INTRACELLULAR MAGNESIUM DEFICIENCY AND EFFECT OF ORAL MAGNESIUM ON BLOOD PRESSURE AND RED CELL SODIUM TRANSPORT IN DIURETIC-TREATED HYPERTENSIVE PATIENTS

Kaoru Hattori, M.D., Komei Saito, M.D., Hiroshi Sano, M.D.*
and Hisashi Fukuzaki, M.D.*

The effects of magnesium supplementation were tested in 20 patients with essential hypertension receiving long-term thiazide diuretic treatment (Th group) and 21 age-matched untreated patients (EHT group). Intraerythrocyte cations, water content and the ouabain-sensitive sodium efflux rate constant were measured. The Th group received magnesium supplementations as MgO (600 mg Mg/day) for 4 weeks. In the Th group intraerythrocyte magnesium and the sodium efflux rate constant were lower and red cell sodium was higher than in the EHT group. During magnesium supplementation, there were significant decreases (p < 0.01) in intra-erythrocyte sodium content and mean blood pressure, and increases (p < 0.005) in red cell magnesium content and the sodium efflux rate constant. These effects of magnesium were more evident in 9 patients who were unresponsive to diuretic therapy, a definite reduction in mean blood pressure, from 104.8 ± 2.7 mmHg to 94.4 ± 2.2 mmHg (p < 0.001), being observed. In the remaining 11 patients, however, blood pressure remained unchanged. The sodium efflux rate constant was positively correlated with red cell magnesium content and negatively correlated with sodium content (r = 0.61, p < 0.005 and r = -0.57, p < 0.01, respectively). These results indicate that long-term diuretic treatment may give rise to intracellular magnesium deficiency and a suppression of cell membrane active sodium transport. The results also suggest that oral magnesium may decrease intracellular sodium, possibly through the activation of Na-K-ATPase, which in turn may contribute to the reduction in blood pressure. Therefore, magnesium supplementation may be a worthwhile additional therapy for diuretics.

The effects of changes in electrolyte balance — particularly of sodium and potassium — on blood pressure have been investigated and established in many studies1,2 and it is now evident that dietary sodium intake plays an important role in the development of human hypertension3. Although diet therapy and thiazide diuretics are commonly initially prescribed to treat arterial hypertension, long-term treatment with thiazide diuretics has been shown to induce urinary losses of potassium and magnesium4,5 accompanied by a deficiency in the intracellular content of these cations4,6–8. A disturbance of magnesium metabolism has recently been reported to be a critical risk factor for the development of coronary heart disease and cerebro-

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Internal Medicine, Hidaka Hospital; *The First Department of Internal Medicine, Kobe University School of Medicine, Kobe, Japan
Mailing address: Kaoru Hattori, M.D., The First Department of Internal Medicine, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650, Japan

Japanese Circulation Journal Vol. 52, November 1988 1249
vascular accidents, and a strong relationship between intracellular magnesium and blood pressure has recently been postulated.

We therefore, conducted an investigation of patients receiving long-term thiazide diuretic treatment for hypertension, and compared them to untreated patients with essential hypertension. We measured intra-erythrocyte magnesium content and sodium transport across the cell membrane, a process requiring magnesium for its adequate function. We also carried out an intervention study to clarify the effects of magnesium supplementation on blood pressure, intracellular electrolyte content and cell membrane sodium transport. Our main objective was to evaluate the mechanism of the blood pressure-lowering effect of oral magnesium and the potentially beneficial effect of oral magnesium as an additional treatment for patients already receiving diuretics.

**MATERIALS AND METHODS**

The subjects included 20 patients receiving long-term treatment with thiazide diuretics for hypertension (Th group), and 21 untreated patients with essential hypertension (EHT group). The Th group consisted of 15 males and 5 females, aged 42 to 66 years; their mean age (±SEM) was 56.5 (±2.0) years. The EHT group consisted of 14 males and 7 females, aged 41 to 65 years; their mean age (±SEM) was 54.9 (±1.5) years. The duration of diuretic treatment (trichlormethiazide 2–4 mg or methyclothiazide 2.5–5.0 mg) was 4.4 ± 3.0 years, during which time no other antihypertensive medications had been administered. Secondary hypertension was ruled out by the usual screening methods, including medical history, physical examination, urinalysis, blood biochemistries, and when appropriate, radiologic evaluation. Early in the morning, after overnight fasting, blood pressure was measured by an automatic sphygmomanometer (Nippon Colin) every 5 min for 30 min in the supine position. Blood samples were taken by venipuncture for the following determinations: intra-erythrocyte sodium, potassium, magnesium and water contents, the sodium efflux rate constant of the ouabain-sensitive component of the red cell membrane, hematocrit
values and serum sodium, potassium and magnesium concentrations. Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were also measured by radioimmunoassay, and 24-hour urinary samples were collected on the same day for evaluation of urinary excretion of sodium, potassium and magnesium.

In the TH group, the patients continued their diuretic treatment while receiving oral supplementation with 1000 mg of MgO (600 mg Mg/day) for 4 weeks and then placebo for an additional 4 weeks. At the end of each period, the same procedures (blood pressure recording and blood sampling) were performed.

**Red cell sodium, potassium, magnesium and water contents**

Intra-erythrocyte electrolyte contents were measured by the method of Kaya et al. Fifty microliters of heparinized blood were injected into a microhematocrit capillary tube (75 mm long) and then centrifuged at 15,000 × g for 5 min at room temperature. After hematocrit determination, the tube was cut at the boundary between packed erythrocytes and the plasma, and the packed cell portion was then diluted in 1 ml of diluted lithium solution. Sodium and potassium concentrations of the hemolysate were determined by a flame photometer (FLM 3, Radiometer), and magnesium by an atomic absorption spectrophotometer (Z-7000, Hitachi).

Intra-erythrocyte cation contents were approximated according to the functions described previously, and the values were corrected by the trapped inter-erythrocyte plasma which was determined using 131I-radioactive human serum albumin at the 3% level. All the measurements were performed in duplicate. The accuracy of this technique was confirmed by a preliminary study which was reported previously. For the present study, the interassay reproducibilities, as determined by the coefficients of variation, were 4.6% for red cell sodium, 2.7% for red cell potassium and 13.0% for magnesium.

Erythrocyte water content was measured by the method of Walter et al. with a slight modification. Fifty microliters of freshly drawn heparinized blood were packed into capillary hematocrit tubes and centrifuged at 6000 g for 5 min. The plasma was discarded and the packed cell portion was dried at 50°C for three days. The weight loss was determined gravimetrically, and erythrocyte water content was calculated by correcting with inter-erythrocyte trapped plasma volume. The interassay coefficient of variation within 7 pairs of each sample was 2.9% for the hematocrit value and 3.4% for the intra-erythrocyte water content.

**Ouabain-sensitive sodium efflux and potassium influx**

The erythrocyte sodium efflux rate constant...
was measured using the method of Cumberbach et al\textsuperscript{14} with a slight modification. Three milliliters of venous blood were admixed with 20 \( \mu l \) of saline buffer containing 10\(^{-4}\) M ouabain, and immediately incubated in a shaking water bath at 37\(^\circ\)C for 120 min. Before and at the end of incubation, 50 \( \mu l \) of the blood were aspirated, cooled immediately at 4\(^\circ\)C, and intra-erythrocyte sodium and potassium were determined as described above. The red cell cation flux rate constants were determined by dividing the difference in red cell sodium or potassium before and after incubation by the initial value for these cations.

All the measurements were performed in duplicate, and the accuracy of this technique was confirmed in the preliminary study through repeated determinations. Within 7 pairs of each sample obtained on different days, the coefficient of variation was 8.9\% for the sodium efflux rate constant.

Data analysis was performed by Student's paired-\( t \) or unpaired-\( t \) test as appropriate. Values were expressed as mean \pm standard error of the mean <SEM>. Differences were considered significant when the \( p \) value was less than 0.05.

**RESULTS**

**Effect of long-term thiazide diuretics in hypertensives**

Age and sex distributions were similar for both the EHT and Th groups. Serum potassium was lower (\( p < 0.01 \)) and PRA was higher (\( p < 0.05 \)) in the Th group than in the EHT group, while average values for the other baseline characteristics showed no intergroup differences (Table I).

Intra-erythrocyte cation contents, water content and red cell cation fluxes are indicated in Table II. The baseline level of red cell sodium in the Th group was significantly higher than that in the EHT group, although red cell potassium showed no difference between the two groups. The red cell magnesium content was significantly lower in the Th group than in the EHT group, while the water content was the same in both groups.

The absolute value and rate constant for red cell membrane sodium efflux were significantly lower in the Th than in the EHT group (Table II), while potassium influx was not different between the two groups. As for the relationship between cell sodium content, plotted on the X axis, and
membrane Na-K-ATPase activity, plotted on the Y axis a negative correlation was noted both in the EHT group (Y = -0.0264 X + 0.420, r = -0.57, p < 0.02) and the Th group (Y = -0.0114 X + 0.253, r = -0.66, p < 0.01).

Effect of magnesium supplementation

In the Th group, magnesium supplementation for 4 weeks resulted in a significant reduction in systolic, diastolic and mean blood pressure levels, with mean reductions of -7.5, -3.0 and -4.5 mmHg, respectively. Pulse rate, hematocrit value, serum concentration and urinary excretion of electrolytes, PRA and PAC showed no significant changes throughout the study periods (Table I).

The effects of oral magnesium supplementation on red cell cation contents and fluxes are presented in Table II. During the magnesium supplementation period, although serum magnesium concentration showed no significant change, red cell magnesium content increased significantly (p < 0.005). The blood pressure level prior to oral magnesium administration was negatively correlated with the overall change in blood pressure after oral magnesium (r = -0.71, p < 0.001). When the supplementation was changed from magnesium to placebo, the red cell magnesium content tended to decrease again. Oral magnesium resulted in a decrease in red cell sodium content (p < 0.01), while the serum potassium concentration and intra-erythrocyte potassium content remained unchanged. The red cell sodium efflux value and the efflux rate constant were significantly increased (p < 0.005), although potassium influx and its rate constant showed no significant change. When the placebo was administered instead of magnesium for 4 weeks, sodium accumulated in the erythrocytes (p < 0.005) and the red cell sodium efflux rate constant was again suppressed (p < 0.05).

Statistical analysis revealed that during magnesium supplementation the red cell sodium efflux rate constant was positively correlated.
with intracellular magnesium concentration and negatively correlated with red cell sodium content (r = 0.61, p < 0.005 and r = −0.57, p < 0.01, respectively, Fig. 1a, Fig. 1b).

The patients were then divided into two groups, responders and non-responders, according to how their blood pressure responded to the oral magnesium supplementation. Nine out of 20 patients showed significant mean blood pressure reduction (ΔMBP) of 3% or more (responder, ΔMBP: −11.1 ± 2.0 mmHg) and the other 11 patients showed no reduction in mean blood pressure (non-responder, ΔMBP: +0.136 ± 0.46 mmHg). Although the two groups did not differ with regard to age, sex, serum and urinary electrolyte values, red cell cation contents or flux values, the average baseline mean blood pressure level was significantly higher in the responder than in the non-responder group (104.8 ± 2.7 mmHg vs 93.1 ± 3.0 mmHg, respectively, p < 0.02). Magnesium supplementation resulted in a significant decrease in red cell sodium, from 10.48 ± 0.40 mEq/l cells to 9.84 ± 0.43 mEq/l cells (p < 0.005, Fig. 1a), and a significant increase in the sodium efflux rate constant, from 0.126 ± 0.009 hr⁻¹ to 0.182 ± 0.013 hr⁻¹ (p < 0.001, Fig. 1b), in the responder, but not in the non-responder sponders who had the highest blood pressures before magnesium supplementation, the greatest increases in sodium efflux rate constants (r = 0.47, p < 0.02) and the greatest decreases in intracellular sodium content (r = −0.64, p < 0.005) occurred.

DISCUSSION

Intra- and extracellular potassium and magnesium concentrations have been reported to decrease during long-term treatment with thiazide diuretics⁴,⁶,¹⁵−¹⁷ as determined from materials obtained by skeletal muscle biopsy and/or by measurements of serum and intraerythrocyte electrolyte concentrations. However, the investigators all seem to agree on a discrepancy between serum and intracellular electrolyte concentrations. Dyckner and Wester⁶,¹⁸ observed successively decreasing values for serum and skeletal muscle magnesium, but found no relation between the two values. Likewise for potassium, long-term diuretic treatment has been reported to induce a decrease in serum potassium without any significant change in muscle⁶ and red cell potassium contents¹⁵. To estimate intracellular magnesium, muscle biopsy might be desirable, though, it is not available as a routine and repeatable clinical method. As for blood since leukocytes consist of many different cell types, and their relative ratios are always changing¹⁶ we have used red blood cells for measurement of intracellular magnesium. In the present study, serum potassium and intra-erythrocyte magnesium were reduced in thiazide-treated patients, while cellular potassium and serum magnesium levels were similar to those in the untreated patients. The serum concentration of magnesium is not considered an adequate measure for assessing the body’s magnesium status, because magnesium is mainly an intracellular cation, and the extracellular amount constitutes only a small percentage of the total body stores.

With regard to cell membrane sodium handling, in patients under long-term diuretic treatment we observed a decrease in the ouabain-sensitive component of the red cell sodium efflux process and an increase in cell sodium content. These effects of thiazide diuretics on red cell sodium transport are consistent with the findings of Kezdi et al¹⁵ who observed a marked decrease in red cell membrane Na⁺-K⁺-ATPase activity and a resultant increase in sodium content without any change in intracellular potassium content. Cell membrane Na⁺-K⁺-ATPase activity is known to be stimulated by extracellular potassium ions¹⁹ and to require magnesium for its adequate function²⁰. Thus, the reduced serum potassium and the cellular magnesium deficiency observed in our patients might have contributed to the suppression of Na⁺-K⁺-ATPase activity and to the decrease in erythrocyte membrane sodium efflux. As we have previously reported¹²,¹¹ that there is an inverse relation between Na⁺-K⁺-ATPase and intra-erythrocyte sodium content in patients with untreated essential hypertension and with uremia, a negative correlation was noted between cell sodium content and membrane Na⁺-K⁺-ATPase activity both in our EHT and the Th groups.

To correct the magnesium deficiency, we administered magnesium supplements to the hypertensives who had been receiving long-term thiazide monotherapy. The present results indicate that magnesium supplementation lowered intra-erythrocyte sodium content and enhanced the sodium efflux rate constant. Simultaneously, intra-erythrocyte magnesium content increased, although the serum concentration was not changed. In addition, when oral magnesium was replaced with a placebo, the intracellular sodium

Japanese Circulation Journal Vol. 52, November 1988
content and the sodium efflux rate returned to their baseline values, while the placebo administration did not appear to affect serum potassium and magnesium concentrations. In the magnesium deficient state, caused by long-term diuretic therapy, magnesium replacement has been found to restore skeletal muscle magnesium values to normal while no significant changes in extracellular potassium and magnesium concentrations have been reported. In the present study, following oral magnesium supplementation, the red cell sodium efflux rate correlated positively with the increased cellular magnesium content and negatively with the decreased red cell sodium concentration. These data are consistent with our previous findings observed in untreated hypertensive patients and suggest that magnesium supplementation activates Na-K-ATPase which, in turn, corrects the intracellular sodium accumulation.

The effects of oral magnesium on blood pressure were significant in the present study, both systolic and diastolic blood pressures being lowered. Dyckner et al. reported the same effect for oral magnesium on blood pressure, although Cappuccio et al. observed no such reduction. This discrepancy may occur, in part, due to differing degrees of magnesium deficiency among subjects.

In the present study, we examined the degree of blood pressure change in more detail and found that about half of the subjects showed a definite blood pressure reduction. In particular, patients whose mean blood pressure was reduced by over 3% while receiving oral magnesium also showed a significant decrease in intra-erythrocyte sodium content, with an increased cell sodium efflux. On the other hand, those whose blood pressures remained unchanged showed no significant changes in either cell sodium content or cellular sodium transport. It has already been reported that elevation of intra-erythrocyte sodium content correlated with blood pressure level that suppression of membrane sodium efflux caused by salt retention increased blood pressure and that intracellular calcium accumulation effected the pressor mechanism of suppressed sodium transport caused by salt retention. In this study patients whose blood pressure were lowered by magnesium supplementation, had previously shown poor blood pressure control with diuretics. In addition their intra-erythrocyte sodium content had been especially high and their cell sodium efflux had been suppressed. However, these findings cannot completely explain the mechanism of oral magnesium's anti-hypertensive effect since some patients whose blood pressure was unchanged by magnesium supplementation still showed decreases in red cell sodium content and increases in intracellular sodium efflux, indicating that improved cellular sodium transport was not the only cause of blood pressure reduction induced by oral magnesium.

Other mechanisms for magnesium effect on blood pressure can be hypothesized, such as some direct effect on the blood vessels, a sympathetic nervous response to magnesium or a variation in intracellular calcium levels among individuals, but it is impossible to clarify any of these in this study. We therefore conclude that an increase in Na-K-ATPase activity with a resultant decrease in cell sodium content may be involved, at least in part, in the blood pressure-lowering effect of oral magnesium. Magnesium supplementation should thus be considered as an additional therapy for patients receiving magnesium-depleting thiazide diuretics, especially for those whose blood pressure is poorly controlled by diuretics, and for those with significant intracellular sodium retention due to impaired membrane sodium transport.

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Japanese Circulation Journal Vol. 52, November 1988