PREVENTIVE EFFECTS OF *CHLORELLA VULGARIS* IN RATS EXPOSED TO 2,3,7,8-TETRACHLORODIBONEZO-*P*-DIOXIN

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ABSTRACT

We investigated the effects of *Chlorella vulgaris* (CV) on fecal excretion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin(TCDD). Fifty rats Sprague-Dawley rats(6 weeks old, n=10 rats/group) were randomly divided into one control group and four TCDD that were orally administered 0.5μg/body weight kg of TCDD dissolved in corn oil once after a period of acclimatization. The dioxin groups included a CV free (TCDD-0CV), a 2% CV (TCDD-2CV), a 5% CV (TCDD-5CV) or a 10% CV (TCDD-2CV) group. All rats had free access to water and diet for 4 weeks, demonstrating that body weight gain was lower in TCDD-0CV group than in TCDD-CV groups ($p<0.05$). The fecal excretion of TCDD was significantly increased in the TCDD-CV groups (540% for TCDD-2CV, 155% for TCDD-5CV, 114% for TCDD-10CV group, $p<0.05$), compared to that in the TCDD-0CV group. These findings suggest that administration of CV may be useful for promoting excretion of TCDD.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype and most potent member of the polyhalogenated aromatic hydrocarbons which exert toxic effects *via* a common mechanism[1]. TCDD has been reported to cause thymic involution, immunosuppression, hyperkeratosis, hepatotoxicity, teratogenicity and diverse tumors in both humans and animals[2,3]. Its biological half-life is known to be about 31 days in rats[4] 5.8~9.7 years in humans[5].

Chlorella vulgaris(CV) is unicellular green algae that grow in fresh water. It contains high concentration of chlorophyll, protein, vitamins, minerals, dietary fiber, and nucleic acid[6]. It is known to have a function as chelate for divalent metal ions and hazardous substance. Due to its characteristics, CV has been drawn much attention to detoxify hazardous compounds as a functional food. Therefore, this study was performed to examine if CV affects fecal excretion of TCDD in rats.

MATERIALS AND METHOD

Chemicals

CV powder was obtained from Daesang Wellife Co. (Seoul, Korea). All chemicals were obtained from Sigma Chemicals Co. (St. Louis. MO, U.S.A.) unless otherwise noted. TCDD has a stated purity >98% as determined by gas chromatography/mass spectrometry. Hexane, acetone, chloroform, methanol,

dichloromethane, anhydrous sodium sulfate and Florisil were purchased from Wako Pure Chemical (Osaka, Japan). These reagents were of the grade used for residual agricultural drug measurements

Animals and housing conditions

Five-week-old male Sprague-Dawley (SD) rats, weighing 90-110 g were allowed to acclimatize for one week prior to commencement of the test. Animal's treatments and procedures were conducted in accordance with Hanyang University Lab Animal Care Committee (HALACC) animal use protocols.

Experimental design

Fifty rats (10 rats/ group) were randomly divided into one control group and four TCDD groups which included a CV free (TCDD-0CV), a 2% CV (TCDD-2CV), a 5% CV (TCDD-5CV) or a 10% CV (TCDD-10CV) group. Rats were orally administered 0.5 μg/body weight kg of TCDD dissolved in corn oil once after a period of acclimatization. All rats were freely access to water and diet for 4 weeks. Composition of the chlorella based diet was presented in Table 1.

Extraction and analysis of TCDD

Fecal samples were homogenized and quantitatively extracted with 150ml of dichlromethane in a cylindrical glass-fiber filter by Soxhlet extraction. The extract of each sample was concentrated to approximately 5ml by evaporation and then diluted with hexane to a final volume of 50ml. The quantification for TCDD was carried out by isotope dilution methods, USEPA 1613, after adding 10 $\mu\ell$ of a $^{13}C_{12}$-labeled 2,3,7,8 PCDD/Fs mix internal standard (EDF8999, Wellington Laboratories, Canada). 10ml of 1M KOH was added to each sample, before hydrolyzing overnight at room temperature. The aqueous layer was extracted twice with 10 ml of hexane, and the entire hexane layer was washed with 5 ml of H_2O and concentrated to approximately 20 ml. After being washed 4 times with 10 ml of conc. H_2SO_4, the hexane extract was concentrated to 2 ml, applied to a column containing 0.8g of silver nitrate (7 mm in diameter) and eluted from the column with 8 ml of hexane, the elute then being concentrated to 1 ml. The resulting elute was applied to a column containing 0.6g of Florisil (7 mm in diameter; U.S. Silica Company, New York, NY, USA) and the TCDD were eluted with 8 ml of dichlromethane after being washed with 4 ml of hexane.

The TCDD were analyzed by HP 6890 Plus High resolution gas chromatography/High resolution mass spectrometry (HRGC/HRMS, JEOL JMS-700, Jeol, U.S.A) with a capillary column (0.25 mm*0.25 um film * 60 m, J & W., U.S.A), setting the resolution mode at 10,000. The TCDD in each sample were quantitatively determined in the selected ion monitoring mode.

Statistical analysis

All data are presented as mean ± standard error (SE). Statistical analysis was performed by ANOVA. When significance was established, differences among the fifth groups of data were tested for significance using Duncan's test. All statistical procedures were performed using SPSS (SPSS Inc. Chicago, IL, USA). The differences were considered significant at $p<0.05$.

RESULTS and DICUSSION

Table 1. Composition of the Chlorella meal-based diet (Unit : g/kg)

Ingredients	Normal diet	Chlorella diet		
		2%	5%	10%
Casein	300	184.6	160.9	125.8
DL-Methionine	3	3	3	3
Cornstarch	150	1 50	150	150
Sucrose	500	495.9	489.2	479.8
Cellulose	50	49.68	49.2	48.4
Coconut oil	50	49.8	49.5	48.0
Mineral Mixture[1]	35	35	35	35
Vitamin Mixture[2]	10	10	10	10
Chlorine Bitartrate	2	2	2	2
Chlorella	0	20	50	100
Total	1,000	1,000	1,000	1,004
Energy	3,862	3,834.2	3,834.2	3,822.6

[1] Mineral mixture (g/100g): $CaHPO_4 \cdot 2H_2O$, 0.43; KH_2PO_4, 34.31; NaCl, 25.06; $Fe(C_6H_5O_7) \cdot 6H_2O$, 0.623; $MgSO_4 \cdot 7H_2O$, 9.98; $ZnCl_2$, 0.02; $MnSO_4 \cdot 4\text{-}5H_2O$, 0.121; $CuSO_4 \cdot 5H_2O$, 0.156; KI, 0.0005; $CaCO_3$, 29.29; $(NH_4)\ 6Mo_7O_2 \cdot 4H_2O$, 0.0025

[2] Vitamim mixture (mg/100g): Vitamin A acetate, 93.2; Vitamin D_3, 0.5825; α-tocopherol-acetate, 1,200; Vitamin K_3, 6.0; Vitamin B_1 hydrochloride, 59.0; Vitamin B_2, 59.0; Vitamin B_6 hydrochloride, 29.0; Vitamin B_{12}, 0.2; Vitamin C, 588; D-Biotin, 1.0; Foric acid, 2; Pantothenic acid, 235; Nicotinic acid, 294; Inositol, 1176; Lactose, 96257

Effect of CV on food intake, body weight, and fecal weight in rats administered TCDD

Food intake and body weight gain were significantly greater in TCDD-CV groups than those in TCDD-0CV group ($p<0.05$). This data were similar to that of Morital et al[6]. Fecal weight did not differ among groups, but it showed increase by feeding CV ($p>0.05$).

Table 2. Food intake, body weight, fecal weight[1,2]

Group[3]	Food intake (g/week)	Body weight gain (g)	Fecal weight ㄴ(g)
Con	421.7±34.0[a]	112.2±24.4[a]	11.1 ± 0.9[NS]
D-0C	322.1±28.4[b]	53.7±15.6[b]	0.5 ± 0.1
D-2C	474.8±34.6[a]	123.2±9.8[a]	0.9 ± 0.6
D-5C	536.4±24.9[a]	127.9±21.6[a]	0.8 ± 0.9
D-10C	484.2±27.1[a]	127.6±16.5[a]	0.7 ± 0.1

[1] Values are means ± S.E., n=10. [2] Significantly different from basal group ($p<0.05$) [3] Con; control group fed without TCDD, D-0C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella free diet, D-2C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella supplementation (2% chlorella diet), D-5C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella supplementation (5% chlorella diet), D-10C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella supplementation (10% chlorella diet).

Effect of CV on fecal excretion of TCDD in rats administered TCDD

The amount of fecal excretion of TCDD is shown in Table 3. Rats in TCDD-CV groups had 114~540% lower ($p<0.05$) TCDD fecal excretion (540% for TCDD-2CV, 155% for TCDD-5CV, 114% for TCDD-10CV group) than those in TCDD-0CV group. It clearly showed that CV had acceleratory effect on elimination of TCDD *via* feces. It is possibly indicated that chlorophyll and dietary fiber contained within chlorella cells may inhibit absorption and reabsorption of dioxins from the intestinal tract by forming

complexes with heterocyclic amines *via* H-bond or electrostatic interactions[7~9] or noncovalent binding with dioxins that have nonpolar structures.

In conclusion, our data suggest that intake of CV may be useful in promoting the fecal excretion of TCDD. Additional studies are required to define the enhancing mechanism of dioxins excretion by CV including the promotion of catabolism of dioxins in the liver.

Table 3. Fecal excretion of TCDD in the TCDD-fed rats[1,2]

Group[3] / Dioxins	Con	D-0C	D-2C	D-5C	D-10C
2,3,7,8-T_4CDD	0.0 ± 0.0[a]	2.16 ± 0.39[b]	11.66 ± 0.77[c]	18.13 ± 0.46[c]	20.70 ± 2.42[c]

[1] Values are means ± S.E., n=10.

[2] Significantly different from basal group (p<0.05)

[3 1] Con; control group fed without TCDD, D-0C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella free diet, D-2C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella supplementation (2% chlorella diet), D-5C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella supplementation (5% chlorella diet), D-10C; 0.5 ㎍/body weight kg of TCDD treatment, chlorella supplementation (10% chlorella diet).

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