PROMOTIVE EXCRETION OF POLYCHLORINATED DIBENZOFURANS AND POLYCHLORINATED DIBENZO-*p*-DIOXINS BY FBRA IN PATIENTS WITH YUSHO

Nagayama J [1], Hirakawa H [2], Kajiwara J [2], Todaka T [3], Shibata S [3], Tsuji H [4], Iwasaki T [5]

[1] Laboratory of Environmental Molecular Epidemiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan; [2] Department of Environmental Sciences, Fukuoka Institute of Health and Environmental Sciences, Fukuoka 818-0135, Japan; [3] Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan; [4] Division of Internal Medicine, Kitakyushu-Tsuyazaki Hospital, Fukuoka 811-3307, Japan; [5] Genmaikouso Corp., Sapporo 001-0012, Japan

Abstract

In this study, we examined the effects of fermented brown rice with *Aspergillus oryzae* (FBRA) on the promotive excretion of PCDFs and PCDDs in two groups of patients with Yusho, that is, groups A and B. Just before starting this study, contamination levels of PCDFs and PCDDs were about 2 times higher in group A than in group B. Group A took FBRA after each meal and tree times a day for the first one year only and group B took FBRA only for the second one year. The concentrations of PCDFs and PCDDs in the blood of groups A and B were also measured at the end of the first and second year. We computed the average amounts in the net reduction of PCDFs and PCDFs/DDs from the body of the patients. As a result, in group A, 85.0 and 99.6 ng-TEQ/patient, respectively, were excreted from the body of the patients. In group B, only 38.1 and 40.0 ng-TEQ/patient were excreted. Accordingly, promotive excretion of PCDFs and PCDDs from the patients with Yusho by FBRA seemed more effective in group A than in group B, namely, in the group with higher contamination.

Introduction

Polychlorinated dibenzofurans (PCDFs) have been the most important etiological agents of Yusho [1) 2)], a mass food poisoning that occurred in western Japan in 1968. At present, namely, 40 years after the outbreak, many patients with Yusho are still suffering from several objective and subjective symptoms.

In order to improve or, if possible, to cure various symptoms of patients with Yusho, the promotive excretion of causative PCDFs congeners, together with polychlorinated dibenzo-*p*-dioxins (PCDDs), from the body of the patients is considered very useful. In rats, dietary fiber and chlorophyll have been shown to promote the fecal excretion of PCDFs and PCDDs, probably due to the restriction or some inhibition of their absorption and re-absorption in the digestive tract and therefore dietary fiber and chlorophyll have reduced their levels in rat liver [3) ~8)].

In DIOXIN 2008, which was held at Birmingham, England, UK on August 17-22, 2008, we presented the promotive excretion of PCDFs by FBRA, which was the brown rice fermented with *Aspergillus oryze* and rich with dietary fiber and chlorophyll (Spirulina) using their concentrations on whole weight basis [9)]. In that presentation, we did not use their concentrations on lipid weight basis, because these were considered not to

adequately precise due to the difficulties of reproducible and quantitative lipid extraction from the blood and breast milk [10) 11)]. We, however, examined the relationship between the concentrations on whole weight basis and those on lipid weight basis in the study of promotive excretion of PCDFs and so on by FBRA in the patients with Yusho and found there was no such problem mentioned above. Therefore, in this study we reevaluated the effects of FBRA on promotive excretion of PCDFs and PCDDs using their concentrations on lipid weight basis.

Materials and Methods

FBRA has been manufactured for almost 40 years with Genmaikouso Corp., Sapporo, Japan, and taken by more than 100,000 people as one of the health foods. Ingredients of FBRA have already been reported in our previous studies [12) 13)] and also indicated in Table 1, together with those of boiled polished rice for the comparison.

Table 1. Ingredients of FBRA and boiled polished rice

Ingredient	Unit	FBRA /21g*	Boiled Polished Rice /600g
Energy	kcal	88	1008
Moisture	g	0.3	360
Protein	g	5.9	15
Fat	g	4.5	1.8
Carbohydrate	g	3.8	223
Dietary Fiber	g	4.7	1.8
Ash	g	1.9	0.6
Mineral			
Sodium	mg	5.3	6.0
Potassium	mg	405	174
Calcium	mg	67	18
Magnesium	mg	162	42
Phosphorus	mg	386	206
Iron	mg	2.7	0.6
Zinc	mg	1.1	3.6
Copper	mg	0.16	0.6
Manganese	mg	2.71	2.1
Selenium	μg	1.47	n.a.
Vitamin			
A	μg	201	0
E	mg	1.1	Trace
K	μg	22.5	0
B1	mg	0.56	0.12
B2	mg	0.20	0.06
Niacin	mg	9.1	1.2
B6	mg	0.55	0.12
B12	μg	2.73	0
Folic acid	μg	48	18
Pantothenic acid	mg	1.32	1.5
Biotin	μg	9.0	n.a.
SOD activity	U/g	650	n.a.
Phytin acid	g	0.87	n.a.
Chlorophyll	mg	12.9	n.a.

*: Analysis was conducted and certified by Foundation of Japan Food Analysis Center on June 17, 2008.
n.a.: Not analyzed.

Eighteen patients with Yusho were voluntarily participated in this study and divided into two groups in compliance with their wishes, namely, groups A and B. Group A consisted of 3 males and 7 females with the mean age of 67.7 years old and group B 4 males and 4 females with that of 64.1 years old. In group A, they took 7.0 to 10.5g of FBRA after each meal and three times a day only for the first one year and didn't for the second one year. In group B, they took FBRA in the same way as group A in the second one year only, but not in the first one year.

Just before starting this study, 20 ml of the peripheral blood was individually taken by venipuncture in both groups A and B, twice at one week intervals. These blood samples were analyzed for PCDFs including 2,3,4,7,8-pentachloro-

dibenzofuran (2,3,4,7,8-PenCDF), 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF) and 1,2,3,6,7,8-HxCDF, which were the most important causative PCDFs congeners for Yusho disease, and PCDDs by HRGC-HRMS technique using a Micromass Autospec Ultima NT mass spectrometer directly interfaced with an Agilent Technologies HP-6890A gas chromatography [14]. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent (TEQ) concentrations of PCDFs and PCDDs were calculated by using 2005 WHO TCDD toxic equivalency factor values [15]. The average concentrations of PCDFs and PCDDs in the two blood samples of the same patient were expressed as the individual original ones in both groups A and B. In order to evaluate the effect of FBRA on their excretion from the patients, their blood concentrations were determined again exactly with the same manner as those measured just before beginning of this study at the end of the first and second years in both groups. The experimental protocol of this study was shown in Fig. 1.

Their concentrations measured at different times in each group were statistically examined by student's t-test.

	First Year		Second Year	
April	May → April		May → April	
Collection of Blood Sample	Intake of FBRA	Collection of Blood Sample	Intake of FBRA	Collection of Blood Sample
2 times/ person·week	Group A (+) Group B (-)	2 times/ person·week	Group A (-) Group B (+)	2 times/ person·week

Fig. 1. Experimental protocol of intake of FBRA on promotive excretion and/or suppressive absorption of PCDFs/DDs in patients with Yusho

Results and Discussion

Changes in concentrations on lipid weight basis of the three PCDF congeners in the blood of patients with Yusho during the period of this study were shown in Table 2. Just before starting this study, concentrations of 2,3,4,7,8-PenCDF, 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF in group A were 413 ± 570, 152 ± 244 and 45.7 ± 65.0 pg/g, respectively, and those in group B 151 ± 122, 42.7 ± 31.0 and 17.3 ± 10.1 pg/g. Although their concentrations were 2.6 to 3.6 times higher in group A than in group B, even in group B these were 3.0 to 8.6 times greater than those in healthy Japanese people at almost the same age, and the concentrations of 2,3,4,7,8-PenCDF, the main causative PCDFs congener of Yusho, were the highest and 18 times over those of healthy people [16]. These results clearly indicate that patients with Yusho are still contaminated with high levels of PCDFs and in order to improve their objective and subjective symptoms, promotive excretion of PCDFs seems quite important.

As indicated in Table 2, in general, mean concentrations of 2,3,4,7,8-PenCDF, 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF decreased after the end of year, in which they had taken FBRA for one year and increased after the end of year, in which they had not for one year. This kind of tendency was also seen in TEQ concentrations of PCDFs and PCDDs, as shown in Table3.

On the assumption that the body fat was contaminated with PCDFs and PCDDs at their blood concentrations on lipid weight basis, as indicated in Tables 2 and 3 and the content of body fat was 20% of body weight (60kg), we calculated their total body burdens and evaluated the effects of the intake of FBRA for one year on the

excretion of PCDFs and PCDDs from the body of patients with Yusho in groups A and B.

Table 2. Effects of the intake of FBRA for one year on changes in concentrations on lipid weight basis of three PCDF congeners in the blood of patients with Yusho

	Concentration (Mean ± S.D.), pg/g lipid weight		
PCDF Congener	Initial	After 1st year	After 2nd year
2,3,4,7,8-PenCDF			
Group / A	413 ± 570	412 ± 569	417 ± 577
B	151 ± 122	157 ± 125	156 ± 126*
1,2,3,4,7,8-HxCDF			
Group / A	152 ± 244	130 ± 205*	137 ± 223**
B	42.7 ± 31.0	41.3 ± 29.5	40.5 ± 31.2
1,2,3,6,7,8-HxCDF			
Group / A	45.7 ± 65.0	39.9 ± 54.0*	42.7 ± 60.3*
B	17.3 ± 10.1	17.2 ± 10.2	16.8 ± 10.9

*: Significantly different from the initial concentration of each group, $p<0.1$.
**: Significantly different from the initial concentration of each group, $p<0.05$.

Table 3. Effects of the intake of FBRA for one year on changes in concentrations on lipid weight basis of PCDFs, PCDDs and PCDFs/DDs in the blood of patients with Yusho

	Concentration (Mean ± S.D.), pg-TEQ/g lipid		
Compound	Initial	After 1st year	After 2nd year
PCDFs			
Group / A	228 ± 317	224 ± 312	228 ± 318
B	82.4 ± 65.0	84.9 ± 66.8	84.3 ± 67.6
PCDDs			
Group / A	30.9 ± 22.8	30.3 ± 21.4	31.0 ± 22.4
B	19.7 ± 5.3	19.7 ± 4.2	19.5 ± 4.3
PCDFs/DDs			
Group / A	258 ± 340	254 ± 333	259 ± 340
B	102 ± 65.5	105 ± 68.6	104 ± 69.2

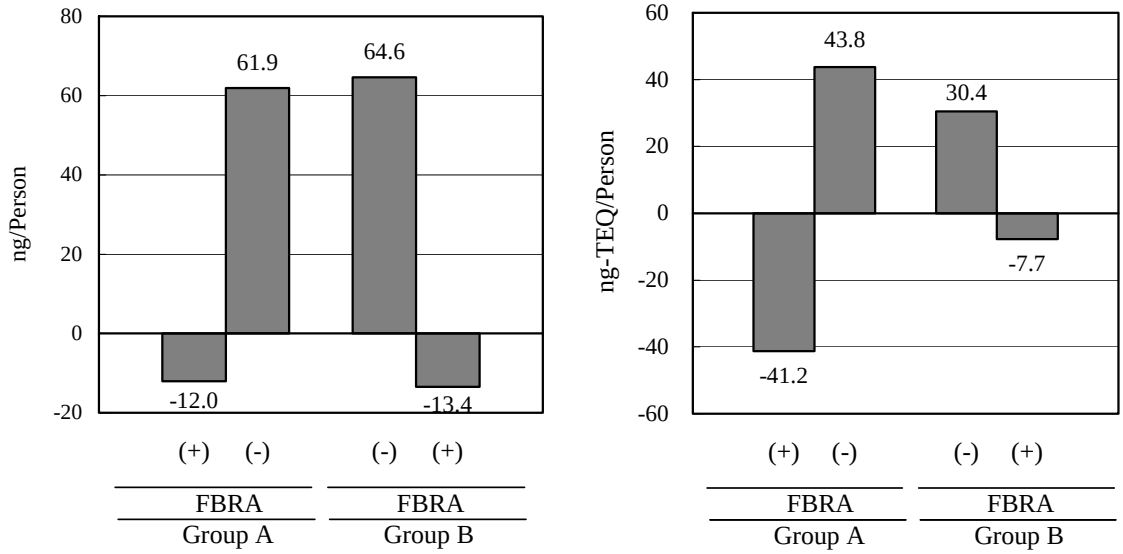

Fig. 2. Effects of the intake of FBRA for one year on the changes of body burden of 2,3,4,7,8-PenCDF in patients with Yusho

Net reduction of 2,3,4,7,8-PenCDF in the body of patients with Yusho were 73.9 and 78.0 ng/ patient in groups A and B, respectively, as shown in Fig. 2. Net reduction of 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDFs were 348 and 102 ng/ patient, respectively, only in group A. We, however, could not find any significant reduction of these two HxCDF congeners in group B, net reduction of PCDFs were 85.0 and 38.1 ng-TEQ/patient in groups A and B, respectively, as shown in Fig. 3. In Fig. 3, effects of FBRA on the excretion of PCDFs/DDs are also indicated and the respective net reductions were 99.6 and 40.0 ng-TEQ/patient in groups A and B.

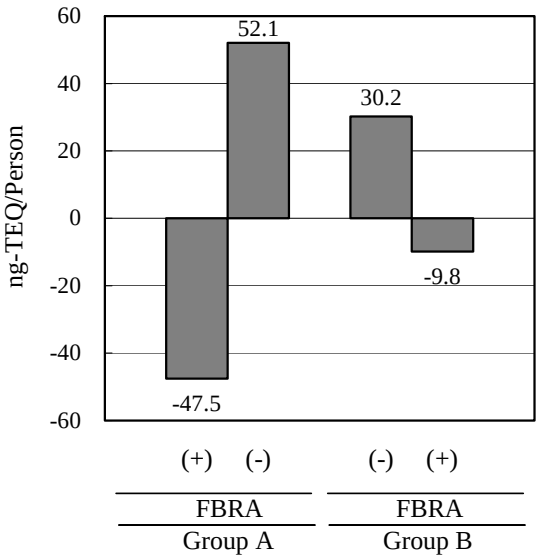

Fig. 3. Effects of the intake of FBRA for one year on the changes of body burden of PCDFs (upper) and PCDFs/DDs (lower) in patients with Yusho

We have already reported the promotive excretion of PCDFs and PCDDs from healthy Japanese people[12) 17) ~19)] and also Yusho patients [9) 13) 20)] by one year intake of FBRA. Results of this study also confirmed the promotive excretion of PCDFs and PCDDs from the patients with Yusho by the intake of FBRA, and showed that this excretion seemed more effective in the patients with higher concentrations of PCDFs and PCDDs, namely, in group A.

References

1. Nagayama J., Masuda Y. and Kuratsune M. *Fd Cosme. Toxicol.* 1977; 15: 195.
2. Iida T., Hirakawa H., Matsueda T. and Nakagawa R. *Fukuoka Acta Med.* 1997; 88: 169. (in Japanese)
3. Morita K., Matsueda T. and Iida T. *Jpn J. Toxicol. Environ. Health* 1997; 43: 35. (in Japanese)
4. Morita K., Matsueda T. and Iida T. *Jpn J. Toxicol. Environ. Health* 1997; 43: 42. (in Japanese)
5. Morita K., Matsueda T., Iida T. and Hasegawa T. *J. Nutr*. 1999; 129: 1731.
6. Morita K., Ogata M. and Hasegawa T. *Environ. Health Perspect.* 2001; 109: 289.
7. Morita K. and Tobiishi K. *Biosci. Biotechnol. Biochem*. 2002; 66; 2306.
8. Morita K. and Nakano T. *J. Agric Food Chem*. 2002; 50: 910.
9. Nagayama J., Hirakawa H. Kajiwara J., Todaka T., Shibata S., Tsuji H. and Iwasaki T. *Organohalogen Compounds* 2008; 70: 1574.
10. Nagayama J., Kohno H., Kunisue T., Shimomura H. and Tanabe S. *Organohalogen Compounds* 2006; 68: 277.
11. Nagayama J., Matsueda T., Hirakawa H., Kunisue T., Tanabe S., Tsuji H. Kohno H., Shimomura H. and Yanagawa T. *Organohalogen Compounds* 2007; 69: 512.
12. Nagayama J., Takasuga T., Tsuji H., Umehara M., Sada T. and Iwasaki T. *Fukuoka Acta Med*. 2003; 94: 118.
13. Nagayama J., Hirakawa H. Kajiwara J., Iida T., Todaka T., Uenotsuchi T., Shibata S., Tsuji H. and Iwasaki T. *Fukuoka Acta Med.* (in Japanese) in press.
14. Todaka T., Hirakawa H., Hori T., Tobiishi K. and Iida T. *Fukuoka Acta Med.* 2005; 96: 249.
15. Van den Berg M., Birnbaum LS., Denison M., Vito M.D., Farland W., Feeley M., Fiedler H., Hakansson H., Hanberg A., Haws L., Rose M., Safe S., Schrenk D., Tohyama C., Tritscher A., Tuomisto J., Tysklind M., Walker N. and Peterson R.E. *Toxicol. Sci*. 2006; 93: 223.
16. Todaka T., Hirakawa H., Tobiishi K. and Iida T. *Fukuoka Acta Med.* 2003; 94: 126.
17. Nagayama J., Takasuga T., Tsuji H. and Iwasaki T. *Organohalogen Compounds* 2001; 52: 293.
18. Nagayama J., Takasuga T., Tsuji H. and Iwasaki T. *Organohalogen Compounds* 2002; 55: 327.
19. Nagayama J., Takasuga T., Tsuji H. and Iwasaki T. *Fukuoka Acta Med.* 2005; 96: 241.
20. Nagayama J., Hirakawa H. Kajiwara J., Iida T., Todaka T., Uenotsuchi T., Shibata S., Tsuji H. and Iwasaki T. *Fukuoka Acta Med.* 2007; 98: 215.