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Effect of Chlorella intake on Cadmium metabolism in rats*

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Abstract

This study was performed to investigate the effect of chlorella on cadmium (Cd) toxicity in Cd- administered rats. Sixty male Sprague-Dawley rats (14 week-old) were blocked into 6 groups. Cadmium chloride was given at levels of 0 or 325 mg (Cd: 0, 160 ppm), and chlorella powder at levels of 0, 3 and 5%. Cadmium was accumulated in blood and tissues (liver, kidney and small intestine) in the Cd-exposed groups, while the accumulation of Cd was decreased in the Cd-exposed chlorella groups. Fecal and urinary Cd excretions were remarkably increased in Cd-exposed chlorella groups. Thus, cadmium retention ratio and absorption rate were decreased in the Cd exposed chlorella groups. Urinary and serum creatinine, and creatinine clearance were not changed in experimental animals. In addition, metallothionein (MT) synthesis in tissues was increased by Cd administration. The Cd-exposed chlorella groups indicated lower MT concentration compared to the Cd-exposed groups. Moreover, glomerular filtration rate (GFR) was not changed by dietary chlorella and Cd administration. According to the results above, this study could suggest that Cd toxicity can be alleviated by increasing Cd excretion through feces. Therefore, when exposed to Cd, chlorella is an appropriate source which counteracts heavy metal poisoning, to decrease the damage of tissues by decreasing cadmium absorption.

Key Words: Chlorella, cadmium, excretion, heavy metal, metallothionein (MT)

Introduction

Cadmium (Cd) is one of the heavy metals and existing very low levels in nature (Chapman *et al*., 2003). However, it is very toxic and an important environmental pollutant in soil, water, air, food and smoke (Järup *et al.,* 1998). Cadmium is mainly used in the industry for coating steel, glass and plastics (including polyvinyl chloride), and for nickel cadmium battery production (Tsalev & Zaprianov, 1993). It has a very long biological half-life (10-30 years) in human body and its toxicity is dependent on the route, amount and the duration of exposure (Goering *et al*., 1987; Goyer & Cherian, 1995; Satarug *et al*., 2003). The chronic Cd exposure in human appears to result in nephrotoxicity and osteoporosis, pulmonary emphysema, liver dysfunction, etceteras (Berglund *et al*., 2000; Goyer & Cherian, 1995; Rikans & Yamano, 2000; Shaikh *et al*., 1999). When cadmium is taken into the body, it is slowly excreted after combining with proteins like albumin and metallothionein (MT), or negative ions of other molecules, especially the -SH group, without the process of metabolism like oxidation, reduction, and alkylation. Also, it is severely toxic in cases of both acute and chronic intoxications; your liver and testis become the target organs during acute intoxication, while your kidney becomes the target organ when chronic Cd intoxication happens (Brzóska *et al*., 2003; Casalino *et al*., 2002).

Metallothionein (MT) has the molecular weight of 6,000~ 7,000 and does not contain aromatic amino acids and histidine, and it is a low molecular weight protein abundant in cysteine (33%) (Kägi & Schäffer, 1988; Manuel *et al*., 1992). Metallothionein has 7 metal binding sites, so it can control the metabolism of several metal ions and mitigates toxicity of heavy metals. It also relates to the immune reaction and prevents tissue damage caused by heavy metal (Nordberg, 1992). The MT synthesis is known to be induced by several metal ions and hormones, stress and cytokine (Hidalgo *et al*., 1990). There is a report that MT subsists in a very low density under normal circumstances, but the MT amount grows when the synthesis is promoted by metals like Cd or Pb in organs such as liver (Nordberg & Nordberg, 1987). MT can be synthesized anywhere inside the body but mostly in the liver and kidney, and can be combined with Cd, which prevents free reactive Cd from making to the body which makes toxic materials (Cousins *et al*., 1973). The half-life of MT differs to the metal it combines with, but is rather short from 1 to 4 days, so the MT must be combined endlessly to counteract poison made by Cd or heavy metals (Revis & Osborne, 1984). The study about Cd and MT synthesis and Cd's removal system through MT has been developed profoundly because Cd's affinity with MT is high compared to other metal ions. As seen above, the

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study about the prevention and the treatment of Cd-intoxication is becoming a field of interest. While the study about the dietary factor which affects absorption of Cd into the body is drawing attention, there is a report that protein, calcium, and fiber reduce the absorption of Cd and the accumulation to the liver and kidney (Omori & Muto, 1977; Revis & Osborne, 1984).

In addition, chlorella has been demonstrated to develop tolerance to Cd polluted environment (Morita *et al*., 1999). Chlorella is a unicellular green algae that reproduces at a rapid rate. It has been known to contain highly nutritious substances and to exert various biological effects (Kojima *et al*., 1973). Chlorella contains about 55~67% protein, 1~4% chlorophyll, 9~18% dietary fiber and an amount of minerals and vitamins (Morita *et al*., 1999). Also, it contains all the essential amino acids required for the nutrition of animals and humans. This algae is considered to be highly resistant to heavy metals such as Cd. The algae can chelate heavy-metal ions such as Cd (Yoshida *et al*., 2006). Recently, chlorella has been used as a health food or functional food in Japan, the U.S. and other countries (Morita *et al*., 1999).

In order to use chlorella as a tool for Cd-removal and recovery, it is necessary to characterize its metal-tolerance and metal- binding capacity. Numerous studies have shown that chlorella intake may reduce Cd absorption and accumulation, and also prevent or reduce the adverse actions of Cd (Han *et al*., 2002; Hwang *et al*., 2006a; Hwang *et al*., 2006b; Morita *et al*., 1999). The aim of our study was to investigate the possible Cd-removal by chlorella intake on Cd metabolism. We measured Cd concentrations (blood, urine, liver, kidney, small intestine and feces), metallothionein (liver, kidney, small intestine) and kidney capacity.

Materials and Methods

Experimental material

The chlorella powder (*chlorella vulgaris*) used in this study was manufactured by Daesang Co. (Korea) and the characteristics of chlorella powder is shown in Table 1.

Table 1. Specifications of chlorella powder extract

Components	Chlorella powder extract (per 100 g powder)
Protein	60.6 g
Carbohydrate	3.7 g
Fat	12.8 g
Dietary Fiber	13.0 g
Ash	4.5 g
Moisture	5.4 g
Calcium	5.1 mg
Vitamin A potency	58,900 IU
Vitamin C	74 mg
Vitamin E	22.8 mg
Total	100 g
Calorie	372 kcal

Animal care and dietary treatment

Sixty 14-week-old male Sprague-Dawley rats (CD (SD)IGS, Outbred, Charles River Laboratory Inc. Origin; Jung-Ang Lab Animal, Inc., Korea) weighing 399.06 ± 0.8 g were blocked into 6 groups according to body weight and raised for 10 weeks on experimental diets. Two-factorial nested classification design, which is shown in Table 2, was used. The independent variables

Table 2. Classification of experimental groups

Groups[1)]	Dietary Cadmium level (ppm)	Dietary chlorella level % (w/w)
NC0	0	0
NC3	0	3
NC5	0	5
CC0	160	0
CC3	160	3
CC5	160	5

[1)] NC0 : No cadmium with 0% chlorella powder
NC3 : No cadmium with 3% chlorella powder
NC5 : No cadmium with 5% chlorella powder
CC0 : Cadmium (160 ppm) with 0% chlorella powder
CC3 : Cadmium (160 ppm) with 3% chlorella powder
CC5 : Cadmium (160 ppm) with 5% chlorella powder

Table 3. Composition of experimental diets (g/kg diet)

Ingredients \ Group[1)]		NC0	NC3	NC5	CC0	CC3	CC5
Corn starch		400.49	388.47	380.46	400.16	388.15	380.14
Dextrinized cornstarch		132.00	128.04	125.40	132.00	128.04	125.40
Sucrose		100.00	97.00	95.00	100.00	97.00	95.00
Casein (>85% protein)		200.00	194.00	190.00	200.00	194.00	190.00
Soybean oil		70.00	67.90	66.50	70.00	67.90	66.50
Fiber		50.00	48.50	47.50	50.00	48.50	47.50
Mineral mix[2)]		35.00	33.95	33.25	35.00	33.95	33.25
Vitamin mix[3)]		10.00	9.70	9.50	10.00	9.70	9.50
Choline bitartrate		2.50	2.43	2.38	2.50	2.43	2.38
TBHQ		0.01	0.01	0.01	0.01	0.01	0.01
chlorella powder		0	30.00	50.00	0	30.00	50.00
$CdCl_2$		0	0	0	0.325	0.325	0.325
Total weight		1000	1000	1000	1000	1000	1000
Energy calories (kcal)		3736.4	3735.9	3735.6	3735.2	3734.7	3734.4
Energy ratio (%)	Carbohydrate	64.0	62.2	61.0	64.0	62.2	61.0
	Protein	19.2	20.5	21.5	19.2	20.5	21.5
	Fat	16.9	17.3	17.6	16.9	17.3	17.6

[1)] See Table 2.
[2)] Mineral mix (AIN-93G-MIX) (g/kg mixture) : anhydrous calcium carbonate, 357; monobasic potassium phosphate, 196; sodium chloride, 74; potassium sulfate, 46.6; tripotassium citrate monohydrate, 70.78; magnesium oxide, 24; ferric citrate, 6.06; zinc carbonate, 1.65; manganous carbonate, 0.63; cupric carbonate, 0.3; potassium iodate, 0.01; anhydrous sodium selenate, 0.01025; ammoniumparamolybdate 4-hydrate, 0.00795; sodium metasilicate 9-hydrate, 1.45; chromium potassium sulfate 12-hydrate, 0.275; boric acid, 0.0815; sodium fluoride, 0.0635; nickel carbonate, 0.0318; lithium chloride, 0.0174; ammonium vanadate, 0.0066; powered sucrose 221.026
[3)] Vitamin mix (*AIN-93-VX*) (g/kg mixture) : Niacin 3, Calcium Pantothenate 1.60, Pyridoxine HCl 0.70, Thiamine HCl 0.60, Riboflavin 0.60, Folic Acid 0.20, Biotin 0.02, Vitamin E Acetate (500 IU/g) 15, Vitamin B12 (0.1%) 2.50, Thitamin A Palmitate (500,000 IU/g) 0.80, Vitamin D3 (400,000 IU/g) 0.25, Vitamin K1/Dextrose Mix (10 mg/g) 7.50, Sucrose 967.23

were dietary Cd level and chlorella level. Animals were placed in individual stainless steel wire-mesh cages in an automatically controlled room. All instruments were treated with 0.4% ethylene diamine tetraacetic acid (EDTA) solution, 10% nitric acid solution and then washed with distilled water to avoid other mineral contaminations. The composition of the experimental diets is shown in Table 3. The chlorella powder (*Chlorella vulgaris*) used in this study was manufactured by Daesang Co. (Korea). The diets were mixed according to the AIN-93G diet (Reeves *et al*., 1993) with slight modifications. Corn starch (Daesang Co., Korea) was the only source of carbohydrate in the mixture. Casein (Murray Goulburn Co., Australia) was used as a source of protein and soybean oil (CJ Co., Korea) was used as a source of lipid. Mineral and vitamin mixtures were purchased from Dyets Inc. (U.S.A). Also, Cd chloride ($CdCl_2$) was substituted for cornstarch and animals were provided with 0.325 g $CdCl_2$ per kg diet. The 0% chlorella diet contained no chlorella in the diet and the 3% chlorella diet contained 3% chlorella per kg diet. The 5% chlorella diet contained 5% chlorella per kg diet. This study was conducted at the nutrition laboratory of Ewha Womans University, in compliance with *the Guide for the Care and Use of Laboratory Animals*. During the experimental period, the rats were allowed free access to the experimental diets and de-ionized water. Body weight was recorded weekly. Food intake was recorded three times per week.

Specimen Collection

To gauge Cd retention ratio, Cd tube feeding was inserted of 0.2 ml $CdCl_2$ solution ($CdCl_2$ 325 ppm (Cd 160 ppm)) daily for the final 2 weeks before the end of the experimental period. The rats were not provided with cadmium diet for the tube feeding period and considering the stress from tube feeding, Cd-free groups had 0.2 ml de-ionized water tube feeding. Feces and urine were collected using the metabolic cages for the final 2 days before the end of the experimental period. Feces were weighed and stored at -80℃ until analysis. Urine was collected in a bottle treated with a few drops of 0.1% HCl and toluene as embalmments. After collection, the bottle was filled up to 100 ml with de-ionized water and centrifuged at 7,000 rpm for 10 minutes (Supra 22K high speed centrifuge, Hanil, Korea). Aliquots of the samples were stored at -80℃ until analysis. At the end of the experimental period, the animals were plundered of food for 12 hr and sacrificed after anesthetization with ethyl ether. After blood samples were collected directly from the heart with a heparinized or non-treated syringe, whole blood samples collected with heparin-treated syringes were transferred to polypropylene tubes, which were frozen at -80℃ in a deep freezer until analysis for cadmium concentration. Blood samples collected using non-treated syringes were placed in an ice bath for 20 minutes and then centrifuged at 2,800 rpm for 30 minutes at 4℃ (Union 55R centrifuge, Hanil, Korea). Obtained serum was for measuring the levels of AST and ALT. The liver, kidney, spleen, epididymal fat pad, perirenal fat pad and femur were removed and weighed. They were cut into small pieces, frozen over dry-ice and stored at -80℃ in a deep freezer until analysis.

AST and ALT

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using a kit (Asan Pharmaceutical, Korea) based on the Reitman-Frankel method (Reitman & Frankel, 1957).

Cadmium concentration in blood and urine

Blood and urine cadmium levels were measured from the method of Zinterhofer (1971). These were determined using an atomic absorption spectrophotometer (AAS, Model 6701F, SHIMADU Co., Japan). The analyses were performed at the 228.8 nm resonance line.

Cadmium concentration in small intestine, liver, kidney and feces

Cd concentrations were measured from the samples using the method of Yeager (1971). These were determined using an atomic absorption spectrophotometer (AAS, Model 6701F, SHIMADU Co., Japan).

Determination of Metallothionein in small intestine, liver and kidney

Metallothionein (MT) concentration was measured from the method of cadmium/hemoglobin affinity assay (Eaton & Cherian, 1991; Eaton & Toal, 1982; Onosaka & Cherian, 1982). The rat red blood cell (RBC) hemolysate was used from the method of Onosaka and Cherian (Yeager *et al*., 1971). The concentration of MT in each tissue was calculated by assuming that 7 g-atom of Cd is bound to each mole of thionein which has a molecular weight of 6050 by amino acid analysis (Kagi & Nordberg, 1979).

Cadmium retention

The rates were calculated using the cadmium absorption rate and retention ratio.

Creatinine clearance

Urinary and serum creatinine were measured using a kit (IVDLAb CO., LTD, Korea) based on Jaff's reaction (Owen, 1954; Simoni *et al*., 2002). Absorbance at 505 nm was determined using a spectrophotometer (Genesys 10 UV, Thermo Electron Co., USA). All procedures followed the manufacturers´ instructions. These were calculated using creatinine clearance (glomerular filtration rate, GFR, ml/min).

Statistical analysis

All results were expressed as means ± standard error (SE). The one-way analysis of variance (ANOVA) and mean differences among the experimental groups were evaluated by Duncan's multiple range tests at the $p<0.05$ level.

Results

Effect of dietary cadmium and chlorella on daily food intake, calorie intake, food efficiency ratio in rats

Daily food intake, body weight gain during the experimental period and food efficiency consumed are shown in Table 4. Daily food intake, body weight gain and food efficiency consumed were significantly influenced by the Cd level. The animals fed Cd administration diets had significantly lower levels of these indexes than animals fed other diets. As shown in Table 4, food intake was slightly decreased in chlorella intake groups compared with that of 0% chlorella group, and these alterations were shown in both Cd-exposed and Cd-free groups. But, food efficiency consumed was slightly increased in chlorella intake groups compared with that of the 0% chlorella group, especially the 5% chlorella diet in Cd-exposed group.

Table 4. Food intake, calorie intake, weight gain and food efficiency ratio in rats fed diets containing chlorella powder

Group[1)]	Food intake (g/day)	Calorie intake (kcal/day)	weight gain (g/10 weeks)	Food efficiency (g/100 kcal)
NC0	25.02 ± 0.68[2)a3)]	93.47 ± 2.55[a]	182.30 ± 11.37[a]	2.82 ± 0.10[a]
NC3	24.46 ± 0.57[a]	91.40 ± 2.13[a]	182.27 ± 5.74[a]	2.92 ± 0.04[a]
NC5	24.09 ± 0.54[a]	89.98 ± 2.01[a]	176.47 ± 10.21[a]	2.84 ± 0.12[a]
CC0	22.90 ± 0.52[ab]	85.55 ± 1.95[ab]	75.81 ± 3.58[b]	1.30 ± 0.08[c]
CC3	21.49 ± 0.95[b]	80.27 ± 3.53[b]	79.33 ± 7.46[b]	1.44 ± 0.13[bc]
CC5	20.87 ± 0.65[b]	77.95 ± 2.42[b]	90.27 ± 8.37[b]	1.69 ± 0.14[b]

[1)] See Table 2.
[2)] Mean ± standard error
[3)] Values with different alphabet within the column are significantly different at α=0.05 by Duncan's multiple range test.

Table 5. The weight of liver, kidney and femur in rats fed diets containing chlorella powder

Group[1)]	Liver (g)	Kidney (g)	Femer (g)
NC0	15.56 ± 0.67[2)a3)]	3.00 ± 0.08[a]	1.70 ± 0.05[NS4)]
NC3	14.12 ± 0.63[a]	2.91 ± 0.10[ab]	1.63 ± 0.05
NC5	14.23 ± 0.41[a]	2.91 ± 0.08[ab]	1.86 ± 0.09
CC0	11.73 ± 0.61[b]	2.65 ± 0.04[c]	1.71 ± 0.05
CC3	11.57 ± 0.34[b]	2.72 ± 0.08[bc]	1.68 ± 0.13
CC5	11.62 ± 0.34[b]	2.72 ± 0.08[bc]	1.67 ± 0.04

[1)] See Table 2.
[2)] Mean ± standard error
[3)] Values with different alphabet within the column are significantly different at α=0.05 by Duncan's multiple range test.
[4)] Values are not significantly different among the groups at α=0.05 using by Duncan's multiple range test.

The weight of liver, kidney and femur

The weight of the liver, kidney and femur are shown in Table 5. In comparison among the chlorella groups, there was no significant difference in all these organ weights. The weights of the liver and kidney were lowest in rats fed the Cd diet. However, these did not show differences among the chlorella groups. Also, femur weight had no significant difference in rats fed both Cd and chlorella diet.

Serum AST and ALT activities

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities are shown in Table 6. The ranges of serum AST and ALT activities were 50.57~60.43 IU/L and 20.19~28.91 IU/L, respectively. These levels were included within the reference range (AST: 39~262 IU/L, ALT: 20~60 IU/L) in Sprague-Dawley rats (Reitman & Frankel, 1957). Therefore all diets were considered to be nontoxic for liver function.

Cadmium concentrations in small intestine, blood, liver and kidney

Cadmium concentrations in small intestine, blood, liver and kidney are shown in Table 7. Cadmium concentrations in blood and organ tissues were significantly affected by Cd administration and dietary chlorella level. Cadmium concentrations in the blood

Table 6. Serum AST and ALT activities of rats fed diets according to different Cd levels and dietary chlorella levels (Unit: IU/L)

Group[1)]	AST (U/L)	ALT (U/L)
NC0	50.57 ± 1.79[2)b3)]	20.41 ± 1.49[b]
NC3	55.81 ± 3.56[ab]	22.48 ± 1.91[ab]
NC5	54.42 ± 1.58[ab]	20.19 ± 1.83[b]
CC0	59.78 ± 2.16[a]	28.91 ± 2.02[a]
CC3	60.43 ± 1.72[a]	27.01 ± 3.30[ab]
CC5	59.10 ± 2.19[a]	25.95 ± 2.03[a]

[1)] See Table 2.
[2)] Mean ± standard error
[3)] Values with different alphabet within the column are significantly different at α=0.05 by Duncan's multiple range test.

Table 7. Cadmium concentrations in small intestine, blood, liver, and kidney

Groups[1)]	Small Intestine (μg/g wet wt)	Blood (μg/100 ml)	Liver (μg/g wet wt)	Kidney (μg/g wet wt)
NC0	0.17 ± 0.04[c2),3)]	0.28 ± 0.03[d]	3.83 ± 0.44[c]	4.80 ± 0.46[d]
NC3	0.07 ± 0.01[c]	0.33 ± 0.03[d]	2.93 ± 0.38[c]	4.78 ± 0.70[d]
NC5	0.08 ± 0.05[c]	0.44 ± 0.05[d]	3.30 ± 0.49[c]	2.54 ± 0.40[d]
CC0	8.24 ± 1.13[a]	36.46 ± 3.45[a]	48.51 ± 6.11[a]	52.35 ± 6.06[a]
CC3	4.05 ± 0.10[b]	18.08 ± 3.19[b]	31.41 ± 1.18[b]	20.50 ± 0.78[b]
CC5	3.64 ± 0.76[b]	11.74 ± 1.31[c]	25.37 ± 4.04[b]	13.99 ± 2.17[c]

[1)] See Table 2.
[2)] Mean ± standard error
[3)] Values with different alphabet within the column are significantly different at α=0.05 by Duncan's multiple range test.

Table 8. Metallothionein concentrations in small intestine, liver and kidney

Group[1)]	Small Intestine (μg/g wet wt)	Liver (μg/g wet wt)	Kidney (μg/g wet wt)
NC0	1.39 ± 0.29[d2)3)]	5.04 ± 0.55[c]	2.85 ± 0.63[c]
NC3	0.97 ± 0.15[d]	4.93 ± 0.70[c]	2.97 ± 0.57[c]
NC5	0.78 ± 0.11[d]	2.31 ± 0.58[c]	2.61 ± 0.28[c]
CC0	7.63 ± 0.62[a]	723.08 ± 47.63[a]	54.32 ± 5.58[a]
CC3	5.72 ± 0.43[b]	138.74 ± 22.16[b]	23.35 ± 3.89[b]
CC5	3.61 ± 0.60[c]	86.89 ± 13.44[b]	18.41 ± 1.73[b]

1) See Table 2.
2) Mean ± standard error
3) Values with different alphabet within the column are significantly different at α=0.05 by Duncan's multiple range test.

Table 9. Urinary and fecal cadmium excretions, Cadmium retention ratio and absorption rate

Group[1)]	Urinary Cd (μg/day)	Fecal Cd (μg/day)	Cadmium retention ratio (%)	Cadmium absorption rate (%)
NC0	2.03 ± 0.53[c2)3)]	17.09 ± 1.81[c]	N.D[4)]	N.D
NC3	7.32 ± 1.53[c]	20.43 ± 3.02[c]	N.D	N.D
NC5	5.93 ± 0.72[c]	8.04 ± 1.33[c]	N.D	N.D
CC0	22.27 ± 3.43[c]	199.29 ± 28.85[b]	86.52 ± 2.47[a]	90.03 ± 1.44[a]
CC3	68.34 ± 6.37[b]	554.33 ± 56.44[a]	65.66 ± 3.91[b]	72.28 ± 2.82[b]
CC5	95.96 ± 17.72[a]	484.72 ± 29.08[a]	71.23 ± 1.96[b]	75.77 ± 1.45[b]

1) See Table 2.
2) Mean ± standard error
3) Values with different alphabet within the column are significantly different at α=0.05 by Duncan's multiple range test.
4) N.D; not detected

Table 10. Urinary creatinine excretions, serum creatinine concentration and creatinine clearance

Group[1)]	Urinary creatinine (mg/day)	Serum creatinine (mg/100 ml)	Creatinine clearance (G.F.R, ml/min)
NC0	22.50 ± 5.53[NS2)]	3.70 ± 0.37[NS]	0.45 ± 0.12[NS]
NC3	27.20 ± 6.62	3.35 ± 0.32	0.64 ± 0.19
NC5	28.00 ± 7.17	3.41 ± 0.42	0.67 ± 0.18
CC0	20.37 ± 4.53	4.54 ± 1.04	0.38 ± 0.11
CC3	23.00 ± 5.17	3.55 ± 0.43	0.53 ± 0.15
CC5	26.26 ± 6.98	3.20 ± 0.42	0.73 ± 0.19

1) See Table 2.
2) Values are not significantly different among the groups at α=0.05 using by Duncan's multiple range test.

and organ tissues of Cd-exposed groups were significantly higher than those of Cd-free groups. However, chlorella intake significantly decreased Cd concentrations in the blood and kidney, especially animals fed 5% chlorella diet.

Metallothionein in small intestine, liver and kidney

Metallothionein, consisted with cysteine, exists at a very low concentration in general environment (Manuel *et al*., 1992; Revis & Osborne, 1984; Tsuritani *et al*., 1992). Metallothionein concentrations in small intestine, liver and kidney are shown in Table 8. Hepatic MT concentration was markedly increased by Cd administration compared with that of control groups. Specially, this study reveals that MT concentration is significantly decreased with chlorella treatment in Cd-exposed groups. In addition, renal MT concentration in Cd-exposed groups was elevated. Moreover, MT concentration in small intestine was higher in Cd-exposed groups than Cd-free groups. When animals were administered cadmium, MT concentration in kidney was remarkably lower in the 5% chlorella group than the 0% chlorella group. These significantly decreased dietary chlorella in a dose-dependent manner. There was a relationship between Cd-administration and dietary chlorella level in the liver, kidney and small intestine.

Urinary and fecal cadmium excretions, Cadmium retention ratio and Cadmium absorption rate

The results of cadmium excretions in urine and feces that were collected during the administration of cadmium through tube feeding and the cadmium retention ratio were showed in Table 9. Urinary and fecal cadmium excretions of Cd-exposed animals were remarkably higher than those of Cd-free animals. However, these were decreased with increasing dietary chlorella levels, particularly animals fed 5% chlorella diet in urinary cadmium excretion. Moreover, Cd excretions in urine and feces were affected by the interaction between dietary chlorella level and Cd level.

The calculated Cd retention ratio using urinary and fecal Cd excretions was significantly affected by dietary chlorella levels. Namely, Cd retention ratio of rats fed 3% chlorella diet and Cd had the lowest among Cd-exposed groups. But, there was no significant difference among Cd-free groups. In addition, calculated Cd absorption rate using fecal cadmium excretions similarly indicated the results of Cd retention ratio.

Renal creatinine clearance

To estimate abnormalities of renal function by Cd toxicity, creatinine clearance was calculated from serum creatinine concentration and urinary creatinine excretion. As shown in Table 10, serum and urinary creatinine, and creatinine clearance were not changed in experimental animals.

Discussion

This study investigated the effect of exposure to Cd and the influence of chlorella intake on Cd administration. Small intestinal absorption of Cd is characterized by high accumulation within the intestinal mucosa and metal-binding proteins were induced in the presence of Cd. Cd-MT form is excreted into the feces with a cell turnover at the same time (Elsenhans *et al*., 1997). After Cd is absorbed from the small intestine into blood plasma, Cd is mainly taken up into the liver. The Cd-albumin is moved to the liver and Cd-MT form is moved

to the kidney (Wang *et al*., 1993). Chronic Cd exposure can cause renal proximal tubular dysfunction resulting from the release of liver Cd-MT and its accumulation and degradation in the renal tubular epithelial cells (Friberg *et al*., 1974). Therefore, renal Cd is made up of free ion form. The reason for this redistribution of Cd has been shown to be its binding to MT that it is readily filtered through the glomerular membrane and is obtained selectively in the renal tubules (Nordberg *et al*., 1975). Moreover, Cd ion induces new renal MT synthesis (Dorian *et al.,* 1992). When much amount of Cd-MT existed in the kidney, Cd-MT in blood was excreted into urine (Friberg, 1984). A damage of the proximal renal tubular cells increases urinary Cd excretion with low molecular weight proteins. Also, Cd increases bone Ca resorption and is accumulated in bone matrix instead of Ca. From the above explanation, Cd affects bone matrix formation and mineralization. Thus, it inhibits growth and maturation of bone tissue. (Wilson *et al*., 1996) During the experimental period, a decrease in body weight was noted in Cd-exposed groups. Consistent with these results, another study (Jemai *et al*., 2007) showed that body weight gain in the Cd-exposed rats was decreased significantly. Also, in numerous other studies (Cousins *et al*., 1973; Toraason & Foulkes, 1984), food intake and food efficiency were significantly depressed in Cd-exposed groups compared to Cd-free groups. In this study, food efficiency was influenced by 5% chlorella diet compared with 0% chlorella diet in rats administered Cd. Chlorella contains CGF (chlorella growth factor) which is water soluble S-nucleotide adenosyl peptide complex and a factor of rejuvenescence. In addition, it helps growth promotion of animal and plant, increase of immune system and improvement of apoplexy (Han *et al*., 2002). Thus chlorella might prevent growth inhibition of Cd because of CGF (chlorella growth factor) which is contained in chlorella. In our study, weight of the liver and kidney, which have been known to be responsible for Cd removal (Toraason & Foulkes, 1984) were decreased by Cd administration, however, these were not influenced by dietary chlorella level. The results of our experiments could be shown in tissue damage or decrease of body weight by Cd-administration, but that were not affected by chlorella groups. In addition, the weight of femur was not influenced by Cd and dietary chlorella. Femur was affected by exposure to Cd in a dose- and duration-dependent manner (Brzóska *et al*., 2005). Finally, rats fed 160 ppm Cd for 10 weeks did not induce intoxication in this study, where Cd concentration in small intestine was decreased in Cd- exposed chlorella groups. Also, fecal Cd excretion was remarkably increased in Cd-exposed chlorella groups compared to the Cd-free groups but there was no difference among the chlorella groups. Dietary fibers contained in chlorella cells may inhibit Cd absorption from the digestive tract by promoting its excretion into feces (Singh *et al*., 1999). Dietary fiber could trap Cd within the epithelial cells of the intestine and eventually excreted via feces with the esquamating cells (Andersen *et al*., 1992; Valberg *et al*., 1976). In addition, intestinal Cd and MT concentrations were lower than the liver and kidney by chlorella in this study. The other study reported that after rats were pretreated with Zn, animals once injected Cd. The result showed that intestinal Cd concentration was elevated after 4 h. but intestinal Cd concentration was decreased within 16~24 h. In consequence, the Cd-MT form in the small intestine was excreted into lumen after sequestering with intestinal lumen cell (Min *et al*., 1991). Considering these facts, the chlorella diet had the effect of Cd-removal by the inhibition of intestinal Cd absorption, due to dietary fiber contained in chlorella. Therefore, the Cd absorption rate and Cd retention ratio were decreased in chlorella diet groups. Cadmium concentrations of liver and kidney were increased in Cd-exposed groups compared to Cd-free groups in this study. In addition, the exposure to Cd increased its concentration in the blood. However, Cd concentrations in liver, kidney and blood were decreased in Cd-exposed chlorella groups. Hwang *et al.* (2006a) showed that hepatic and renal Cd concentrations were significantly decreased in chlorella supplementation groups. Thus, it was speculated that chlorella intake decreased Cd concentrations in liver, kidney and blood. In mammals, most of the total body burden of Cd is associated with MT. One molecule of MT can bind with 7 atoms of Zn or Cd. Metallothinein has been proposed to play important roles in the removal of heavy metals such as Cd and in the scavenging of oxygen-free radicals (Goering *et al*., 1995; Sato & Bremner, 1993). Cadmium in the cell is classified into two forms; MT-bound form and non-MT-bound form. Also, several studies reported that the MT-bound form is not toxic. However, the non-MT-bound form is having toxicity (Manuel *et al.*, 1992; Nordberg, 1984). A non-MT bound form is produced when MT is not synthesized yet or when Cd, more than the inducible MT amounts, is inserted. Therefore, Cd concentrations in tissues did not reflect in all Cd toxicity. Shim et al., showed that hepatic MT II was more expressed in the Cd-5C and Cd-10C groups than in the Cd-0C group (Shim *et al*., 2008). In other words, Cd concentration is associated with MT synthesis. In fact, the basal MT level in the liver and kidney plays an important role in removal of Cd toxicity. The amount of synthesized MT in the liver and kidney of Cd-administered groups was significantly higher than those of Cd-free groups in this study. Absorbed Cd in human body induced MT synthesis in accordance with Cd dosage and duration of Cd feeding (Park *et al*., 1994). Other studies showed that MT synthesis was increased in proportion to Cd accumulation in the body (Goering & Klaassen, 1984; Hwang *et al*., 2006b). Furthermore, it has been reported that Cd induces the upregulation of cytoprotective and metal-scavenging proteins such as MT (Thevenod, 2003; Thijssen *et al*., 2007). In addition, newly synthesized MT was not induced by chlorella intake in this study. Conversely, the chlorella diet was not effective in increasing the basal MT level when the Cd was taken up. Also, in the other study, 46 male SD rats were given dietary supplementation with 1%, 5%, and 10% dried chlorella and 40 ppm of Cd for 4 weeks. The result showed that total MT synthesis

in the liver and kidney was lower in the group of rats on the supplementation with chlorella and 40 ppm of Cd than the control group of rats on the supplementation without chlorella (Hwang *et al*., 2006a). This result is conflicted with the other study which reported that capacity of Cd-MT synthesis to remove Cd was increased by chlorella vulgaris (Friberg, 1984). Therefore, this may be due to an inhibition of Cd absorption by chlorella in the small intestine, resulting in declines in MT synthesis. In this study, urinary Cd excretions were increased in Cd exposed chlorella groups. Although the accurate mechanism related to this result was unknown. Based on the evidence that urinary and fecal Cd in Itai-Itai disease is excreted by chlorella intake (Hagino & Ichimura, 1975), it is thought that excretion of Cd through urine is one of the important ways of Cd removal. Thus, chlorella intake could be predicted elimination effect of Cd in human body. Urinary and serum creatinine concentrations in Cd-exposed groups were not changed compared to those in Cd-free groups in this study. However, these concentrations were slightly influenced by dietary chlorella. Renal glomerulus was not affected by Cd and dietary chlorella intake, and glomerular filtration rate (GFR) was not different among experimental groups. Therefore, it seems that Cd exposure did not exert severe renal toxicity. In summary, fecal and urinary excretions for Cd were remarkably increased in the Cd-exposed chlorella groups than in controls. Thus, Cd concentrations in tissues and blood were decreased in the Cd-exposed chlorella groups and thereby MT concentrations in organ tissues were reduced by chlorella supplementation in a dose-dependent manner. According to the results above, this study could suggest that chlorella inhibited Cd absorption, promoting the excretion of Cd from the body into feces. Also, it seems that small amount of MT was synthesized in this study due to decreased Cd absorption in the small intestine.

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