Thrombotic microangiopathy: A role for magnesium?

Steven Van Laecke; Evi V. T. Nagler; Raymond Vanholder
Department of Nephrology, Ghent University Hospital, Ghent, Belgium

Summary
Despite advances in more recent years, the pathophysiology and especially treatment modalities of thrombotic microangiopathy (TMA) largely remain enigmatic. Disruption of endothelial homeostasis plays an essential role in TMA. Considering the proven causal association between magnesium and both endothelial function and platelet aggregability, we speculate that a magnesium deficit could influence the course of TMA and the related haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. A predisposition towards TMA is seen in many conditions with both extracellular and intracellular magnesium deficiency. We propose a rationale for magnesium supplementation in TMA, in analogy with its evidence-based therapeutic application in pre-eclampsia and suggest, based on theoretical grounds, that it might attenuate the development of TMA, minimise its severity and prevent its recurrence. This is based on several lines of evidence from both in vitro and in vivo data showing dose-dependent effects of magnesium supplementation on nitric oxide production, platelet aggregability and inflammation. Our hypothesis, which is further amenable to assessment in animal models before therapeutic applications in humans are implemented, could be explored both in vitro and in vivo to decipher the potential role of magnesium deficit in TMA and of the effects of its supplementation.

Keywords
ADAMS/ADAMTS 13, thrombotic thrombocytopenic purpura (TTP / HUS), inflammation, clinical studies, magnesium

Introduction
Forty-five years ago, the Lancet published a case-report of thrombotic microangiopathy (TMA) resolving after treatment with heparin and magnesium (1). At that time the authors could not provide a solid explanation for their observation. With the current knowledge of the role of magnesium in especially endothelial homeostasis and platelet aggregation, we believe we can provide a rationale and pathophysiologic basis for what these investigators found.

TMA is caused by direct endothelial injury. It is characterised by microangiopathic haemolytic anaemia, thrombocytopenia and microvascular thrombosis (2, 3). It usually presents as thrombotic thrombocytopenic purpura (TTP) with neurologic symptoms or as haemolytic uraemic syndrome (HUS) when acute renal failure is present due to platelet-fibrin thrombi predominantly occluding the renal circulation. TTP and HUS share similar pathophysiologic mechanisms, but despite scientific advances in recent years, the exact pathways have not been fully unravelled. It is generally accepted that deficiency of plasmatic von Willebrand factor (vWF)-cleaving protease (ADAMTS13) and abnormalities of complement regulatory proteins are crucial (2, 4). Recent data have focused on the role of disruption of the alternative complement cascade in HUS, stressing the necessity of appropriately functioning plasma proteins such as factor H, I, B, thrombomodulin, C3 and membrane-cofactor protein (MCP) in the absence of mutations or auto-immune deficiency (5–7). Yet these factors do not seem to be solely responsible or sufficient for provoking TMA (3). This suggests the presence of environmental or genetic triggers and could explain why some patients with hereditary complement regulation defects present with HUS only late in life.

The effect of nitric oxide (NO) in preventing endothelial damage and subsequent TMA is well recognised. NO inhibits the exocytosis of endothelial cellular vacuoles (Weibel-Palate bodies) with consecutive release of p-selectin, an indicator of platelet reactivity (8) next to vWF, both of which are capable of initiating local inflammation and thrombosis (9). Not only do endothelial NO synthase (eNOS) knockout mice develop TMA with aging (10), but increasing NO by L-arginine protects mice from developing TMA in a model of Shiga-toxin induced HUS (11). Also, administration of vascular endothelial growth factor (VEGF) hastens renal recovery in experimental models of TMA (12).

Those observations suggest ancillary modulation of endothelial function could prevent TMA or at least alter its course. We suggest that a magnesium deficit could play a part in the development of TMA through multiple pathways (Table 1, Fig. 1). To the best of our knowledge, these have never been explored, unless indirectly. In this publication we review and discuss each of these elements in detail and join them together into one pathophysiologic concept emanating also in suggestions for future therapeutic approach.
Magnesium affects endothelial function

In vitro, low magnesium concentrations reversibly inhibit endothelial proliferation, and are associated with up-regulation of vascular cell adhesion molecule-1 (VCAM-1) and plasminogen activator-inhibitor (PAI)-1 (13) and increased endothelial secretion of platelet derived growth factor BB next to matrix metalloprotease-2 (MMP-2) and MMP-9 (14). Exposure to high magnesium concentrations leads to increased eNOS expression, promoting endothelial repair of injured blood vessels (15). Magnesium stimulates the endothelial release of prostacyclin from human umbilical arteries and cultured umbilical vein endothelial cells (HUVEC) (16) and decreases the activity of MMP-9 (17), whose plasma levels inversely correlate with markers of NO formation in healthy volunteers (18). Inbred mice with low concentrations of intracellular magnesium have impaired mesenteric arterial endothelial

Table 1: Magnesium and its potential role in TMA.

<table>
<thead>
<tr>
<th>Endothelial (dys)function</th>
<th>Platelet aggregation</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro magnesium supplementation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Nitric oxide synthesis and release</td>
<td>↓ Thromboxane-A2 production</td>
<td>↓ Production of pro-inflammatory cytokines</td>
</tr>
<tr>
<td>↑ Expression eNOS</td>
<td>↓ Platelet aggregation</td>
<td></td>
</tr>
<tr>
<td>↑ Release of prostacyclin (PGI2)</td>
<td>↑ cAMP production</td>
<td></td>
</tr>
<tr>
<td>↓ Endothelin-1 induced vasoconstriction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ Endothelin-1 levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ Secretion ultra-large vWF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ ICAM-1 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ MMP-9 activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro low magnesium status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Endothelin-1 levels</td>
<td>↓ Platelet guanyl cyclase (cGMP)</td>
<td>↑ Expression of NF-κB</td>
</tr>
<tr>
<td>↑ Phosphorylation of MAPK</td>
<td>↑ Platelet aggregation</td>
<td>↑ Production of IL-1/TNF-alpha</td>
</tr>
<tr>
<td>↓ Endothelial proliferation</td>
<td>↑ Thromboxane-A2 production</td>
<td>↑ Oxidative stress</td>
</tr>
<tr>
<td>↑ VCAM-1 expression</td>
<td>↑ Release of beta-thromboglobulin</td>
<td>↓ Cellular anti-oxidant properties</td>
</tr>
<tr>
<td>↑ PAI-1 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Secretion of MMP-2 and MMP-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo magnesium supplementation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Arterial endothelial function</td>
<td>↓ Up-regulation of p-selectin</td>
<td>↓ Inflammatory markers (including CRP)</td>
</tr>
<tr>
<td>↑ Lipid profile</td>
<td>↓ Stent thrombosis (dogs)</td>
<td>↑ Insulin sensitivity</td>
</tr>
<tr>
<td>↓ Aldosterone production</td>
<td>↓ Platelet-dependent thrombosis (humans)</td>
<td></td>
</tr>
<tr>
<td>↓ Thromboxane-A2 production</td>
<td>↓ Intracellular calcium mobilisation</td>
<td></td>
</tr>
<tr>
<td>↓ Platelet aggregation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo low magnesium status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Expression of NF-κB</td>
<td>↑ Thromboxane-A2 production</td>
<td>↑ Release of pro-inflammatory markers</td>
</tr>
<tr>
<td>↓ eNOS expression</td>
<td>↑ Platelet-dependent thrombosis (humans)</td>
<td>↑ Leukocyte and macrophage activation</td>
</tr>
<tr>
<td>↓ Arterial endothelial function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Aldosterone secretion</td>
<td>↑ Free radical production</td>
<td></td>
</tr>
<tr>
<td>↑ Phosphorylation of MAPK</td>
<td>↓ Insulin sensitivity</td>
<td></td>
</tr>
<tr>
<td>↑ Oxidative stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Complement C3 level</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The potential role of magnesium in the pathophysiology of thrombotic microangiopathy and the effect of magnesium supplementation. Both associations with low magnesium status and effects of magnesium supplementation in both in vivo and in vitro studies are depicted. eNOS= endothelial nitric oxide synthase; PG2= prostaglandin 2; vWF= von Willebrand factor; ICAM-1= intercellular adhesion molecule-1; MMP-9= matrix metalloprotease-9; MAPK= mitogen-activated protein kinase; VCAM-1= vascular cell adhesion molecule-1; PAI-1= plasminogen-activator inhibitor-1; NF-κB= nuclear factor kappa-B; cGMP= circulating adenosine monophosphate; cGMP= circulating guanosine monophosphate; IL-1= interleukin-1; TNF-alpha= tumour necrosis factor alpha; CRP=C-reactive protein.
function, decreased expression of eNOS and increased levels of pro-inflammatory markers (19). In post-menopausal women, magnesium intake has been associated with improved endothelial function and decreased inflammatory markers (20). When compared with placebo in a randomised trial, in patients with coronary artery disease, magnesium proved to enhance brachial artery endothelial function, especially in those with the lowest intracellular magnesium concentrations (21). Also, in diabetic patients aged over 65, magnesium supplementation improved endothelial function (22). Finally, hyperlipidaemia-induced endothelial dysfunction might be attenuated by magnesium supplementation at the same time beneficially altering serum lipid levels (23–25).

Changes in intracellular magnesium modify the endothelial synthesis and release of NO (26). The role of endothelial NO in TMA is well recognised (3). A magnesium deficiency with its negative influence on NO production and endothelial function might increase the vulnerability to microvascular injury and reduce endothelial regenerative capacity. Also see Tables 1–3 for detailed information.

Magnesium and intracellular signalling

Magnesium is the second most common intracellular cation with a free cytosolic concentration of approximately 0.2–1 mM (27). As a cofactor, it is involved in numerous enzymatic reactions and especially in those involving protein kinases (28). Although the binding of magnesium to proteins or other molecules generally is weaker than that of calcium (29), its extra-to intracellular gradient is moderate in comparison with that of calcium. Consequently, whereas calcium is an ideal agent for immediate signal transduction, magnesium has been attributed rather with long-term regulatory properties, in view of the extracellular concentration of ionised magnesium of 0.7 mM. Magnesium also regulates the function of ion channels and transcellular calcium current (28) and as such potentially affects both endothelial and platelet intracellular signalling.

Recently, Shiga toxin 1B-induced vWF secretion in HUVEC was associated with activation of different signalling pathways and a transient rise in intracellular calcium levels (30). In HUVEC, the rise in intracellular calcium levels with potential effect on calcium-dependent cellular reactions such as endothelial hyperpermeability and apoptosis was inhibited dose-dependently by the extracellular magnesium concentration (31). NO is an inhibitor of calcium entry through transient receptor potential channels (TRPC) in endothelial cells (32). Considering the role of magnesium as a physiological calcium antagonist, it can be speculated that higher intracellular magnesium concentrations in endothelial cells might convey protection against detrimental effects of excessive calcium. Higher intracellular calcium and lower intracellular magnesium concentrations were observed in platelets of non-treated hypertensives as compared to those of controls and this effect was even more pronounced after exposure of these platelets to 1 nM angiotensin II (33). Conversely, increasing their extracellular magnesium concentration significantly decreased intracellular calcium concentration while increasing the intracellular magnesium concentration (33).

In another HUVEC model, Shiga toxin 2 exerted a number of negative effects that could contribute to altered endothelial cell-leukocyte interactions and the development of microangiopathic lesions as observed in TMA (34). These processes, which included the expression of chemokines such as fractalkine were dependent on phosphorylation of p38 mitogen-activated protein kinase.
(MAPK) through activation of the pro-inflammatory transcription factor nuclear factor-kappa B (NF-κB) (30, 34). Of note, phosphorylation of MAP-kinases was increased two- to three-fold in magnesium deficient rats (35). Also, a magnesium deficit increased the expression of NF-κB in endothelial cells (14). As such, it can be assumed that, although intricate and with still many questions unresolved, intracellular magnesium interferes with essential biological processes that are involved in the pathophysiology of TMA.

**Magnesium increases proteolysis of ultra-large vWF and reduces platelet aggregation**

In *vitro*, magnesium sulfate increases cleavage of newly released vWF by ADAMTS13, decreases the endothelial secretion of ultra-large vWF and inhibits the interaction of vWF and platelets (36). It inhibits platelet aggregation dose-dependently (37, 38) through inhibition of thromboxane A2 (TxA2) production (39), increases formation of cyclic adenosine monophosphate (cAMP) and reduces intracellular calcium mobilisation (40). It markedly reduced platelet aggregation under flow conditions mimicking *in vivo* blood circulation (36). The infusion of magnesium sulfate significantly reduced acute stent thrombosis in dogs (41). In rats, magnesium sulfate attenuated the upregulation of P-selectin (8) and magnesium oxide decreased platelet aggregation while decreasing the intracellular calcium concentration (42). Additionally, in a randomised trial in men with coronary artery disease, magnesium oxide reduced platelet-dependent thrombosis (43) which was shown to be promoted by a low platelet magnesium concentration in both coronary artery disease and diabetes (44, 45).

Subsequently, a magnesium deficit seems to have the potential to increase platelet activation and aggregation in TMA, while magnesium supplements could have the potential to reduce the severity of TMA and thus improve clinical outcomes. Of note, increased platelet reactivity is observed in obese patients (46), patients with acute coronary syndrome or diabetes (47) and renal transplant recipients, whose vWF levels are higher versus controls (48). Interestingly, these subpopulations also have a well-recognised propensity to hypomagnesaemia (49–55). Also, NO-sensitive activation of platelet guanylcyclase with consecutive increase in cyclic guanosine monophosphate (cGMP) and decreased platelet aggregation was markedly enhanced by increments in free magnesium levels within the physiological range (56). Of note, intraplatelet free magnesium concentrations vary greatly and are increased significantly by insulin administration (57, 58). Insulin has been reported to exert a protective effect against platelet aggregation while increasing NO production (59), whereas insulin resistance is associated with increased platelet aggregability and an inherent increased prothrombotic risk (59).

Deficiency of plasmatic ADAMTS13 is a frequent epiphenomenon of pregnancy and malignant hypertension, two conditions with particular vulnerability to both the development of TMA and (intracellular) magnesium deficit. Magnesium might alleviate this negative effect by enhancing vWF cleavage (36).

<table>
<thead>
<tr>
<th>Study</th>
<th>Mg concentration in media</th>
<th>Cut-off level of action</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferre S et al. (14)</td>
<td>0.1 vs. 1 mM (MgSO₄)</td>
<td>NA</td>
<td>HUVEC</td>
</tr>
<tr>
<td>Maier JA et al. (15)</td>
<td>2–10 mM vs. 1 mM</td>
<td>NA</td>
<td>HUVEC</td>
</tr>
<tr>
<td>Dolinsky BM et al. (17)</td>
<td>0.07–7 mg/ml (MgSO₄)</td>
<td>NA</td>
<td>HUVEC</td>
</tr>
<tr>
<td>Howard AB et al. (26)</td>
<td>1.0, 3.16, 10.0 mM (MgCl₂)</td>
<td>NA</td>
<td>PAEC</td>
</tr>
<tr>
<td>Satake K et al. (31)</td>
<td>0.3–3 mM (MgSO₄)</td>
<td>1mmol/l</td>
<td>Human platelets</td>
</tr>
<tr>
<td>Ravn HB et al. (37)</td>
<td>0.5–8.0 mM (MgSO₄)</td>
<td>1mmol/l</td>
<td>Human platelets</td>
</tr>
<tr>
<td>Corsonello A et al. (39)</td>
<td>0.25–8.8 mM (MgSO₄)</td>
<td>1mmol/l</td>
<td>Human platelets</td>
</tr>
<tr>
<td>Sheu JR et al. (39)</td>
<td>0.6–3 mM (MgSO₄)</td>
<td>1.5mmol/l</td>
<td>Human platelets</td>
</tr>
<tr>
<td>Hsiao G et al. (40)</td>
<td>0.6–3 mM (MgSO₄)</td>
<td>1.5mmol/l</td>
<td>Human platelets</td>
</tr>
<tr>
<td>Acevedo F et al. (60)</td>
<td>0–1 mM</td>
<td>NA</td>
<td>C3</td>
</tr>
<tr>
<td>Shogi T et al. (74)</td>
<td>0.390 mM vs. 0.021 mM</td>
<td>NA</td>
<td>Rat alveolar macrophages</td>
</tr>
<tr>
<td>Martin H et al. (75)</td>
<td>0–0.4 mM vs. 0.8 mM (MgSO₄)</td>
<td>NA</td>
<td>Rat/human hepatocytes</td>
</tr>
<tr>
<td>Wolf FI et al. (76)</td>
<td>0.1 mM vs. 1 mM (MgSO₄)</td>
<td>NA</td>
<td>HUVEC</td>
</tr>
<tr>
<td>Rochelson B et al. (100)</td>
<td>1–20 mM (MgSO₄) or 1–10 mM (MgCl₂)</td>
<td>NA</td>
<td>HUVEC</td>
</tr>
</tbody>
</table>

PAEC = porcine aortic endothelial cells; HUVEC = human umbilical vein endothelial cells; Mg = magnesium; NA = not applicable or no cut-off level mentioned; VSMC = vascular smooth muscle cells; C3 = complement factor 3.
Table 3: Magnesium dosage in in vivo studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Mg dosage</th>
<th>Mg concentration serum</th>
<th>Cell/patient model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ying SQ et al. (8)</td>
<td>IV MgSO₄ 2 mmol/ml from 30 min before coronary occlusion until the onset of reperfusion</td>
<td>No exact data</td>
<td>Rats</td>
</tr>
<tr>
<td>Shechter M et al. (21)</td>
<td>Oral 365 mg Mg* vs. placebo (6 months)</td>
<td>IC Mg 36.2 ± 5.0 vs. 32.7 ± 2.7 meq/l (placebo) (p&lt;0.02)</td>
<td>Sublingual cells/CAD</td>
</tr>
<tr>
<td>Barbagallo et al. (22)</td>
<td>Oral 368 mg Mg* vs. control (1 month)</td>
<td>0.42 ± 0.05 mM to 0.49 ± 0.06 mM (ionised) (p&lt;0.02)</td>
<td>Diabetics &gt;65 years</td>
</tr>
<tr>
<td>Ruksin V et al. (41)</td>
<td>Pulse dose 2 g MgSO₄ followed by 2 g/h infusion over 40 min</td>
<td>1.3 ± 0.2 meq/l to 4.8 ± 1.1 meq/l</td>
<td>Dogs</td>
</tr>
<tr>
<td>Kh R et al. (42)</td>
<td>Oral 1g/kg MgO (6 weeks)</td>
<td>No exact data</td>
<td>Rats</td>
</tr>
<tr>
<td>Shechter et al. (43)</td>
<td>Oral 800–1,200 mg MgO vs. placebo (3 months)</td>
<td>2.11 ± 0.14 vs. 2.08 ± 0.16 mg/dl (NS)</td>
<td>CAD</td>
</tr>
<tr>
<td>Bussiere FI et al. (63)</td>
<td>Mg-deficient vs. control diet (8 days)</td>
<td>0.33 ± 0.03 vs. 0.94 ± 0.04 mM (p&lt;0.001)</td>
<td>Rats</td>
</tr>
<tr>
<td>Freedman AM et al. (77)</td>
<td>Mg-deficient vs. control diet (14 days)</td>
<td>1.38 ± 0.4 vs. 5.02 ± 0.47 meq/l (p&lt;0.01)</td>
<td>Hamsters</td>
</tr>
<tr>
<td>Nadler JL et al. (79)</td>
<td>Mg-deficient diet (&lt;0.5 mmol/day) (4 weeks)</td>
<td>0.78 ± 0.08 to 0.53 ± 0.08 mM (p&lt;0.01)</td>
<td>Healthy humans</td>
</tr>
<tr>
<td>Almoznino-Sarafian D et al. (88)</td>
<td>Oral Mg citrate 300 mg/day vs. control (5 weeks)</td>
<td>0.74 ± 0.04 to 0.88 ± 0.08 meq/l (p&lt;0.001)</td>
<td>Humans with heart failure</td>
</tr>
<tr>
<td>Ichihara A et al. (114)</td>
<td>IV MgSO₄ (0.72% in 3.24% glucose solution) 5 meq/l (6 h)</td>
<td>1.58 ± 0.07 to 2.32 ± 0.09 meq/l (maximum)</td>
<td>Healthy humans</td>
</tr>
<tr>
<td>Yuan J et al. (118)</td>
<td>Ca²⁺+Mg-rich diet 0.6% Mg enriched by MgCl₂ vs. Ca²⁺+normal Mg diet (4 weeks)</td>
<td>1.46 ± 0.12 mM vs. 0.97 ± 0.12 mM (p&lt;0.05)</td>
<td>Rats</td>
</tr>
</tbody>
</table>

Mg=magnesium; IV=intravenous; PAEC=porcine aortic endothelial cells; HUVEC=human umbilical vein endothelial cells; Mg*=elementary magnesium; CAD=coronary artery disease; IC=intracellular; MgO=magnesium oxide; NS=not significant; CsA=cyclosporine A.

Of note, numerous in vitro and in vivo studies have assessed the effect of various extracellular magnesium concentrations on the physiology of both endothelial cells and platelets (Tables 2 and 3). Exposure to an extracellular magnesium concentration of 0.3 mM corresponds to the lowest physiological plausible level measurable in the serum, of 1 mM to the physiological level and of 3 mM to the level reached by therapeutic magnesium supplementation in arrhythmia and pre-eclampsia (31). Notably, effects on platelet function are most pronounced beyond levels of 1–1.5 mM of extracellular magnesium concentration, which can be considered as physiological to slightly supra-physiological (Table 2).

Magnesium and its role in the complement cascade

Magnesium has an established role as a natural cofactor in the activation of the alternative complement cascade by promotion of the conversion from C3 to C3b, the cleavage of factor B upon binding to C3b by factor D and stabilisation of the labile C3 convertase (60, 61). In vitro experiments demonstrated that incubation of C3 with increasing concentrations of magnesium up to 1 mM dose-dependently increased its activation (60) or increased the formation of C3 convertase (62). Consequently and somewhat in contradiction to our previous argumentation, magnesium deficiency could from a theoretical point of view exert a beneficial impact on the pathways emanating in TMA. However, this might be hard to reconcile with our hypothesis. In contrast with this theoretical benefit, the dietary induction of hypomagnesaemia in rats increased the production of complement factor C3 and generated complement haemolytic activity as part of a generalised inflammatory reaction (63). Moreover, as far as we are aware, no in vivo study has ever assessed the effect of higher versus lower extra- or intracellular magnesium concentrations on the alternative complement cascade in general let alone in HUS/TMA. As such, it still remains an open question whether and when the potentially beneficial but unproven modifying effects of a magnesium deficit on the alternative complement cascade can offset its overall potentially deleterious effects on endothelial function, platelet aggregability and inflammation (Table 1).

Magnesium has anti-inflammatory and anti-oxidant properties

Increasing evidence points to an essential role of inflammation in TMA (2, 64–66). Patients developing TMA have elevated levels of pro-inflammatory cytokines (66) stimulating endothelial release of ultra-large vWF (65). The ratio of vWF antigen level and ADAMTS13 activity is significantly higher in sepsis and inflam-
mation (67), supposedly due to endothelial activation. Recently, increased oxidative stress was observed in the early phase of HUS (68). Additionally, many observational and interventional studies have demonstrated an association between inflammation and endothelial dysfunction (69). Exposure of healthy volunteers to interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) resulted in prolonged endothelial dysfunction (70). As such, it seems reasonable that anti-inflammatory therapeutic strategies might improve endothelial function and consecutively TMA.

Interestingly, anti-inflammatory and anti-oxidant properties have extensively been attributed to magnesium (71, 72). Conversely, a magnesium deficit dose-dependently increased the expression of the transcription factor NF-kB in vascular smooth muscle cells (73) and enhanced the production of IL-1β and TNF-α in alveolar macrophages in vitro (74). Furthermore, hypomagnesaemia was associated with an increase in oxidative stress (75, 76) and a decrease in cellular anti-oxidant capacity (77). In vivo, dietary induction of magnesium deficiency in rodents lead to early leukocyte and macrophage activation, release of pro-inflammatory cytokines and excessive production of free radicals (78). In healthy humans, dietary induced isolated magnesium deficits decreased insulin sensitivity and increased the production of TxA2 (79). On the other hand, magnesium supplementation in these patients decreased TxA2 production to levels beneath those of normomagnesaemic subjects (79). In a large cohort, a low serum magnesium was associated with both decreased insulin sensitivity and increased high-sensitivity C-reactive protein (CRP) concentrations (80). Considering the established relationship between inflammation, insulin resistance, glucose metabolism disturbances and vascular stiffness (81–83), this might at least partially explain why hypomagnesaemia in observational studies is associated with vascular stiffness (84) or the later development of diabetes in both transplant and non-transplant patients (51, 85–87). In a small prospective open-label intervention trial in patients with heart failure, magnesium supplementation decreased CRP (88). Yet, its therapeutic use as an anti-inflammatory or anti-oxidative agent is limited by the scarcity of large and methodologically sound trials.

Support from clinical data

Pre-eclampsia is a leading cause of perinatal morbidity and mortality in both mother and child. Its pathophysiology is complex and still not fully unraveled. Established features are hypertension, vasoconstriction, oxidative stress, platelet aggregation, insulin resistance and endothelial dysfunction, all of which are associated with a cellular or plasmatic magnesium deficit. Low circulating NO levels have been observed in pre-eclampsia even after recovery (89). TMA can complicate pre-eclampsia and is even more distinct in the HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome where activity of ADAMTS13 typically is decreased (90). Importantly, a magnesium deficit, as assessed by brain and muscular nuclear magnetic resonance spectroscopy or determination of intra-erythrocytic levels, is more common during pregnancy (91). It is also more profound in pre-eclamptic than in healthy pregnant women (91, 92). Obesity and diabetes, conditions often associated with hypomagnesaemia (49–51, 53), are risk factors for the development of pre-eclampsia (93). In a landmark study in pre-eclamptic women, magnesium sulfate halved the risk of eclampsia in comparison with placebo, and a trend towards reduced maternal death was observed (94). However, this unequivocal benefit (95) is not well-outlined from a pathophysiological perspective. It mainly has been attributed to vasodilatory properties. Interestingly, treatment of pre-eclamptic women with magnesium sulfate improved endothelial function with higher placental expression of eNOS (96) and lower concentrations of endothelin-1 (97). It is fair to assume that magnesium exerts its beneficial effects largely through its endothelial tropism with amplification of vasodilation (NO) and inhibition of vasoconstriction (endothelin-1) in the microcirculation, especially of the placenta. Moreover, a beneficial effect on platelet aggregability and responsiveness, which are altered in proportion to the severity of the disease (98), seems likely. Irrespective of pre-eclamptic status, an increase in markers of platelet activation during pregnancy was observed (99). Finally, in lipopolysaccharide (LPS)-treated HUVEC, magnesium sulfate dose-dependently inhibited endothelial cell activation, and decreased IL-8 and ICAM-1 levels, both of which are enhanced via NF-κB, thus pointing to intrinsic anti-inflammatory properties of magnesium (100) as discussed before.

Interestingly, defective complement regulatory proteins recently have been associated with the development of pre-eclampsia or HELLP (101–103) suggesting an ethiological overlap between atypical HUS (aHUS) and pre-eclampsia. This is further supported by recent findings in a mouse model of pre-eclampsia where targeted inhibition of complement activation by a C3-inhibitor prevented pathological features of pre-eclampsia (102).

Also, pregnancy-associated aHUS (p-aHUS), a frequently-encountered subtype of aHUS which is triggered by pregnancy (103), mostly occurs in the immediate postpartum and frequently is associated with mutational dysregulation of the alternative C3 convertase. It can be speculated that lower postpartum magnesium levels increase the vulnerability to endothelial dysfunction and platelet aggregability or alternatively that the interaction between magnesium and the dysfunctional C3 convertase is altered.

Also malignant hypertension was associated with endothelial and platelet dysfunction, and increased soluble P-selectin and VWF levels versus normotensive controls (104), whereas also this condition is often complicated by TMA. In rats with malignant hypertension, extracellular and intracellular magnesium concentrations were markedly reduced (28). In humans with malignant hypertension, blood pressure correlated inversely with intracellular magnesium concentrations (105). Endothelial dysfunction and renal ischaemia play a key role in the pathophysiology of malignant hypertension. Deficiency of NO seems to be the common factor explaining the causal relation with TMA. Magnesium deficiency with its negative influence on vasodilatory NO production (19) or its stimulation of vasoconstrictive endothelin-1, might potentiate the development of TMA in malignant hypertension. Conversely, the
association between magnesium and suppressed endothelin-1 (97) or increased NO synthesis (15, 26) seems equally relevant. Magnesium attenuated the effects of endothelin-1 in rats (106).

Hyperaldosteronism which is considered a feature of malignant hypertension (107) is even more pronounced in the subgroup of patients with TMA (108). Aldosterone infusion in a murine model leads to impairment of endothelial progenitor cell function and endothelial dysfunction in a blood pressure-independent manner (109). Flow-mediated dilatation (FMD) as an index of endothelial function correlated negatively with plasma aldosterone levels in patients with resistant hypertension (r=-0.38; p=0.0006) (110). Interestingly, hyperaldosteronism leads to hypomagnesaemia and/or intracellular magnesium deficiency (111–113) while magnesium supplementation both decreases aldosterone production in healthy volunteers (114) and counteracts the damaging cardiovascular and renal effects of aldosterone in both normomagnesaemic and hypomagnesaemic mice (71, 113). In sharp contrast to thiazides and loop diuretics, spironolactone increases intracellular magnesium levels (115).

Subsequently, magnesium supplementation could possibly be beneficial in malignant hypertension associated TMA through different mechanisms of action.

Calcineurin-inhibitor (CNI)-related TMA is another distinct clinical entity with histological signs of CNI toxicity often accompanying the TMA lesions (116). Cyclosporine causes endothelial injury by decreasing the production of prostacyclin and NO and increasing both TxA2 and endothelin-1 synthesis (117). Hypomagnesaemia is a frequent complication of CNI (54, 55), mainly through renal magnesium wasting. In rats exposed to cyclosporine, magnesium supplements improved the cyclosporine-induced histological lesions, provoked an increase in renal NO (118) and attenuate the upregulation of renal cortical mRNA expression of endothelin-1 (119). Considering the proven effects of magnesium on endothelin-1 production, endothelial dysfunction and NO synthesis, and based on the animal experiments mentioned above (118, 119), we expect a protective effect of its magnesium supplementation in the development of CNI-related TMA.

VEGF inhibition-related TMA is one of the novel drug-related entities which have been described after exposure to bevacizumab (120), a humanised monoclonal antibody against VEGF, the oral tyrosine kinase receptor blocker suinitinib (121) and after intravitreal injection of the VEGF-inhibitor ranibizumab (122). VEGF is an essential factor in the regulation of endothelial NO production and its administration can protect against renal injury in animal models of TMA (12, 123). Interestingly, VEGF dose-dependently increases the intracellular magnesium content in HUVEC through tyrosine-kinease signalling pathways (124). In pre-eclampsia in a rat model, the VEGF antagonist placental soluble fms-like-tyrosine kinase 1 (sFlt1) was upregulated and responsible for hypertension, proteinuria and glomerular TMA lesions, while circulating levels of free VEGF were decreased (125). One might speculate that the propensity to TMA by lowering of circulating VEGF levels partially relates to the possible lowering of (intracellular) magnesium levels, which is indeed what is observed in pre-eclamptic patients (91, 92).

**Further areas of research and barriers to investigation**

We speculate that a magnesium deficit or hypomagnesaemia negatively influences the course and severity of TMA. Unfortunately, observational studies have never evaluated the magnesium status in patients with TMA, let alone have correlated it with their clinical course. The purpose of this publication is to draw attention to this problem, and to stimulate research in this field.

Clinical trials to evaluate both benefits and harms of magnesium supplements for treating and preventing TMA flares should be considered. However, HUS, TTP, malignant hypertension and CNI-related TMA are rare and clinically heterogeneous. As such, evaluation of any treatment in a randomised controlled trial will be difficult. A potential hazard also might be the incidental occurrence of severe and sometimes rapidly progressive kidney failure, potentially causing a risk of hypermagnesaemia, if supplementation is not discontinued timely. Also, poor gastro-intestinal tolerance or malabsorption of magnesium supplementation might hamper its use in a therapeutic setting.

In light of these hurdles, one might first consider using existing animal models of TMA. Unfortunately, differences in human and rodent physiology make the extrapolation of findings to humans cumbersome. More recent models with genetically modified mice overexpressing human renin and angiotensin or mice with conditional knockout of ADAMTS13 or complement factor H seem to provide a better basis for mimicking human disease. Also, models with Shiga-toxin induced HUS (126) and TTP (127) have been developed in baboons. These new animal models seem apt for unravelling the precise role of magnesium in TMA, if any, and will especially be of help for developing new treatment approaches aimed at preventing or limiting TMA, which with the currently available options remains difficult to accomplish (128).

**Conclusion**

We propose a link between magnesium deficiency and the development of TMA and its related diseases. This relationship is based on *in vitro* and *in vivo* data pointing to pleiotropic endothelium-modifying and anti-inflammatory properties of magnesium. Our hypothesis is supported by data suggesting that magnesium decreases platelet aggregability and thrombosis. Further investigation in animal models is warranted to validate the proposed assumptions and to assess the potential of magnesium supplementation. The heterogeneity and rarity of the TMA-associated diseases might limit the possibility to evaluate any therapeutic approach in humans. Nevertheless, given the potential of magnesium to act on various pathways, the low cost and toxicity involved and bearing in mind that no specific treatment aimed at preventing or limiting TMA has been successful so far, it should be worth exploring its therapeutic use, especially in case of proven deficiency and in the absence of rapidly progressive kidney failure.

© Schattauer 2012

Thrombosis and Haemostasis 107.3/2012
Conflict of interest
None declared.

References


50. Lima Mde L, Cruz T, Rodrigues LE, et al. Serum and intracellular magnesium defi-
ciency in patients with metabolic syndrome—evidences for its relation to insulin
53. Pham PC, Pham PM, Pham SV, et al. Hypomagnesemia in patients with type 2
emia and its relation with immunosuppression as predictors of new-onset diabetes
55. Barton CH, Vaziri ND, Martin DC, et al. Hypomagnesemia and renal magnesium
wasting in renal transplant recipients receiving cyclosporine. Am J Med 1987; 83:
693–699.
56. Kempfert J, Behrends S. Analysis of nitric oxide-sensitive guanylyl cyclase in hu-
57. Hwang DL, Yen CF, Nadler JL. Insulin increases intracellular magnesium trans-
59. Randriamboavonjy F, Fleming J. Insulin, insulin resistance, and platelet signaling
60. Acevedo F, Vesterberg O. Nickel and cobalt activate complement factor C3 faster
61. Maeda S, Nagasawa S. Effect of sodium chloride concentration on fluid-phase as-
sembly and stability of the C3 convertase of the classical pathway of the comple-
62. Fishelson Z, Müller-Eberhard HJ. C3 convertase of human complement: en-
hanced formation and stability of the enzyme generated with nickel instead of
64. Van Laecke S, Desideri F, Geerts A, et al. Hypomagnesemia and the risk of new-
65. Koo WH, Folsom AR, Nieto FJ, et al. Serum and dietary magnesium and the risk for
type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study. Arch
66. Guerrero-Romo F, Rascón-Pacheco RA, Rodríguez-Morán M, et al. Hypomag-
nesemia and risk for metabolic glucose disorders: a 10-year follow-up study. Eur
67. Almoznino-Sarafian D, Berman S, Mor A, et al. Magnesium and C-reactive pro-
tein in heart failure: an anti-inflammatory effect of magnesium administration? Eur
cerepeptin, recurrent pregnancy loss, and future cardiovascular events? Hypertension
69. Lattuada A, Rossi E, Calzarossa C, et al. Mild to moderate reduction of a von
Willebrand factor cleaving factor protease (ADAMTS-13) in pregnant women
in brain and muscle of normal and pre eclamptic pregnant: a nuclear magnetic
71. Adam B, Malayatioliou E, Albur M, et al. Magnesium, zinc and iron levels in pre-
1244–1246.
73. Altman DJ, Carroll G, Duley L, et al. Do women with pre-eclampsia, and their
babies, benefit from magnesium sulphate? The Magpie Trial: a randomised place-
74. Duley L, Gülmezoglu AM, Henderson-Smart DJ, et al. Magnesium sulphate and
other anticonvulsants for women with pre-eclampsia. Cochrane Database Syst
75. Ariza AC, Bobadilla N, Díaz L, et al. Placental gene expression of calciitonin gene-
related peptide and nitric oxide synthases in pre eclampsia: effects of magnesium
gastro motility and gastrointestinal symptoms in women with pre eclampsia. Am
77. Norris LA, Glesson SC, Shepherd BL, et al. Whole blood platelet aggregation
78. Robb AO, Dine JN, Mills NL, et al. The influence of the menstrual cycle, normal
pregnancy and pre-eclampsia on platelet activation. Thromb Haemost 2010; 103:
372–378.
79. Rochelson B, Dowling O, Schwartz N, et al. Magnesium sulfate suppresses in-
flammatory responses by human umbilical vein endothelial cells (HUVECs)