Aging impairs the protective effect of magnesium supplementation on hypertension in spontaneously hypertensive rats

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Abstract. Preeclampsia is a hypertensive disorder that is unique to pregnancy. Magnesium (Mg²⁺) supplementation is a potential new therapy to ameliorate development of hypertension. The aim of this work was to compare the effects of Mg²⁺ supplementation on systolic blood pressure (SBP) in young and aged rats. Spontaneously hypertensive rats (SHR) were divided into young (6-week-old male, n = 10) and old (16-week-old male, n = 10) groups. Each group of rats comprised two subgroups made of a control subgroup fed with normal rat chow (0.2% Mg²⁺, n = 5) and a high Mg²⁺ subgroup nourished with a Mg²⁺ rich diet (0.8% Mg²⁺, n = 5). Age-matched Wistar-Kyoto rats (WKY) were also allocated into two groups. SBP was assessed weekly for 12 weeks indirectly by the tail-cuff method. SBP increased progressively in SHR-young rats after 7 weeks. This increase was greater in the control subgroup compared to high Mg²⁺ subgroup at 7 weeks (p < 0.05). No difference in the SBP was registered between old SHR subgroups. Mg²⁺ supplementation does not exert antihypertensive effects in the WKY rats. In conclusion, Mg²⁺ supplementation may provide beneficial effect in the developmental phase of hypertension but not in established hypertension.

Key words: aging, hypertension, magnesium

Preeclampsia, a syndrome of pregnant women, is one of the leading causes of maternal and fetal morbidity and mortality [1]. Severe dysfunction of multiple organs including lung, kidney, liver, and brain as a consequence of preeclampsia warrants aggressive multidisciplinary management and treatment. The treatment of the major complication of this disorder, hypertension, has been the focus of much research. Magnesium sulfate is the first line drug for acute treatment and prophylaxis of hypertension-induced eclampsia. There was some evidence of a reduced risk of preeclampsia with a high intake of Mg²⁺ [2]. Previous studies have shown that dietary Mg²⁺ supplementation reduces arterial blood pressure in young (developing hypertension) SHR rats [3, 4] but that Mg²⁺ supplementation does not exert antihypertensive effects in aged rats with established hypertension [5]. Although the mechanisms whereby Mg²⁺ influences increased blood pressure have not been fully elucidated, these previous models independently imply that animal age may be a fully controlling limiting factor with respect to SBP, suggesting that the absence of the antihypertensive effect of Mg²⁺ supplementation in aged SHR may be related to...
microvascular damage rather than autonomic dysfunction that occurs with development of hypertension.

The goal of this study was to evaluate the effect of Mg²⁺ supplementation on SBP in SHR and WKY rats. In the present study, both rats were divided into young (6 weeks old) and old (16 weeks old) groups. Our results, that Mg²⁺ reduces SBP in an age-specific manner only in the SHR, may strengthen the previous hypothesis.

Materials and methods

Male SHR (n = 20) and age-matched Wistar-Kyoto rats (WKY) (n = 20) were obtained through the Nagoya Institutes (Aichi, Japan) and housed on a 12L:12D cycle, temperature, + 22°C. At 6 weeks (young) and 16 weeks (old) of age, rats were divided into two groups: control group (normal rat chow containing Mg²⁺ 0.2% of the dry weight of the chow) and Mg²⁺-supplemented group (high Mg²⁺, chow containing 0.8% Mg²⁺). Namely, SHR were randomly divided into four groups of five animals each: young control SHR (young-SHR), young Mg²⁺ treated SHR (young-Mg-SHR), old control SHR (old-SHR), and old Mg²⁺ treated SHR (old-Mg-SHR). WKY were also randomly divided into four groups of five animals each. Mg²⁺ was given in the form of magnesium oxide. Experimental diet and drinking water were available ad libitum for 12 weeks. The supplementations and indirect blood pressure measurements were continued for 12 more weeks. All animal studies were conducted in accordance with the principles and procedures outlined in A Guide to the Care and Use of Experimental Animals prepared by the Nara Medical University.

The SBP of conscious animals held in plastic restrainers were measured at +28°C using the tail-cuff method (Model UR-5000, Ueda Co., Ltd., Tokyo, Japan) with an acclimation period of about 30 min preceding the measurements [6]. Values for SBP were obtained by averaging readings from three to five measurements.

Data are expressed as means ± SEM. Comparisons between groups were made using non-paired Student t-test. All statistical analysis was performed using StatView (HULINKS, Tokyo) for Windows. Differences were considered as significant at p < 0.05.

Results

At 6 weeks of age, SBP was similar in the two groups (young-SHR, 156 ± 8 mmHg; young-Mg-SHR, 150 ± 7 mmHg). SBP increased gradually with age in the young-SHR group: in rats at 18 weeks of age, SBP was 241 ± 7 mmHg (figure 1, left). In the young-Mg-SHR group fed a high Mg²⁺ diet, SBP increased for the first 6 weeks and then reached a plateau at ~ 210 mmHg, which was significantly lower than that in age-matched rats in the young-SHR group. Dietary

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**Figure 1.** Line graphs show systolic blood pressures in untreated young-SHR (young-SHR), Mg²⁺-supplemented young-SHR (young-Mg-SHR), untreated old-SHR (old-SHR), and Mg²⁺-supplemented old-SHR (old-Mg-SHR). Values represent mean ± SEM, n = 5 in each group; * P < 0.05. Open circle, normal chow; filled circle, Mg²⁺-supplemented chow.
Mg\textsuperscript{2+} supplementation decreased SBP by ≈ 25 mmHg at 8 weeks and by ~20 mm Hg at 12 weeks in young rats. At 16 weeks of age, SBP was similar in the two groups (old-SHR, 216 ± 9 mmHg; old-Mg-SHR, 220 ± 7 mmHg). SBP plateau-ed at ≈ 240 mmHg both in the old-SHR and old-Mg-SHR group at 21 weeks of age (figure 1, right). During the 12-week-long follow up, no protection against SBP was observed in the old-SHR even in the presence of Mg\textsuperscript{2+} supplementation.

The SBP in WKY (115 ± 5 mmHg in 6 week-old rats; 130 ± 5 mmHg in 16 week-old rats) were already lower at the beginning of the study than in SHR. As shown in figure 2, SBP still remained lower in all WKY groups than in the SHR groups. No significant alteration in SBP was observed in the young (figure 2, left) and old-WKY (figure 2, right) during the 12-week-long follow up. Mg\textsuperscript{2+} supplementation did not affect hypertension in the young and aged WKY.

Discussion

It has long been recognized that Mg\textsuperscript{2+} is associated with several important diseases, including diabetes, hypertension, cardiovascular, and cerebrovascular diseases [7]. Using an established animal model of hypertension, we tested the hypothesis that Mg\textsuperscript{2+} would have reduced efficacy in young compared with aged rats. Long-term Mg\textsuperscript{2+} supplementation may attenuate the development of hypertension in the young SHR during the developmental phase of hypertension, but not in the aged SHR with an established phase of hypertension. Thus, Mg\textsuperscript{2+} supplementation during an early phase in the development of hypertension confers long-term protection against developing defined hypertension. No effects on SBP were observed in age-matched normotensive WKY rats.

Our study was able to confirm the previous independent findings that the Mg\textsuperscript{2+} intake decreases SBP in the 6-week-old SHR [8] and that Mg\textsuperscript{2+} supplementation has no antihypertensive effect in 17-week-old SHR [5]. Wolf et al., [9] reported that the SBP was lower in young and mature SHR fed with a high Mg\textsuperscript{2+} diet than in the young and mature SHR fed with a normal Mg\textsuperscript{2+} diet. They also confirmed that the hypotensive effect is not related to an inhibition of the renin release. Although clinical and experimental investigations have been contradictory [8, 10, 11], these conflicting results may depend on the age of animals used in the studies and be partially attributable to the dose and duration of Mg\textsuperscript{2+} supplementation. Taken together, cellular magnesium handling may be disturbed in old SHR, but not in young rats.

Two pathways have been proposed for Mg\textsuperscript{2+} transport across the plasma membrane in smooth muscle: 1) Na\textsuperscript{+} (gradient)-dependent Mg\textsuperscript{2+} extrusion (Na\textsuperscript{+}-Mg\textsuperscript{2+} exchange); 2) Na\textsuperscript{+}-independent passive Mg\textsuperscript{2+} flux, depending on the Mg\textsuperscript{2+} concentration gradient, and blocked by extracellular Ca\textsuperscript{2+} [12]. Na\textsuperscript{+}-Mg\textsuperscript{2+}
exchange is considered to be the major Mg\(^{2+}\) pathway [13]. Taken together, it is suggested that the Na\(^{+}\)-Mg\(^{2+}\) exchange mechanism may be impaired in the older SHR.

The mechanisms underlying the Mg\(^{2+}\)-dependent antihypertensive effects may involve increased sensitivity of vascular smooth muscle to nitric oxide (NO) or decreased production of vasoconstrictor prostanoids. Aging is associated with an overall increase in oxidative stress [14] and changes in the levels of antioxidants and antioxidant enzymes [15]. Mg\(^{2+}\) supplementation in the young rats may reduce the formation of peroxynitrite and increased bioavailability of NO [8]. Additionally, Mg\(^{2+}\) is able to improve endothelium-dependent vascular relaxation in response to acetylcholine [6]. Small arteries undergo autonomic dysfunction and microvascular damage which occur with aging and the development of hypertension [16]. If these changes are already evident in 16-week-old rats, Mg\(^{2+}\) supplementation would not improve the established hypertension. Further studies are required to elucidate why Mg\(^{2+}\) supplementation lowers blood pressure only in younger SHR.

Conclusion

The present data suggest that Mg\(^{2+}\) supplementation may provide beneficial effects in younger SHR and lead us to propose that the clinical design of Mg\(^{2+}\) supplementation may offer protection in pregnancy-induced hypertension. Clinical studies in mild pre-eclamptic patients (during the developmental phase of hypertension) to assess the possible protective effects of Mg\(^{2+}\) supplementation would appear to be worthwhile.

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References