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ScienceDirect
Chemistry and Physics of Lipids 148 (2007) 70–76
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

Changes in the components of biliary and plasma lipids in selenium-deficient rats

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Received 23 January 2007; accepted 7 April 2007
Available online 19 April 2007

Abstract

We constructed a chronic oxidative stress model in which Se-deficient diet was fed to male Wister rats for 8 weeks. As expected, effects of oxidative damage, including Fe accumulation and increase in peroxidized lipids, were identified in the liver owing to the lack of glutathione peroxidase. Although the oxidative stress caused Fe accumulation in the liver, the Fe concentration in bile of the SeD rat was almost the same as that in the control rats. The constant excretion of Fe into bile supported the Fe accumulation in the liver. No differences were observed in the principal components of biliary lipids, i.e., bile acids, phospholipids, and cholesterol, between the two groups; moreover, these trends were also reflected in the plasma. Due to the trapping of reactive oxygen species, only bilirubin concentrations in the bile and plasma were decreased in the SeD group, when compared with those in the control group. Measurement of bilirubin concentration may be used as a supplemental oxidative stress marker.

Keywords: Selenium; Oxidative stress; Bile; Blood plasma; Bilirubin

1. Introduction

It has been known that Se-deficiency during a certain period exposes a living body to weak chronic oxidative stress (Agay et al., 2005; Chareonpong-Kawamoto and Yasumoto, 1995; Matsumoto et al., 2005, 2006; South et al., 2000). Se forms the active center of glutathione peroxidase (GSH-Px) that plays a role in relieving severe oxidative stress (Burk, 1983; Rotruck et al., 1973). GSH-Px reduces hydrogen peroxide (H_2O_2) to water by using the reduced form of GSH. Therefore, the weak chronic stress originating from Se-deficiency may result in the development of some abnormal oxidation–reduction systems in organs or cells. Several relevant study on Se-deficiency in animal livers have reported increases in Fe accumulation (Chareonpong-Kawamoto and Yasumoto, 1995; South et al., 2000), H_2O_2 (Hostetler and Kincaid, 2004; Ueda et al., 2000), and peroxidized lipids (Agay et al., 2005; Giray et al., 2004; Wu and Huang, 2004), but decreased GSH-Px (Agay et al., 2005) and catalase activities (Giray et al., 2004). Therefore, the liver has been

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0009-3084/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2007.04.005

considered as one of the internal organs that the most prone to damage by oxidative stress in Se-deficiency. Hepatic bile is directly influenced by the condition of the liver in oxidative stress. On the other hand, the blood was thought to indirectly reflect the oxidative stress in the internal organs and the liver due to the absorption of bile salts and lipids in the intestine (Agay et al., 2005). The physiological role of bile is mainly related to digestion and the intestinal absorption of various lipids and fats. In general, the bile is roughly constituted of 97% water and 3% solid materials in humans and animals (Klaassen and Watkins, 1984). The bile acids, phospholipids, cholesterol, and bilirubin are the main constituents of the biliary lipids, which form the majority of solid materials (Klaassen, 1974). On the other hand, the solid materials include small amount of inorganic materials, and the composition of the inorganic electrolytes is similar to that in the plasma.

The aim of the present study is to investigate the changes in the components of biliary and plasma lipids by using the Se-deficiency rat model. The components of biliary and plasma lipids are thought to be closely linked to the chronic oxidative stress. It would be very useful to identify lipids showing marked changes as oxidative stress markers in relation to plasma and liver. For this purpose, key minerals related to oxidative stress have to be concurrently investigated. This study on the oxidative stress-induced changes in lipid components does not clarify the detailed mechanisms of the changes in individual lipids; however, it demonstrates the great importance of the marked changes in lipids in response to oxidative stress.

2. Materials and methods

2.1. Animals and materials

The Se-deficient diet was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan) and the basal ingredients of this diet are summarized in Table 1. The principal mineral constituents of the diet (AIN-93G base) were examined by instrumental neutron activation analysis. The concentration of Se was 0.1 ± 0.09 (0) mg/kg; Fe, 327 ± 32 (235) mg/kg; Zn, 49 ± 4 (30) mg/kg, where the values in parenthesis are those mentioned in the catalog ones. Ion-exchanged water used for rat breeding was further purified using the Mili-Q System (Millipore Co.).

Pregnant Wistar rats (gestation period: 2 weeks) were purchased from Japan Laboratory Animals, Inc. These rats were fed a Se-deficient diet and ultrapure water until their offspring were weaned. The newborn male rats were weaned from their mothers after 4 weeks and divided into two groups—Se-deficiency (SeD) group and Se-control (SeC) group. The rats in the SeD group (SeD) were continued to be fed with the Se-deficient diet and ultrapure water until 8 weeks of age, whereas the SeC group received a 0.8 ppm solution of a mixture of Se compounds (seleno-L-methionine:sodium selenite:sodium selenate = 8:1:1) instead of ultrapure water. The individual Se compounds were purchased from Wako Pure Chemical Industries, Ltd. In this experiment, the 8-week-old rats that were fed the Se-deficient diet were considered to be under severe oxidative stress (Matsumoto et al., 2005). At 8 weeks of age, the average body weight of eight rats was 314 ± 35 g in the SeD group and 319 ± 20 g in the SeC group.

Table 1
The basal ingredients of the diet (AIN-93G mineral base)

Ingredient	Amount (g/kg diet)
Casein	200
L-Cystine	3
Corn starch	397
α-Corn starch	132
Sucrose	100
Soybean oil	70
Cellulose	50
Vitamin mix (AIN-93)	10
Choline bitartrate	3
Mineral mix (AIN-93G base)	35

The animal experiments were carried out in compliance with the Guidelines for Animal Care and Use (2001) and approved by the relevant Ethical Committee of Showa Pharmaceutical University.

2.2. Assay of lipids and metals in bile, plasma, and liver

2.2.1. Bile extraction and liver sample preparation

Eight-week-old rats were anesthetized using a 50 mg/kg phenobarbital injection of Nembutal (Dinabot, Japan) in proportion to the body weight. The upper right quadrant of the abdomen was opened with a 1.5 cm vertical incision. The bile duct was quickly cannulated with a polyethylene tube, and the bile was subdivided into the small vessels at proper time intervals. The bile collected between 60 and 120 min was used as measurement samples for the lipid assay because the period of time was almost unchanged in the bile flow and the lipid concentration in the present as well as another experiment (Galan et al., 1990).

The liver samples were prepared as follow. Whole blood was collected from the abdominal aorta into a test