Effect of Magnesium Deficiency on Blood Pressure and Mechanical Properties of Rat Carotid Artery

Pascal Laurant, Daniel Hayoz, Hans R. Brunner, Alain Berthelot

Abstract—The purpose of this study was to determine the effect of dietary Mg deficiency (80 mg/kg versus control diet: 960 mg/kg) on blood pressure and mechanical properties of the rat common carotid artery. The internal diameter and intra-arterial pressure of carotid artery were measured continuously with an echo-tracking device. At 19 weeks, systolic, diastolic, and mean blood pressures were higher in Mg-deficient rats. Histological examination showed an increase in cross-sectional area, intima-media thickness, and media-to-lumen ratio in carotid artery of Mg-deficient rats. Mg deficiency did not modify the arterial distensibility–blood pressure curve. At mean blood pressure, arterial distensibility was significantly less in 19-week-old rats than in 5-week-old rats of both control and Mg-deficient groups. A significant interaction between age and Mg-deficient diet on arterial distensibility (P<0.04) indicates an accelerated age-dependent decreased arterial distensibility with Mg deficiency. At 19 weeks, the artery was stiffer in hypertensive Mg-deficient rats, as illustrated by a shift to higher levels of the incremental elastic modulus–stress curve. In conclusion, the increased blood pressure and the vascular morphological alterations observed in Mg-deficient rats may contribute to an accelerated alteration of the wall material, which in turn leads to a stiffening of the carotid artery. (Hypertension. 1999;33:1105-1110.)

Key Words: ultrasonography ■ carotid arteries ■ elastic modulus ■ calcium ■ magnesium deficiency

Epidemiologic and experimental evidence indicates that Mg deficiency may be considered a risk factor for cardiovascular diseases and hypertension.1,2 Several epidemiological studies have shown that Mg consumption is inversely related to blood pressure (BP).3 In humans, one study has shown that dietary Mg deprivation elevates BP.4 Experimental investigations in rats also indicate that long-term dietary Mg deficiency increases BP and induces sustained hypertension.5–7 In vitro, decreasing Mg concentration results in increased vascular tone, increased vascular reactivity, and reduction in peripheral blood flow.1,2 Mg deficiency–induced hypertension in rats is associated with reduced arteriolar, venular, and precapillary lumen size, suggesting an increase in myogenic tone.5 Early studies have shown that Mg deficiency induces vascular lesions, including wall thickness, endothelial and smooth muscle cell hyperplasia, inflammation of the media and the intima, and fibrinoid necrosis of the blood vessels.8,9 Epidemiologically, an inverse relationship between serum Mg concentration and intima-media thickness (IMT) of the carotid artery has been recently demonstrated.10 All these findings suggest that Mg deficiency contributes to structural modifications of blood vessels, mainly arteries, that should alter their viscoelastic properties.

BP levels seem to be closely correlated to vascular structure.11 Chronic elevation of BP in both animals and humans is characterized by an increase in arterial wall thickness.11 These structural modifications should decrease the buffering function and modify the wall elastic properties of the conductance arteries. Whether Mg deficiency contributes to modify elastic properties of the conductance arteries is not known. The purpose of the present investigation was to study the effect of long-term dietary Mg deficiency intake on structural and elastic properties of the rat common carotid artery (CCA).

Methods

Thirty Wistar male rats (3 weeks old) weighing 60 g (IFFA CREDO, L’Arbresle, France) were used. Animal care, surgical preparation, and experimental procedures were approved by the government review committee. The rats were housed in plastic cages with a constant temperature of 23°C, constant humidity (50% to 60%), and a daily 12-hour light/dark cycle. They were randomly divided into pair-fed with the appropriate diets for 19 weeks. The synthetic diets contained the following (%): casein 20, starch 40, sucrose 21, cellulose 6, groundnut oil 2.5, corn oil 2.5, mineral mixture 7, vitamin mixture 1. Mg was given in the form of MgO.

Systolic BP was measured in unanesthetized restrained prewarmed rats by the indirect tail-cuff method with a sphygmomanometer (PE-3000, Narco Biosystem). The lowest and the highest values were...
Results

Effect of Mg-Deficient Diet on BP

During the first 6 weeks of magnesium deficiency, the Mg-deficient rats presented hyperemia of the ears, alopecia, and ulceration of the skin. Until week 8, BP was similar in both control and Mg-deficient rats. After 8 weeks, until the end of the experimental period, the BP of the Mg-deficient rats was significantly higher than in the control rats (P<0.01) (Figure 1). Body weight increased with time, and Mg-deficient diet significantly altered growth of the rats (0.33±0.01% versus 0.26±0.01%; P<0.05). When the rats were anesthetized at 5 weeks, systolic, diastolic, and mean BPs of the control and the Mg-deficient rats were not significantly different. At 19 weeks, systolic, diastolic, and mean BPs were significantly higher in Mg-deficient rats than in control rats (P<0.05). Two-way ANOVA, however, indicated significant interaction between age and Mg-deficient diet on BPs (P<0.02). Pulse pressures were similar in the 2 groups (Table 1).

Effect of Mg-Deficient Diet on Mechanical Parameters of CCA

Mg deficiency did not affect the ID-BP curve at 5 weeks but induced a significant downward shift of the curve at 19 weeks (P<0.01) (Figure 2). At mean BP, ID of the CCA of the Mg-deficient rats treated for 19 weeks was significantly smaller (P<0.05) than in those from control rats (Table 1). The increase in BP significantly decreased arterial distensibility of the CCA from both control and Mg-deficient rats. Mg deficiency did not modify the arterial distensibility–BP curves at 5 and 19 weeks (Figure 2). At mean BP, arterial distensibility was less in the aged rats from both groups than in the young rats of the same groups, respectively (P<0.05). At 19 weeks, arterial distensibility was slightly, but not significantly, decreased in the Mg-deficient rats compared with the age-matched control rats. Two-way ANOVA, however, indicated significant interaction between age and Mg-deficient diet on arterial distensibility (P<0.04), suggesting that distensibility had decreased more with age in the Mg-deficient rats than in the control rats (Table 1). Intergroup linear regression showed that distensibility was inversely related to systolic, diastolic, and mean BP (5 weeks: P<0.01, r = −0.6319, r² = 0.3993; 19 weeks: P<0.0001, r = −0.9199, r² = 0.8462).

At mean BP, wall stress was significantly less in CCA from the Mg-deficient rats than in those from the control rats at 5 and 19 weeks. When E inc was plotted against wall stress, the curves were not different for Mg-deficient and control rats at 5 weeks. In the small range of nearly overlapping stress values, the E inc values obtained in Mg-deficient rats treated for 19 weeks was significantly greater than in control rats (P<0.05) (Figure 2). At mean BP, E inc was not different between the Mg-deficient and the control rats at 5 and 19 weeks (Table 1).
Effect of Mg-Deficient Diet on Histomorphometric Characteristics of CCA

The IMT, CSA, and M/L ratio of the fixed CCA artery were significantly greater (P<0.05) in Mg-deficient rats at 5 and 19 weeks. Mg deficiency did not affect the external diameter (Table 2).

Effect of Mg-Deficient Diet on Biochemical Parameters

When the rats were fed the Mg-deficient diet for 5 and 19 weeks, the plasma total Mg concentration was significantly lower, whereas total Ca and cholesterol concentrations were significantly higher than in the control rats. Triglyceride concentration was significantly higher (P<0.05) in the Mg-deficient rats at 19 weeks (Table 3). At mean BP, plasma Ca concentration was positively correlated with IMT of the CCA of both Mg-deficient and control rats (5 weeks: P<0.0023, r²=0.7239, r²=0.5241; 19 weeks: P<0.0018, r²=0.7562, r²=0.5718). At 19 weeks, an inverse correlation was found between plasma Ca and ID (P<0.01, r=-0.6525, r²=0.4257) and plasma Ca and wall stress (P<0.006, r=-0.6917, r²=0.4784) (Figure 3). At 19 weeks, plasma triglycerides were positively correlated with IMT (P<0.0001, r²=0.6503) and negatively correlated with wall stress (P<0.0005, r=-0.8064, r²=0.6503) (Figure 3). No significant relation was found between distensibility or Einc with the biochemical parameters studied. Multiple regression analysis for IMT or wall stress at 19 weeks showed that the effect of plasma Ca was no longer significant when added to plasma triglycerides (IMT; r²=0.919, P=0.003 for triglycerides, P=0.1872 for Ca; wall stress; r²=0.704, P=0.0035 for triglycerides, P=0.1872 for Ca), implying an interaction between triglycerides and Ca on IMT and wall stress.

Discussion

The data of the present study provide the first in vivo evidence that dietary Mg deficiency induces changes in the mechanical properties of the rat CCA. The increased thickness of the carotid artery wall, concomitant with the chronic elevation of BP observed in Wistar rats fed with the Mg-deficient diet for 19 weeks, was associated with an increase in
arterial stiffness and an accelerated age-dependent decrease in arterial distensibility. No measurable mechanical alterations appeared at the early phase of dietary Mg deficiency (when BP did not change), despite an increase in IMT. These findings first suggest that the mechanical properties of large arteries depend on BP level and arterial wall structure.13

Increased BP and sustained chronic hypertension have been previously reported in rats fed a Mg-deficient diet.5–7 The fact that Mg deficiency elevated both systolic and diastolic BP, as previously described,6 suggests that Mg deficiency increases ventricular ejection and peripheral resistance. The greater cardiac weight index, indicating the existence of ventricular hypertrophy, would support an increase in isotropic activity in hypertensive Mg-deficient rats. In addition, chronic Mg deficiency, or hypomagnesemia, elevates vascular tone, potentiates vasoconstrictor activity to various agonists, and attenuates responses to various dilator agents,4,6 leading to increased peripheral resistance and thus to increased BP.

In the present study the CCA of Mg-deficient rats had a greater CSA, IMT, and M/L ratio, whereas external diameter did not change. These findings indicate both vascular remodeling and wall hypertrophy. The smaller ID of CCA observed in vivo in Mg-deficient rats may reflect an enhanced arterial tone, as will be discussed below. However, a confounding factor is that the body weight gain in Mg-deficient rats was lower than in control rats. The difference in ID therefore might also be accounted for by the difference in growth.

Increasing BP elevates stress of the arterial wall. To counteract the rise in wall tension, chronic hypertension induces outward hypertrophic remodeling of the large blood vessels. As a consequence of chronic increased BP, wall thickening normalizes circumferential wall stress.13 Our findings, however, report that the Mg-deficient diet induced thickening in the arterial wall before elevation of BP. In addition, wall stress was lower in Mg-deficient than in control rats before and after elevation in BP. These findings suggest that the adaptive process that tends to maintain optimal wall tensile stress in response to BP levels was ineffective in Mg-deficient rats and that Mg deficiency may stimulate growth and/or proliferation of arterial wall constituents independently of BP elevation. Early studies have shown that Mg deficiency induces morphological changes in arteries, including hyperplasia and proliferation of endothelial and smooth muscle cells, calcification, fibrinoid necrosis, and edema with inflammatory infiltration.8,9 Furthermore, Mg deficiency increases the production of inflammatory and mitogenic factors by vascular smooth muscle and endothelial cells.2

The present study demonstrates, at mean BP, an intergroup positive linear relationship between IMT of CCA and plasma total Ca or triglyceride concentrations and a negative linear relationship between wall stress and the same plasma parameters. These findings strongly support the hypothesis that the elevation of circulating Ca and triglyceride levels induced by Mg deficiency1,2,9,14,15 may contribute to increased IMT of CCA of the rat. Ca stimulates growth of various cells and mediates migration, proliferation, matrix production, and necrosis of vascular smooth muscle cells.16–18 Extracellular Mg concentration influences Ca entry, binding, translocation, and intracellular mobilization in vascular smooth muscle cells.1,19,20 Hence, the decrease in extracellular Mg concentration, or hypomagnesemia, will lead to an enhanced intracellular Ca level. Ca overloading in heart and blood vessels occurs as a general consequence of Mg deficiency.6,9

TABLE 2. Histomorphometric Characteristics of Carotid Arteries From Control and Mg-Deficient Rats at 5 and 19 Weeks

<table>
<thead>
<tr>
<th></th>
<th>5 Weeks</th>
<th>19 Weeks</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=8)</td>
<td>Mg-Deficient (n=8)</td>
<td>Control (n=7)</td>
</tr>
<tr>
<td>IMT, μm</td>
<td>22.2±0.8</td>
<td>27.2±0.7*</td>
<td>25.7±0.4‡</td>
</tr>
<tr>
<td>ED, μm</td>
<td>601±11</td>
<td>615±19</td>
<td>632±15</td>
</tr>
<tr>
<td>CSA, 10^-3 · μm²</td>
<td>40.5±1.9</td>
<td>50.3±2.3*</td>
<td>48.9±1.3‡</td>
</tr>
<tr>
<td>M/L, %</td>
<td>8.0±0.3</td>
<td>9.8±0.3*</td>
<td>8.9±0.3</td>
</tr>
</tbody>
</table>

ED indicates external diameter. n=number of rats. Values are mean±SEM.

*P<0.05 vs age-matched control rats.
†P<0.05 vs 5-week treated Mg-deficient rats.
‡P<0.05 vs 5-week control rats.

TABLE 3. Biochemical Parameters Obtained in Plasma From Control and Mg-Deficient Rats at 5 and 19 Weeks

<table>
<thead>
<tr>
<th></th>
<th>5 Weeks</th>
<th>19 Weeks</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=8)</td>
<td>Mg-Deficient (n=8)</td>
<td>Control (n=7)</td>
</tr>
<tr>
<td>Mg, mmol/L</td>
<td>0.87±0.05</td>
<td>0.36±0.06*</td>
<td>0.75±0.02</td>
</tr>
<tr>
<td>Ca, mmol/L</td>
<td>2.69±0.04</td>
<td>2.89±0.03*</td>
<td>2.85±0.02‡</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>1.65±0.05</td>
<td>1.87±0.06*</td>
<td>1.93±0.10</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.13±0.11</td>
<td>1.14±0.06</td>
<td>1.32±0.09</td>
</tr>
</tbody>
</table>

n=number of rats. Values are mean±SEM.

*P<0.05 vs age-matched control rats.
†P<0.05 vs 5-week treated Mg-deficient rats.
‡P<0.05 vs 5-week control rats.
vascular diseases. Recently, it has been shown that phenomena may be deleterious to the function, composition, lipid metabolism in the arterial wall. Recent experimental findings suggest that Mg deficiency may contribute to arterial stiffness in hypertension. Furthermore, the same authors demonstrate that intracellular free Mg depletion observed with age may be one possible cellular mechanism mediating the age-related decrease in arterial distensibility.

Arterial isobaric distensibility decreases with age. In our study distensibility significantly decreased with age in both groups of rats, when assessed under isobaric conditions and at mean BP. Two-way ANOVA indicated that the age-dependent decrease in arterial distensibility was more pronounced in the carotid artery of Mg-deficient rats. Furthermore, it was demonstrated that an intergroup negative linear relationship exists between arterial distensibility (assessed at mean BP) and BP, whereas under isobaric conditions, arterial distensibility was similar in both hypertensive Mg-deficient and control rats. These findings suggest that distensibility is highly related to operating BP levels and that, at mean BP, hypertensive Mg-deficient rats would exhibit an accelerated age-dependent decrease in arterial distensibility, which would contribute to an increase in arterial stiffness. Recent clinical reports have shown a positive linear relationship between aortic distensibility and intracellular free Mg concentration in hypertensive subjects, suggesting that intracellular Mg deficiency may contribute to arterial stiffness in hypertension. Furthermore, the same authors demonstrate that intracellular free Mg depletion observed with age may be one possible cellular mechanism mediating the age-related decrease in arterial distensibility.

Distensibility is dependent on the geometry of blood vessels and the stiffness of vascular wall components defined by $E_{inc}$. In hypertensive Mg-deficient rats, $E_{inc}$ plotted against wall stress, which is recognized as the best determinant of wall stiffness, showed a significant increased stiffness of the wall constituent of the CCA. In contrast, at the early phase of dietary treatment, before the elevation of BP in Mg-deficient rats, $E_{inc}$ was similar for a given level of stress for the 2 groups. It appears that with a longer duration of dietary Mg deficiency, the wall constituents of the arteries become significantly more rigid than those of the arteries of control rats. Mg deficiency causes changes in the composition of the arterial wall between hypertensive Mg-deficient and control rats. Mg deficiency causes changes in the composition of blood vessels, including calcification, increased collagen, and decreased elastin content. Thinning, fragmentation of the elastic membranes, and modification in synthesis, turnover, increased intracellular Ca concentration results in vascular smooth muscle contraction and increases vascular tone. Increasing vascular tone contributes to a thickening of the vascular wall and to a reduction in the lumen diameter. Our study demonstrated an intergroup negative linear relationship between plasma total Ca concentration and ID of CCA. These findings suggest that CCA of hypertensive Mg-deficient rats for equivalent wall stress, and plasma Ca or triglyceride concentrations within the groups of rats fed control and Mg-deficient diets for 19 weeks.

Increased intracellular Ca concentration results in vascular smooth muscle contraction and increases vascular tone. Increasing vascular tone contributes to a thickening of the vascular wall and to a reduction in the lumen diameter. Our study demonstrated an intergroup negative linear relationship between plasma total Ca concentration and ID of CCA. These findings suggest that CCA of hypertensive Mg-deficient rats may exhibit enhanced arterial tone and that the well-known antagonistic properties between Mg and Ca on smooth muscle contraction may be fundamental.

Experimental and clinical evidence suggests that alterations of lipid metabolism induced by Mg deficiency are linked to the development of atherosclerosis and that dietary Mg intake plays an important modulatory role in controlling lipid metabolism in the arterial wall. Recent experimental findings also demonstrate that Mg deficiency increases lipid peroxide and oxygen-derived free radical production in vascular and cardiac tissues, alters membrane phospholipid, and changes membrane fatty acid saturation. All or any of these phenomena may be deleterious to the function, composition, and structure of blood vessels, causing atherogenesis and vascular diseases. Recently, it has been shown that hypertriglyceridemic serum from Mg-deficient rats stimulates cultured vascular smooth muscle cell proliferation and causes lipid accumulation in these cells and lipoprotein oxidation in the arterial wall. These findings are consistent with our data and confirm that lipids, particularly triglycerides, contribute to increase IMT in Mg-deficient rats. In our study multiple regression analysis demonstrated that plasma Ca and triglycerides dependently contribute to an increase in wall thickness. Although the cellular basis of the stimulatory effect of Mg deficiency on the development of atherosclerosis remains unclear, Ca might play a crucial role in the development of vascular atherosclerotic lesions.
and composition of elastin have also been reported. All these findings indicate that Mg deficiency may alter wall elasticity. In conclusion, chronic Mg deficiency induced elevation in BP and growth of the rat CCA. Chronic BP elevation and arterial growth contribute to an alteration of elastic properties of vascular wall, including an accelerated age-dependent decrease in distensibility and an increased wall stiffness with Mg deficiency.

Acknowledgment
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References
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