Cardiovascular Mystery Series *Series Editor:* Karl T. Weber

Role of free radicals and substance P in magnesium deficiency

W.B. Weglicki *, I.T. Mak, J.H. Kramer, B.F. Dickens, M.M. Cassidy, R.E. Stafford, T.M. Phillips

The George Washington University Medical Center, Department of Medicine and Physiology, 2300 Eye Street NW, Washington DC, 20037, USA

Received 22 February 1995; accepted 22 August 1995

Keywords: Free radicals; Substance P; Magnesium; Magnesium deficiency

0008-6363/96/$15.00
SSDI 0008-6363(95)00196-4

1. Historical perspective

In the United States the literature contains only sporadic references to clinical disorders of Mg-deficiency, compared to more recent interest in the benefits of magnesium infusion in myocardial infarction and other acute clinical conditions [1,2]. In Europe the clinical interest in Mg-deficiency was pioneered by Durlach in his book entitled *Le Magnesium en Pratique Clinique*; the English edition was entitled *Magnesium in Clinical Practice* [3]. In the conclusion of his book, Durlach stated: "This ion which is present in all the cells is involved in many different pathologies. Integrating a search for the disorders of magnesium metabolism in daily diagnostic processes allows determination of the indications and precise methods of magnesium therapy." In the United States, Seelig authored a text in 1980 entitled *Magnesium Deficiency in the Pathogenesis of Disease* [4] and reviewed the literature concerning magnesium requirements in human nutrition and the association of magnesium deficiency with cardiovascular disease [5]. That same year Wacker published an excellent book entitled *Magnesium and Man* in which he emphasized the clinical relevance of magnesium [6].

Six decades ago MacCollum [7] studied the effects of Mg-deficiency on development, reproduction, neuromuscular and humoral abnormalities in animals. In 1959 Bajusz and Selye published a paper describing the influence of electrolytes in the process of myocardial injury [8]. More recently, Lehr focused attention on magnesium and the process of cardiac necrosis [9], or cardiomyopathic lesions which had been described earlier [10]. B.T. Altura and B.M. Altura published a series of papers which postulated a working hypothesis for the detrimental effect of Mg-deficiency in cardiac pathology and heart failure [11–13] based upon the hypothesis that Mg-deficiency results in calcium overload, vasospasms, and increased vascular reactivity. Recent studies to elaborate on their model demonstrate that perfusion of isolated working rat hearts with low concentrations of extracellular Mg (0.3 mM) resulted in myocardial injury consistent with irreversible myocyte injury, which they conclude is probably a result of coronary vasoconstriction [14]. Other investigators have contributed abundantly to the literature of the field for the past three decades [15–18]. Thus, interest in the study of Mg-deficiency in animals began early in this century, but our understanding of the complex mechanisms leading to injury of the heart, and other organs remains incomplete.

2. Free radical mechanisms

When our research group entered the field in the late 1980's, through collaboration with Dr. S. Bloom, we initially focused on whether the pathobiology of dietary Mg-deficiency might be related to free radical mechanisms. Using both the Mg-deficient hamster and rat, we embarked upon a series of antioxidant intervention studies. Animals were placed on Mg-deficient diets and concurrently treated with subcutaneously implanted pellets containing probucol [19] vitamin E [20], and other antioxidants [21–23]. This diet induced a severe deficiency in total serum Mg within a week of the animals being placed upon the diet, with serum levels below 0.3 mM after 1–2 weeks on the diet. However, total tissue Mg is relatively conserved during this time [24]. Using morphological end-

* Corresponding author. Tel.: (+1-202) 994-0501; fax: (+1-202) 994-3553.

Time for primary review 40 days.

points we observed that the myocardial lesions were diminished significantly by concurrent treatment with antioxidants. We postulated that this was evidence that free radicals contributed to the injury process. However, these radicals are known to be associated with many injury processes including inflammation and ischemia/reperfusion.

Mammalian tissues contain numerous defenses against oxidative stress, some of which have been shown to be compromised during Mg-deficiency. Glutathione, a thiol-containing tripeptide present in millimolar amounts in most mammalian cells, plays an important protective role against cellular oxidative injury by detoxifying free radicals and lipid peroxides. We demonstrated in rats that the level of glutathione in the red blood cells (RBCs) was significantly reduced after 2–3 weeks on a Mg-deficient diet; changes were observed in the Mg-deficient hamster [25] in which the level of RBC glutathione decreased 62% (from 1.22 to 0.36 μmol GSH/ml packed cells) within 2 weeks. In the swine, the GSH levels decreased $\sim 45\%$ after 7 weeks on the Mg-deficient diet [26]. Mg-deficiency also leads to a decrease in ascorbate in liver [27] brain [28], and other tissues. Ascorbate is known to regenerate reduced vitamin E from oxidized vitamin E, and loss of ascorbate during Mg-deficiency suggests a possible subsequent loss of the reduced form of vitamin E [29].

Other evidence that endogenous antioxidant defense is compromised during Mg-deficiency includes the accumulation of oxidation products. Rats fed a Mg-deficient diet have a 40% increase in lipid peroxidation in the liver [30]. In our study with the rat, after 2 weeks on the deficient diet, the circulating plasma oxidized lipids, determined as thiobarbutaric-acid-reactive substances, increased from $0.82 \pm .20$ to $1.92 \pm .25$ nmol malondialdehyde/ml ($P < 0.01$) [31]. Concomitantly, significant ($P < 0.05$) accumulation of protein oxidation products, determined as protein-carbonyls, occurred in the kidney (increased from 2.7 to 5.2 nmol/mg protein) and in the brain (from 1.6 to 3.2 nmol/mg protein) [32].

Plasma catecholamine levels also increase during Mg-deficiency, and auto-oxidation of catecholamines results in the generation of free radicals [33]. Free radicals may play a major role in the development of cardiomyopathies in other experimental models: hyperoxia-induced toxicity, myocardial iron overload, and selenium-deficiency which causes inhibition of a key antioxidant enzyme.

Myocardial lesions due to Mg-deficiency in the hamster [20] and rat [21,34] models were blocked by long-term treatment with the lipid-soluble antioxidant, vitamin E. In a separate study, we showed that epi-captopril, an SH-containing agent, provided effective protection against the Mg-deficiency cardiomyopathic lesions [35]. In these antioxidant studies, the animals remained Mg-deficient even with antioxidant supplements, so the protection against lesion formation was not associated with correction of the hypomagnesemia. Epi-captopril is water-soluble and has no significant ACE-inhibitory activity. Since both captopril and epi-captopril (10–50 μM) displayed 20–50% inhibition of OH radical level formation monitored by electron spin resonance (ESR) spin-trapping using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as the spin trap, we suggested that the protective effect was due to a free radical scavenging action of the SH-moiety [36]. The inhibition of lesion formation by antioxidant therapy and the increase in oxidation products during Mg-deficiency provided indirect evidence that the *endogenous* antioxidant defenses may be compromised during Mg-deficiency. This finding may result from either oxidative loss of endogenous antioxidants during Mg-deficiency, or may indicate that antioxidants are insufficient to cope with increased levels of oxidative stress during Mg-deficiency.

Table 1
Effect of dietary Mg-deficiency on functional recovery and lipid free radical production of postischemic rat hearts

Dietary group	Recovery of cardiac work (%) Ischemic duration		Total PBN/alkosyl (nM/g × min)	
	30 min	40 min	30 min	40 min
Mg-S	54.5 ± 7.1	42.1 ± 2.6	75.6 ± 17	129.2 ± 24
Mg-D	31.6 ± 5.4 *	24.1 ± 7.0 *	148.8 ± 18 *	263 ± 46 *
Mg-S + vit. E	ND	67.1 ± 6.0 *	ND	52.9 ± 20 *
Mg-D + vit. E	ND	53.7 ± 8.0 * *	ND	135.0 ± 35 * *

Rats treated with vitamin E received 3-week continuous release pellets (1.2 mg/day, s.c.). Values are expressed ± s.e. of 8-12 hearts from untreated groups, from 4-8 rats for the vitamin-E-treated group.
* $P < 0.05$ vs, untreated Mg-sufficient and * * $P < 0.05$ vs, Mg-deficient group, following comparable ischemic durations. ND = non-detectable.

Mg-deficiency may predispose cells to oxidative injury. We showed that red blood cells (RBC) from Mg-deficient hamsters were more susceptible than those from Mg-sufficient hamsters to exogenous free radicals [25]. Moreover, we found that RBC's from Mg-deficient animals treated with vitamin E recovered their resistance to exogenously-generated free radicals. Further studies suggested that Mg-deficient tissues were less tolerant to oxidative stress (Table 1). Isolated working rat hearts from Mg-deficient rats were exposed to ischemia/reperfusion injury and they displayed a significant further impairment of functional recovery compared to Mg-sufficient animals following either 30 or 40 min of ischemia [37]. Mg-deficiency also caused significantly higher myocardial production of lipid peroxidation-derived free radicals following ischemia/reperfusion [37,38], as detected by electron spin resonance spin trapping using α-phenyl-*N*-*tert*-butylnitrone (PBN) as the trapping agent. The elevated production of alkoxyl radicals (as PBN-RO $\cdot$), in Mg-deficient compared to Mg-sufficient hearts (Table 1), suggested that lipid peroxidation above that usually expected was occurring during reperfusion of previously ischemic Mg-deficient hearts. Again, vitamin E supplementation was found to attenuate free radical production in the ischemic/reperfused Mg-de-

ficient rat heart and improve myocardial functional recovery [37]. As presented earlier, increased protein oxidation also occurred during Mg-deficiency. Therefore, it is reasonable to anticipate that, in addition to lipid peroxidation, protein sulphydryl oxidation might also occur and thus affect related protein function as well.

These results allowed us to conclude that free radicals mediated much of the tissue injury in Mg-deficiency since: oxidative products accumulate during Mg-deficiency [30,31]; Mg-deficiency reduces antioxidant status in tissues and cells so that they are more susceptible to ischemia/reperfusion [37,38] or exogenous oxidative stress [25,31]; and antioxidant treatment can prevent much of the pathology associated with Mg-deficiency by either preventing the loss of endogenous antioxidants or the replenishment of endogenous antioxidant capacity [19–23,35,36]. In a recent study of the effect of acute exposure of working rat hearts to low extracellular Mg (0.3 mM), Wu et al. [39] provided evidence that Mg-deficiency induced ferrylmyoglobin formation and caused cardiac failure. The oxidation of myoglobin was inhibited by incubation with ascorbate, which improved cardiac function. Furthermore, this injury reported for cardiac exposure to modified Krebs-Henseleit bicarbonate buffer containing low Mg (0.3 mM) was not reversible by subsequent perfusing with the same buffer supplemented with normal (1.2 mM) Mg [14]. However, in our laboratory no difference could be observed in cardiac function from hearts isolated from rats on a Mg-deficient or Mg-sufficient diet for up to 4 weeks when isolated hearts from both were perfused with normal (1.2 mM) levels of Mg. These differences in response of acute versus chronic hypomagnesemic hearts indicate the importance of assessing pathobiological responses in both kinds of models.

3. Inflammation: cytokines and neuropeptides

In recent studies we have shown that Mg-deficiency has a profound effect on the process of inflammation, causing high circulating amounts of interleukins (IL-1, IL-6) and tumor necrosis factor-alpha (TNFα) [40]. These inflammatory modulators are elevated in rats, mice, and swine, and we have not found evidence of viral or bacterial infection in the animals. These findings suggest that dietary Mg-deficiency induces an unexpected rise in these mediators. These peptides are known to elicit free radical production in a variety of cell types, including endothelial cells and circulating white blood cells. Following activation of endothelial cells by these inflammatory cytokines, cytotoxic processes involving the production of oxygen free radicals can be ameliorated by antioxidant therapy both in vitro and in vivo [41,42].

We decided to study the temporal events of the Mg-deficiency-mediated inflammatory process, and found that specific cytokines, (IL1, IL6 and TNFα) were elevated

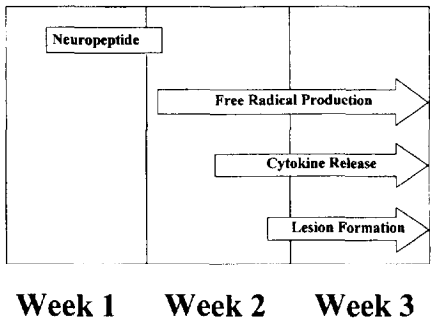

Fig. 1. Time course in the rat model of changes in neuropeptides, cytokines, free radicals, and cardiac lesions during 3 weeks on a Mg-deficient diet.

only at the end of 2 weeks on the Mg-deficient diet [40,43]. They were associated temporally with the increasing occurrence of cardiomyopathic lesions. We were puzzled by the fact that free-radical-related changes (increase in malonyldialdehyde and lipid hydroperoxide production) and decrease in RBC glutathione occurred only during the second week of Mg-deficiency [31,37], and that these changes preceded significant cardiac lesion production and significant elevations of inflammatory cytokines (Fig. 1). We hypothesized that there should be an earlier "trigger" mechanism which induced the later changes in free radical stress, cytokine levels, and lesion formation.

In an effort to determine the mechanism responsible for this early trigger mechanism we considered the possibility that neuropeptides, and specifically substance P, could trigger this inflammation via various cell types of the immune system [44,45]. Substance P was chosen based upon several known responses to Mg-deficiency. Mg-deficiency results in enhanced convulsive activity in response to auditory stimuli. We reasoned that there might be a relationship between the neuronal events in these animals and the observed cardiovascular pathology. Of the neuropeptides which could be possible involved, substance P was deemed a likely candidate because it is a known initiator of free radical production and neurogenic inflammation; it also causes histamine release, and proliferation

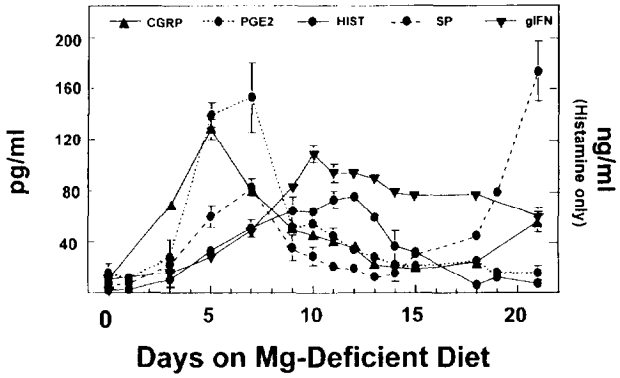

Fig. 2. Plasma levels of substance P, CGRP, PGE_2, histamine, and γ-interferon during 3-week period of Mg-deficiency in the rat model.

Table 2
Effect of substance P receptor blockade on the indices of oxidative injury during Mg-deficiency in the rat

Treatment	Plasma BARS (nmol/ml)	RBC GSH (μmol/ml RBC)	Cardiac lesion	
			Size (#/cm^2 × 10^{-2})	Frequency (area × 10^{-3})
MgS control	0.8 ± 0.1	1.65 ± 0.2	ND	ND
MgD	1.8 ± 2$^+$	0.73 ± .1$^+$	17.8 ± 4.0$^+$	3.7 ± 1.4$^+$
MgD + CP96345	1.0 ± .2 *	1.35 ± .2 *	3.2 ± 1.2 *	0.7 ± .25 *

ND = non-detectable; $^+ P < 0.01$ vs. MgS control; $^* P < 0.05$ vs. MgD alone.

of WBC's, both of which occur early during Mg-deficiency. To our satisfaction we found that both plasma substance P and calcitonin-gene-related peptide (CGRP) were elevated during the first week of Mg-deficiency in association with substantial hypomagnesemia (Fig. 2). These elevations coincided with increases in PGE_2, a prostaglandin involved in inflammation [43], and preceded elevation of histamine and γ-interferon.

Based on the fact that substance P (and CGRP) increased before other changes observed in our Mg-deficient model (Fig. 2), we reasoned that it must be playing a role in the subsequent pathology. To test this hypothesis, we inhibited the action of substance P in the Mg-deficient animals by the use of a specific substance P receptor blocker [38,46,47]. Up until that time, other investigators reported depletion of substance of P using capsaicin as a strategy to study the role of this neuropeptide in pathology [48,49]. Fortunately, a newly developed specific substance P NK-1 receptor blocker, CP-96,345 [50], became available from work by investigators from the Pfizer laboratories. We were provided with this agent and proceeded to treat Mg-deficient and Mg-sufficient animals with sustained release pellets containing CP-96,345. In those Mg-deficient animals treated continuously with the substance P receptor blocker, we found significant reductions in cardiac lesion number and size (Table 2), as well as significant decreases in plasma PGE_2 and histamine [46]. Additional data indicated that RBC glutathione, a good indicator of oxidative stress, was preserved in those animals pretreated with CP-96,345 (Table 2) [38]. From these observations we were able to postulate a neurogenic inflammation mechanism that resulted from early release of neuropeptides, which induced inflammatory responses in Mg-deficient animals, with subsequent free radical production and lesion formation. Since the circulating levels of substance P were not affected by substance P receptor blockade, we directly analyzed the cardiac lesions by a microdissection technique to ascertain whether there were any effects of the blocker on tissue concentrations of substance P. The methodology involved preparing frozen sections of cardiac tissue, identifying lesions, microdissecting them, solubilizing the samples, and injecting aliquots

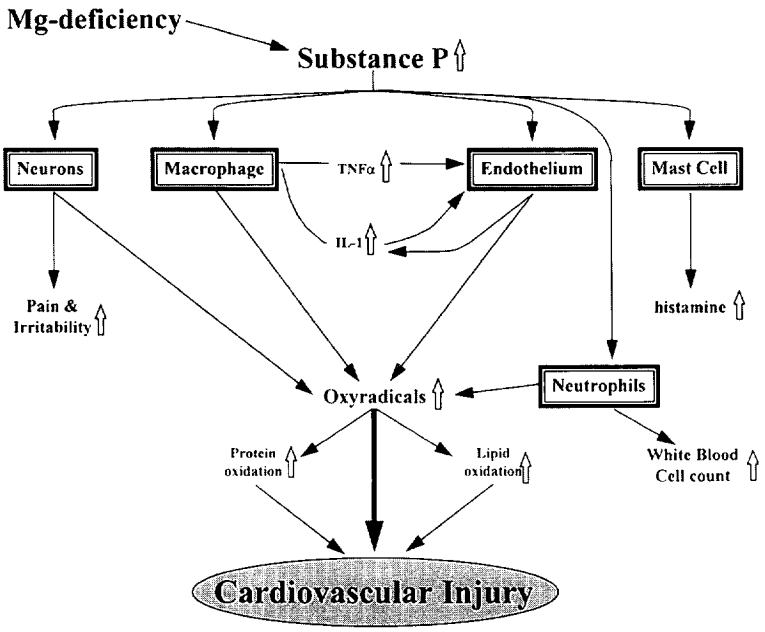

Fig. 3. Scheme of interrelated changes due to Mg-deficiency, emphasizing the central role of substance P influencing endothelium, mast cells, neutrophils, macrophage, neuronal and cardiovascular tissues. The cascade results in oxyradical production which leads to protein and lipid oxidation and cardiovascular injury.

Table 3
Accumulation of substrance P and CGRP in lesion areas from MgD rat hearts with or without treatment of substance P blocker

Treatment	Substance P (pg/ μg protein)	CGRP
MgS control	ND	ND
MgD	61 ± 8	27 ± 2
MgD + CP96345	16 ± 2 **	15 ± 2 **

ND = non-detectable; ** $P < 0.01$ vs. MgD alone.

into high-performance capillary electrophoresis columns for separation. We were able to show significant accumulation of neuropeptides in the microvascular lesions of Mg-deficient hearts (Table 3); however, in the normal-appearing cardiac tissue of deficient animals we saw only minimal neuropeptide elevations [47]. Thus, these studies localized neuropeptides within the cardiac lesions, and in those animals treated with the substance P receptor blocker we confirmed significant diminution of neuropeptides (Table 3). We viewed this as strong evidence for the involvement of neuropeptides in myocardial lesion formation.

These experiments raised new questions and provided some answers to explain the mechanisms leading to the Mg-deficiency cardiomyopathy. Our ability to reverse the pathobiology with neuropeptide receptor blockade convinced us that neuropeptides were a significant initiator of the pathogenic cascade leading to cardiac lesion formation, and that they probably involved other tissues such as the lungs, brain and kidneys. Fig. 3 depicts our expanded concept of the role of neuropeptides in the cascade of systemic cellular injury leading to enhanced free radical production, oxidation of proteins and lipids, and ultimately, cardiovascular injury.

4. Clinical relevance

The case of a patient presenting with profound Mg-deficiency due to alcoholism points to some causal role for magnesium in the observed multifaceted clinical pathology. The fact that the patient endured cardiovascular injury, pancreatic injury, and hepatic injury suggests a systemic process. Does the mechanism of injury include neurogenic peptide-triggered free radical production that results in multiorgan injury? We do not know at this time, but as our clinical diagnostic tools improve in the future we may be able to characterize the pathobiology of Mg-deficiency in man more accurately than we are able to do so today with atomic absorption analysis of plasma samples, which, while it measures total magnesium, does not provide information about ionized magnesium. New studies report the ability to detect levels of free magnesium rather than measuring total (bound plus free) magnesium in the plasma, using electrodes that are more specific for ionized magnesium in plasma. These techniques may help to enhance the diagnostic ability of clinicians who suspect Mg-deficiency as they become more widely utilized. Another interesting diagnostic modality is the use of a photofluorescent indicator of various ions within cells. The intravenous administration of magnesium and the subsequent quantification of the excreted portion of the magnesium is another test that identifies patients with a chronic deficit of magnesium, since they retain a much higher percentage of the intravenous magnesium [51]. The present difficulty in diagnosing Mg-deficiency is not unlike that of the problems detecting cholesterol disorders prior to the 1960's when more sophisticated electrophoretic techniques were established. With increasing fractionation of lipoproteins, a whole new therapeutic approach to patients with hypercholesterolemia was adopted by clinicians over the ensuing decades. Perhaps the field of Mg-deficiency-associated pathobiology will be advanced more rapidly when newer, more sophisticated diagnostic methodologies are applied routinely in the clinic and hospital. Only then will we be able to establish more firmly the multiple role of deficiency of this mineral in the etiology of disease in man.

Acknowledgments

This work was supported by USPHS grants HL-49232 and HL36418.

References

[1] Casscells W. Mg and myocardial Infarction. Lancet 1994; 343:807–809.
[2] Woods KL, Fletcher S. Long-term outcome after intravenous magnesium sulfate in suspected acute myocardial infarction: the second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2), Lancet 1994; 343:816–819.
[3] Durlach J. Magnesium in Clinical Practice. London, UK John Libbeg and Co., 1988.
[4] Seelig MS. Magnesium Deficiency in the Pathogenesis of Disease. New York: Plenum Medical Book Co., 1980.
[5] Seelig MS. Cardiovascular consequences of magnesium deficiency and loss. Am. J Cardio 1981, 63:4–21.
[6] Wacker WEC. Magnesium and Man. Cambridge, MA–London: Harvard University Press, 1980.
[7] Day HG. E.V. MacCollum and magnesium in nutrition. Trends Biochem. Sci 1962; 7(3):112–113.
[8] Bajusz E, Selye H. Codetermining factors for cardiac necrosis. Trans. NY Acad. Sci Ser 1959; II 1:659–667.
[9] Lehr D. Magnesium and cardiac necrosis. Magnesium Bull 1981; 3(1a):178–191.
[10] Heggtveit HA, Herman L, Mishra RK. Cardiac necrosis in experimental magnesium deficiency. A light and electron microscopic study. Am J Pathol 1964; 45:757–782.
[11] Altura BM. Sudden-death ischemic heart disease and dietary magnesium intake: Is the target site coronary smooth muscle? Med Hypotheses 1979; 5:843–848.

[12] Turlapaty PDMV, Altura BM. Magnesium deficiency produced spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease. Science 1980; 208:198–200.
[13] Altura BM, Altura BT. New perspective on the role of Mg in the pathophysiology of the cardiovascular system. Magnesium 1985; 4:226–244.
[14] Altura BM, Barbour RL, Dowd TL, Wu F, Altura BT, Gupta RK. Low extracellular magnesium induces intracellular free Mg deficits, ischemia, depletion of high-energy phosphates and cardiac failure in intact working rat hearts: a ^{31}P-NMR study. Biochim Biophys Acta 1993; 1182(3):329–332.
[15] Rayssiguier Y, Gueux E, Bussiére J, Durlach J, Mazur A: Dietary magnesium affects susceptibility of lipoproteins and tissues to peroxidation in rats. J Am Coll Nutr 1993; 12:133–137.
[16] Classen HG, Fischer G, Jacob R, Marx J, Schimatschek H, Stein C. Prenecrotic electrolyte alterations of the adrenergic cardiopathy: potentiation by magnesium depletion and prevention by high dietary magnesium levels and rerapaniel. Magnesium Bull. 1986; 8:82–92.
[17] Bloom S. Magnesium deficiency cardiomyopathy Am J Cardiovasc Pathol 1988;2:7–17.
[18] Günther T, Vormann J, Hollriegl V, Disch G, Clossen HG. Role of lipid peroxidation and vitamin E in magnesium deficiency. Magnesium Bull 1992;14:57–66.
[19] Atrakchi AH, Bloom S, Dickens BF, Mak IT, Weglicki WB: Hypomagnesemia and isoproterenol cardiomyopathies: protection by probucol. J Cardiovas Pathol 1992;1:155–160.
[20] Freedman AM, Atrakchi AH, Cassidy MM, Weglicki WB. Magnesium deficiency-induced cardiomyopathy: protection by vitamin E. Biochem Biophys Res Commun 1990;1770:1102–1106.
[21] Weglicki WB, Freedman AM, Bloom S, et al. Antioxidants and the cardiomyopathy of Mg-deficiency. Am J Cardiovasc Pathol 1992;4: 210–215.
[22] Weglicki WB, Stafford RE, Cassidy MM, Phillips TM. Modulation of cytokines and cardiomyopathic lesions in a magnesium-deficient rodent model: effects of vitamin E and chloroquine. Am J Physiol 1993;264:C723–C726.
[23] Freedman AM, Cassidy MM, Weglicki WB. Propranolol reduces cardiomyopathic injury induced by magnesium deficiency. Magnesium Trace Elem 1992;10:348–354.
[24] Kimura M, Itokawa M. Inefficient utilization of iron and minerals in magnesium deficient rats. In Itokawa Y, Durlach J, eds. Magnesium in Health and Disease London: John Libbey & Co. Ltd., 1989;95–102.
[25] Freedman AM, Mak, IT, Stafford RE, et al. Erythrocytes from magnesium-deficient hamsters display an enhanced susceptibility to oxidative stress. Am J Physiol Cell Physiol 1992;262:C1371–C1375.
[26] Herzog WR, Atar D, Mak IT, Alyono D, MacCord C, Weglicki WB. Magnesium deficiency prolongs myocardial stunning in an open-chest swine model. Int J Cardiol 1994;47:105–115.
[27] Hsu JM, Smith JC Jr, Yunice AA, Kepford S. Impairment of ascorbic acid synthesis in liver extracts of magnesium-deficient rats: J Nutr 1983;113:2041–2047.
[28] Kassouny, ME, Coen CH, Bebok ST. Influence of vitamin C and magnesium calcium, magnesium, and copper contents of guinea pig tissues. Int J Vitam Nutr Res 1985;55:295–300.
[29] Niki E. Vitamin C as an antioxidant. World Rev Nutr Diet 1991;64:1–30.
[30] Günther T, Vormann J, Höllriegl V, Gossrau R. Effect of magnesium deficiency and salicylate on lipid peroxidation in vivo. Magnesium Bull 1991;13:26–29.
[31] Mak IT, Stafford RE, Weglicki WB. Loss of red cell glutathione during Mg deficiency: prevention by vitamin E. D-propranolol, and chloroquine. Am J Physiol 1994;267(Cell Physiol. 36):C1366–C1370.
[32] Stafford RE, Mak IT, Kramer JH, Weglicki WB. Protein oxidation in magnesium-deficient rat brains and kidneys. Biochem Biophys Res Commun 1993;28:569–600.
[33] Singal P, Kapur N, Dhillon K. Beamish R, Dhalla N. Role of free radicals in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol 1982;60:1390–1397.
[34] Freedman AM, Cassidy MM, Weglicki WB. Magnesium-deficient myocardium demonstrates an increased susceptibility to an in vivo oxidative stress. Magnesium Res 1991;4:185–189.
[35] Freedman AM, Cassidy MM, Weglicki WB. Captopril protects against myocardial injury by magnesium deficiency. Hypertension 1991;18:142–147.
[36] MaK IT, Freedman AM, Dickens BF, Weglicki WB. Protective effects of sulfhydryl-containing angiotensin converting enzyme inhibitors against free radial injury in endothelial cells. Biochem Pharmacol 1990;40:2169–2175.
[37] Kramer JH, Misík V, Weglicki WB. Magnesium-deficiency potentiates free radical production associated with postischemic injury to rat hearts: vitamin E affords protection. Free Radical Biol Med 1994;16(6):713–723.
[38] Weglicki WB, Phillips TM, Mak IT, et al. Cytokines, neuropeptides, and reperfusion injury during magnesium deficiency. Ann NY Acad Sci 1994;723:246–257.
[39] Wu F, Altura BT, Gao J, Barbour RL, Altura BM. Ferrylmyoglobin formation induced by acute magnesium deficiency in perfused rat heart causes cardiac failure. Biochim Biophys Acta 1994;1225(2): 158–164.
[40] Weglicki WB, Phillips TM, Freedman AM, Cassidy MM, Dickens BF. Magnesium-deficiency elevates circulating levels of inflammatory cytokines and endothelin. Mol Cell Biochem 1992;110:169–173.
[41] Henning B, Chow CK. Lipid peroxidation and endothelial cell injury: implications in atherosclerosis. Free Radical Biol Med 1988;4:99–106.
[42] Gannon DE, Varani J, Phan SH, et al. Source of iron in neutrophil-mediated killing of endothelial cells. Lab Invest 1987;57:37–44.
[43] Weglicki WB, Phillips TM. Pathobiology of magnesium deficiency: a cytokine/neurogenic inflammation hypothesis. Am J Physiol 1992;263:R734–R737.
[44] Mantyh PW. Substance P and the inflammatory and immune response. Ann NY Acad Sci 1991;632:263–271.
[45] Baraniuk JN, Kowalski ML, Kaliner MA. Relationships between permeable vessels, nerves, and mast cells in rat cutaneous neurogenic inflammation. J Appl Physiol 1990;68:2305–2311.
[46] Weglicki WB, Mak IT, Stafford RE, Dickens BF, Cassidy MM, Phillips TM. Neurogenic peptides and the cardiomyopathy of Mg-deficiency: effects of substance P-receptor inhibition. Mol Cell Biochem 1994;130:130–109.
[47] Weglicki WB, Mak, IT, Phillips TM: Blockade of cardiac inflammation Mg^{2+} deficiency by substance P receptor inhibition. Circ Res 1994;74:1009–1013.
[48] Ralevich V, Milner P, Hudlicka O, Kristek F, Burnstock G. Substance P is released from the endothelium of normal and capsaicin-treated rat hind-limb vasculature, in vivo, by increased flow. Circ Res 1990;66:1178–1183.
[49] Papka RE, Furness JB, Della NG, Murphy R, Costa M. Time course of effect of capsaicin on ultrastructure and histochemistry of substance-P immunoreactive nerves associated with the cardiovascular system of the guinea-pig. Neuroscience 1984;12:1277–1292.
[50] Schmidt AW, McLean S, Heym J. The substance P receptor CP-96345 interacts with Ca^{2+} channels. Eur J Pharmacol 1992;215: 351–352.
[51] Gullestad L, Dolva LØ, Waage E, Falch D, Fagerthun H, Kjekshus J. Magnesium deficiency diagnosed by an intravenous loading test. Scand J Clin Lab Invest 1992;52:245–253.