., Dexmedetomidine controls systemic cytokine levels through the cholinergic anti-inflammatory pathway, Inflammation 37 (2014) 1763–1770.
[27] J. Gu, P. Sun, H. Zhao, et al., Dexmedetomidine provides renoprotection against ischemia-reperfusion injury in mice, Crit. Care 15 (3) (2011, Jun 24).
[28] D.J. van Westerloo, I.A. Giebelen, S. Florquin, et al., The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis, J. Infect. Dis. 191 (2005) 2138–2148.
[29] U. Protzer, M.K. Maini, P.A. Knolle, Living in the liver: hepatic infections, Nat. Rev. Immunol. 12 (2012) 201–213.
[30] I. Dardalas, E. Stamoula, P. Rigopoulos, et al., Dexmedetomidine effects in different experimental sepsis in vivo models, Eur. J. Pharmacol. 856 (2019) 172401.
[31] Z. Liu, Y. Wang, Y. Wang, et al., Dexmedetomidine attenuates inflammatory reaction in the lung tissues of septic mice by activating cholinergic anti-inflammatory pathway, Int. Immunopharmacol. 35 (2016) 210–216.
[32] K. Wang, Molecular mechanisms of hepatic apoptosis, Cell Death Dis. 5 (2014) e996.
[33] N.A. Thornberry, Y. Lazebnik, Caspases: enemies within, Science 281 (1998) 1312–1316.
[34] A. Kanashiro, F. Sonego, R.G. Ferreira, et al., Therapeutic potential and limitations of cholinergic anti-inflammatory pathway in sepsis, Pharmacol. Res. 117 (2017) 1–8.
[35] K. Yamada-Nomoto, O. Yoshino, I. Akiyama, et al., Alpha-7 nicotinic acetylcholine receptor (nAChR) agonist inhibits the development of endometriosis by regulating inflammation, Am. J. Reprod. Immunol. 76 (2016) 491–498.

[36] M. Kox, J.F. van Velzen, J.C. Pompe, et al., GTS-21 inhibits pro-inflammatory cytokine release independent of the Toll-like receptor stimulated via a transcriptional mechanism involving JAK2 activation, Biochem. Pharmacol. 78 (2009) 863–872.
[37] Y. Yue, R. Liu, W. Cheng, et al., GTS-21 attenuates lipopolysaccharide-induced inflammatory cytokine production in vitro by modulating the Akt and NF-kappaB signaling pathway through the alpha7 nicotinic acetylcholine receptor, Int. Immunopharmacol. 29 (2015) 504–512.
[38] J.L. Cedillo, F. Arnalich, C. Martin-Sanchez, et al., Usefulness of alpha7 nicotinic receptor messenger RNA levels in peripheral blood mononuclear cells as a marker for cholinergic antiinflammatory pathway activity in septic patients: results of a pilot study, J. Infect. Dis. 211 (2015) 146–155.
[39] Y. Chang, X. Huang, Z. Liu, et al., Dexmedetomidine inhibits the secretion of high mobility group box 1 from lipopolysaccharide-activated macrophages in vitro, J. Surg. Res. 181 (2013) 308–314.
[40] J. Shen, L. Li, L. Jiang, et al., Effect of dexmedetomidine hydrochloride on H2O2-induced oxidative stress and inflammatory response in Kupffer cells, J. Cent. South Univ. Med. Sci. 41 (2016) 477–481.
[41] R.F. Schwabe, E. Seki, D.A. Brenner, Toll-like receptor signaling in the liver, Gastroenterology 130 (2006) 1886–1900.
[42] Y. Wang, S. Wu, X. Yu, et al., Dexmedetomidine protects rat liver against ischemia-reperfusion injury partly by the alpha2A-adrenoceptor subtype and the mechanism is associated with the TLR4/NF-kappaB pathway, Int. J. Mol. Sci. 17 (2016).
[43] L. Di Candia, E. Gomez, E. Venereau, et al., HMGB1 is upregulated in the airways in asthma and potentiates airway smooth muscle contraction via TLR4, J. Allergy Clin. Immunol. 140 (2017) 584–587 (e588).
[44] I. Zila, D. Mokra, J. Kopincova, et al., Vagal-immune interactions involved in cholinergic anti-inflammatory pathway, Physiol. Res. (2017) 66 Sep 22.
[45] Y.J. Zhu, K. Peng, X.W. Meng, et al., Attenuation of neuroinflammation by dexmedetomidine is associated with activation of a cholinergic anti-inflammatory pathway in a rat tibial fracture model, Brain Res. 1644 (2016) 1–8.
[46] L.V. Borovikova, S. Ivanova, M. Zhang, et al., Vague nerve stimulation attenuates the systemic inflammatory response to endotoxin, Nature 405 (6785) (2000) 458–462 May 25.
[47] J. Park, J.W. Kang, S.M. Lee, Activation of the cholinergic anti-inflammatory pathway by nicotine attenuates hepatic ischemia/reperfusion injury via heme oxygenase-1 induction, Eur. J. Pharmacol. 707 (2013) 61–70.