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Octacosanol Attenuates Disrupted Hepatic Reactive Oxygen Species Metabolism Associated with Acute Liver Injury Progression in Rats Intoxicated with Carbon Tetrachloride

Yoshiji Ohta[1],*, Koji Ohashi[2], Tatsuya Matsura[3], Kenji Tokunaga[4], Akira Kitagawa[5], and Kazuo Yamada[3]

[1]*Department of Chemistry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan*

[2]*Department of Clinical Biochemistry, School of Health Sciences, Fujita Health University, Toyoake, Aichi 470-1192, Japan*

[3]*Division of Medical Biochemistry, Department of Pathophysiological and Therapeutic Science, Totorri University Faculty of Medicine, Yonago, Tottori 683-8503, Japan*

[4]*Department of Clinical Medical Technology, Kagawa Prefectural College of Health Science, Mure-cho, Kagawa 761-0123, Japan*

[5]*Department of Nutrition, Faculty of Wellness, Chukyo Women's University, Ohbu, Aichi 474-8651, Japan*

Summary We examined whether octacosanol, the main component of policosanol, attenuates disrupted hepatic reactive oxygen species metabolism associated with acute liver injury progression in rats intoxicated with carbon tetrachloride (CCl_4). In rats intoxicated with CCl_4 (1 ml/kg, i.p.), the activities of serum transaminases increased 6 h after intoxication and further increased at 24 h. In the liver of CCl_4-intoxicated rats, increases in lipid peroxide (LPO) concentration and myeloperoxidase activity and decreases in superoxixde dismutase activity and reduced glutathione (GSH) concentration occurred 6 h after intoxication and these changes were enhanced with an increase in xanthine oxidase activity and a decrease in catalase activity at 24 h. Octacosanol (10, 50 or 100 mg/kg) administered orally to CCl_4-intoxicated rats at 6 h after intoxication attenuated the increased activities of serum transaminases and the increased hepatic myeloperoxidase and xanthine oxidase activities and LPO concentration and the decreased hepatic superoxide dismutase and catalase activities and GSH concentration found at 24 h after intoxication dose-dependently. Octacosanol (50 or 100 mg/kg) administered to untreated rats decreased the hepatic LPO concentration and increased the hepatic GSH concentration. These results indicate that octacosanol attenuates disrupted hepatic reactive oxygen species metabolism associated with acute liver injury progression in CCl_4-intoxicated rats.

Key Words : liver injury (rat), octacosanol, oxidative stress

Introduction

*To whom correspondence should be addressed.
Tel: +81-562-92-2573 Fax: +81-562-93-4593
E-mail: yohta@fujita-hu.ac.jp

Policosanol is a mixture of high molecular weight aliphatic alcohols, such as octacosanol, triacosanol, and hexacosanol, isolated and purified from sugar cane

(*Saccharum officinarum* L.) wax. Fraga *et al.* [*1*] have shown that the concentration of lipid peroxide (LPO) in liver microsomes of rats administered orally with policosanol is less than that of rats not administered and that liver microsomes prepared from rats treated with octacosanol is more resistant to lipid peroxidation induced by Fe^{2+}/ADP/NADPH or carbon tetrachloride (CCl_4)/NADPH than liver microsomes from untreated rats. Menézden *et al.* [*2*] have reported that low-density lipoprotein (LDL) prepared from octacosanol-treated rats is more resistant to lipid peroxidation induced by copper ion than LDL from untreated rats. Noa *et al.* [*3*] have shown that policosanol protects against the histological changes characteristic of CCl_4-induced acute liver injury in rats.

It is well known that CCl_4 induces hepatotoxicity in humans and experimental animals. According to the present views of the initial developmental process of CCl_4-induced hepatotoxicity, the process is dominated by factors such as CCl_4 activation to trichloromethyl radical ($^{\cdot}CCl_3$) and trichloromethyl peroxy radical ($CCl_3O_2^{\cdot}$), the covalent binding of $^{\cdot}CCl_3$ to membrane lipids and proteins, and the hydrogen abstraction from polyunsaturated fatty acids by the $CCl_3O_2^{\cdot}$ and the $^{\cdot}CCl_3$ to initiate lipid peroxidation [*4*, *5*]. It has been shown that an increase in hepatic lipid peroxidation, which occurs via reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical, contributes to not only the formation but also the progression of CCl_4-induced acute liver injury in rats [*6–14*]. It has also been shown that a single treatment of rats with CCl_4 causes disruption of hepatic antioxidant defense systems associated with antioxidants such as reduced glutathione (GSH) and antioxidant enzymes such as superoxide dismutase (SOD), an enzyme to scavenge $O_2^{\cdot-}$ to form hydrogen peroxide (H_2O_2) and O_2 and catalase, an enzyme to decompose H_2O_2 to H_2O and O_2, and that the disruption of hepatic antioxidant defense systems in rats with CCl_4 contributes to liver injury progression rather than liver injury formation [*7–15*]. In addition, we have shown that ROS, such as $O_2^{\cdot-}$ and H_2O_2, derived from xanthine oxidase (XO) contributes to the progression of acute liver injury in rats treated with CCl_4 and that ROS derived from infiltrated neutrophils in the liver of CCl_4-treated rats plays an important role in liver injury progression through disruption of hepatic ROS metabolism [*10–12*]. Octacosanol [$CH_3(CH_2)_{26}CH_2OH$] is the major component of policosanol and occupies 60–70% of total aliphatic alcohols present in policosanol. We have reported that orally administered octacosanol prevents the progression of CCl_4-induced acute liver injury in rats through improvement of hepatic triglyceride accumulation [*16*]. However, it is still unclear whether orally administered octacosanol attenuates disrupted hepatic ROS metabolism associated with acute liver injury progression in rats intoxicated CCl_4.

Therefore, we examined whether octacosanol administered orally to rats intoxicated once with CCl_4 attenuates progressive liver injury and the changes in hepatic GSH and LPO levels and the hepatic activities of SOD, catalase, Se-glutathione peroxidase (Se-GSHpx), XO, and myeloperoxidase (MPO), an index of tissue neutrophil infiltration [*17–19*], with acute liver injury progression.

Materials and Methods

Chemicals

Octacosanol, leupeptin, 2,2',5,5'-teramethylbenzidine (TMB), xanthine, bovine erythrocyte Cu,Zn-SOD, and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO); dithiothreitol (DTT), phenylmethyl-sulfonylfloride, yeast glutathione reductase, and milk XO from Roche-Diagnostic Co. (Tokyo, Japan); CCl_4, ethylenediaminetetraacetic acid (EDTA), GSH, 2-thiobarbituric acid, NADPH, and other reagents, of the highest grade, were purchased from Wako Pure Chemical Industry Ltd. (Osaka, Japan). All chemicals were used without further purification.

Animals

Male Wistar rats aged 5 weeks were purchased from Nippon SLC Co. (Hamamatsu, Japan). The animals were maintained under a daily controlled 12 h-light, 12 h-dark lighting cycle at 23°C and 50% humidity with free access to rat chow (Oriental MF, Oriental Yeast Co., Tokyo, Japan) and water. All animals received humane care in compliance with the guidelines of the Management of Laboratory Animals in Fujita Health University, Japan.

Administration of CCl_4 and octacosanol

CCl_4 was diluted two-fold with olive oil. Fed rats (six weeks old) received a single intraperitoneal (i.p.) injection of CCl_4 at a dose of 1 ml/kg body weight (BW), that is, 2 ml/kg BW of the 50% CCl_4 solution. Rats without CCl_4 intoxication received an equal volume of olive oil in the same manner. These animals were starved with free access to water after CCl_4 intoxication, as described in our previous reports [*9–12*, *16*]. Octacosanol (1, 5 or 10 mg) was suspended in 1 ml of olive oil. Each suspension of octacosanol was orally administered to rats with and without CCl_4 intoxication at a dose of 1 ml/100 g BW at 6 h after intoxication. The doses of octacosanol were 10, 50, and 100 mg/kg BW. Rats not administered with octacosanol received olive oil at a dose of 1 ml/100 g BW at the same time point.

Sample preparation

Rats were sacrificed after collecting blood from the inferior vena cava under ether anesthesia at 6 or 24 h after CCl_4 intoxication. Immediately after sacrifice, livers were

perfused with ice-cold 0.9% NaCl through the portal vein to remove residual blood in the tissue as much as possible and then removed from the body. The isolated liver was washed well in ice-cold 0.9% NaCl, clotted on a filter, weighed, and frozen on dry ice as soon as possible. The collected blood was kept on ice for 30 min and then separated into serum by centrifugation at 4°C. The isolated liver and serum were kept at −80°C until use. For the assays of hepatic LPO, GSH, SOD, catalase, Se-GSHpx, and MPO, a part of the right large lobe of each liver was homogenized in 9 volumes of ice-cold 0.15 M KCl containing 1.0 mM EDTA using a glass homogenizer with a Telfon pestle. For SOD, catalase, Se-GSHpx, and MPO assays, the prepared homogenate was sonicated on ice twice × 30 s using a Handy Sonic model UR-20P (Tomy Seiko Co., Tokyo, Japan). The sonicated homogenate was centrifuged at 12,000 × g for 20 min at 4°C. The supernatant was dialyzed against 100 volumes of the same buffered solution at 4°C for 60 min using a microdialysis device (MWCO = 3,500 daltons) (Bio-Tec International Inc., Belleuve, WA). For hepatic XO assays, another part of the right large lobe of each liver was homogenized in 9 volumes of an ice-cold buffered solution (pH 7.8) containing 0.05 M potassium phosphate, 0.1 M EDTA, 0.5 mM DTT, 0.5 mg/ml of leupeptin, and 0.2 mM phenylmethylsulfonylfluoride using a glass homogenizer with a Telfon pestle as described previously [10–12]. The homogenate was dialyzed against 100 volumes of the same buffered solution without DTT at 4°C for 60 min using the above-described microdialysis device.

Assays of serum and hepatic components and enzymes

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using a commercial kit, Iatorzyme TA-L$_Q$ (Dai-Iatron Co., Tokyo, Japan). Both activities are expressed as an international unit (IU/l). Hepatic LPO was determined by the method of Ohkawa *et al.* [20] using the thiobarbituric acid reaction except that 1.0 mM EDTA was added to the reaction mixture. The concentration of LPO is expressed as the amount of malondialdehyde (MDA) equivalents. Hepatic GSH was determined by the method of Sedlak and Lindsay [21] using Ellman's reagent and GSH as a standard. Hepatic SOD, catalase, and Se-GSH-Px were assayed by the methods of Oyanagui [22], Bergmeyer [23], and Hochstein, and Utley [24], respectively. SOD activity was determined at 37°C by the xanthine oxidase-NH$_2$OH method using purified bovine erythrocyte SOD (5000 units/mg solid) as a standard. SOD activity is expressed as the amount of the erythrocyte SOD showing activity equivalent to the determined activity. Catalase activity was measured at 37°C by recording H$_2$O$_2$ decomposition at 240 nm. One unit (U) of this activity is defined as the amount of enzyme decomposing 1 μmol H$_2$O$_2$ per min. Se-GSH-Px activity was determined at 37°C by

recording the decrease in absorbance at 340 nm following the oxidation of NADPH in the presence of H$_2$O$_2$, GSH, yeast GSSG-R, and NaN$_3$ as a catalase inhibitor. One unit (U) of this activity is defined as the amount of enzyme oxidizing 1 μmol NADPH per min. Hepatic XO was assayed at 30°C by the method of Hashimoto [25] using xanthine as a substrate. XO activity was assessed by measuring the increase in absorbance at 292 nm following the formation of uric acid. One unit (U) of XO activity is defined as the amount of enzyme forming 1 μmol uric acid per min. Hepatic MPO was assayed as follows: the dialyzed supernatant was incubated at 60°C for 2 h to increase the recovery of MPO in liver tissues according to the method of Schierwagen *et al.* [18]. This heat treatment caused a complete inactivation of catalase, which is known to influence the assay of MPO in liver tissues. MPO activity in the heat-treated supernatant was assessed by measuring H$_2$O$_2$-dependent oxidation of TMB at 37°C according to the method of Suzuki *et al.* [26]. This TMB oxidation was measured spectrophotometrically at 655 nm. One unit of this enzyme activity is expressed as the amount of enzyme causing a change in absorbance of 1.0 per min at 655 nm. Protein in liver tissue samples was measured by the method of Lowry *et al.* [27] using bovine serum albumin as a standard.

Statistical analysis

All values obtained are expressed as the mean ± SD. All data were statistically analyzed by computerized statistical packages (StatView). Each mean value is compared by one-way analysis of variance and Fisher's protected significant difference for multicomparison as the post hoc test. The level of significance was set at $p<0.05$.

Results

In rats intoxicated with CCl$_4$ (1 ml/kg BW), serum ALT and AST activities significantly increased 6 h after intoxication and further increased at 24 h (Fig. 1). When octacosanol (10, 50 or 100 mg/kg BW) was administered to CCl$_4$-intoxicated rats at 6 h after intoxication, the increases in serum ALT and AST activities found at 24 h after CCl$_4$ intoxication were significantly depressed in a dose-dependent manner (Fig. 1). The serum ALT and AST activities in the CCl$_4$-intoxicated group post-administered with octacosanol (100 mg/kg BW) were significantly higher than those in the control group but were not significantly different from those in the CCl$_4$-intoxicated group found at the time point of the alcohol administration (Fig. 1). The same does of octacosanol had no effect on serum ALT and AST activities in CCl$_4$-untreated rats (Fig. 1).

In CCl$_4$-intoxicated rats, a significant increase in hepatic LPO concentration and a significant decrease in hepatic
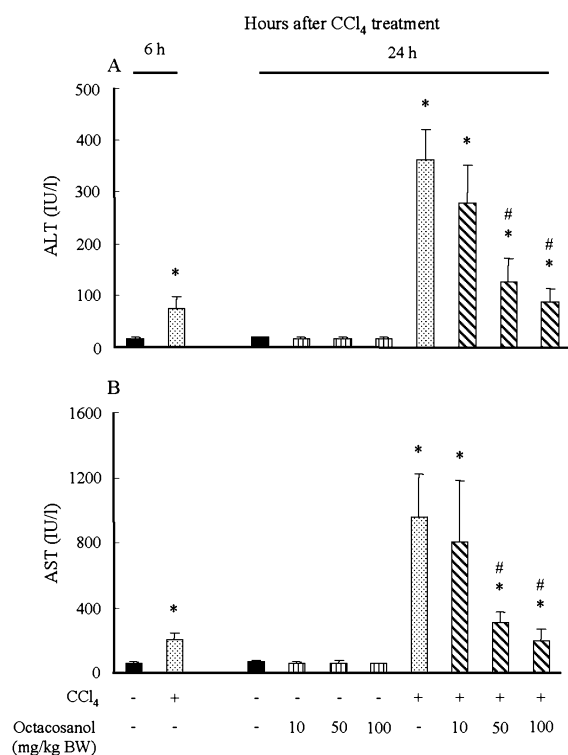
Fig. 1.   Effect of post-administered octacosanol on serum ALT (A) and AST (B) activities in rats intoxicated with CCl₄. Rats with and without a single intoxication of CCl₄ (1 ml/kg BW, i.p.) were orally administered with octacosanol (10, 50 or 100 mg/kg BW) or olive oil (vehicle) 6 h after intoxication. Serum ALT and AST were assayed 6 and 24 h after CCl₄ intoxication as described in Materials and Methods. Each vale is a mean ± SD ($n = 5$ for CCl₄-untreated rats with and without octacosanol administration; $n = 10$ for CCl₄-intoxicated rats with and without octacosanol administration). *$p<0.05$ (vs control group); #$p<0.05$ (vs CCl₄-intoxicated group).
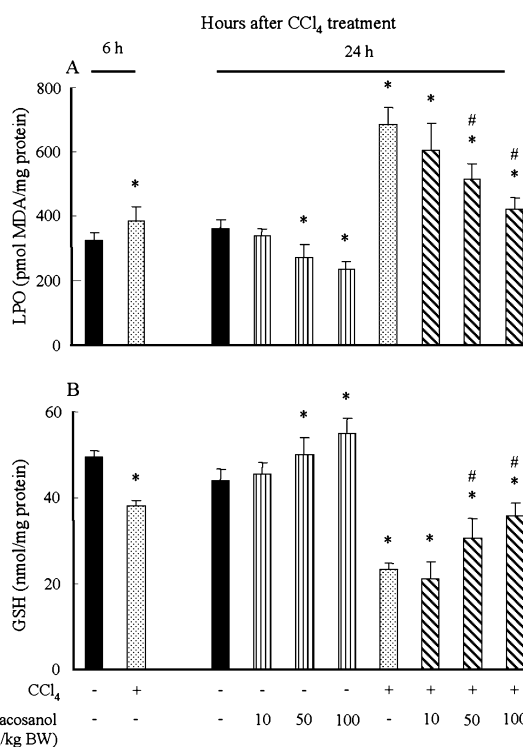
Fig. 2.   Effect of post-administered octacosanol on hepatic LPO (A) and GSH (B) concentrations in rats intoxicated with CCl₄. Experiment condition and explanation are the same as described in the legend for Fig. 1 except that hepatic LPO and GSH were assayed as described in Materials and Methods.

GSH concentration occurred 6 and 24 h after intoxication and these changes were larger at 24 h than at 6 h ($p<0.05$) (Fig. 2). Octacosanol administered at 6 h after CCl₄ intoxication significantly attenuated the increase in hepatic LPO concentration and the decrease in hepatic GSH concentration found at 24 h after intoxication in a dose-dependent manner (Fig. 2). The CCl₄-intoxicated group post-administered with octacosanol (100 mg/kg BW) had significantly higher hepatic LPO concentration and lower hepatic GSH concentration than the control group (Fig. 2). However, the hepatic LPO and GSH concentrations in the CCl₄-intoxicated group post-administered with octacosanol (100 mg/kg BW) were not significantly different from those in the CCl₄-intoxicated group found at the time point of the alcohol administration (Fig. 2). When the same doses of octacosanol (50 or 100 mg/kg BW) were given to CCl₄-untreated rats, both a significant decrease in LPO concentration and a

significant increase in GSH concentration were found in the liver tissue (Fig. 2).

CCl₄-intoxicated rats had significantly lower hepatic SOD activity than control rats at 6 and 24 h after intoxication and this decrease in activity was larger at 24 h than at 6 h ($p<0.05$) (Fig. 3). The CCl₄-intoxicated group had significantly lower hepatic catalase activity than the control group at 24 h after intoxication but showed no change in hepatic Se-GSHpx activity at 6 and 24 h (Fig. 3). Octacosanol (10, 50 or 100 mg/kg BW) post-administered to CCl₄-intoxicated rats significantly attenuated the decrease in hepatic SOD and catalase activities found at 24 h after intoxication in a dose-dependent manner but had no effect on the hepatic Se-GSHpx activity (Fig. 3). The hepatic SOD activity in the CCl₄-intoxicated group post-administered with octacosanol (100 mg/kg BW) was significantly lower than that in the control group but was not significantly different from that in the CCl₄-intoxicated group found at the time point of the alcohol administration (Fig. 3A). The hepatic catalase activity in the CCl₄-intoxicated group post-administered with octacosanol (100 mg/kg BW) was significantly lower than that in the control group, although there was a little difference in that activity between both groups (Fig. 3B).
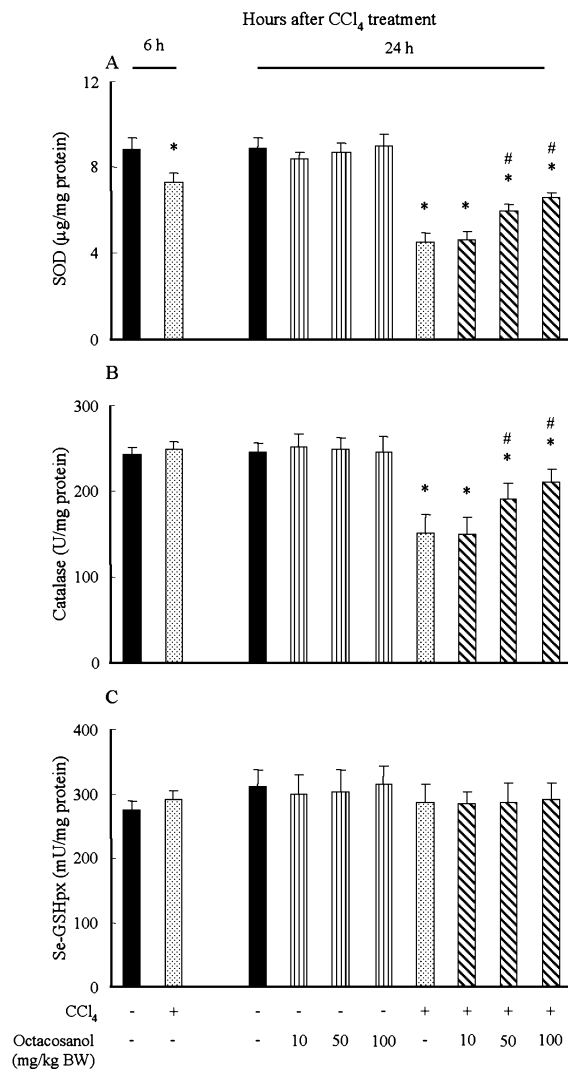
Fig. 3.   Effect of post-administered octacosanol on hepatic SOD (A), catalase (B), and Se-GSHpx (C) activities in rats intoxicated with CCl₄. Experiment condition and explanation are the same as described in the legend for Fig. 1 except that hepatic SOD, catalase, and Se-GSHpx were assayed as described in Materials and Methods.
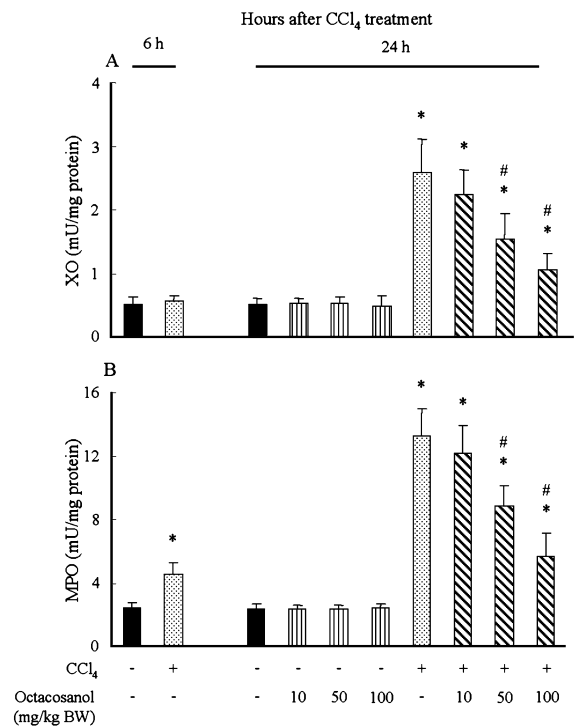

Fig. 4.   Effect of post-administered octacosanol on hepatic XO (A) and MPO (B) activities in rats intoxicated with CCl₄. Experiment condition and explanation are the same as described in the legend for Fig. 1 except that hepatic XO and MPO were assayed as described in Materials and Methods.

The same doses of octacosanol given to CCl₄-untreated rats did not affect the hepatic SOD, catalase, and Se-GSHpx activities (Fig. 3).

CCl₄-intoxicated rats had significantly higher XO activity than control rats at 24 h after intoxication and had significantly higher hepatic MPO activity than control rats at 6 and 24 h, although the increased hepatic MPO activity was significantly higher at 24 h than at 6 h ($p<0.05$) (Fig. 4). Octacosanol (10, 50 or 100 mg/kg BW) post-administered to CCl₄-intoxicated rats significantly attenuated the increase in hepatic XO and MPO activities found 24 h after intoxication in a dose-dependent manner (Fig. 4). The hepatic MPO activity in CCl₄-intoxicated group post-administered with octacosanol (100 mg/kg BW) was significantly higher than that in the control group but was not significantly different from that in the CCl₄-intoxicated group found at the time point of the alcohol administration (Fig. 4B). The same doses of octacosanol given to CCl₄-untreated rats did not affect the hepatic XO and MPO activities (Fig. 4).

Discussion

In the present study, rats intoxicated once with CCl₄ (1 ml/kg BW) showed apparent liver injury at 6 h after intoxication and progressed liver injury at 24 h, judging from the serum levels of ALT and AST, indices of liver cell damage, as shown in our previous reports [7, 9–12, 16]. When octacosanol (10, 50 or 100 mg/kg BW) was orally administered to CCl₄-intoxicated rats at 6 h after intoxication, the severity of progressed liver injury found at 24 after intoxication was reduced in a dose-dependent manner. The severity of liver injury in CCl₄-intoxicated rats post-administered with octacosanol (100 mg/kg BW) was almost equal to that in rats intoxicated with CCl₄ alone found at the time point of the alcohol administration, as shown in our previous report [16]. Thus, orally administered octacosanol

can prevent the progression of acute liver injury in CCl₄-intoxicated rats almost completely. This finding indicates clearly that orally administered octacosanol prevents the progression of acute liver injury in rats intoxicated with CCl₄.

In the present study, CCl₄-intoxicated rats showed an increase in hepatic LPO concentration and a decrease in hepatic GSH concentration at an early stage of liver injury followed by enhanced changes in the levels of both components at a progressed stage of the injury, as reported previously [7–12]. Octacosanol (10, 50 or 100 mg/kg BW) administered at an early stage of CCl₄-induced liver injury attenuated the changes in hepatic LPO and GSH concentrations found at a progressed stage of the injury in a dose-dependent manner. The LPO and GSH concentrations in CCl₄-intoxicated rats given octacosanol (100 mg/kg BW) were almost equal to those in CCl₄-intoxicated rats found at the time point of the alcohol administration. In addition, octacosanol given to rats without CCl₄ intoxication at a dose of 50 or 100 mg/kg BW caused a significant decrease in the hepatic LPO concentration and a significant increase in the hepatic GSH concentration. However, it is known that tetracosanol, hexacosanol, and octacosanol, which are the main components of policosanol, have little activity to scavenge 2,2'-diphenyl-1-picrylhydrazyl free radical and no activity to inhibit copper ion-induced LDL oxidation when they are added to each reaction medium directly [28]. We have observed that octacosanol has no inhibitory effect on lipid peroxidation induced by a free radical generator, 2,2'-azobis(2-amidinopropane), in rat liver microsomes when the alcohol at concentrations of 10 to 1000 μg/ml is added to the reaction medium directly (unpublished data). Therefore, these findings suggest that orally administered octacosanol attenuates hepatic oxidative stress occurring with liver injury progression in rats intoxicated once with CCl₄ through its indirect antioxidant action by increasing tissue GSH level rather than its direct antioxidant action to scavenge free radicals participating in lipid peroxidation. The activation of CCl₄ to ·CCl₃ is caused via the cytochrom P450 system in the liver [4, 5]. However, it is known that policosanol has no inhibitory effect on the metabolism of drugs via the cytochrom P450 system in the liver of rats [3]. It is also known that when rats are intoxicated with CCl₄ (5 ml/kg BW, i.p.), the activation of CCl₄ to ·CCl₃ and CCl₃O₂·, the covalent binding of ·CCl₃ to membrane lipids and proteins, and the hydrogen abstraction from polyunsaturated fatty acids by the CCl₃O₂· and the ·CCl₃ to initiate lipid peroxidation terminate within a few hours after intoxication [29, 30]. Therefore, it seems unlikely that octacosanol administered orally to CCl₄-intoxicated rats at 6 h after intoxication affects the activation of CCl₄ to ·CCl₃ and CCl₃O₂· via the cytochrome P450 system followed by the covalent binding of ·CCl₃ to membrane lipids and lipid

peroxidation mediated by ·CCl₃ and CCl₃O₂· in the liver.

In the present study, CCl₄-intoxicated rats showed a decrease in hepatic SOD activity at an early stage of liver injury and had further decreased hepatic SOD activity and decreased hepatic catalase activity at a progressed stage of the injury, although there was no change in hepatic Se-GSHpx activity, as shown in previous reports [7, 9–12]. Oral administration of octacosanol (10, 50 or 100 mg/kg BW) to CCl₄-intoxicated rats at an early stage of liver injury attenuated the further decrease in hepatic SOD activity and the decrease in hepatic catalase activity found at a progressed stage of the injury in a dose-dependent manner but had no effect on the hepatic Se-GSHpx activity. The hepatic SOD activity in the CCl₄-intoxicated rats given octacosanol (100 mg/kg BW) was almost equal to that in CCl₄-intoxicated rats found at the time point of the alcohol administration. Orally administered octacosanol (100 mg/kg BW) prevented the decrease in hepatic catalase activity found at a progressed stage of CCl₄-induced liver injury almost completely. The same doses of octacosanol given to rats without CCl₄ intoxication had no effect on the hepatic SOD, catalase, and Se-GSHpx activities. These results indicate that orally administered octacosanol can attenuate the disruption of hepatic ROS-metabolizing system associated with SOD and catalase following acute liver injury progression in rats intoxicated with CCl₄.

In the present study, CCl₄-intoxicated rats had increased hepatic XO activity at a progressed stage of liver injury, as shown in our previous reports [10–12]. Octacosanol (10, 50 or 100 mg/kg BW) administered orally to CCl₄-intoxicated rats at en early stage of liver injury attenuated the increase in hepatic XO activity found at a progressed stage of the injury in a dose-dependent manner, although the same doses of octacosanol given to CCl₄-untreated rats did not affect the hepatic XO activity. The hepatic XO activity in the CCl₄-intoxicated rats post-administered with octacosanol (100 mg/kg BW) was still higher than that in control rats. The hepatic activity of MPO, an index of tissue neutrophil infiltration [17–19], in CCl₄-intoxicated rats increased at an early stage of liver injury and further increased at a progressed stage of the injury, as reported previously [12]. Octacosanol (10, 50 or 100 mg/kg BW) administered orally at an early stage of CCl₄-induced liver injury reduced the increase in hepatic MPO activity found at a progressed stage of the injury in a dose-dependent manner. The hepatic MPO activity in the CCl₄-intoxicated rats post-administered with octacosanol (100 mg/kg BW) was almost equal to that in CCl₄-treated rats found at the time point of the alcohol administration. However, the same doses of octacosanol given to rats without CCl₄ intoxication had no effect on the hepatic MPO activity. In addition, we have observed that octacosanol does not inhibit XO and MPO activities in the liver homogenate prepared from CCl₄-intoxicated rats

when the alcohol is added to the reaction medium at concentrations of 10–1000 µg/ml directly (unpublished data). Thus, octacosanol administered orally to rats intoxicated once with CCl$_4$ can attenuate increased hepatic XO activity and enhanced neutrophil infiltration in the liver tissue.

It has been suggested that an increase in hepatic XO activity in rats treated once with CCl$_4$ is due to the conversion of xanthine dehydrogenase (XD) to XO in the ischemic or hypoxic liver [11]. Our recent report has shown in rats treated once with CCl$_4$ that neutrophils accumulated in the liver tissue contribute to an increase in XO activity in the liver tissue [12]. The conversion of XD to XO in tissues is known to be caused by limited proteolysis and/or the oxidation of sulfhydryl groups of the protein [31, 32]. Activated neutrophils not only produce ROS but also release proteases from the cells [33, 34]. As described above, octacosanol administration attenuated the enhanced hepatic GSH depletion and neutrophil infiltration found in CCl$_4$-intoxicated rats and increased the hepatic GSH concentration in rats without CCl$_4$ intoxication. Therefore, one can think a possibility that orally administered octacosanol reduces an increase in hepatic XO activity in CCl$_4$-intoxicated rats by inhibiting the conversion of XD to XO *via* the GSH depletion-mediated oxidation of sulfhydryl groups present in the protein of XD and/or *via* limited proteolysis mediated by proteases released from infiltrated neutrophils in the liver tissue.

It has been shown that when octacosanol (60 mg/kg BW) is orally administered to rats, not only octacosanol but also octacosanoic acid, an oxidation product of octacosanol, appears in the liver and the hepatic amount of octacosanoic acid is a few times higher than that of octacosanol [35]. Noa *et al.* [36] reported that oral administration of D-003, a mixture of high molecular weight primary acids, such as octacosanoic acid, purified from sugar cane wax, protected against CCl$_4$-induced acute liver injury in rats. In addition, it is known that LDL prepared from rats administered with D-003 is less susceptible to in vitro lipid peroxidation induced by copper ion and a free radical generator, 2,2'-azobis(2-amidinopropane), than LDL from rats without D-003 administration [37], like the case of administered policosanol [1, 2]. Therefore, there seems to be a possibility that octacosanoic acid produced by oxidation of orally administered octacosanol in the liver of rats intoxicated once with CCl$_4$ contributes to the attenuating effect of the administered octacosanol on both liver injury progression and disrupted hepatic ROS metabolism associated with the injury progression by exerting an antioxidant action.

In conclusion, the results of the present study indicate that orally administered octacosanol attenuates disrupted hepatic ROS metabolism associated with acute liver injury progression in rats intoxicated with CCl$_4$ through its antioxidant action and its inhibitory action on neutrophil infiltration. These results also suggest that these actions of orally administered octacosanol are involved in the mechanism by which the administered alcohol prevents the progression of CCl$_4$-induced acute liver injury.

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