Bull Environ Contam Toxicol (2007) 78:485–488
DOI 10.1007/s00128-007-9098-6

Effect of Daidzein in Rats on Cadmium Excretion

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Received: 26 June 2006 / Accepted: 15 March 2007 / Published online: 31 May 2007

Cadmium (Cd) is a very toxic contaminant that has a long biological half-life in both humans and animals. This toxic metal can lead to itai-itai disease (Friberg et al., 1986), kidney tubular dysfunction (Friberg et al., 1984, 1986; Om et al., 2002) and cancer (Waalkers et al., 1999; Itokawa et al., 1978). Among these characteristic symptoms, Cd typically gives rise to renal dysfunction since the kidneys are a main route of excretion. Recently, there have been several reports on Cd exposure to humans in Korea (http://www.hani.co.kr). As a result, there has been increased public awareness and concern for cadmium exposure among the public.

There have been many studies on Cd detoxification and excretion by protein (Ryu and Kim, 1996), dietary fiber (Bae et al., 1997), and minerals such as calcium (Walter et al., 2000; Whelton et al., 1997), copper (Glover and Hogstrand, 2003; Reeves and Chaney, 2001), and zinc (Glover and Hogstrand, 2003; Reeves and Chaney, 2001) as well as vitamins (Brzoska et al., 2001; Kurata et al., 2001). Moreover, some natural products have been potentially used to inhibit heavy metal accumulation and intoxication by stimulating excretion from the body. Recently, green tea catechin has shown effects on reduction in heavy metal accumulation and intoxification by promoting excretion from the body (Choi et al., 2003). Like catechin, genistein has polyphenol rings that are believed to play a role in binding them to hydroxyl groups in its structure. Paik et al. (2003) had a study on the effect of genistein on female OVX rats treated with 50 ppm of Cd. It demonstrated that genistein has detoxification effects on inhibiting the absorption of Cd as well as increasing its excretion.

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Cd can act as an endocrine disruptor by altering the homeostatic balance of a variety of hormones (Takiguchi and Yoshihara, 2006). In particular, acute exposure of female rats to Cd can also decrease progesterone production, and this effect is dependent on the stage of the estrus cycle (Piasek and Laskey, 1994, 1999). More recent work has shown that Cd may have estrogenic properties (Hall et al., 2001) that share certain similar cellular pathways to estrogen. Furthermore, Cd can also alter the hormonal balance and may exert estrogenic effects via interaction with ERs. Accordingly, Pillet et al. (2006) suggest that females may be at a greater risk than males for Cd-induced immunomodulation.

Most studies on soy isoflavones are done with genistein. Genistein (5,7,4-trihydroxyisoflavone) and daidzein (7,4-dihydroxyisoflavone) have weak estrogen activity as phytoestrogens which have a similar structure to estrogen. It is believed that daidzein may bind with metal ions to form an insoluble complex-ionic salt used to remove heavy metals by a hydroxyl group and polyphenol (Joseph and Michael, 1999). However, few studies have been conducted with daidzein to examine its detoxification effect particularly using OVX rats.

Therefore, this study was undertaken to investigate the influence of daidzein on Cd excretion in 50 ppm of Cd-treated OVX rats. The data obtained may provide basal information about prevention of cadmium toxicity in the interest of public health.

Materials and Methods

Animal treatment

Female Wistar rats (25 days old) were purchased from Samtako Inc. (Osan, Korea) and hosed in a controlled

environment animal facility at 22 ± 2°C with a 12 hour light–dark cycle at Hanyang University, Seoul, Korea. All the rats received water and food ad libitum. After three days of acclimation, the rats were assigned to either the sham-operated (SH) group or three ovariectomized (OVX) groups. One week after survey, the OVX rats were randomly assigned to OVX, OVX-Cd without daidzein (OVX-Cd50), and OVX-Cd treated with daidzein at a daidzein concentration of 10 mg/100g of feed (OVX-Cd50-D). Experimental diets were prepared by mixing the powdered daidzein (90.1% pure, BioSpectrum, Yongin, Korea) with AIN-76 modified diet (Bifido, Seoul, Korea, American Institute of Nutrition, 1997). All diets were provided in powdered form. The rats in Cd-treated groups were given drinking water containing 50 ppm of Cd ($CdCl_2$, Sigma-Aldrich Inc.).

Rat growth

Body weights were monitored every three days for eight weeks. The composition of the diet is shown in Table 1.

Blood, urine and feces samples

Blood was collected by heart puncture, and the serum was centrifuged (3,000 rpm, 20 min at 4°C), aliquoted, and stored at -70°C. Urine and feces were collected in separators in the metabolic cages for 16 hours and stored at -20°C until they were analyzed.

Cd measurement

Feces were dried at 60 ± 10°C for 24 hours and then ashed at 550°C for 8 hours. The ashed samples were dissolved in 1 N HCl and diluted with 1% lanthanum oxide (Association of Official Analytical Chemists, 1984). The Cd concentrations were quantified with an atomic absorption spectrophotometer (Model 400, Perkin Elmer, Norwalk, CT, U.S.A.). The Cd concentrations in urine and blood were measured by the same method as that of the feces samples after using trichloroacetic acid solution to remove protein (Yeager et al., 1971; Zinterhofer et al., 1971).

Statistical analysis

Means and standard deviations (S.D.) of all variables were computed for each of the groups. Analysis of variance (ANOVA) was first performed on the means to determine whether there were significant differences ($p < 0.05$). When ANOVA indicated statistical significance, Duncan's multiple range test was used to determine which means were significantly different. SPSS (Chicago, IL) software was used for all statistical analyses.

Table 1 Composition of the soy protein free powdered semipurified diet [1]

Ingredient	g/kg Diet
Casein	200
Cornstarch	600
α-Cellulose	50
Corn oil	100
Vitamin mixture [a]	10
Mineral mixture [a]	35
DL-Methionine	3
Choline chloride	2
Total	1,000

[1] From BIFIDO (Seoul, Korea), containing: casein (Screma, Coudeville, France), cornstarch (Daesang, Seoul, Korea), cellulose (Sigma-Aldrich, Yongin, Korea), corn oil (Shindongbang, Seoul, Korea), vitamin mixture (Dyets Inc., Bethlehem, US), mineral mixture (Dyets Inc., Bethlehem, US), DL-methionine (Sigma-Aldrich, Yongin, Korea), and choline chloride (Sigma-Aldrich, Yongin, Korea)

[a] Prepared according to AIN-76 formulation (Dyets, Inc., Bethlehem, PA)

Results and Discussion

In the present study, we examined the effect of daidzein in Cd-treated OVX rats. The sex hormone estrogen is very important for sex development in both males and females. The characteristics of estrogen deficiency include disorder of sex development, hypertension, lack of pubertal development, and bone disorder. Recent data from rats show that Cd can act as an estrogen mimic in the whole animal, inducing conditions ranging from uterine hyperplasia to early onset of puberty (Safe, 2003). Cd has potent estrogen- and androgen-like activities in vivo and in vitro, by directly binding to estrogen and androgen receptors. However, the precise mechanisms underlying the effects of Cd as an endocrine disruptor remain to be elucidated.

Likewise, phytoestrogens such as genistein and daidzein can bind to estrogen (Kuiper and Xu, 1997) and other (Barnes and Peterson, 1995) receptors in vitro and can exert estrogenic effects in vivo (Levy et al., 1995; Santell et al., 1997). Phytoestrogens may act through diverse mechanisms, including inhibition of enzymes, increased synthesis of sex hormone binding globulin, and antioxidation. Phytoestrogens may competitively bind to an estrogen receptor with Cd. Hence, the inclusion of phytoestrogens in rodent diets might affect the outcome of bioassays designed to detect developmental toxicity or carcinogenity (Pillet et al., 2006).

Body weight gain had significantly ($P < 0.05$) increased by ovariectomy compared with the SH (Table 2). It is well known that estrogen is involved in the differentiation of the mesenchymal stromal cell. Estrogen deficiency after OVX

Table 2 Body weight gain[1]

Group[2]	Body weight gain (g for eight weeks)
SH	144.56 ± 6.0[a]
OVX	224.15 ± 9.5[b]
OVX + Cd 50	216.38 ± 5.6[b]
OVX + Cd50 + D	183.44 ± 8.3[c]

[1] Values are mean ± S.E. (n = 9)

[2] SH: sham, OVX: ovariectomy, OVX + Cd50: ovariectomy + Cd50 ppm, OVX + Cd 50 + D: ovariectomy + Cd50 ppm + daidzein (10ug/g b.w.)

[a,b,c] Values with different letters are significantly different among groups at $p < 0.05$ by Duncan's multiple range test

Table 3 Blood, urinary and fecal cadmium excretion of Cd-treated rats[1]

Group[2]	Blood Cd (ug/L)	Urinary Cd (ug/L)	Fecal Cd (ug/g)
SH	0.05 ± 0.01[a]	0.22 ± 0.02[a]	1.55 ± 0.21[a]
OVX	0.05 ± 0.01[a]	0.24 ± 0.02[a]	1.50 ± 0.37[a]
OVX + Cd 50	0.25 ± 0.12[b]	7.10 ± 1.30[b]	84.00 ± 23.60[b]
OVX + Cd50 + D	0.10 ± 0.04[b]	13.30 ± 0.66[c]	157.82 ± 44.33[c]

[1] Values are mean ± S.D. (n = 9)

[2] SH: sham, OVX: ovariectomy, OVX + Cd50: ovariectomy + Cd50 ppm, OVX + Cd 50 + D: ovariectomy + Cd50 ppm + daidzein (10ug/g b.w.)

[a,b,c] Values with different letters are significantly different among groups at $p < 0.05$ by Duncan's multiple range test

may result in body weight gain leading to stimulating accumulation of adipocytes (Grigoriadis et al., 1988). However, the group fed with daidzein shows that the body weight may be recovered to that in the SH group to a certain degree. Thus, more investigation is needed to examine this effect in a long-term experiment.

Blood Cd concentration is shown in Table 3. Rats in OVX-Cd-D had significantly ($p < 0.05$) lower blood Cd concentration than those in OVX-Cd. It showed that the blood Cd concentration may decreased by feeding daidzein. However, it is unclear whether absorption is inhibited and/or excretion is stimulated. Urinary and fecal excretion of Cd at the end of the experiment were significantly ($p < 0.05$) increased in the OVX-Cd-D group compared with OVX-Cd (Table 3). These findings indicate that daidzein may be involved in stimulating the excretion of Cd. Joseph and Michael (1999) demonstrated that daidzein may be a good ligand for ionizing metal due to electronic properties by the position of the hydroxyl group and ring system. This may be due to hydroxyl groups in daidzein that form insoluble compounds with Cd leading to an accelerated Cd excretion. Thus, daidzein has a high affinity to divalent metals such as Cd and may stimulate excretion of Cd. Kim et al. (1999) reported that hesperidin extracted from orange, a flavonoid that has phenolic rings in its structure, did not affect urinary excretion of Cd but it exceeded Cd excretion through feces. This data was similar to the current data.

In conclusion, daidzein may alleviate Cd toxicity in OVX rats by increasing excretion of Cd via urine and feces. Thus further study is needed to compare daidzein with genistein in regard to Cd excretion and detoxification. Furthermore, there is a necessity to examine these effects in a long-term study and elucidate the molecular mechanisms by which daidzein and genistein excrete Cd.

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