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Low magnesium and atherosclerosis: an evidence-based link

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Abstract

Several data indicate that magnesium deficiency caused by poor diet and/or errors in its metabolism may be a missing link between diverse cardiovascular risk factors and atherosclerosis. Experimentally induced low plasma levels of magnesium accelerate atherogenesis by increasing LDL concentrations and their oxidative modifications, and by promoting inflammation. In vitro studies have shown that low magnesium determines endothelial dysfunction, the initiating event leading to the formation of the plaque. Moreover, oral magnesium therapy has been shown to improve endothelial function in patients with coronary artery disease.

Magnesium, which is an inexpensive, natural and rather safe element, could be useful in preventing atherosclerosis and as an adjuvant therapy in patients with clinical manifestations of the disease.

1. Introduction

Recently, the concept of atherogenesis has evolved from the vague ideas of inevitable degeneration to a much better defined scenario of molecular and cellular events. Thanks to the understanding of its fundamental mechanisms, atherogenesis is approached as a modifiable rather than ineluctable process through the development of novel strategies for mitigating the disease. In a broad outline, atherosclerosis is a form of chronic inflammation resulting from interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal components of the arterial wall. This inflammatory process ultimately leads to the development of

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complex lesions, the plaques, that protrude into the arterial lumen (Glass and Witzum, 2001).

Recruitment of mononuclear leukocytes to the intima is one of the earliest events in the formation of the atherosclerotic lesion. This is reached through the expression on the surface of the endothelium of specific adhesion molecules which mediate first leukocyte rolling and then their definitive sticking. In particular, vascular cell adhesion molecule-1 (VCAM-1) binds precisely the types of leukocytes found in early human and experimental atheroma, the monocyte and the T lymphocyte. Once adherent, the leukocytes penetrate the artery wall directed by certain chemokines, a particular role being played by monocyte chemoattractant protein-1 (MCP1). When resident in the arterial wall, the blood-derived cells participate in promoting and perpetuating a local inflammatory response. In response to macrophage-colony stimulating factor (M-CSF), the monocytes differentiate into macrophages, which express scavenger receptors for modified lipoproteins, so that they ingest lipids and become foam cells, the hallmark of the early atheromatous precursor, the fatty streak. All these events are reversible and do not cause clinical consequences. However, macrophage accumulation in the intima sets the stage for progression of atheroma and evolution into more fibrous and eventually more complicated plaque that can indeed cause clinical disease. Accumulation of smooth muscle cells and their elaboration of extracellular matrix proteins importantly contribute to the progression of the fibrous lesions (Libby et al., 2002).

Among the many genetic and environmental risk factors that have been identified by epidemiologic studies (Table 1), elevated levels of serum cholesterol are unique in being sufficient to drive the development of atherosclerosis in humans and experimental animals, even in the absence of other known risk factors. However, even among individuals with the same cholesterol levels, there is a great disparity in the expression of the clinical disease, thus indicating that other factors, eventually underestimated, contribute to atherogenesis.

Plasma levels of magnesium represent an interesting candidate which may explain the variable response to risk factors reported in different individuals. Indeed, low

Table 1
Risk factors for the development of atherosclerosis

Environmental factors
Smoke
Diet
Lack of exercise
Infections
Factors with significant genetic component
Diabetes
Hyperlipemia ↑LDL, ↑VLDL, ↑Lp(a), ↓HDL
Hypertension
Obesity
Hyperhomocysteinemia
Male gender

plasma concentrations of magnesium promote inflammation and atherosclerosis is an inflammatory disease. Moreover, low plasma magnesium potentiates the detrimental effects of several risk factors and induces endothelial dysfunction, the initiating step in atherosclerosis. Last but not least, hypomagnesemia is frequently associated with hypertension, diabetes and aging, known risk factors in atherosclerosis.

2. Magnesium: a puzzling, albeit forgotten, electrolyte

Magnesium is the second most abundant intracellular cation, after potassium, and is a cofactor in more than 300 enzymatic reactions involving energy metabolism, protein and nucleic acid synthesis. Indeed, it activates a large array of enzymes either by bridging distinct molecules or by functioning as an allosteric modulator through its interaction with negatively charged moieties (Romani and Scarpa, 1992). In addition, magnesium is considered the physiologic calcium antagonist; at the cellular level, it has been proposed to act as a chronic regulator of cell functions, opposed to calcium which is responsible for acute events (Iseri and French, 1984). Approximately 40% of the magnesium contained in the adult human body resides in the muscles and soft tissues, about 1% in the extracellular fluid and the remainder in the skeleton (Aikawa, 1981). The serum level of the cation is maintained remarkably constant in healthy individuals by poorly understood hemostatic mechanisms. Although the measurement of magnesium in serum does not always reflect the overall status of its metabolism, it well correlates with intracellular free magnesium, the physiologically active form of the element (Resnick et al., 1993). This is one of the reasons why serum magnesium is still the most commonly used parameter for assessing disorders of magnesium metabolism in clinical practice (Elin, 1994).

Epidemiological studies have indicated a direct relation between atherosclerosis and serum magnesium (Ma et al., 1995), which, in turn, depends on dietary intake. The importance of this electrolyte has further been emphasized by autopsy studies demonstrating more coronary atherosclerosis in men from soft water areas (Crawford and Crawford, 1967). Indeed, as the magnesium level in drinking water increases, the incidence of ischemic heart disease, one of the clinical patterns of atherosclerosis, declines (Rubenowitz et al., 1996). In parallel, supplementation with magnesium has beneficial effects on plasma lipids (Rasmussen et al., 1989) and platelet reactivity (Nadler et al., 1992) in patients with ischemic heart disease. It has also been observed that coronary artery disease is associated with excessive magnesium loss and a relative magnesium-deficient state (Seelig, 1964), thus generating a loop of events in which it is difficult to identify the initiating step.

Evidence suggests that the occidental "American diet" is relatively deficient in magnesium, whereas the "Oriental diet," which is characterized by a greater intake of fruits and vegetables, is rich in magnesium (Seelig, 1989). This different dietary intake of the cation may contribute to explaining the lower incidence of atherosclerosis in oriental than in Western populations. Moreover it should be recalled that magnesium deficiency is a significant clinical complication arising in patients treated

with some classes of diuretics, as well as patients with diabetes mellitus and alcoholism, both condition being linked to a high incidence of atherosclerotic lesions.

On these bases, it is conceivable to suggest magnesium supplementation a safe and inexpensive tool useful in prevention and in therapy.

In the next sections, experimental evidence about the mechanisms behind the antiatherogenic effect of magnesium will be discussed.

3. The antiatherogenic effect of magnesium: lessons from animal models

Experimental induced magnesium deficiency promotes atherosclerosis in several animal models, because it activates an inflammatory response and causes hyperlipemia. In rabbits, dietary magnesium restriction, compatible with the daily intake of magnesium in western population, is sufficient to exacerbate atherogenesis by upregulating serum levels of triglycerides and cholesterol. In high cholesterol-fed rabbits, magnesium deficiency increases lipid deposition in the intima thus leading to intimal thickening. In addition, magnesium deficient diet activates macrophages, which accumulate huge amounts of lipids contributing to the progression of the lesion (Altura et al., 1990). Interestingly, higher than normal magnesium intake is a very useful ameliorative agent in experimental atherogenesis in rabbits because it reduces macrophage activation and corrects the lipid profile (Altura et al., 1990).

In rats, dietary magnesium restriction increases the plasma levels of LDL and VLDL and reduces HDL. The lower concentrations of HDL is determined by a decrease in the availability of apo E and apo A-1, the major apoproteins of HDL. In magnesium deficient rats, hepatic apo E synthesis is downregulated at the transcriptional level, while the origin of the reduction in plasma apo A-1 concentration remains unclear (Nassir et al., 1995). It is noteworthy that magnesium deficiency also stimulates the peroxidation of lipoproteins (Rayssiguier et al., 1993). This is explained by the finding that dietary restricted rats are less protected against oxidative stress. Oxidized LDL is more atherogenic than the wild type lipoprotein; moreover, HDL loses its effect to stimulate efflux of cholesterol from foam cells after oxidative modifications (Nagano et al., 1991). In addition to hyperlipemia and oxidative stress, another event plays a crucial role in promoting atherosclerosis in rats on a low magnesium diet: inflammation. Elevated levels of cytokines have been observed in these animals (Malpuech-Brugère et al., 2001), in particular IL-6 is primarily induced by magnesium depletion and is responsible for the induction of acute phase protein synthesis. The increased circulating concentrations of proinflammatory cytokines, such as interleukin 1, activate macrophages and endothelial cells. In brief, the inflammatory response in magnesium deficient rats may contribute to atherogenesis by modifications of lipoprotein metabolism, oxidation of lipoproteins, inflammatory cell recruitment and release of cytokines and growth factors that induce cell migration and proliferation (Nassir et al., 1995; Rayssiguier et al., 1993). Interestingly, increased magnesium intake counteracts all these effects (Laurant et al., 2000).

Murine models are increasingly used to study the mechanisms involved in atherogenesis. With the introduction of transgenic and knockout techniques, a number of

new murine strains have been developed with altered lipoprotein metabolism and accelerated atherosclerosis. Two of these models generate extensive atherosclerosis in the entire arterial system, namely the LDL receptor deficient and the apo E deficient mice (Carmeliet et al., 1998). Severe hypercholesterolemia and advanced atherosclerosis are induced in the LDL receptor deficient mice by a high fat diet. These detrimental events are retarded after magnesium fortification of the drinking water which, however, does not affect the lipid profile (Sherer et al., 1999). This result is puzzling, since it suggests that magnesium acts on targets other than hyperlipemia. We can speculate that it retards inflammatory dependent events, such as the adhesion of monocytes to the endothelium, the first of a long list of steps leading to the formation of the plaque.

In contrast, the apo E knockout mice spontaneously develop hypercholesterolemia and mature atherosclerotic plaques (Nakashima et al., 1994). Oral magnesium induces favorable antiatherogenic changes in apo E knockout mice and this parallels with a significant reduction in cholesterol and triglyceride levels (Ravn et al., 2001).

Magnesium has proven useful also in acute conditions, since it reduces infarct size after ischemia/reperfusion injury (Ravn et al., 1999). Two mechanisms are implicated in this protective effect: (i) magnesium reduces the generation and release of free oxygen radicals; and (ii) magnesium inhibits platelet reactivity. This finding is relevant because, a part from being protagonists in arterial thrombosis, platelets are also involved in atherogenesis since they release growth factors for smooth muscle and endothelial cells.

4. The antiatherogenic effect of magnesium: what cells teach us

Endothelial cells maintain the functional integrity of the vascular wall. Beyond their role as a permeability factor, they are involved in the maintenance of a non-thrombogenic blood-tissue interface, in the modulation of blood flow and vascular resistance, in the regulation of immune and inflammatory reactions (Maciag, 1984). A huge amount of experimental evidence supports the paradigm of endothelial dysfunction as the common link between risk factors (Table 1) and atherosclerotic burden (Ross, 1999). Endothelial dysfunction actively participates in the process of lesion formation by promoting early and late mechanisms of atherosclerosis. These include the upregulation of adhesion molecules with consequent leukocyte adherence, increased chemokine secretion, increased cell permeability to lipids, enhanced LDL oxidation, cytokine elaboration, vascular smooth muscle cell proliferation and migration and platelet activation.

Most of these alterations of endothelial behavior have been described in cells grown in medium containing low magnesium (Fig. 1). Magnesium deficiency increases LDL transport across the endothelial monolayer (Yokoyama et al., 1994). In the first hours this is mainly due to the stimulation of energy-dependent transport, while later also energy-independent transport plays a role because of the formation of intercellular gaps. Once in the subendothelial space, LDL is going to be oxidized by endothelial cells. Indeed, while LDL is protected from oxidation in the plasma

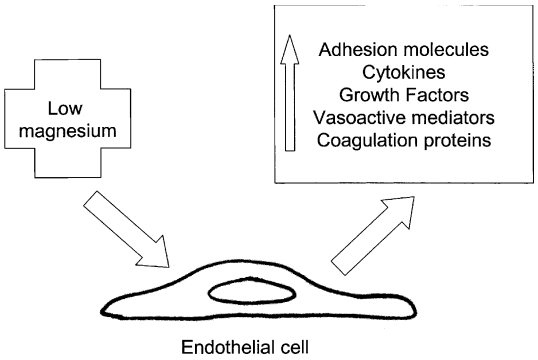

Fig. 1. How low magnesium affects endothelial cell behavior.

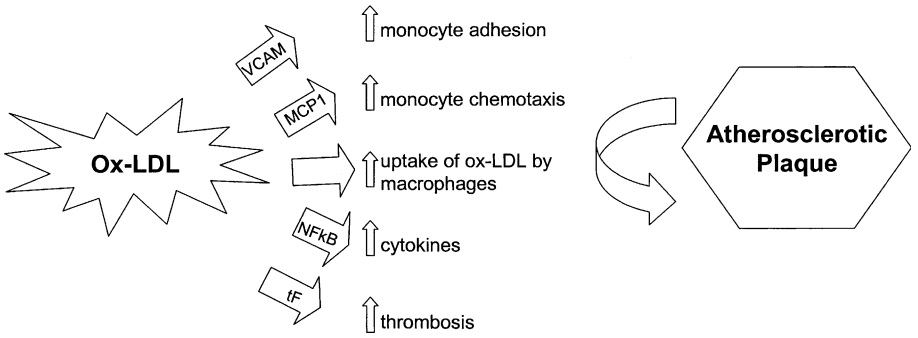

Fig. 2. The proatherosclerotic effects of ox-LDL. VCAM: vascular cell adhesion molecule; MCP1 monocyte chemoattractant protein-1; tF: tissue factor.

compartment, it is more susceptible to enzymatic and non-enzymatic modifications when retained by extracellular proteins in the artery wall. Several pro-atherogenic properties have been ascribed to oxidized LDL (Fig. 2). A number of potential oxidant-generating systems could target LDL lipids, including myeloperoxidase, nitric oxide synthase and 15-lipoxygenase (Steinberg and Witzum, 2001). Interestingly, endothelial cells demonstrate oxidative activation following magnesium deficiency (Zhou et al., 1999). In addition, under these culture conditions, endothelial cells are more susceptible to oxidative injury (Dickens et al., 1992). A vicious circle is therefore established: injured endothelial cells oxidize LDL which becomes more atherogenic and further impairs endothelial function (Fig. 3).

It has also been demonstrated that low extracellular magnesium markedly and reversibly enhances endothelial cell/monocyte interactions (Maier et al., 2001), thus establishing another important step towards the formation of the atherosclerotic lesion. An early event in atherosclerosis is the elaboration by endothelial cells of growth factors and cytokines that perpetuate their dysfunction, the accumulation and activation of monocyte macrophages, have effects on smooth muscle cells and, eventually, influence platelets' functions. Among others, low magnesium increased

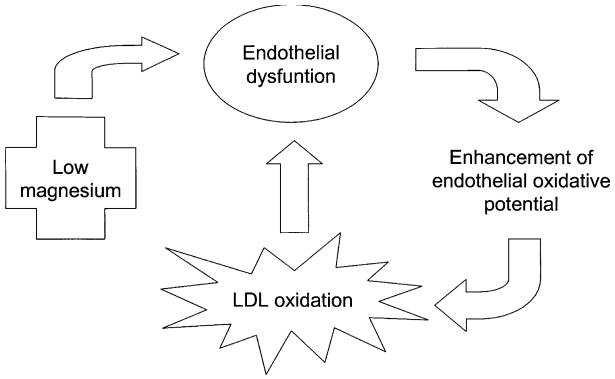

Fig. 3. The loop existing between low magnesium, ox-LDL and endothelial dysfunction (see the text).

endothelial secretion of platelet derived growth factor (PDGF)-AA and -BB (Kawano et al., 1995), molecules that stimulate medial smooth muscle cells to migrate into the subendothelial space and proliferate (Ross, 1993). Similar effects are determined by interleukin (IL) 1, which is also upregulated endothelial cells cultured in low magnesium (Maier et al, submitted). IL-1 has multiple effects on all the cell types involved in atherogenesis and, because it is pro-inflammatory, plays a role in promoting and maintaining chronic inflammation.

Low magnesium also impairs endothelial proliferation, and this may be another contributing factor to atherogenesis: if a severe endothelial damage has occurred, healing of the injury will be delayed (Banai et al., 1990). It is likely that this growth inhibition is related to the release of cytokines by endothelial cells themselves, but also by other cell types infiltrating the lesion, in particular macrophages and lymphocytes that elaborate IL-1, TGF beta, interferon gamma, among others, all known inhibitors of endothelial growth and modulators of their behavior (Cines et al., 1998).

Several studies have shown that homocysteine (HC) causes endothelial injury, proliferation of smooth muscle cells and altered blood coagulation, thus indicating that hyperhomocysteinemia contributes to atherogenesis (Stamler et al., 1993). Indeed, an inverse relation between magnesium and HC has been demonstrated (Li et al., 1999). Since the major enzymes involved in HC metabolism are magnesium dependent, it is noteworthy that exposure of smooth muscle cells to reduced concentrations of this cation inhibits the conversion of HC to cysteine and methionine and this metabolic blockage increases circulating HC levels. This is not the only direct effect of magnesium deficiency on smooth muscle cells, another being the induction of calcium flux and, therefore, contraction. To this purpose, it is noteworthy to recall the possible role of magnesium in the pathogenesis and treatment of hypertension (Laurant and Berthelot, 2001), a major risk factor for atherosclerosis. The role of this cation in regulating blood pressure is rather complex, since magnesium directly antagonizes calcium and is a second messenger in angiotensin II signaling in smooth muscle cells (Touyz, 2001); it also interferes with endothelial production of

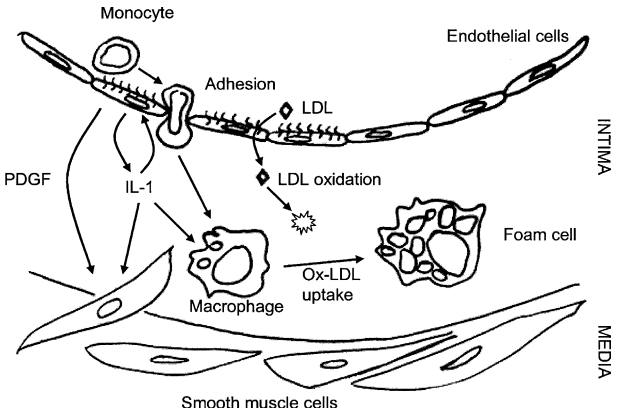

Fig. 4. Hypothetical scheme of the proatherogenic effects of low magnesium. Several changes take place in the endothelium: increased permeability to LDL, upregulation of adhesion molecules, migration of monocytes in the artery wall, release of platelet derived growth factor (PDGF) and interleukin (IL)-1.

nitric oxide (Pearson et al., 1998), a potent endogenous nitrovasodilator and inhibitor of platelet aggregation and adhesion, which is reduced in atherosclerosis. Interestingly, experimental work has shown that hypercoagulability and increased platelet aggregation are associated to hypomagnesemia (Gawaz et al., 1994), which stimulates platelet activation also by enhancing platelet stimulating factors such as thromboxane A2 (Schecter, 2001). Moreover, when cultured in low magnesium, endothelial cells upregulate plasminogen activator inhibitor (PAI)-1 (Maier et al., submitted), frequently overexpressed in atherosclerotic arteries (Schneiderman et al., 1992).

All the in vitro experiments indicate that low magnesium contributes to atherosclerosis since it profoundly affects endothelial and smooth muscle cells function (Fig. 4).

5. Conclusions

The accumulated theoretical, experimental and clinical data suggest that magnesium protects against atherosclerosis and thrombosis. Indeed, exposure of endothelial cells to low magnesium mimics some of the crucial events involved in the pathogenesis of the disease and its complications. Moreover, different animal models have demonstrated that hypomagnesemia induces inflammation and hyperlipemia thus accelerating atherogenesis. In addition, oral magnesium supplementation reduces plasma concentrations of triglycerides, VLDL and apo B, and enhances the apo AI/apo B ratio in patients who suffered myocardial infarction (Rasmussen et al., 1989), and communities with low concentrations of magnesium in the drinking water tend to have an increased incidence of ischemic heart disease. Recently oral magnesium therapy has been shown to revert endothelial dysfunction in patients with

coronary artery disease (Schecter et al., 2000). All these studies demonstrate the rational of the use of magnesium in the prevention and treatment of atherosclerotic lesions.

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