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Impairment of α_2 -Macroglobulin Synthesis in Experimental Hepatopathic Rats Treated with Turpentine Oil

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Abstract: The aim of this study was to investigate the synthesis of α_2 -macroglobulin (α_2 M) in hepatopathic rats injected with turpentine oil to induce acute inflammation. Hepatopathy was induced by oral administration of acetaminophen at a dose of 1 g/kg daily for 2 weeks or a 25% solution of carbon tetrachloride (CCl_4) at 2 ml/kg body weight three times per week for 7 weeks. Acute inflammation was induced by intramuscular injection of turpentine oil at a dose of 1.0 ml/kg body weight. Serum concentrations of α_2 M were measured by enzyme-linked immunosorbent assay. Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total protein differed significantly between acetaminophen or CCl_4 -induced hepatopathic rats and acetaminophen control (AA-control) or CCl_4 control (CC-control) rats. Furthermore, pathological examination confirmed hepatopathy in rat livers. Peak serum concentrations and area under the time-concentration curve for α_2 M showed significant differences between hepatopathic rats and AA-control or CC-control rats. Thus, serum concentrations of α_2 M did not increase when compared with nontreated rats.

Key words: α_2 M, acetaminophen, CCl_4 , hepatopathy, rat

Introduction

α_2 -Macroglobulin (α_2 M) and α_1 -acid glycoprotein (α_1 -AG) are typical acute-phase proteins in rats [8, 9]. Serum concentrations of α_2 M and α_1 -AG increase by 50- to 290-fold and 34-fold, respectively, compared with pretreatment values in rats during induced acute inflammation [8]. In this way, α_2 M responds more sensitively than α_1 -AG as a marker of acute inflammation and is thus considered to be a useful inflammatory marker in rats [8, 9, 11–14].

The typical acute-phase protein in humans is C-reactive

protein (CRP) [3–6]. CRP is reportedly lower in hepatopathic patients, and the production of acute-phase proteins is considered to be influenced by hepatopathy [1, 20]. α_2 M is also synthesized in the liver [4]. However, there have been few reports on the influence of hepatopathy on the production of α_2 M in rats. In the present study, we prepared experimental hepatopathic rats by administering acetaminophen or carbon tetrachloride (CCl_4) [2, 18–20], and we then investigated the changes in serum α_2 M after injection of turpentine oil to induce acute inflammation.

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Materials and Methods

Animals

Twenty male Sprague-Dawley rats (age, 6 weeks) were purchased from CLEA Japan, Inc. (Tokyo, Japan). Hepatopathy was induced in 10 rats; 5 rats were administered acetaminophen and 5 rats were administered CCl₄. The rats were kept in isolators at a temperature of 23 ± 2°C and a relative humidity of 55 ± 10% with a 12/12 h dark/light cycle (6:00–18:00) and the air exchanged 12 times or more per hour. They were fed MF (Oriental Yeast Co., Ltd., Tokyo, Japan) and were allowed free access to water.

Animal experiments

High-dose administration of acetaminophen is known to induce hepatopathy [1, 15]. Bruck *et al.* reported that hepatopathy is induced by a one-time administration of acetaminophen at 1 g/kg in rats [2]. We therefore used repeated administration of acetaminophen to induce hepatopathy. The 5 rats in the hepatopathy group were administered acetaminophen (Calonal Fine Granules 50%; Showa Yakuhin Kako Co., Ltd., Tokyo, Japan) at 2 g/kg daily for 2 weeks, and 5 rats were administered sterilized water (AA-controls).

CCl₄ is also known to induce hepatopathy [19]. In the present study, hepatopathy was induced by administration of CCl₄ in accordance with the procedure of Nakayama *et al.* [19]. A 25% solution of CCl₄ (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was prepared with olive oil (Wako Pure Chemical Industries, Ltd.). Ten rats were divided into two groups; 5 rats were administered a 25% solution of CCl₄ at 2 ml/kg body weight, and 5 rats were administered olive oil (CC-control group). The rats were administered CCl₄ three times per week for 7 weeks.

Turpentine oil is known to induce acute inflammation and has been used to induce acute inflammation in rats and dogs [1, 3, 6, 8, 9, 12]. In this study, turpentine oil (Wako Pure Chemical Industries, Ltd.) was thus used to induce acute inflammation by intramuscular injection at 1.0 ml/kg body weight at 2 days after the end of acetaminophen or CCl₄ administration. Blood (0.3 ml) was collected from the venae cervicalis superficialis under pentobarbital anesthesia at six time points (pretreatment, and at 12, 24, 48, 72 and 96 h after injection of turpentine oil). Serum was obtained by centrifugation (1,600 × g, 15 min) and was stored at –80°C until use.

All experiments were approved by the Institutional Review Board of Azabu University and were conducted in accordance with the Institute's Animal Experimentation Guidelines (Japanese Association for Laboratory Animal Science, JALAS, 1987).

Measurement of serum concentrations of α 2M

Serum concentrations of α 2M were measured by an enzyme-linked immunosorbent assay (ELISA) according to the procedure described by Honjo *et al.* [8].

Blood chemistry

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by the ultraviolet method. Alkaline phosphatase (ALP) was measured by the Kind-King method. Total protein was measured by the Biuret method.

Evaluation of histopathology

Livers were removed after blood collection in the hepatopathy and control groups, cut into small specimens and fixed in a 10% neutral formaldehyde solution. The specimens were embedded in paraffin, sliced at 5 μ m and stained with hematoxylin and eosin for observation by microscopy.

Statistics

All values are expressed as means ± SD. Area under the concentration-time curve (AUC) for α 2M was calculated according to the trapezoidal rule. Variations in serum concentrations of α 2M, AST, ALT, ALP and TP were assessed using the unpaired Student's *t*-test. *P*-values of <0.05 were considered to be significant.

Results

Blood chemistry

AST, ALT, ALP and TP concentrations in the hepatopathy group and control group are shown in Table 1. One rat in each of the acetaminophen-induced hepatopathy and control groups died due to adverse events related to the anesthetic for blood collection. The mean values of AST, ALT and ALP in the acetaminophen and CCl₄-induced hepatopathy groups were significantly higher than in the control group. TP in both hepatopathy groups was significantly lower than in the control group.

Table 1. Blood chemistry parameters in rats after administration of acetaminophen or carbon tetrachloride

Administration compound	ALP (U/l)	ALT (U/l)	AST (U/l)	TP (g/dl)
Acetaminophen	1,050.6 \pm 321.1*	42.4 \pm 7.9*	225.0 \pm 11.2*	6.4 \pm 0.2*
AA-Control	427.0 \pm 24.0	30.5 \pm 24.0	104.5 \pm 17.7	7.0 \pm 0.1
Carbon tetrachloride	1,875.5 \pm 176.1*	683.5 \pm 352.8*	652.0 \pm 486.5*	5.8 \pm 0.4*
CC-Control	829.4 \pm 292.0	35.4 \pm 7.2	74.4 \pm 25.1	6.5 \pm 0.2

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TP, total protein. Data are expressed as means \pm SD (n=5). However, one rat in each of the acetaminophen-induced hepatopathy and AA-control groups died due to adverse events related to the anesthetic for blood collection. Differences were compared using the unpaired Student's *t*-test. *: Significantly different from the control group ($P < 0.05$). Rats were administered acetaminophen or carbon tetrachloride.

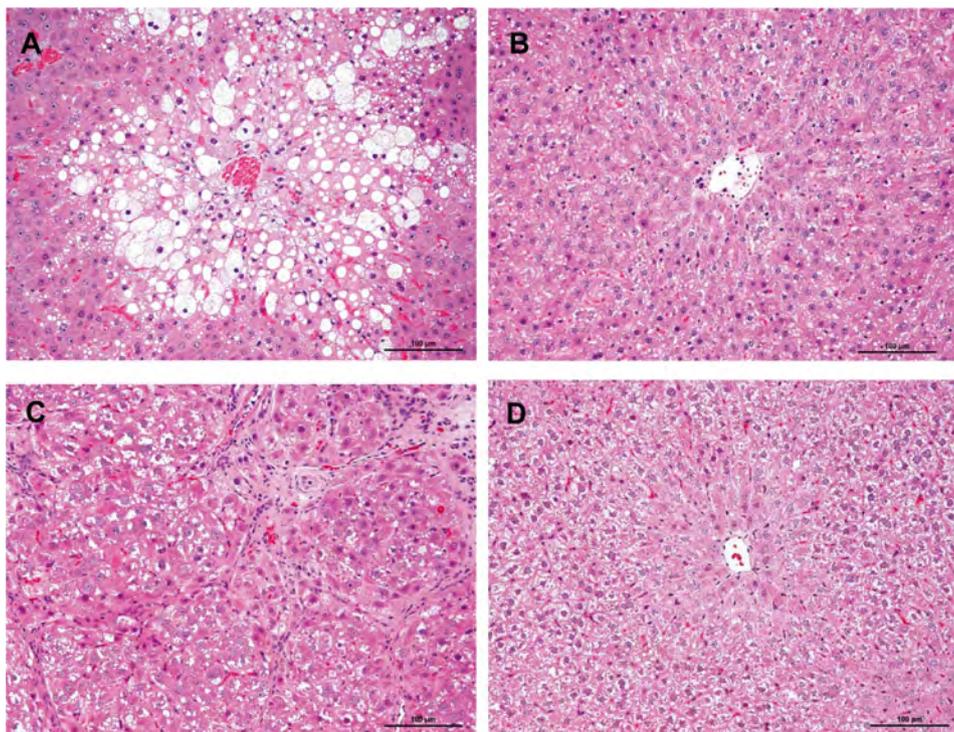


Fig. 1. Hematoxylin-eosin staining of liver specimens from rats in the acetaminophen (A), acetaminophen-control (B), carbon tetrachloride (CCl₄) (C), and CCl₄-control (D) groups.

Evaluation of histopathology

Figure 1 shows representative images of hematoxylin and eosin-stained liver specimens from rats administered acetaminophen or CCl₄. We observed severe vacuolation in numerous hepatocytes around the central vein and various degrees of hepatic steatosis in the acetaminophen-treated liver. Degenerating hepatocytes showed swelling, while other hepatocytes without vacuolation were of irregular size.

There were no normal lobular structures in the CCl₄-treated liver. We confirmed advancing fibrosis and pseudoblobule formation and observed vacuolated and ne-

crotic hepatocytes. Regeneration by small, basophilic hepatocytes was also present. AA-control and CC-control rats showed no abnormal findings. Thus, treatment with acetaminophen or CCl₄ induced hepatopathy.

Serum concentrations of α 2M

The changes in serum concentrations of α 2M in the acetaminophen- and CCl₄-induced hepatopathy groups are shown in Fig. 2. The peak serum concentrations (C_{max}) of α 2M in the acetaminophen-induced hepatopathy and AA-control groups were 67.2 μ g/ml and 780.3 μ g/ml, respectively, and the differences were sig-

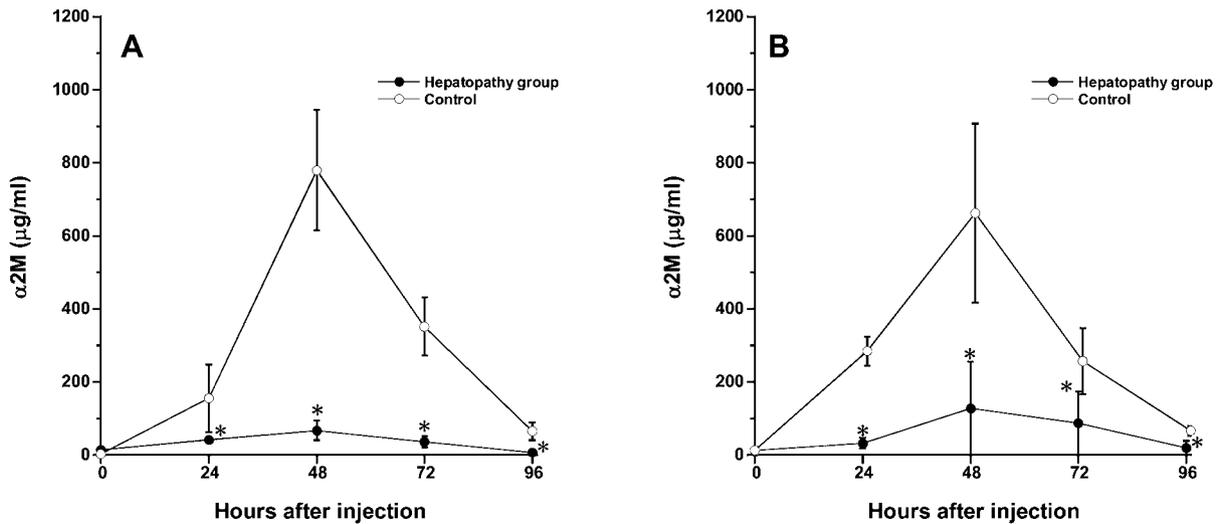


Fig. 2. Changes in serum concentrations of α_2 -macroglobulin in rats after administration of acetaminophen (A) or carbon tetrachloride (CCl_4) (B). Rats were injected with turpentine oil (0.4 ml/rat) to induce acute inflammation. Data are expressed as means \pm SD ($n=5$). However, one rat in each of the acetaminophen-induced hepatopathy and AA-control groups died due to adverse events related to the anesthetic for blood collection. Differences were compared using the unpaired Student's *t*-test. *: Significantly different from the control ($P < 0.05$).

nificant. The AUC^{0-96} was 3.8 mg-h/ml in the hepatopathy group and 31.7 mg-h/ml in AA-control group. The AUC^{0-96} in the hepatopathy group was significantly lower than that in the AA-control group. The C_{max} of $\alpha_2\text{M}$ was 128.0 $\mu\text{g/ml}$ in the CCl_4 -induced hepatopathy group and 662.0 $\mu\text{g/ml}$ in the CC-control group, and the difference was significant. The AUC^{0-96} was 6.3 mg-h/ml in the hepatopathy group and 29.9 mg-h/ml in the CC-control group. AUC^{0-96} in the hepatopathy group was significantly lower than that in the CC-control group.

Discussion

We have previously reported the kinetics of $\alpha_2\text{M}$ in rats stimulated by various methods. Most acute-phase proteins are known to be synthesized in the liver [4, 21, 24]. We thus investigated $\alpha_2\text{M}$ synthesis in experimental hepatopathic rats treated with turpentine oil.

The C_{max} of $\alpha_2\text{M}$ was observed at 48 h after inflammatory stimulation, e.g., injection of turpentine oil, inoculation with microorganisms or surgical treatment, before decreasing slowly [8–10, 13]. In the present study, The C_{max} was also observed at 48 h after injection of turpentine oil before decreasing slowly in hepatopathic rats. The kinetics of $\alpha_2\text{M}$ in experimental hepatopathic

rats were similar to those in previous reports [8–10, 13]. This study clarified that serum concentrations of $\alpha_2\text{M}$ did not increase in hepatopathic rats when compared with nontreated rats. CRP should not be used as a marker of infection in fulminant hepatic failure patients [21, 22], as serum concentrations of CRP are undetectable in patients with severe liver injury. Thus, caution is required when using $\alpha_2\text{M}$ as an acute inflammation marker in cases of severe hepatopathy in rats.

CCl_4 -induced hepatopathic rats were prepared according to a previously reported chronic hepatic failure model [19], and the exposure period for acetaminophen was short, while that for CCl_4 was long. The values for AST, ALT and ALP in CCl_4 -induced hepatopathic rats were further outside of the normal range when compared with acetaminophen induction. However, the AUC^{0-96} ratios between the hepatopathy group and control group were 8.3 for acetaminophen-induced hepatopathy and 4.7 in CCl_4 -induced hepatopathy. The differences in serum levels between CCl_4 -induced hepatopathy and the control group were smaller than with acetaminophen induction. Hepatocytes are known to have greater regeneration capacity than cells in other human organs [7, 23]. Some new hepatocytes were observed in CCl_4 -induced hepatopathic rats, and synthesis of $\alpha_2\text{M}$ appeared to have recovered slightly in these rats.

On the other hand, the levels of interleukin-6 (IL-6) are considered to be related to the synthesis of acute-phase proteins [5, 13]. It was reported that the levels of IL-6 are elevated in liver injury patients, as well as in mice and rats with induced liver damage [15–17, 25]. IL-6 levels are thus considered to be affected by factors other than liver damage. The cause of the reduction in α 2M synthesis was presumed to be hepatocyte dysfunction, irrespective of whether IL-6 is related to α 2M synthesis.

In conclusion, serum concentrations of α 2M are lower in rats with hepatic injury, even during acute inflammation.

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