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SCIENTIFIC ARTICLE

Dexmedetomidine preconditioning protects against lung injury in hemorrhagic shock rats

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KEYWORDS
Dexmedetomidine;
Hemorrhagic shock;
Preconditioning;
Lung injury;
Rat

Abstract
Background and objectives: Dexmedetomidine has demonstrated protective effects against lung injury in vitro. Here, we investigated whether dexmedetomidine preconditioning protected against lung injury in hemorrhagic shock rats.
Methods: Male Sprague-Dawley rats were randomly divided into four groups ($n = 8$): control group, hemorrhagic shock group, 5 ug.kg^{-1} dexmedetomidine (DEX1) group, and 10 ug.kg^{-1} dexmedetomidine (DEX2) group. Saline or dexmedetomidine were administered over 20 min. 30 min after injection, hemorrhage was initiated in the hemorrhagic shock, DEX1 and DEX2 group. Four hours after resuscitation, protein and cellular content in bronchoalveolar lavage fluid, and the lung histopathology were measured. The malondialdehyde, superoxide dismutase, Bcl-2, Bax and caspase-3 were also tested in the lung tissue.
Results: Compare with hemorrhagic shock group, 5 ug.kg^{-1} dexmedetomidine pretreatment reduced the apoptosis (2.25 ± 0.24 vs. $4.12 \pm 0.42\%$, $p < 0.05$), histological score (1.06 ± 0.12 vs. 1.68 ± 0.15, $p < 0.05$) and protein (1.92 ± 0.38 vs. 3.95 ± 0.42 mg.mL^{-1}, $p < 0.05$) and WBC (0.42 ± 0.11 vs. $0.92 \pm 0.13 \times 10^9$/L, $p < 0.05$) in bronchoalveolar lavage fluid. Which is correlated with increased superoxide dismutase activity (8.35 ± 0.68 vs. 4.73 ± 0.44 U.mg^{-1} protein, $p < 0.05$) and decreased malondialdehyde (2.18 ± 0.19 vs. 3.28 ± 0.27 nmoL.mg^{-1} protein, $p < 0.05$). Dexmedetomidine preconditioning also increased the Bcl-2 level (0.55 ± 0.04 vs. 0.34 ± 0.05, $p < 0.05$) and decreased the level of Bax (0.46 ± 0.03 vs. 0.68 ± 0.04, $p < 0.05$), caspase-3 (0.49 ± 0.03 vs. 0.69 ± 0.04, $p < 0.05$). However, we did not observe any difference between the DEX1 and DEX2 groups for these ($p > 0.05$).

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Conclusion: Dexmedetomidine preconditioning has a protective effect against lung injury caused by hemorrhagic shock in rats. The potential mechanisms involved are the inhibition of cell death and improvement of antioxidation. But did not show a dose-dependent effect.

PALAVRAS-CHAVE
Dexmedetomidina;
Choque hemorrágico;
Pré-condicionamento;
Lesão pulmonar;
Rato

Pré-condicionamento com dexmedetomidina protege contra lesão pulmonar em ratos com choque hemorrágico

Resumo
Justificativa e objetivos: Dexmedetomidina demonstrou efeitos protetores contra a lesão pulmonar *in vitro*. Neste estudo, investigamos se o pré-condicionamento com dexmedetomidina protege contra a lesão pulmonar em ratos com choque hemorrágico.
Métodos: Ratos machos, Sprague-Dawley, foram aleatoriamente divididos em quatro grupos ($n = 8$): grupo controle, grupo com choque hemorrágico, grupo com 5 $\mu g.kg^{-1}$ de dexmedetomidina (DEX1) e grupo com 10 $\mu g.kg^{-1}$ de dexmedetomidina (DEX2). Solução salina ou dexmedetomidina foi administrada durante 20 minutos. Trinta minutos após a injeção, a hemorragia foi iniciada nos grupos choque hemorrágico, DEX1 e DEX2. Quatro horas após a ressuscitação, a proteína e o conteúdo celular no lavado broncoalveolar e a histopatologia pulmonar foram medidos. Malondialdeído, superóxido dismutase, Bcl-2, Bax e caspase-3 também foram testados no tecido pulmonar.
Resultados: Na comparação com o grupo choque hemorrágico, o pré-tratamento com 5 $ug.kg^{-1}$ de dexmedetomidina reduziu a apoptose ($2,25 \pm 0,24$ *vs.* $4,12 \pm 0,42\%$, $p < 0,05$), escore histológico ($1,06 \pm 0,12$ *vs.* $1,68 \pm 0,15$, $p < 0,05$) e proteína ($1,92 \pm 0,38$ *vs.* $3,95 \pm 0,42$ $mg.mL^{-1}$, $p < 0,05$) e leucócitos ($0,42 \pm 0,11$ *vs.* $0,92 \pm 0,13 \times 10^9/L$, $p < 0,05$) no lavado broncoalveolar; o que está correlacionado com o aumento da atividade da superóxido dismutase ($8,35 \pm 0,68$ *vs.* $4,73 \pm 0,44$ $U.mg^{-1}$ de proteína, $p < 0,05$) e diminuição do malondialdeído ($2,18 \pm 0,19$ *vs.* $3,28 \pm 0,27$ $nmoL.mg^{-1}$ de proteína, $p < 0,05$). O pré-condicionamento com dexmedetomidina também aumentou o nível de Bcl-2 ($0,55 \pm 0,04$ *vs.* $0,34 \pm 0,05$, $p < 0,05$) e diminuiu o nível de Bax ($0,46 \pm 0,03$ *vs.* $0,68 \pm 0,04$, $p < 0,05$), caspase-3 ($0,49 \pm 0,03$ *vs.* $0,69 \pm 0,04$, $p < 0,05$). No entanto, não houve diferença entre os grupos DEX1 e DEX2 para essas proteínas ($p > 0,05$).
Conclusão: O pré-condicionamento com dexmedetomidina tem um efeito protetor contra a lesão pulmonar causada por choque hemorrágico em ratos. Os potenciais mecanismos envolvidos são a inibição da morte celular e a melhora da antioxidação. Porém, não mostrou um efeito dose-dependente.

Introduction

Hemorrhagic shock is a common clinical phenomenon and the main pathophysiology is that vital tissues and organs cannot receive enough blood and oxygen to maintain basic metabolic activity, which often aggravates damage after reperfusion.[1] Despite improvements in treatment, hemorrhagic shock and its complications are still maintained high mortality.[2,3] Recent studies have found that lung is one of the important target organs in hemorrhagic shock.[3] The complication, such as cute Lung Injury (ALI) and its most serious form, Acute Respiratory Distress Syndrome (ARDS), usually with higher mortality.[3] Although the pathophysiology of ALI/ARDS has been intensively studied during hemorrhagic shock, but no effective prevention and treatment methods have been found, so the morbidity and mortality rates remain high.[4]

Ischemic preconditioning has been reported to offer an effective protection against injury of vital tissues and organs through a previous stimuli in hemorrhagic shock.[5] Due to the predictability of hemorrhagic shock during perioperation, ischemic preconditioning is a reasonable strategy to improve outcome for patients. To date, the mechanism of ischemic preconditioning has been extensively studied and found that its protective effect can be mimicked by many drugs more safely, such as Dexmedetomidine (DEX).[6,7]

DEX is often used as a sedative in clinical and the researchers recently found that DEX can simulate the effect of pretreatment, and showed a protective effect on human alveolar epithelial cell in vitro.[7] There is evidence that DEX may have a protective effect on lung injury in vivo. Here we use a hemorrhagic shock model to investigate whether DEX pretreatment produces protection against lung injury. At the same time, we also observed the effect of DEX pretreatment

on cell death and anti-oxidative function, and explored the protective mechanism of DEX.

Materials and methods

The study was approved by the Bioethics Committee of Wuhan University and the procedures were carried out according to the routine animal-care guidelines. Male Sprague-Dawley rats were supplied by the Center of Experimental Animals at Wuhan University. Fasting for 12 hours before the experiment.

Experimental protocol and hemorrhage shock model

SD rats were anesthetized with 2% sodium pentobarbital (50 mg.kg^{-1}) and placed in supine position. Then tracheal intubation was performed and maintained breathe spontaneously. The right femoral artery was dissected by aseptic technique and cannulated with a polyethylene tubing (PE-24G) containing heparinized saline (10 U.mL^{-1}) for blood withdrawal. At the same time, a tail vein channel was established for injection of drugs and resuscitation. At the end of the surgical procedure, the rats were randomly assigned to 4 different groups ($n = 8$): Control; Hemorrhagic Shock (HS); 5 ug.kg^{-1} DEX (DEX1; Jiangsu Hengrui Medicine Co; Ltd, Jiangsu, China) and 10 ug.kg^{-1} DEX (DEX2) group (5 μg.kg^{-1} in rats is equivalent to 1 μg.kg^{-1} in humans).[8] Control and HS groups were perfused with 0.5 mL of saline through the tail vein within 20 min; whereas DEX1 and DEX2 groups were given 0.5 mL of the dilutions containing the corresponding doses of DEX. 0.5 mL heparinized saline (10 U.mL^{-1}) was used to ensure that saline or drug were injected into the blood. 30 min later, hemorrhagic shock was induced in all group except the control group by uniformly withdrawing 32 mL.kg^{-1} blood (volume shock model, approximately 45% of the total blood volume) via the femoral arterial catheter within 10 min.[9] After maintaining this ischemic state for 60 min, the rats were resuscitated by transfusion of the shed blood and Ringer's lactate (32 mL.kg^{-1}) at a constant rate within 30 min. Four hours after resuscitation, the left lung of the rats was subjected to bronchoalveolar lavage and the right lung was taken for testing.

Superoxide dismutase (SOD) activity and malondialdehyde (MDA) formation

The contents of SOD and MDA were determined by microplate reader according to the instructions of the assay kits (Jiancheng Biologic Project Co., Nanjing, China) in lung tissue. The protein content was measured by the BCA protein assay kit (BestBio Co., Shanghai, China) according to the manufacturer's protocol.

Western blotting

The lung tissues were homogenized and the protein concentration of the supernatant was measured by the BCA method. Supernatants containing 50 μg of protein were separated by SDS-PAGE and transferred to PVDF membrane. The membranes were incubated with primary antibodies to caspase-3, Bcl-2, Bax (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and β-actin (Abcam, Cambridge, MA, USA), at 4 °C overnight, followed by Alexa Fluor secondary antibody (1:10,000 dilution, Thermo Fisher Scientific, Inc.) for 1 h at room temperature. Signals were detected using an Odyssey fluorescence imaging scanner and quantified using Odyssey software v.3.0.29.

Bronchoalveolar lavage (BAL)

Four hours after resuscitation, left lung BAL was performed by injection of 5 mL Phosphate buffer saline into the left main bronchus followed by gentle aspiration. The procedure was performed twice, and the recovered fluid processed for protein and WBC count as previously described.[9]

Histological observation

The lung tissue is stained using a standard HE procedure. A pathologist unknown to the experimental procedure scored for HE sections (0 = normal, 3 = most severe).[9] The extent of lung apoptosis was also determined using Terminal dUTP Nick-Labeling (TUNEL), as indicated by kit instructions (Roche, Germany).

Statistical analysis

Data are expressed as mean ± SEM. ANOVA and Student–Newman–Keuls (SNK) were used for statistical analysis to compare measurement data among all groups. Significant differences were established at $p < 0.05$.

Results

The weight of the rats

Sprague-Dawley rats weighing 300 ± 20 g were used in this study, and there was no significant difference between the four groups ($p > 0.05$).

SOD and MDA

Compared with the control group, ischemia and reperfusion significantly reduced the SOD activity in the lung tissue (11.52 ± 0.65 U.mg^{-1} protein vs. 4.73 ± 0.44 U.mg^{-1} protein, $p < 0.05$) (Fig. 1A) and increased MDA concentration (1.36 ± 0.16 nmoL.mg^{-1} protein vs. 3.28 ± 0.27 nmoL.mg^{-1} protein, $p < 0.05$) (Fig. 1B). The medium-dose DEX pretreatment significantly increased SOD activity (8.35 ± 0.68 U.mg^{-1} protein, $p < 0.05$ vs. HS group) and decreased MDA concentration (2.18 ± 0.19 nmoL.mg^{-1} protein, $p < 0.05$ vs. HS group) in the lung tissue. The high-dose DEX pretreatment also increased SOD (8.58 ± 0.55 U.mg^{-1} protein, $p < 0.05$ vs. HS group) and decreased MDA (2.22 ± 0.26 nmoL.mg^{-1} protein, $p < 0.05$ vs. HS group).

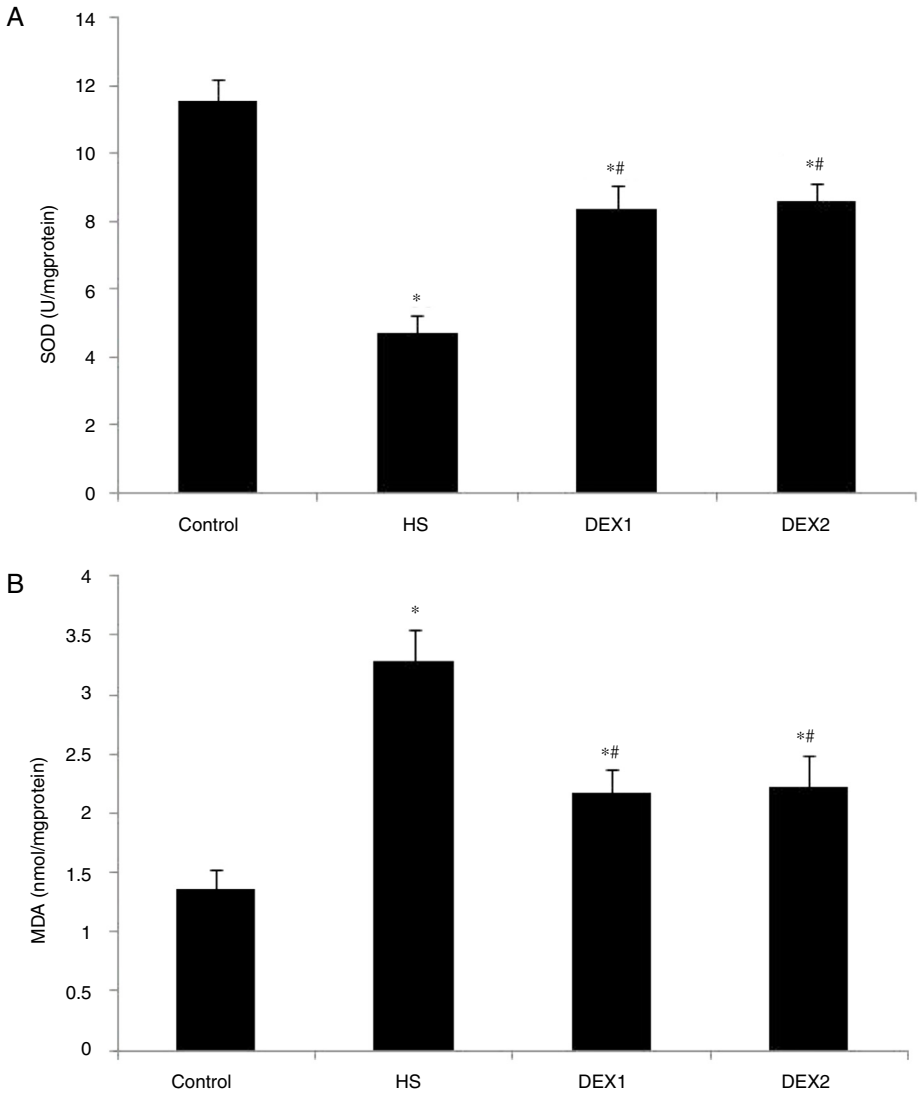

Figure 1 (A) SOD activity in the lung tissue. (B) MDA levels in the lung tissue. SOD, superoxide dismutase; MAD, malondialdehyde; Control, without hemorrhage; HS, hemorrhage shock; DEX1, 5 ug.kg^{-1} dexmedetomidine; DEX2, 10 ug.kg^{-1} dexmedetomidine. Values were presented as mean ± SEM, $n = 8$ for each group. *$p < 0.05$ vs. control group and #$p < 0.05$ vs. HS group.

However, compared with medium-dose DEX, there is no obvious advantage in high-dose DEX pretreatment ($p > 0.05$).

Effect of DEX on the expression of Bcl-2, Bax and caspase-3

Compared with the control group, the Bcl-2 level in HS group was decreased (0.72 ± 0.03 vs. 0.34 ± 0.05, $p < 0.05$), and Bax was up-regulated (0.25 ± 0.04 vs. 0.68 ± 0.04, $p < 0.05$) (Fig. 2A and B). The medium-dose DEX pretreatment significantly attenuated this effect (0.55 ± 0.04 and 0.46 ± 0.03, $p < 0.05$ vs. HS group). Compared with the control group, ischemia and reperfusion also increased caspase-3 expression in the lung tissue (0.29 ± 0.04 vs. 0.69 ± 0.04, $p < 0.05$) (Fig. 2C and D). The medium-dose DEX pretreatment significantly attenuated this effect (0.49 ± 0.03 vs. $p < 0.05$ vs. HS group). However, we did not detect any difference between the high and medium dose DEX groups for these ($p > 0.05$).

BAL protein and WBC content

Almost no protein level was detected in the BAL of the control group (0.42 ± 0.06 mg.mL^{-1}) (Fig. 3A), while ischemia and reperfusion resulted in a significant increase (3.95 ± 0.42 mg.mL^{-1}, $p < 0.05$ vs. control group). Such an effect was significantly attenuated by medium-dose DEX preconditioning (1.92 ± 0.38 mg.mL^{-1}, $p < 0.05$ vs. HS group). High-dose DEX pretreatment also significantly reduced protein levels in BAL (2.05 ± 0.46 mg.mL^{-1}, $p < 0.05$ vs. HS group). However, compared with medium-dose DEX, high-dose DEX pretreatment had no obvious advantage ($p > 0.05$).

Almost no WBC was detected in the BAL of the control group (0.10 ± 0.02 × 10^9/L) (Fig. 3B), while ischemia and reperfusion resulted in a significant increase in WBC count (0.92 ± 0.13 × 10^9/L, $p < 0.05$ vs. control group). Such an effect was significantly attenuated by medium-dose DEX preconditioning (0.42 ± 0.11 × 10^9/L, $p < 0.05$ vs. HS group).

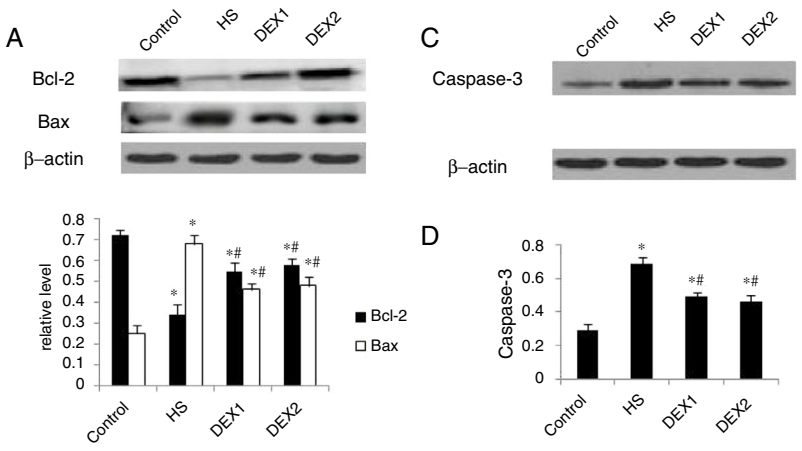

Figure 2 (A) Representative picture of Bcl-2 and Bax. (B) The level of Bcl-2 and Bax. (C) Representative picture of caspase-3. (D) The level of caspase-3. Control, without hemorrhage; HS, hemorrhage shock; DEX1, 5 ug.kg^{-1} dexmedetomidine; DEX2, 10 ug.kg^{-1} dexmedetomidine. Values were presented as mean ± SEM, $n = 8$ for each group. *$p < 0.05$ vs. control group and #$p < 0.05$ vs. HS group.

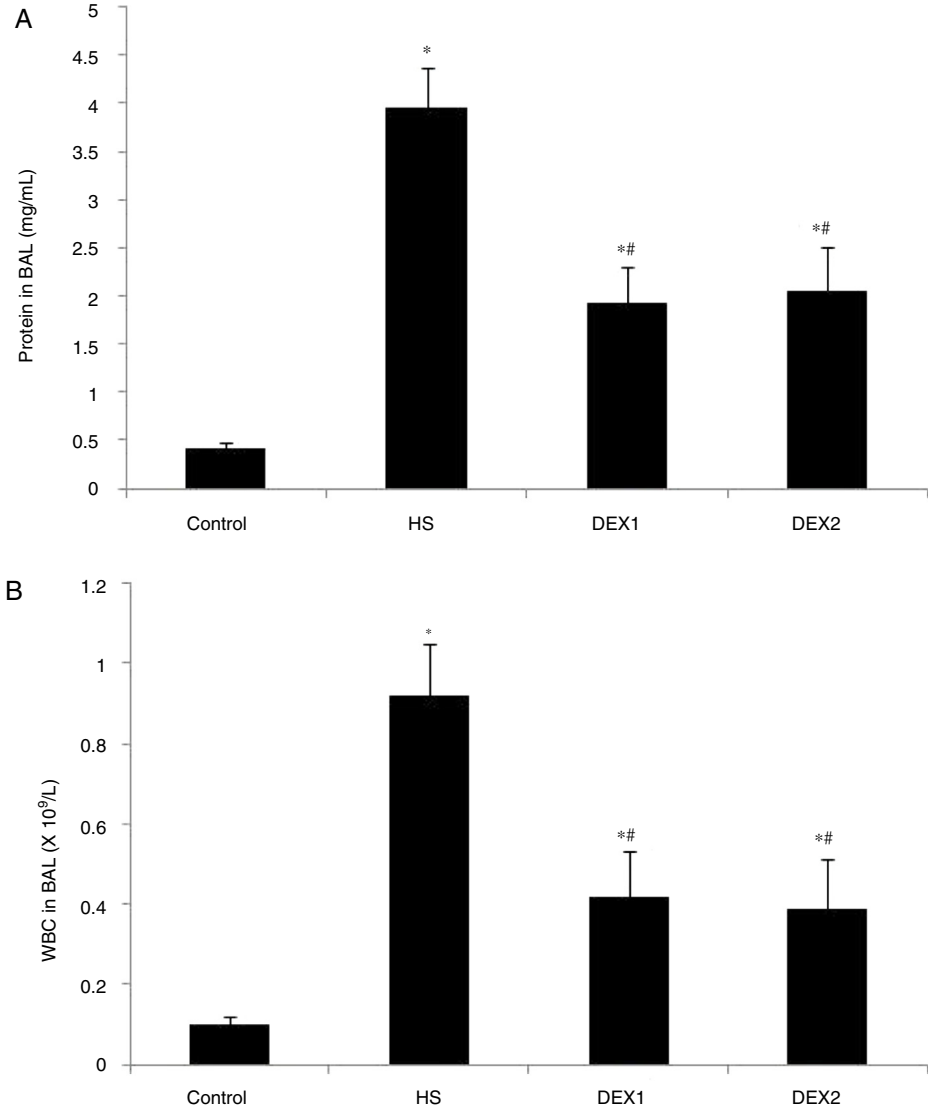

Figure 3 (A) Protein in BAL. (B) WBC in BAL. BAL, bronchoalveolar lavage; Control, without hemorrhage; HS, hemorrhage shock; DEX1, 5 ug.kg^{-1} dexmedetomidine; DEX2, 10 ug.kg^{-1} dexmedetomidine. Values were presented as mean ± SEM, $n = 8$ for each group. *$p < 0.05$ vs. control group and #$p < 0.05$ vs. HS group.

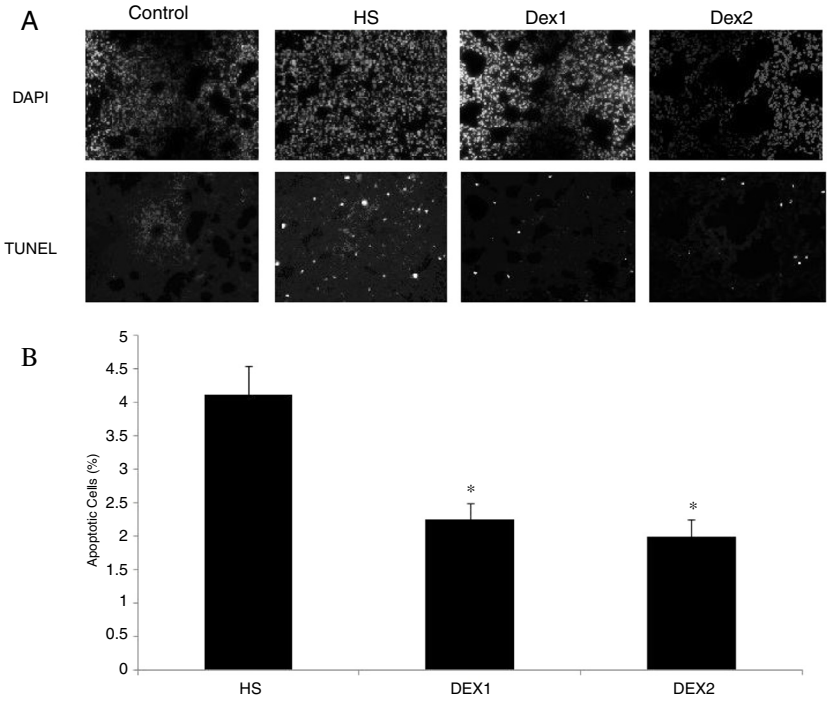

Figure 4 (A) Representative TUNEL-sections of the lung tissue. (B) The cell apoptosis of the lung tissue. Control, without hemorrhage; HS, hemorrhage shock; DEX1, 5 ug.kg^{-1} dexmedetomidine; DEX2, 10 ug.kg^{-1} dexmedetomidine. Values were presented as mean ± SEM, $n = 8$ for each group. *$p < 0.05$ vs. HS group. Original magnification − 400×.

High-dose DEX pretreatment also significantly reduced the WBC content in BAL ($0.39 \pm 0.12 \times 10^9$/L, $p < 0.05$ vs. HS group). However, compared with medium-dose DEX, high-dose DEX pretreatment had no obvious advantage ($p > 0.05$).

Histological changes

TUNEL

Ischemia and reperfusion significantly increased the percentage of apoptotic cells in lung tissue (4.12% ± 0.42%) (Fig. 4A and B). Medium-dose DEX pretreatment inhibited the apoptotic cells in lung tissue after hemorrhagic shock (2.25% ± 0.24%, $p < 0.05$ vs. HS group). And high-dose DEX pretreatment also reduced lung apoptotic cells (1.98% ± 0.26%, $p < 0.05$ vs. HS group). However, compared with medium-dose DEX, high-dose DEX pretreatment had no obvious advantage ($p > 0.05$).

HE

The score was significantly higher (1.68 ± 0.15) (Fig. 5A and B) in HS group. Medium-dose DEX pretreatment had protective effect on lung tissue after hemorrhagic shock (1.06 ± 0.12, $p < 0.05$ vs. HS group). And high-dose DEX pretreatment also significantly reduced the score (1.12 ± 0.13, $p < 0.05$ vs. HS group). However, compared with medium-dose DEX, high-dose DEX pretreatment had no obvious advantage ($p > 0.05$).

Discussion

In this experiment, we found that DEX pretreatment significantly reduced the lung injury in the hemorrhagic shock rats. The main findings are as follows:

(1) DEX pretreatment can reduce the content of MDA and increase the activity of SOD in lung tissue.
(2) DEX pretreatment can increase the ratio of Bcl-2/Bax and decrease the level of caspase-3.
(3) DEX pretreatment can effectively reduce the histological changes in lung tissue.
(4) Increasing the dose of DEX pretreatment did not accordingly increase the protective effect.

It has been reported that oxidative stress plays an important role in ischemia-reperfusion injury.[10] Consistent with previous studies, we also found that oxidative stress was an increase with increased MDA and decreased SOD levels in hemorrhagic shock rats. MDA is a degradation product of oxygen free radicals and lipid peroxidation. Therefore, an increase of MDA implies damage of the normal membrane structure and oxidative damage.[11] However, SOD is considered as an important intracellular antioxidant enzyme with multiple biological functions that prompts cells to scavenge oxygen free radicals.[12] Previous studies have also reported that DEX prevents peroxidation by increasing SOD and decreasing MDA.[7] Our results also show that DEX preconditioning results in an increase of SOD activity and a decrease of MDA content in lung tissue during ischemia and reperfusion. These findings suggest that DEX pretreatment can

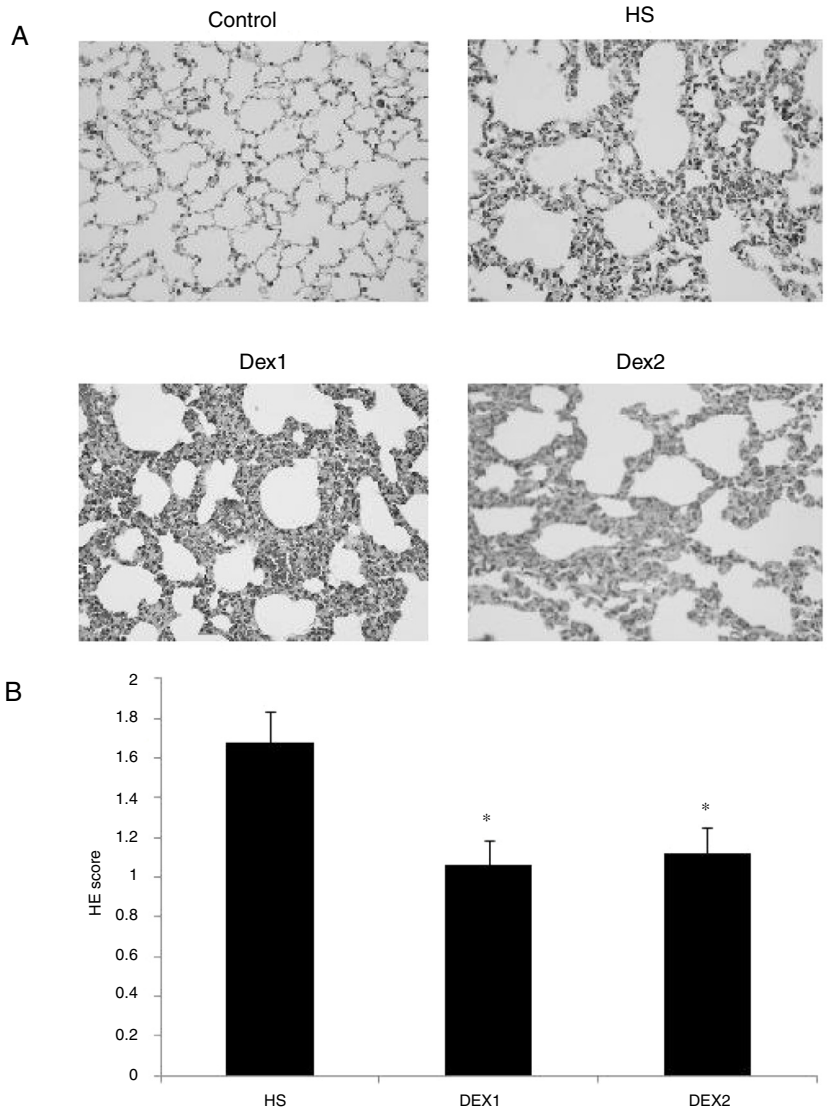

Figure 5 (A) Representative histological sections of the lung tissue. (B) The grade of the lung tissue. Control, without hemorrhage; HS, hemorrhage shock; DEX1, 5 ug.kg^{-1} dexmedetomidine; DEX2: 1 ug.kg^{-1} dexmedetomidine. Values were presented as mean ± SEM, $n = 8$ for each group. *$p < 0.05$ vs. HS group. Original magnification − 400×.

prevent the oxidative damage of lung tissue during ischemia and reperfusion.

There are some evidences that oxidative stress is involved in apoptosis caused by ischemia and reperfusion.[13,14] In this experiment, we also found that the level of MDA content and SOD activity was closely related to the ratio of Bcl-2/Bax in lung tissue. Both Bcl-2 and Bax belong to the Bcl-2 family and have been identified as one of the key factors in initiating apoptosis. However, Bcl-2 may be considered as an important cellular component that not only prevents cell apoptosis but also affects a variety of cellular events. In recent studies, Bcl-2 was found to prevent ischemia and reperfusion injury in some organs including lung tissue.[15,16] In contrast, Bax shows a pro-apoptotic effect; when Bax is over-expressed, it may form apoptotic channels or pores, and promote mitochondrial release of cytochrome c and other factors.[17] Therefore, the ratio of Bcl-2/Bax protein may be the key to cell survival after injury.[17] In the present study, our results show that DEX increases the expression of Bcl-2, decreases the expression of Bax, and results in an increase in the Bcl-2/Bax ratio.

Caspase-3 activation is a key point in the apoptotic cascade and can be regulated by the ratio of Bcl-2/Bax.[18] Previous studies have shown that excessive oxidative stress may lead to caspase-3 activation and the process can be inhibited by Bcl-2.[19,20] Activated caspase-3 is involved in chromatin condensation, DNA fragmentation and cytoskeletal destruction of downstream cellular targets, thereby expressing significant morphological changes in apoptosis.[20,21] It has been confirmed that ischemia and reperfusion is one of the ways to induce the activation of caspase-3.[14] In this study, our data also show a significant increase in caspase-3 in lung tissue during ischemia and reperfusion, consistent with previous studies. In addition, we also observed that caspase-3 activation was inhibited by DEX pretreatment.

Cell death is thought to be one of the mechanisms by which ischemia and reperfusion may lead to organ dysfunction or failure.[1] And apoptosis is the basic process of cell death, which involves down-regulation of Bcl-2/Bax and activation of caspase-3 by activating different signaling pathways.[22,23] Eventually, the cells are damaged and form apoptotic bodies.[24] It has been shown that ischemia and reperfusion initiates this apoptotic cascade in cells.[25] When cell death is inhibited, organ function is significantly improved during ischemia and reperfusion.[6,25] Some studies has been reported that DEX preconditioning attenuates cell death and improves organ function during ischemia-reperfusion injury.[6] In this experiment, consistent with attenuated apoptosis, we also found that DEX preconditioning reduced protein and cell content in BAL and decreased the pathology score in lung tissue, indicating that DEX pretreatment significantly improves lung injury caused by ischemia and reperfusion.

However, there are some obvious limitations to this study that need to be addressed. First, the doses of DEX, we investigated, is very large to the clinical practice. Compared with clinical, a large dose is usually used in laboratory. These may be related with different species, such as some studies even reported that 5 $\mu g.kg^{-1}$ DEX in rats is equivalent to 1 $\mu g.kg^{-1}$ in humans.[8] Second, we did not detect the difference between the two doses of DEX. This may be related to the fact that the receptor has been fully occupied on 5 $\mu g.kg^{-1}$ DEX. Third, the limitations also include a sampling time point, brief observation time, and lack of correlation with clinical measurements of lung injury. Therefore, the relevant research needs to be further explored in the model.

Summary

Our data show that DEX pretreatment can effectively protect against lung injury in hemorrhagic shock rats. The protective effect was related to reducing the oxidative stress and cell death. The experimental results suggest that DEX may be effective in the treatment of lung injury induced by ischemia and reperfusion.

Conflicts of interest

The authors declare no conflicts of interest.

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