Effects of Choline Deficiency and Different Fats on the Hepatic Glutathione-Dependent Enzyme Activities in 2-Acetylaminoflavorene Treated Rats

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Abstract: Weaning Sprague-Dawley male rats were fed the diet containing either 15% beef tallow or 15% corn oil with or without 0.075% choline chloride for 10 weeks. At 3rd and 5th week, animals were given injection of 2-acetylaminoflavorene (AAF) at a dose of 50 mg/kg body weight twice a week (1st and 3rd day of the week). At 11th week, all animals were decapitated. Microsomal lipid peroxide values, microsomal enzyme activities of glucose-6-phosphatase (G6Pase), cytosolic enzyme activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione S-transferase (GST) were determined. Microsomal lipid peroxide values measured as malondialdehyde (MDA) were increased significantly in choline deficient corn oil group, but not significantly in any other groups. Microsomal G6Pase activities were decreased in corn oil groups compared with beef tallow groups, and choline deficient corn oil group had the lowest value. Cytosolic GSH-Px activities tended to be elevated by choline deficiency in both fat groups. Cytosolic GR activities tended to be decreased by choline deficiency in beef tallow group, but significantly increased in corn oil group. Activities of cytosolic GST were tended to be increased by choline deficiency in both fat groups. All glutathione-dependent enzyme activities were significantly higher in corn oil groups than in beef tallow groups and the highest in choline deficient corn oil group. These results suggest that polyunsaturated fatty acid (PUFA) diet and choline deficiency may increase hepatic microsomal lipid peroxidation. Although lipid peroxidation induces protective enzymes, destruction of membrane integrity is inevitable. When 2 factors were compared on lipid peroxidation, induction of protective enzymes, and destruction of membrane integrity, PUFA diet was more effective than choline deficient diet.

The nutritional state of an animal may appreciably influence its response to toxic substances and on tumor induction by various chemical carcinogens (Ames, 1984; Willet & MacMahon, 1984).

Chronic administration of semisynthetic diets singly deficient in choline, a major dietary source of labile methyl groups, resulted in the development of hepatocellular carcinomas in rats (Yokoyama, 1985). And it was also revealed that sequential administration of carcinogen followed by a choline deficient diet enhances both induction of enzyme-altered foci and subsequent progression to hepatocellular carcinomas (Sawada et al., 1990; Schrager et al., 1990).

The mechanisms by which a choline deficient diet...
exerts its promoting and/or carcinogenic effects are not known. But it is proposed that choline deficiency induce phosphatidylcholine deficiency, and change membrane composition or physical properties. Then it can stimulate membrane lipid peroxidation (Rushmore, 1986).

On the other hand, dietary fats, both in quantity and quality, play important roles in carcinogenesis of many organs. Diets rich in fat enhance the development of tumors (Chan et al., 1983; Reddy et al., 1985) and unsaturated fats induce tumorigenesis more than do similar dietary levels of saturated fats (King & McCay, 1983; Sundram et al., 1989). Dietary fat influence tissue fatty acid composition, and high intake of unsaturated fatty acid increase the unsaturation of biomembrane, and may enhance possibility of lipid peroxidation. Products of lipid peroxidation such as lipid peroxides or free radicals interact many macromolecules in cells and promote changes of microstructure or fluidity of membrane (Gower et al., 1986; Jordan & Schenkman, 1982). Therefore activities of membrane bound enzymes such as glucose-6-phosphatase (G6Pase) may also be affected.

Choline deficient diet with a high-fat content exerted a stronger tumor promoting action than the diet with a low-fat content. The unsaturated fatty acid with choline deficiency exerted a higher tumor promoting efficacy (Perera et al., 1985).

The cell employs several lines of defense against the toxic products of lipid peroxidation. These are antioxidant enzymes such as glutathione peroxidase (GSH-Px: EC 1.11.19), glutathione reductase (GR: EC 1.6.4.2), and glutathione S-transferase (GST: EC 2.5.1.18).

In the present study, we investigated the effect of choline deficiency with different dietary fats (beef tallow and corn oil) on extent of membrane lipid peroxidation and damage, and glutathione-dependent enzyme activities in 2-acetylamino fluorene (AAF) treated rat liver.

Materials and Methods

Animals

Male Sprague Dawley rats (50—60 g body wt.) from Animal Breeding Laboratory of Seoul National University were housed in plexiglas cages and were exposed to light during 07:00~19:00 h a day.

They were fed the diets containing 15% beef tallow or 15% corn oil with vitamin fortification mixture (75.0 g choline chloride/kg mixture, ICN, Cleveland, Ohio) or choline free vitamin mixture (ICN, Cleveland, Ohio) for 10 weeks.

At 3rd and 5th week, 2-AAF (50 mg/kg body wt., Sigma, USA) was injected twice each week (1st and 3rd day of the week) intraperitoneally. Total 2-AAF injection was four times. At 11th week, animals were decapitated.

Dietary composition and experimental designs have been described in detail in a previous publication (Kim & Choi, 1990).

Preparation of microsomes and cytosols

At 11th week, animals were decapitated after 12 h fasting. Liver was homogenized in Tris-HCl buffer (pH 7.4), centrifuged at 12,000×g, 4°C for 20 min, and the supernatant was centrifuged again at 105,000×g, 4°C for 1 h to separate cytosolic upper fraction from the lower microsomal fraction. Microsomes were resuspended in Tris-HCl buffer, and frozen with liquid nitrogen and stored at −20°C until used.

Biochemical assay

Microsomal lipid peroxides were measured by TBA method of Bidlack and Tappel (1973) and microsomal G6Pase activities by method of Baginski et al. (1983). Cytosolic GSH-Px activities were measured by methods of Tappel (1978) using H₂O₂ as a substrates, cytosolic GR activities by methods of Carberg and Mannervick (1985), and cytosolic GST activities by method of Habig et al. (1974).

Statistical analysis

The data were analyzed by Duncan's multiple range test and two way ANOVA test at α=0.05 (Ott, 1984).

Results and Discussion

Lipid peroxidation is regarded as one of the mechanisms of cellular damage and has been implicated in liver cell injury and even in tumor promotion (Minotti, 1988). Peroxidation of fatty acids containing th-