Original Article

Endocrine Disruption of Cadmium in Rats Using the OECD Enhanced TG 407 Test System*

WANG Heng Juan, LIU Zhao Ping#, JIA Xu Dong, CHEN Hao, and TAN Yan Jun

Key Laboratory of China National Center for Food Safety Risk Assessment, NHFPC, Beijing 100022, China

Abstract

Objective To evaluate the endocrine disrupting effects of cadmium (Cd) using OECD enhanced TG407 test guideline.

Methods Sprague-Dawley (SD) rats were randomly divided into six groups and accordingly administered with 0, 1, 2.5, 5, 10, 20 mg/kg·BW/day of Cd by gavage for 28 days. Body weight, food consumption, hematology, biochemistry, sex hormone levels, urinary β2-microglobulin, organ weights and histopathology and estrous cycle were detected.

Results Cd could significantly decrease animals' body weight (P<0.05). Serum luteinizing hormone (LH) at 10-20 mg/kg·BW groups and testosterone (T) at 2.5 and 10 mg/kg·BW groups decreased significantly (P<0.05). However, no statistically significant change was found in urinary β2-microglobulin among Cd-treatment groups (P>0.05). Endpoints related to female reproduction including uterus weight and histopathological change at 10-20 mg/kg·BW groups showed significant increase (P<0.05). While among male rats in 2.5, 10, 20 mg/kg·BW groups, weight of prostate, thyroids, and seminal vesicle glands significantly decreased (P<0.05). Moreover, no histopathological change was observed in kidney.

Conclusion Results suggested that Cd can cause endocrine disrupting effects in SD rats. Comparing with possible renal toxicity of Cd, its toxicity on endocrine system was more sensitive.

Key words: OECD Enhanced Test Guideline 407; Endocrine Disrupting; Cadmium; Renal toxicity

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INTRODUCTION

Cadmium is one of the vital industrial and environmental pollutants, ranked as the sixth toxic substances of human health hazards by Agency for Toxic Substances and Disease Registry (ATSDR). In nature, cadmium can exist in both inorganic and organic forms and each form possesses varied toxicity. Inorganic cadmium salts, such as cadmium nitrate and cadmium chloride, are proved more harmful to animals, plants and humans due to their easy solubility in water[1]. Most people are exposed to cadmium through non-occupational situations, such as drinking water, consuming food, and smoking tobacco[2-3].

Researchers have begun to pay attention to the reproductive toxicity of cadmium since 1950s. Available research data indicated that cadmium was toxic to mammal reproductive system, including structural and function-degenerative changes in testis, epididymis, seminal vesicle, ovarian follicle development, ovulation, ovum transport, and fertilization process[4-12]. It seems that the fact that cadmium can exert endocrine disruption on animals is out of doubt. So far, the endocrine disruption of cadmium has been extensively studied; and the

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#Correspondence should be addressed to LIU Zhao Ping, E-mail: liuzhaoping2010@163.com

Biographical note of the first author: WANG Heng Juan, born in 1986, Master, majoring in food hygiene and nutrition.

results suggested that its mechanism involved in estrogen and androgen receptors binding and activation[13-15]. But most studies were conducted on parenteral exposure routes or used estrogen deficiency animal models, such as immature or ovariectomized rats. In these studies, cadmium was administered to animals by non-oral exposure, such as intraperitoneal or subcutaneous injection. Great caution should be exercised when extrapolating results from above animal models and dosing approach to normal exposure scenarios in nature. However, few studies follow the OECD enhanced TG407 test system to evaluate endocrine disrupting effect of cadmium exposed orally in intact young rats.

OECD initiated a high-priority activity in 1998 to revise existing guidelines and to develop new guidelines for the screening and testing of potential endocrine disruptors. One element of the activity was to update the existing OECD guideline for 'repeated dose 28-day oral toxicity study in rodents' (TG 407) by parameters suitable to detect endocrine activity of test substances. The rationale for updating the OECD TG 407 with a number of endocrine and reproductive end points is to serve as an *in vivo* method providing data about multiple endocrine mechanisms and effects. This update procedure underwent an extensive international program to test for the relevance and practicability of the additional parameters, the performance of these parameters for chemicals with (anti) oestrogenic, (anti) androgenic, and (anti) thyroid activity, the intra- and interlaboratory reproducibility, and the interference of the new parameters with those required by the prior TG 407[16]. Currently, the OECD enhanced TG407 is contained in level 4 under the OECD Conceptual Framework for the testing and assessment of endorine disruting chemicals[17].

Currently, most attention is paid to renal toxicity and other general organs or tissues toxicity like bone, lung etc. Even the latest provisional tolerable monthly intake (PTMI) for cadmium was set according to parameters reflecting renal toxicity by the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Expert Committee on Food Additives (JECFA)[18]. As researches comparing endocrine disrupting effect and renal toxicity of cadmium to intact young animals or human are almost unavailable, it is no strange that almost no consideration was given to the effect of endocrine disrupting when setting a PTMI for cadmium. So the two main objectives of the present study were: 1) to detect endocrine disrupting effect of cadmium using OECD enhanced test guideline 407; 2) to compare sensitivity of endocrine disruption and renal toxicity in the same testing system based on the preliminarily researched endpoints.

MATERIALS AND METHODS

Chemicals Tested

Cadmium chloride ($CdCl_2$, purity >99%, CAS No. 10108-64-2) was purchased from Beijing Bailingwei Science and Technology Limited Company. The dosing formulations were prepared by dissolving the test substance in double distilled water. Dosing formulations were stored in room temperature.

Animals, Housing, Diet, and Water

This study was performed according to standard operating procedures which were previously accepted and periodically inspected by Organization for Economic Co-operation and Development (OECD). Male and female Sprague-Dawley (SD) rats were purchased from Vital River Laboratories (Beijing China). The rats aged six weeks old were acclimatized to laboratory conditions for 7 d before treatment. After acclimation, rats were weighed ranging from 212 to 230 g for male rats and 167 to 183 g for female rats. Then they were randomly divided into six groups based on their weights, with 8 female and 8 male animals in each group. The laboratory conditions in the animal room were controlled and monitored by an automatic system with temperature of (22±2) °C, relative humidity of approximately 55%, a 12-h light/dark cycle, and air exchange rate of 15 times per hour. Certified rodent pelleted and irradiated diet and filtered and softened tap water from the municipal water supply were available ad libitum.

Experimental Design

The study design followed the enhanced OECD test guide line 407. Briefly, 5 groups of rats received $CdCl_2$ at 1, 2.5, 5, 10, or 20 mg/kg·BW (calculated as Cd) by gavage once daily, while rats in the left group were administered with double distilled water as a control. The exposure duration was lasted for 28 d. Rats were housed in the metabolism cage for 24 h and urine was collected to detect urinary β2-microglobulin with the method of radioimmunoassay and urinary creatinine (CRE) with the picric acid method on the day 28. Animals were

not fasted prior to sacrifice. All animals were sacrificed by exsanguination under pentobarbital anesthesia after gavage was finished. Blood samples were obtained from the abdominal aorta immediately prior to necropsy for hematology, clinical chemistry, and hormonal analysis. Liver, kidney, ovary etc. were collected for further histopathological observations.

General Observation

Clinical signs were recorded at least once daily for all animals. Detailed physical examinations were performed at least once per week during the treatment period. All clinical impairments were recorded in detail including: nature, the onset, severity, reversibility, and duration.

Body Weight and Food Consumption

Individual body weight and food consumption were measured at weekly intervals throughout the treatment period. In addition, body weight was recorded prior to sacrifice.

Estrous Cycle Staging

At the 4th week of the study, the stage of the estrous cycle of all females was determined daily between 8:00 AM and 11:00 AM by vaginal smears for at least 5 consecutive days. The specific method is wetting the medical sterile cotton swab with saline firstly, inserting it into animals' vagina and rotating gently, then taking out the swab with vagina inclusions and smearing equally on a glass slide with a drop of saline, lastly observing the morphology of vaginal shedding cells at low magnification to determine the stage of estrous cycle.

Clinical Pathology and Hormone Analysis

Standard hematological and clinical chemistry determinations were used to determine the hematological parameters, enzymes, electrolytes, substrates, and products of metabolism. The levels of LH, T, follicle-stimulating hormone (FSH), estradiol (E2), progestogen (P), 5, 3′-triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH) were measured by radioimmunoassay.

Postmortem Examinations and Organ Weights

All animals were subjected to necropsy after study on day 29 and 30. The necropsy included examination of all major organs, tissues, and body cavities. Gross lesions were recorded and sampled along with all major organs and tissues. Adrenal gland, epididymis, kidney, liver, heart, brain, ovary, pituitary gland, prostate gland, seminal vesicle (with coagulating gland), testis, thyroid gland (with parathyroid gland), uterus (including cervix), spleen, and thymus were weighed freshly. Paired organs were weighed together.

Histopathology

The following organs and tissues were fixed by immersion in neutral buffered 10% formalin and examined after hematoxylin-eosinstaining: adrenal gland, brain, heart, kidney, liver, mammary gland, ovary, parathyroid gland, pituitary gland, prostate gland, seminal vesicle with coagulating gland, spleen, thymus, thyroid gland, uterus, vagina, testis, and epididymis. In addition, part of the testis, ovary, uterus, and kidney in the highest dose group and control group were fixed by immersion in glutaraldehyde after cut into 1 mm^3 blocks for electron microscopic examination.

Statistics

Means and standard deviations (STD) were calculated according to gender and dosage respectively. The single factor variance analysis (ANOVA) was used to compare the means among groups. If one-way ANOVA result was significant ($P \leq 0.5$), each treated group was compared to the control group using Dunnett-*t* test.

RESULTS

General Observation, Body Weight, and Food Consumption

No deaths were observed during the study. Rats in 5, 10, 20 mg/kg·BW dose groups showed treatment-related clinical signs, which consisted of listlessness, anorexia, low hair-gloss, irritation, and their severity showed a dose-dependent trend. Female animals in all cadmium-treatment groups showed normal estrous cycle.

Terminal body weight and total food consumption of female rats in 2.5, 5, 10, 20 mg/kg·BW dose groups and male rats in 5, 10, 20 mg/kg·BW dose groups were significantly lower than those in control group ($P<0.05$). Furthermore, female food utilization in 20 mg/kg·BW dose group was significantly lower than that in control group ($P<0.01$). (Table 1 and Table 2).

Organ Weights

There were no significant gross changes observed related to cadmium treatments. Comparing with control group, absolute uterus weight and relative uterus weight in 10 and 20 mg/kg·BW dose groups increased significantly (P<0.05) (Table 1). Besides, no significant changes were observed for the weight of other organs, including kidney (P>0.05).

The relative weight of testis and adrenal of male rats in 20 mg/kg·BW dose group was significantly higher than that in control group (P<0.05). Thyroids absolute weight of male rats in 2.5, 5, 10, and 20 mg/kg·BW dose groups and their relative weight of male rats in 2.5 and 10 mg/kg·BW dose groups were significantly lower than that in control group (P<0.05). And compared to control group, male rats in 2.5, 10, and 20 mg/kg·BW dose groups had significantly lower prostate absolute and relative weight (P<0.05) (Table 2). No other significant changes of organ weights, including kidney were observed compared with control males (P>0.05).

Changes in Hormone Concentration

There were no significant changes in serum hormone concentrations in cadmium-treated female rats. However, in male rats, serum LH and T in 10 and 20 mg/kg·BW dose groups were significantly lower than those in control group (P<0.05) (Table 3).

Table 1. Effect of Cd Treatment on Body Weights and Organ Weights in Female Rats

Items	Control (n=8)	1 mg/kg·BW (n=8)	2.5 mg/kg·BW (n=8)	5 mg/kg·BW (n=8)	10 mg/kg·BW (n=8)	20 mg/kg·BW (n=8)
Body Weight						
Initial	176±7.4	174.3±3.8	170.8±2.8	172.8±4.8	174.1±6.7	173.6±6.1
Terminal	260±14.4	258.6±6.8	242±8.6*	244±10.3*	231.1±11.5**	214.6±7.7**
Total Food Consumption	419.9±9.7	415.1±47.0	377.3±8.4**	386.9±5.5*	363.2±15.6**	348.6±7.8**
Total Food Utilization	18.95±2.36	20.52±1.68	17.77±1.62	18.61±1.86	17.34±2.09	11.51±0.63**
Uterus						
Absolute (g)	0.34±0.08	0.34±0.11	0.33±0.03	0.42±0.17	0.48±0.09*	0.50±0.24*
Relative (%)	0.14±0.05	0.14±0.09	0.14±0.02	0.17±0.08	0.21±0.05*	0.23±0.12*
Ovary						
Absolute (g)	0.11±0.03	0.11±0.03	0.09±0.01	0.11±0.02	0.12±0.02	0.12±0.02
Relative (%)	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.05±0.01	0.05±0.01
Pituitary						
Absolute (mg)	14.53±2.96	14.32±2.86	14.01±1.46	12.69±3.07	13.40±2.06	12.63±1.82
Relative (mg%)	5.97±1.38	5.63±1.10	5.84±0.46	5.19±1.25	5.57±0.56	5.72±0.82
Adrenal gland						
Absolute (mg)	65.19±10.39	59.38±6.19	57.18±3.00	61.01±5.88	74.69±8.05	62.91±5.54
Relative (mg%)	26.73±4.94	23.31±1.98	23.85±1.36	25.04±2.75	31.22±3.29	28.62±3.73
Thyroid						
Absolute (mg)	21.98±3.84	21.60±3.21	25.40±4.26	19.80±2.56	24.16±3.51	17.97±3.08
Relative (mg%)	9.05±2.00	8.48±1.15	10.63±1.96	8.14±1.22	10.14±1.74	8.16±1.55
Kidney						
Absolute (g)	1.68±0.14	1.73±0.24	1.64±0.14	1.65±0.08	1.71±0.13	1.64±0.10
Relative (%)	0.69±0.06	0.68±0.10	0.68±0.06	0.68±0.03	0.71±0.05	0.75±0.05
Liver						
Absolute (g)	9.12±0.89	10.86±4.26	9.42±1.12	10.15±1.00	9.99±1.67	9.77±1.94
Relative (%)	3.72±0.19	4.26±1.63	3.91±0.23	4.14±0.18	4.15±0.45	4.39±0.6

Note. Means and standard deviations are given. Statistical results indicate significance compared with the corresponding control group or subgroup: $^{*}P$<0.05; $^{**}P$<0.01.

Table 2. Effect of Cd Treatment on Body Weights and Organ Weights in Male Rats

Items	Control (*n*=8)	1 mg/kg·BW (*n*=8)	2.5 mg/kg·BW (*n*=8)	5 mg/kg·BW (*n*=8)	10 mg/kg·BW (*n*=8)	20 mg/kg·BW (*n*=8)
Body Weight						
Initial	223.4±5.1	220.5±8.6	220.5±6.8	220.6±6.5	224.1±6.1	224.4±4.1
Terminal	393.6±15.9	389.8±25.5	374.8±13.2	357.5±16.2*	348.2±12.8**	317.5±20.4**
Total Food Consumption	576.83±9.63	589.17±20.57	556.41±15.05	514.83±20.36**	431.41±20.94**	389.58±56.62**
Total Food Utilization	28.59±1.02	28.77±0.65	28.08±0.43	27.03±1.76	28.53±1.38	23.06±3.57
Testis						
Absolute (g)	3.21±0.29	3.12±0.37	3.24±0.23	3.15±0.27	3.08±0.22	3.00±0.36
Relative (%)	0.87±0.07	0.83±0.11	0.89±0.07	0.93±0.10	0.91±0.05	1.00±0.15*
Epididymis						
Absolute (g)	0.95±0.07	0.91±0.07	0.91±0.08	0.92±0.04	0.88±0.09	0.87±0.08
Relative (%)	0.26±0.02	0.24±0.02	0.25±0.03	0.27±0.03	0.26±0.02	0.29±0.02
Prostate and seminal vesicles						
Absolute (g)	2.47±0.28	2.21±0.29	1.98±0.21*	2.10±0.28	1.88±0.20*	1.63±0.38**
Relative (%)	0.67±0.09	0.58±0.05	0.55±0.05*	0.62±0.09	0.56±0.06*	0.54±0.10*
Pituitary						
Absolute (mg)	10.80±1.91	11.36±1.03	12.41±2.99	11.13±1.26	10.33±0.88	10.04±1.61
Relative (mg%)	2.91±0.53	3.01±0.34	3.44±0.94	3.27±0.30	3.08±0.33	3.31±0.35
Adrenal						
Absolute (mg)	55.61±7.36	55.03±12.03	56.99±5.30	58.73±12.92	60.33±6.49	59.80±13.43
Relative (mg%)	14.98±1.90	14.54±3.23	15.72±1.28	17.25±3.61	18.01±2.52	20.34±3.79**
Thyroid						
Absolute (mg)	28.62±5.56	27.36±4.11	18.93±1.35**	23.88±3.31*	20.51±2.33**	21.24±2.65**
Relative (mg%)	7.69±1.31	7.23±0.98	5.23±0.41**	7.04±1.08	6.11±0.75*	7.02±0.61
Kidney						
Absolute (g)	2.52±0.18	2.43±0.58	2.49±0.18	2.27±0.19	2.31±0.19	2.25±0.16
Relative (%)	0.68±0.04	0.64±0.14	0.69±0.03	0.67±0.09	0.69±0.07	0.75±0.03
Liver						
Absolute (g)	13.97±1.18	14.27±1.38	12.42±0.95	13.04±1.39	13.24±0.7	12.77±2.20
Relative (%)	3.76±0.23	3.77±0.26	3.43±0.18	3.83±0.31	3.94±0.13	4.21±0.48

Note. Means and standard deviations are given. Statistical results indicate significance compared with the corresponding control group or subgroup: $^{*}P<0.05$; $^{**}P<0.01$.

Table 3. Effect of Cd on Serum Sex Hormone and Thyroid Hormone Level in Enhanced TG 407 Test

Gender	Dose (mg/kg·BW)	*N*	T3 (ng/mL)	T4 (ng/mL)	TSH (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)	E2 (pg/mL)	P (ng/mL)	T (ng/mL)
F	0	8	2.19±0.19	76.90±6.84	1.88±0.57	3.96±0.39	4.21±1.31	44.52±17.53	6.19±1.84	-
	1	8	2.13±0.19	80.77±14.61	2.50±0.97	4.09±0.87	3.88±0.89	50.79±10.51	6.13±3.14	-
	2.5	8	2.01±0.05	79.12±9.61	1.71±1.54	4.58±1.11	3.70±0.88	49.69±15.00	7.44±2.77	-
	5	8	2.04±0.15	80.24±17.36	1.61±1.25	3.85±0.77	3.71±1.09	31.55±6.59	10.13±5.86	-
	10	8	1.90±0.19	79.68±13.83	1.59±1.50	4.59±0.83	3.99±0.36	36.39±9.40	6.37±3.74	-
	20	8	1.90±0.26	76.81±24.48	1.74±1.70	4.45±1.37	3.98±0.98	58.66±15.76	4.63±3.19	-
M	0	8	1.67±0.19	67.01±16.28	0.39±0.30	5.03±0.86	4.92±1.12	-	-	2.56±1.27
	1	8	1.75±0.19	74.13±8.57	0.47±0.32	5.36±1.28	4.99±0.90	-	-	1.85±1.07
	2.5	8	1.68±0.16	67.29±18.03	0.57±0.17	5.16±0.55	4.22±0.65	-	-	1.86±0.67
	5	8	1.56±0.13	61.69±10.15	0.50±0.05	5.51±0.88	4.20±0.44	-	-	1.73±0.90
	10	8	1.52±0.25	63.24±14.74	0.63±0.49	4.96±0.52	3.29±0.79*	-	-	1.10±0.54*
	20	8	2.78±1.55	79.63±15.39	0.78±0.39	4.80±0.48	3.05±1.18**	-	-	0.89±0.26**

Note. Means and standard deviations are given. Statistical results indicate significance compared with the corresponding control group or subgroup: $^{*}P<0.05$; $^{**}P<0.01$.

Hematology and Clinical Biochemistry

Hematology results showed that red blood cell (RBC) and hemoglobin (HGB) in 10 and 20 mg/kg·BW male rats groups were significantly lower than those in control group (P<0.05). Platelets (PLT) in 20 mg/kg·BW female group and 5-20 mg/kg·BW male groups were significantly higher than those in control group (P<0.05) (Table 4). Clinical chemistry results revealed that serum alanine aminotransferase (ALT) in 5, 10, and 20 mg/kg·BW dose groups female rats was significantly higher than that in control group (P<0.05), while albumin (ALB) and total protein (TP) were significantly lower (P<0.05). Male rats in 2.5, 5, 10, and 20 mg/kg·BW dose groups as well as male rats in 10 and 20 mg/kg·BW dose groups showed higher serum ALT than those in control (P<0.05). Male rats in 20 mg/kg·BW dose group also showed significantly higher glucose (GLU), alkaline phosphatase (ALP) and triglycerides (TG) and lower serum aspartate aminotransferas (AST) (P<0.05), comparing with those in control group (P<0.05). However, no statistically significant changes were observed for CRE and blood urea nitrogen (BUN) (Table 5).

Table 4. Effect of Cd on Hematology in Enhanced TG407 Test

Gender	Dose (mg/kg·BW)	*N*	WBC ($\times10^9$/L)	RBC ($\times10^{12}$/L)	HGB (g/L)	PLT ($\times10^9$/L)
F	0	8	7.29±1.61	6.97±0.31	142.50±5.01	302.13±71.06
	1	8	6.45±1.95	6.79±0.31	140.75±4.77	304.50±60.77
	2.5	8	7.67±1.68	7.00±0.46	149.50±8.73	298.50±52.06
	5	8	7.36±1.99	6.76±0.20	146.00±2.92	254.25±35.51
	10	8	9.50±2.20	7.11±0.52	146.50±11.98	375.25±65.36
	20	8	9.97±2.82	7.09±0.36	141.00±7.62	521.37±166.79*
M	0	8	9.91±2.06	7.16±0.29	147.75±8.08	461.25±142.85
	1	8	10.86±1.81	7.04±0.26	146.12±6.49	455.75±136.74
	2.5	8	11.21±2.30	7.10±0.39	144.88±11.75	740.88±394.21
	5	8	12.12±3.13	7.15±0.39	140.87±9.61	1067.63±421.17**
	10	8	12.93±1.64	6.72±0.43*	132.00±10.95*	1351.75±468.37**
	20	8	14.81±3.05*	6.32±0.27**	123.10±24.91**	1675.12±219.13**

Note. Means and standard deviations are given. Statistical results indicate significance compared with the corresponding control group or subgroup: $^{*}P$<0.05; $^{**}P$<0.01.

Table 5. Effect of Cd on Clinical Biochemistry in Enhanced TG407 Test

Gender	Dose (mg/kg·BW)	*N*	ALT (U/L)	AST (U/L)	TP (g/L)	ALB (g/L)	GLU (mmol/L)	BUN (mmol/L)	CRE (μmol/L)	CHO (mmol/L)	TG (mmol/L)
F	0	8	23.75±3.53	158.25±20.78	72.88±3.63	48.56±1.71	4.26±0.52	5.77±0.55	58.03±14.87	1.68±0.39	0.33±0.07
	1	8	27.75±4.68	165.25±21.75	68.71±3.60	46.48±1.97	4.81±0.49	5.36±0.89	57.61±3.17	1.93±0.48	0.37±0.08
	2.5	8	29.38±3.77	158.13±13.36	68.23±4.94	46.59±2.80	4.36±0.34	5.59±1.06	54.1±16.40	1.71±0.19	0.30±0.05
	5	8	39.13±6.94*	181.00±18.01	64.16±4.28**	44.41±2.35**	4.19±0.51	5.04±0.58	57.55±6.66	1.82±0.31	0.40±0.06
	10	8	42.75±8.58**	180.63±26.35	60.69±4.32**	43.31±2.06**	4.49±1.14	4.95±0.74	59.58±8.38	1.68±0.32	0.41±0.03
	20	8	69.25±12.96**	181.75±30.84	61.63±2.62**	44.10±1.55**	4.64±0.55	5.13±1.14	53.54±6.79	1.69±0.35	0.39±0.04
M	0	8	28.63±3.02	184.88±27.73	62.74±2.86	42.31±1.28	4.69±0.48	5.98±0.70	55.83±6.38	1.47±0.25	0.50±0.18
	1	8	35.88±3.91	182.50±16.52	62.06±2.32	42.06±1.67	4.45±0.67	5.57±0.72	54.59±5.69	1.52±0.30	0.49±0.15
	2.5	8	36.88±6.81*	190.00±34.04	62.61±3.52	43.15±1.63	4.92±0.62	5.63±0.50	60.94±7.31	1.28±0.29	0.50±0.13
	5	8	48.75±7.27**	187.63±26.94	60.16±3.08	42.46±1.98	4.43±0.42	5.65±0.85	60.06±3.64	1.55±0.25	0.38±0.08
	10	8	53.25±5.75**	154.88±31.26	64.83±6.93	43.64±2.11	5.74±1.43	5.33±1.49	58.19±7.94	1.23±0.35	0.98±0.57*
	20	8	44.25±7.80**	133.00±22.53*	66.88±2.99	43.40±1.39	6.09±0.87*	5.85±1.04	55.51±6.43	1.20±0.49	1.21±0.10**

Note. Means and standard deviations are given. Statistical results indicate significance compared with the corresponding control group or subgroup: $^{*}P$<0.05; $^{**}P$<0.01.

Urinary β2-microglobulin Level

Compared with those in control group, cadmium-treatment groups showed no significant changes in urinary β2-microglobulin level (P>0.05). (Figures 1 and 2).

Histopathology

Treatment-related changes were only confined to uterus. Uterus cavity expansion, endometrial epithelial cell and/or interstitial cell hyperplasia were found in rats treated with 5, 10, and 20 mg/kg·BW cadmium; and the changes showed dose-dependent relationship. Furthermore, uterus endometrial cells in cadmium-treatment groups were manifested with more mitochondria, rough endoplasmic reticulum and ribosome than in control group in electron microscopic examination (Figure 3). No significant changes were observed in ovary, testis, kidney, and other organs.

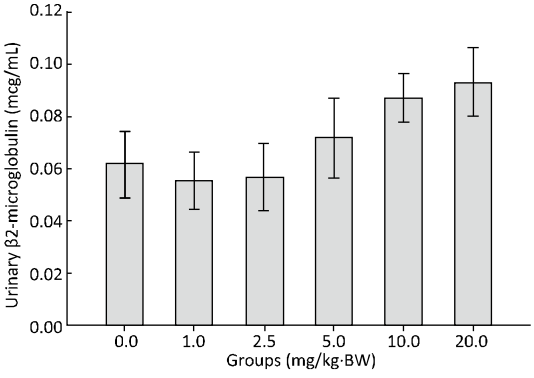

Figure 1. Effect of Cd on urinary β2-microglobulin level in female rats.

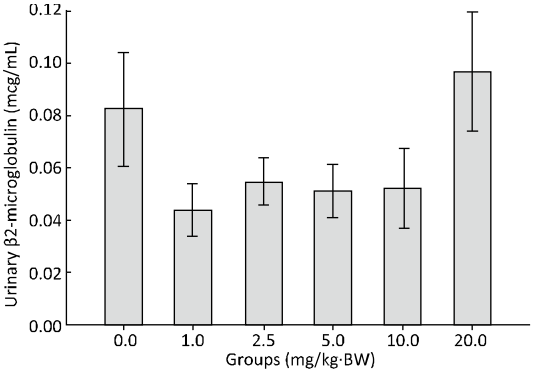

Figure 2. Effect of Cd on urinary β2-microglobulin level in male rats.

DISSCUSION

Several available animal studies and epidemiological studies showed that cadmium has endocrine disrupting effect[4,8,19]. Besides, recent experimental studies also found that cadmium has uterotrophic effect[15,20-21]. In the present experiment, the results indicated that oral exposure to cadmium could increase the absolute and relative weight of uterus, starting at dose 10 mg/kg·BW/day. At the low dose of 5 mg/kg·BW/day, uterus histopathological changes of cadmium were observed including endometrial epithelial, interstitial cell hyperplasia, and increased intracellular organelles. However, the estrogen-like effects observed in our oral-exposure sub-acute toxicity study may be less sensitive than that in premature rat or ovariectomized adult rat models by subcutaneous or intraperitoneal injection. At least two reasons involved: firstly, absorption rate of cadmium by oral exposure is lower than by subcutaneous or intraperitoneal injection; secondly, the hypothalamus-pituitary-gonadal axis feedback mechanism of complete adult rats make a part of compensation on the endocrine disrupting effects[22].

This experiment showed that cadmium could decrease the serum T and LH levels of male rats at

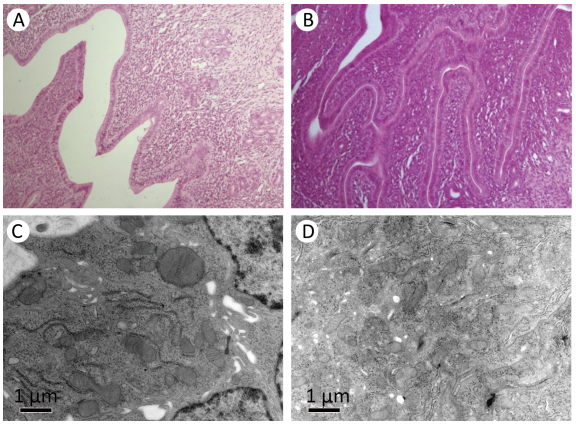

Figure 3. Effect of cadmium on rat uterus. Comparing with normal uterus endometrial epithelial cell (A, H.E.100×), uterus in treatment group showed endometrial epithelial cell hyperplasia, tall columnar, pseudostratified, Leydig cell hyperplasia (B, H.E.100×). Comparing with normal uterus (C), uterus endometrial cells in treatment group were manifested with rich mitochondria, rough endoplasmic reticulum and ribosome (D).

10-20 mg/kg·BW groups. In male rats, T is mainly synthesized by the Leydig cells, while LH has important regulatory role in this process. LH, a kind of gonadotropin secreted by pituitary, can induce cholesterol changed into T by activating Leydig cells[23-24]. Decreased LH level may be caused by the inhibition of cadmium on pituitary, which resulted in impaired regulatory function on Leydig cells. Thus, serum T level was accordingly decreased due to reduced synthesis of Leydig cells. Furthermore, exposure to cadmium may also have direct adverse effect on testis function so that the T synthesis was reduced[25]. Changes of serum T and LH in this study were similar to those caused by 17β-ethynyl estradiol, which may indicate the oestrogenic effect of cadmium. However, study results of cadmium exposure on sex hormones were not consistent. For example, cadmium can inhibit T secretion of testicular cells both *in vivo* and *vitro*[19]; high cadmium intake by smoking can reduce plasma T and LH levels[26]; chronic cadmium exposure did not change rat plasma FSH and LH level, but it could decrease T level at the third and the sixth week of the study[25]; low dose exposure of cadmium can rapidly reduce serum T level of rats[27]. In view of above studies, exposure routes, doses, duration of exposure, and experimental animal species all might play a role in such discrepancy.

It was reported that, compared with testis, male accessory sex glands might be less sensitive to cadmium exposure[26]. However, in the present study, weights of prostate and of seminal vesicle were significantly decreased in 2.5, 10, 20 mg/kg·BW dose groups, while no significant changes were observed in testis. Some other studies also find the similar results as well as ultra-structural changes of seminal vesicle and pathological changes of prostate in rats[13,27]. These effects might be caused by joint action by several mechanisms consisting of: 1) testis impairment leading to T synthesis decline[28]; 2) T decline resulting in reduction of androgen receptor expression on accessory sex organs[29]; 3) activation of estrogen receptors on accessory sex organs[30].

Besides endocrine disrupting effect of cadmium in our enhanced TG 407 test, adverse effects in other body systems related to cadmium were also observed in the present study. Effects of cadmium on body weight, RBC, HCG, serum AST, and serum TP were consistent to those findings obtained by Fatma M. et al.[31].

To back up the facts of kidney toxicity caused by cadmium, urinary protein content such as CRE and BUN levels, urinary β2-microglobulin level, kidney weight, and kidney histopathology are special parameters to corroborate the results[31-32]. We found that cadmium exposure under current conditions of this study did not change CRE and BUN levels, urinary β2-microglobulin concentration, kidney weight, and kidney histopathology. Although, it is generally recognized that kidney is the primary target organ of cadmium exposure with the main lesion including tubular swelling, degeneration, necrosis, cast of proximal tubule epithelial cell, dilatation of the distal convoluted tubule, and even glomerular damage when serious kidney lesion is caused[33-35]. As cadmium accumulates primarily in the kidneys, and its biological half-life in humans is 10-35 years, long-term exposure to cadmium even with low level can lead to substantive accumulation of cadmium in kidney and result in kidney lesion[35]. However, no adverse effect on kidney was found in this study likely due to the short exposure period, relatively low exposure dose as well as the exposure way.

JECFA recently established a provisional tolerable monthly intake for cadmium of 25 μg/kg·BW according to relevant urinary biomarkers, such as urinary β2-microglobulin and CRE level[18]. There are some evidences suggesting that endocrine system is more sensitive to cadmium exposure, if it is true, then attention should be paid when establishing a provisional tolerable monthly intake for cadmium in future. The present study provided some clues about which kind of system is more sensitive to oral cadmium sub-acute exposure, endocrine system or urinary system. Treatment with $CdCl_2$ by gavage for 28 d did not lead to any special changes in urinary β2-microglobulin concentration, serum CRE and BUN levels, kidney weight and kidney histopathology, which meant cadmium might not raise adverse effects on kidney under this experimental condition. However, after oral exposure to 5, 10, 20 mg /kg·BW of cadmium, female rats presented significant uterotrophic effects and histopathology changes, and male rats showed serum sex hormone levels affected together with prostate plus seminal vesicle weight decreased. These findings demonstrated that, under this experimental condition, cadmium could lead to obvious endocrine disruption rather than renal toxicity, which suggests the endocrine system, is more sensitive to cadmium exposure by oral for 28 d in rats than kidney. At present, a few studies also compared sensitivity of endocrine disruption and

renal toxicity of cadmium. Some showed that gonad toxicity of cadmium was more sensitive than renal toxicity (acute or sub-acute toxicity) by intraperitoneal or subcutaneous injection[36-37], while other reported that kidney is the most sensitive target organ to cadmium exposure[38]. Given that human exposure occurs mainly from consumption of contaminated food and drinking water, active and passive inhalation of tobacco smoke and inhalation by workers in the non-ferrous metal industry. For most populations, consumption of contaminated food and drinking water is the primary route of exposure to cadmium. Thus, exposure by oral is the most necessary direction for research. However, based on current available literatures, there is almost no research designed to use the method of oral exposure. So, comparing with above mentioned researches, the significant different results we got in this study may indicate that different exposure routes, animal species and ages, exposure dose, and exposure period etc. can result in different levels of adverse effects of cadmium on endocrine system and urinary system.

Sub-acute exposed to cadmium by oral may cause endocrine disruption in young animals, which is more sensitive than renal toxicity. This conclusion needs to further verify since only a few similar studies available. Potentially, as young children is more sensitive to cadmium exposure and short term exposure to cadmium may cause endocrine disruption, endocrine disruption of cadmium can be taken into consideration when set a provisional tolerable monthly intake for cadmium in future.

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