Prevention of Dioxin-induced Toxicity in Mice by Increasing Dietary Nutrient and Dietary Fiber

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Introduction

Our bodies are continuously exposed via food, ambient air, water and soil to toxic dioxins (PCDDs, PCDFs and Co-PCBs). Biological half lives for most of the extremely toxic 2,3,7,8-substituted isomers have been estimated to range from 3 to 15 years[1]. It is suggested the dioxins accumulated in the body transfer from the mother to the baby, and alter thyroid hormone regulation and an immune function in the baby[2]. Moreover, pollution of the human body by these compounds has attracted attention as a factor in the increased incidence of atopy in breast-fed babies and the rapidly increasing incidence of endometriosis in women. It is necessary to hasten the excretion of extremely toxic and highly persistent dioxins from our bodies to maintain health. As an effective means of hastening the excretion of dioxins, ingesting dietary fibers capable of adsorbing mutagens and carcinogens is recognized[3]. However, few data are available on the extent to which hastening the excretion of dioxins prevents their toxic influence. This study assessed whether enhanced excretion of dioxins by dietary fiber reduces the toxic influence (immune suppression, hepatic hypertrophy, splenic atrophy and enzyme induction) in mice after oral administration of 1,2,3,4,7,8-HxCDD (HxCDD). In addition, the effect of increased nutrient (protein, lipid, vitamins and minerals) absorption on the HxCDD-induced toxicity was investigated to clarify the additive effect of nutrient absorption and dietary fiber.

Materials and Methods

Experiment 1. Prevention of HxCDD-induced toxicity in mice by increasing dietary

nutrient

Six groups (4 or 5 mice per group) of 10-week-old C57BL/6 female mice were used for the experiment. Basal diet and four high nutrient diets containing the nutrients shown in Table 1 were respectively given to five groups of mice for 14 days. All five groups were treated with a single oral administration of HxCDD dissolved in ethanol : Tween 80 : saline (1:10:89) at a dose of 10 μ g/kg body weight on day 8. An additional group (control animals) was maintained on the basal diet for 14 days and received only the vehicle on day 8. All the mice were challenged with antigen (DNP-dextran) 1 day following the single administration. On day 14, all the mice were sacrificed, and whole blood was collected. The anti-DNP-IgM antibody in the separated serum was determined using enzyme-linked immunosorbent assay. The liver and spleen were removed and weighed. Hepatic microsomes were prepared from the liver and EROD activity was measured fluorometrically according to the procedures described by Phol and Fouts[4].

Experiment 2. Enhanced excretion of HxCDD and the prevention of induced toxicity by increased intake of dietary fiber

Mice were fed the basal fiber-free diet shown in Table 1 and diets containing 10 % following dietary fiber *ad lib.* for 10 days : cellulose, chitin, locust bean gum, pectin, guar gum, and alginic acid. These mice were received HxCDD orally on day 4. Then on day 10, anti-DNP-IgM antibody in serum and the EROD activity in hepatic microsomes was determined as experiment 1. In addition, feces collected every day after exposure were analyzed for HxCDD using a gas chromatograph-mass spectrometer. The analytical conditions were previously described elsewhere[5].

Table 1. Compositions of basal diet , diets containing high nutrients and basal fiber-free diet (g)

Constituent	Basal	High protein	High lipid	High mineral	High vitamin	Basal fiber-free
Casein	25	**50**	25	25	25	25
Oil, soy	6.0	6.0	**30**	6.0	6.0	6.0
Mineral mix.	3.5	3.5	3.5	**17.5**	3.5	**6.0**
Vitamin mix.	1.0	1.0	1.0	1.0	**5.0**	**2.0**
Starch, corn	41.5	41.5	41.5	41.5	41.5	41.5
Granulated sugar	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose	8.0	8.0	8.0	8.0	8.0	8.0

Mineral and vitamin mixture were identical with AIN-76 in the basal diet.

Results and Discussion

HxCDD-induced toxicity in mice

Fig.1 shows a comparison of dose-response curves for anti-DNP-IgM antibody level in the serum, hepatic microsomal EROD activity and ratios of tissues/body weight in the mice receiving HxCDD. The anti-DNP-IgM antibody level in the serum was significantly decreased at a dose of 10 μ g /kg body weight compared to that in the vehicle-treated control group. Treatment at a dose of 10 μ g/kg increased the ratio of liver/body weight, reduced the ratio of spleen and elevated EROD activity. From these results, a dosage of 10 μ g/kg was confirmed to be suitable for this experiment, because this amount induced immune suppression as well as hepatic hypertrophy, splenic atrophy and induction of enzymes in mice.

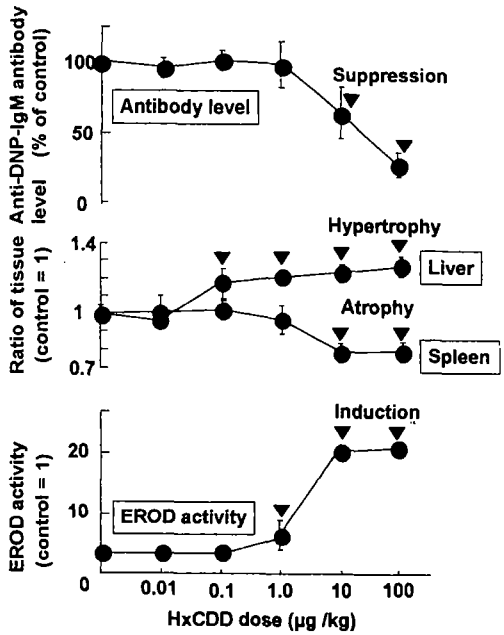

Fig. 1 Anti-DNP-IgM antibody level, EROD activity and ratios of tissues/weight in mice treated with HxCDD

Prevention of HxCDD-induced toxicity in mice by the increasing dietary nutrient

HxCDD administration decreased the anti-DNP-IgM antibody level in the serum to 22% relative to that in the control group (Fig. 2). When mice were maintained on a high mineral diet containing a 5-fold increase in mineral mixture for 14 days, there was a 51% decrease in anti-DNP-IgM antibody level. Intake of a high vitamin diet caused a 34% reduction in the antibody level. Moreover, HxCDD-induced splenic atrophy in mice was inhibited by high mineral diet. Thses results suggest that increased minerals and vitamins in the diet inhibited immune suppression of HxCDD. Therefore, in following experiment, enhancement of fecal HxCDD excretion by adding dietary fiber to diet was investigated using basal fiber-free diet containing a 2-fold increase in mineral and vitamin contents, to clarify the additive effect of increased nutrient absorption and dietary fiber.

Enhanced excretion of HxCDD and the prevention of induced toxicity by addition of the dietary fiber to diet.

Fig.3 shows the effects of selected dietary fiber on fecal excretion in mice treated with HxCDD.

The following dietary fibers were selected based on the adsorption rate for HxCDD shown during *in vitro* experiments : cellulose, chitin, locust bean gum, pectin, guar gum, and alginic acid. Except for alginic acid, the other five dietary fibers added to the diet increased the excretion of HxCDD in a range between 33% and 57% as compared to that of mice eating the basal fiber-free diet (22%). Moreover, locust bean gum, pectin and guar gum reduced the decrease in anti-DNP-IgM antibody level relative to that of mice eating the basal fiber-free diet (Data not shown). Especially, guar gum showed the highest potency in hastening excretion among the selected dietary fibers and decreased HxCDD-induced induction of hepatic microsomal EROD activity.

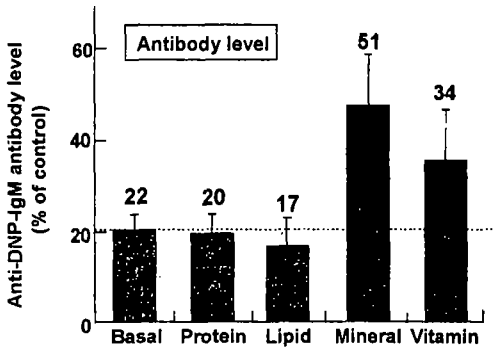

Fig. 2 Effects of high nutrients on anti-DNP-IgM antibody levels in mice treated with HxCDD

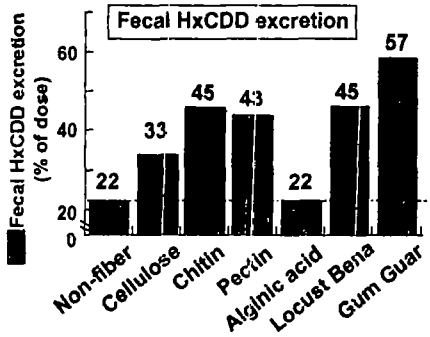

Fig. 3 Effects of dietary fibers on fecal excretions in mice treated with HxCDD

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