Magnesium and the inflammatory response: potential pathophysiological implications in the management of patients with aneurysmal subarachnoid hemorrhage?

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Abstract. Cerebral vasospasm and delayed cerebral ischemia remain an unsolved problem in patients with aneurysmal subarachnoid hemorrhage (SAH). In theory, high-dose magnesium sulfate (MgSO₄) therapy offers vascular and neuroprotective benefits and is therefore currently under evaluation. The intensity of the inflammatory response after SAH is associated with the outcome. The aim of the current study was to evaluate a possible link between the inflammatory response and MgSO₄ therapy, since magnesium (Mg²⁺) has anti-inflammatory properties. In 15 patients with SAH, inflammatory cytokine levels in the cerebrospinal fluid (CSF) and peripheral blood were determined daily using an enzyme-linked immunosorbent assay between day 4 and day 12. Eight patients were treated with standard therapy alone (group 1) and seven patients were treated with an additional, high-dose of MgSO₄ (group 2). Serum Mg²⁺ levels in group 2 were significantly higher compared to group 1: 1.48 ± 0.04 mmol/L versus 0.90 ± 0.01 mmol/L, p < 0.001. Interleukin-6 (IL-6) in the CSF was significantly lower in group 2 compared to group 1: 6680 ± 989 vs. 11079 ± 1277 pg/mL, p = 0.021. A trend towards lower systemic IL-6 levels was found in group 2: 58 ± 7 versus 104 ± 21 pg/mL, p = 0.052. Systemic IL-1β levels were significantly lower in group 2: 0.66 ± 0.11 and 0.15 ± 0.01 pg/mL (p < 0.001), while the CSF levels did not differ. Tumor necrosis factor-α levels did not differ between the two groups. Although there were more patients with favorable outcome in group 2, the difference was not statistically significant. This was probably due to the small sample size. The results indicate a suppression of inflammatory cytokine release, in particular IL-6, in patients treated with high-dose MgSO₄. These results call for further studies of the effect of Mg²⁺ on the inflammatory signaling pathway with regard to delayed cerebral ischemia following SAH.

Key words: magnesium, magnesium sulfate, aneurysmal subarachnoid hemorrhage, inflammation, interleukin-6
Subarachnoid hemorrhage (SAH) due to a ruptured intracranial aneurysm is a devastating and life-threatening condition. Its incidence is approximately 7-20 per 100,000 people. Considerable advances in diagnostic and treatment strategies have reduced the morbidity and mortality following SAH [1]. Nevertheless, the secondary stroke syndrome, i.e. delayed cerebral ischemia (DCI), remains a difficult and only partially understood issue. Classically, cerebral vasospasm (CVS) has been associated with DCI. However, current evidence suggests that CVS is not the only cause of DCI and that in reality, DCI might be multifactorial [2, 3]. Magnesium (Mg\textsuperscript{2+}) has been shown to have a neuroprotective effect in experimental, transient, focal ischemia [4]. Further, it reversed cerebral vasospasm (CVS) after experimental SAH [5]. Therefore, administration of magnesium sulfate (MgSO\textsubscript{4}) has been suggested to reduce the incidence of CVS/DCI and improve outcome [5, 6]. Clinical trials have revealed controversial results, and their evaluation is currently ongoing [7, 8]. An inflammatory response with cytokine release, in particular interleukin-6 (IL-6), has been linked with the occurrence of DCI and a poorer outcome [9-11]. Since Mg\textsuperscript{2+} has been reported to have a profound effect on the process of inflammation [12], the question arises as to whether high-dose MgSO\textsubscript{4} therapy and inflammation might be related in patients suffering from SAH. The goal of this case series was to compare cytokine levels in patients with and without high-dose MgSO\textsubscript{4} therapy after SAH, and discuss the potential pathophysiological implications.

**Materials and methods**

Of the 38 patients with aneurysmal SAH, admitted to the Neurocritical Care Unit, University Hospital of Zurich, during a six-month period, 15 were included in the current study. All 15 patients were suffering from an angiographically-confirmed aneurysmal SAH, and all had a ventricular catheter for cerebro-spinal fluid (CSF) drainage. The clinical severity grade of the SAH was determined in accordance with the World Federation of Neurological Surgeons (WFNS) scale [13]. The radiological severity grade was assessed using the Fisher scale [14]. All patients were treated according to a standardized treatment protocol for patients with SAH [15, 16]. Selected in chronological order, the first eight patients received standard therapy alone (group 1). Based on the results of a previously conducted trial at the Neurocritical Care Unit of the University Hospital Zurich [17], the remaining seven received additional high-dose MgSO\textsubscript{4} therapy (group 2). The high-dose MgSO\textsubscript{4} therapy was started at time of admission. MgSO\textsubscript{4} was administered by continuous, intravenous infusion of 64 mmol/d, to maintain the serum Mg\textsuperscript{2+} at twice the baseline level the, with a maximum of 2.0 mmol/L until day 12 after the SAH. Subsequent dosage adjustments of the MgSO\textsubscript{4} infusion were made every 12 hours. Measurement of C-reactive protein (CRP) and a systemic leukocyte count was performed daily as part of the clinical routine. Between days 4 and 12 following onset of the bleedling, IL-6, tumor-necrosis-factor-\(\alpha\) (TNF-\(\alpha\)) and interleukin-1\(\beta\) (IL-1\(\beta\)) levels were measured daily in cerebrospinal fluid (CSF) and blood samples using an enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Human IL-6: BioSource, Camarillo, CA, USA; TNF-\(\alpha\) and IL-1\(\beta\): R&D Systems, Minneapolis, MN, USA). All concentrations below the mean minimal detection limit of 2 pg/mL (IL-6), 0.5 pg/mL (TNF-\(\alpha\)), and 0.12 pg/mL (IL-1\(\beta\)) were assigned as the detection limit. Clinical outcome after one year was assessed in the outpatient clinic using the Glasgow outcome scale (GOS). DCI was defined as any new cerebral hypodensity based on comparison of the CT scan at time of discharge from the Neurocritical Care Unit with the initial, postoperative CT scan. The study was strictly observational, and was approved by the local ethics committee. Values are given as mean ± SEM. Nominal variables were compared using the Fisher’s exact test. Scaled variables were compared using the independent t test, if necessary with logarithmic transformation to achieve normal distribution. The Mann-Whitney U test was used, where normal distribution could not be assumed. A \(p\)-value \(<0.05\) was regarded as statistically significant. Owing to the limited sample size and wide variance, time-courses for the inflammatory parameters were shown for descriptive purpose only.

**Results**

There were no statistically significant differences in age, gender or severity grade between the two
Table 1. Patient characteristics and results.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 8)</th>
<th>Group 2 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age [years]</strong></td>
<td>53.8 ± 3.9</td>
<td>51.9 ± 4.0</td>
</tr>
<tr>
<td><strong>Sex [male:female]</strong></td>
<td>2:6</td>
<td>1:6</td>
</tr>
<tr>
<td><strong>WFNS grade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>2 (25%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>4-5</td>
<td>6 (75%)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td><strong>Fisher grade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 (38%)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>4</td>
<td>5 (62%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td><strong>CSF parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 [pg/mL]</td>
<td>11,079 ± 1,277</td>
<td>6,680 ± 989</td>
</tr>
<tr>
<td>TNF-α [pg/mL]</td>
<td>2.0 ± 0.3</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>IL-1β [pg/mL]</td>
<td>1.57 ± 0.32</td>
<td>1.85 ± 0.40</td>
</tr>
<tr>
<td><strong>Systemic parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 [pg/mL]</td>
<td>104 ± 21</td>
<td>58 ± 7</td>
</tr>
<tr>
<td>TNF-α [pg/mL]</td>
<td>1.8 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>IL-1β [pg/mL]</td>
<td>0.66 ± 0.11</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Mg²⁺ [mmol/L]</td>
<td>0.90 ± 0.01</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>Lc counts [x1/mL]</td>
<td>12.24 ± 0.55</td>
<td>10.63 ± 0.49</td>
</tr>
<tr>
<td>CRP [mg/L]</td>
<td>53.41 ± 6.078</td>
<td>33.07 ± 4.118</td>
</tr>
<tr>
<td><strong>Infectious complications</strong></td>
<td></td>
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<tr>
<td>Outcome</td>
<td></td>
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</tr>
<tr>
<td>GOS 1-3</td>
<td>6 (75%)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>GOS 4-5</td>
<td>2 (25%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>DCI</td>
<td>4 (50%)</td>
<td>3 (43%)</td>
</tr>
</tbody>
</table>

Group 1: patients receiving standard therapy alone. Group 2: patients receiving standard + high-dose MgSO₄ therapy; * statistically significant; # a trend was observed; CRP: c-reactive protein; CSF: cerebrospinal fluid; DCI: delayed cerebral ischemia; GOS: Glasgow outcome scale; IL: interleukin; Lc: leukocyte; TNF: tumor necrosis factor; WFNS: World Federation of Neurosurgical Societies.

Patient groups (table 1). In the current case series, all patients presented with Fisher grade 3 or 4. All patients underwent early aneurysm clipping, with the one exception of a patient in group 1, who was treated conservatively, for a spontaneously thrombosed aneurysm. During the course of the illness, serum Mg²⁺ levels in group 2 were statistically significantly higher compared to group 1: 1.48 ± 0.04 mmol/L versus 0.90 ± 0.01 mmol/L, p < 0.001 (table 1). Almost all cytokine levels were higher in the CSF than the peripheral blood, most prominent being IL-6, with a ten-fold increase. Mean IL-6 levels in the CSF were significantly lower in group 2 compared to group 1 during the course of the illness: 6680 ± 989 versus 11079 ± 1277 pg/mL, p = 0.021 (table 1). Systemic IL-6 levels were also lower in group 2. However, the difference fell short of significance: 58 ± 7 versus 104 ± 21 pg/mL, p = 0.052 (table 1). There were no differences between groups 1 and 2 as regards systemic and CSF levels of TNF-α: 1.8 ± 0.2 in group 1 versus 2.1 ± 0.3 pg/mL in group 2, p = 0.692 and 2.0 ± 0.3 versus 2.0 ± 0.3 pg/mL, p = 0.216 (table 1). There were no differences in IL-1β levels in the CSF between groups 1 and 2: 1.57 ± 0.32 versus 1.85 ± 0.40 pg/mL, p = 0.809. In 59% of all blood samples, IL-1β was below the detection limit. In group 2, IL-1β was below the detection limit in 84% of the blood samples. The mean systemic values were 0.66 ± 0.11 and 0.15 ± 0.01 pg/mL in group 1 and group 2 respectively (p < 0.001) (table 1). The mean CRP level was significantly lower in group 2: 33.07 ± 4.118 versus 53.41 ± 6.078 mg/L, p = 0.043. A trend towards lower peripheral leukocyte counts was observed in group 2 compared to group 1: 66 ± 0.49 versus 104 ± 21 pg/mL, p = 0.052. The time-courses for CSF and systemic IL-6 levels, and systemic IL-1β levels are shown as box plots in figure 1 for descriptive purposes. With regard to CSF and systemic IL-6 levels, the differences between groups 1 and 2 could be seen in the first week post-SAH, while
Figure 1. Results shown as box plots. A) CSF IL-6 levels. The differences were more pronounced between day 4 and day 8 following SAH. B) Systemic IL-6 levels. Differences were more pronounced between day 4 and day 7. C) Systemic IL-1β levels. Differences were more pronounced between day 6 and day 12.

the differences in systemic IL-1β levels were pronounced in the second week following the SAH. Although more patients in group 2 presented with a favorable outcome, defined as GOS 4-5, this was not statistically significant. The incidence of DCI did not differ either. Furthermore, the occurrence of infectious complications did not differ between the groups. The results are summarized in table 1.
Discussion

The fact that patients presenting with hypomagnesemia following SAH might be at risk of DCI has been described earlier [18]. Therefore, maintaining Mg\(^{2+}\) within normal limits in patients with SAH is a reasonable approach [8]. It has been suggested that the beneficial effects seen in patients with SAH are caused by the fact that: 1) Mg\(^{2+}\) is a natural, noncompetitive antagonist of voltage-dependent L-type calcium (Ca\(^{2+}\)) channels and therefore has a dilatory effect on cerebral arteries; 2) Mg\(^{2+}\) inhibits calcium influx by blocking N-methyl-d-aspartate receptors, thereby limiting neuronal damage. Therefore, high-dose MgSO\(_4\) therapy has been proposed in patients with SAH in terms of CVS/DCI-prophylaxis [5, 6]. Results from phase 2 studies indicated a beneficial effect in patients with SAH [3, 7, 8, 17]. However, the IMASH (intravenous magnesium sulfate for aneurysmal subarachnoid hemorrhage) trial, the only phase 3 trial to date, could not reproduce a beneficial effect in terms of a better functional outcome [19]. The most recent meta-analysis could not lend support to a beneficial effect either. However, the authors pointed out that owing to the limited sample size, a beneficial effect cannot be ruled out [7]. Results from another phase 3 study, the MASH-II (magnesium in aneurysmal subarachnoid hemorrhage) study are expected soon [20]. The latter study might shed more light on the efficacy of intravenous MgSO\(_4\) infusion in patients with SAH.

An additional rationale for MgSO\(_4\) therapy in patients with SAH is the fact that a beneficial effect has been shown in women with pre-eclampsia and eclampsia [21, 22]. In the latter, cerebral vasoconstriction similar to the CVS following a SAH has been reported as part of its pathophysiological characteristics [21, 23]. However, the exact pathophysiology as to how Mg\(^{2+}\) acts in these patients remains controversial. A common theory suggests that a defective placentation during the early stage of pregnancy triggers an increased release of factors into the maternal circulation, leading to maternal endothelial dysfunction and the onset of the maternal symptoms of pre-eclampsia. IL-6, a potent proinflammatory cytokine, is one of those factors suggested to be involved [24, 25]. Interestingly, Amash et al. was able to show ex vivo that pre-eclamptic placenta secretes increased levels of IL-6 into the maternal circulation and that MgSO\(_4\) normalizes these IL-6 levels [24]. The authors pointed out that the results might be significant while considering the ongoing discussion about the mechanism of action of MgSO\(_4\) in pre-eclampsia.

Increasing evidence suggests that there is a relationship between the local inflammatory response in the central nervous system and the severity of illness, clinical outcome and occurrence of DCI following SAH [9-11]. In SAH, the presence and breakdown of erythrocytes in the subarachnoid space is suggested to trigger an inflammatory response, with expression and release of a variety of proinflammatory factors. These factors include leukocyte adhesion molecules, such as intracellular adhesion molecules (ICAM)-1 and vascular cell adhesion molecules (VCAM)-1, inflammatory cytokines, such as IL-6, TNF-\(\alpha\) and IL-1\(\beta\) [9, 11]. Furthermore, in experimental animal studies, a couple of signal transduction pathways or transcription factors in the inflammatory cascade were found to be involved. These include, among others, the nuclear factor (NF)-\(\kappa\)B and p38 mitogen-activated protein kinase (MAPK) [26, 27]. The clinical outcome in patients with SAH has been linked to the occurrence of a systemic inflammatory response [28, 29], and pathologically high levels of inflammatory markers have been found in the peripheral blood of patients with SAH [9, 10]. In addition, systemic IL-6 has been shown to be an independent predictor of outcome in unselected, critically ill patients [30]. Interestingly, MgSO\(_4\) has been shown to play a distinct role in the pathogenesis of the inflammatory response [12]. In animal experiments, Mg\(^{2+}\) deficiency has been linked to a marked increase in total circulating leukocytes [31, 32] and it enhanced the production of proinflammatory factors, including IL-6 [31-34], TNF-\(\alpha\) [33], IL-1\(\beta\) [33], and VCAM-1 [34]. Nakagawa et al. reported enhanced production of IL-6 and IL-1\(\beta\), but not TNF-\(\alpha\), in endotoxin-triggered inflammatory responses in rats with an Mg\(^{2+}\) deficiency [35]. In vitro, MgSO\(_4\) suppressed the endotoxin-triggered inflammatory response in terms of reducing ICAM-1 expression and NF-\(\kappa\)B activation [36]. In a convincing study, Lee et al. reported the activation of the inflammatory pathways p38 MAPK and NF-\(\kappa\)B with consecutive IL-6 and TNF-\(\alpha\) release by purinergic receptor stimulation in microglia, while Mg\(^{2+}\) attenuated these effects [37]. Lin et al. reported a significant inhibition of endotoxin-induced, up-regulation of inflammatory
molecules, including IL-6 and IL-1β, and NF-κB activation in vitro. The authors concluded that the effects of MgSO₄ on inflammatory molecules and NF-κB might involve antagonizing Ca²⁺, inhibiting the L-type Ca²⁺-channels, or both [38]. In this context, it is worth considering that an endotoxin-triggered inflammatory response and SAH-triggered inflammation might share a common, pathogenic pathway as recently shown in an experimental animal study [39].

With regard to the above-mentioned facts the question arises as to whether treatment with MgSO₄ might be beneficial in terms of suppressing the inflammatory response in patients with SAH. In the current case series, patients treated with continuous, intravenous, high-dose MgSO₄ showed statistically significant lower CSF IL-6, systemic IL-1β and CRP levels during the course of the illness. Furthermore, a trend towards lower systemic IL-6 and peripheral leukocyte counts was seen. In particular, our results indicate a decrease in IL-6 in the early phase, i.e. the first week following SAH, as a result of treatment with high-dose MgSO₄. With respect to the inflammatory parameter, we could not show any statistically significant benefit in terms of improved outcome or reduction of DCI. This might be for two reasons: 1) the small number of patients included in the current study, hence a poor statistical perspective; 2) only patients with a clinical indication for a ventricular drainage were included in the study. The latter explains the high proportion of patients with severe bleeding. Further limitations of the study are the fact that patients were not randomized and that it was not placebo-controlled. Finally, the actual mechanism by which the MgSO₄ therapy influenced the inflammatory parameters, in particular the cytokine levels cannot be answered from this study.

Conclusion

In this case series, we observed significantly decreased CSF IL-6 and systemic IL-1β levels in patients treated with high-dose MgSO₄ following SAH. Although a conclusion cannot be drawn because of the limited sample size, the current results are of interest since the effect of high-dose MgSO₄ therapy on the inflammatory response following SAH has not, to date, been considered. These results call for further examination of the effect of Mg²⁺ on the inflammatory signaling pathway in SAH, in both the experimental and clinical setting.

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Disclosure

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