

Long-Period-Effect of Moderate Mg Deficiency on Circulation Parameters in Rats Treated with Cadmium

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The present study was carried out to elucidate the effect of a moderately Mg-deficient diet on circulation parameters such as blood pressure and cardiac functions in rats treated with Cd. Forty male Wistar rats were divided into the following groups to evaluate the effect of two different dietary Mg concentrations in regard to Cd treatment: normal dietary Mg and no Cd treatment (group N); normal Mg plus Cd treatment (group N + Cd); moderately Mg-deficient diet defined as 40% concentration of normal level and no Cd treatment (group D); and moderate Mg-deficient diet plus Cd treatment (group D + Cd). Subcutaneous injection of Cd in the backs of the animals at 1 mg/kg body weight was performed twice a day for 7 consecutive days and the animals then were maintained with two different diets and without Cd treatment for an additional period of 203 days before analysis. Comparison of the two groups of N + Cd and D + Cd showed that ingestion of the Mg-deficient diet markedly increased cardiac output and decreased total peripheral resistance without altering blood pressure. The Ca concentration in the heart of D + Cd rats was not increased compared with that from N rats, although there was significant interaction between Mg deficiency and Cd treatment on the Ca concentration in the heart, suggesting that moderate Mg deficiency did not cause myocardial necrosis or impair cardiac contraction in the rats treated with Cd.

Key words — Mg deficiency, Cd treatment, circulation parameter

INTRODUCTION

Various epidemiological studies have reported that high cadmium (Cd) concentration in normal environmental conditions is associated with increased blood pressure in humans^{1,2} and experimental animals treated with Cd.^{3,4} In addition, decreased magnesium (Mg) intake may increase in blood pressure in humans.^{5,6} In contrast, Mg deficiency appears to have variable effects on blood pressure in experimental animals. In rats, whereas moderate Mg deficiency at 83% of control elevated blood pressure,⁷

severely Mg-deficient diet at 2% of control produced decreased blood pressure.⁸ We have recently reported that decreased blood pressure was observed in severe Mg-deficient rats fed Cd.⁹ In light of these reports, we speculated that the effects of moderate Mg-deficiency and Cd treatment might interact to affect circulation parameters in rats, which results in increased blood pressure.

The present study was carried out to elucidate the long-period-effect of a moderately Mg-deficient diet at 40% of control level on circulation parameters in rats treated with Cd.

MATERIALS AND METHODS

Diets — The basal composition of the experimental diet is given in Table 1. To minimize spillage the two different diets were prepared in solid form. The “normal diet” (N diet) was supplemented with MgO at a rate of 0.8 g per kg of diet. The moderately Mg-

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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, vitamin B₁₂ 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, K₂C₆H₅O₇·H₂O 22 g, K₂SO₄ 5.2 g, CaHPO₄ 50 g, MgO 2.4 g, FeC₆H₅O₇·5H₂O 0.6 g, MnCO₃ 0.35 g, CuCO₃ 30 mg, CrK(SO₄)₂·12H₂O 55 mg, CoCl₂·6H₂O 10 mg, KI 1 mg, ZnCO₃ 160 mg.

deficient diet (D diet) was supplemented with MgO at a concentration of 0.4 g per kg of diet. Observed concentrations of Mg²⁺ in the N and D diets were 500 and 200 mg/kg diet, respectively. The Ca²⁺ concentration in both diets was 2.1 g/kg of diet. The Zinc, Iron, Copper, and Phosphorus concentrations in the basal experimental diet corresponded to those recommended by the American Institute of Nutrition (AIN-76).¹⁰⁾

Experimental Design — Male STD-Wistar rats (8-week-old, average weight, 210 g) were purchased from Nippon SLC Co. Ltd. (Shizuoka, Japan) and allocated 40 rats into four groups according to the two different diets and two Cd treatment options: animals receiving the N diet without parenterally Cd (N group); those fed the N diet and treated with parenterally Cd (N + Cd group); rats fed the Mg-deficient diet without parenterally Cd (D group); and animals fed the D diet and treated with parenterally Cd (D + Cd group). The rats were placed in groups of five in plastic cages. 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 210 days. Body weights and food consumption were recorded every month.

Cd Treatment — The rats were treated with parenterally Cd to maintain a constant body burden of Cd in each rat. Subcutaneous injection of 0.1 ml Cd at a dose of 1.0 mg/kg body weight beneath the skin over the backs of the animals was performed

twice daily (12-hr intervals) (time-zero) for 7 consecutive days. The animals were maintained without Cd treatment for an additional 203 days with two different diets before analysis, for a total experimental period of 210 days.

Analysis of Blood Pressure, Total Peripheral Resistance, Cardiac Output, Heart Rate, and Regional Blood Flow in the Kidney, Liver, and Brain

— All animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) on day 210. Cardiac output (CO), total peripheral resistance (TPR), and heart rate were determined by the method of Fujioka *et al.*¹²⁾ Catheters (PE-50) were inserted into the right carotid artery and right femoral artery of the anesthetized animals for the measurement of the mean blood pressure and reference flow. Then the catheters tubing was advanced into the left ventricle, the location being confirmed by pressure tracing.

To obtain the reference flow rate necessary to determine the regional blood flow, carbonized microspheres labeled with ¹⁴¹Ce (NEN TRAC; specific activity, 9.7 Ci/g; diameter, $16 \pm 0.1 \mu\text{m}$) were resuspended in physiological saline containing 0.01% Tween 80. A vial containing this suspension was shaken vigorously for at least 5 min to permit mechanical movement of the microspheres. An aliquot of the suspension solution (0.25 ml) containing 80000 microspheres and corresponding to 0.2 μCi radioactivity, was injected. Withdrawal of the reference sample was started 5 sec before the injection of the radioactive tracer. Left ventricular pressure and heart rate were monitored continuously throughout this procedure. The reference sample was collected from the right femoral artery at a constant rate of 0.41 ml/min with a constant-withdrawal Harvard pump (Holliston, MA, U.S.A.). Sampling did not affect the heart rate or arterial blood pressure, and the arterial microsphere concentration was 0 at the end of the collection time. The reference sample was transferred to a counting tube, and 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 remaining in the arterial catheter was measured.

The net radioactivity (cpm) of the injected dose was calculated as: average cpm of the total radioactivity in the 0.25 ml of injection solution — the residual cpm of the arterial catheter and injection syringe. CO (ml/min) was calculated as: blood withdrawal rate (ml/min) \times total cpm injected/total cpm

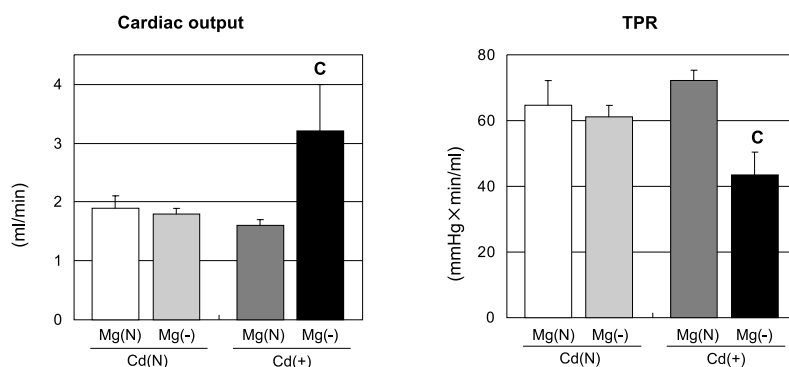


Fig. 1. CO and TPR in Mg-Deficient Rats Treated with Parenterally Cd

Each value is expressed as ml/min of cardiac output and mmHg × min/ml of TPR. The data are shown as mean ± S.E. of 10 animals. C: The interaction between Mg deficiency and Cd treatment was significant at $p < 0.05$ and substituting the symbol for the “C” in the figure.

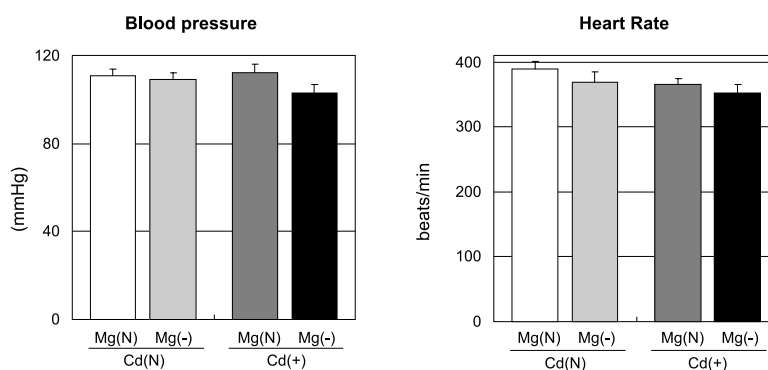


Fig. 2. Blood Pressure and Heart Rate in Mg-Deficient Rats Treated with Parenterally Cd

Each value is expressed as millimeters of mercury of systolic blood pressure and beats per min of heart rate. The data are shown as mean ± S.E. of 10 animals.

in reference sample. TPR was determined as: mean blood pressure (mm Hg)/CO (ml/min). Systolic blood pressure was measured between 0900 and 1200 in conscious rats by using a programmed electro-sphygmomanometer system (PE-300, Narco) after each animal rested for 10 min in a 30°C environment. The values were the average of five consecutive measurements for each animal, as previously reported.¹¹⁾ Systolic blood pressure was recorded by the tail-cuff method as described earlier.

Metal Concentrations in the Organs — The heart and abdominal aorta of each rat were removed on day 210, weighed, and quickly frozen in liquid N₂. Concentrations of Ca, Mg, and Cd in those organs were assayed using a flame atomic absorption spectrophotometer. Cd concentration in the heart and aorta was assayed using a flameless atomic absorption photometer (model 180-80 polarized Zeeman atomic absorption spectrophotometer, Hitachi, Tokyo, Japan) as previously reported.¹¹⁾

Statistical Analysis — Data were evaluated by means of one- and two-way analysis of variance for a 2 × 2 factorial design in a randomized block by using the IBM-BMDP 7D program as previously reported.¹³⁾

RESULTS AND DISCUSSION

The moderately Mg-deficient diet markedly increased CO and decreased TPR in the rats treated with Cd (Fig. 1), and significant interaction between Mg deficiency and Cd treatment in CO and TPR was observed (Fig. 1). In those rats, decreased TPR with a concomitant increase in CO might be a factor of the lack of significant effects on blood pressure and heart rate between the four groups (Fig. 2). Itokawa *et al.*⁸⁾ have shown that a severely Mg-deficient diet significantly lowered blood pressure in rats. These investigators postulated that the peripheral vasodi-

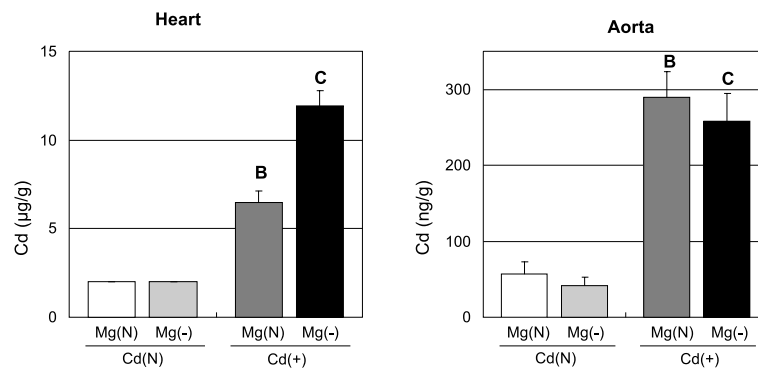


Fig. 3. Cd Concentration in Mg-Deficient Rats Treated with Parenterally Cd

Each value is expressed as μg per wet tissue (g) of the heart and ng per wet tissue (g) of the aorta in Mg-deficient rats parentally treated with Cd. The data are shown as mean \pm S.E. of 10 animals. B: The effect of Cd treatment is significant at $p < 0.05$. C: The interaction between Mg deficiency and Cd treatment was significant at $p < 0.05$ and substituting the symbol for the "C" in the figure.

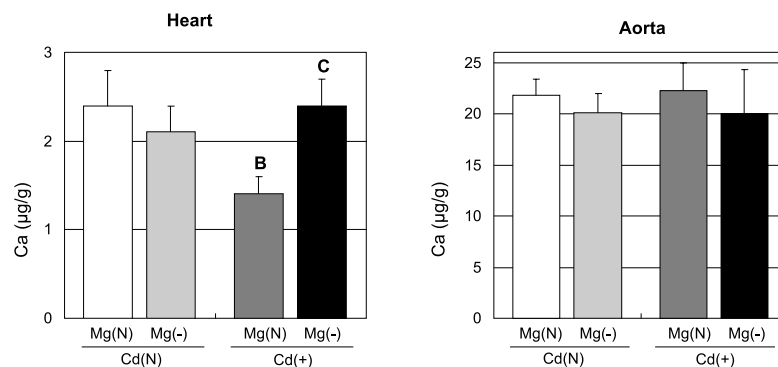


Fig. 4. Ca Concentration in Mg-Deficient Rats Treated with Parenterally Cd

Each value is expressed as μg per wet tissue (g) in Mg-deficient rats parentally treated with Cd. The data are shown as mean \pm S.E. of 10 animals. B: The effect of Cd treatment is significant at $p < 0.05$. C: The interaction between Mg deficiency and Cd treatment was significant at $p < 0.05$ and substituting the symbol for the "C" in the figure.

latation symptoms associated with their Mg-deficient rats may have been due to an increase in blood serotonin level. It is well known that an increased serotonin level leads to vasodilating effects, which results in marked erythema of the ears and fingers. The marked erythema of the ears and toes that we noted in our rats that received the moderately Mg-deficient diet and Cd treatment (data not shown) might have involved an increase in the blood serotonin level, which resulted in the observed marked decrease in TPR.

The moderately Mg-deficient diet in our present study had a concentration of 40% of control level whereas the severely Mg-deficient diet in our previous study had observed concentration of Mg at 2% of control.⁹ We have also reported that the route of administration and body burden of Cd used in present study produced increase in blood pressure and plasma aldosterone level.^{11,14} Present study by us-

ing the moderately Mg-deficient diet demonstrated significant interaction between Mg deficiency and Cd treatment on Cd concentrations in the heart and aorta (Fig. 3) and on Ca concentration in the heart (Fig. 4). These results might be ascribed to nonspecific competition between Ca and Cd in these tissues since it is well known that high concentrations of Cd^{2+} (0.1 to 0.3 mM) block cardiac entry of activator Ca^{2+} .¹⁵ Investigators recently have found that micromolar concentrations of Cd^{2+} directly affect the cardiac sarcoplasmic reticulum Ca^{2+} -release channel.¹⁶ This previous report and our present results prompt us to speculate that moderate Mg deficiency might impair cardiac contraction in the rats treated with Cd, because moderate dietary Mg deficiency markedly increased Cd concentrations in the hearts from rats treated with Cd (Fig. 3). However, the markedly increased CO in the absence of increased heart rate in the rats fed the moderately Mg-defi-

cient diet and treated with Cd (Fig. 2) suggests that moderate Mg deficiency in fact did not impair cardiac contraction in the rats treated with Cd. Although the regional blood flow in the kidney was significantly decreased in the Mg-deficient rats treated with Cd, regional blood flow in the heart and brain were not affected by the two different diets on day 210 (data not shown). The reasons why marked increased Cd in the heart did not impair cardiac contraction in the animals fed the moderately Mg-deficient diet remains unknown.

We have reported that Cd at a low concentration (0.28 $\mu\text{g/g}$) in the heart induced cardiotoxic effects manifested by decreases of the heart rate and weight and histopathological changes in the presence of severe Mg deficiency, whereas Cd supplementation of a normal diet did not induce any cardiotoxic effect.¹³⁾ In that study, the Ca concentration and ratio of Ca to Mg in the heart were increased significantly in rats fed the severely Mg-deficient diet compared with those fed the normal diet. However, in the current study, the Ca concentration in the heart of rats fed the Mg-deficient diet and treated with Cd was not increased compared with that of animals given the normal diet, although significant interaction between Mg deficiency and Cd treatment in Ca concentration in the heart occurred (Fig. 4). This result suggests that in contrast to the condition of severe Mg deficiency, moderate Mg deficiency did not produce myocardial necrosis in the rats treated with Cd.

In the present study, significant interaction between moderate Mg deficiency and Cd treatment on CO and TPR in rats occurred without concomitant changes in blood pressure. In addition the markedly increased CO in the absence of increased heart rate in the rats fed a moderately Mg-deficient diet and treated with Cd suggests that Mg deficiency did not impair cardiac contraction in these animals.

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