CLINICAL TRIALS OF CHOLESTYRAMINE AND A COMBINATION OF RICE BRAN FIBER AND CHOLESTYRAMINE FOR PROMOTION OF FECAL EXCRETION OF RETAINED POLYCHLORINATED DIBENZOFURANS IN YUSHO PATIENTS

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Two incidents of mass food poisoning, one in Japan in 1968[1] and the other in Taiwan in 1979[2], due to the ingestion of rice oil contaminated with polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) have been reported. Yusho disease, the disease caused by PCBs and PCDFs, is characterized by various symptoms including acneform eruptions, hypersecretion of the Meibomian glands, hyperpigmentation of the face, eyelids, gingiva and nails, and general fatigue[3]. In Japan, most of the victims still suffer from various chronic symptoms, and the number of cases detected over the past 22 years now totals 1,860[4]. We have previously reported that the level of PCDFs in the subcutaneous adipose tissue of Yusho patients is 100 times higher than that in normal subjects, and that the fecal excretion of PCDFs in these patients corresponds to this concentration in subcutaneous adipose tissue as well as that in blood[5]. The most effective therapy for the disease is thought to be removal of the toxic agents remaining in the body, but so far no method of doing so has been found. The elimination of these chlorinated compounds using squalane, liquid paraffin[6], and activated charcoal beads[7] has been investigated in experimental animals. On the other hand, it has been reported that cholestyramine, an anion-exchange resin, binds chlordecone in the rat intestine, and increases its excretion into the feces[8]. The resin appears to offer a practical means of treating chronic poisoning involving lipophilic toxins. Takenaka et al reported that, in a group of rats fed a diet containing 10% rice bran fiber (RBF) and cholestyramine, fecal excretion of PCB was stimulated 5.4 times more than that in the control group[9]. In the present study we investigated whether the excretion of residual PCDFs in Yusho patients is enhanced by the administration of cholestyramine and a combination of RBF and cholestyramine.

MATERIALS AND METHODS

Cholestyramine obtained from Bristol Myers Co. Ltd. and RBF refined with the Prosky method containing 85 % dietary fiber (23.5% cellulose, 43.2% hemicellulose and 18.4% lignin) were used.

Administration of drugs and collection of stool samples

Cholestyramine

Six patients were orally administered 4 g of cholestyramine suspended in a cup of water three times a day (after each meal) for six months. Patients in this investigation consisted of three couples with classic Yusho disease, which had been diagnosed on the basis of analysis of gas chromatographic profiles of PCBs in the blood[10]. All stool excreted by the patients during a 6-day period was collected in a 2 L plastic container for 6 days before administration of cholestyramine and two , four and six months after the start of continuous administration.

A combination of RBF and cholestyramine

The patients were administered orally 10g of RBF and 4 g of cholestyramine suspended in a cup of water three times a day (after each meal) for two weeks. Yusho patients in this investigation consisted of two couples with classic Yusho disease as defined above. All stool excreted by the patients was collected in a 2 L plastic container for a 7-day period before and for a 13-day period after the start of administration of RBF and cholestyramine.

Preparation of specimens for determination of PCDFs

PCDFs in stool were extracted 3 times with chloroform / methanol (1:1, v/v). Extracts were filtered through filter paper, and after the addition of distilled water, the chloroform layer was collected. To the chloroform extracts, ^{13}C labelled internal standards were added. Extracts were washed by shaking with conc. sulfuric acid and subjected to column chromatography ($AgNO_3$-silica gel, charcoal-silica gel, Florisil column chromatography). PCDFs were analyzed using gas chromatography / mass spectrometry with selected ion monitoring mode.

RESULTS AND CONCLUSION

Effect of cholestyramine on the fecal excretion of PCDFs in stool

Table 1 summarizes the results for fecal excretion of PCDFs by Yusho patients who consumed cholestyramine. The stool weight increased 1.7 and 1.4 times after the administration period in Patients B and D, respectively. On the other hand, while in the other four, stool weight was almost the same before and after the administration period. In Patient A, the fecal excretion of 2,3,4,7,8-pentachlorodibenzofuran (PnCDF) before and after the administration period was 1,050 and 1,300 pg/day, respectively. Similarly, the fecal excretion of 1,2,3,4,7,8- and 1,2,3,6,7,8-hexachlorodibenzofurans (HxCDFs) before and after the administration period was 1,030 and 1,290 pg/day, respectively; this was the only patient in whom the excretion of PnCDF and HxCDFs in stool was increased, and the increase

Table 1 The excretion levels of PCDFs into the stool from Yusho patients before and after administration of cholestyramine

Patient	Sex	Age		Sample Weight(g)	PnCDF pg/g	PnCDF pg/day	HxCDFs pg/g	HxCDFs pg/day
A	M	60	before	112	9.9	1050	8.6	1030
			after	118	11.0	1300	9.9	1290
B	F	56	before	137	10.3	1380	11.2	1530
			after	235	6.7	1550	5.1	1190
C	M	53	before	75	2.8	200	2.6	190
			after	76	1.7	123	1.5	113
D	F	49	before	64	5.1	330	3.6	220
			after	90	3.8	330	2.7	240
E	M	55	before	126	1.7	200	1.7	200
			after	146	1.3	190	1.4	200
F	F	53	before	213	5.4	1160	7.3	1590
			after	225	3.3	727	3.9	930

PnCDF:2,3,4,7,8-pentachloro-dibenzofuran, HxCDF: 1,2,3,4,7,8- and 1,2,3,6,7,8-hexachlorodibenzofuran

Table 2 The excretion levels of PCDFs into the stool from Yusho patients before and after administration of a combination of rice bran fiber and cholestyramine

Patient	Sex	Age		Sample Weight(g)	PnCDF pg/g	PnCDF pg/day	HxCDFs pg/g	HxCDFs pg/day
A	M	60	before	68	16.6	1130	20.4	1390
			after	127	7.1	870	8.8	1050
B	F	56	before	210	5.4	1130	5.6	1170
			after	387	3.9	1490	4.8	1810
G	M	63	before	75	5.6	420	4.1	310
			after	172	3.8	630	2.6	425
H	F	49	before	80	29.4	2350	30.9	2470
			after	151	15.2	2280	14.9	2250

PnCDF:2,3,4,7,8-pentachloro-dibenzofuran, HxCDF: 1,2,3,4,7,8- and 1,2,3,6,7,8-hexachlorodibenzofuran

was slight (about 20%). In the other five patients, no increase of excretion of PnCDF and HxCDFs in stool was observed. Thus, following administration of cholestyramine, the fecal excretion of these toxic agents showed no significant increase. It was concluded, therefore, that the excretion of PCDFs is not promoted by the consumption of cholestyramine alone.

Effect of combined administration of RBF and cholestyramine on fecal excretion of PCDFs in stool

Table 2 summarizes the results for fecal excretion of PCDFs by the four patients who consumed RBF and cholestyramine. The stool weight after the administration period was 1.9 to 2.3 times greater than that before this period. The amount of PnCDF in stool before and after the administration period was 1,130 and 1,490 pg/day in Patient B, and 420 and 640 pg/day in Patient G. Similarly, the quantity of HxCDFs before and after the administration was 1,390 and 1,780 pg/day in Patient B and 310 and 430 pg/day in Patient G. In these two patients, the excretion of PnCDF and HxCDFs in the stool increased 30 - 50 % and 40 - 50 % respectively, after the administration period. In the other two patients, no increased excretion of PnCDF and HxCDFs in the stool was observed. We think that this finding may be related to the fact that not all samples were collected; we were able to collect only 13 evacuation of 18 evacuation samples for patient A, and only 13 of 16 for patient H. We tentatively consider that our results indicate that the administration of a combination of cholestyramine and RBF is effective for promotion of the excretion of PCDFs in stool, but further research is required.

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