

# Why vasculitis probably can be ameliorated with magnesium and antagonists of ceramides and platelet-activating factor

## Introduction

Vasculitis is characterized as an inflammatory disease of the body's small blood vessels, particularly in the lungs and kidneys.<sup>1-4</sup> Many other organ regions are usually affected which often induces morbidity and mortality.<sup>1-4</sup> Although the exact causes of vasculitis are not known, it appears to be an autoimmune disease even though physical, chemical injuries and infections can result in vasculitis.<sup>1-4</sup> It is classified as a rare disease in the USA because there are only about 200,000 cases. Although numerous treatments have been advocated, there is no known cure or preventative treatment. Vasculitis often leads to difficulties in breathing and renal shut-down. In addition, vasculitis leads to cardiac malfunctions, cardiac failure and strokes. Vasculitis is clearly more common in the aged.<sup>1-4</sup> Recently, we have found that a few patients that were diagnosed with vasculitis appear to have a magnesium deficiency (MgD), particularly in the serum ionized Mg<sup>2+</sup> fraction [unpublished findings].

Unlike atherosclerosis that takes decades to develop, vasculitis of small and medium sized arterial vessels, as well as microscopic arterioles, venules and capillaries, progresses rapidly, thus producing tissue ischemia via lumen-occlusive intimal hyperplasia and inflammatory syndromes.<sup>1-4</sup> Whether the end result is giant cell arteritis (GCA), polyarteritis nodosa (PAN), Churg- Strauss vasculitis (CSV), Wegner granulomatosis (WG), polymyalgia rheumatica (PR), Behcets disease (BD), or other vascular diseases, invasion of the arterial and microcirculatory walls by macrophages, leukocytes and CD4 T-cells seems to be pivotal.<sup>1-4</sup> In addition, most of these patients appear to demonstrate coagulationopathies. The degrees of luminal stenoses vary from patient to patient. Usually, degradation of the internal elastic laminae (EL) follow suit.<sup>1-3</sup> It has been hypothesized that the latter stenoses are due to concentric growth of the intima which seems to be related to several angiogenic growth factors, e.g., platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF).<sup>4</sup> What stimulates the production of these growth factors is not completely known but is thought to involve activation of nuclear-factor-KB (NF-kB) in the macrophages and leukocytes.<sup>4</sup>

As stated, the macrophages and leukocytes clearly play key roles in the development of vasculitis. They are activated by NF-kB to produce a host of cytokines and chemokines which are needed for tissue remodeling and granuloma formation in development of vasculitis.<sup>2,4</sup> What activates the macrophages and leukocytes to induce production of NF-kB is not known.

## Why magnesium deficiency is most likely a key player in development of vasculitis

While we were routinely investigating the potential role of magnesium deficiency (MgD) in numerous cardiovascular- diseased patients, who presented with coronary arterial diseases, coronary vasospasm, acute myocardial infarctions (AMIs), congestive heart

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failure, and strokes, we noted that several of these patients had an underlying vasculitis together with significant deficits in serum ionized Mg, but not necessarily total serum Mg levels.<sup>5</sup>

More than 50 years ago, two of us found that reduction in the concentration of extracellular free Mg ions (Mg<sup>2+</sup>) resulted in vasospasm of coronary, cerebral, and peripheral arterial vessels; the lower the [Mg<sup>2+</sup>]<sub>0</sub>, the more the intense the arterial vasospasm.<sup>6-13</sup> In addition, our laboratories found that microscopic blood vessels in skeletal, cutaneous, and cerebral vascular beds of intact rats, mice, rabbits, guinea-pigs, dogs, and piglets exhibited similar phenomena as dietary Mg intake was reduced over three to 12 weeks.<sup>14,15</sup> Moreover, vascular reactivity to circulating humoral and hormonal vasoconstrictor agents (i.e., angiotensin II, norepinephrine, serotonin, numerous peptide mediators, etc.) was intensified when [Mg<sup>2+</sup>]<sub>0</sub> was reduced; the lower the [Mg<sup>2+</sup>]<sub>0</sub>, the greater the humoral and hormonal-induced vasoconstriction.<sup>6-10,13</sup> It is important to note, here, that these agents are often present at increased, circulating levels in cases of vasculitis.

It is now clear that all cases of vasculitis are associated with increased levels of various cytokines and chemokines (e.g., IFN-alpha, IL-1-beta, IL-2, IL-8, IL-10, IL-4, IL-17, TNF-alpha, MCP-1, among others) which are pro-inflammatory in nature.<sup>1-4</sup> We have found that rats placed on MgD diets for 21 days generate all of these pro-inflammatory cytokines and chemokines in the blood, cardiac

tissues and arterial vessels.<sup>16,17</sup> Other investigators have also reported finding many of these cytokines and chemokines in MgD animals.<sup>18</sup> Furthermore, we have found that these MgD animals generate growth factors similar to those found in patients presenting with various forms of vasculitis.<sup>4,5</sup> All of these cytokines, chemokines, and growth factors, we found in the MgD animals, were associated with microvascular wall remodeling and pathological alterations in the postcapillary venules, resulting in reduced lumen sizes, increased vascular reactivity, and adherence of leukocytes and macrophages on the endothelial cell walls,<sup>19-21</sup> thus, in many respects, similar to what is seen in vasculitis.

Last, but not least, we have found that the MgD state that we produced in the rats resulted in activation of NF- $\kappa$ B in cardiac, cerebral, and peripheral vascular smooth muscle cells.<sup>16,17,19,20-26</sup> In view of our findings, we believe, collectively, it is difficult to dismiss the probable role of MgD in the etiology and sustenance of a state of inflammation and vasculitis. Thus, we recommend that our hypothesis should be tested in two ways: 1. Use a Mg<sup>2+</sup>-ion selective electrode similar to those we helped to pioneer<sup>27-31</sup> in order to carefully measure the levels of ionized free Mg; and 2. Administer Mg salts, initially, intravenously, then orally, for extended periods of time.

Low Mg<sup>2+</sup> induces leukocyte and macrophage sticking, increased adhesiveness to venular endothelial walls, and increased postcapillary permeability in the microcirculation. Approximately 40 years ago, Ross et al advanced the hypothesis that atherosclerosis is an inflammatory disease brought about by injury to the endothelial surfaces of the macro- and microcirculations,<sup>32</sup> for summary of their hypothesis]. The hypothesis stated that different forms of injury (e.g., ischemic events) will result in numerous dysfunctions in the homeostatic properties of the endothelium, e.g., increases in adhesiveness of macrophages and leukocytes and/or platelets, alteration in the procoagulant properties, formation/release of cytokines/chemokines and growth factors. Usually, inflammation is defined as a response of microcirculatory blood vessels and the tissues they perfuse to infections and damaged tissues which bring cells and host-defence factors/molecules directly from the circulation to all the diverse sites where they are required in order to eliminate/degrade the offending agents.<sup>33</sup> The mediators of the defense mechanisms include white blood cells, macrophages, phagocytic leukocytes, chemokines, antibodies, and complement proteins.<sup>33</sup> The inflammatory process brings these cells and molecules to the damaged or necrotic tissues. During the normal inflammatory process, macrophages, leukocytes, and monocytes migrate across the venous capillary walls through holes in between the endothelial cells due to increases in permeability and move to the site(s) of injury via chemotaxis. This sequence of events is thought to take place in all types of inflammatory events and in developing vasculitis.<sup>34</sup> The normal mediators for these processes to take place are adhesion molecules, cytokines, and chemokines, all of which we have found in patients with different forms of vasculitis and in MgD.<sup>5,26</sup>

### Probable contributing roles of ceramides and platelet-activating factor as a consequence of MgD to aetiology of vasculitis

In the late 1990's, working with proton-nuclear magnetic resonance spectroscopy (1H-NMRS), and arterial vessels exposed to low Mg<sup>2+</sup> levels, two of us found an increased synthesis of several sphingolipids

(namely, ceramides, sphingosine, and sphingosine-1-phosphate) along with an increased formation of platelet-activating factor (PAF).<sup>35,36</sup> We and others have reported that many of these sphingolipids (particularly ceramides) and PAF promote vasoconstriction and vasospasm of different types of arterial blood vessels as well as arterioles and muscular venules in the living microcirculation *in situ*.<sup>22,23,25,26,37-41</sup> In addition, three of us found that ceramides and PAF cause increases in postcapillary permeability, leukocyte and macrophage adhesion to the endothelial linings of the postcapillary venules, and migration of these latter cell types to the extravascular tissue spaces.<sup>41</sup> What we found, of particular interest, is that vascular smooth muscle cells (of different types), when exposed, in primary cell culture, to low Mg<sup>2+</sup> caused a synthesis of both ceramides and PAF, which could be selectively inhibited using specific antagonists of ceramides and PAF.<sup>42</sup> More than 30 years ago, Cunningham and colleagues reported that sera from rheumatoid vasculitis patients contained platelet-releasing activity.<sup>43</sup> Two years later, Warren and his colleagues, using a rat model of immune complex vasculitis, found that a receptor blocker of PAF inhibited an Arthus reaction.<sup>44</sup> Sera taken from patients in our hospitals which had an underlying vasculitis (of diverse origins), and lowered serum ionized Mg, demonstrated increased levels of both ceramides and PAF.<sup>5</sup> We do not believe these findings are merely coincidental. It is our contention that low Mg coupled to increased cellular and serum levels of ceramides and PAF are causal agents in many types of vasculitis.

### Conclusion

Although the exact cause(s) of vasculitis is not known, Mg depletion appears to be a presence in different types of vasculitis. When several of our cardiovascular- diseased patients were admitted to our hospitals, a number of them exhibited an underlying vasculitis coupled with an ionized Mg deficiency along with elevated serum levels of ceramides and PAF. Mg-deficient animals, in our labs, exhibited elevated serum and tissue levels of ceramides and PAF which could be reduced/inhibited with specific antagonists of ceramide and PAF synthesis. Elevated dietary levels of Mg also reduced the synthesis of both ceramides and PAF, at least in experimental animals. Experimental animals fed Mg deficient diets exhibited, *in-vivo*, inflammatory alterations in the microcirculation similar to those patients presenting with different forms of vasculitis (e.g., elevated cytokines, elevated chemokines, elevated adhesion molecules, elevated tissue levels of NF- $\kappa$ B, along with other substances). In view of these new findings from our laboratories, it is our belief that patients exhibiting vasculitis should be treated with oral Mg supplements along with inhibitors of ceramide and PAF synthesis in order to determine if our hypothesis is valid.

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## Conflicts of interest

The authors declare there are no conflicts of interest.

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