Systemic Magnesium Deficiency Disclosed by Magnesium Loading Test in Patients with Essential Hypertension

Ryoji Ozono, Tetsuya Oshima*, Hideo Matsuura, Yukihito Higashi, Takafumi Ishida, Mitsuaki Watanabe, Mitsuisa Yoshimura, Hiroyuki Hiraga, Norihisa Ono, and Goro Kajiyama

The present study was designed to determine whether magnesium (Mg) deficiency is present in patients with essential hypertension. We measured the retention of an intravenously administered Mg load (0.2 mmol/kg MgSO₄ over 4 h), and serum and erythrocyte Mg concentrations in 17 inpatients with essential hypertension and in 15 normotensive controls. There was no significant difference between the two groups in erythrocyte Mg concentration (normotensives *vs.*, hypertensives: 2.0 ± 0.5 *vs.* 2.1 ± 0.4 mmol/l cells), serum Mg concentration (normotensives *vs.* hypertensives: 2.1 ± 0.2 *vs.* 2.1 ± 0.2 mg/dl), or in urinary Mg excretion (normotensives *vs.* hypertensives: 65.8 ± 25.5 *vs.* 73.7 ± 26.7 mg/day). However, Mg retention was significantly higher in hypertensives than in normotensives (normotensives *vs.* hypertensives: 31.8 ± 12.1 *vs.* $41.9 \pm 13.3\%$). These results suggest that a systemic Mg deficiency, which is undetectable by serum or erythrocyte Mg determination, may exist in patients with essential hypertension. (*Hypertens Res* 1995; 18: 39-42)

Key Words: magnesium, essential hypertension, magnesium retention

Although numerous pathophysiological abnormalities have been described, the underlying mechanisms of essential hypertension remain unknown. Magnesium (Mg) is essential to many cell functions (1), including transmembrane Ca²⁺ flux and plasma membrane Na⁺-K⁺ ATPase activation. Experimental studies have demonstrated that hypomagnesemia caused vasoconstriction (2), and Mg-deficient rats developed systemic hypertension (3). These observations indicate that Mg deficiency may be an important contributory factor to the development of hypertension. In support of this hypothesis, several investigators have demonstrated an inverse correlation between blood pressure and serum Mg (4) or intracellular free Mg concentration (5), and others (6, 7) have shown a blood pressure lowering effect of oral Mg supplementation. However, conflicting results have been also reported (8, 9), and recent clinical trials (10, 11) of oral Mg treatment in patients with essential hypertension failed to show any effect of Mg on blood pressure. Thus, whether or not Mg deficiency, either at the cellular or systemic level, is involved in the onset and maintenance of essential hypertension still remains controversial.

To study the role of Mg in the pathogenesis of

essential hypertension, intracellular free Mg concentration in vascular smooth muscle should be examined (12). Since specimens of vascular smooth muscle cannot be readily obtained from humans, total Mg concentrations in blood cells, including lymphocytes (1, 13) and erythrocytes (1, 4, 9, 14), have been widely used and are considered to reflect the intracellular Mg status. Serum Mg concentration (15) and 24-h urinary Mg excretion (1, 9) may provide the bases for estimating systemic Mg balance, but extracellular fluid contains only 1% of total body Mg and urinary Mg excretion is largely affected by Mg intake. The best estimate of Mg status in man is thus debatable.

The retention of intravenously loaded Mg, calculated using urinary Mg excretion before and after Mg loading, has been demonstrated to inversely correlate with total body Mg (16, 17), and this Mg retention is considered to be one of the most reliable indices of systemic Mg deficiency. Increased Mg retention has been reported in patients with congestive heart failure (18), myocardial infarction (19) and variant angina pectoris (20), as well as in patients with chronic alcoholism (21, 22) who had extreme Mg deficiency. Furthermore, repletion of Mg resulted in a reduction in the Mg retention (18,

From the First Department of Internal Medicine and *Department of Clinical Laboratory Medicine, Hiroshima University School of Medicine, Japan.

This study was supported in part by a Grant-in-Aid for Scientific Research (no.02670396 and 06672293) from the Ministry of Education, Science and Culture, Japan and from the Kurozumi Medical Foundation.

Address for Reprints: Tetsuya Oshima, M.D., Department of Clinical Laboratory Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan.

Received April 16, 1994; accepted in revised form August 4, 1994.

21) accompanied by an improvement in clinical manifestations of Mg deficiency (21). To date, however, Mg retention has not been studied in untreated patients with essential hypertension.

In the present study, we compared systemic and intracellular Mg status between essential hypertensives and normotensive controls by the Mg loading test and by measuring Mg concentrations in serum and erythrocytes.

Subjects and Methods

Seventeen patients with mild to moderate essential hypertension (7 men and 10 women, mean age 55.7 ± 12.6 years) and 15 normotensive control subjects (7 men and 8 women, mean age 52.3 ± 16.8 years) were studied. Hypertension was defined as a diastolic blood pressure more than 90 mmHg in the sitting position on at least three different occasions in the outpatient clinic. Patients with severe organ damage and secondary forms of hypertension were excluded. Three of the 17 subjects had never received antihypertensive drugs. For the others, all antihypertensive medication was discontinued for at least 3 weeks before the study. Among the 14 patients who were receiving antihypertensive therapy, 10 were prescribed calcium channel blockers, 3 β -blockers, and 1 both a calcium channel blocker and an angiotensin converting enzyme inhibitor. None had received any diuretics for a year before the study. Normotensive controls were enrolled from subjects with atypical chest pain in whom no evidence of diseases was detected by coronary angiography, thallium-201 myocardial scintigraphy, and echocardiography. All the normotensive controls had blood pressure less than 140/90 mmHg and received no medication. There was no significant difference between the two groups in mean body weight (normotensives vs. hypertensives: 57.9 ± 9.0 vs. 62.2 ± 9.0 kg), and or mean serum creatinine concentration (0.80 ± 0.23 vs. 0.78 ± 0.18 mg/dl). Informed consent was obtained from all subjects.

All subjects were admitted to the Hospital of Hiroshima University School of Medicine (Hiroshima, Japan) and placed on a regular hospital diet containing 170 mEq/day of sodium chloride. Baseline 24-h urine samples were collected for determination of Mg and creatinine excretion for 3-7 days after admission, and mean values for the last three days were used for analysis. Urine collection was started at 9:00 AM. On the morning of the Mg loading test, the subjects, who had fasted overnight, remained supine for 30 min after voiding, and blood pressure was measured 5 times at 1-min intervals with a mercury sphygmomanometer, and the mean value was used for analysis. Venous blood was drawn for determination of serum and erythrocyte total Mg concentrations and plasma renin activity. Subsequently, subjects received an intravenous dose of MgSO_4 (0.2 mmol/kg body weight) in 250 ml of 5% dextrose in water at 9:00 AM over 4 h. Serum Mg concentration was again determined at the end of Mg infusion. Twenty-four-hour urine samples were again collected, starting from

the time of initiating the infusion, for determination of 24-h urinary excretion of Mg and creatinine. The baseline 24-h urinary Mg excretion was subtracted from postinfusion 24-h urinary Mg excretion. The ratio of the difference in urinary Mg excretion to the total amount of Mg infused was expressed as a percent of loaded Mg retention at 24 h after infusion. Thus, Mg retention (%) was calculated according to the following equation: $\{\text{total amount of Mg infused} - (\text{post infusion 24-h urinary Mg} - \text{baseline 24-h urinary Mg})\} \times 100 / \text{total amount of Mg infused}$.

A preliminary study revealed that the coefficient of variance of baseline 24-h urinary Mg excretion within one hospitalized individual was $36 \pm 15.0\%$ (average of 13 subjects). Based on these findings, the postinfusion 24-h urinary Mg excretion was corrected for baseline-related variations in baseline Mg excretion.

Serum Mg, urine Mg and urine creatinine concentrations were determined by auto-analyzer. Erythrocyte Mg was calculated as previously described (23) from the Mg concentration, measured by atomic absorption (Shimadzu, Kyoto, Japan) in hemolysates prepared from erythrocytes that had been washed three times with ice-cold isotonic choline-chloride solution, packed in a microcapillary tube (Hematolon, Kayagaki Irikakogyo, Kumamoto, Japan), and centrifuged at 15,000 *g* for 5 min. Plasma renin activity was measured by radioimmunoassay (Gamma Coat PRA, Baxter Travenol Ltd, Tokyo, Japan) as previously described (23).

All data were expressed as mean $\pm$ SD. Student's unpaired *t*-test and simple regression analysis were used for statistical analysis. A *p* value < 0.05 was accepted as significant.

Results

Systolic and diastolic blood pressure were 122.8 ± 19.7 and 75.9 ± 7.0 mmHg in normotensives, and 152.6 ± 21.7 and 92.4 ± 9.7 mmHg in hypertensives, respectively. As shown in Table 1, there was no significant difference in baseline 24-h urinary excretions of Mg and creatinine, serum Mg concentration, erythrocyte Mg concentration, plasma renin activity or creatinine clearance between the normotensives and hypertensives. Twenty-four-hour urinary creatinine excretion was similar preinfusion and postinfusion in each group.

Mg infusion resulted in a significant ($p < 0.01$) elevation in serum Mg concentration both in normotensives and hypertensives, and there was no significant difference in the postinfusion serum Mg concentration between the two groups (normotensives vs. hypertensives: 3.4 ± 0.2 vs. 3.5 ± 0.4 mg/dl). However, the hypertensives retained a significantly greater amount of Mg than did the normotensives (normotensives vs. hypertensives: 31.8 ± 12.1 vs. $41.9 \pm 13.3\%$, $p < 0.05$, Fig. 1) although there was a relatively wide overlap between the groups. Furthermore, in the combined group, the Mg retention was significantly and positively correlated with mean blood pressure ($r =$

Table 1. Variables in Normotensive and Hypertensive Subjects

		Normotensives	Hypertensives
Systolic blood pressure	(mmHg)	122.8±19.7	152.6±21.7*
Diastolic blood pressure	(mmHg)	75.9±7.0	92.4±9.7*
Preinfusion serum Mg	(mg/dl)	2.1±0.2	2.1±0.2
Postinfusion serum Mg	(mg/dl)	3.4±0.2	3.5±0.4
Erythrocyte Mg	(mmol/l cells)	2.1±0.4	2.0±0.5
Preinfusion 24-h urinary Mg	(mg/day)	65.8±25.5	73.7±26.7
Postinfusion 24-h urinary Mg	(mg/day)	243.2±35.0	254.1±38.9
Preinfusion 24-h urinary Cr	(mg/day)	1,079±175	1,045±217
Postinfusion 24-h urinary Cr	(mg/day)	1,082±113	1,053±175
Plasma renin activity	(ng AI/ml/h)	1.6±1.9	1.7±2.4
Cr clearance	(ml/min)	92.6±20.5	95.0±15.3

* $p < 0.01$ vs. normotensives

Cr, creatinine.

Fig. 1 Magnesium retention in normotensive subjects (NT) and essential hypertensive patients (EHT).

0.35, $p < 0.05$). In the combined group, the Mg retention had no significant correlation with age ($r = -0.29$), serum creatinine concentration ($r = 0.01$), serum Mg concentration ($r = -0.23$), erythrocyte Mg concentration ($r = -0.16$), or with plasma renin activity ($r = 0.07$). In addition, no difference in the Mg retention was detected between men and women in the combined group (men vs. women: 36.8 ± 12.6 vs. $37.4 \pm 14.6\%$) or in each group.

Discussion

In the present study, we demonstrated that essential hypertensive patients retained a larger proportion of loaded Mg than did normotensive subjects, indicating the presence of systemic Mg deficiency in

essential hypertensive patients. However, we could detect no significant difference in serum Mg concentration, erythrocyte Mg concentration, or in 24-h urinary Mg excretion between the normotensive and hypertensive subjects.

Although the difference was statistically significant, there was a substantial overlap in the Mg retention between the hypertensive and normotensive subjects. Resnick *et al.* (5) have demonstrated a striking difference in intracellular free Mg level between "low-renin" essential hypertensive patients and control subjects but not between "high renin" patients and the control subjects. In the present study, the Mg retention in our hypertensive subjects was unrelated to plasma renin activity or other factors that may affect Mg retention, including age, sex, and renal function. The overlap between the two groups in the Mg retention suggests that the Mg deficiency alone does not account for the pathogenesis of essential hypertension, but it is one of the significant factors contributing to the elevation of blood pressure.

Since only 1% of total body Mg is in the extracellular space (1), serum Mg concentration may not accurately reflect total body Mg. Previous studies (1, 5, 9, 24) also have failed to find a difference in serum levels of Mg between hypertensive and control subjects. Hypomagnesemia is observed only when the Mg deficiency is severe (18, 21, 22). It is likely that the Mg deficiency in our hypertensive subjects was not severe enough to affect the serum Mg level.

Although the Mg retention was greater in the hypertensive subjects showing a systemic Mg deficiency, we could not detect a decrease in erythrocyte total Mg concentration in the hypertensives. Although this erythrocyte Mg concentration is widely used as an index for intracellular Mg status (1, 4, 9, 14), there is no convincing evidence that it reflects intracellular Mg levels in other cells such as vascular smooth muscle cells. In addition, the intracellular free Mg account for only approximately 10% of total Mg content (4, 5, 8). We cannot discard the possibility that total Mg concentration in erythrocytes may not be a suitable index to detect

differences in cellular Mg status between hypertensive and normotensive subjects.

Previously, Mg deficiency in patients with cardiovascular diseases such as vasospastic angina (20), myocardial infarction (19), arrhythmia (18) and congestive heart failure (18) was detected using the Mg loading test. Gullestad *et al.* (18) reported that patients with essential hypertension had increased Mg retention, but most of the patients in those studies were on diuretic treatment, established as the most common cause of systemic Mg deficiency (1). Our study is the first to demonstrate an increased Mg retention in patients with untreated essential hypertension.

If systemic Mg deficiency exists, urinary Mg excretion may be decreased. However, we could not find any difference in urinary Mg excretion between the groups. The coefficient of variance of the 24-h urinary Mg excretion was 36% in one subject, since it is difficult to control the Mg intake. Thus, such relatively large day-to-day variations may mask small group differences in basal urinary Mg excretion.

In summary, we demonstrated that the Mg loading test disclosed a systemic Mg deficiency in patients with essential hypertension. This deficiency was not detected based on serum and intraerythrocyte concentrations of Mg or 24-h urinary Mg excretion. Our data support the hypothesis that systemic Mg deficiency plays a role in the pathogenesis of essential hypertension.

References

1. Reinhart RA: Magnesium metabolism: a review with special reference to the relationship between intracellular content and serum levels. *Arch Intern Med* 1988; **148**: 2415-2420.
2. Altura BM, Altura BT, Carella A: Magnesium deficiency-induced spasm of umbilical vessels: relation to preeclampsia, hypertension, growth retardation. *Science* 1983; **221**: 376-378.
3. Altura BM, Altura BT, Gebrewold A, Ising H, Gunther T: Magnesium deficiency and hypertension: correlation between magnesium-deficient diets and microcirculatory changes *in situ*. *Science* 1984; **223**: 1315-1317.
4. Petersen B, Schroll M, Christiansen C, Transbøl I: Serum and erythrocyte magnesium in normal elderly Danish people. *Acta Med Scand* 1977; **201**: 31-34.
5. Resnick LM, Gupta RK, Laragh JH: Intracellular free magnesium in erythrocytes of essential hypertension: relation to blood pressure and serum divalent cations. *Proc Natl Acad Sci USA* 1984; **81**: 6511-6515.
6. Motoyama T, Sano H, Fukuzaki H: Oral magnesium supplementation in patients with essential hypertension. *Hypertension* 1989; **13**: 227-232.
7. Dickner T, Wester PO: Effect of magnesium on blood pressure. *Br Med J* 1983; **286**: 1847-1849.
8. Woods KL, Walmsley D, Heagerty AM, Turner DL, Lian LY: ^{31}P nuclear magnetic resonance measurement of free erythrocyte magnesium concentration in man and its relation to blood pressure. *Clin Sci* 1988; **74**: 513-517.
9. Kjeldsen SE, Sejersted OM, Frederichsen P, Leren P, Edie IK: Increased erythrocyte magnesium content in essential hypertension. *Scand J Clin Lab Invest* 1990; **50**: 395-400.
10. Cappuccio FP, Markandu ND, Beynon GW, Shore AC, Sampson B, MacGregor GA: Lack of effect of oral magnesium on blood pressure: a double blind study. *Br Med J*, 1985; **291**: 235-238.
11. The trials of Hypertension Prevention Collaborative Research group: The effects of nonpharmacologic interventions on blood pressure of persons with high normal levels. *JAMA* 1992; **267**: 1213-1220.
12. Ng LL, Davies JE, Ameen M: Intracellular free-magnesium levels in vascular smooth muscle and striated muscle cells of the spontaneously hypertensive rat. *Metabolism* 1992; **41**: 772-777.
13. Wong NLM, Sutton RAL, Dirks JH: Is lymphocyte magnesium concentration a reflection of intracellular magnesium concentration? *J Lab Clin Med* 1988; **112**: 721-726.
14. Touyz RM, Milne FJ, Reinach SG: Platelet and erythrocyte Mg^{2+} , Ca^{2+} , Na^{+} , K^{+} and cell membrane adenosine triphosphatase activity in essential hypertension in blacks. *J Hypertens* 1992; **10**: 571-578.
15. Whang R, Oei TO, Aikawa JK, *et al*: Predictors of clinical hypomagnesemia. Hypokalemia, hypophosphatemia, hyponatremia, and hypocalcemia. *Arch Intern Med* 1984; **144**: 1794-1796.
16. Jones JE, Shane SR, Jacobs WH, Flink BF: Mg balance studies in chronic alcoholism. *Ann N Y Acad Sci* 1969; **162**: 934-945.
17. Caddel JL, Reed GF: Validity of the parenteral magnesium load test for mature mammals. *Magnesium* 1989; **8**: 65-70.
18. Gullestad L, Dolva Lø, Waage A, Falch D, Fagerthun H, Kjekshus J: Magnesium deficiency diagnosed by an intravenous loading test. *Scand J Clin Lab Invest* 1992; **52**: 245-253.
19. Rasmussen HS, McNir P, Gøransson L, Balsløv S, Larsen OG, Auruo P: Magnesium deficiency in patients with ischemic heart disease with and without acute myocardial infarction uncovered by an intravenous loading test. *Arch Intern Med* 1988; **148**: 329-332.
20. Goto K, Yasue H, Okumura K, *et al*: Magnesium deficiency detected by intravenous loading test in variant angina pectoris. *Am J Cardiol* 1990; **65**: 709-712.
21. Ryzen E, Elbaum N, Singer FR, Rude R: Parenteral magnesium tolerance testing in the evaluation of magnesium deficiency. *Magnesium* 1985; **4**: 137-147.
22. Bøhmer T, Mathiesen B: magnesium deficiency in chronic alcoholic patients uncovered by intravenous loading test. *Scand J Clin Lab Invest* 1982; **42**: 633-636.
23. Oshima T, Matsuura H, Ishibashi K, *et al*: Familial influence upon NaCl sensitivity in patients with essential hypertension. *J Hypertens* 1992; **10**: 1089-1094.
24. Handwerker SM, Altura BT, Royo B, Altura BM: Ionized magnesium and calcium levels in umbilical cord serum of pregnant women with transient hypertension during labor. *Am J Hypertens* 1993; **6**: 542-545.