Regulated Ferroptosis Cell Death is Produced in Cardiovascular Tissues and Cells in Dietary Magnesium Deficiency: Initiation of Roles of Glutathione, Mitochondrial Alterations and Lipid Peroxidation in Inflammation and Atherogenesis

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Approximately 40 years ago, one of us suggested that a progressive dietary and/or metabolic-induced loss of magnesium (Mg) during early developmental stages of life, particularly in coronary arteries, could lead to coronary arterial spasm (CAS), ischemic heart diseases (IHD), and sudden cardiac death (SCD) later in life [1,2]. After these first reports, a number of clinical studies appeared in the literature in support of our hypothesis, at least in adults [3-8]. Autopsies in a number of children, who died due to accidental causes, have been reported to demonstrate early signs of atherogenesis (i.e. fatty streaks on the walls of the aortas and carotid arteries in young children as early as six years of age) [9]. Atherosclerosis, preceded by inflammatory lesions, is the prime cause of premature death in developing countries and even in the United States, which is known to be a forerunner of hypertension, IHD, SCD and strokes.

Numerous irregularities in diets are well-known to induce inflammatory lesions, which are believed to mediate the initiation process of atherogenesis. Such dietary irregularities have been reported to promote lipid deposition and accelerate the growth and transformation of the smooth muscle cells in the vascular walls [10-16]. Dietary deficiency of Mg has been experimentally shown to cause hypertension [12-14,17-21], atherogenesis [12,15,22], coronary vasospasm [1,2,12,22,23], cardiac failure and simulated heart attacks [12,13,18-29], atrial fibrillation, cerebral vasospasm and strokes [11,28-35]. In contrast, hypermagnesemia diets have been demonstrated to ameliorate hypertension, atherogenesis, atrial fibrillation, coronary vasospasm, and strokes [11,16,18-20,22,26-30,34]. The myocardial level of Mg has been observed to be lower in subjects dying from IHD and SCD in soft-water areas than those individuals living in hard-water areas [1,2,11,12,37-41]. Low Mg content in drinking water found in areas of soft-water and Mg-poor soil is associated with high incidences of IHD, coronary vasospasm, atherosclerosis, and SCD. Current evidence indicates that almost 80% of the populations in North America, the UK, and Europe, consuming Western-type diets, are only ingesting about 35 - 50% of the RDA for Mg (i.e. approximately 135 - 235 mg Mg/day vs. 450 - 500 mg Mg/day [11,16].

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Using specifically-designed Mg electrodes to detect ionized Mg in plasma, serum, whole blood, and body fluids [44], our laboratories have shown that patients that present with hypertension, IHD, cardiac failure advanced atherosclerosis, and strokes all demonstrate deficits in plasma, serum and whole blood ionized Mg in more than 65% of the cases; usually either no or little change in total Mg levels is seen in many of these subjects [11,42,43]. Even using rat and rabbit model systems, our group and others have shown that dietary deficiency of Mg (approximating levels seen in most Western diets), results in elevations in systolic and diastolic blood pressures, cardiac failure, and remodeling in the peripheral microvasculature (i.e. arteriolar wall hypertrophy and alterations in the matrices of the vascular walls), which was concomitant with alterations of arterial blood pressure, signs of inflammation, and microvascular vasospasm [11-15,18-27,33,43-46]. Close examination of the vascular smooth muscle cells (VSMc), lipid-laden macrophages, and cardiac cells, using transmission electron microscopy (TEM), revealed many cells undergoing various stages of cell death [45]. Further close inspection of the cells revealed areas of apoptosis and necroptosis [47]. In addition, we noted a form of cell death termed “ferroptosis” [48,49]. Up until recently, it has been thought that cell death is a major component of remodeling in IHD, atherosclerosis, hypertension, and cardiac diseases involving only apoptosis and necroptosis. Recently, we reported that both apoptosis and necroptosis, unlike necrosis, are forms of “regulated cell death” in Mg-deficient animals [48-50]. Another form of regulated cell death, i.e. ferroptosis, is an iron-dependent form of regulated cell death which has gained considerable attention [52,53]. Ferroptosis has been shown to play roles in the pathogenesis of numerous human diseases such as cancers, tissue injuries, inflammations, T-cell immunity, and cell death [49-53]. Unlike apoptosis and necroptosis, morphologically, there are no membrane ruptures or nuclear condensations [49-53]. Ferroptosis is characterized by mitochondrial (MT) abnormalities such as smaller than normal MT, dissolution of multiple cristae with ruptures, and increased MT outer membrane density with rupture [57,58]. Using specific histochemical stains to detect nonheme-iron (i.e. acid-ferrocyanide and ammonium sulfide), we noted areas of the Mg-deficient cells and tissues to contain deposits of nonheme-iron, a clear sign of ferroptosis [52,53]. Closer examination of the Mg-deficient cells and tissues, using fluorescence histochemical methods devised by Petrat, et al. [59], we demonstrated chelatable iron in VSMc, macrophage-laden cells, endothelial cells and cardiac myocytes [49].

Nonheme-iron signifies heterogeneous species of iron complexes where iron is found to be bound to low-molecular weight organic bases and proteins such as phosphates, ascorbates, carboxylates, nucleotides, transferrin, ferredoxin, aconitases, ferritin in cytosol and MT, and divalent metal transporters [60]. Interestingly, Mg\(^{2+}\) can either inhibit or attenuate binding of nonheme-iron to these molecules [60]. It is important to note, here, that a major property of iron in cells is to cause accumulation and release of hydroxyl free radicals (-OH) through the Fenton reaction or iron-catalyzed Haber-Weiss reaction in the presence of H\(_2\)O\(_2\) or O\(_2^-\) [61]. We have shown that experimental Mg deficiency in VSMc (i.e. aortic, mesenteric, and cerebral arterial cells) and cardiomyocytes causes accumulation of numerous free radicals, including lipoperoxides and -OH [62]. The latter, most likely, being a result of iron accumulation.

Earlier studies from our laboratories indicated that production of Mg deficiency in intact animals, and VSMc in primary culture, resulted in an upregulation of the tumor suppressor protein p53 [63] which is known to initiate ferroptosis [52-54]. When we utilized specific inhibitors of p53 activation in the primary-cultured VSMc ferroptosis formations and MT alterations were curtailed drastically [49] It has also been demonstrated in several studies using other types of cells and tissues that lipophilic antioxidants and iron chelators can prevent ferroptotic cell death [52-56]. Using similar agents, we also found that VSMc, exposed to Mg -deficient environments, exhibited marked reductions in ferroptosis, but apoptosis and necroptosis in these primary cells were not inhibited [49].
Oxidation of cellular lipids causes degradation by free radicals (e.g. -OH) taking electrons from the lipid molecules to lose its electrons by oxidation. This process is thought to make ferroptosis a regulatory form of cell death [52-56]. The process of oxidative degradation of the lipid molecules is due to the fact that glutathione peroxidase 4 (GXP4), a lipid repair enzyme found in mitochondria [55], undergoes loss of activity [52-55]. This suggests that mitochondria must perform play a vital role in ferroptosis.

It has been demonstrated in diverse cell types that blocking of GXP4 can induce ferroptosis [55]. Recent experiments from our group have shown that primary cells, incubated in excess levels of extracellular Mg$^{2+}$, with drugs that supposedly block GXP4 will prevent ferroptosis [58]. Furthermore, we found that inhibition of GSH, which is critical for the function of GXP4 (necessary for inducing a ferroptotic response) [55] inhibits ferroptosis in the primary VSMc exposed to low extracellular Mg$^{2+}$ [49]. These inhibitors, however, do not prevent low Mg$^{2+}$-induced apoptosis or necroptosis [unpublished findings].

Interestingly, recent studies from our group, have shown that the ferroptosis inhibitors ferrostatin-1 and liproxstatin-1, prevent mitochondrial dysfunction and cell death induced by low Mg$^{2+}$, thus linking ferroptosis to the mitochondrial damage we have observed in VSMc and cardiac myocytes [49].

Conclusions and Future Thoughts

Since our studies in rabbits and rats given low dietary Mg (and elevated cholesterol) demonstrate ferroptosis in the arterial VSMc, lipid-laden macrophages, and cardiac myocytes [48,49], we believe that it is probable that Mg deficiency may result in inflammatory lesions and atherogenesis not only via apoptosis and necroptosis [44-46,48-52], but ferroptosis as well thus suggesting that iron-overload may be an overlooked factor in the etiology of these disease processes. Our results do, and importantly, point to major roles for MT, glutathione, and GXP4 in Mg deficiency-induced cell death. We believe studying ferroptotic events in inflammation and atherogenesis may prove to be important not only in the etiology of these processes but in the etiology of and treatment of IHD, hypertension, SCD, and strokes.

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