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Why it is difficult to treat and prevent drug-resistant hypertension and elevated pulse pressure: the unrecognized roles of ionized hypomagnesemia, nitric oxide, ceramides, platelet-activating factor,and epigenesis

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Introduction

In today's world, there is a growing prevalence of drug-resistant hypertension (DRH). Although numerous anti-hypertensive drugs keep-being designed by pharmaceutical and biotech companies, there is not, as yet, any one, two, or three drugs when taken together that will consistently lower and maintain both a near-normal systolic (SBP) and diastolic (DBP) arterial blood pressure in DRH patients.[1,2] This failure to be able to consistently lower SBP and DBP, in DRH patients, has been attributed to the latter being of precise, unknown origin. Many of these patients are obese, diabetic, and/or demonstrate defective kidneys hence are usually treated with diuretics, anti-diabetic drugs, beta-blockers, calcium channel blockers and renal drugs, told to undergo lifestyle changes, and placed on strict weight-loss diets.[1,2] But despite these measures and therapies, they rarely return SBP and DBP towards normal.

It is generally agreed that age-related increases in arterial blood pressure (ABP) are mainly a reflection of an increase in SBP while maintaining or exhibiting a slight increase in DBP. This situation results in a widening of pulse pressure (PP) which is usually a cardinal sign seen in DRH patients. Whatever the exact cause, this often leads to atrial fibrillation (AF), stiffness of arterial vessels, and is associated with developing atherogenesis, and coronary arterial disease, a heart attack, or a stroke.

Approximately 60 years ago, two of us, using diverse mammalian isolated arterial blood vessels, found that lowering the magnesium ion (Mg^{2+}) concentration in oxygenated Krebs-Ringer bicarbonate solution, surprisingly, would cause contraction; the lower the Mg^{2+}, the greater the development of contractile force and vasoconstriction.[3–17] After several of our findings were published, numerous other laboratories corroborated our reports.[18,19] Approximately 35 years ago, our laboratories, using rats subjected to dietary deficiency of Mg, observed a progressive elevation in ABP with a progressive widening of PP.

Importance of dietary mg deficiency and mg-deficient environments to elevation in sbp, dbp and pp

Our group and others have reported, in several studies, that aging human subjects (from infants to the elderly) demonstrate a progressive decline in both total Mg and total ionized serum Mg($[Mg^{2+}]_0$); with the ionized fraction showing the greatest, progressive decline.[20–26] The older the test subject, the lower the $[Mg^{2+}]$ in both cells and sera. The serum ionized Mg is the most significant as it parallels that of many tissue and cellular levels of free ionized Mg.[26] Those subjects with either type 2 diabetes mellitus, coronary arterial disease, on renal dialysis, gestational diabetes, renal transplant recipients (with hypertension), orthotopic liver transplantation, pregnant women with transient hypertension during labor, or DRHs demonstrate the greatest declines in $[Mg^{2+}]_0$ and intracellular free Mg ($[Mg^{2+}]_i$).[26–36] Previously, in multiple human studies, we documented that serum ionized Ca/Mg ratios are indicative of peripheral vasoconstriction and increased vascular reactivity to circulating neuro-humoral vasoconstrictor amines and peptides.[37–40] The results of these pathophysiological alterations could cause significant elevations in PP and stiffening of arterial vessels, particularly in the elderly.

At the turn of the last century, the North American and European populations were ingesting about 450-550 mg Mg/day.[24,38–42] Today, about 70-75% of these populations are ingesting only about 165-

235mg Mg/day.[24,43] It has been shown, in multiple studies, that people drinking soft-water (e.g., <10 mg/l of Mg/day) demonstrate a high risk for coronary arterial diseases, hypertension, hyper lipidemias, atherogenesis, and strokes, whereas those peoples drinking hard-waters (with high Mg levels, i.e., >25 mg/l) have low risks for these cardiovascular diseases.[24,43]

Importance of low mg environments in generation of ceramides and platelet-activation factor and inhibition of nitric oxide formation: relationship to rises in sbp and pp in drhs

Approximately 35 years ago, two of us reported that endothelial relaxation of isolated coronary arterial vessels required Mg^{2+}, the greater the $[Mg^{2+}]_0$, the greater the relaxation of the coronary vessels.[44] In 1978,1984,and 1992, respectively, studying the intact intestinal and skeletal muscle microvasculatures in the rat, we reported that lowering the dietary level of Mg or the perfusate

$[Mg^{2+}]_0$ resulted in vasoconstriction of arterioles (18-30 um), metarterioles (10-16 um) and precapillary sphincters (3-6 um).[5,16,17] Some years later, studying the same intact microvasculatures, with low Mg levels, use of chemical inhibitors of nitric oxide synthases, resulted in vasodilation, reduced SBP and DBP, and reduced vasodilation when challenged with endothelial-dependent vasodilators such as acetylcholine and bradykinin, thus proving that Mg^{2+} is a key regulator of nitric oxide synthases and vasomotor tone in the peripheral microcirculation and PP.[45]

About 25 years ago, three of us, working with isolated cerebral and aortic vascular smooth muscle cells, and ^{31}P-nuclear magnetic spectroscopy and ^{1}H-nuclear magnetic resonance spectroscopy, we found that decreased extracellular Mg resulted in an increase in sphingolipids, particularly ceramides, sphingosine, and sphingosine-1-P.[46,47] Further examination of the moieties revealed that low $[Mg^{2+}]_0$ resulted in synthesis and release of platelet-activating factor (PAF) and PAF-like lipids.[46] All of these sphingolipids and PAF can cause vasoconstriction/contraction of microvascular arterioles and muscular venules, as well as cerebral and coronary blood vessels.[48–52]

Approximately 25 years ago, using rats placed on Mg-deficient diets for 21 days, we noted an increased release of ceramides, PAF, and PAF-like lipids into the blood stream.[25,38,39,46,47,51,52] Some years later, we found that cardiovascular tissues and cells excised from these Mg-deficient animals demonstrated increased levels of ceramides, SP-1-P; the greater the deficit in Mg^{2+}, the greater the synthesis of sphingolipids, PAF and PAF-like lipids.[51–60] Use of ceramide and PAF antagonists, in these Mg-deficient animals, not only reduced the ABP and PP, but reduced the vasoconstriction of intact arterioles and venules in the microcirculation.[61] Overall, we believe that these experiments surely point to an important, if not critical role of Mg, sphingolipids, PAF and PAF-like lipids. Recently, we found that elderly DRH and type 2 diabetic patients (>65 years old) demonstrate increased serum levels of ceramides, SP-1-P, and PAF.[62,63]

Progressive decrease of ionized magnesium results in a progressive down-regulation of telomerase: relation to epigenetic changes in the elderly

Working with rats fed low Mg diets for 21 days, we have reported that cardiovascular tissues and cells showed peroxidation of vascular smooth muscle cells, a down-regulation of telomerases and oxidation of DNA.[51–53,64–66] Using specific ELISA assays, we found that the extirpated cardiovascular tissues and cells showed that, as the ionized Mg fell in both the blood and cardiovascular tissues and cells, there was a progressive peroxidation, a down-regulation in telomerases and increases in oxidation of the DNA.[51–53,64–66] Examination of elderly DRH subjects with elevated PP, also showed a down-regulation of telomerase in the sera along with deficits in ionized Mg.[63] Twenty-five years ago, using Mg-deficient rabbits, we found that the atherogenesis (and plaques) that developed on the aortic and coronary arterial vessels became greater and greater with time on the Mg-deficient diets; invasion of the arterial walls showed alterations in the morphology of the macrophages and monocytes.[70] We believe, overall, our experiments and elderly DRH -patient data suggest that even short-term Mg deficiency, in the aging process, could cause alterations in the genome that would result in atherogenesis, stiffening of arterial and arteriolar vessels, DRH and increases in PP in the elderly.

Roles of DNA oxidation and fragmentation, inflammation, and genotoxicity in mg deficiency and drh: importance in epigenetic processes

Recently, we have reported that cardiovascular tissues and cells obtained from rats placed on Mg-deficient diets for 21 days demonstrate a marked increase in 8-hydroxydeoxyguanosine, a reliable indicator of DNA oxidation; the lower the serum and cellular Mg^{2+}, the greater the oxidation of DNA.[51,53,59,64–69] These results clearly support our contention that Mg deficiency probably leads to multiple mutations in the genomes of tissues and cells throughout the cardiovascular system.[69] Previously, we found that only 21 days of Mg deficiency can result in DNA fragmentation and lipid peroxidation.[51,53,59,67]

Although all cells in the body (except for red blood cells) continue to develop strategies to preserve the integrity of DNA structure, numerous factors can alter the structure of DNA, such as reactive oxygen (ROS) and nitrogen species (RNS), numerous extrinsic molecules, and UV radiation, to name a few.[71–73] Each day, DNA of diverse mammalian tissues and cells (including cardiovascular components) receive multiple assaults from, for example, guanine methylations,

cytosine deaminations, spontaneous depurinations, single strand breaks, double strand breaks, and oxidative lesions, among others.[71–78] Everyone of these insults induces specific damage of DNA. For example, external molecules can cause alkylation of DNA by methyl-nitrosourea.[72,79] Cancer therapeutic agents such as mitomycin C and cisplatin can induce very-specific base modifications in the DNA,[72,79] not surprisingly, both of these chemo-therapeutics cause profound depletion of body Mg.[80,81] ROS and RNS can also induce very-specific base modifications in DNA.[71–73,76–79,82–89] these agents also induce bodily depletion of Mg.[24,25,38, 39, 47, 51,52,53,67,80,81] UV light is known to induce strand breaks in DNA; wave lengths of this radiation can also induce bodily depletion of Mg.[79] Interestingly, each one of these injurious assaults cause different DNA aberrations leading to different DNA-repair processes.[73–79] Thus, each one of these assault species not only lead, in different ways, to alterations in DNA, but to different DNA-repair processes.[73–79]

Several years ago, using a short-term 21-day dietary Mg -deficient rat model, we reported that excised cardiovascular tissues and cells showed marked reductions in glutathione content and activation of eNOS and iNOS.[88] Since eNOS-activation has been shown to activate

synthesis of PAF in endothelial cells, our findings of low Mg^{2+} -induced synthesis/generation of eNOS, in cardiovascular tissues and cells may trigger (and be linked), in part, to activation of the sphingomyelinase-ceramide pathways.[88] DNA damage and synthesis of DNA damage has been identified in human atherosclerosis.[90,91] Thus, it is important to keep in mind, that both DNA damage and synthesis could be expected in diverse tissues and cells, depending upon time in low plasma Mg^{2+} environments to exert multiple phenotypic alterations in the biochemical machinery of endothelial and vascular smooth muscle cells, resulting in inflammatory responses, stiffening of blood vessels and elevations in ABP, SBP, and PP. In this context, we have shown that short-term Mg deficiency in rats leads to up-regulation of NF-kB and proto-oncogenes c-fos and c-jun,[51,52,58,60,92] molecular pathways required for any alterations in phenotypic alterations of endothelial and vascular smooth muscle cells.

Approximately, ten years ago, we suggested that magnesium-deficiency can act as a genotoxic agent.[54,55] One of ceramide's major physiological actions is its ability to induce cell differentiation and cell transformation,[52,93–96] short-term Mg deficiency activates five different enzymatic pathways responsible for synthesis of ceramide in vascular smooth muscle cells.[47,51,52,54–60,88] Such synthesis and release in Mg-deficient humans could aid in explaining, to a large extent, transformation of contractile vascular smooth muscle cells to a new phenotype in atherogenesis i.e., vascular muscle cells that no longer contract or relax, but become synthetic machines for a variety of cytokines, chemokines, and growth factors.[52] Abnormal cell differentiation, transformation, and growth are pivotal events in the development of atherogenesis, hypertension, hyperlipidemia, and progressive cardiac failure.

Conclusions and future thoughts

Why there is an elevation in arterial blood pressure and a progressive rise of ABP and PP in drug-resistant hypertensive subjects (DRHs) has resulted in numerous hypotheses over the past 30 years. Why these DRHs cannot even be consistently treated with three different drugs also is not known. More than 40 years ago, our team, working with isolated arterial and arteriolar blood vessels found hat exposure of these vessels to physiologic salt solutions with reduced Mg^{2+} concentrations caused contraction and vasospasm which is dependent on extracellular and release of intracellular free Ca^{2+}; the lower the free Mg^{2+}, the greater the development of contractile force. Some years later, using intact rats placed on low Mg diets, we noted intestinal and skeletal microvessels went into spasm concomitant with increased constrictor activity to circulating neuro-humoral constrictor agents; ABP, SBP and PP rose the longer the animals ingested the low Mg diets., the greater the vasoconstriction of the microvessels, the greater the rise in PP. A combination of a low Mg diet and administration of nitric oxide inhibitors resulted in a potentiation of the vasospasms and potentiation of the arteriolar, metarteriolar and precapillary vasoconstrictions with markedly reduced capillary blood flows. Use of ^{31}P-NMR spectroscopy and ^{1}H-NMR spectroscopy on diverse vascular smooth muscle cells in low Mg^{2+}caused a synthesis and release of sphingolipids, PAF and PAF-like lipids. Use of inhibitors of ceramide and PAF in intact rats, given low Mg diets, ameliorated/inhibited the rises in SBP and PP.

Measurement of serum ceramides, sphingosine-1-P and PAF in DRHs revealed rises in these moieties, particularly in elderly patients (i.e., >65 years old). Rabbits placed on low Mg diets resulted in atherosclerotic arterial vessels (i.e., coronaries and aorta) with morphological alterations in the endothelial, monocytic and macrophage cells on the arterial walls. Cardiac and vascular smooth muscle cells excised from animals placed on low Mg diets show a down-regulation of telomerases, oxidation and fragmentation of DNA, activation of NF-kB, activation of proto-oncogenes, and an up-regulation of five different enzymes leading to synthesis of ceramides Since the North American and European diets are low in dietary Mg, we believe our results on animals, cardiovascular tissues and cells and on human patients characterized as DRHs provide enough evidence to posit that low dietary intake of Mg probably plays an important role in DRH and elevated PP, particularly as these subjects age.

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Conflicts of interest

The author declares there are no conflicts of interest.

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