ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY
Commentary

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

Burton M Altura[1-7]*, Asefa Gebrewold[1], Anthony Carella[1], Aimin Zhang[1], Nilank C Shah[1,6], Gatha J Shah[1,6] and Bella T Altura[1,3-7]

[1]Department of Physiology and Pharmacology, The State University of New York Downstate Medical Center, Brooklyn, New York, USA

[2]Department of Medicine, The State University of New York Downstate Medical Center, Brooklyn, New York, USA

[3]The Center for Cardiovascular and Muscle Research, The State University of New York Downstate Medical Center, Brooklyn, New York, USA

[4]The School of Graduate Studies in Molecular and Cellular Science, The State University of New York Downstate Medical Center, Brooklyn, New York, USA

[5]Bio-Defense Systems, Inc, Rockville Centre, New York, USA

[6]Orient Biomedica, Estero, Florida, USA

[7]Magnesium for Health Foundation, Patterson, California, USA

***Corresponding Author:** Burton M Altura, Professor, Department of Physiology and Pharmacology, SUNY Downstate Medical Center, Brooklyn, New York, USA.

Received: April 27, 2018; **Published:** June 15, 2018

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-p00r 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].

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 cardiomyocytes and VSMc obtained from living rats exposed to only 21 days of short-term Mg deficiency [50], we noted that the MT membranes were broken in many cells and there was evidence of loss of cristae in numerous MT, characteristic of ferroptosis [48,49].

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 ($^{\cdot}OH$) through the Fenton reaction or iron-catalyzed Haber-Weiss reaction in the presence of H_2O_2 or $O_{2\cdot}$ [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 perforce play a vital role in ferroptosis.

It has been demonstrated in diverse cell types that blocking of GPX4 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 GPX4 will prevent ferroptosis [58]. Furthermore, we found that inhibition of GSH, which is critical for the function of GPX4 (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.

Acknowledgements

The authors acknowledge that many of our experiments and human studies, over five decades, were supported in part, by Research Grants from The N.I.H. (to B.M.A. and B.T.A.) and unrestricted grants from several pharmaceutical companies as well as kind donors.

Bibliography

1. Altura BM. "Sudden-death ischemic heart disease and dietary magnesium intake: is the target site coronary vascular smooth muscle?" *Medical Hypotheses* 5.8 (1979): 843-849.

2. Turlapaty PDMV and Altura BM. "Magnesium deficiency produces spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease". *Science* 208.4440 (1980): 198-200.

3. Kimura T., *et al*. "Effects of magnesium on the tone of isolated human coronary arteries. Comparison with diltiazem and nitroglycerin". *Circulation* 79 (1989): 1118-1124.

4. Goto K., *et al*. "Magnesium deficiency detected by intravenous loading test in variant angina pectoris". American *Journal of Cardiology* 65.11 (1990):709-712.

5. Simko F. "Pathophysiological aspects of the protective effect of magnesium in myocardial infarction (review)". *Acta Medica Hungarica* 50.1-2 (1994): 55-64.

6. Satake K., *et al*. "Relation between severity of magnesium deficiency and frequency of angina attacks in men with variant angina". *Journal of the American College of Cardiology* 28.4 (1996): 897-902.

7. Sueda S., *et al*. "Magnesium deficiency in patients with myocardial infarction and provoked by coronary artery spasm". *Japanese Circulation Journal* 65.7 (2001): 643-648.

8. Minato N., *et al*. "Perioperative coronary artery spasm in off pump coronary bypass grafting and its possible relation with perioperative hypomagnesemia". *Annals of Thoracic and Cardiovascular Surgery* 12.1 (2006):32-36.

9. Berenson GS., *et al*. "Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults, The Bogalusa Heart Study". *New England Journal of Medicine* 338.23 (1998): 1650-1656.

10. Kumar V., *et al*. "Robbins and Cotran Pathologic Basis of Disease". (8th edition), Saunders, Philadelphia, (2010): 105-109.

11. Seelig MS. "Magnesium Deficiency in the Pathogenesis of Disease Early Roots of Cardiovascular, Skeletal, and Renal Abnormalities". *Plenum Corporation* (1980).

12. Altura BM and Altura BT. "Magnesium: forgotten mineral in cardiovascular biology and angiogenesis". In: *New Perspectives in Magnesium Research*. Springer, London (2007): 239-260.

13. Altura BM, *et al*. "Magnesium deficiency and hypertension: correlation between magnesium deficiency diet and microcirculatory changes *in situ*". *Science* 223 (1984): 1315-1317.

14. Altura BM., *et al*. "Noise-induced hypertension and magnesium in rats: relationship to microcirculation and calcium". *Journal of Applied Physiology* 72.1 (1992): 194-202.

15. Altura BT., *et al*. "Magnesium dietary intake modulates blood lipid levels and atherogenesis". *Proceedings of the National Academy of Sciences of the United States of America* 87.5 (1990): 1840-1844.

16. Dean C. "The Magnesium Miracle". (3rd edition.) Ballantine Books, New York (2014).

17. Luthringer C., *et al*. "Effect of moderate magnesium deficiency on serum lipids, blood pressure and cardiovascular reactivity in normotensive rats". *British Journal of Nutrition* 59.2 (1988): 243-250.

18. Altura BM and Altura BT. "Role of magnesium in the pathogenesis of hypertension updated: relationship to its actions on cardiac, vascular smooth muscle, endothelial cells". In: Hypertension: Pathophysiology, Diagnosis, and Management, 2nd edition. Laragh JH, Brenner Bm, editors. Raven Press, New York (1995): 1213-1242.

19. Altura BM and Altura BT. "Magnesium and cardiovascular biology: an important link between cardiovascular risk factors and atherogenesis". *Cellular and Molecular Biology Research* 41.5 (1995): 347-359.

20. Altura BM and Altura BT. "Magnesium in cardiovascular biology". *Scientific America* 2.1 (1995): 28-37.

21. Laurant P., *et al*. "Effect of magnesium deficiency on blood pressure and mechanical properties of rat carotid artery". *Hypertension* 33.5 (1999): 1105-1110.

22. Ravin HB., *et al*. "Oral magnesium supplementation induces favorable atherogenic changes in cholesterol-fed rabbits". *Arteriosclerosis, Thrombosis, and Vascular Biology* 21.5 (2001): 858-862.

23. Altura BM and Turlapaty PDMV. "Withdrawal of magnesium enhances coronary arterial spasms produced by vasoactive agents". *British Journal of Pharmacology* 77.4 (1982): 649-659.

24. Murakawa T., *et al*. "Importance of magnesium and potassium concentration on basal tone and 5-HT induced contractionsin canine coronary artery". *British Journal of Pharmacology* 94.2 (1988): 325-334.

25. Altura BM and Altura BT. "Magnesium, electrolyte transport and coronary vascular tone". Drugs 28 (1984): 120-142.

26. Altura BT and Altura BM. "Cardiovascular actions of magnesium: Importance in etiology and treatment of high blood pressure". *Magnesium-Bulletin* 9 (1987): 6-21.

27. Altura BM and Altura BT. "Cardiovascular risk factors and magnesium: relationships to atherosclerosis, heart disease and hypertension". *Magnesium and Trace Elements* 10.2-4 (1991-1992): 182-192.

28. Wu F., *et al*. "Low extracellular magnesium results in cardiac failure in isolated perfused rat hearts". *Magnesium and Trace Elements* 10.5-6 (1992): 364-373.

29. Wu F., *et al*. "Ferrylmyoglobin formation in acute magnesium deficiency in perfused rat heart causes cardiac failure". *Biochimica et Biophysica Acta* 1225.2 (1994): 158-164.

30. Altura BT and Altura BM. "Withdrawal of magnesium causes vasospasm while elevated magnesium produces relaxation of tone in cerebral arteries". *Neuroscience Letters* 20.3 (1980): 323-327.

31. Altura BM., *et al*. "Low extracellular magnesium induces intracellular free Mg deficits, depletion of high-energy phosphates and cardiac failure in intact working rat hearts: A 31P-NMR srudy". *Biochimica et Biophysica Acta* 1182.3 (1993): 328-332.

32. Altura BT and Altura BM. "The role of magnesium in etiology of strokes and cerebrovascspasm". *Magnesium* 1 (1982): 277-201.

33. Altura BM and Altura BT. "Role of magnesium in alcohol-induced hypertension and strokes as probed by in-vivo television microscopy, digital-image microscopy, optical spectroscopy and a unique magnesium ion-selective electrode". *Alcoholism: Clinical and Experimental Research* 18.5 (1994): 1057-1068.

34. Altura BT., *et al*. "Low levels of serum ionized magnesium are found in stroke patients early after stroke which results in rapid elevation in cytosolic free calcium and spasm in cerebral vascular smooth muscle cells". *Neuroscience Letters* 230.1 (1997): 37-40.

35. Vink R., *et al*. "Magnesium in acute and chronic brain injury: an update. *Magnesium Research* 22.3 (2009): S158-S162.

36. Seelig MS and Rosanoff A. "The Magnesium Factor". Ballantine Books, New York (2003).

37. Crawford T and Crawford MD. "Prevalence of pathological changes of ischaemic heart disease in a hard-water and in a soft-water area". *Lancet* 1 (1967): 229-232.

38. Anderson TW., *et al*. "Sudden death and ischemic heart disease. Correlation with water hardness of local water supply". *New England Journal of Medicine* 280 (1969): 805-807.

39. Anderson TW. "Water hardness, magnesium, and ischemic heart disease". *Nova Scotia Medical Bulletin* (1977): 58.

40. Marier JH. "Cardio-protective contribution of hard waters to magnesium in-take". *Revue Canadienne De Biologie* 37.2 (1978): 115-125.

41. Marier JH and Neri LC. "Quantifying the role of magnesium in the interrelationship between human mortality/morbidity and water hardness". *Magnesium Journal* 4.2-3 (1985): 53-59.

42. Altura BM and Altura BT. "Importance of ionized magnesium measurements in physiology and medicine and the need for ion-selective electrodes". *Journal of Clinical Case Studies* 1 (2016): 1-4.

43. Altura BM., *et al*. "Sudden cardiac death infants, children and young adults: Possible role s of dietary magnesium intake and generation of platelet-activating factor in coronary arteries". *Journal of Heart Health* 2.2 (2016).

44. Altura BM., *et al*. "Expression of PAF is induced by low Mg in aortic, cerebral and piglet coronary arterial vascular smooth muscle cells: cross-talk with ceramide production, DNA, nuclear factor -kB and proto-oncogenes: possible links to inflammation, atherogenesis, hypertension, sudden cardiac death in children and infants, stroke, and aging: hypothesis and review". *International Journal of Cardiology and Research* 3.1 (2016): 47-67.

45. Altura BM., *et al*. "Genotoxic effects of magnesium deficiency in the cardiovascular system and their relationship to cardiovascular diseases and atherogenesis". *Journal of Cardiovascular Diseases and Diagnosis* S1 (2016): 1.

46. Altura BM., *et al*. "Magnesium deficiency results in oxidation and fragmentation of DNA, downregulation of telomerase activity, and ceramide release in cardiovascular tissues and cells: Potential relationship to atherogenesis, cardiovascular diseases and aging". *International Journal of Diabetology and Vascular Disease Research* 4.1 (2016): 1-5.

47. Altura BM., *et al*. "Regulated RIPK3 necroptosis is produced in cardiovascular tissues and cells in dietary magnesium deficiency: Roles of cytokines and their potential importance in inflammation and atherogenesis". *Journal of Medical and Surgical Pathology* 2 (2017): 3.

48. Shah NC., *et al*. "Short-term magnesium deficiency upregulates RIPK3 kinase in cardiovascular tissues and cells: cross-talk with cytokines, acid sphingomyelinase, and ceramide" (2018).

49. Altura BM., *et al*. "Regulated ferroptosis is produced in cardiovascular cells and tissues: Roles of lipoperoxides, hydroxyl radicals, and hydroperoxides and their potential importance in inflammation and atherogenesis" (2018).

50. Li JF., *et al*. "Peroxynitite induces apoptosis and decline of intracellular free Mg with concomitant elevation in {Ca| in rat aortic smooth muscle cells: possible roles of extracellular and intracellular magnesium ions in peroxynitrite-induced cell death". *Drug Metabolism Letters* 1.3 (2007): 85-89.

51. Altura BM., *et al*. "Short-term magnesium deficiency results in decreased levels of serum sphingomyelin, lipid peroxidation and apoptosis in cardiovascular tissues". *American Journal of Physiology-Heart and Circulatory Physiology* 297.1 (2009): H86-H92.

52. Dixon SJ., *et al*. "Ferroptosis: An iron-dependent form of nonapoptotic cell death". *Cell* 149.5 (2012): 1060-1072.

53. Gao M., *et al*. "Ferroptosis is an autophagic cell death process". *Cell Research* 26.9 (2016): 1021-1032.

54. Wu D and Chen L. "Ferroptosis: a novel cell death form will be a promising therapy target for diseases". *Archives of Biochemistry and Biophysics* 47.10 (2015): 857-859.

55. Cao JY and Dixon SJ. "Mechanisms of ferroptosis". *Cellular and Molecular Life Sciences* 73.11-12 (2016): 2195-2209.

56. Yu H., *et al*. "Ferroptosis, a new form of cell death, and its relationships with tumourous diseases". *Journal of Cellular and Molecular Medicine* 21.4 (2017): 648-657.

57. Neitemeier S., *et al*. "BID links ferroptosis to mitochondrial cell death pathways". *Redox Biology* 12 (2017): 558-570.

58. Wu C., *et al*. "Induction of ferroptosis and mitochondrial dysfunction by oxidative stress in PC12 cells". *Scientific Reports* 8.1 (2018): 574.

59. Petrat F., *et al*. "Selective determination of mitochondrial chelatable iron in viable cells with a new fluorescent sensor". *Biochemical Journal* 362 (2002): 137-147.

60. Meguro R., *et al*. "Nonheme-iron histochemistry for light and electron microscopy: a historical, theoretical and technical review". *Archives of Histology and Cytology* 70.1 (2007): 1-19.

61. Crichton RR., *et al*. "Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells". *Journal of Inorganic Chemistry* 91.1 (2002): 9-18.

62. Altura BM., *et al*. "Magnesium deficiency, sphingolipids and telomerase and aging in the cardiovascular system". In: Famine, Starvation and Nutrient Deprivation. Preedy VL, editors. Springer, Berlin, in press (2018).

63. Altura BM., *et al*. "Short-term magnesium deficiency upregulates sphingomyelinase synthase and p53 in cardiovascular tissues and cells: relevance to the de novo synthesis of ceramide". *American Journal of Physiology-Heart and Circulatory Physiology* (2010): H2046-H2055.

Volume 6 Issue 7 July 2018