Ursodeoxycholic Acid (UDCA) Increases Lactate Clearance and Reduces Liver Injury by Inhibiting Farnesoid X Receptor (FXR) in Hemorrhagic Shock (HS)

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Research

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Abstract

Background: Hemorrhagic shock (HS) is one of the leading causes of death worldwide. Acute liver injury (ALI) is an important manifestation of HS. Farnesoid X receptor (FXR) plays an important role in the liver protection of bile acids. Therefore, we designed this experiment to study the effect of ursodeoxycholic acid (UDCA) on acute liver injury and its relationship with the FXR pathway in HS.

Methods: In this study, eighteen adult male C57BL/6 mice were randomly divided into 3 groups of six mice per group applying a table of random numbers: sham group; HS group and HS + UDCA group. Six FXR (-/-) adult male C57BL mice were classified as FXR (-/-) + HS + UDCA group. As to model of HS, MAP of 40 ± 5 mmHg was maintained for 1 hour. As to UDCA intervention, UDCA (300mg/kg) was given nasally fed. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) were quantified with chromatographic quantitative kits according to the manufacturer's instructions. Lactate concentration was determined by an automatic biochemical analyzer. The liver tissues were stained with hematoxylin and eosin (H&E) and scored histopathologically.

Results: Plasma ALT, AST and lactate levels decreased significantly after treatment with UDCA under HS conditions. Pathological scores of liver tissues decreased significantly after treatment with UDCA under HS conditions. In the case of FXR (-/-), the influence of UDCA was inhibited.

Conclusions: UDCA can reduce ALT, AST and lactate levels and alleviate liver injury during HS through the FXR pathway.

Background

Hemorrhagic shock (HS) is one of the leading causes of death worldwide in people aged 5–44 years (1, 2). Patients with HS often die of post-shock multiple organ dysfunction syndrome (3). Acute liver injury (ALI) is defined by a rapid development of hepatic dysfunction (loss of 80–90% of liver) (4), complicated by coagulopathy and hepatic encephalopathy in patients without preexisting chronic liver disease(5). ALI is one of the important manifestations of post-shock multiple organ dysfunction syndrome. Liver ischemia-reperfusion injury during shock not only causes decreased liver function (6), but also is closely related to the dysfunction of distant organs such as kidney and lung (7), and eventually leads to increased mortality of patients (8–10). A large number of literatures have shown that bile acids can protect the liver(11–13), and farnesoid X receptor (FXR) plays an important role in the liver protection of bile acids(14, 15). We designed this experiment to study the effect of ursodeoxycholic acid (UDCA) on liver injury in HS.

Materials And Methods

Ethics Statement

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Peking Union Medical College, Beijing, China. (IACUC protocol number: 2017050125). Animal surgical procedures were carried out in strict accordance with the guidelines for the care and use of laboratory animals established by the Animal Use and Care Committee of the Beijing Committee on Animal Care.

The animal protocol was designed to minimize pain or discomfort to the mice. The mice were acclimatized to laboratory conditions (25°C, 12h/12h light/dark, 50% humidity, ad libitum access to food and water) for one week prior to experimentation. All mice were intraperitoneally anesthetized with 3 % sodium pentobarbital (0.2 ml/100 g) prior to surgery and decapitation.

Animal Model

Eighteen adult male C57BL/6 mice (SPF, 8wk) were purchased from the Fangyuan Yuan Animal Centre of Beijing, and the Chinese People's Liberation Army Military Academy of Medical Sciences maintained animal quality control. Six FXR (-/-) adult male C57BL mice were purchased from the Cyagen Biosciences (Suzhou, China). These mice are FXR full knock-out mice.

After a one-week adaption period during which food and water were available ad libitum, eighteen adult male C57BL/6 mice were randomly divided into 3 groups of six mice per group applying a table of random numbers: sham group; HS group and HS + UDCA group. Six FXR (-/-) adult male C57BL mice were classified as FXR (-/-) + HS + UDCA group. Mice in the HS + UDCA group and FXR (-/-) + HS + UDCA group were intraperitoneally anesthetized with sodium pentobarbital. The mean arterial pressure (MAP) was detected by applying noninvasive blood-pressure monitors for animals (BP-98A, Softron, Japan). The right carotid artery was ligated at the distal end of the heart with cannulation using a 30-G detaining needle. The mice were exsanguinated slowly to shock while using a noninvasive blood-pressure monitor (MAP: 40 ± 5 mmHg). A MAP of 40 ± 5 mmHg was maintained for 1 hour. Mice were resuscitated using an equal volume of normal saline with their shed blood at the end of shock period. The right carotid artery was ligated following resuscitation. Then, UDCA (300mg/kg) was given nasally fed. HS mice underwent pentobarbital anesthesia, laparotomy, vascular cannulation, blood withdrawal and suturing but no UDCA. Sham mice underwent pentobarbital anesthesia, laparotomy, vascular cannulation and suturing but no blood withdrawal. The blood sample was collected by tail vein after the tail cut 6 hours after resuscitation. The blood samples were stored in a heparinized eppendorf tube (1.5 mL). Samples were centrifuged at 2000 g for 5 min at 4°C, and the supernatant was placed in an eppendorf tube and stored at -80°C for further study. Then mice in every group were euthanized by rapid decapitation and liver was harvested.

The Chromatographic Quantitative

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using chromatographic quantitative kits according to the manufacturer's instructions. The absorbance from each sample was normalized for the actual concentration.

Determination of Lactate Concentration

Lactate concentration was determined by an automatic biochemical analyzer.

Hematoxylin and Eosin (H&E) Staining

The liver tissues samples in every group were obtained and immediately fixed in 4% paraformaldehyde solution. The samples were rinsed with phosphate-buffered saline and dehydrated with ethanol. Vitrification was performed using a mixture of pure ethanol and xylene, and the samples were then immersed in paraffin. The paraffinembedded samples were cut into thick histological sections, baked, dewaxed, hydrated, stained with H&E, dehydrated with ethanol, vitrified with xylene, and sealed with gum. Finally, the stained sections were stored at room temperature. Sections were observed under a light microscope. The severity of liver lesions was assessed according to Suzuki criteria, and each specimen was scored by two experienced pathologists using a double-blind method(16). All evaluations were made on six fields per section and six sections under 100 x magnification.

Reagents

UDCA was purchased from J&K Scientific (970735, Beijing, China). The mouse chromatographic quantitative kit of ALT was purchased from the Nanjing Jiancheng BioengineeringInstitute (C009-2-1, Jiangsu, China). The mouse chromatographic quantitative kit of AST was purchased from the Nanjing Jiancheng BioengineeringInstitute (C010-2-1, Jiangsu, China).

Statistics

Data were analyzed using SPSS 16.0 software. All data are expressed as means $\pm$ SD of mean values, and results were compared using one-way analysis of variance followed by Tukey's test. A $P < 0.05$ was considered to be statistically significant.

Results

Plasma ALT, AST and Lactate Levels

Plasma ALT, AST and lactate levels in the HS group increased significantly compared with the sham group ($P < 0.05$). Plasma ALT, AST and lactate levels in the HS + UDCA group decreased significantly compared with the HS group ($P < 0.05$). Plasma ALT, AST and lactate levels in the FXR (-/-) + HS + UDCA group increased significantly compared with the HS + UDCA group ($P < 0.05$) (Fig. 1).

Histomorphology of the Liver

No obvious tissue damage of the liver was shown in the sham group. Histological images of liver tissues showed decreased levels of in the HS + UDCA group as compared to the HS group ($P < 0.05$). Histological images of liver tissues increased in the FXR (-/-) + HS + UDCA group as compared to the HS + UDCA group ($P < 0.05$) (Fig. 2).

Discussion

This study demonstrated that plasma ALT, AST and lactate levels decreased significantly after treatment with UDCA under HS conditions. Liver tissues damages significantly reduced after treatment with UDCA under HS conditions. In the case of FXR (-/-), the influence of UDCA was inhibited.

First, we observed effects of UDCA on ALT and AST in HS. The mechanism of acute hepatic injury is complex. Some studies suggest that tissue inflammatory immune response and oxidative stress play key roles in the pathogenesis of ALI(17, 18). Inflammation hinders liver microcirculation and causes liver ischemia, hypoxia and oxidative stress, leading to liver injury. The content of ALT and AST in serum is an important indicator to measure liver injury in clinic. The activity of ALT and AST shows the pathophysiology of liver. Our study demonstrated that plasma ALT and AST levels decreased significantly after treatment with UDCA under HS conditions. These above findings suggest that UDCA can reduce liver injury during HS.

Second, we observed effects of UDCA on lactate in HS. Clearance of lactate occurs primarily in the liver. During shock, when tissue perfusion is reduced, the productive pathway of anaerobic fermentation is activated, leading to the formation of a large amount of lactate. Some studies have shown that the level of lactate can reflect the severity of diseases(19). Dysfunction of lactate metabolism occurs after ischemia reperfusion. Early lactate clearance is an independent risk factor for inpatient death in shock patients (20–22). Our study demonstrated that plasma lactate levels decreased significantly after treatment with UDCA under HS conditions. These above phenomena indicate that UDCA can promote the metabolism of lactate and increase lactate clearance under HS conditions.

Third, we observed effects of UDCA on the pathological damage of the liver in HS. The liver show disordered hepatic architecture, pronounced lobular edema, and sinusoidal congestion, heavy centrilobular ballooning as well as extensive hepatocellular necrosis under HS conditions. Our study demonstrated that Suzuki's scores of liver significantly reduced after treatment with UDCA under HS conditions. These above results further confirm the liver-protective effects of UDCA.

Fourth, we observed the relationship between effects of UDCA and FXR pathway in HS. A large number of literatures have shown that FXR, as a receptor for bile acids, plays an important role in reducing liver injury and promoting liver repair(23–25). Our study demonstrated that in the case of FXR (-/-), the influence of UDCA was inhibited. These above results indicate that UDCA can promote lactate clearance and alleviate liver injury during HS through the FXR pathway.

There are some limitations in our study. First of all, the observation time was limited so we cannot ensure the relationship between time and effects of UDCA on liver function in HS. Secondly, liver has a very complex function. We only studied ALT, AST and lactate clearance. Other factors such as coagulation and immunity were not included in this study, which may lead to bias of the study results.

Conclusions

In summary, UDCA can reduce ALT, AST and lactate level and alleviate liver pathological damage during HS through the FXR pathway. The relationship between time and effects of UDCA in HS require further research.

Abbreviations

Hemorrhagic shock = HS, acute liver injury = ALI, farnesoid X receptor = FXR, ursodeoxycholic acid = UDCA, Institutional Animal Care and Use Committee = IACUC, mean arterial pressure = MAP, alanine aminotransferase = ALT, aspartate aminotransferase = AST, Hematoxylin and Eosin = H&E.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Peking Union Medical College, Beijing, China. (IACUC protocol number: 2017050125). Animal surgical procedures were carried out in strict accordance with the guidelines for the care and use of laboratory animals established by the Animal Use and Care Committee of the Beijing Committee on Animal Care.

The animal protocol was designed to minimize pain or discomfort to the mice. The mice were acclimatized to laboratory conditions (25C°, 12h/12h light/dark, 50% humidity, ad libitum access to food and water) for one week prior to experimentation. All mice were intraperitoneally anesthetized with 3 % sodium pentobarbital (0.2 ml/100 g) before surgery.

Consent for publication

All authors have seen and agreed with the contents of the manuscript, and the manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere.

Availability of data and material

All data are fully available without restriction.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LW, XR, HWH, XZ and YL drafted the manuscript. LW, XR and HWH participated in the surgical procedure. LW, XR and HWH performed the statistical analysis. XZ and YL conceived of the study, and participated in the design of the study. All authors read and approved the final manuscript.

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Figures

Figure 1

Effects of ursodeoxycholic acid (UDCA) on plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate levels and its relationship with farnesoid X receptor (FXR) in hemorrhagic shock (HS). Results are presented as mean $\pm$ SD ($n = 6$). * = $P < 0.05$ compared to the sham group, † = $P < 0.05$ compared to the HS group, ‡ = $P < 0.05$ compared to the HS + UDCA group.

Figure 2

Effects of ursodeoxycholic acid (UDCA) on histological images of liver and its relationship with farnesoid X receptor (FXR) in hemorrhagic shock (HS). Images with magnifications 100×. Results are presented as mean ± SD (n = 6). * = $P < 0.05$ compared to the sham group, † = $P < 0.05$ compared to the HS group, ‡ = $P < 0.05$ compared to the HS + UDCA group.