CAN URSODEOXYCHOLİC ACİD BE AN ALTERNATIVE OF N-ACETYLCESTEİN İN ACUTE ACETAMİNOPHEN İNTOXİCATİON?

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ABSTRACT

Acetaminophen is a commonly used antipyretic and analgesic agent which may cause severe hepatic failure in overdoses. The only approved agent used in the treatment of overdoses is still N-acetylcysteine. Ursodeoxycholic acid, is a well known chemical approved in the treatment of especially cholestatic liver diseases but may it also have an affect in acetaminophen intoxication? Our aim was to study the effect of ursodeoxycholic acid on outcome of acetaminophen overdose on a rat model. 32 male Sprague Dawley albino mature rats were randomly assigned into 4 groups. Group 1 (n=8) was control group and was administrated no drug. Remaining 24 rats were applied single dose 300 mg/kg paracetamol intraperitoneally and divided randomly into 3 groups (n=8). Group 2, 3 and 4 were applied 1 ml/kg/day of %0,9 NaCl saline, 300 mg/kg/day of N-acetylcysteine and 100 mg/kg/day of ursodeoxycholic acid respectively intraperitoneally for three days. Then, the animals were decapitated and blood samples were collected by cardiac puncture for biochemical analysis and heptectomy was performed for histopathological and biochemical examinations. Alanine aminotransferase, aspartate aminotransferase and malondialdehyde levels in group 4 were decreased and tissue glutathione levels were increased significantly in a similar pattern with the group 3 when compared to group 2. According to these results we can conclude that ursodeoxycholic acid ameliorates liver function and tissue destruction in acute acetaminophen intoxication.

KEYWORDS: acetaminophen, intoxication, liver failure, n-acetylcysteine, ursodeoxycholic acid.

INTRODUCTION

Acetaminophen is a worldwide used analgesic and antipyretic drug which is usually safe at therapeutic doses but its overdose (> 150 mg/kg) is the most common cause of drug induced liver injury causing fulminant hepatic failure.[1,2] Hepatocytes injured by acetaminophen release inflammatory mediators like interferon-γ, interleukin-1β and tumor necrosis factor (TNF)-α which chemoattract mononuclear phagocytes leading to hepatocyte death in centrilobular regions.[3,4] Acetaminophen also causes mitochondrial injury in hepatocytes that triggers apoptosis.[5,6] N-acetylcysteine (NAC) is still used today as the only approved drug to treat acetaminophen overdose patients.[7]

Ursodeoxycholic acid (UDCA) a dihydroxy bile acid, was proposed for treating chronic cholestatic liver diseases after it was reported to dissolve cholesterol gall stones long after its first identification in China.[8-10] The mechanisms of action seem to be; protection of cholangiocytes against cytotoxicity of hydrophobic bile acids, stimulation of hepatobiliary secretion, and protection of hepatocytes against bile acid–induced apoptosis due to experimental data. One or all of these mechanisms may be of relevance in individual cholestatic disorders and/or different stages of the cholestatic liver disease.

Since 1989, a number of controlled trials on the use of UDCA in primary biliary cirrhosis and primary sclerosing cholangitis were published in the literature.[11]
To date, UDCA is widely used for the treatment of primary biliary cirrhosis for which it is the only drug approved by the U.S. Food and Drug Administration. In the literature, UDCA, being a beneficial treatment in chronic liver diseases has not been studied in acute drug induced liver failure. In this study we aimed to study the effect of UDCA in acute acetaminophen intoxication on a rat model.

**MATERIALS AND METHODS**

**Animals**
In this study 32 male Sprague Dawley albino mature rats weighing 200–220 g, were used. Animals were fed ad libitum and housed in pairs in steel cages having a temperature-controlled environment (22 ± 2 °C) with 12-h light/dark cycles. The experimental protocol was approved by the Committee for Animal Research of Gaziosmanpasa University. All animal studies are strictly confirmed to the animal experiment guidelines.

**Experimental design**
32 male Sprague Dawley albino mature rats were randomly assigned into 4 groups. Group 1 (n=8) was control group and was administered no drug. Remaining 24 rats were given single dose 300 mg/kg paracetamol (PCT) (Parol, Atabay, 10 mg/mL) intraperitoneally (i.p.) and divided randomly into 3 groups (n=8). Group 2 rats (PCT+saline) were administered %0,9 NaCl saline at a dose of 1 ml/kg/day i.p. for three days. Group 3 rats (PCT+NAC) were administered 300 mg/kg/day NAC (Asist, Husnu Arsan, 100 mg/mL) i.p. for three days. Group 4 (PCT+UDCA) were administered 100 mg/kg/day UDCA i.p. for three days. Then, the animals were decapitated and blood samples were collected by cardiac puncture for biochemical analysis and hepatectomy was performed for histopathological and biochemical examinations.

**Determination of plasma ALT and AST levels**
Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit (USCN, Life Science Inc.)

**Determination of lipid peroxidation**
Lipid peroxidation was determined in tissue samples by measuring malondialdehyde (MDA) levels as thiobarbituric acid reactive substances (TBARS).[12] Briefly, trichloroacetic acid and TBARS reagent were added to the tissue samples, then mixed and incubated at 100°C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve using tetraethoxypropane and expressed as nmol/mgr protein.

**Determination of tissue glutathione (GSH) levels**
GSH content in tissue samples was measured spectrophotometrically according to Ellman’s method.[13] In this method, thiols interact with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and form a colored anion with maximum peak at 412 nm. GSH levels were calculated from the standard calibration curve and expressed as nmol/mgr protein.

**Histopathological studies of liver**
After decapitation hepatectomy was performed and the liver samples were perfused with 200 ml of 4% formaldehyde in 0.1 M phosphate-buffer saline (PBS). Formalin-fixed liver sections (4 μm) were stained with hematoxylene & eosine. All sections were photographed with Olympus C-5050 digital camera mounted on Olympus BX51 microscope.

Morphological evaluation was done by computerized image analysis system (Image- Pro Express v1.4.5, Media Cybernetics, Inc. USA) on 10 microscopic fields per section examined at a magnification of ×100, by the observer blind to the study group.

**TNF-α immunoe expression**
For immunohistochemistry, sections were incubated with H2O2 (10%) for 30 min to eliminate endogenous peroxidase activity and blocked with 10% normal goat serum (Invitrogen) for 1 hour at room temperature. Subsequently, sections were incubated in primary antibodies (TNF-α, Santa Cruz Biotechnology; 1/1000) for 24 h at 4 °C. Antibody detection was performed with the Histostain-Plus bulk kit (Invitrogen) against rabbit IgG, and 3,3’ dianinobenzidine (DAB) was used to visualise the final product. All sections were washed in PBS and photographed with an Olympus C-5050 digital camera mounted on Olympus BX51 microscope. Brown cytoplasmic staining hepatocyte was scored positive for TNF-α.

The number of TNF-α positive cell (%) was assessed by systematically scoring at least 100 hepatocyte cell per field in 10 fields of tissue sections at a magnification of 100x.

**Statistical analysis**
Data are presented as mean values ± standard error of the mean (SEM). Data analyses were performed using SPSS version 15.0 for Windows. All data were analyzed by non-parametric (Mann-Whitney U) test. p values of 0.05 or less were regarded as statistically significant.

**RESULTS**
The ALT, AST, MDA and GSH levels measured in all groups are outlined in Table 1. According to these results the ALT (p<0.001), AST (p<0.0001) and MDA (p<0.0001) levels in saline group are extremely increased and GSH (p<0.0001) levels depleted when compared to the normal group as expected. ALT, AST and MDA levels in the NAC group were found to be decreased (p<0.05) and GSH levels increased (p<0.005) remarkably when compared to the saline group. The ALT, AST and MDA levels in the UDCA group were decreased (p<0.05) and GSH levels were increased...
(p<0.05) in a similar pattern with the NAC group when compared to the saline group. The MDA levels measured in the UDCA group being remarkably lower than the saline group displayed a higher value when compared to the NAC group (p<0.05).

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>MDA (nmol/L)</th>
<th>MDA (nmol/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.03 ± 4.48</td>
<td>122.2 ± 7.15</td>
<td>107.2 ± 7.1</td>
<td>1.19 ± 0.10</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>PCT+saline</td>
<td>146.5 ± 18.9*</td>
<td>279.1 ± 22.3**</td>
<td>249.9 ± 21.2**</td>
<td>4.8 ± 0.53**</td>
<td>1.23 ± 0.12**</td>
</tr>
<tr>
<td>PCT+NAC</td>
<td>65.2 ± 7.6#</td>
<td>158.03 ± 18.6#</td>
<td>147.1 ± 24.5#</td>
<td>1.99± 0.20#</td>
<td>4.13 ± 038##</td>
</tr>
<tr>
<td>PCT+UDCA</td>
<td>80.8 ± 10.1#</td>
<td>175.6 ± 31.2#</td>
<td>198.8 ±15.6# †</td>
<td>2.18 ± 0.29#</td>
<td>3.05 ± 0.70#</td>
</tr>
</tbody>
</table>

* p<0.001, ** p<0.0001 (compared to control group);
# p<0.05, ## p<0.005 (compared to PCT+saline);
† p<0.05 (compared to PCT+NAC)

The evaluation of liver tissues by histopathological findings
The liver specimens of all groups are shown in Figure 1. There was severe fatty change and necrosis in centrilobular areas of liver specimens in the saline group. In the paracetamol and UDCA groups there was no remarkable destruction.

The percentage of TNF-α immunoexpression in liver specimens (Table 2, Figure 2.) were extremely elevated in the saline group when compared to the normal group (p<0.001). There was remarkable decline in the percentage of TNF-α immunoexpression in liver specimens of the NAC group and the UDCA group when compared to the saline group (p<0.001) but in the UDCA group the percentage of immunoexpression was higher than the NAC group (p<0.01).
Hematoxylin & Eosine (H & E) staining of sections from rat liver (X 40 and X 100 magnification), cv: central vein. 

(a) and (b) Normal group liver; 
(c) and (d) paracetamol and saline group liver; 
(e) and (f) paracetamol and NAC group liver, no fatty change and necrosis in centrilobular area of liver; 
(g) and (h) paracetamol and UDCA group liver, no fatty change and necrosis in centrilobular area of liver

Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-alfa immunoexpression (% percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.6 ± 0.95</td>
</tr>
<tr>
<td>PCT+saline</td>
<td>71.5 ± 4.6 *</td>
</tr>
<tr>
<td>PCT+NAC</td>
<td>21.8 ± 2.1 #</td>
</tr>
<tr>
<td>PCT+UDCA</td>
<td>48.8 ± 5.3 # †</td>
</tr>
</tbody>
</table>

* p<0.001 (compared to control group); 
# p<0.001 (compared to PCT+saline group); 
† p<0.01 (compared to PCT+NAC group)

Figure 2.

Immunohistochemical staining for TNF alfa (x 100 magnification)

(a) Normal group liver; (b) paracetamol and saline group liver; (c) paracetamol and NAC group liver; (d) paracetamol and UDCA group liver
DISCUSSION

Acetaminophen is a weak acid which is absorbed rapidly from the duodenum after ingestion. The half-life of this molecule after therapeutic doses is 1.5–3 hours but is prolonged in intoxication cases and liver injury.[14] The major elimination site is liver via glucuronidation or sulfation of the molecule. UDP-glucuronosyl transferase enzymes transfer the glucuronosyl group of uridine 5-diphosphoglucuronic acid to target molecules converting them to more water-soluble forms. Relatively less amount of acetaminophen is recovered by sulfation. The metabolic activation of acetaminophen is mainly catalyzed by cytochrome P450 enzymes and the main reactive molecule that is believed to be responsible of hepatotoxicity is N-acetyl-p-benzoquinone imine (NAPQI).[15] NAPQI reacts with nucleophilic sulphhydryl groups. In case of acetaminophen overdose, NAPQI is formed in excess amount which can bind to the cysteine thiol of glutathione. This results in depletion of glutathione reserves of the liver and NAPQI binding to liver proteins.[16] The recognition that glutathione is a critical defence against the reactive metabolite led to the introduction of N-acetylcysteine (NAC) as an antidote against acetaminophen hepatotoxicity in clinical practice.[17]

Apoptosis of hepatocyte was reported to be a significant cause of cell death in patients with cholestatic liver diseases due to the accumulation of toxic hydrophobic bile acids.[18] UDCA is a hydrophilic non-toxic bile acid that competitively inhibits the ideal absorption of toxic endogenous bile acids and dominates them. This competition seems to be the most important mode of action of UDCA.[19-21]

UDCA conjugates have been shown to decrease cytolyis of hepatocytes induced by more hydrophobic bile salts.[22] UDCA, reverses the membrane damaging effect of lipophilic bile acids at mitochondrial level via stabilizing the membrane.[23] As in our study this leads to less apoptosis and necrosis documented by liver enzymes and histopathological examination.

UDCA has antioxidative effects on liver cells by increasing GSH levels in hepatocytes.[24] In our study we also reached similar results indicating the GSH level increase in the UDCA group when compared to the saline group.

Although NAC still being the most effective treatment option in acetaminophen intoxication; our results suggest that UDCA is also remarkably beneficial and may be an alternative. Further investigation is needed to be done.

REFERENCES

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