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Mice in the early stage of liver steatosis caused by a high fat diet are resistant to thioacetamide-induced hepatotoxicity and oxidative stress

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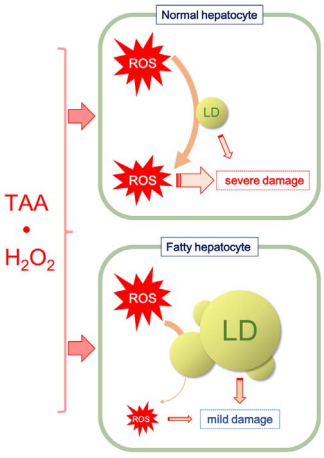
GRAPHICAL ABSTRACT

Schematic diagram of the effects of accumulated lipid in hepatocytes. Treatment with TAA or hydrogen peroxide caused oxidative damage to hepatocytes. Accumulated lipids in the cells were partly peroxidized but also caused the elimination of ROS. Thus, cellular damage are mitigated by the accumulated lipids at an early steatosis.



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ABSTRACT

Lipogenesis is stimulated in the liver by an unfolded protein response (UPR) to endoplasmic reticulum stress under a variety of pathological conditions and results in the accumulation of lipids in hepatocytes. Assuming that UPR is a protective mechanism against stress, we hypothesized that the accumulated lipids might have a beneficial function. We prepared mice with fatty livers by feeding two types of high-calorie diets; a lard-rich high-calorie diet (LHD) or a menhaden oil-containing high-calorie diet (MHD), for two weeks and treated them, as well as control diet (CD)-fed mice, with thioacetamide (TAA), a liver toxicant. When a lethal dose (500 mg/kg) of TAA was administered, the LHD-fed mice and the MHD-fed mice survived longer than those fed with CD. The accumulated lipids appeared to be associated with protecting the liver against TAA toxicity (200 mg/kg). Consistently, lipid-loaded Hepa 1–6 cells showed a partial resistance to hydrogen peroxide toxicity compared to those cultured in conventional media. In conclusion, while sustained steatosis impairs liver function and leads to

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; CD, control diet; HFD, high fat diet; LHD, lard-rich high calorie diet; MHD, menhaden oil-containing high-calorie diet; SOD, superoxide dismutase; ROS, reactive oxygen species; TAA, thioacetamide; ALT, alanine aminotransferase; ER, endoplasmic reticulum; CYP, cytochrome P450; SREBP, sterol regulatory element-binding protein; PBS, phosphate-buffered saline; H & E, hematoxylin and eosin; TG, triglyceride; HNE, 4-hydroxyl 2-nonenal; UPR, unfolded protein response; PUFA, polyunsaturated fatty acids; PA, palmitic acid; OA, oleic acid; MeOH, methanol; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate

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hazardous conditions, lipids that transiently accumulate as the result of UPR or other stimuli may exert a beneficial function in the liver at least partly through scavenging reactive oxygen species.

1. Introduction

Continuing hypernutrition, notably triglyceride (TG)-rich food, or an excessive intake of alcohol result in the accumulation of fat and the development of steatosis in the liver (Cave et al., 2007). When the fat content in the liver exceeds 5–10% by weight, without a history of an excess ingestion of alcohol, the condition is defined as non-alcoholic fatty liver disease (NAFLD) (Serviddio et al., 2013). NALFD is associated with a variety of metabolic diseases including diabetes, dyslipidemia, and the metabolic syndrome, and in some instances, degenerates to non-alcoholic steatohepatitis (NASH), a condition that involves insulin resistance, inflammation, and fibrotic liver damage (Anstee et al., 2013).

Hepatotoxic compounds such as thioacetamide (TAA) is administered to rodents in order to generate animal models of acute hepatic damage and hepatic fibrosis (Wallace et al., 2015). Many hepatotoxic compounds, such as TAA, are converted into more toxic oxygenized metabolites by cytochrome P450 (CYP) enzymes in the liver (Kang et al., 2008), which simultaneously trigger the production of reactive oxygen species (ROS) as byproducts (Yasui et al., 2005). Elevated levels of ROS and exposure to liver toxicants associated with endoplasmic reticulum (ER) stress stimulates the *de novo* synthesis and accumulation of TG and cholesterol in certain instances and simultaneously inhibits the secretion of lipoproteins (Malhotra and Kaufman, 2007). Sterol regulatory element-binding proteins (SREBPs), which regulate genes that are involved in steroidogenesis and lipogenesis, are proteolytically activated by Golgi body-resident proteases, site-1 protease (S1P) and site-2 protease (S2P), under physiological conditions with a cholesterol insufficiency and also under pathological conditions including ER stress (Ye et al., 2000). Hence, ROS result in lipid accumulation in the liver as the result of ER stress by activating SREBPs (Sekiya et al., 2008). Elevated oxidative stress due to a SOD1 deficiency, in fact, causes the activation of SREBP1c and the accumulation of lipid droplets in primary cultured hepatocyte via ER stress (Lee et al., 2015).

Considering the unfolded protein response (UPR) as primarily a protective mechanism against ER stress (Walter and Ron, 2011), we hypothesized that lipid droplets that accumulate in response to such stress may have a role in protecting against hepatotoxic compounds and/or ROS. The finding that feeding a lard-containing high-calorie diet (LHD) improves the longevity of the SOD-deficient mice compared to a normal diet-fed mice (Ito et al., 2017) provides support for this hypothesis. Among the various lipids, ω-3 polyunsaturated fatty acids (PUFA) have been reported to be effective in maintaining liver homeostasis and the treatment of NASH (Bouzianas et al., 2013). Fish oil is rich in ω-3 PUFA and, hence, an intake of the fish oil instead of lard, a saturated fatty acid-rich fat, may exert more advantages.

In this study we report on an attempt to clarify this issue by examining mice with liver steatosis that were produced by feeding two types of high-calorie diets, LHD and a menhaden oil-containing high-calorie diet (MHD), to C57BL/6N male mice for two weeks and then examining TAA-induced hepatotoxicity in these animals compared to mice that were fed a normal control diet (CD). Lipid droplets function to protect against oxidative stress, which is a likely cause of the TAA-induced hepatotoxicity, in fatty acid-loaded Hepa 1–6 cells under culture conditions. Based on the results we propose that accumulated lipids, notably PUFA, play protective roles against an oxidative insult and toxicant-induced liver damage at an early stage of liver steatosis.

2. Materials and methods

2.1. Mice

C57BL/6N mice housed in our institution were used in this study. Male mice were either fed a control diet (CD) (4.5 kcal/g, Picolab Rodent Diet 20 5053, LabDiet, St. Louis, MO), a lard-rich high-calorie diet (LHD) (D12492, 774 kcal/g containing 54% lard, Research Diets, New Brunswick, NJ), or a menhaden oil-containing high-calorie diet (MHD) (D16010609, 774 kcal/g, 49% lard and 5% menhaden oil, Research Diets). The animal room was maintained under specific pathogen-free conditions at a constant temperature of 20–22 °C with a 12 h alternating light-dark cycle. Animal experiments were performed in accordance with the Declaration of Helsinki under protocols approved by the Animal Research Committee at Yamagata University.

2.2. TAA treatment of mice

In preliminary experiments, male mice at 8, 10, or 11 weeks of age were fed an LHD for 4, 2, or 1 weeks, respectively, sacrificed at 12 weeks of age, and then subjected to analyses (4 mice in each group, total of 16 mice). To decide the timing for the TAA treatment, mice fed with the CD or LHD for 2 w or 8 w were administered TAA (4 mice in each group, total of 24 mice). In the following experiment, male mice at 10 weeks of age were fed the corresponding diets for 2 weeks and were injected intraperitoneally with TAA (either 200 or 500 mg/kg body weight; Wako, 202-00882, Osaka, Japan) dissolved in phosphate-buffered saline (PBS) as described in a previous study (Shirato et al., 2017). The mice were injected with 1/20 vol. of the reagent solutions, PBS or TAA (10 mg/ml for 200 mg/kg or 25 mg/ml for 500 mg/kg), equivalent to their body weight. The same volume of PBS was used as the vehicle control. To examine the effects of a lethal TAA dose, mice (12 mice in each group, total of 36 mice) were intraperitoneally injected with 500 mg/kg TAA and observed for 5 days. To investigate the acute effects of a sublethal dose of TAA on the liver, mice (3–9 mice in each group, total of 37 mice) were treated with TAA at a dose of 200 mg/kg of body weight once and blood and liver samples were collected at 24 h after the TAA injection.

2.3. Blood tests

Blood was collected from the heart under ether anesthesia in the presence of ethylenediaminetetraacetic acid. After centrifugation at 800 × *g* for 3 min, blood plasma was collected. The levels of alanine transaminase (ALT), glucose (Glc), and TG in the blood plasma were determined using Fuji DRI-CHEM slides on Fuji DRI-CHEM 3500 V (Fuji Film, Tokyo, Japan).

2.4. Histological analyses of the liver

Livers dissected from the mice were fixed in 15% buffered formalin followed by embedding in paraffin, sectioned, and were then stained with hematoxylin and eosin (H & E). To visualize hepatic lipid accumulation, the frozen liver sections were stained with an Oil Red O solution as described previously (Kurahashi et al., 2015).

2.5. Western blotting

The liver tissues were homogenized with a glass-teflon homogenizer in lysis buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 1%