Effect of Chronic Alcohol Consumption on Phosphatidylcholine Hydroperoxide Content of Rat Liver and Brain

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Abstract

Purpose: To investigate the correlation between alcohol-induced oxidative stress and tissue phosphatidylcholine hydroperoxide (PC-OOH) content of rat liver and brain.

Methods: Ten Wistar rats were divided into two groups: one group was given 20% ethanol (5 g/kg) and the other the same volume of normal saline, orally once a day for 6 weeks. Catalase activity, malondialdehyde (MDA) content, total antioxidant capacity (TAC) and PC-OOH content of liver and brain were determined.

Results: The ethanol-treated group had lower catalase activity and total antioxidant capacity. MDA level in the liver was 0.33 ± 0.07 μM/mg protein which is significantly (p < 0.05) higher than that of the control group (0.17 ± 0.06 μM/mg protein), but in brain, there was no significant difference. PC-OOH level in the ethanol-treated group was 46.91 ± 12.87 pmol/mg in liver and 71.97 ± 26.12 pmol/mg protein in brain while PC-OOH level of control group was 21.40 ± 10.71 pmol/mg protein in liver and that in brain was 25.29 ± 5.67 pmol/mg protein pmol. Thus, PC-OOH levels in both liver and brain were significantly (p < 0.05) higher than that of control group. PC-OOH content in the liver and brain correlated significantly (p < 0.05) with catalase activity and total antioxidant capacity (TAC).

Conclusion: The study demonstrates that PC-OOH content in liver and brain tissues may be a marker for alcohol-induced oxidative stress.

Keywords: Alcoholic toxicity, Oxidative stress, Phosphatidylcholine hydroperoxide, Liver, Brain, Biomarker

INTRODUCTION

Alcohol is consumed worldwide as a drink by humans. Regular and unlimited use of alcohol leads to toxicity and alcohol-induced pathological problems and can constitute a menace in the society. Therefore, several studies have focused on the mechanism of cell or tissue injury caused by alcohol-induced oxidative stress and protective methods. Alcohol-induced oxidative stress leads to various diseases like hepatic diseases, cardiovascular diseases, and nervous diseases [1-3]. The role of reduced nicotinamide adenine dinucleotide (NADH) is one of the more reliable hypotheses of ethanol-induced oxidative stress. The increased level of NADH, during metabolizing ethanol, leads to increase respiratory rate and the absorption of oxygen is increased. Eventually, the production of reactive oxygen species are elevated [4]. Another reliable
The common methods for measuring the effect of oxidative stress are determination of the level of malondialdehyde (MDA), reactive oxygen species (ROS), glutathione (GSSG/GSH) or measuring the activity of antioxidant enzyme [6-9]. In addition, protein carbonyl content in the tissue samples is also used for tissue injury [10]. Phosphatidylcholine (PC) and phosphatidylyl-ethanolamine (PE), which are the main composition of cell wall are oxidized by oxidative stress-induced tissue injury and remains in the tissue or flow in the plasma. Miyazawa et al developed the method for simultaneous determination of PC-OOH and PE-OOH by using the chemiluminescence analyzer-high performance liquid chromatography (CL-HPLC) system [11].

The aim of this study was to investigate the correlation between the alcohol-induced oxidative stress and PC-OOH content in rat liver and brain.

**EXPERIMENTAL**

**Animal studies**

Ten male Wistar rats (7 weeks old, mean body weight approx 150 g) were purchased from the Central Laboratory Animal Inc (Seoul, Korea) and allowed a normal diet for one week while adapting to the new raising environment. After the adaptation period, rats were divided in two groups. One group was treated with 20 % ethanol (5 g ethanol/kg body weight) and the other group was treated with the same volume of 8.5 % saline solution, via oral administration by using the gavage feeding needle once a day. High fat diet (45 % of energy divided from fat) and water were administered by ad libitum feeding. After 6 weeks, rats were fasted for 24 h and anesthetized with intraperitoneal injection of Zoletil (Zoletil 50, Virbac, Carros, France), blood was collected from the abdominal aorta, and the liver and brain were extracted for measuring antioxidant capacity and PC-OOH levels.

All experimental protocols and schedules involving animals were approved (ref no. KU11101) by Institutional Animal Care and Use Committee of Konkuk University and the international guideline for the care and use of laboratory animals was followed [12].

**Organ weight**

The main organs (liver, kidney, spleen, adipose tissues, and brain) were removed, rinsed twice with phosphate buffered saline solution (PBS), and dried with filter paper. Their weights were measured immediately using electronic balance (AR2140, Ohaus, Pine Brook, NJ). Organ weight, except that of brain, was presented as organ-to-body weight ratio.

**Determination of malondialdehyde**

The level of malondialdehyde (MDA) was determined by using MDA quantitation TBARS assay kit (Cell Biolabs, San Diego, CA). The tissue sample was homogenized on ice with BHT containing 1X phosphate buffered saline (1X PBS) solution. The homogenate was centrifuged at 10,000 g for 5 min to collect the supernatant. A 100 μL of tissue lystate or MDA standard were moved on microcentrifuge tubes. A 100 μL of Sodium dodecyl sulfate (SDS) lysis solution (150 mM of NaCl and 0.1 mM of EDTA in 10 mM Tris–buffer, pH 7.5) was added and mixed, and then, a 250 μL of 2-thiobarbituric acid (TBA) reagent was added and incubated at 95 °C for 60 min. The absorbance of the reactant was read at 532 nm. MDA content was expressed as nmol MDA/mg protein. Protein content of the tissue was determined by the biuret method.

**Evaluation of antioxidative enzyme activity and total antioxidant capacity**

The catalase activity (Cell Biolabs, San Diego, CA), superoxide dismutase activity (Cell Biolabs, San Diego, CA), and total antioxidant capacity (BioAssay Systems, Hayward, CA) was determined by using a colorimetric assay kit. The liver and brain tissues were homogenized with 1X phosphate buffered saline (1X PBS) solution. The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C. The supernatant was collected and stored at -80 °C before the assay.

**Determination of PC-OOH**

The photoirradiation method for preparation of the PC-OOH standard was used [11]. The amount of peroxide in the standard solution was determined by the method of the American Oil Chemist’s Society [13]. The hydroperoxide concentration of the photo-oxidized PC was 19.1 μmol hydroperoxide-O_2/g of PC. Total lipids of the liver and brain tissue were extracted by using a modification of the method of Folch et al [14]. The standard solution and lipid extract of the tissue were determined by using chemiluminescence analyzer-high performance...
liquid chromatography (CL-HPLC) system [11]. The detail condition of CL-HPLC is shown in Table 1. PC-OOH content was expressed as mg/g tissue protein. The total protein of liver and brain was determined by biuret method [14].

**Statistical analysis**

All data are presented as the mean ± standard error of the mean (S. E. M.). The differences between the groups were calculated by the Duncan’s multiple range test (p < 0.05). The Pearson’s correlation efficiency between the PC-OOH level and other antioxidant capacity was calculated, and p-value was calculated for statistical hypothesis testing (p < 0.05). All statistical analyses were performed using the Statistical Analysis Software (Statistics Analytical System Institute, Cary, NC, 1988).

**RESULTS**

**Food intake, body weight and organ weight**

Daily food intake and changes in body weight are shown in Table 2. The food intake and bodyweight gain of the ethanol-treated group was significantly (p < 0.05) less than that of the control group. Weights of perirenal (PAT) and epididymal adipose tissue (EAT) decreased remarkably in alcohol treated rats.

**Level of malondiadehyde in liver and brain**

The level of hepatic MDA significantly (p < 0.05) increased in alcohol treated rats which is in similar pattern as observed in previous study [5]. In liver samples, MDA level of ethanol treated group (0.33 ± 0.07 μM/mg) was significantly higher than that of control group (0.17 ± 0.06 μM/mg).

However, in the brain samples, there were no significant differences in the level of MDA between control (0.05 ± 0.02 μM/mg) and ethanol treated group (0.11 ± 0.05 μM/mg). The previous study has reported that alcohol consumption affects the MDA level of particular part in rat brain [16]. They reported that only the cerebellum MDA level was affected by alcohol consumption, while the hippocampus and cortex were not affected.

**Table 1:** Chemiluminescence analyzer-high performance liquid chromatography conditions for phosphatidylcholine hydroperoxide determination

<table>
<thead>
<tr>
<th>Column</th>
<th>ZORBAX NH2 (4.6 × 250 mm, 5 μm, Agilent Technologies, USA) column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>2-propanol-methanol-water (1350:450:200, v/v/v), flow rate: 1 mL/min.</td>
</tr>
<tr>
<td>Chemiluminescence analyzer</td>
<td>CLD-1100 (Tohoku Electronic Industries Co., Sendai, Japan)</td>
</tr>
<tr>
<td></td>
<td>Reaction temperature: 40 °C</td>
</tr>
<tr>
<td>Luminescence reagent</td>
<td>10 mg/L of cytochrome c (from bovine heart, Sigma-Aldrich) and</td>
</tr>
<tr>
<td></td>
<td>2 mg/L of luminol (Wako pure chemical) in 50 mM borate buffer (pH 10.0). flow rate: 0.8 mL/min</td>
</tr>
</tbody>
</table>

**Table 2:** Daily food intake, body weight and organ weight of control and treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily food intake (gram/day)</td>
<td>17.4 ± 1.4</td>
<td>13.7 ± 3.6*</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>296.2 ± 9.4</td>
<td>299.6 ± 7.2</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>464.8 ± 24.8</td>
<td>415.6 ± 16.2*</td>
</tr>
<tr>
<td>Change in body weight (%)</td>
<td>56.9</td>
<td>38.7</td>
</tr>
<tr>
<td>Liver (g/kg body weight)</td>
<td>23.1 ± 3.2</td>
<td>21.9 ± 2.0</td>
</tr>
<tr>
<td>Spleen (g/kg body weight)</td>
<td>1.16 ± 0.12</td>
<td>1.18 ± 0.06</td>
</tr>
<tr>
<td>Kidney (g/kg body weight)</td>
<td>4.86 ± 0.52</td>
<td>5.03 ± 0.20</td>
</tr>
<tr>
<td>EAT (g/kg body weight)</td>
<td>22.3 ± 5.5</td>
<td>14.3 ± 0.8*</td>
</tr>
<tr>
<td>PAT (g/kg body weight)</td>
<td>30.8 ± 4.9</td>
<td>11.7 ± 2.3*</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>1.97 ± 0.03</td>
<td>1.89 ± 0.05</td>
</tr>
</tbody>
</table>

Each value is mean ± SEM; Control: high fat diet (45 % of calories derived from fat) + saline solution (0.85 % NaCl); Treatment: high fat diet + ethanol (20 %) @ 5 g/kg body weight; *Significantly different (p < 0.05); EAT, epididymal adipose tissue, PAT, perirenal adipose tissue
Activity of antioxidant enzymes and total antioxidant capacity

Total antioxidant capacity (TAC) and the activity of antioxidative enzymes in the rat liver and brain were decreased \((p < 0.05)\) in alcohol treated rats (Table 3). This result suggests that the enzymatic antioxidant system has been broken down by ethanol consumption, as previously estimated \([18]\).

PC-OOH content of tissue

The PC-OOH level of control group was \(21.40 \pm 10.71\) pmol/mg in liver and \(25.29 \pm 5.67\) pmol/mg in brain. Ethanol treated rats shows significantly \((p < 0.05)\) higher level in both liver \((46.91 \pm 12.87\) pmol/mg) and brain \((71.97 \pm 26.12\) pmol/mg in brain). This result confirms the fact that alcohol-induced oxidative stress by chronic ethanol consumption may accelerate the tissue injury of the liver and brain. The correlation between PC-OOH content and other oxidative indices was shown in Table 4.

DISCUSSION

The weights of perirenal (PAT) and epididymal adipose tissue (EAT) decreased remarkably in alcohol treated rats. These results show the similar pattern of decreased body weight caused by the prevention of decline in the plasma leptin, which was induced by the ethanol consumption as reported by the previous study \([16]\).

The increased level of hepatic MDA in alcohol treated rats is in similar pattern as observed in previous study \([5]\). However, in the brain samples no difference in the level of MDA between control and treated group was observed. A previous study has reported that alcohol consumption affects the MDA level of particular part in rat brain \([16]\). They reported that only the cerebellum MDA level was affected by alcohol consumption, while the hippocampus and cortex were not affected.

The decreased total antioxidant capacity (TAC) and the activity of antioxidant enzymes in rat liver and brain in alcohol treated rat suggests that the enzymatic antioxidant system may have been broken down by ethanol consumption, which is in agreement with the earlier report \([16]\). The results in the present study indicate that the ethanol treated rats were sufficiently exposed to alcohol-induced oxidative stress.

The higher levels of PC-OOH contents in liver and brain of the alcohol treated rats compared to control group confirms the fact that alcohol-induced oxidative stress by chronic ethanol consumption may accelerate the tissue injury of the liver and brain. All data were statistically correlated with PC-OOH content, except the MDA level in the brain. PC-OOH contents in both of the liver and brain were increased by the

Table 3: The effect of ethanol on the activity of catalase, total antioxidant capacity (TAC) and total glutathione in rat liver and brain

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Brain</td>
</tr>
<tr>
<td>Catalase activity (U/mg protein)</td>
<td>27.94 ± 0.61</td>
<td>21.66 ± 0.79</td>
</tr>
<tr>
<td>SOD activity (inhibition %/mg protein)</td>
<td>3.21 ± 0.17</td>
<td>2.68 ± 0.13</td>
</tr>
<tr>
<td>TAC (μM trolox equivalents)</td>
<td>504.9 ± 17.66</td>
<td>470.3 ± 31.52</td>
</tr>
</tbody>
</table>

*Each value is mean ± S. E. M; Control: high fat diet (45 % of calories derived from fat) + saline solution (0.85 % NaCl); Treatment: high fat diet + ethanol (20 % EtOH, @5 g/kg body weight); *significantly different \((p < 0.05)\)*

Table 4: Pearson’s correlation coefficient between PC-OOH content in tissue and oxidant markers

<table>
<thead>
<tr>
<th>Oxidant marker</th>
<th>Pearson’s correlation efficiency</th>
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<tbody>
<tr>
<td></td>
<td>Liver PC-OOH</td>
</tr>
<tr>
<td>MDA</td>
<td>0.749</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>-0.912*</td>
</tr>
<tr>
<td>SOD activity (Inhibition %)</td>
<td>-0.759</td>
</tr>
<tr>
<td>Total antioxidant capacity</td>
<td>-0.679</td>
</tr>
</tbody>
</table>

\* \(P < 0.05\) \((n = 10)\); \textsuperscript{NC} no statistical correlation between each crossed data
Fig 1: Typical chromatograms of PC–OOH in rat liver. (a) PC–OOH standard, (b) lipid extract of saline solution treated rat liver, (c) alcohol treated rat liver, (d) saline solution treated rat brain, and (e) alcohol treated rat brain ethanol treatment. The present study has demonstrated that PC-OOH content in the tissue as a new marker for alcohol-induced oxidative stress.

The positive correlation between PC-OOH content and other oxidative index in both of the liver and brain has demonstrated that PC-OOH content in the tissue as a new marker for alcohol-induced oxidative stress.

Since the method of determination PC-OOH has improved, there are several reports about the relationship between oxidative stress-induced diseases and PC-OOH content in tissues or blood. Since metabolism of ethanol accelerates the formation of oxidative stress, some studies have been carried out to ascertain the relationship between them. Adachi et al [11] has reported that blood PC-OOH level is suitable as a new marker of alcoholic diseases.
Based on these theories, the current study investigated the relationship between alcohol induced-oxidative stress and tissue PC-OOH content. These indices were statistically correlated and PC-OOH content showed more sensitive differences between control and alcohol treated group. Therefore, PC-OOH content in tissue samples will be a more sensitive and effective index for in vivo alcohol-induced oxidative stress related research in future.

**CONCLUSION**

The findings of this study show that the PC-OOH content liver and brain tissues may be useful as a marker for alcohol-induced oxidative stress or tissue degradation, and may be a more accurate marker than MDA content in alcohol-related studies. Further studies are, however, required to compare tissue PC-OOH content as a marker with protein oxidation (carbonated protein), in addition to histological and immunological investigations.

**ACKNOWLEDGEMENT**

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