Protective effects of ursodeoxycholic acid on ceftriaxone-induced hepatic injury in rats

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Abstract Ceftriaxone is a broad-spectrum semisynthetic cephalosporin antibiotic that causes partial damage in the liver manifested by transient elevation in some biochemical parameters. In this study, our aim was to investigate the use of ursodeoxycholic acid (UDCA) in prevention of the hepatotoxic effect and biochemical changes induced by ceftriaxone in rats. Rats were divided into six groups (control, UDCA 20 mg/kg, ceftriaxone 180 mg/kg, UDCA + ceftriaxone 180 mg/kg, ceftriaxone 360 mg/kg, and UDCA + ceftriaxone 360 mg/kg). Ceftriaxone was injected intraperitoneally, and UDCA was given orally daily for four consecutive weeks. Then liver functions (serums AST, ALT, ALP, direct bilirubin, and total protein) were assessed. Histopathological examination was performed. Treatment of animals with ceftriaxone caused elevated activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as total bilirubin level. These elevations in liver enzymes were decreased by combination ceftriaxone with UDCA. In addition, ceftriaxone caused a significant increase in malondialdehyde (MDA) and nitric oxide (NO) content but significant decrease in glutathione (GSH) content. Combination of UDCA and ceftriaxone resulted in a significant decrease in MDA, NO content and significantly elevated GSH content. It could be concluded that UDCA acts as an effective hepatoprotective agent against liver dysfunction caused by ceftriaxone, and this effect might be related to its antioxidant properties. Hepatic functions should be monitored, and the dose should be adjusted during ceftriaxone therapy.

1. Introduction

Liver injury caused by drugs ranges from mild biochemical abnormalities to acute and chronic liver failure. The majority of adverse liver reactions is idiosyncratic, and occurs in most instances 5–90 days after the causative medication was last taken.1

Some antibiotics are considered a common cause of drug-induced liver injury.2 Hepatotoxicity that occurs is usually
asymptomatic, transient and associated with hepatic impairment.³ Ceftriaxone is a broad-spectrum parenteral cephalosporin with potent activity against gram-positive and gram-negative bacteria.⁴ It widely used, because of its prolonged terminal half-life that allows its prescription as a single dose per day.⁵ Hepatotoxicity caused by ceftriaxone appears after 9–11 days.⁶⁻⁷ Previous studies have reported high aspartate aminotransferase (ALT) and alanine aminotransferase (AST) activities with the administration of ceftriaxone.⁸⁻⁹

Ceftriaxone causes partial damage in the liver as a result of transient elevation in some biochemical parameters such as AST, ALT, total bilirubin, cholesterol, triglyceride (TG) and Low-density lipoprotein (LDL) as well as transient decrease in albumin and High-density lipoprotein (HDL) concentrations.¹⁰

Ursodeoxycholic acid (UDCA) is one of the secondary bile acids, which are metabolic byproducts of intestinal bacteria and it has antioxidative properties.¹¹ It has extensively been used in clinical practice as a first-line therapy for cholestatic liver diseases. However, in recent years, a number of clinical and experimental data have shown the beneficial effects of UDCA in noncholestatic liver injury. UDCA prevents damaging the liver mitochondrial functions and preserve its structure in chronic alcohol intoxication.¹² UDCA has been confirmed to improve liver functions in primary biliary cirrhosis (PBC), primary sclerosing cholangitis, pediatric cholestatic disorders, and cystic fibrosis.¹³

UDCA has been reported to protect against hepatotoxicity caused by amoxicillin-clavulanic acid in rats through its antioxidative properties.¹⁴ In addition, another study showed that UDCA has protective effect against isoniazid plus rifampicin induced liver injury in mice.¹⁵ The mechanism of the UDCA hepatoprotective effect could be mediated by displacement of toxic bile acids from the bile acid pool as well as choleretic, immunomodulatory and cytoprotective properties.¹⁶

The present work has been designed to evaluate the potential role of UDCA in prevention of hepatotoxic effect and biochemical alterations that are induced by ceftriaxone in albino male rats.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats (150–200 g) were obtained from the animal house colony of the National Research Center (Giza, Egypt). Rats were placed in a temperature (22 ± 2 °C) and humidity (50 ± 5%) controlled room in which 12 h light/dark cycles were maintained for one week before the start of the experiment. A standard diet and tap water were provided ad libitum. This study was approved by the Animal Research Ethics Committee, Faculty of Pharmacy, Cairo University (PT 309).

2.2. Drugs

UDCA was purchased from SEDICO (6th October, Egypt) and was dissolved in 1% tween 80 shortly before administration to animals. Ceftriaxone was obtained from Novartis Pharma Company (Cairo, Egypt) and was freshly dissolved in distilled water immediately before administration. Other chemicals were obtained from local sources and were of analytical grade.

2.3. Experimental design

The rats were divided randomly into six experimental groups, each consisting of eight rats, that were treated as follows: group 1 received vehicle and served as a control, group 2 received UDCA (20 mg/kg), group 3 received ceftriaxone (180 mg/kg), group 4 received combined oral doses of UDCA 20 mg/kg and ceftriaxone 180 mg/kg, group 5 received ceftriaxone (360 mg/kg) and finally group 6 received combined oral doses of UDCA 20 mg/kg and ceftriaxone (360 mg/kg). Ceftriaxone was i.p. injected while UDCA was orally administered for 4 weeks.

At the end of the experiment blood samples were collected from the retro-orbital plexus and used for serum separation. All the rats were sacrificed by decapitation and the livers of rats were immediately dissected out. Part of the liver tissues was homogenized in ice-cold 0.9% w/v saline using a homogenizer to obtain 20% homogenate. Aliquots of the liver homogenate were stored at −4 °C prior to biochemical analysis. The other part of the liver was preserved in 10% formalin solution for histopathological examination.

2.4. Determination of biochemical parameters

Hepatic enzymes in the serum such as AST and ALT were used as biochemical markers for hepatotoxicity and assayed by the method of Reitman and Frankel.¹⁷ Serum alkaline phosphatase (ALP) was determined according to the method of Bellfield and Goldberg¹⁸ using colorimetric kit obtained from Diamond Co., Egypt. Total serum bilirubin was determined spectrophotometrically according to the method of Walter and Gerade.¹⁹

Glutathione (GSH) content was measured spectrophotometrically using the method of Sedlak and L’ Hanus.²⁰ Results were calculated as μM/g tissue. Lipid peroxidation was determined in liver homogenates as thiobarbituric acid reactive species (TBARS) using malondialdehyde (MDA) as a reference standard of oxidative stress according to the method described by Buege and Aust²¹ and measured in nmol/g.²² Nitric oxide was determined according to the method described by Miranda et al.²³

2.5. Preparation of sections for histopathological examination

Liver was dissected out and the liver samples were excised from the experimental animals of each group and were fixed in 10% saline buffered formalin. Tissues were then embedded in paraffin subsequently; 5 μm sections were cut on a microtome and examined microscopically for the evaluation of histopathological changes.

2.6. Statistical analysis

Data are presented as mean ± SE. Statistical analysis of the data was carried out using one way analysis of variance (ANOVA) followed by Tukey’s test for post hoc analysis. Statistical significance was acceptable to a level of p < 0.05.
Data analysis was accomplished using the software program graphpad prism (version 5).

3. Results

3.1. Effect of ceftriaxone 180 mg/kg (cef1) and ceftriaxone 360 mg/kg (cef2) with or without UDCA 20 mg/kg on serum levels of ALT, AST, ALP, total protein, and direct bilirubin

Results of biochemical tests (ALT, AST, ALP, total protein, and direct bilirubin) are summarized in Table 1. Serum ALT activity significantly increased in rats treated with ceftriaxone 180 mg/kg (cef1) and ceftriaxone 360 mg/kg (cef2) by 40% and 48% respectively as compared with control group. However groups which were treated with cef1 + UDCA and cef2 + UDCA showed significantly low activity of ALT by 42% and 30% when compared with cef1 and cef2 respectively. As well groups treated with cef1 and cef2 have significantly increased AST activity by 30% and 48% respectively as compared with the control group. Concurrent administration of cef1 and cef2 simultaneously with UDCA induced a significant decrease in AST activity by 35% and 38% when compared with groups treated with cef1 and cef2 respectively. ALP activity in rats treated with cef1 and cef2 was found to be higher than the control group by 15% and 17% respectively. However groups which were treated with cef1 + UDCA and cef2 + UDCA showed significantly low activity of ALP by 19% and 14% when compared with cef1 and cef2 respectively. Total protein and direct bilirubin were significantly decreased in the group treated with cef2 + UDCA by 31% and 37% respectively when compared with group treated with cef2.

3.2. Effect of ceftriaxone 180 mg/kg (cef1) and ceftriaxone 360 mg/kg (cef2) with or without UDCA 20 mg/kg on hepatic oxidative stress: GSH, MDA contents, and NO contents

As shown in Table 2, groups were treated with ceftriaxone 180 mg/kg (cef1) and ceftriaxone 360 mg/kg (cef2) showed significantly low levels in GSH contents by 20% and 31% respectively as compared with control group and the level of hepatic GSH in the groups cef1 + UDCA and cef2 + UDCA was found significantly highly elevated by 31% and 57% when compared with cef1 and cef2 respectively. Finally, MDA and nitric oxide contents were significantly increased in cef1 and cef2 groups when compared with the control group and the levels of MDA and nitric oxide were significantly decreased in the groups treated with cef1 + UDCA, and cef2 + UDCA when compared with cef1 and cef2 respectively.

3.3. Histopathological study

Histological studies, showed normal hepatic lobular architecture in the liver of rats treated with UDCA 20 mg/kg (Fig. 2) in comparison with the control group (Fig. 1).

In the Ceftriaxone 180 mg/kg treated group the liver showed diffuse hydropic degeneration in the hepatocytes of perportal (peripheral) area (zone 3) with shrunk, dark nuclei (pyknosis) (Fig. 3). The group treated with ceftriaxone 180 mg/kg in combination with UDCA showed mild focal hydropic degeneration in the perportal area in limited areas in comparison with the group treated with ceftriaxone alone (Fig. 4).

Ceftriaxone 360 mg/kg treated rats (group 5) showed nearly complete lobular degeneration in the form of hydropic degeneration of hepatocytes with pyknosis nuclei, sparing a small area surrounding the central zone (Fig. 5). Group 6 treated by ceftriaxone 360 mg/kg in combination with UDCA showed degeneration in hepatocytes of the perportal area with normal hypotocytes in the central lobular area which were protected from damage (Fig. 6).

4. Discussion

It is well-known that ceftriaxone is widely used as a third generation cephalosporin antibiotic that has a broad spectrum of bactericidal activity. However, an increasing number of evidence indicates that it has risk of elevation of the liver enzyme, cholestatic abnormalities and liver injury as adverse effect and the mechanism of its hepatotoxicity appears to be immunologically mediated.

UDCA stabilizes the mitochondrial and plasma membranes of hepatocytes that protect them from various other injuries and it constitute an antiapoptotic action. This protective effect is probably due to its antioxidant action. Previous study showed that UDCA protected mice from liver injury induced by ceftriaxone.

### Table 1 Effect of ceftriaxone (cef1) 180 mg/kg and ceftriaxone (cef2) 360 mg/kg with or without of ursodeoxycholic acid (UDCA) 20 mg/kg on serum activity of ALT, AST, ALP, direct bilirubin and total protein.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ALT(U/L)</th>
<th>AST(U/L)</th>
<th>ALP(U/L)</th>
<th>Direct bilirubin(μmol/l)</th>
<th>Total Protein mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>25.1 ± 2.16</td>
<td>61.5 ± 2.77</td>
<td>326 ± 7.06</td>
<td>1.04 ± 0.194</td>
<td>8.65 ± 0.570</td>
</tr>
<tr>
<td>UDCA (20 mg/kg)</td>
<td></td>
<td>24.3 ± 2.33</td>
<td>52.6 ± 4.34</td>
<td>359 ± 8.62</td>
<td>1.27 ± 0.258</td>
<td>10.3 ± 1.10</td>
</tr>
<tr>
<td>Cef1 (180 mg/kg)</td>
<td></td>
<td>35.0 ± 1.35a</td>
<td>80.4 ± 5.70a</td>
<td>376 ± 1.72</td>
<td>1.31 ± 0.141</td>
<td>8.11 ± 0.379</td>
</tr>
<tr>
<td>Cef1 + UDCA</td>
<td></td>
<td>20.3 ± 1.22bc</td>
<td>51.9 ± 3.17e</td>
<td>298 ± 15.8e</td>
<td>1.3 ± 0.0609</td>
<td>8.84 ± 0.430</td>
</tr>
<tr>
<td>Cef2 (360 mg/kg)</td>
<td></td>
<td>37.2 ± 1.62a</td>
<td>91.3 ± 4.36a</td>
<td>382 ± 6.85a</td>
<td>1.91 ± 0.100a</td>
<td>9.07 ± 0.707</td>
</tr>
<tr>
<td>Cef2 + UDCA</td>
<td></td>
<td>26.1 ± 2.89bc</td>
<td>55.9 ± 4.414b</td>
<td>330 ± 16.0</td>
<td>1.20 ± 0.102c</td>
<td>11.9 ± 0.733c</td>
</tr>
</tbody>
</table>

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase.

All data were expressed as means ± SE (n = 8–10/group).

Data comparison was performed using one way ANOVA followed by Tukey’s Multiple Comparison Test.

* Significantly different from the control group at p < 0.05.

b,c Significantly different from (cef1) group values at p < 0.05.

a,c Significantly different from (cef2) group values at p < 0.05.
by isoniazid plus rifampicin. In addition, UDCA acts an effective hepatoprotective agent against liver dysfunction caused by the broad spectrum antibiotic combination amoxicillin-clavulanic acid and protected rats from liver injury induced by methotrexate, an immunosuppressant drug.

The serum bilirubin, AST, ALT, and ALP are the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction. In this study we found significant elevation of serum ALT and AST activity in rats treated with ceftriaxone which is similar to the results found in previous studies. However combination of ceftriaxone plus UDCA causes significant improvement in liver function and significant reduction in liver enzyme activities such as ALT and AST. These results provide evidence that UDCA could protect against drug-induced liver injury. The normalization of serum markers by UDCA suggests that it is able to protect the membrane integrity against ceftriaxone that induces leakage of marker enzymes into the circulation. Also in this study we found that high dosages of ceftriaxone were associated with higher percentages of hepatocellular enzyme elevations.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Control</th>
<th>UDCA (20 mg/kg)</th>
<th>Cef1 (180 mg/kg)</th>
<th>Cef1 + UDCA</th>
<th>Cef2 (360 mg/kg)</th>
<th>Cef2 + UDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH (μM/g)</td>
<td>4.56 ± 0.0592</td>
<td>4.84 ± 0.237</td>
<td>3.65 ± 0.0320</td>
<td>4.75 ± 0.0793</td>
<td>3.14 ± 0.199</td>
<td>4.93 ± 0.155</td>
</tr>
<tr>
<td></td>
<td>MDA (nM/mg)</td>
<td>216.5 ± 7.10</td>
<td>216.2 ± 11.7</td>
<td>279.8 ± 9.01</td>
<td>205.8 ± 4.77</td>
<td>314 ± 19.9</td>
<td>237.7 ± 2.43</td>
</tr>
<tr>
<td></td>
<td>NO (μmol/l)</td>
<td>420.8 ± 2.85</td>
<td>358.9 ± 11.11</td>
<td>528.3 ± 31.77</td>
<td>437.6 ± 20.85</td>
<td>688.1 ± 35.05</td>
<td>480.1 ± 41.94</td>
</tr>
</tbody>
</table>

GSH: glutathione, MDA: malondialdehyde, NO: nitric oxide.

All data were expressed as means ± SE, (n = 8–10/group).

Data comparison was performed using one way ANOVA followed by Tukey’s Multiple Comparison Test.

a Significantly different from the control group at p < 0.05.

b Significantly different from (cef1) group values at p < 0.05.

c Significantly different from (cef2) group values at p < 0.05.
induced the expression of detoxifying enzymes and antioxidative stress genes via the Nrf2 transcriptional pathway and protective effect of UDCA against different liver injuries was confirmed by several previous studies. 

UDCA has a protective role in the secondary biliary cirrhosis through counteracting mitochondrial oxidative stress and the synthesis of endogenous antioxidant defenses, including glutathione synthesis and antioxidant enzymes. It protected liver mitochondria from abnormalities induced by lipid peroxidation and minimized the elevation of lipid peroxides induced by hydrogen peroxide. The antioxidative effect and immunomodulatory effects of UDCA can explain its hepatoprotective effects observed in this study.

Histopathological examination in this study confirmed the biochemical results. Liver specimens obtained from groups treated with ceftriaxone plus UDCA showed mild hydropic degeneration in the periportal (peripheral) area and normal hepatocytes in the central lobular area compared to ceftriaxone which showed nearly complete lobular degeneration.

5. Conclusion

In conclusion; the results of the present study demonstrate that UDCA has a hepatoprotective effect against liver injury caused by ceftriaxone owing to its antioxidant and immunomodulatory properties. Further, clinical studies are required to confirm this effect.

6. Conflict of interest

The authors declare that there is no conflict of interest.

References


