Effect of Magnesium Deficiency on Blood Pressure in Normal Rats Fed Cadmium

Shoji Nishiyama,*[, a, 1] Satomi Onosaka,[b] Noboru Saito,[c] Yuko Konishi,[d] and Toshio Nakadate[a]

[a]Department of Hygiene and Preventive Medicine, School of Medicine, Showa University, 1–5–8 Hatanodai, Shinagawa, Tokyo 142–8555, Japan, [b]Faculty of Nutrition, Kobe-Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 651–2180, Japan, [c]Department of Internal Medicine and Center for Lifestyle-related Disease, Miyazaki Medical Center Hospital, 2–16, Takamatsucho, Miyazaki 880–0003, Japan, and [d]Medical Research Center, Department of Medicine, Kochi University, Kohasu, Oko, Nankoku, Kochi 783–8505, Japan

(Received January 5, 2005; Accepted April 11, 2005)

We attempted to further define the effects of an Mg-deficient diet on blood pressure in rats fed Cd for long periods. Twenty male Wistar rats were grouped according to four different diets: normal diet (N rats); normal diet supplemented with Cd at a dose of 50 μg/g of diet (N rats fed Cd); Mg-deficient diet (D rats); and Mg-deficient diet supplemented with Cd at a dose of 50 μg/g of diet (D rats fed Cd). Each diet was given for 60 days. N rats fed Cd developed a duration-limited increase in blood pressure: by day 60, increased blood pressure returned to the level seen in N rats. Mg deficiency lowered the Cd-induced increase in blood pressure and this response was more pronounced on day 60. A variety of independent blood pressure regulatory mechanisms was investigated. Although Mg deficiency tended to occur at lower concentrations of Cd and metallothionein in the heart of N rats fed Cd and the Cd concentration was less than 5 μg/g of tissue, Mg deficiency increased the Ca concentration in the hearts of N rats fed Cd while a decrease in urinary Na excretion and an increase in water retention were not observed. Diet-related toxic clinical signs in D rats fed Cd induced marked blush on the ears and fingers, indicating vasodilatation had occurred. These findings may have been a factor in the pronounced reduction in blood pressure seen.

Key words —— cadmium, blood pressure, magnesium, deficiency, calcium

INTRODUCTION

Some epidemiological studies have reported that high cadmium (Cd) concentrations are associated with a rise in blood pressure, and that this rise is particularly pronounced in subjects with hypertensive disease.[1,2] However, other epidemiological studies in Belgium produced evidence inconsistent with the hypothesis that environmental exposure to Cd leads to an increase in blood pressure and a higher prevalence of hypertension.[3,4] Although many reports have shown an increase in blood pressure in experimental animals treated parentally with Cd,[5,6] the effects of dietary administration of Cd over long periods and at low levels on blood pressure remain unknown.

Some workers have suggested that there is a relationship between magnesium (Mg) intake and the incidences of increased blood pressure.[7,8] In experimental animals, the alteration of blood pressure has been observed in Mg-deficient rats.[9,10]

These reports have speculated that dietary Cd supplementation and Mg deprivation leads to synergistic or additive effects on blood pressure in experimental animals. The aim of the present study was to further define the effects of an Mg-deficient diet on blood pressure in rats fed Cd for long periods.

MATERIALS AND METHODS

Diets —— The basal composition of the experimental diet is given in Table 1. The normal diet (N diet) was supplemented with magnesium oxide (MgO) at a rate of 0.8 g per kg of diet. The Mg-deficient diet did not contain MgO (D diet). Cd was added to the

[1]Present address: Project Planning and Development Pharmaceuticals, Meiji Seika Kaisha, Ltd., 2–4–16, Kyobashi, Chuo, Tokyo 104–8002, Japan

*To whom correspondence should be addressed: Project Planning and Development Pharmaceuticals, Meiji Seika Kaisha, Ltd., 2–4–16, Kyobashi, Chuo, Tokyo 104–8002, Japan. Tel.: +81-3-3273-3437; Fax: +81-3-3273-3439; E-mail: shoji_nishiyama@meiji.co.jp

Table 1. Composition of Purified Basal Diet

Ingredients	Percentage in diet (%)
Sucrose	50.0
Casein	20.0
Purified starch	15.0
Cellulose	5.0
Olive oil	5.0
Vitamin mix[a)]	1.0
Mineral mix[b)]	3.5
DL-methionine	0.3
Choline hydrochloride	0.2

a) Vitamin per 100 g diet: thiamine 100 mg, riboflavin 150 mg, pyridoxine HCl 100 mg, nicotinamide 1000 mg, D-panthenate 500 mg, folic acid 50 mg, vitamine B_{12} 0.1 mg, vitamin A 2.5×10^5 IU, vitamin E 100 mg, calciferol 2×10^4 IU, vitamin C 3.7×10^3 mg. *b*) Minerals per 100 g diet: NaCl 7.4 g, $K_2C_6H_5O_7 \cdot H_2O$ 22 g, K_2SO_4 5.2 g, $CaHPO_4$ 50 g, MgO 2.4 g, $FeC_6H_5O_7 \cdot 5H_2O$ 0.6 g, $MnCO_3$ 0.35 g, $CuCO_3$ 30 mg, $CrK(SO_4)_2 \cdot 12H_2O$ 55 mg, $CoCl_2 \cdot 6H_2O$ 10 mg, Kl 1 mg, $ZnCO_3$ 160 mg.

N and D diets at a concentration of 50 mg per kg of diet as $CdCl_2$, to give two more diets, the N + Cd diet and D + Cd diet. To minimize spillage the four different diets were prepared in solid form. The levels of Cd, Mg, calcium (Ca), iron (Fe), zinc (Zn), and copper (Cu) in the four diets were determined by flame atomic absorption spectrophotometry, as previously reported.[11)] The Cd^{2+} concentration in the N and D diets was less than 0.02 μg/g of diet, and that in the N + Cd and D + Cd diets was 50 μg/g of diet. The Mg^{2+} concentration in the N diet was 500 mg/kg of diet, and in the D diet it was 10 mg/kg diet. The Ca^{2+} concentration in the four diets was 1.2 g/kg of diet. The Zn, Fe, Cu, and phosphorus (P) concentrations in the basal experimental diet corresponded to those recommended by the American Institute of Nutrition (AIN-76).[12)]

Experimental Design —— Eight-week-old male STD-Wistar rats with a mean weight of about 210 g were purchased from Nippon SLC Co. Ltd. (Shizuoka, Japan). Twenty animals were grouped according to the four different diets: rats fed the N diet (N rats); rats fed the N diet supplemented with Cd (N rats fed Cd); rats fed the Mg-deficient diet (D rats); and rats fed the D diet supplemented with Cd (D rats fed Cd). The rats were placed in plastic cages with stainless-steel tops in groups of five. They were housed in a temperature- (25°C) and light-controlled room (12 hr light), as previously reported.[11)] Food and distilled water were provided ad libitum for the entire experimental period of 60 days. Body weights and food consumption were recorded on days –1 (before dietary Cd supplementation), 15, 30, 45, and 60.

Regional Blood Flow (RBF) —— All the animals were anesthetized with pentobarbital-Na (50 mg/kg, *i.p.*) on day 60. Regional blood flow (RBF) was determined by the method of Fujioka *et al.*[13)] In brief, under anesthesia, catheters (PE-50) were inserted into the right carotid and right femoral arteries to measure the mean blood pressure and reference flow. Then the former tubing was advanced into the left ventricle, the location being confirmed by pressure tracing. A reference flow rate was required for measurement of RBF. Carbonized microspheres (NEN-TRAC, specific activity 9.7 Ci/g), 16 ± 0.1 μm in diameter and labeled with ^{141}Ce, were suspended in physiological saline with 0.01% Tween 80. The suspension (0.25 ml), containing 80000 microspheres and corresponding to a 0.2-μCi source of radioactivity for ^{141}Ce, was injected and a reference sample collected from the right femoral artery at a constant rate of 0.41 ml/min with a constant withdrawal Harvard pump (Holliston, MA, United States). The reference sample was transferred to a counting tube and then the collection syringe and arterial catheter were rinsed with physiological saline. This washing was added to the counting tube and the radioactivity in the sample and in the arterial catheter was measured.

Blood Pressure —— Systolic blood pressure was measured with a programmed electro-sphygmomanometer system (Narco PE-300) in conscious rats between 0900 and 1200 after 10 min in a 30°C environment. The values were the averages of five consecutive measurements for each animal, as previously reported.[11)] Systolic blood pressure was recorded by the tail-cuff method, using the above mentioned system, on days –1, 30, 45, and 60.

Urinary Na Excretion and Water Retention —— The daily water intake and urine excretion in each group were measured in a metabolic cage. Percentage water retention was expressed according to the formula of Doyle *et al.*[14)]

Percent water retention = water intake (ml) – urine output (ml)/water intake (ml) × 100%.

Metal Concentrations in the Organs —— The heart, abdominal aorta, kidney, and liver of each rat were removed on day 60, weighed, and quickly frozen in liquid N_2. The concentrations of Ca, Mg, and Cd in these organs were assayed by flame atomic absorp-

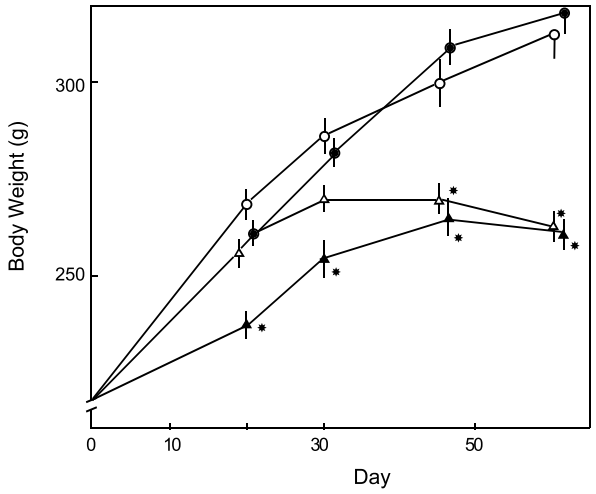

Fig. 1. Effect of an Mg-Deficient Diet on Body Weight Gain in Normal Rats Fed Cd

Symbols represent body weights of rats fed a normal diet (○), N rats fed Cd (●), D rats (△), and D rats fed Cd (▲). Each value is expressed as body weight (g) ± S.E. of five animals. *Significantly different from normal rats at $p < 0.05$, evaluated statistically by Bonferroni's test.

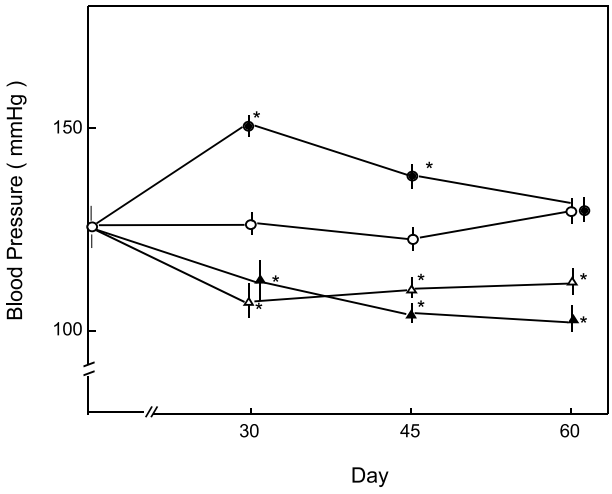

Fig. 2. Effect of an Mg-Deficient Diet on Blood Pressure in Normal Rats Fed Cd

Symbols represent blood pressures of rats fed a normal diet (○), N rats fed Cd (●), D rats (△), and D rats fed Cd (▲). Each value is expressed as blood pressure (mm Hg) ± S.E. of five animals. *Significantly different from normal rats at $p < 0.05$, evaluated statistically by Bonferroni's test.

tion spectrophotometry. Cd concentrations in the heart, aorta, lung, kidney, and liver were assayed by flameless atomic absorption photometry (Hitachi polarized Zeeman atomic absorption spectrophotometer 180-80), as previously reported.[11]

Metallothionein Concentration —— Metallothionein (MT) concentrations in the heart, lung, kidney, and liver were assayed by the method of Onosaka.[15]

Statistical Analyses —— All data were evaluated statistically by Bonferroni's test.

RESULTS AND DISCUSSION

Body weight gain in the D rats fed Cd decreased significantly from day 15 onwards when compared with those of N rats and N rats fed Cd (Fig. 1). However, no significant difference in food consumption in any of the four groups was found during the experimental period (data not shown). The diet-related toxic clinical sign observed in D rats fed Cd but not N rats was marked blush on the ears and fingers.

Dietary intake of Cd produced an increase in blood pressure in N rats at a limited term and dose, because blood pressure in the N rats fed Cd was elevated on days 30 and 45 but had returned to normal by day 60 (Fig. 2). Our previous study showed that parental administration of Cd at a dose of 1 mg/kg of body weight in rats lowered blood pressure, whereas a dose of 0.5 mg Cd/kg of body weight increased blood pressure.[11] These results suggest that in rats treatment with Cd at lower doses induces hypertension, whereas treatment at higher doses induces hypotension.

Mg deficiency lowered the increase in blood pressure on days 30 and 45, and this response was more pronounced on day 60 (Fig. 2). The regulation of blood pressure is a complex process involving a variety of independent regulatory mechanisms such as Ca concentration, RBF in the heart and aorta, and water retention in the body, which play important roles in the regulation of blood pressure. Cd and MT concentrations in the heart, kidney and liver of D rats fed Cd tended to be lower compared to N rats fed Cd, except for the Cd concentration in the kidney (Table 2). Some workers have observed cardiotoxicity in rats that had about 5 μg/g Cd in their cardiac tissues.[16] Compared to the present study, this concentration is higher than that in the N rats fed Cd and D rats fed Cd. These results indicate that cardiotoxicity may not occur in N rats fed Cd and D rats fed Cd. However, Jamall and Smith[17] suggested that the absolute Cd concentration in the heart may not be as critical to the development of cardiotoxicity as the concentration of Cd relative to the concentrations of Se and Ca, and perhaps other essential trace elements, even though the Cd dose was less than 5 μg/g. Myocardial calcification through Ca influx into cardiac myocytes may be an important mechanism of cardiac injury.[18] An increase in the Ca/Mg

Table 2. Cd and MT Concentrations in Cd-Fed Rats, Mg-Deficient Rats and Mg-Deficient Rats Fed Cd on day 60

Organ (μg per g)	Normal	Cd-fed	Depleted Mg	Depleted Mg and Cd-fed
Heart (Cd)	0.73 ± 0.5	3.0 ± 0.45[a)]	0.61 ± 0.4	1.9 ± 0.9
Heart (MT)	16.9 ± 4.2	22.1 ± 7.9[a)]	14.0 ± 1.8	14.2 ± 2.0
Aorta (Cd)	0.10 ± 0.02	0.21 ± 0.03	0.04 ± 0.004	0.16 ± 0.03
Kidney (Cd)	0.06 ± 0.02	10.8 ± 1.6[a)]	0.07 ± 0.01	12.9 ± 1.8[a)]
Kidney (MT)	28.7 ± 6.2	260 ± 7.7[a)]	49.3 ± 11.4	189 ± 24.1[a,b)]
Liver (Cd)	0.60 ± 0.4	11.5 ± 1.1[a)]	1.1 ± 0.2	8.1 ± 0.9[a)]
Liver (MT)	15.7 ± 6.3	164 ± 31[a)]	31.8 ± 7.7	126 ± 12.3[a)]

Values are mean ± S.E. in micrograms per wet tissue (g) in rats (n = 5) fed one of the four different diets. *a*) significantly different from normal group at $p < 0.05$, evaluated by Bonferroni's test. *b*) significantly different from N rats fed Cd group at $p < 0.05$, evaluated by Bonferroni's test. MT was not detected in the aorta in any group.

Table 3. Ca and Mg Concentrations in Cd-Fed Rats, Mg-Deficient Rats and Mg-Deficient Rats Fed Cd on day 60

Organ (μg per g)	Normal	Cd-fed	Depleted Mg	Depleted Mg and Cd-fed
Heart (Ca)	11.2 ± 0.4	10.5 ± 0.5	30.8 ± 8.6[a)]	15.3 ± 0.6[a,b)]
Heart (Mg)	212 ± 7	219 ± 5	200 ± 2	219 ± 8
Aorta (Ca)	78.3 ± 4.4	91.4 ± 4.2	66.4 ± 5.6	88.1 ± 15.4
Aorta (Mg)	275 ± 22	289 ± 28	194 ± 48	225 ± 25
Kidney (Ca)	19.2 ± 1.0	18.0 ± 3.0	806.7 ± 176[a)]	837.3 ± 138[a,b)]
Kidney (Mg)	224 ± 12	218 ± 29	200 ± 9	232 ± 19

Values are mean ± S.E. in micrograms per wet tissue (g) in rats (n = 5) fed one of the four different diets. *a*) significantly different from normal group at $p < 0.05$, evaluated by Bonferroni's test. *b*) significantly different from N rats fed Cd group at $p < 0.05$, evaluated by Bonferroni's test.

Table 4. RBF in Cd-Fed Rats, Mg-Deficient Rats and Mg-Deficient Rats Fed Cd on day 60

Reginal blood flow (ml/g per min)	Normal	Cd-fed	Depleted Mg	Depleted Mg and Cd-fed
Heart	4.48 ± 0.62	4.67 ± 0.72	5.03 ± 1.3	3.30 ± 0.69
Lung	0.39 ± 0.1	0.33 ± 0.09	0.68 ± 0.23	0.42 ± 0.29
Brain	0.61 ± 0.11	0.41 ± 0.04	0.54 ± 0.08	0.43 ± 0.04
Kidney	5.80 ± 0.7	4.95 ± 0.62	2.76 ± 0.68[a)]	2.40 ± 0.68[a)]
Liver	0.24 ± 0.05	0.24 ± 0.04	0.39 ± 0.07	0.46 ± 0.13

Values are mean ± S.E. of ml per wet tissue (g) per min. in rats (n = 5) fed one of the four different diets. *a*) significantly different from normal group at $p < 0.05$, evaluated by Bonferroni's test.

ratio in the heart produces myocardial necrosis, and a decrease in this ratio prevents the development of cardiac necrosis. In the present study, Mg deficiency increased the Ca concentration in the hearts of the D rats fed Cd compared with N rats fed Cd (Table 3). There were no significant differences in Mg concentration in the heart compared to N rats fed Cd and N rats while urinary Mg excretion in D rats and D rats fed Cd was decreased (44 and 80% of normal). We have already reported that Cd ameliorates histopathological changes due to Ca overload in the hearts of D rats but may also inhibit the release of Ca^{2+}, which is a major determinant of the level of contractile force.[18)] In the present study, Mg deficiency also markedly increased Ca concentration in the kidney of D rats fed Cd, suggesting that severe Mg deficiency produced renal damage in D rats fed Cd, which corresponds to a marked decrease in RBF of the kidney (Table 4). It is well known that chronic renal failure was induced by surgical 5/6 renal ablation and that these 5/6 nephrectomy (NTX) rats showed elevated blood pressure 12 weeks after surgery.[19)] This NTX evidence supports our speculation that renal damage induced in D + Cd rats may not contribute to the lower blood pressure. A decrease in urinary Na excretion and an increase in water re-

tention, which are important factors for the regulation of blood pressure, were not observed in D rats fed Cd compared to N rats and N rats fed Cd (data not shown). The diet-related toxic clinical manifestation observed in D rats fed Cd was marked blush on the ears and fingers, indicating that vasodilatation had occurred, which may have been caused by an increase in plasma serotonin.[20)]

From these results, it is concluded Ca overload in the heart might be ascribed to myocardial toxicity and vasodilatation in D rats fed Cd, which may be a factor for lowered blood pressure in these rats.

Acknowledgements We thank Professors Keiichi Tanaka and Hiroshi Yamamoto of Osaka University for their critical review of the manuscript.

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