Clinical Trial of a Combination of Rice Bran Fiber and Cholestyramine for Promotion of Fecal Excretion of Retained Polychlorinated Dibenzofuran and Polychlorinated Biphenyl in Yu-Cheng Patients

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We have previously reported that the level of PCDF in the subcutaneous adipose tissue of Yusho patients is 100 times higher than that in normal subjects, and that the fecal PCDF excretion by these patients corresponds to its concentration in subcutaneous adipose tissue as well as in blood.[1] Removal of the toxic chemicals that still remain in the body is thought to be the most effective therapy for the disease. A clinical trial of the combination of rice bran fiber (RBF) and cholestyramine (CHO) was carried out to promote the excretion of PCDF retained in Yusho patients. Upon administration, a tendency of excess fecal excretion was observed, but, the effect of the therapy could not be confirmed.[2] In the present study, a similar trial was carried out on Yu-Cheng patients in Taiwan to examine the degree of enhancement of excretion of residual PCDF and PCB.

Materials and Methods

Cholestyramine obtained form Bristol Myers Co. Ltd. and RBF refined by 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.

Six Yu-Cheng patients were administered orally 6g of RBF and 4g of cholestyramine suspended in a cup of water three times a day after meals for two weeks. Prior to the administration, all samples of stool excreted by the patients were collected for a 7-day

period. During the administration, all samples of stool excreted by the patients were collected for a 14-day period. Twenty milliliters of blood was obtained from each patient before and after the clinical trial.

Preparation of specimens for determination of PCDF and PCB

Blood sample

For determination of PCB in the blood, to a portion of sample (2g) was added 2 ml of 1 N KOH-ethanol. The mixture was heated at 80 °C for one hour, then extracted with hexane. The extract was cleaned up with a silica gel column and analyzed with ECD/GC. For PCDF determination in the blood, a portion of the sample (10g) was extracted three times with acetone/hexane (2:1, v/v), and after the addition of distilled water, the hexane layer was collected. Then, the extract was concentrated to dryness and weighed. The extract was dissolved with hexane and was cleaned up with an $AgNO_3$-silica gel column, charcoal column, Florisil column and concentrated sulfuric acid, and analyzed with GC/MS.

Stool sample

Stool samples of each patient were pooled together for each period separately (7-day period prior to administration, 6-day period from the second to the seventh day, and 7-days period from the eighth to fourteenth day after beginning of administration). Samples for each period were homogenized. For determination of PCB and PCDF, 100g of each sample was used, and about 300g of sample was stored in a freezer at -20°C. PCB and PCDF in the stool were extracted 3 times with chloroform/methanol (1:1, v/v). The extracts were filtered through glass filter paper, and after dilution with distilled water, the chloroform layer was collected. Then, the chloroform layer was concentrated to dryness and weighed. A portion (20ml) of 1N KOH-ethanol was added to the extracts. Then, it was mixed and left at room temperature over night, and extracted with hexane. The hexane extracts were concentrated and adjusted to 10 ml. A portion (2ml) of hexane solution was cleaned up with Florisil column for the determination of PCB with ECD-GC, and another portion of the solution (8 ml) was cleaned up with concentrated sulfuric acid, an $AgNO_3$-silica gel column, charcoal column and Florisil column for the determination of PCDF with GC/MS.

Determination of cholestyramine excreted into stools

One to four grams of stool were weighed into 50 ml centrifuge tube. To it 2N KOH-ethanol was added and then it was heated at 80°C for one hour. After mixture with distilled water, it was centrifuged at 2000 rpm. The water-ethanol supernatant was removed and the pellet was washed with acetone. Subsequently, to the residue was added 2.5 % of NaOCl and was left at room temperature overnight. Distilled water was added and the mixture was centrifuged at 2000 rpm. The water supernatant was removed and the pellet was washed with acetone. Subsequently, cold 72% of sulfuric acid was added to the pellet and mixed, then it was left at 4°C overnight. Finally, the mixture was

filtered with a G-4 glass filter and the trapped material was washed with distilled water, hot water, acetone and hexane. G-4 glass filter and the trapped material were let to dryness at 105°C for 4 hours and after cooled they were weighed (A). Then, they were heated at 525°C for 4 hours, and weighed after cooled (B). The difference in weight between A and B was considered as the weight of cholestyramine.

Results and Conclusions

PCDD, PCDF, CoPCB and PCB in blood

Table 1 shows the concentrations of PCDD, PCDF, CoPCB and PCB in the blood of Yu-Cheng patients on a lipid base. Among dioxins, the concentration of 1,2,3,4,7,8-HxCDF was the highest (1390±497 ppt) and next was 2,3,4,7,8-PeCDF (487±207 ppt). Concentration of PCB was 3190±1690 ppb, and that of the fat was 0.43±0.07%.

Table 1 Concentrations of PCDD, PCDF, coplanar PCB and PCB in blood of Yu-Cheng patients (pg/g, lipid base)

Patient	A01	A02	A03	D04	D05	D12
2378TCDD	<3	4	4	<2	<3	2
12378PeCDD	10	15	16	7	11	7
123678HxCDD	66	96	114	54	89	66
1234678HpCDD	45	30	36	68	56	46
OCDD	368	667	574	372	300	372
2378TCDF	11	17	8	7	11	5
12378PeCDF	10	14	10	13	9	7
23478PeCDF	553	809	467	395	522	177
123478HxCDF	1073	2138	1858	1052	1314	904
123678HxCDF	82	123	103	75	78	67
1234678HpCDF	42	93	55	46	57	31
33'44'TCB	10	4	5	<2	14	15
33'44'5PeCB	135	171	225	314	482	384
33'44'55'HxCB	90	78	146	53	88	116
TEQ(Inter National)	425	679	485	356	472	244
Total PCB(ppb)	2410	2368	1151	3805	6097	3308
Fat(%)	0.46	0.43	0.44	0.34	0.37	0.54

PCDF and PCB levels in stools before and after administration of RBF and CHO

Table 2 shows the fecal excretion levels of PeCDF (2,3,4,7,8-PeCDF), HxCDF (1,2,3,4,7,8- and 1,2,3,4,7,8-HxCDF) and PCB into stools during the three periods of measurements. Stool weight was 212±45 g/day before treatment, 245±50 g/day during the first week of administration, and 215±61 g/day during the second week. The fecal

excretion level of PeCDF was 837±449, 919±516 and 945±461 pg/day, respectively. The fecal excretion level of HxCDF was 2894±1421, 2556±1183 and 2536±1006 pg/day, respectively. On the other hand, the fecal excretion level of PCB was 565±267, 716±394 and 613±223 ng/day, respectively. These findings indicate that due to the administration of RBF and CHO, the quantity of stools, and the fecal PeCDF and PCB excretion level became slightly increased. However, the fecal excretion of HxCDF during administration was less than that before treatment.

Table 2 PCDF and PCB fecal excretion of Yu-Cheng patients

Patient	Administration	Stool (g/day)	PeCDF (pg/day)	HxCDF (pg/day)	PCB (ng/day)
	Before	280	1027	2822	504
A01	First week	304	976	2518	601
	Second week	328	1134	2633	645
	Before	217	1311	3323	415
A02	First week	236	1608	3350	497
	Second week	210	1459	2875	394
	Before	214	902	4103	536
A03	First week	202	840	2897	589
	Second week	230	1203	3463	575
	Before	209	427	1443	384
D04	First week	297	520	1327	466
	Second week	188	465	1303	432
	Before	210	1189	4615	1098
D05	First week	224	1358	4159	1510
	Second week	181	1120	3599	1020
	Before	139	166	1055	453
D12	First week	180	214	1083	634
	Second week	155	286	1341	614

Determination of cholestyramine excreted into stools

Table 3 shows the results of the determination of CHO excreted into daily stools by Yu-Cheng patients during the administration period. CHO is an anion exchange resin, and it is not absorbed or metabolized. Moreover, over 96 % of CHO was excreted into feces within 24 hours on rat. As 12 g/day CHO was administered to every patient, it is obvious that similar amounts should be excreted into the stools. As to Patient A01, the amount regarded as CHO was 15.35 g/day in the first week, and 14.55 g/day in the second week of administration. At present, we have no explanation regarding the higher amount of CHO. In Patient A03, the amount regarded as CHO coincided with 12 g. In

Patients A02, D04, D05 and D12, the amount ranged from 3.6 to 9.8 g/day. It is suggested that the patients had failed to collect all the stools they discharged.

Table 3 Determination of CHO in the stools of Yu-Cheng patients (g/day)

Patient	A01	A02	A03	D04	D05	D12
First week	15.35	7.85	12.30	8.70	6.50	9.75
Second week	14.55	9.20	11.70	3.90	3.60	9.25

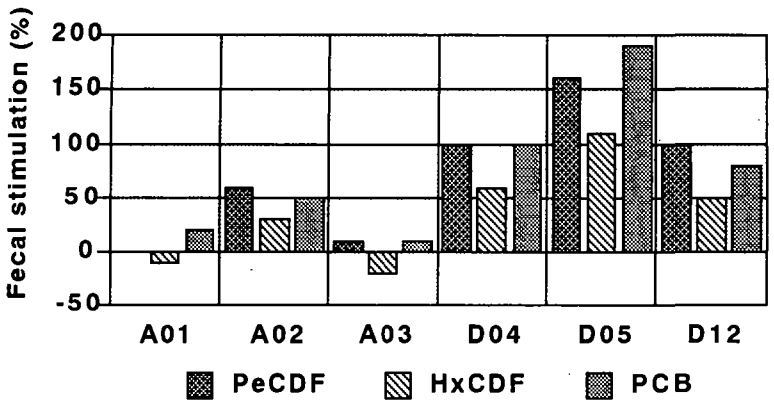
The data in Table 2 were calibrated according to the data shown in Table 3, and the new data are shown in Table 4.

Table 4 Calibrated data of PCDF and PCB fecal excretion of Yu-Cheng patients by the cholestyramine in the stools

Patient	Administration	Stool (g/day)	PeCDF (pg/day)	HxCDF (pg/day)	PCB (pg/day)
A01	Before	280	1027	2822	504
	First week	304	976	2518	601
	Second week	328	1134	2633	645
A02	Before	217	1311	3323	415
	First week	361	2458	5121	760
	Second week	274	1903	3750	514
A03	Before	214	902	4103	536
	First week	202	840	2897	589
	Second week	230	1203	3463	575
D04	Before	209	427	1443	384
	First week	410	717	1830	643
	Second week	578	1431	4009	1329
D05	Before	210	1189	4615	1098
	First week	413	2507	7678	2788
	Second week	603	3733	11997	3400
D12	Before	139	166	1055	453
	First week	222	263	1333	780
	Second week	201	371	1740	797

Figure 1 shows the increase in the percentage of excretion of PeCDF, HxCDF and PCB into stool compared with that before the treatment. In Patients A02, D04, D05 and D12, the increase in excretion was 60 to 160 % for PeCDF, 30 to 110 % for HxCDF, and 50 to 190 % for PCB. However, no increase in excretion was noticed in Patients A01 and

A03 regarding PeCDF, HxCDF and PCB. Administration of 18 g/day of RBF, and 12 g/day of CHO for two weeks caused an increase in excretion of PeCDF, HxCDF and PCB from 30 to 190 % in four out of six treated Yu-Cheng patients.



PeCDF	0	60	10	100	160	100
HxCDF	-10	30	-20	60	110	50
PCB	20	50	10	100	190	80

Figure 1 Stimulation of PeCDF, HxCDF and PCB in the stools by administration of RBF and Cholestyramine

References

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