ENHANCED EXCRETION OF DIOXIN BY MICE FROM INTAKE OF DIETARY FIBER BEARING LINKED PORPHYRINS

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Introduction

Enhancing the excretion of dioxin, which is an extremely toxic environmental contaminant that accumulates in our body, has been examined as a means of reducing the body burden induced by exposure in animal experiments. In previous studies, the means for reducing the body burden was the intake of an evacuation substance capable of adsorbing dioxin such as activated charcoal[1]. Recently, it was reported that dietary fiber extracted from vegetables etc. and porphyrins hastened the fecal excretion of dioxin in rodents[2]. The dietary fiber contained in our daily meals and porphyrins showed a high potency in hastening excretion.

The aim of this study is the development of a new evacuation substance to excrete dioxin more efficiently. This investigation assessed several porphyrins and dietary fibers for their potency in hastening the fecal excretion of dioxin (1,2,3,4,7,8-HxCDD) in mice. The porphyrins and dietary fiber having the highest ability were selected from those tested, and the dietary fiber bearing linked porphyrins was prepared. We assessed whether the intake of the prepared dietary fiber bearing linked porphyrins enhanced the fecal excretion of dioxin.

Material and Methods

Experiment 1. Selection of porphyrins based on the their potency to enhance excretion of HxCDD

Five groups (4 or 5 mice) of 10-week-old C57BL/6 female mice were used in the experiment. The basal fiber-free diet and four diets containing 1% of the following porphyrins shown in Fig. 1 were respectively given to five groups of mice for 7 days : metal-free chlorophyllin, iron-chlorophyllin, copper-chlorophyllin and copper-phthalocyanine. All five groups were given a single oral administration of HxCDD

dissolved in ethanol : Tween80 : saline (1:10: 89) at a dose of 10 μ g/kg body weight on day 4. Feces collected daily after exposure for 3 days were analyzed for excreted HxCDD using a gas chromatograph-mass spectrometer. The analytical conditions were previously described [3].

Experiment 2. Selection of dietary fiber

Mice were fed the basal fiber-free diet and diets containing 10% dietary fiber *ad lib*. for 7 days. These mice were given HxCDD orally on day 4. The amount of HxCDD excreted in the feces after oral administration was measured for 3 days as in experiment 1.

Experiment 3. Enhanced excretion of HxCDD by intake of the prepared dietary fiber bearing linked porphyrins

The dietary fiber bearing linked porphyrins was prepared as follows: First, the dietary fiber (1.0g) and the porphyrins (1.3g) were dissolved in 200ml methanol/0.5% hydrochloric acid solution (1:1); and dicyclohexylcarbodiimide (0.41g) and dimethylaminopridine (0.06g) were added to solution. This mixture was reacted at room temperature for 4 days. The reacted solution was washed with ethanol to dissolving the dietary fiber and remove free porphyrins. The washed solution was filtered, and the residue dried and powdered in a motor.

The dietary fiber bearing linked porphyrins was mixed with the diet at 1%. The mixed diet was given to the mice, and the excreted amount of HxCDD given orally in the feces was measured as in experiment 1.

Results and Discussion

1. Selection of porphyrins based on the their potency to enhance excretion of HxCDD

Table 1 shows the effects of the four porphyrins on fecal excretion for 3 days in mice treated with HxCDD orally. The metal-free chlorophyllin supplemented diet increased the excretion of HxCDD to 26% of dose as compared to that (18%) of the basal fiber-free diets. Intake of the iron-chlorophyllin diet caused an enhancement to 33% of dose, and the maximum level (44%) of the fecal excretion was observed in mice given the copper-chlorophyllin diet.

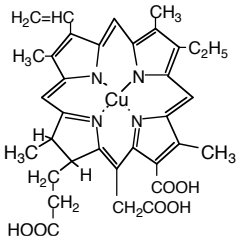
From this result, it appeared that the potency in hastening the fecal excretion of the derivative chlorophyllin was dependent on the metal coordinating in the porphyrin ring, and when the coordinating metal was copper, the administrated HxCDD was discharged into the feces more efficiently. However, in case of copper-phthalocyanine, although copper is coordinated to the porphyrin ring, no significant elevation of the HxCDD level in feces was observed. This finding indicated the reduced ability of copper-phthalocyanine because of is

different functional groups. In other words, it is expected that the dioxin excretion enhancement increased by changing the functional group bound to the porphyrin ring.

(1) Copper-chlorophyllin

Table 1 Effects of the four porphyrins on fecal excretion for 3 days in mice given HxCDD orally.

	Fecal HxCDD Excretion (% of dose)
Non-fiber diet	18
Metal-free chlorophyllin	26
Iron-chlorophyllin	33
Copper-chlorophyllin	44
Copper-phthalocyanine	19



(2) Copper-phthalocyanine

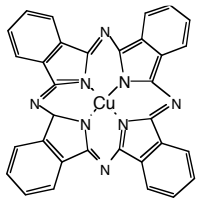


Fig .1 Structures of copper-chlorophyllin and phthalocyanine

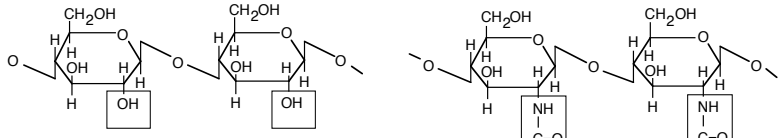
2. Selection of dietary fiber

We have already reported that the intake of the five dietary fibers, which showed high adsorption rate for HxCDD in *in vitro* experiments, were selected from among the 16 dietary fibers observed to increase the amount of the administered HxCDD in feces of mice. In the mice given 10% cellulose, the HxCDD was excreted at a clearly higher level (33% of dose) in feces than in mice fed a non-fiber diet (22%). On the other hand, chitin having the acetyl amino group substituted from the hydroxyl of cellulose shown in Fig. 2 led to an increase in fecal discharge, up to 45% of dose. In this study, in addition, the mice bred with chitosan had an amino group instead of an acetyl amino group of chitin (data not shown). Compared to chitin, the daily intake of chitosan removed the HxCDD given orally in feces more rapidly. However, a marked reduction in the elevated fecal excretion observed in chitosan was induced by varying water-insoluble high molecular weight chitosan to water-soluble low molecule weight. From this result, water-insoluble high molecular weight chitosan was considered to be available for promoting elimination of dioxin from the body.

3. Enhanced excretion of HxCDD by intake of the prepared dietary fiber bearing linked porphyrins

The water-insoluble high molecular weight chitosan bearing linked copper-chlorophyllin was prepared by reacting the carboxyl group of water-soluble copper-chlorophyllin, which had the highest potency in fecal dioxin excretion among the tested porphyrins, with the high molecular weight chitosan hydroxyl group.

(1) Cellulose (2) Chitin



(3) Chitosan

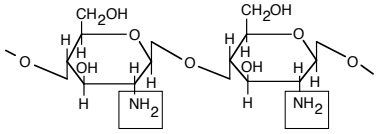


Fig. 2 Structures of cellulose, chitin and chitosan

Table 2 Intake of diet, weight of feces and fecal HxCDD excretion for 3 days in mice given HxCDD orally

	Intake of Diet (g/mouse/3days)	Weight of Feces (g/mouse/3days)	Fecal HxCDD Excretion (Control=100/mouse/3days)
1 % Bound chitosan	10.4	0.53	137
0.3% Copper-chlorophyllin +0.7% Chitosan (Non-bound)	11.9	0.53	101
Control (Non-fiber)	10.6	0.44	100

The prepared bound chitosan added to the diet increased the 3-day excretion of HxCDD given orally to 137% as compared to that (100%) of mice bred with the basal fiber-free diets. The prepared binding chitosan contained copper-chlorophyllin and chitosan at a rate of 30% and 70%, respectively. In contrast, the intake of the diet mixed with copper-chlorophyllin and chitosan non-bound (3:7) showed no significant increase in excretion (101%). These

findings indicated that the binding of copper-chlorophyllin and chitosan enhanced their potency in increasing fecal dioxin excretion.

References

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