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Application of Benchmark Dose (BMD) in Estimating Biological Exposure Limit (BEL) to Cadmium

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Objective To estimate the biological exposure limit (BEL) using benchmark dose (BMD) based on two sets of data from occupational epidemiology. **Methods** Cadmium-exposed workers were selected from a cadmium smelting factory and a zinc product factory. Doctors, nurses or shop assistants living in the same area served as a control group. Urinary cadmium (UCd) was used as an exposure biomarker and urinary β2-microgloburin (B2M), N-acety1-β-D-glucosaminidase (NAG) and albumin (ALB) as effect biomarkers. All urine parameters were adjusted by urinary creatinine. Software of BMDS (Version 1.3.2, EPA.U.S.A) was used to calculate BMD. **Results** The cut-off point (abnormal values) was determined based on the upper limit of 95% of effect biomarkers in control group. There was a significant dose response relationship between the effect biomarkers (urinary B2M, NAG, and ALB) and exposure biomarker (UCd). BEL value was 5 μg/g creatinine for UB2M as an effect biomarker, consistent with the recommendation of WHO. BEL could be estimated by using the method of BMD. BEL value was 3 μg/g creatinine for UNAG as an effect biomarker. The more sensitive the used biomarker is, the more occupational population will be protected. **Conclusion** BMD can be used in estimating the biological exposure limit (BEL). UNAG is a sensitive biomarker for estimating BEL after cadmium exposure.

Key words: Benchmark dose; Biological exposure limit; Biomarker

INTRODUCTION

The benchmark dose (BMD) has been defined by Crump[1] as a statistical lower confidence limit to the dose that increases the predetermined response rate defining benchmark dose response (BMR, e.g., 1%-10%). Recently, Gaylor *et al*.[2] have redefined the benchmark dose as the point estimate of the dose corresponding to a specified low risk. This method was developed in an attempt to overcome some notable shortcomings of the use of an unobserved adverse effect level (NOAEL) in the default approach. The main strength of the BMD method is that an explicit response level can be associated with it. The dose serving as a starting point for human risk assessment is based more on toxicological judgement (choice of benchmark response level) than on statistical characteristics of the data (sometimes more in assessing a NOAEL). Generally, the quantal linear logistic regression model is used in the BMD procedure and in risk assessment of cadmium. We have used the BMD procedure to estimate the urinary Cd (UCd) critical concentration in a general population exposed to cadmium in China. Recently, Kobayashi *et al*.[3] have estimated the threshold levels of urinary Cd as BMDL, using the BMD approach. The results are in accordance with the values of UCd concentration in other studies[4-5].

Based on the relationship between exposure, internal dose, and effects, biological exposure limits (BEL) can be proposed. The concept is expressed mainly as "an alteration and/or maximum concentration of an endogenous substance in the body which must not surpass…". It entails measurement of the concentration of a chemical determinant in exposed biological media and is an indicator of the uptake of a substance[6]. Apart from a large number of reports on studies employing biological monitoring, many articles and textbooks on the method are available. The biological exposure indices (BEI) in the USA and *Biologische Arbeitstofftoleranzwerte* (BAT) values in Germany are also available. These indices can be used as guidelines for the evaluation of potential health hazards in occupational hygiene. In China, BEL of six substances has been issued[7]. In this study, BMD

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was used in estimating BEL .

We used BMD to estimate the BEL values for cadmium (Cd) in two selected factories located in two regions. Since in study of health effects of Cd exposure, urinary Cd concentration is commonly used as an indicator of an internal dose, urinary cadmium was used as an exposure biomarker, and urinary B2M, NAG, and ALB as indicators of renal effects of cadmium in the present study.

MATERIALS AND METHODS

Study Population

Factory A is situated in the middle south of China, the number of workers is relatively stable, and preventive work goes on regularly. Historical data from the local industrial health inspector show that the cadmium concentration in the air of the workplace is in the range of 0.013-5.410 mg/m^3 (the average concentration is 1.896 mg/m^3), about 20 times the maximal concentration of national hygienic standard (calculated on CdO, 0.1 mg/m^3). A total of 150 cadmium-exposed workers were selected from this factory as the exposure group. The control group consisted of 46 subjects with no history of cadmium exposure. There were no significant differences in age, gender, education, and economic status between the exposure and control groups.

Factory B is located in the south of China. Zinc is its main product. A total of 58 cadmium-exposed workers were selected from its frit workshop (the average concentration of air cadmium is 0.61 mg/m^3) and 47 persons from its electrolysis workshop (the average concentration of air cadmium is 3.54 mg/m^3). The control group consisting of 101 subjects matched for age and gender was selected from the shop assistants in this area. There were no significant differences in age, gender, education, and economic status between the exposure and control groups.

Sampling and Analysis

Urine samples were collected from all participants into acid-washed containers with cadmium measured by atomic absorption spectrometry (AAS). The samples were frozen at -20℃ until analysis. Each sample was divided into three parts immediately after collection. Of the samples, the first was assayed for cadmium after acidification with concentrated nitric acid, the second was used for the measurement of β_2-microglobulin (B2M), and the third was used for the determination of albumin, N-acety1-β-D-glucosaminidase (NAG), and creatinine without pretreatment.

Urinary cadmium concentrations were measured by graphite-furnace atomic absorption spectrometry (AAS). UB2M was measured by radioimmunoassay. UB2M kits were purchased from the Chinese Academy of Sciences. Urinary albumin was measured by ELISA. NAG and creatinine were measured as previously described[8-9]. Substrate and standard were purchased from Sigma. All urinary parameters were adjusted for creatinine in urine[10].

Statistical Analysis

Data were entered into a database on a microcomputer using Epi Info (Version 6.04) and transferred to another formal database according to the requirements of the analysis. Chi-square test and chi-square trend test were performed by the StatCalc program of the Epi Info package. The analysis of regression and curve estimation were performed using the Benchmark dose software (Version 1.3.2) from US Environment Protection Agency (EPA). Distributions of the biological measurements were normalized by logarithmic transformation. The data were expressed in geometric means.

RESULTS

Increased Excretion of Proteins and Enzymes in Urine

We defined the normal cut-off point based on the 95% limit value in the control group. If the value was higher than the normal cut-off point, we defined the renal function as abnormal (positive). The cut-off points of UB2M, UNAG, and UALB, in factory A were 0.36 mg/g creatinine, 9.4 U/g creatinine, and 11.9 mg/g creatinine, and those in factory B were 0.50 mg/g creatinine, 12.0 U/g creatinine, and 15.0 mg/g creatinine. We determined the increased excretion of proteins and enzymes at the different levels of urinary cadmium (Tables 1 and 2), indicating that there was a significantly increased excretion of proteins and enzymes in urine when the levels of Cd excretion were increased in urine. The increase was statistically significant in chi-square test for each relationship between urinary cadmium level and renal dysfunction. There was also a significant dose-response relationship between cadmium exposure (UCd) and renal dysfunction (Tables 1 and 2). The dose-response curves were plotted based on the BMD procedure (Figs. 1 and 2).

Values of BMDL for Different Urinary Indicators of Renal Dysfunction

BMDL was calculated using the Benchmark dose Software (Version 1.3.2) from US Environment Protection Agency (EPA), the estimated parameters and corresponding values of BMD are presented in Tables 3 and 4.

TABLE 1

Increased Excretion of Proteins and Enzymes in Urine of Workers in Factory A

Urinary Cadmium (μg/g creatinine)	UB2M		UNAG		UALB	
	+/−	%	+/−	%	+/−	%
0-	6/64	8.57	13/57	18.57	2/68	2.86
2-	8/62	11.43	18/52	25.71	6/64	8.57
5-	5/25	16.67	12/18	40.00	3/27	10.00
10-	9/17	34.62	13/13	50.00	8/18	30.77
Linear Trend	χ^2=10.70		χ^2=11.08		χ^2=15.69	
Test	*P*=0.0011		*P*=0.0009		*P*=0.0001	

TABLE 2

Increased Excretion of Proteins and Enzymes inUrine of Workers in Factory B

Urinary cadmium (μg/g creatinine)	UB2M		UNAG		UALB	
	+/−	%	+/−	%	+/−	%
0-	2/11	15.38	2/11	15.38	1/12	7.69
2-	10/51	16.39	15/46	24.59	11/50	18.03
5-	9/41	18.00	23/27	46.00	12/38	24.00
10-	15/23	39.47	24/14	63.16	15/23	39.47
20-	27/17	61.36	31/13	70.45	21/23	47.73
Linear Trend	χ^2=30.80		χ^2=25.59		χ^2=15.09	
Test	*P*=0.0001		*P*=0.0001		*P*=0.0001	

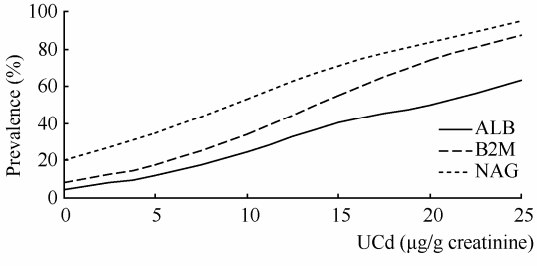

FIG. 1. Dose-response curves of UCd and urinary ALB, B2M, and NAG.

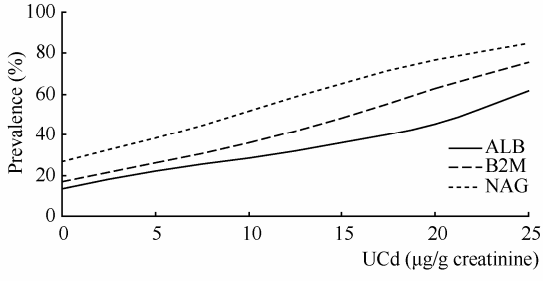

FIG. 2. Dose-response curves of UCd and urinary ALB, B2M, and NAG.

TABLE 3

BMDL Estimates of UCd (μg/g creatinine) for Urinary Indicators of Renal Dysfunction

Indicators	*n*	b_0	b_1	BMD-0.10	BMDL-0.10	*P* Values*
UB2M	196	−2.396	0.173	4.89	3.63	0.98
UNAG	196	−1.385	0.151	2.92	2.13	0.75
UALB	196	−3.146	0.233	5.60	4.37	0.62

Note. Model: ln (P/1-P) = b_0+b_1d; Excess risk at BMD is 0.10. **P* values were obtained from chi-square test, with the Pearson goodness of fit test; if *P*>0.05, the equation is a good fit.

TABLE 4

BMDL Estimates of UCd (μg/g Creatinine) for Urinary Indicators of Renal Dysfunction

Indicators	*n*	b_0	b_1	BMD-0.10	BMDL-0.10	*P* Values*
UB2M	206	−1.877	0.120	5.07	4.20	0.78
UNAG	206	−1.021	0.110	3.18	2.58	0.11
UALB	206	−1.606	0.083	6.16	4.85	0.54

Note. Model: ln (P/1-P) = b_0+b_1d; Excess risk at BMD is 0.10. **P* values were obtained from Chi-square test, with the Pearson goodness of fit test; if *P*>0.05, the equation is a good fit.

DISCUSSION

The main advantage of the benchmark dose methodology is that it uses all dose-response information by fitting a mathematical model to the data[11]. BMD can be used in both quantal and continuous data, and extrapolated using data on population health effect epidemiology. In this study, BMD weakened the error caused by using an uncertainty factor and extrapolated from animals to humans. Moreover, when applied to occupational population, it had a great reference value suggesting that using occupational epidemiological data collected from occupational workers in China to estimate their BEL is of great significance in China.

UB2M and UNAG have been suggested as sensitive biomarkers for testing early renal tubular dysfunction caused by cadmium. UALB is now widely used as an index of glomerular damage. In this study, these biomarkers were found to be continuous data. Therefore, we selected them as a cut-off point, and the data could be dichotomized based on it. We defined the normal cut-off point based on the 95% limit value in the control group, and determined the increased excretion of proteins and enzymes at different levels of urinary cadmium. Following characterization of the hazards and selection of appropriate endpoints was used for the dose-response assessment. When there was at least a statistically or biologically significant dose-related trend in the selected endpoints, BMD analysis could be evaluated. In this study, a significant dose-response relationship was found between urinary cadmium levels (Figs. 1 and 2) and used in BMD analysis. BMD was directly determined by the selection of BMR. For quantal data, an excess risk of 10% is the default BMR, since 10% response is just or near the limit of sensitivity in most cancer bioassays and in some non-cancer bioassays as well.

In this study, 10% BMR was selected to calculate BMD (Tables 3 and 4), indicating that different biomarkers of renal dysfunction have different BMD and BMDL values of UALB, UB2M, and UNAG. The value of UALB was higher than that of others, suggesting that renal tubular dysfunction occurs earlier than renal glomerular dysfunction. In the present study, the diagnostic biomarker of occupational cadmium poisoning was defined based on UB2M, but not on urinary enzymes. BEL was defined based on estimated urinary protein. In the two groups with UB2M as an effect biomarker, BMD value was 4.89 μg/g creatinine and 5.07 μg/g creatinine, respectively, consistent with the recommendation of WHO[12] and current occupational exposure limit in China, suggesting that using UB2M as an effect biomarker can estimate the BEL value.

NAG is mostly generated by the kidneys and excreted by their tubular cells . When the kidneys are damaged, the activity of urinary NAG significantly increases, aggravating the damage of their tubular cells. It has been recommended as an early sensitive biomarker of renal dysfunction caused by cadmium[13]. Measurement of urinary NAG has no other special requirement for urine samples, and is not disturbed by urinary color. However, UB2M is easily affected by age, liver disease and urinary pH. In this study, BMDL for urinary NAG was lower than BMDL for urinary B2M, indicating that BMDL is more sensitive. These values agree well with the reported values[14]. Therefore, using urinary NAG as an effect biomarker of cadmium exposure should be recommended for estimating BEL. In this study, the calculated values of BMD were 2.92 μg/g creatinine and 3.18 μg/g creatinine, with UNAG as an effect biomarker, suggesting that it is more reasonable to adjust BEL of urinary cadmium to 3 μg/g creatinine.

In conclusion, BMD can be used in calculating BEL value. UNAG is more sensitive than UB2M as an effect biomarker of cadmium exposure, and can be used in estimating BEL.

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