The Effect of Ethanolamine on Acute Carbon Tetrachloride Intoxication

Takaya Murakami, Yoichi Nagamura, and Kazuyuki Hirano

Department of Pharmaceutics, Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502, Japan and Department of Clinical Chemistry School of Health Sciences, Fujita Health University, Toyoake, Aichi 470-11, Japan.

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The effects of ethanolamine on injured liver were investigated by oral administration of ethanolamine to male ddY mice 24 h after carbon tetrachloride (CCl₄) injection. The serum aminotransferase activities in mice with liver injury were reduced by ethanolamine treatment (10–30 mg/kg body weight). Drastically increased regenerative reaction of ethanolamine treated-CCl₄ injured liver was also observed through an increase in 5-bromo-2′-deoxyuridine uptake. ATP concentration in liver tissue was recovered by administration of ethanolamine. These results suggest that oral administration of ethanolamine accelerates recovery from CCl₄-induced liver injury.

Key words: ethanolamine; liver injury; carbon tetrachloride; hepatocellular regeneration; 5-bromo-2′-deoxyuridine; ATP

Ethanolamine is a precursor of cell membrane phospholipids such as phosphatidylethanolamine and phosphatidylcholine, and plays a specific role not only as a metabolite of phospholipids, but also as one of the intracellular signal transductants that is closely connected with regulation of membrane bounded enzymes. The regenerative ability of intraperitoneally administered ethanolamine was demonstrated by its effects on the liver following partial hepatectomy. Ethanolamine thus plays a key role in hepatocellular regeneration.

The aim of the present study was to investigate the effects of oral administration of ethanolamine on CCl₄-induced hepatitis, since it is contained in foods such as milk and is also generated in the intestine.

MATERIALS AND METHODS

CCl₄ Induced Hepatitis Mouse Model Mice (6-week-old male ddY mice, 28–30 g body weight, Chubu Kagaku Shizai, Nagoya, Japan) were housed for at least one week prior to the study and were maintained on a 12 h light–dark cycle at 23°C. Food (MF; Oriental Yeast, Osaka, Japan) and distilled water were freely available during this acclimation period. CCl₄ was dissolved in Panacetone 810 (Nishiyu Loposome Co., Ltd., Tokyo, Japan) at a concentration of 10% (v/v). After deprivation of food, 10% CCl₄ was injected intraperitoneally at a dose of 10 ml/g body weight.

Treatment of Ethanolamine Twenty-four hours after the CCl₄ treatment, indicated doses of ethanolamine were orally administered. The control groups received saline or Panacetone 810, respectively. Twenty-four hours later, blood samples were collected from ether anesthetized mice via the heart, and liver samples were taken. Alamine aminotransferase (ALT) activity in the serum samples was measured with a commercial reagent and autoanalyzer COBAS MIRA (Japan Roche, Tokyo).

Measurement of ATP Levels The liver was rapidly removed and washed with cold 0.1 M Tris–HCl buffer, pH 7.5 containing 4 mM EDTA. The 10% (w/v) homogenates were mixed with the same volume of 5% trichloroacetic acid (TCA) and centrifuged at 7000×g for 10 min; 0.7 ml of the supernatant was mixed with 75 μl of 20% KHCO₃. ATP concentration was determined by bioluminescence assay kit II (Boehringer Mannheim, Mannheim, Germany). ATP levels in tissue were expressed as nmol ATP/mg protein. Protein was determined by Lowry method.

Analysis of Hepatocellular Proliferation 5-bromo-2′-deoxyuridine/5-fluoro-2′-deoxyuridine (BrdU/FdU; RPNI 210, Amersham International Plc, Buckinghamshire, England) was injected (1 ml/100 g body weight) 2 h before the mice were sacrificed. The liver was fixed in cold methanol for 24 h at 4°C, and then processed for paraffin sections. Immunohistochemistry for BrdU/FdU incorporation was carried out by treating sectioned samples with anti-BrdU antibody as primary antibody. Thereafter the peroxidase labeled method was followed according to the manufacturer’s instructions. The number of anti-BrdU antibody stained nuclei was counted using light microscopy at a magnitude of 400X. One field corresponded to an area 2.74 mm², and anti-BrdU antibody stained nuclei counted in 10 randomly selected fields of each sample (a total area of 27.4 mm²) and tallied. Each group had 3–5 samples.

Statistical Analysis The values of serum aminotransferase activities and DNA synthesis are all expressed as mean±standard deviation (S.D.). For all analyses, p<0.05 was the minimal requirement for statistically significant difference in ANOVA test.

RESULTS

To elucidate the effect of ethanolamine on recovery from CCl₄-induced liver injury, ethanolamine (10 to 30 mg/kg body weight) was administered orally 24 h after the CCl₄ administration, since we had previously reported that maximum serum aminotransferase activity was observed at 24 h after the CCl₄ treatment. As shown in Fig. 1, the levels of serum ALT were drastically decreased compared with those of CCl₄-treated mice. However, dose dependency was not observed, and there was no significant difference between 15 mg/kg body weight and 30 mg/kg body weight of ethanolamine administration. BrdU incorporation into hepatocellular nuclear DNA in ethanolamine-treated groups was higher than that in the CCl₄-treated group (Table 1). Figure 2 shows typical microscopic photographs of the BrdU uptake in the control, Panacetone 810, CCl₄-injected, and CCl₄-ethanolamine-administered mice. The stained gene granules
Fig. 1. Effects of Ethanolamine on CCl₄ Injured Mouse Liver

Aminotransferase activity (ALT) at 24 h after different doses of ethanolamine treatment were determined by the COBAS MIRA automatic analyzer. The results represent the mean ± S.D. of ten mice. Asterisks indicate significant differences between the ethanolamine-treated group and CCl₄-treated group (* p<0.05, ** p<0.01, * p<0.01 as CCl₄ treated group compared with control and Panacete 810 treated groups).

Dose of orally administered ethanolamine

Fig. 2. Typical Examples of Immunohistochemistry for BrdU/FdU Incorporation
The experimental procedure is described in the text. (a) no plus CCl₄ treated; (b) Panacete 810 plus saline; (c) CCl₄ plus saline; (d, e, f) CCl₄ plus different dose of ethanolamine.

could hardly be identified in the control, Panacete 810 and CCl₄-injected mice (Fig. 2a, b, c), while the liver sections from ethanolamine-administered mice showed an increased number of gathered (Fig. 2d) or scattered (Fig. 2e, f) positive gene granules in the surviving hepatocytes. A few positive reactions of BrdU uptake were identified in the liver necrosis; however, a slight increase of hepatocyte necrosis was observed in the administration of 30 mg/kg body weight of
ethanolamine compared with the 10 mg/kg body weight treatment. Table 2 shows the liver ATP level of tested groups. The ATP level in the CCl₄-treated group was lower than other groups. Furthermore, the recovery of ATP levels in ethanolamine-treated groups was observed. These data strongly suggest that the oral administration of ethanolamine plays a role in hepatocellular regeneration.

DISCUSSION

It is widely accepted that CCl₄ initiates liver injury through the production of free radicals following bioactivation by the liver drug metabolizing cytochrome P450 enzyme system. Radicals lead to disruption of hepatocyte homeostasis. These reactions, occurring during liver injury, are responsible for all membrane damage. Cell membranes are composed of phospholipids. It is well demonstrated that the hepatocytes during the regeneration cycle have higher tolerance to stress, compared with the resting condition. Therefore, the progression of these reactions in the liver is dependent on the hepatocellular regeneration and hepatolobular restoration.

In the present study, we attempted to elucidate the contribution of ethanolamine to the hepatocellular repair process after administration of a toxic dose of CCl₄. Oral administration of ethanolamine decreased the activity of aminotransferase detected in serum (Fig. 1) and remarkably increased the uptake of BrdU into hepatocyte nuclei (Fig. 2, Table 1). Moreover, increased ATP levels in the liver (Table 2) compared to the control group was observed. Since a strong relationship between epidermal growth factor (EGF) cell-binding ability and the presence of ethanolamine had been reported, our finding suggests that orally administered ethanolamine contributes to this bioactivation. From these data and typical photos (Fig. 2) of the liver section, administration of more than 30 mg/kg of ethanolamine was rather harmful for the repair process; thus, the maximal beneficial effect in our experiment was recognized as a single dose of ethanolamine of 10–30 mg/kg body weight. Oral administration of ethanolamine therefore protects or repairs liver injury by CCl₄. This information is potentially very important for nutrition and health, since ethanolamine is contained in foods such as milk and is generated in the intestine.

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REFERENCES