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ORIGINAL ARTICLE

Dexmedetomidine Attenuates Total Body Radiation-Induced Acute Liver Injury in Mice Through the Nrf2/HO-1 Pathway

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SUMMARY

Background: **The purpose of this study was to investigate the protective effects of dexmedetomidine (DEX) on total body radiation-induced acute liver injury in mice and to explore the possible mechanisms.**
Methods: **A total of 40 mice were randomly divided into the Control group (Group C), Dexmedetomidine group (Group Dex), Radiation group (Group R), and Group R+Dex. Mice in Group Dex and Group R+Dex were intraperitoneally injected with 10 μg/mL Dex at 50 mg/kg. Both Group C and Group R received normal saline instead of Dex. Mice were treated via continuous administration for 10 days and injection once a day (pre-administration for 3 days and 7 days after radiation). One hour after administration on the third day, the mice in Group R and R+Dex received total body radiation with a total dose of 6 Gy at a rate of 2 Gy/min. Group C received sham radiation. Levels of aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and liver levels of tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), reactive oxygen species (ROS), superoxide dismutase (SOD), malondialdehyde (MDA) were measured. HE staining was employed to evaluate the pathological changes in liver tissues, and the expressions of Nrf2 and HO-1 proteins in the liver were measured by western blot.**
Results: **Compared with group C, serum levels of AST and ALT, liver TNF-α, IL-1β, MDA, and ROS levels increased, and SOD decreased in Group R. Group R mice had higher liver injury scores, and the protein expressions of Nrf2 and HO-1 proteins were lower ($p < 0.05$). Compared with Group R, the levels of AST, ALT, TNF-α, IL-1β, MDA, and ROS decreased, SOD increased, liver injury scores were lower, and the expressions of Nrf2 and HO-1 proteins were higher in the Group R+Dex group (all $p < 0.05$).**
Conclusions: **Dex exhibits a protective effect on reducing acute radiation-induced liver injury and oxidative stress, and the mechanism may be associated with the activation of Nrf2/HO-1 pathways.**
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KEY WORDS

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INTRODUCTION

Organ injury caused by total body radiation is a serious complication after radiotherapy for malignant tumor patients. The liver is one of the most sensitive organs to radiation damage, so it is easy to cause radiation-induced liver injury (RILI). However, there are no good prevention and treatment measures at present. RILI initially caused late pathological changes in hepatocytes, which gradually developed into liver tissue damage [1, 2]. The degree of injury depends on comprehensive factors, such as exposure dose, exposure area, exposure time, and liver function capacity before radiation. The pathogenesis of RILI is complex. Studies suggested that the occurrence of RILI is related to the imbalance of oxidative stress and inflammatory cascade [3,4].

The nuclear factor erythroid-2-related actor 2 (Nrf2) signaling pathway is one of the most important defense systems of intracellular oxidative stress. When oxidetive stress occurs in cells, the Nrf2 signaling pathway is activated first, which then leads to the large expression of antioxidants and related enzymes, to resist cell damage caused by oxidative stress and reduce ROS production [5]. Nrf2 achieves protective purposes by regulating heme oxygenase-1 (HO-1) with important antioxidant and anti-inflammatory activities. The Nfr2/HO-1 signaling pathway is associated with anti-inflammatory, antioxidant, reduction of mitochondrial damage, regulation of Ca^{2+} influx, regulation of cell death, and other mechanisms, and is an indispensable signaling pathway of the anti-oxidative stress response.

Dex, as a novel highly selective α_2 adrenergic receptor agonist, has a series of anti-inflammatory, antioxidant, analgesic, sedative, and anti-apoptotic effects, which is widely used in clinical practice and has protective effects on many organs in the body. Antioxidant activity is one of the important mechanisms of Dex in the treatment of a variety of inflammatory diseases [6,7]. At present, the research on Dex on liver injury mainly focuses on its protective effect on liver injury caused by sepsis and ischemia-reperfusion [8-11]. Whether Dex is protective against radioactive liver injury has not been reported. In this study, we investigated the protective effects and mechanisms of Dex on RILI in the mouse model of RIL, to provide new potential targets for intervention in radiation-induced liver injury.

MATERIALS AND METHODS

Medicines and reagents

Dex (Jiangsu Hengrui Medicine Co, Ltd, Jiangsu, China); kits for AST and ALT detection (Beckman Coulter Life Sciences, San Jose, CA, USA); TNF-a and IL-1b ELISA Kits (Boster, Wuhan, China); ROS, MDA, and SOD kits (Beyotime, Nanjing, China), nuclear factor E2-related factor 2 (Nrf2) protein, heme oxygenase-1(HO-1), and β-actin detection kits (ABclonal, Nanjing, China).

Mice and radiation

All of the animal experiments were performed following the Guidelines for Care and Use of Laboratory Animals of Bengbu Medical College. This study was reviewed and approved by the Animal Ethics Committee of Animal Experiments at Bengbu Medical College (Permit No. 2021275). Female KM mice, 6 - 7 weeks of age, were obtained by the Experimental Animal Center of Bengbu Medical College.

Grouping and administration

After adaptive feeding for 7 days, 40 mice with a bodyweight of 25 ± 1 g were selected and were randomly divided into Group C, Group Dex, Group R, and Group R+Dex. Each group contained 10 mice. Dex hydrochloride solution (10 μg/mL) was prepared before administration each day. Mice of Dex and R+Dex groups were administered via intraperitoneal injection of Dex at a dose of 50 mg/kg. Group C and Group R were administered an intraperitoneal injection of normal saline. Mice were treated via continuous administration for 10 days and injection once a day (3 days pre-radiation + 7 days post-radiation). The feeding and experimental processes of animals followed the 3R principle.

One hour after administration on the third day, mice were anesthetized with 3% pentobarbital sodium. Mice in groups R and R+Dex were exposed to 6 Gy whole-body radiation using an SL18 electron linear accelerator X-ray source (Siemens, Germany) with a dose of 2 Gy/min in the First Affiliated Hospital radiotherapy of Bengbu Medical College. Mice in groups C and Dex received sham radiation. Survival was observed daily up to 14 days post-radiation. Two weeks after radiation, blood was taken from the eyeball vein, left at room temperature for 2 hours, centrifuged at 3,000 r/min for 10 minutes, and the serum was taken and stored at -80℃. A portion of the mouse livers was fixed in 10% neutral formaldehyde for 24 hours and sent to the Department of Pathology, the First Affiliated Hospital of Bengbu Medical College. The remaining livers were collected in sterile tubes, flash-frozen in liquid nitrogen, and stored at -80℃.

Liver pathology

Pathological changes in the liver tissues were scored by three experienced pathologists. Hepatocyte necrosis, inflammatory cell infiltration, sinusoidal congestion, and portal edema were the focus of this study. The specific grading standards were as follows: 0 points: no or very slight liver lesions; 1 point: mild lesion; 2 points: moderate lesion; 3 points: severe lesion; 4 points: overwhelming lesions. The total scoring of liver injury was obtained by adding the four evaluation scores (range 0 - 16 points).

Liver inflammation and oxidative stress

RIPA (1 mL) was added to 100 mg liver tissues and the tissues were homogenized, triturated, filtered, and centrifuged at 12,000 g for 10 minutes at 4℃. ELISA was

employed to detect TNF-α and IL-1β in the liver supernatants, and the levels of oxidative stress indexes ROS, SOD, and MDA were measured according to kit instructions. All tests were carried out following the kit instruction manuals.

Western blot analysis of Nrf2 and HO-1 expression in liver tissues

Liver homogenates were centrifuged, and total protein was extracted. Proteins were separated by SDS-PAGE and transferred to PVDF membranes (EMD Millipore, Burlington, MA, USA). The membranes were blocked in Western Blocking Solution (Beyotime Biotechnology, China) for 2 hours and incubated with Nrf2, HO-1 (ABclonal, China), and β-actin (Affinity Biosciences, Cincinnati, OH, USA) antibodies at 4℃ overnight. The membranes then were washed and incubated with HRP conjugated secondary antibodies (Affinity) for 2 hours at room temperature. The target bands were visualized using a chemiluminescent imaging system (5200, Tanon, China) combined with a Beyo ECL Plus kit (Beyotime Biotechnology) and quantified using Image J software (NIH, Bethesda, MD, USA).

Statistical analysis

All statistical analyses were performed using SPSS 19.0 software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Quantitative data were shown as $\bar{x} \pm s$. The *t*-test and ANOVA were applied to compare quantitative data between groups.

RESULTS

RILI mice liver function

The levels of AST and ALT were higher in Group R than those in Group C ($p < 0.05$). Compared with Group R, the levels of AST and ALT decreased prominently in Group R+Dex ($p < 0.05$), (Table 1). The results showed that Dex could alleviate liver injury and improve liver function in rats induced by total body radiation.

Table 1. The effects of Dex on ALT and AST in the RILI mice.

Group	ALT (U/L)	AST (U/L)
C	49.63 ± 4.25	45.96 ± 3.31
Dex	58.12 ± 10.01	54.32 ± 9.971
R	202.68 ± 30.57 •	156.23 ± 13.69 •
R+Dex	122.51 ± 24.71 •★	176.31 ± 67.86 •★

Note: Compared with Group C, • $p < 0.05$; Compared with Group R, ★ $p < 0.05$.

Hepatic inflammation in RILI mice

Levels of TNF-α and IL-1β were higher in Group R than those in Group C ($p < 0.05$). Compared with Group R, the levels of TNF-a and IL-1b were decreased in group R+Dex ($p < 0.05$), (Table 2). These results suggested that Dex can reduce the inflammatory response induced by total body radiation in rats.

Table 2. The effects of Dex on TNF-α, IL-1β in the RILI mice.

Group	TNF-α (ng/g)	IL-1β (ng/g)
C	19.02 ± 0.89	14.88 ± 1.27
Dex	20.35 ± 2.60	19.98 ± 1.68
R	55.26 ± 1.01 •	59.62 ± 1.05 •
R+Dex	38.15 ± 0.94 •★	43.83 ± 1.34 •★

Note: Compared with Group C, • $p < 0.05$; compared with Group R, ★ $p < 0.05$.

Levels of MDA, SOD, and ROS in RILI mice livers

The levels of MDA and ROS were higher and SOD was lower in Group R than those in Group C (all $p < 0.05$). Compared with Group R, the levels of MDA and ROS were decreased and SOD increased in the R+Dex group (all $p < 0.05$), (Table 3). This experiment proved that Dex can reduce the oxidative stress response of liver injury induced by total body radiation.

Table 3. The effects of Dex on MDA, SOD, and ROS in the RILI mice.

Group	MDA (mmoL/g)	ROS (U/g)	SOD (U/g)
C	6.02 ± 0.53	53.45 ± 7.64	12.21 ± 0.18
Dex	6.14 ± 0.98	11.53 ± 1.05	11.53 ± 1.05
R	10.82 ± 0.45 •	105.36 ± 20.82 •	9.82 ± 0.29 •
R+Dex	9.67 ± 0.24 •★	74.65 ± 13.11 •★	11.73 ± 0.24 •★

Note: Compared with Group C, • $p < 0.05$; compared with Group R, ★ $p < 0.05$.

The pathological changes of liver in RILI mice

A large number of inflammatory cells and fatty steatoses were observed in Group R. Meanwhile, the degree of inflammation and steatosis was attenuated in Group R+Dex compared with Group R (Figure 1A). The score of liver injury in Group R was higher compared with Group C ($p < 0.05$). Additionally, the score of pathological injury in Group R+Dex was reduced, compared with Group R ($p < 0.05$), (Figure 1B). The results showed that Dex could significantly reduce liver injury induced by total body radiation in rats.

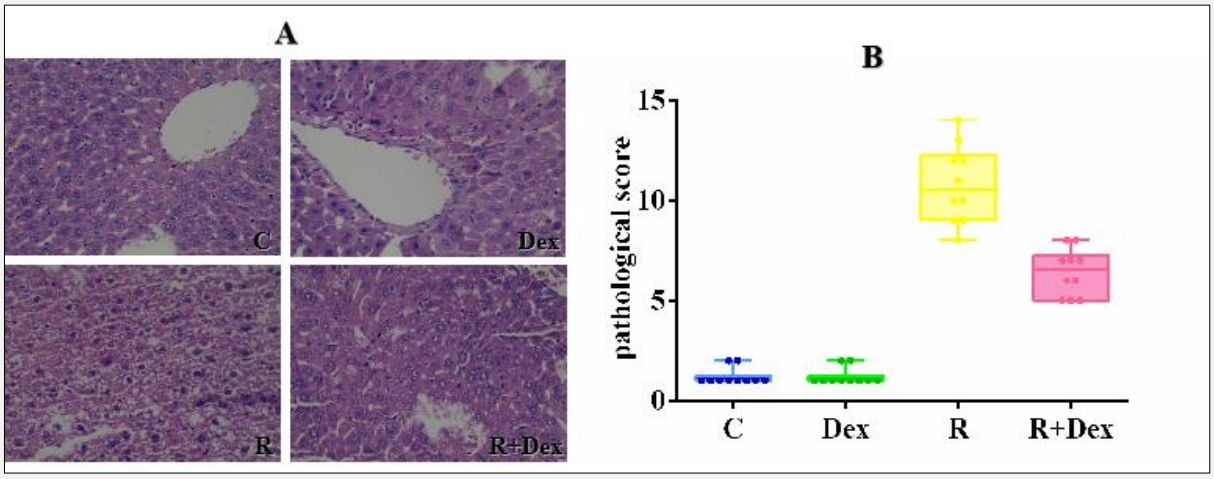

Figure 1. The pathological changes of liver in the RILI mice.

A. Pathological changes of the liver under the light microscope (HE, × 200); B. Pathological score of liver injury.
Note: Compared with Group C, • p < 0.05; compared with Group R, ★ p < 0.05.

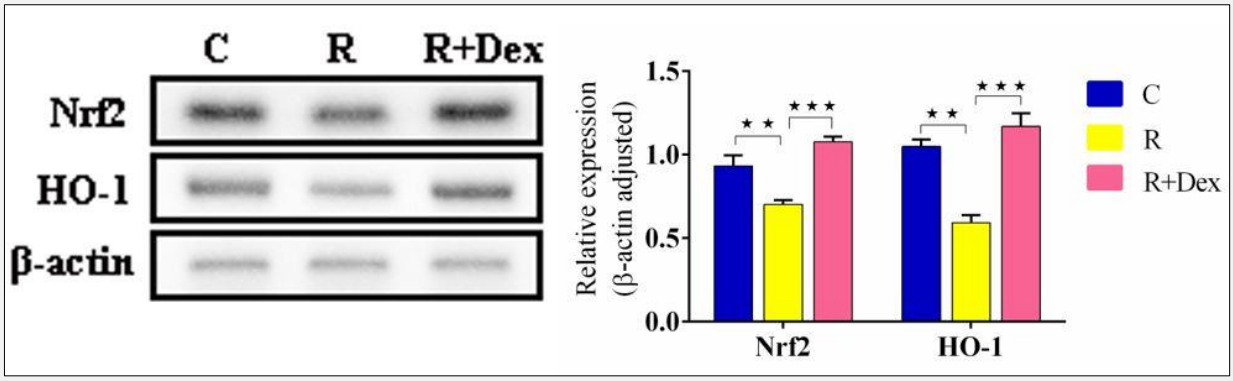

Figure 2. The effects of Dex on the expressions of Nrf2 and HO-1 in the RILI mice liver tissues.

Note: Compared with Group C, ** p < 0.05; compared with Group R, *** p < 0.05.

The expressions of Nrf2 and HO-1 RILI mice livers

The expression of HO-1 in the liver tissues in Group R was reduced, compared with Group C ($p < 0.05$). The expression of Nrf2 and HO-1 protein in Group R+Dex was higher than in Group R ($p < 0.05$), (Figure 2). The results showed that Nrf2/HO-1 pathway is a mechanism by which Dex alleviates liver injury induced by total body radiation.

DISCUSSION

Clinically, during radiotherapy, target volumes are typically large enough that other organs of the body are subjected to low-dose radiation, even when advanced technologies and instrumentation are used [12,13]. As a radiation-sensitive organ, the liver cannot avoid damage from radiation exposure. Due to formidable compensatory repairability, the liver may not present radiation-

induced injury until weeks or months after radiation. An animal model of radiation-induced liver injury found that, although there were signs of radiation-induced injury at the subcellular level, microscopic examination showed no pathological changes in hepatocytes on the third day after radiation [14].

The mechanisms by which exposure to radiation causes liver injury include mainly direct damage to hepatocyte DNA and the formation of free radicals. Free radical-induced damage includes ionization of water molecules within liver tissues and secondary damage caused by oxygen free radicals, peroxides, and hydroxyl groups to surrounding tissues. Until recently, there were no effective treatments or related drugs available for treating radiation-induced liver injury.

In recent years, Dex has been widely used in surgical and ICU patients. Basic and clinical studies have confirmed that Dex has significant anti-inflammatory and antioxidant effects in reducing oxygen free radicals, and has shown significant protective effects in multiple organs. Thus, Dex is favored by anesthesiologists and ICU doctors. This paper investigated mainly the protective effect and mechanism of Dex on acute radiation-induced liver injury in mice and discussed the application value in clinical radiotherapy.

As an α_2-AR agonist, Dex can reduce the level of radiation-induced inflammatory damage to the liver by depressing the levels of serum inflammatory cytokines, such as TNF-α and IL-1β, in mice with acute radiation liver injury [15-19]. These results were consistent with our study, which suggested that Dex could reduce liver tissue injury and improve liver function in RILI mice. In the clinical sense, the study of this experiment provides a certain theoretical basis to reduce the liver damage in tumor patients receiving radiotherapy.

MDA and SOD are important markers of oxidative stress. In the present study, MDA and ROS increased in the R group, but the levels of the anti-oxidant SOD decreased, compared with Group C, suggesting that there was oxidative stress in radiation-induced acute liver injury mice. In addition, the levels of MDA and ROS decreased in Group R+Dex and the activity of SOD increased, compared with Group R, indicating that Dex helps reduce oxidative stress in RILI mice. Previous studies affirmed that Dex can eliminate excessive free radicals, reduce oxidative stress by reducing MDA, and increase the activity of superoxide dismutase by activating the Nrf2/HO-1 pathway [20].

Intraperitoneal injection of Dex 30 minutes ahead of liver ischemia could increase the levels of SOD, catalase, and glutathione and reduce injury of liver tissues [21-23]. Interestingly, lipopolysaccharide-induced oxidative stress and apoptosis in rat livers also confirmed that Dex enhanced GSK-3/MKP-1/Nrf2 pathway activity to reduce liver injury [7,24]. In brief, Dex affected inflammatory cell apoptosis and oxidative stress in liver injury.

Nrf2 takes part in regulating several genes related to oxidative damage. When cells are in the state of oxidative stress-activated, Nrf2 shuttles into the nucleus under the action of ROS or other electrophilic groups to induce and modulate the expression of multiple antioxidant and protective genes. Nrf2 plays an important role in the response to cellular antioxidant and oxidative stress and is a key component in the regulation of oxidative stress and ferroptosis *in vivo* [25-27]. HO-1 has anti-apoptotic, anti-inflammatory, and antioxidative functions, which play important roles in mediating oxidative stress in the body [28-30]. In our study, Dex had a significant effect on upregulating the expressions of Nrf2 and HO-1 in liver tissue of acute RILI mice. This suggested that Dex plays an antioxidant role in acute RILI by activating the Nrf2/HO-1 signaling pathway.

CONCLUSION

Dex could eliminate excessive oxygen free radicals in acute radiation-induced liver injury mice by promoting the expression of Nrf2 and HO-1 in liver tissue, and improve liver tissue cell injury and liver dysfunction in mice. Its mechanism may be related to the activation of the Nrf2/ARE/HO-1 signaling pathway. However, this study provided only a preliminary discussion of the possible protective mechanism of Dex in acute radiation-induced liver injury mice, and its specific mechanism requires study further.

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Ethics Statement:

The animal study was reviewed and approved by the Animal Ethics Committee of Bengbu Medical College (Permit No. 2021275).

Declaration of Interest:

No potential conflicts of interest concerning this article were declared.

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