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Neutral sphingomyelinase-induced ceramide accumulation by oxidative stress during carbon tetrachloride intoxication

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ABSTRACT

Ceramide is a biologically active lipid causing apoptosis in a variety of cells. In this study, we examined the effect of CCl_4 on the ceramide metabolism and indicators of oxidative stress. After 12 h of oral administration of CCl_4 (4 ml/kg body weight as a 1:1 mixture of CCl_4 and mineral oil) to rats, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were increased. Antioxidants such as vitamins C and E were decreased in the liver and kidney. In addition, the ratio of GSH/GSSG in the liver, plasma, kidney, and brain decreased at 2 h. The total ceramide in the liver significantly increased as early as 2 h after CCl_4 administration. After 24 and 36 h, the total ceramide in plasma and the kidney was also augmented. In the brain, the total ceramide dramatically increased at 36 h. These results suggested that the increased ceramide in plasma was transferred to the kidney and the brain. The activity of neutral sphingomyelinase (SMase), which was reported to be enhanced by the decrease of GSH, was significantly increased after CCl_4 treatment in the liver, kidney, and brain. However, acid SMase activities were not increased in the liver and kidney. Thus, the activation of neutral SMase via oxidative stress induced the increase of ceramide during CCl_4 intoxication in not only the liver but also other tissues. These results suggested that the excess accumulation of ceramide causes damage in other organs including the kidney and brain during fulminant hepatic failure.

1. Introduction

Carbon tetrachloride (CCl_4) is metabolized by P450-dependent dehalogenation to trichloromethyl radical, which deranges the cellular metabolism, including alteration of membrane proteins and lipids by their covalent binding to cellular macromolecules (Racknagel et al., 1989). CCl_4 intoxication has been used as an animal model of fulminant hepatic failure to develop artificial liver support (Soloviev et al., 2003; Shnyra et al., 1991). The mechanism of liver injury by CCl_4 has received more attention than that by any other chemical (Bruckner and Warren, 2001).

Ceramide has been implicated in regulating cell-cycle arrest, apoptosis, and cell senescence (Hannum and Luberto, 2000; Merrill, 2002; Hannun and Obeid, 2002) and serves as an intracellular second messenger (Kolesnick, 1992). Ceramide is generated by sphingomyelin (SM) hydrolysis by sphingomyelinase (SMase) (Merrill and Jones, 1990). SMases are classified based on pH optima, subcellular localization, and cation dependence (Goni and Alonso, 2002). Neutral SMase (nSMase) is localized in the plasma membrane and exhibits an optimum pH of about 7.5 and Mg^{2+}-dependence. Acid SMase (aSMase), with an optimum pH of about 4.8, operates at the endosomal-lysosomal compartments or plasma membrane. Some studies report that a negative correlation exists between nSMase activity and reduced glutathione (GSH) (Tsyupko et al., 2001; Rutkute et al., 2007) and partially purified nSMase is dose-dependently inhibited by GSH (Liu and Hannun, 1997). Because GSH is the major antioxidant defense system that eliminates toxic oxygen radical (Han et al., 2006), the activation of SMase might be accompanied with the enhancement of oxidative stress by CCl_4 administration.

Oxidative stress such as UV-light and irradiation induced the ceramide accumulation in the injured cells (Farrell et al., 1998; Santana et al., 1996). In addition, the hydrophobic nature of CCl_4 allows it to permeate cell membranes and CCl_4 is distributed and accumulated in organs such as the liver, brain, kidney, and heart (Melin et al., 2000). Therefore, the radical derived from CCl_4 might affect the synthesis of ceramide in these organs. In fulminant hepatic failure, which induces massive necrosis of the liver via oxidative stress, toxic substances and cytokines released into the circulation are assumed to cause encephalopathy and renal dysfunction (Laleman et al., 2006). Our previous study showed that hepatic and

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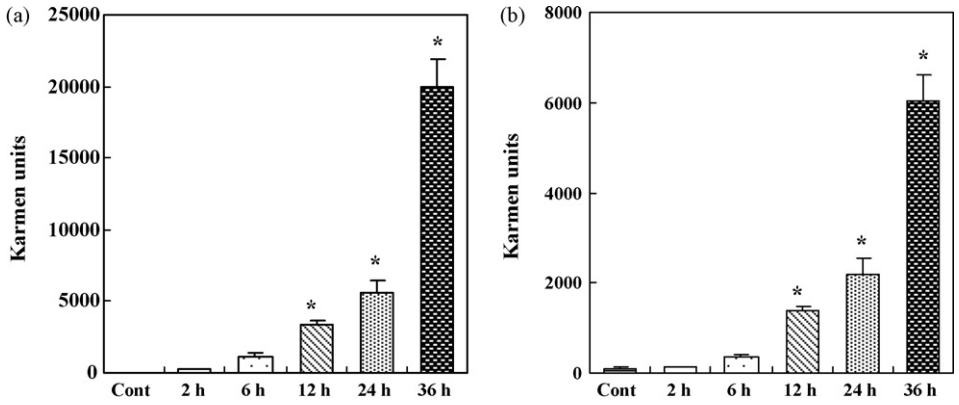

Fig. 1. CCl_4 (4 ml/kg; as a mixture of CCl_4:mineral oil = 1:1) was orally administered to rats. After 1, 2, 3, 6, 12, and 24 h, plasma AST (a) and ALT (b) activities were assayed as described in the text. Control rats received mineral oil (4 ml/kg) and the enzyme activity was determined after 12 h. Values are means ± S.E. for 4–5 rats and asterisks indicate significant differences from the corresponding control group (ANOVA Fisher's protected least significant difference test (PLSD), $^*P < 0.05$).

plasma ceramides were increased by the administration of CCl_4 to rats (Ichi et al., 2007a). Therefore, it is possible that the increased ceramide in the liver and plasma during fulminant hepatic failure is one of the important toxins causing damage in other organs including the kidney and the brain. In the present study, we show that oxidative stress via CCl_4 administration induces the changes of ceramide metabolism in not only the liver but also other organs such as the kidney and brain.

2. Materials and methods

2.1. Animals

This study was approved by the Animal Care Committee of Nara Women's University. Eight-week-old male rats (SLC: Wistar strain) were obtained from Japan SLC Co. (Hamamatsu, Shizuoka, Japan). The animals were housed in a room at 24 ± 2 °C, with a 12 h/12 h light–dark cycle. Animals were fed commercial laboratory chow (MF, Oriental Yeast Co., Osaka, Japan) and water *ad libitum*. Eight-week-old rats were administered a mixture of CCl_4 and mineral oil (1:1, 4 ml/kg body weight) through an

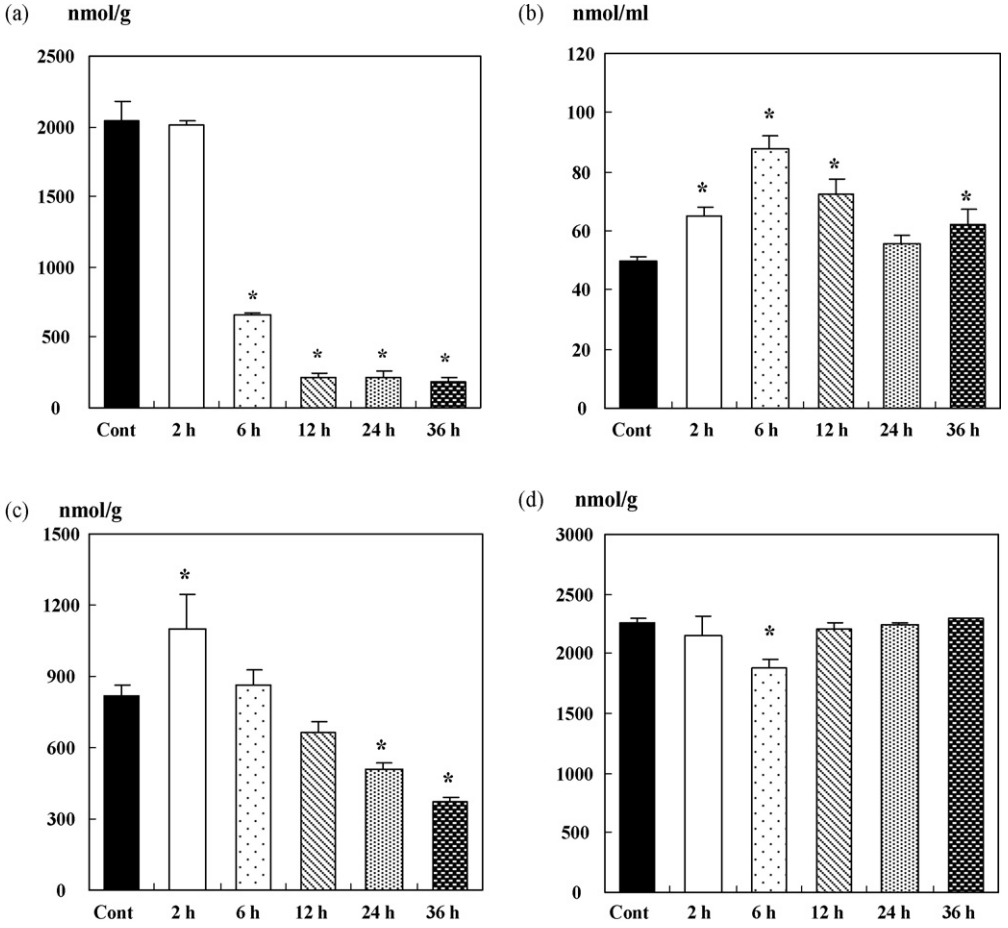

Fig. 2. The level of vitamin C in the liver (a), plasma (b), kidney (c), and brain (d) of rats after CCl_4 administration and the control group. After 2, 6, 12, 24, and 36 h, the level of vitamin C in these tissues were determined as described in the text. After 12 h of the administration of mineral oil, determinations were made for control rats. Values are means ± S.E. for 4–5 rats and asterisks indicate a significant difference from the control group (ANOVA Fisher's protected least significant difference test (PLSD), $^*P < 0.05$).