Taurine Treatment Reduces Hepatic Lipids and Oxidative Stress in Chronically Ethanol-Treated Rats

Jale BALKAN, Öznr KANBAĞLI, Gülçin AYKAÇ-TOKER, and Müjdat UYSAL*

Department of Biochemistry, Istanbul Medical Faculty, University of Istanbul; Çapa, Istanbul 34390, Turkey.
Received March 28, 2002; accepted April 30, 2002

In this study, we evaluated whether taurine treatment has a protective effect on the prooxidant-antioxidant state following chronic ethanol treatment in rats. Rats were given water containing 20% ethanol (v/v) as drinking water for 3 months. Chronic ethanol treatment in drinking water resulted in increased oxidative stress in the liver of rats. Taurine treatment was performed by adding 1% taurine (w/v) to the drinking water plus injection (400 mg/kg body weight) intraperitoneally 3 times/week for 28 d after ethanol cessation in chronically ethanol-treated rats. This treatment starting after ethanol cessation caused a significant decrease in serum transaminase activities and hepatic total lipid, triglyceride, malondialdehyde, and diene conjugate levels and significant increases in hepatic glutathione, vitamin E, and vitamin C levels, but did not alter the activities of superoxide dismutase, glutathione peroxidase, and glutathione transferase in the liver as compared with chronically ethanol-treated rats. Accordingly, we propose that taurine has a restorative effect on ethanol-induced hepatic damage by decreasing oxidative stress.

Key words taurine; chronic ethanol; lipid peroxide; antioxidant; liver; rat

MATERIALS AND METHODS

Rats and Treatment Male Wistar rats (180—200 g) were used for all experiments. Animals were obtained from the Experimental Medical Research Institute of Istanbul University. Rats were fed a standard diet and had free access to water. Ethanol was added to drinking water 20% (v/v) for 3 months (approximately 8.5 g ethanol/kg body weight/d). Control rats (n=8) were given tap water as drinking fluid.

At the end of this period, some rats were killed after overnight fasting. Other rats were divided into two groups. The first group did not receive any treatment, but the second group received taurine for 28 d after ethanol cessation. Taurine treatment was performed by adding taurine to the drinking water 1% (w/v) plus injection (400 mg/kg body weight) intraperitoneally 3 times per week.

Experimental Procedure and Determinations Blood samples were collected from animals by cardiac puncture and serum transaminase (ALT and AST) activities were determined using kits from Sigma. The livers were rapidly removed, washed in 0.9% NaCl and kept in ice. Liver portions were homogenized in ice-cold 0.15 m KCl 10% (w/v). Lipids were extracted by chloroform:methanol (2:1) and hepatic total lipid levels were measured according to the methods of Chiang et al.17) Hepatic triglyceride levels were determined in the lipid extracts with kits from Sigma. The degree of lipid peroxidation was assessed measuring malondialdehyde (MDA) levels using the thiobarbituric acid test18) and diene conjugate levels.19) Liver glutathione (GSH) levels were measured with 5,5′-dithiobis-(2-nitrobenzoate) at 412 nm according to the method of Beutler.20) Liver vitamin E and vitamin C levels were determined in homogenates by the method of Desai21) and Omaye et al.,22) respectively. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione transferase (GST) activities were determined in the hepatic postmitochondrial fractions that were separated by sequential centrifugation. In brief, tissue homogenates were centrifuged at 6000×g for 10 min at 4 °C to remove crude fractions. Then the supernatants were centrifuged at 10000×g for 20 min to obtain postmitochondrial fractions. Hepatic SOD activities were assayed by its ability to increase the effect of riboflavin-sensitized photoreduction of ortho-dinitrobenzene.23) GSH-Px activities were measured using cumene hydroperoxide as a substrate.24) GST activities were assayed using the spectrophotometric method using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate.25) Protein levels were determined by the method of Lowry et al.26) using bovine serum albumin as a standard. Statistical Analysis Results are expressed as mean±S.D. Statistical analysis was performed using Student’s t-test for unpaired data, and values of p<0.05 were considered statistically significant.

* To whom correspondence should be addressed. e-mail: mujdatuysal@hotmail.com © 2002 Pharmaceutical Society of Japan
RESULTS AND DISCUSSION

There is increasing evidence that ethanol-induced liver injury is associated with free radical injury and oxidative stress.\(^8,9\) Oxidative stress is characterized by increased lipid peroxidation and/or altered nonenzymatic and enzymatic antioxidant systems. However, conflicting results are reported in the literature concerning oxidative stress following chronic ethanol treatment in rats.\(^3,10,12,13,17,28\) These discrepancies may be due to the techniques used for assessing oxidative stress as well as the conditions of ethanol administration. Several types of rat model are used for alcoholic liver disease studies such as the Lieber–DeCarli liquid diet or by adding ethanol to rats’ drinking water.\(^29,30\) The Lieber–DeCarli diet has been widely used to cause significant liver damage in rats.\(^29,30\) However, some investigators have proposed that ethanol administration in drinking water may be a model closer to human conditions than a liquid diet.\(^31\) Our previous studies have shown that chronic ethanol treatment in drinking water resulted in increased oxidative stress in the liver of rats.\(^10,11,32\) Therefore in the present study, rats were given water containing 20% (v/v) ethanol as drinking water for 3 months. We found that chronic ethanol treatment caused significant increases in plasma ALT and AST activities and hepatic total lipid, triglyceride, MDA, and diene conjugate levels and significant decreases in GSH, vitamin E, and vitamin C levels, but hepatic SOD, GSH-Px and GST activities remained unchanged as compared with those in controls (Fig. 1). Twenty-eight days after alcohol cessation in chronically ethanol-treated rats, we detected significant decreases in liver triglyceride levels and significant increases in GSH and vitamin E levels. However, there were no changes in total lipid, MDA, and vitamin C levels as well as serum transaminase activities after ethanol cessation. Taurine treatment after the cessation of ethanol in chronically treated rats caused a further decrease in hepatic triglyceride levels and further increases in GSH and vitamin C levels as well as significantly decreased diene conjugate and MDA levels and serum transaminase activities. No changes in antioxidant enzyme activities were observed with and without taurine treatment following alcohol cessation in chronically treated rats (Table 1). Based on these data, taurine treatment has a restoring effect on lipid peroxidation and nonenzymatic antioxidants without affecting antioxidant enzyme activities after chronic ethanol ingestion. Similar effects were also demonstrated by Kerai et al.\(^5\) However, the important difference between these two studies is that in their studies, alcohol and taurine were coadministered to rats, that is, they examined the preventive effect of taurine during the production of alcohol-induced hepatic damage, whereas we tried to detect the reverse effect of taurine on preexisting ethanol-induced hepatic damage by administrating taurine after the cessation of ethanol.

The mechanism of the restorative effect of taurine has not been clarified. It has been suggested that taurine acts as an antioxidant\(^33\) and has a scavenger effect.\(^3\) In our recent investigations, we showed that taurine had preventive effect on the acute\(^6\) and chronic\(^7\) damage induced by thioacetamide in rats by decreasing oxidative stress. Accordingly, we propose that taurine has a restorative effect on ethanol-induced oxidative stress.

![Fig. 1. Changes in Serum ALT and AST Activities and Hepatic Lipids and Prooxidant–Antioxidant Status in Chronically Ethanol-Treated Rats (% of Control Values)](image)

Table 1. The Effect of Taurine Treatment on Plasma Transaminase Activities and Hepatic Lipids, and Prooxidant and Antioxidant Status in Chronically Ethanol Treated Rats (Mean±S.D.)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ethanol (n=8)</th>
<th>Ethanol cessation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No treatment</td>
<td>Taurine treatment</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td>(n=8)</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>56±7</td>
<td>52±6</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>135±21</td>
<td>136±20</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipid (mg/g tissue)</td>
<td>46.8±4.27</td>
<td>45.6±5.25</td>
</tr>
<tr>
<td>Triglyceride (mg/g tissue)</td>
<td>14.1±3.15</td>
<td>9.59±1.62(^a)</td>
</tr>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>199.1±31.3</td>
<td>185.4±21.9</td>
</tr>
<tr>
<td>Diene conjugates (µmol/g tissue)</td>
<td>3.05±0.27</td>
<td>2.87±0.16</td>
</tr>
<tr>
<td>GSH (µmol/g tissue)</td>
<td>5.21±0.71</td>
<td>6.07±0.45(^a)</td>
</tr>
<tr>
<td>Vitamin E (nmol/g total lipid)</td>
<td>804.8±143.6</td>
<td>936.5±90.7(^a)</td>
</tr>
<tr>
<td>Vitamin C (nmol/g tissue)</td>
<td>392.3±56.7</td>
<td>450.2±60.2(^a)</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>8.62±2.09</td>
<td>9.32±1.35</td>
</tr>
<tr>
<td>GSH-Px (nmol/mg protein/min)</td>
<td>661.7±129.3</td>
<td>714.2±44.4(^a)</td>
</tr>
<tr>
<td>GST (nmol/mg protein/min)</td>
<td>644.2±234.1</td>
<td>590.7±105.0(^a)</td>
</tr>
</tbody>
</table>

\(^a\) p<0.05 as compared with the ethanol-treated group. \(^b\) p<0.05 as compared with the ethanol-ceased group.
Acknowledgment This work was supported by the Research Fund of the University of Istanbul (Project no. Ö-998/030552001).

REFERENCES