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The role of oxidative stress in NASH and fatty liver model

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Summary

The aim of this study was to investigate whether oxidative stress is related to the development of liver injury and an iron chelator, deferoxamine (DFO) can prevent lipid peroxidation resulting in reduced liver injury as well as reduce preneoplastic lesions induced by a choline-deficient L-amino acid-defined (CDAA) diet. CDAA diet administration resulted in an increased serum ALT level after one week. Hepatocytes in rat liver fed a CDAA diet showed malondialdehyde (MDA) accumulation.

But simultaneous DFO treatment for one week reduced this elevation of ALT as well as MDA accumulation in the liver. Feeding rats a CDAA diet for 14 weeks led to the development of severe liver fibrosis and preneoplastic lesions detected as enzyme-altered lesions. DFO treatment also prevented the expression of activated stellate cells, resulting in the reduction of liver fibrosis as well as reducing the development of preneoplastic lesions. These results indicate that iron chelation can reduce the development of preneoplastic lesions in a CDAA diet model.

Keywords: Oxidative stress; Iron; Liver injury; Preneoplastic lesion; Liver fibrosis

1. Introduction

A cellular pool of transient iron, chelatable by deferoxamine (DFO) and distinct from ferritin is required to catalyze the generation of active oxygen species [1]. Oxidative stress can initiate membrane lipid peroxidation related to the loss of cell viability not only in cultured hepatocytes [2,3] but also in vivo as in alcoholic or drug-induced liver injury, which can be protected against by an iron chelator, deferoxamine [4,5].

On the other hand, some reports suggest that hepatocellular carcinoma in the rat liver induced by prolonged exposure to a diet with a low concentration of choline without any carcinogens is presumably the result of oxidative stress [6,7].

In the present study, we induced the development of liver cirrhosis with a CDAA diet and using this model we investigated whether a CDAA diet can cause oxidative stress and the effect of an iron chelator, deferoxamine, on lipid peroxidation.

2. Methods

2.1. Animals

Male Wistar rats (180–200 g) were obtained from Nippon SLC Co. Ltd. (Shizuoka, Japan). The animals were fed ad libitum for one week before use and kept in a climate-controlled room with a 12 h dark–light cycle.

2.2. Experimental protocol

For the experiments, rats were fed a CDAA diet for one week or 14 weeks with administration of 0, 200, or 400 mg/kg DFO (Ciba Pharmaceutical Co., Summit, NJ, USA) once a day by intraperitoneal injection of a 250 mg/mL solution in 0.9% NaCl. Each group consisted of six rats.

All rats were killed under ether anesthesia and blood was drained from the aortic bifurcation, allowed to clot at room temperature, then centrifuged for 15 min at 3000 × *g*. After drainage of blood, the liver was excised and immediately frozen for the assessment described below, or fixed in neutralized 10% formalin for 24 h and embedded in paraffin for immunohistochemical examination other than GSTP staining.

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2.3. Serum markers

Serum ALT and AST were measured as described elsewhere.

2.4. Histology and immunohistochemical examination

Sections of the right lobe of all rat livers 5 μm thick were processed routinely for hematoxylin and eosin (HE), Azan-Mallory and Sirius red staining. Alpha-smooth muscle actin for the detection of activated stellate (Ito) cells and glutathione *S*-transferase placental form (GSTP) for the detection of enzyme-altered (preneoplastic) lesions were immunohistochemically examined by the advidin–biotin–peroxidase complex method as previously described. Anti-alpha smooth muscle actin and anti-rat GSTP antibodies (Dako Japan Inc., Kyoto, Japan) were employed.

2.5. Assay of lipid peroxidation, iron and hydroxyproline content in the liver

Hepatic lipid peroxidation was quantified by measuring MDA in liver using the modified method described by Yagi as previously described [5]. Hydroxyproline content was determined by a modification of the method of Kivirikko as previously reported [8,9].

2.6. Statistical analysis

All results are given as mean ± S.D. The statistical significance of observed differences was assessed using one-way ANOVA. Differences were considered statistically significant at the 0.05 level.

2.7. Ethical considerations

This experiment was reviewed by the Ethics Committee for Animal Experiments of Yamaguchi University School of Medicine and was carried out under the control of the Guidelines for Animal Experiments of Yamaguchi University School of Medicine and Law No. 105 and Notification No. 6 of the Japanese government.

3. Results

3.1. The role of oxidative stress in a CDAA diet model

One week after a CDAA diet administration, serum ALT increased up to 283 ± 52 U/L as well as MDA production compared to that fed a choline supplemented diet (53 ± 8 U/L). Liver histology showed prominent fatty liver (Fig. 1) without fibrosis.

3.2. Effect of DFO on CDAA diet-induced liver injury after one week

DFO shows the dose-dependent protective effect up to 400 mg/kg on CDAA diet-induced liver injury after one week of dietary intake as assessed by the serum ALT level. One-week administration of the CDAA with 400 mg/kg DFO treatment significantly reduced the serum ALT level to 203 ± 48 U/L compared to the CDAA diet alone.

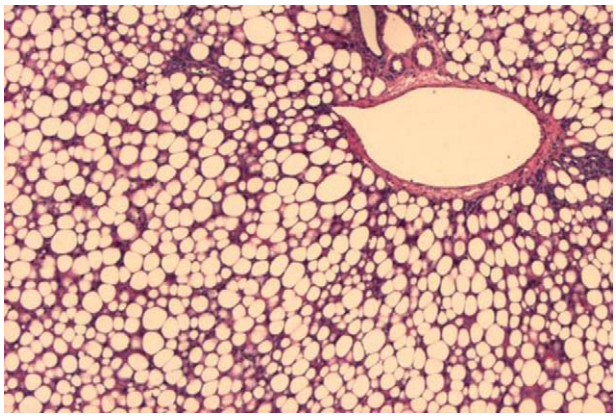

Fig. 1. Photomicrograph of liver section stained with Azan-Mallory from a male Wistar rat fed a CDAA diet for one week. (Magnification: 100×).

3.3. Hydroxyproline content of the liver

Based on the above results, we carried out a long-term experiment (14 weeks). Rats fed a CDAA diet for 14 weeks showed an increased liver hydroxyproline content of 617 ± 87 μg/g wet weight, compared with 146 ± 13 for CSAA fed rats as control. The treatment with DFO at 400 mg/kg reduced the increase in hydroxyproline content to 485 ± 53 ($P < 0.01$).

3.4. Histological findings

The livers of rats fed the CDAA diet for 14 weeks showed extensive accumulation of extracellular matrix with Sirius red staining (Fig. 2) including preneoplastic lesions detected as glutathione *S*-transferase placental form (GSTP)-positive lesions (Fig. 3). The treatment with 400 mg/kg

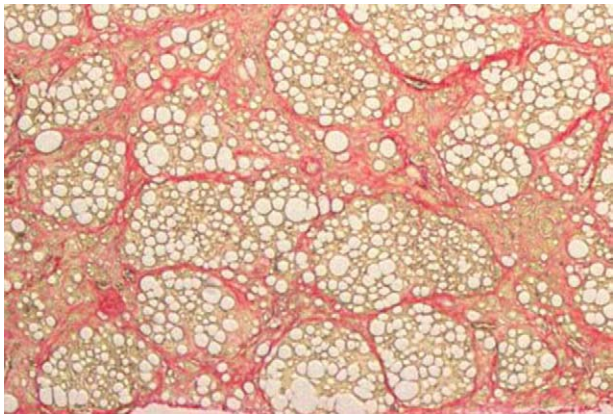

Fig. 2. Photomicrograph of liver section stained with sirius red from a male Wistar rat fed a CDAA diet for 14 weeks. (Magnification: 100×).