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Genetic polymorphism of heme-oxygenase-1 in Thai population with low environmental cadmium exposure

Kanyarat Boonprasert[1*], Ronnatrai Ruengweerayut[2], Soisanwan Satarug[3], Kesara Na-Bangchang[1**]

[1]Pharmacology and Toxicology Unit, Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences Thammasat University, Pathumthani, Thailand.
[2]Mae Sot Hospital, Tak, Thailand.
[3]Department of Pathology, University of North Dakota School of Medicine and Health Sciences, USA.
*Presenting Author, ** Corresponding Author

Abstract

The aims of the study were to investigate (i) the effects of environmental cadmium (Cd) on high blood pressure development, (ii) the association between genetic polymorphism of heme oxygenase-1 (HO-1) and high blood pressure development, and cadmium-induced renal injury in exposed Thai population. The study was a case-control study in adults residing in a Cd contaminated area (Mae Sot District, Thailand). All subjects were randomly selected and consistently distributed for sex, age and residential areas. Blood and urinary Cd levels were not significantly different between the case (hypertensive subjects) and control (healthy subjects) groups. While other kidney dysfunction parameters were comparable between the two groups, microalbumin and serum creatinine showed significant difference (p=0.008 and p=0.02, respectively). With respect to HO-1 polymorphism, the frequencies of S (short), M (medium) and L (long) alleles in this group of subjects were 49.7, 44.4 and 5.9%, respectively. The frequencies of S, M and L alleles between the matched pair case and the control groups were not significantly different.

Keywords: Cadmium, hypertension, heme oxygenase-1, genetic polymorphism, renal biomarkers

Introduction

Cadmium (Cd) is an environmental pollutant. Food and smoking is the major sources of Cd exposure from the environment for general population. Cd-induced hypertension and renal diseases are the health problems in population that reside in Cd-contaminated areas. In individuals with chronic exposure, Cd accumulates, particularly in the kidney and can cause renal toxicity. The early sign of Cd induced kidney damage is tubular dysfunction demonstrated by an increased excretion of low molecular weight proteins, such as β_2-microglobulin, retinol binding protein (RBP), α_1-microglobulin, or *N*-acetyl-β-glucosaminidase (NAG) enzyme (1, 2, 3). Several animal studies have demonstrated the increase in blood pressure levels in animal exposing to chronic low-dose of Cd. Effects of Cd on blood pressure depend on Cd dose levels. In rats, 0.1-5 *ppm* of cadmium induces hypertension, but 10-50 *ppm* does not or only does during the early exposure before toxicity becomes evident (4). Nomiyama *et al*. reported that chronic Cd exposure may slightly elevate blood pressure, whereas a higher dose may depress blood pressure only in hypertensive humans and animals (5). Study on relationship between Cd and hypertension in human population is however, limited and has several methodological errors (6).

Heme oxygenase 1 (HO-1) enzyme has also been proposed to play important role in modulating Cd -induced renal toxicity and hypertension. It is a renal protector from a wide variety of cellular stressors including Cd, and on the other hand, also possesses depressure ability. Several single nucleotide polymorphisms (SNPs) in human HO-1 gene result in a

variety of HO-1 expression. Individuals with reduced HO-1 activities are more susceptible to Cd-induced renal toxicity and severe hypertension than those with normal HO-1 activities (7). The aims of the study were to investigate (i) the effects of environmental Cd on high blood pressure development, (ii) the association between genetic polymorphism of HO-1 and high blood pressure development, and Cd-induced renal injury in exposed Thai population.

Methods

The study protocol was approved by the Ethics Committee of the Ministry of Public Health of Thailand. The study was conducted in local residents of 10 Cd-contaminated villages in Mae Sot District, Tak province (Tambon Mae Tao, Mae Ku and Prathadphadaeng). These Cd contaminated areas were defined based on urinary cadmium levels in a total of 7,697 subjects (8). All subjects were randomly selected and consistently distributed for sex, age and residential areas, and were divided into three groups as follows: control (healthy subjects), pre-hypertensive and hypertensive groups. Hypertensive group was classified based on clinical diagnosis, and/or the level of systolic blood pressure (SBP) of more than or equal to 140 mmHg, and/or diastolic blood pressure (DBP) of more than or equal to 90 mmHg. Those genetic-inherited hypertensive cases or those hypertensive cases from other diseases especially renal dysfunction were excluded. The pre-hypertensive group was classified based on the level of systolic blood pressure (SBP) between 120-139 mmHg, and/or diastolic blood pressure (DBP) between 80-89 mmHg (9). Blood and second morning urine samples were collected from each subject for analysis of blood and urinary Cd concentrations, renal toxicity biomarkers [β_2-microglobulin, *N*-acetyl-β-glucosaminidase (NAG), microalbumin and creatinine]. Cadmium concentrations in blood and urine were determined by electrothermal (graphite furnace) atomic absorption spectrometer. Urinary NAG was determined by a colorimetric assay using NAG test kit. gDNA was prepared from buffy coat of blood samples for analysis of $(GT)_n$ repeat of HO-1 by using laser-based automate DNA sequencer.

Results

Table 1 shows demographic characteristics, smoking status, blood and urinary Cd levels, and biomarkers of kidney dysfunction. Blood cadmium concentration was significantly different between the control and hypertensive group, whereas urinary Cd concentrations were similar in the two groups. It is noted however that the normatensive group had higher propertion of current smokers than the hypertensive group. Current smokers had significantly higher blood Cd concentrations than non-smokers and ex-smokers. Kidney dysfunction biomarkers including serum creatinine, β_2-microglobulin and NAG in the three groups were similar, but the level of microalbumin was significantly higher in the hypertensive group. When the hypertensive subjects (cases) were matched for sex, age, residential areas and smoking habit with normatensive subjects (control), no differences in blood (median 2.87 *vs* 3.08 μg/l) or urinary Cd (median 0.39 *vs* 0.43 μg/g creatinine) levels was found. The levels of microalbumin (median 0.66 *vs* 0.33 mg/g creatinine) was however, significantly higher in the case group. The levels of other kidney dysfunction parameters were similar between the two groups.

The number of $(GT)_n$ repeats of HO-1 gene in all subjects varied between 16-39. HO-1 gene allele was categorized into three groups as: short (S) with 21-27 repeats, intermediate (M) with 28-33 repaets and long (L) alleles with 34-39 repreats (10). The frequencies of S, M and L alleles were 49.7, 44.4 and 5.9% respectively (Table 3). The frequencies of S, M and L alleles in the case and control matched pair groups were similar. The frequencies of genotypes (SS, SM, SL, MM, ML, LL) of $(GT)_n$ repeat in all subjects and case-control matched pair groups were shown in Table 2.

Table 1 Demographic data, smoking habits, blood and urinary cadmium levels, and markers of kidney dysfunction in all subjects included in the study.

	Hypertension	Pre-hypertension	Normatension
Males:Females (n:n)	35:112	17:40	35:76
Age (years)[b]	47.34 ± 4.50	47.68 ± 4.79	46.86 ± 4.91
BMI (kg/m^2)[b]	25.11 ± 3.60 (n=147)	23.85 ± 3.38 (n=55)	23.91 ± 3.35 (n=109)*
Smoking status[a]			
Non-smoke**rs**	95 (50.8%)	30 (16.0%)	62 (33.2%)
Current smokers	21 (28.8%)	20 (27.4%)	32 (43.8%)
Former smokers	31 (56.4%)	7 (12.7%)	55 (30.9%)
Cadmium levels [c]			
Blood cadmium (μg/l)	2.35 (0.47-14.64)	2.97 (0.52-17.95)	3.11 (0.40-19.50)**
Urinary cadmium (μg/g creatinine)	0.38 (0.08-1.83)	0.40 (0.03-2.93)	0.42 (0.09-3.12)
Markers of kidney dysfunction[c]			
Serum creatinine (mg/dl)	0.80 (0.50-2.30)	0.80 (0.50-1.20)	0.90 (0.50-1.30)
Microalbumin (mg/g creatinine)	0.63 (0.08-42.73) (n=129)	0.47 (0.06-19.98) (n=49)	0.37 (0.06-29.34) (n=91)**
NAG (U/ g creatinine)	1.06 (0.16-8.21)	1.22 (0.00-11.33)	0.95 (0.44-4.22)
β2-MG (ug/g creatinine)	4.64 (0.00-1728.35)	4.31 (0.04-4035.14)	6.33 (0.06-1766.76)

[a]Data are presented as number (percentage).; [b]Data are presented as mean ± SD.; [c]Data are presented as median (range).
*Statistically significant difference between normatensive group and hypertension group with $p<0.05$ (One-way ANOVA)
**Statistically significant difference between normatensive group and hypertension group with $p<0.05$ (Kruskal-Wallis test)

Table 2 Distribution of HO-1 promoter genotypes and allele frequecies

Characteristic	Total	Case-control match paired	
		Case	Control
HO-1 phenotype (allenes), n (%)			
- **S**	313 (49.7%)	79 (51.3%)	78 (50.6%)
- **M**	280 (44.4%)	67 (43.5%)	68 (44.2%)
- **L**	37 (5.9%)	8 (5.2%)	8 (5.2%)
Total	630 (100%)	154 (100%)	154 (100%)
HO-1 phenotype, n (%)			
- **SS**	73 (23.2%)	19 (24.7%)	17 (22.1%)
- **SM**	147 (46.7%)	41 (53.2%)	36 (46.8%)
- **SL**	20 (6.3%)	0	8 (10.4%)*
- **MM**	60 (19.0%)	10 (13.0%)	16 (20.8%)
- **ML**	13 (4.1%)	6 (7.8%)	0*
- **LL**	2 (0.6%)	1 (1.3%)	0
Total	315 (100%)	77 (100%)	77 (100%)

*Statistically significant difference between case and control with $p<0.05$ (Chi-square test)

Discussion

Despite the abundance of evidences showing the inductive effect of Cd to high blood pressure in animals, there are limited information on Cd-exposed workers (1). Moreover, available data on the relationship between Cd and hypertension in human population are controversial and debatable due to methodological errors (6). Several confounding factors such as smoking status, other air pollutants, as well as other environmental factors make it difficult to draw conclusions concerning the effects of Cd to hypertension (1). In 1984, Staessen *et al*., showed negative correlation between blood pressure and urinary Cd or $ß_2$-microglobulin in some groups after the confounding variables age, sex, body weight, and cigarette smoking were adjusted in a multiple regression analysis. As there was a very strong age effect on both blood pressure and urinary Cd, the meaning of the negative correlation is not clear (1, 11). In the present study, although hypertensive subjects were matched with the normatensive subjects for sex, age, residential areas of Cd-exposure and smoking status, Cd concentrations in blood and urine in both groups were similar. This could be due to

inadequate sample size. Apart from microalbumin, the levels of biomarkers for kidney dysfunction in both hypertensive and normatensive subjects were comparable and lied within the range of expected value for a healthy population. A wide ranges of tests with different sensitivities and levels of significance have been used to determine Cd nephrotoxicity. For occupationally populations, 2 μg/g creatinine of urinary Cd is associated with biochemical alterations. The albumin level in urine as a marker of glomerular dysfunction and urinary NAG as a marker of renal tubule injury are associated with 4 μg/g creatinine of urine cadmium. The 10 μg/g creatinine of urinary Cd is associated with β_2-MG: a tubular proteinuria resulting from an impaired tubular reabsorption (12).

HO-1 can be induced by of Cd and act as renal protector. It also has depressure effect *via* ability of HO-1 to generate CO which cause vasodilation. Several polymorphisms have been found in HO-1 genes of human which lead to a variety of HO-1 expression. A $(GT)_n$ repeat in the 5'flanking region of the HO-1 gene is highly polymorphic and its expression of may be altered according to the number of $(GT)_n$ repeat. The large size of a $(GT)_n$ repeats in the HO-1 gene promoter may reduce HO-1 activity (13). Therefore, individuals with reduced HO-1 activities are expected to be more susceptible to Cd-induced renal toxicity and severe hypertension than those with normal HO-1 activities. Nevertheless, no such association was observed in this study.

Conclusion

Results from the present study suggest no clear evidence on the effects of environmental Cd nor HO-1 polymorphism on high blood pressure development, and Cd-induced renal injury in exposed Thai population.

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References

1. International Programme on Chemical Safety. Cadmium. Environmental Health Criteria, vol.134. Geneva: World Health Organization 1992.
2. Lauwerys RR, Bernard AM, Roels HA and Buchet JP. Cadmium: Exposure markers as predictors of nephrotoxic effects. Clin Chem 1994; 40/7: 1391-4.
3. Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure-a review of the literature and a risk estimate. Scand J Work Environ Health 1998; 24 (Suppl 1): 1–51.
4. Perry HM, Erlander M and Perry EF. Increase in the systolic pressure of rats chronically fed cadmium. Am J Physiol 1979; 235: H385-H91.
5. Nomiyama K and Nomiyama H. Cadmoum-induced elevation of blood pressure. J trace Elemem Exp Med 2000; 13: 155-63.
6. Houston MC. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infraction. Alternative Therapies in Health and Medicine 2007; 13: S128-S33.
7. Satarug S, Nishijo M, Lasker JM, Edwards RJ and Moore MR. Kidney dysfunction and hypertension: role for cadmium, P450 and heme oxygenase? Tohoku J Exp Med 2006; 208: 179-202.

8. Swaddiwudhipong W, Limpatanachote P, Mahasakpan P, Krintratun S and Padungtod C. Cadmium-exposed population in Mae Sot District, Tak Province: 1.Prevalence of high urinary cadmium levels in the adults. J Med Assoc Thai 2007; 90: 143-8.
9. JNC7. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. NIH Publication No. 03-5233. December 2003.
10. Takeda M, Kikuchi M, Ubalee R, *et al*. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to cerebral malaria in Myanmar. Jpn J Infect Dis 2005; 58: 268-71.
11. Staessen J, Bulpitt CJ, Roels H, *et al*. Urinary cadmium and lead concentrations and their relation to blood pressure in a population with low exposure. Br J Ind Med 1984; 41: 241-8.
12. Trzcinka-Ochocka M, Jakubowski M, Razniewska G, Halatek T and Gazewski A. The effects of environmental cadmium exposure on kidney function: the possible influence of age. Environ Res 2004; 95: 143-50.
13. Yamada N, Yamaya M, Okinaga S, *et al*. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am J Hum Genet 2000; 66: 187-95.