The effect of liver hydrolysate on chronic ethanol-induced hepatic injury in normal rats

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Summary

The mechanism underlying the improvement in hepatic function by liver hydrolysate (LH) after ethanol-induced hepatic injury is unclear. Therefore, we investigated the effects of LH administration on chronic ethanol-induced hepatic injury in normal rats and the mechanism underlying the improvement of its symptoms by LH.

LH attenuated liver damage and reduced oxidative stress after chronic ethanol-induced hepatic injury in normal rats. LH treatment reduced hepatic injury biomarkers of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT). LH treatment also decreased levels of 8-hydroxy-deoxyguanosine (8-OHdG) as oxidative stress marker. LH may prove beneficial to prevent the liver damage of chronic ethanol, at least in part, by alleviating oxidative stress.

Key words Chronic ethanol; Liver damage; Liver hydrolysate; Oxidative stress
INTRODUCTION

Chronic ethanol ingestion can lead to various alcoholic liver diseases, including liver disease,1) diabetes mellitus,2) and cancer.3)

LH primarily comprises peptides and amino acids.4) LH has attracted attention because it can enhance hepatic function.4) Kishimoto et al. suggested that LH suppresses the increased serum concentration of acetaldehyde after ethanol ingestion in mice, and LH is relevant for symptom improvement in acute ethanol intoxication.5) In addition, Okuyama et al. have shown that LH improves hepatic function in alcoholic liver disease in humans.6)

However, the mechanism underlying the amelioration in hepatic function by LH after ethanol-induced hepatic injury is unclear. Thus, we examined the effects of LH administration on chronic ethanol-induced hepatic injury in normal rats, and the mechanism underlying the amelioration of its symptoms by LH was investigated.
MATERIALS AND METHODS

Materials and experimental diets

We used LH from Zeria Pharmaceutical Co. Ltd.\textsuperscript{4} LH was obtained by enzymatic degradation of the liver.\textsuperscript{4} The Lieber–DeCarli diet has been widely used to cause significant liver damage in rats.\textsuperscript{7} Experimental diets were prepared by the Lieber–DeCarli liquid diet with slight modification.\textsuperscript{8,9} These diets have been resulted in increased oxidative stress in the liver of rats.\textsuperscript{10} The levels of carbohydrate and protein in the ethanol + LH group diet were kept equivalent to those of the other two groups by referring to the ingredients comprising LH (Table 1). The diets were prepared daily and provided to the rats in plastic bottles with nozzles made of glass that were designed to minimize spillage.

Animals and biochemical marker assays in plasma

Because it was demonstrated binge alcohol is more injurious to liver in female than in male rats,\textsuperscript{11} we used female Wistar rats aged 7 weeks (Japan SLC, Shizuoka, Japan) in same as our previous study.\textsuperscript{10} The rats were housed individually under steady condition (23° ± 2°C, with a 12h light-dark cycle with lights on at 8:00). On day 14 after
the feeding of LH, rats scarified after 14 h of fasting. To determine the plasma biomarker levels, blood was obtained from the rats by decapitation. The blood samples were collected in tubes with Na₂EDTA. The blood samples were centrifuged at 1,870 g for 15 min at 4°C, and the plasma was divided into aliquots and stored at −30°C.

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by enzymatic colorimetric methods (Wako Pure Chemical Corporation) according to the manufacturer’s instructions. The concentration of 8-OHdG was determined by EIA with a commercially available kit purchased from JaiCA (Japan Institute for the Control of Aging, Shizuoka, Japan). The assay was performed according to the manufacturer’s instructions.

**Statistical analysis**

Results are expressed as the mean ± standard error of the mean. One-way analysis of variance followed by the Fisher least significant difference test was used to evaluate the differences between groups (Fig. 1, Fig. 2). Significant difference was defined as p<0.05.
For comparing groups in Fig.3, the significance of the differences was determined by the Tukey–Kramer test. A significant difference was defined as $P < 0.05$.

**Ethical guidelines**

All protocols approved by the Committee in Tohoku University (No. 2013AgA-024, and 2014AgA-006).

**RESULTS**

**Effects of LH on the body weight and food intake**

A significantly lower body weight was found in both ethanol groups from the sixth day than that in controls (Control: 180.5 ± 1.3 g, Ethanol: 163.7 ± 2.1 g, Ethanol+LH: 164.0 ± 2.2 g) (Fig. 1A). It was also observed in the total food intake that ethanol fed groups consumed lower liquid diet, thus partly explaining the lower weight gain. This observation on growth due to reduced food intake and is a commonly observed phenomenon. The total food intake of the ethanol-fed groups was significantly lower liquid diet than that of controls (Control: 804.4 ± 8.2 g, Ethanol: 537.0 ± 12.9 g, Ethanol+LH: 516.8 ± 14.3 g)
(Fig. 1B). There were no significant in the body weight and food intake between the ethanol group and ethanol + LH group.

Effects of the activities of plasma AST and ALT activities

ALT and AST are liver injury biomarkers and their significant elevation often reflects liver injury. Primary indicators of liver injury that may be detected efficiently is the high concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. In the current study, LH treatment decreased the activities of plasma AST (Control: 90.7 ± 5.8 IU/L, Ethanol: 232.6 ± 25.9 IU/L, Ethanol+LH: 135.1 ± 30.6 IU/L) (Fig. 2A) and ALT (Control: 35.7 ± 3.9 IU/L, Ethanol: 111.5 ± 15.8 IU/L, Ethanol+LH: 64.2 ± 10.8 IU/L) (Fig. 2B). These results demonstrate that LH protect against alcohol-induced liver injury.

Effects of the serum 8-OHdG level

8-OHdG level was used as the index of oxidative stress marker. Acute and chronic ethanol intake has been shown to increase the production of reactive oxygen species (ROS)
and lower cellular antioxidant levels in many tissues especially the liver.\textsuperscript{14-18} A consequence of increased ROS production is oxidation of mitochondrial DNA reflected by increased amounts of 8-OHdG. It is also reported that 8-OHdG may be reliable marker of oxidative stress in patients with chronic liver disease.\textsuperscript{19} As shown in Fig. 3, LH treatment reduced the levels of 8-OHdG (Control: 508.7 ± 24.8 pg/mL, Ethanol: 616.4 ± 7.7 pg/mL, Ethanol+LH: 500.7 ± 25.8 pg/mL).

**DISCUSSION**

The present study showed that LH attenuates hepatic damage together with the antioxidative effect on chronic ethanol-induced hepatic injury in normal rats. The mechanism underlying the amelioration of hepatic injury in ethanol exposure by LH has not been clarified; however, an antioxidative effect may be involved.

Chronic ethanol consumption causes injury to hepatocytes. The blood activities of AST and ALT are used biomarkers for hepatic injury.\textsuperscript{13} AST is found in the skeletal muscle, liver, lung, leukocytes and kidney. Alternatively, ALT is largely found in the liver.\textsuperscript{20} The present study showed that chronic ethanol exposure caused liver impairment,
as evidenced by the elevated activities of plasma AST and ALT. LH treatment offered significant protection against the chronic exposure of ethanol in normal rats by attenuating the elevation of AST and ALT activities. Okuyama et al. showed that LH ameliorated hepatic function in patients with alcoholic liver disease. We found that LH attenuates the elevated activities of plasma AST and ALT in an ethanol-induced hepatic injury model. LH may attenuate the elevated activities of blood AST and ALT in chronic ethanol-induced hepatic injury in healthy humans.

Oxidative stress is associated with the pathogenesis of ethanol-induced hepatic damage, and chronic ethanol exposure increases the production of reactive oxygen species (ROS) in the hepatocyte. The oxidation of mitochondrial DNA by increased ROS production increased amounts of 8-OHdG. In the current study, treatment with LH attenuated increased oxidative stress, as shown by the reduction of 8-OHdG. It was reported that acetaldehyde has increase of intracellular ROS and oxidative stress. Because LH suppresses the increased serum concentration of acetaldehyde after ethanol ingestion, the antioxidative effect of LH may also include a decrease in acetaldehyde.

LH contains a significant amount of proteins and peptides (>70%). Some peptides and proteins have antioxidant activity. In addition, peptides are known for higher
activity than proteins. Because LH is prepared by enzymatic digestion of the liver, peptides in LH may have large capacity against excess oxidative stress under conditions of chronic ethanol exposure.

In conclusion, our data indicated that LH attenuated hepatic damage in a chronic ethanol-induced animal model in normal rats, at least in part, by the antioxidative activity of LH. Further extensive experiments of the identification of the active constituent will be presented in a subsequent paper.

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Conflicts of interest

KY, SH and HS are employees of Zeria Pharmaceutical Co.,Ltd.
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Table 1. Formulation of experimental diets

| Ingredient (g/L)                  | Control (negative control 0% ethanol) | Ethanol (positive control 5% ethanol) | Ethanol + LH (5% ethanol + LH) |
|----------------------------------|---------------------------------------|---------------------------------------|---------------------------------|
| Na saccharin                     | 0.29                                  | 0.29                                  | 0.29                            |
| DL-methionine                    | 0.3                                   | 0.3                                   | 0.3                             |
| Cellulose                        | 1.1                                   | 1.1                                   | 1.1                             |
| Vitamin mixture*                 | 2.4                                   | 2.4                                   | 2.4                             |
| Xanthan gum                      | 2.5                                   | 2.5                                   | 2.5                             |
| Mineral mixture**                | 8.3                                   | 8.3                                   | 8.3                             |
| Sucrose                          | 124.4                                 | 32.7                                  | 32.7                            |
| Protein                          |                                       |                                       |                                 |
| Soy protein isolate              | 46.5                                  | 46.5                                  | 46.27                           |
| LH                               | 0                                     | 0                                     | 0.31                            |
| Corn oil                         | 53                                    | 53                                    | 53                              |
| Ethanol                          | 0                                     | 50                                    | 50                              |
| Dist. water                      | 759.7                                 | 759.7                                 | 759.7                           |

* : To 100 g of the AIN-93 vitamin mixture, 57.3 mg of retinyl acetate was added.

** : AIN-93M mineral mixture.
Fig. 1. Body weight and food intake

Each value represents the mean ± SE; n = 5. Significantly different from control, **p < 0.01.
Fig. 2. Plasma AST and ALT activities

LH treatment decreased biochemical markers for hepatic injury.

LH treatment suppressed both plasma (A) AST and (B) ALT activities. Each value represents the mean ± SE; n = 5. Significantly different from ethanol, *p < 0.05, **p < 0.01.
Fig. 3. Serum 8-OHdG concentration.

LH treatment decreased the level of serum 8-OHdG concentration.

Each value represents the mean ± SE; n = 3-4. Significantly different from ethanol, *p < 0.05