Letter to the Editor

Benchmark Dose Estimation for Cadmium-Induced Renal Effects Based on a Large Sample Population from Five Chinese Provinces*

KE Shen[1,‡], KE Qin Mei[2,‡], JIA Wen Jing[1], CHENG Xi Yu[1,#], LI Hao[1], ZHANG Jie Ying[1], LUO Hui Fang[1], HE Jin Sheng[1], and CHEN Zhi Nan[1,#]

A survey involving 6103 participants from five Chinese provinces was conducted to evaluate the threshold value of urinary cadmium (UCd) for renal dysfunction as benchmark dose low (BMDL). The urinary N-acetyl-β-D-glucosaminidase (UNAG) was chosen as an effect biomarker. The UCd BMDLs for UNAG ranged from 2.18 μg/g creatinine (cr) to 4.26 μg/g cr in the populations of different provinces. The selection of the sample population and area affect the evaluation of the BMDL. The reference level of UCd for renal effects was further evaluated based on the data of all 6103 subjects. With benchmark responses (BMR) of 10%/5%, the overall UCd BMDLs for males in the total population were 3.73/2.08 μg/g cr. The BMD was slightly lower in females, thereby indicating that females may be relatively more sensitive to Cd exposure than are males.

Cadmium (Cd) is a non-essential element commonly used in different industrial processes; however, the popular use of this element has resulted in Cd contamination in soil, water, and air[1]. With a half-life of 10 to 30 years in humans, Cd can be accumulated and transferred by various edible plants and animals, which can then threaten human health through the food chain. To protect people from the adverse effects of Cd exposure, it is quite important to evaluate the reference level of Cd. The benchmark dose (BMD) represents the dose that corresponds to a specific level of increased benchmark response (the benchmark response, BMR). Previous studies have suggested that the lower 95% confidence limit of the BMD can replace the no observed adverse effect level (NOAEL)[2-4]. The reference level for Cd-induced damage to the kidneys has been previously assessed using the BMD method[2-4]. However, the possible effect of the study population selection on the BMDL evaluation remains unclear. Moreover, information on a reference level for Cd-induced renal effects for the entire Chinese population is still lacking. The Chinese population used in these limited studies was significantly smaller than the Japanese population used in other studies (374 to 790 participants *vs.* 1270 to 3103 participants)[2-7].

In the present study, a survey involving 6103 participants from five Chinese provinces was conducted to identify the UCd reference level for renal dysfunction with the BMD method. Our study objectives were as follows: (1) to evaluate the possible effect of the study population and gender on the BMDL and (2) to obtain reliable data on the critical UCd concentration for renal dysfunction in the general population based on the results of surveying a large sample population from different Cd-polluted areas.

The five study provinces included one slightly exposed area (Xiaogan, Hubei) and four highly exposed areas (Shaoguan, Guangdong; Gejiu, Yunnan; Baiyin, Gansu; and Hezhang, Guizhou). Hubei had relatively less industrial Cd contamination than the other provinces. Compared to Hubei, more smelting and mining sites that contribute to serious Cd pollution are located in the above highly exposed areas. The average values of soil Cd concentrations in these polluted areas reached 6 mg/kg to 10 mg/kg[1].

Subjects who had been diagnosed with kidney and/or liver disease were excluded from the study. Participants who were occupationally exposed to

doi: 10.3967/bes2015.054

*This study was financially supported by Special Funds of the State Environmental Protection Public Welfare Industry (201009049; 201309049), National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2013BAI12B03), and the Fundamental Research Funds for the Central Universities (2015JBM108).

1. College of Life Sciences and Bioengineering, School of Science, Beijing Jiaotong University, Beijing 100044, China; 2. Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430074, Hubei, China

heavy metals were also excluded. Morning urine samples were collected from all of the eligible participants to measure the UCd, N-acetyl-β-D-glucosaminidase (UNAG), and creatinine (cr) levels. The UCd and urinary cr level were measured using the standard methods described in previous studies[5-6]. All urinary parameters were normalized to the creatinine concentration and expressed as μg/g creatinine (μg/g cr)[5-6]. SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) were used to perform statistical analyses. BMD studies were performed using BMDS (Version 2.3.1, Environmental Protection Agency, USA). The UNAG level of 17 U/g cr was used as the cut-off value[8]. In most cases, the BMDs and BMDLs for Cd-induced renal dysfunction were calculated using a Gamma model with a good fit. When the equation was not a good fit, the Log-logistic model was chosen, as specified in the text.

The characteristics of the subjects are shown in Table 1. These subjects were 35 years or older. The mean Cd levels of the subjects from Guangdong were statistically lower than the levels of the subjects from other provinces (P<0.05). The highest mean value of 6.60 μg/g cr was observed in the subjects from Guizhou, which had more serious Cd pollution than that of the other areas. The mean UNAG levels of the male and female subjects from Guangdong were also the lowest, whereas the highest level of 12 U/g cr was observed in both the male and female subjects from Guizhou (Table 1). Subjects for each area were divided into the following four groups: UCd at 0 to 2, 2 to 6, 6 to 12, and >12 μg/g cr. The percentages that exceeded the UNAG limit, which ranged from 2.4% to 65.3% (Table 2), were calculated accordingly. The prevalence of renal dysfunction, indicated by percentages that exceeded the standard of UNAG, was correlated with increasing UCd, as verified by the chi-square linear trend test (P<0.05). The prevalence of renal dysfunction reflected by UNAG was significantly higher when the UCd was higher than 2 μg/g cr compared to when it was lower than 2 μg/g cr. Hence, an obvious dose-response relationship (Table 2) existed between the UCd and the prevalence of renal dysfunction, which is consistent with the findings of previous studies[3-5].

Next, the UCd BMDL for UNAG in different populations from the five Chinese provinces was investigated. The estimated UCd BMDL was close to 3 μg/g cr in males from all of the areas except Guizhou. The UCd BMDL of 4.26 μg/g cr in males from Guizhou was obviously higher than in the males from the other areas (Table 3). At a BMR of 10%, the UCd BMDLs for UNAG were 4.05, 2.98, 2.18, 3.33, and 2.39 μg/g cr in females from Gansu, Hubei, Guizhou, Guangdong and Yunnan, respectively (Table 3). The reference point of Cd damage to the kidneys has been assessed in several studies, the results of which showed that the estimated BMD values have displayed large variations[2-6]. Sakuragi et al. reported that both BMD and BMDL for the Cd-induced renal effect varied by four to seven folds, even when the analysis was conducted in a single

Table 1. Characteristics of the Populations Involved in the Study

Area	Sex	No.	Age (y) (Mean, Range)	UCd (μg/g cr) (GM±GSD, Range)	UNAG (U/g cr) (GM±SD, Range)
Gansu	Male	663	59.2 (35-87)	4.44[b]±2.47 (0.10-36.79)	10.73[b]±2.06 (0.30-29.11)
Hubei		724	60.5 (35-89)	4.69[b]±1.97 (0.21-33.32)	10.22[b]±2.27 (0.58-53.49)
Guizhou		548	59.3 (36-89)	6.60[a]±2.18 (0.08-56.99)	11.83[a]±3.55 (1.10-68.48)
Guangdong		321	62.4 (35-89)	4.06[c]±1.93 (0.81-24.86)	6.23[c]±3.09 (0.51-46.39)
Yunnan		459	61.1 (35-89)	4.37[b]±2.08 (0.56-31.96)	9.63[b]±2.15 (0.88-42.83)
Gansu	Female	833	60.4 (35-89)	4.34[b]±2.64 (0.11-48.47)	11.27[b]±2.01 (0.29-36.75)
Hubei		841	60.7 (35-89)	4.84[b]±2.02 (0.17-42.05)	10.54[b]±2.30 (0.49-46.22)
Guizhou		691	58.5 (35-89)	6.50[a]±2.26 (0.05-57.27)	12.18[a]±4.25 (1.08-70.95)
Guangdong		415	61.2 (35-89)	3.94[c]±2.12 (0.13-45.62)	6.86[c]±3.10 (0.48-67.59)
Yunnan		608	56.4 (35-88)	4.76[b]±2.25 (0.07-47.90)	10.12[b]±2.36 (0.49-45.85)
All areas	Male	2715	60.3 (35-89)	4.82±2.19 (0.08-56.99)	9.95±2.77 (0.30-68.48)
	Female	3388	59.5 (35-89)	4.87±2.33 (0.05-57.27)	10.39±2.88 (0.29-70.95)

Note. GM, geometric mean; GSD, geometric standard deviation. Values in the same column followed by different superscript capital letters are significantly different at P<0.05 according to Duncan's test.

Table 2. Prevalence of Renal Dysfunction at Different UCd Levels in Males and Females

Sex	UCd (μg/g cr)	Gansu			Hubei			Guizhou			Guangdong			Yunnan			All Regions		
		-	+	%	-	+	%	-	+	%	-	+	%	-	+	%	-	+	%
Male	0-2	99	7	6.6	57	9	13.6	31	5	13.9	38	1	2.6	45	3	6.3	270	25	8.5
	2-6	262	30	10.3	331	61	15.6	159	31	16.3	178	15	7.8	269	35	11.5	1199	172	12.5
	6-12	139	56	28.7	162	69	29.9	165	50	23.3	50	16	24.2	40	16	28.6	556	207	27.1
	12-	38	32	45.7	15	20	57.1	53	54	50.5	9	14	60.9	24	27	52.9	139	147	51.4
	χ^2		63.399			37.826			36.556			47.658			55.209			240.495	
	P^*		0.000			0.000			0.000			0.000			0.000			0.000	
Female	0-2	147	13	8.1	72	6	7.7	40	1	2.4	64	4	5.9	58	8	12.1	381	32	7.7
	2-6	299	47	13.6	384	52	11.9	209	43	17.1	204	19	8.5	288	58	16.8	1384	219	13.7
	6-12	179	54	23.2	179	99	35.6	189	73	27.9	74	23	23.7	79	42	34.7	700	291	29.4
	12-	37	57	60.6	17	32	65.3	53	83	61.0	10	17	63.0	39	36	48.0	156	225	59.1
	χ^2		91.638			104.09			89.051			49.319			43.621			391.678	
	P^*		0.000			0.000			0.000			0.000			0.000			0.000	

Note. *Chi-squared linear trend test, $P<0.05$.

Table 3. BMDL Estimates of the UCd for UNAG in Different Regions in All of Regions

Area	Sex	AIC	Chi-Square	*P* Value	BMD_{10} (μg/g cr)	BMDL10 (μg/g cr)	BMD5 (μg/g cr)	$BMDL_5$ (μg/g cr)
					BMR=10%		BMR=5%	
Gansu	Male	584.1	2.81	0.09	4.37	2.64	-	-
Hubei		727.3	0.32	0.57	5.66	3.24	-	-
Guizhou		585.6	0.01	0.91	8.11	4.26	-	-
Guangdong		225.1	0.40	0.53	4.87	3.27	-	-
Yunnan		384.1	0.99	0.32	4.08	2.43	-	-
Gansu	Female	750.4	0.93	0.34	6.10	4.05	-	-
Hubei		796.1	3.86	0.05	3.99	2.98*	-	-
Guizhou		740.3	2.73	0.10	3.33	2.18	-	-
Guangdong		308.5	0.26	0.61	5.41	3.33	-	-
Yunnan		629.1	3.49	0.17	3.14	2.39	-	-
All areas	Male	2502.6	1.55	0.21	4.89	3.73	3.09	2.08
	Female	3226.2	1.05	0.31	4.28	3.41	2.66	1.93

Note. Confidence level, 95%. *The BMDL value was calculated based on the Log-Logistic model. BMD_{10} ($BMDL_{10}$) and BMD_5 ($BMDL_5$) are the BMDs (BMDLs) corresponding to a 10% and 5% additional risk, respectively.

nation[7]. The BMDLs may be affected by many factors, such as race, sampling differences and effect biomarkers. However, these did not affect the BMDLs in the sample population of the current study. In this study, higher UCd BMDLs for UNAG were observed in males from Guizhou and in females from Gansu than those of the other provinces. The selection of the sample population affected the final BMD values when the same race, effect biomarker, sampling and analytical procedures were considered. The high BMDLs observed in some study areas of this study may be partly attributed to high Cd exposure levels in these areas, which was indicated by environmental[1] and food (e.g., rice) Cd pollution (detailed information will be reported in another study). However, the BMDLs of UCd in females from Guizhou and males from Gansu were not as high as those in males and females from the corresponding areas despite the similarities in the environmental Cd exposure levels. These results showed that BMDLs in populations from different areas may have also been affected by other factors apart from Cd exposure levels. Age or the exposure time may influence the BMD values[9]. In addition, there are as many as 56 ethnic groups in China. Large differences in the ethnic group, lifestyle, and eating habits of the study population may also be important factors related to Cd's toxic effects. Further studies are needed to obtain conclusive results.

Based on the Benchmark Dose Technical Guidance, datasets that are statistically and biologically compatible may be combined, resulting in increased confidence, both statistical and biological, in the calculated BMD. Therefore, data obtained from all of the participants from the five provinces were used to evaluate the reference level for Cd-induced kidney effects. The overall BMDLs in females from the five provinces were slightly lower than those of males. Specifically, the UCd BMDLs for UNAG with BMRs at 10% and 5% were 3.73 and 2.08 μg/g cr in males, and 3.41 and 1.93 μg/g cr in females, respectively (Table 3). In terms of statistical power, the number of subjects in the present study (n=6103) was much larger than the number of Chinese subjects in the populations used by Jin et al. (n=790) and Shao et al. (n=402)[5-6]. The population size was also relatively larger in the present study than that of the Japanese populations[2-4]. Given the quality of the subjects and the large sample population in the current study, the UCd BMD for UNAG in the Chinese population is relatively exact and reliable.

Overall, a slightly higher UCd BMDL for UNAG was observed in males than in females. The selection of the sample population and area affected the evaluation of the BMDL. A large number of subjects from typical areas are recommended for future studies. Doing so can help researchers obtain a more accurate and reliable reference concentration for Cd, which induces public health problems. The WHO reference concentration for Cd-related kidney effects is 5 μg/g cr. The lowest observed adverse effect level of 2 μg/g cr has been proposed in a European risk assessment report (EU RAR, 2007). The Scientific Committee on Toxicity, Ecotoxicity and the Environment reported that effects may occur even at Cd level as low as 0.5 μg/g cr[10]. The findings of the current study provide additional scientific support to a low biological limit value for Cd exposure to prevent potential health risks to residents, especially those vulnerable subjects.

ACKNOWLEDGMENTS

We wish to express our thanks to the staffs and/or students in the College of Life Sciences and Bioengineering, Beijing Jiaotong University and Tongji Medical College, Huazhong University of Science and Technology for their efforts to collect and analyze samples. We also thank all participants for their enthusiasm.

‡These authors contributed equally to this work.

#Correspondence should be addressed to CHEN Zhi Nan, professor, PhD, Tel: 86-10-51684351-210, Fax: 86-10-51683887, E-mail: chen_zhinan56@163.com; CHENG Xi Yu, PhD, E-mail: xycheng@bjtu.edu.cn

Biographical notes of the first authors: KE Shen, male, born in 1968, associate professor, PhD, majoring in environmental pollution and public health; KE Qin Mei, female, born in 1971, associate professor, PhD, majoring in public health.

Received: November 15, 2014;

Accepted: April 5, 2015

REFERENCES

1. Ke S, Qiao S. Cadmium toxicity is much more terrible than the beast: Preliminary studies of adverse health effects of cadmium pollution in China. Huaxia Press, Beijing. 2013.
2. Kobayshi E, Suwazono Y, Uetani M, et al. Estimation of benchmark dose as the threshold levels of urinary cadmium, based on excretion of total protein, β_2-microglobulin, and N-acetyl-β-d-glucosaminidase in cadmium nonpolluted regions in Japan. Environ Res, 2006; 101, 401-6.
3. Suwazono Y, Nogawa K, Uetani M, et al. Reassessment of the threshold of urinary cadmium by using hybrid approach in a cadmium non-polluted area in Japan. Int J Hyg Environ Health,

2011; 214, 175-8.
4. Suwazono Y, Nogawa K, Uetani M, et al. Application of the hybrid approach to the benchmark dose of urinary cadmium as the reference level for renal effects in cadmium polluted and non-polluted areas in Japan. Environ Res, 2011; 111, 312-4.
5. Jin TY, Wu XW, Tang YQ, et al. Environmental epidemiological study and estimation of benchmark dose for renal dysfunction in a cadmium-polluted area in China. Bio Metals, 2004; 17, 525-30.
6. Shao B, Jin TY, Wu XW, et al. Application of benchmark dose (BMD) in estimating biological exposure limit (BEL) to cadmium. Biomed Environ Sci, 2007; 20, 460-4.
7. Sakuragi S, Takahashi K, Hoshuyama T, et al. Variation in benchmark dose (BMD) and the 95% lower confidence limit of benchmark dose (BMDL) among general Japanese populations with no anthropogenic exposure to cadmium. Int Arch Occup Environ Health, 2012; 85, 941-50.
8. MOHC (Ministry of Health of the People's Republic of China). Standard of China, GB/T 17221-1998, Discriminant standard for health hazard area caused by environmental cadmium pollution. Standards Press of China, Beijing. 1998.
9. Chen X, Gan CH, Zhu GY, et al. Benchmark dose for estimation of cadmium reference level for osteoporosis in a Chinese female population. Food Chem Toxicol, 2013, 55, 592-5.
10. EC (European Commission). Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE). Opinion on the results of the risk assessment of cadmium metal human health, cadmium oxide human health. C7/VR/csteeop/ Cdmet-ox hh/080104 D(04). 2004. Adopted by the CSTEE during the 41st plenary meeting of 8 January 2004.