Is magnesium neuroprotective following global and focal cerebral ischaemia? 
A review of published studies

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Abstract. Neuroprotective activity with magnesium associated with animal models of cerebral ischaemia, seizure, perinatal hypoxia/ischaemia, subarachnoid haemorrhage and traumatic brain injury has provided the justification for clinical stroke trials. However, the recent IMAGES stroke clinical trial found magnesium to be largely ineffective. Hence, due to the negative stroke trial outcome, current FAST-MAG trial and our own experience with magnesium in cerebral ischaemia animal models, we thought it prudent to review these preclinical and clinical studies. We reviewed nine studies describing the use of magnesium following global cerebral ischaemia and fourteen following focal cerebral ischaemia. Four global ischaemia and six focal ischaemia studies did not show a significant neuroprotective effect with magnesium. In the majority of positive magnesium studies animal body temperature was not monitored post-ischaemia. Thus the effects of post-ischaemic hypothermia cannot be ruled out as a confounding factor in positive magnesium cerebral ischaemia studies. Moreover, data from our own laboratory indicates that magnesium is only neuroprotective when combined with post-ischaemic hypothermia. These data provide a possible explanation of why the IMAGES trial was largely unsuccessful, as current stroke patient management does not involve hypothermia induction. Future preclinical and clinical cerebral ischaemia trials with magnesium should consider combining treatment with mild hypothermia.

Key words: magnesium, global cerebral ischaemia, focal cerebral ischaemia, hypothermia, neuroprotection, IMAGES trial

Magnesium is the fourth most abundant cation in the body and the second most abundant cation in intracellular fluid. Magnesium is essential for cell functions such as preservation of membrane integrity, protein synthesis, energy metabolism, maintenance of ionic gradients, smooth muscle tone, regulation of calcium transport and reduction of calcium accumulation. The mean free plasma magnesium concentration in humans is 0.85 mmol/L with a reference interval of 0.7-1.0 mmol/L [1]. Interestingly, the concentration of magnesium in the CSF (1.21-1.45 mmol/L) is about 40% higher than plasma magnesium levels. Moreover, the amount of calculated free ionic Mg\(^{2+}\) in CSF is three times greater than that in plasma (1.02-1.23 mmol/L in CSF versus 0.33-0.47 mmol/L in plasma) [2]. Since magnesium is important in maintaining many cellular processes, changes in magnesium status before, during and after a brain insult are likely to have a profound effect on neurological outcome. Indeed, clinical and experimental studies have shown subjects with low CSF or serum magnesium
have worsened neurological outcomes following ischaemia and traumatic brain injury [3-5]. There is also ample evidence demonstrating marked changes in intracellular and extracellular free brain magnesium concentrations following ischaemic and traumatic insults [6-9]. Consequently, the restoration of magnesium homeostasis in the brain, along with magnesium’s known anti-excitotoxic actions and vascular effects have been the rationale for the administration of magnesium as a neuroprotective treatment following traumatic brain injury, seizure, subarachnoid haemorrhage and cerebral ischaemia [10-15]. However, despite the optimism surrounding its potential as a neuroprotective agent, the recent IMAGES acute stroke clinical trial found magnesium to be largely ineffective [16]. Therefore, in the light of previous conflicting animal cerebral ischaemia studies, the current Field Administration of Stroke Therapy-Magnesium (FAST-MAG) trial [17] and our own experience with magnesium in cerebral ischaemia animal models, we thought it prudent to review these preclinical and clinical studies.

**Efficacy of magnesium in global (forebrain) cerebral ischaemia animal models**

The results obtained from nine studies with magnesium in global models are summarised in table 1 and will be discussed in chronological order below. As can be seen in table 1, there is considerable variability in study design making it difficult to directly compare outcomes. Broadly, however, five studies found a neuroprotective effect, two studies did not report a neuroprotective effect, while two studies reported a positive outcome only when magnesium treatment was combined with post-ischaemic hypothermia.

In the first study by Blair et al. [18], magnesium chloride administered intravenously immediately before ischaemia was not neuroprotective. In fact, CA1 neuronal injury was higher in magnesium treated rats than in rats treated with saline. In this case, the dose of magnesium given (5 mmol/kg/476 mg/kg) would be considered high compared to subsequent studies, and additionally was believed to be responsible for raising serum glucose levels from 150 mg/dL to 220 mg/dL immediately after administration. The authors attributed the increased level of CA1 injury to the high serum glucose levels observed in these rats. When glucose levels were controlled by the simultaneous administration of magnesium and insulin the level of CA1 injury was no different to saline treated controls. One potential confounding factor in this study was the high magnesium dose used, which raised serum magnesium levels 10 fold when measured 5 minutes after infusion.

Tsuda et al. [19] observed a neuroprotective effect with magnesium chloride when applied directly to the CA1 sector (1 μL; 50 mM solution) 10 minutes before ischaemia or 0, 2, 12 and 24 hours after ischaemia, but not at 48 hours post-ischaemia in rats. Interestingly, a lower dose of magnesium chloride (1 μL; 10 mM) administered at the 24 hour time point showed a neuroprotective trend, but was not significant at the p < 0.01 level. In this study a potential confounding factor was the possibility of animals becoming hypothermic after ischaemia, as animal body temperature was not monitored during recovery. The potential for hypothermia may have been further compounded as the animals were re-anaesthetised for post-ischaemia magnesium administration. The implications of post-ischaemia hypothermia confounding the findings of magnesium studies will be discussed later.

Next, Okawa [20] evaluated the effects of magnesium sulphate administered as an intravenous loading dose (0.664 mmol/kg) followed by an infusion (0.332 mmol/kg/h for 3 hours followed by 0.083 mmol/kg/h for 45 hours) immediately after ischaemia in dogs. Magnesium treated dogs showed improvement of post-ischaemic (cerebral) hypoperfusion at 48 hours and improvement in neurological outcome at 7 days. The loading dose and initial infusion dose of magnesium used in this study are relatively high compared with other reports, while the 45 hour infusion dose was low. However, Okawa [20] showed a significant increase in magnesium levels in the CSF in magnesium treated ischaemic dogs after 48 hours. Like the previous study, animal body temperature was not monitored post-ischaemia and histological examination of CA1 neurons was not performed.

Sirin et al. [21] used a subcutaneous dose of magnesium sulphate (600 mg/kg or 5 mmol/kg) administered 48 hours before ischaemia and examined the neurological outcome and CA1 and CA3 neuronal injury 4 days post-ischaemia in rats. Magnesium treated rats showed a slightly higher neurological score at 3 and 4 days post-ischaemia, while CA1 and CA3 neuronal damage was decreased from 47% to 36% and 22% to 14% respectively after 4 days. Although Sirin et al. [21] showed positive outcomes in the magnesium treated rats, they were relatively modest and no measures were taken to ensure normal animal body temperature was maintained post-ischaemia. The authors refer to a previous study
Table 1. Summary of studies using magnesium following global cerebral ischaemia.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal model</th>
<th>Magnesium salt</th>
<th>Route</th>
<th>Magnesium dose</th>
<th>Time of treatment</th>
<th>Post-ischaemic temperature monitoring</th>
<th>Neuroprotection and assessment method</th>
</tr>
</thead>
<tbody>
<tr>
<td>[18] Blair et al. (1989)</td>
<td>10 min: 2VO rat</td>
<td>MgCl₂</td>
<td>IV</td>
<td>5.0 mmol/kg</td>
<td>Immediately before ischaemia</td>
<td>No</td>
<td>No; based on CA1 injury</td>
</tr>
<tr>
<td>[19] Tsuda et al. (1991)</td>
<td>20 min: 4VO rat</td>
<td>MgCl₂</td>
<td>CA1 region</td>
<td>1 μl of 50 mM</td>
<td>10 min before, 0, 2, 12, 24 or 48 h after ischaemia</td>
<td>No</td>
<td>Yes; at 10 min before and 0, 2, 12, 24 h after ischaemia</td>
</tr>
<tr>
<td>[20] Okawa (1992)</td>
<td>18 min: aorta occlusion dog</td>
<td>MgSO₄</td>
<td>IV</td>
<td>0.66 mmol/kg + 0.33 mmol/kg/h for 3 h + 0.083 mmol/kg/h for 45 h</td>
<td>Immediately after ischaemia</td>
<td>No</td>
<td>Yes; based on neurological outcome</td>
</tr>
<tr>
<td>[21] Sirin et al. (1998)</td>
<td>15 min: 4VO rat</td>
<td>MgSO₄</td>
<td>SC</td>
<td>5 mmol/kg</td>
<td>48 h before ischaemia</td>
<td>No</td>
<td>Yes; based on CA1 injury &amp; neurological outcome</td>
</tr>
<tr>
<td>[22] Milani et al. (1999)</td>
<td>15 min: 4VO rat</td>
<td>MgCl₂</td>
<td>SC</td>
<td>2.5 mmol/kg x 4 + 5.0 mmol/kg x 4 + 7.5 mmol/kg x 4 + 5.0 mmol/kg x 1</td>
<td>1, 2, 4, 8 &amp; 24 h after ischaemia</td>
<td>No</td>
<td>No; based on CA1 &amp; subiculum injury</td>
</tr>
</tbody>
</table>

Dose in mg/kg

Dose in mmol/kg/h

MAGNESIUM AND CEREBRAL ISCHAEMIA
<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal model</th>
<th>Magnesium salt</th>
<th>Route</th>
<th>Magnesium dose</th>
<th>Dose in mg/kg</th>
<th>Time of treatment</th>
<th>Post-ischaemic temperature monitoring</th>
<th>Neuroprotection and assessment method</th>
</tr>
</thead>
<tbody>
<tr>
<td>[23] Miles et al. (2001)</td>
<td>8 min: 2VO rat MgSO₄·7H₂O</td>
<td>IV</td>
<td>0.36 mmol/kg + 0.06 mmol/kg/h or 0.12 mmol/kg/h or 0.24 mmol/kg/h or 0.48 mmol/kg/h for 48 h</td>
<td>90 mg/kg 15 mg/kg/h or 30 mg/kg/h or 60 mg/kg/h or 120 mg/kg/h</td>
<td>Before, 4, 8, 12 or 24 h after ischaemia</td>
<td>No</td>
<td>Yes; based on CA1 injury</td>
<td></td>
</tr>
<tr>
<td>[24] Zhu et al. (2004)</td>
<td>8 min: 2VO rat MgCl₂</td>
<td>IV</td>
<td>0.36 mmol/kg + 0.12 mmol/kg/h for 48 h</td>
<td>34 mg/kg 11.4 mg/kg/h</td>
<td>Before or 8 h after ischaemia</td>
<td>Yes; before ischaemia. No; at 8 h after ischaemia; based on CA1 injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[25] Zhou et al. (2003)</td>
<td>10 min: 2VO gerbil MgSO₄</td>
<td>IP</td>
<td>16.6 mmol/kg</td>
<td>2000 mg/kg</td>
<td>30 min before ischaemia</td>
<td>No</td>
<td>Yes; based on Tunel staining</td>
<td></td>
</tr>
<tr>
<td>[26] Zhu et al. (2005)</td>
<td>8 min: 2VO rat MgSO₄·7H₂O</td>
<td>IV</td>
<td>0.36 mmol/kg + 0.06 mmol/kg/h or 0.12 mmol/kg/h or 0.24 mmol/kg/h or 0.48 mmol/kg/h for 48 h</td>
<td>90 mg/kg 15 mg/kg/h or 30 mg/kg/h or 60 mg/kg/h or 120 mg/kg/h</td>
<td>Immediately before or 2 h after ischaemia</td>
<td>Hypothermia induced 2 h after ischaemia</td>
<td>No when animals maintained normothermic; based on CA1 injury</td>
<td></td>
</tr>
</tbody>
</table>

B.P. MELONI, ET AL.
reporting that a 600 mg/kg subcutaneous dose of magnesium sulphate can significantly increase brain magnesium levels after 48 hours.

In a study by Milani et al. [22] different subcutaneous doses of magnesium chloride (2.5 mmol/kg, 5.0 mmol/kg, 7.5 mmol/kg) were administered to rats at multiple time points post-ischaemia (1, 2, 24 and 48 hours). The 5 mmol/kg dose was evaluated as a single dose administered 2 hours post-ischaemia. In addition, the single and multiple 5 mmol/kg magnesium doses were combined with the CNS suppressant diazepam. In an attempt to minimise post-ischaemia hypothermia, animal body temperatures were maintained between 37-38°C during the first 3 hours after ischaemia by placing rats in a warming box at 30°C when necessary. Seven days after ischaemia, magnesium treatments alone or in combination with diazepam did not significantly decrease neuronal loss in the subiculum and CA1 regions. Interestingly, body temperature in magnesium treated rats remained relatively normal while rats treated with magnesium and diazepam recorded a drop in body temperature of between 1-2.5°C during the 3 hours post-ischaemia monitoring period. Although the authors did not measure magnesium levels in serum or brain they referred to several studies using similar dosage regimens that indicated that their magnesium doses would have increased levels in the brain, especially after ischaemia.

The next report by Miles et al. [23] originated from our own laboratory and evaluated the neuroprotective efficacy of magnesium sulphate when administered intravenously following global cerebral ischaemia in rats. In this study, magnesium treatment initially involved a loading dose at 0.36 mmol/kg, commencing immediately before ischaemia either alone or followed by an infusion of magnesium at 0.06, 0.12, 0.24 or 0.48 mmol/kg/h over 48 hours. We observed that the loading dose alone increased CA1 neuronal survival from 5% to 33%, while rats receiving the magnesium loading dose followed by the infusions at 0.06, 0.12, 0.24 or 0.48 mmol/kg/h demonstrated 30%, 80%, 16% and 5% CA1 neuronal survival respectively. Based on the dose response data for magnesium on CA1 neuronal survival, the loading dose and 0.12 mmol/kg/h infusion dose was selected for assessment of efficacy in post-ischaemic treatment protocols. In these experiments, the magnesium loading dose followed by the infusion was commenced 4, 8, 12 or 24 hours after cerebral ischaemia and resulted in 82%, 71%, 55% and 33% CA1 neuronal survival respectively.

We also assessed the efficacy of the magnesium 0.36 mmol/kg loading dose and 0.12 mmol/kg/h infusion dose in the form of magnesium chloride when administered immediately before ischaemia or at 8 hours after ischaemia. Magnesium chloride administration before ischaemia resulted in 50% CA1 neuronal survival, while 8 hour post-ischaemia administration resulted in 5% CA1 survival.

Our findings confirmed a neuroprotective effect for magnesium that appeared to follow a dose response pattern. We also confirmed that all doses were capable of increasing serum magnesium levels. Hence, based on these results we hypothesised that the failure of previous magnesium studies may have been due to an inappropriate magnesium dose. There are, however, two issues that need to be addressed with respect to our findings. The first is the unexpected ineffectiveness of magnesium chloride when administered 8 hours post-ischaemia. Secondly, we did not monitor animal body temperature post-ischaemia, allowing the possibility that the combination of magnesium treatment and post-ischaemic hypothermia was responsible for the observed neuroprotective effects. The lack of neuroprotective effect with magnesium chloride when administered 8 hours post-ischaemia (5% CA1 survival) was surprising, since magnesium sulphate had been highly effective (71% CA1 survival). Animals treated with the magnesium chloride loading and infusion dose do not experience hyperglycaemia (unpublished observation), therefore the negative finding could not be attributable to elevated blood glucose levels. One possible explanation is that the animals treated with magnesium sulphate, but not those treated with magnesium chloride, experienced a significant period of post-ischaemic hypothermia. In subsequent experiments [24] we have obtained evidence to support the likelihood that post-ischaemic hypothermia was a confounding factor in our study, and this is discussed below.

Zhou et al. [25] administered magnesium sulphate (16.6 mmol/kg) intraperitoneally 30 minutes before global ischaemia in gerbils. At 12, 24 and 48 hours post-ischaemia neuronal apoptosis was assessed in the hippocampus using TUNEL staining. Immunohistochemistry was also used to assess the levels of Bax, Bcl2 and caspase-3. TUNEL staining and levels of Bax and caspase-3 were significantly reduced in magnesium treated animals. Bcl2 levels remained unchanged in treated and untreated animals. While this study has provided evidence that magnesium treatment reduced the level of pro-apoptotic markers, the neuronal outcome after an extended post-ischaemic period (7 days) is unknown and the possibility of post-ischaemic hypothermia has not been adequately accounted for.
The next two studies [26, 27] that assessed magnesium treatment following global ischaemia are from our laboratory. The first evaluated the efficacy of an intravenous magnesium sulphate loading dose at 0.36 mmol/kg administered immediately before ischaemia followed by an infusion of magnesium at 0.06, 0.12 or 0.24 mmol/kg/h over 48 hours. In these experiments we carefully monitored animal body temperature post-ischaemia to ensure animals did not experience any significant hypothermia. At 7 days post-ischaemia we assessed CA1 neuronal survival and observed that none of the magnesium treatments increased CA1 neuronal survival.

We next assessed the 0.36 mmol/kg loading dose followed by an infusion of magnesium at either 0.12 or 0.24 mmol/kg/h over 48 hours. In these experiments we monitored animal body temperature, but made no attempt to keep animals normothermic. In control and magnesium treated animals that self regulated their body temperature a drop in rectal temperature of 0.5-1.5°C during the immediate 4 hour post-ischaemic period was recorded. Moreover, we observed a significantly increased level of CA1 neuronal survival (34%) in animals treated with the loading dose and the 0.12 mmol/kg/h infusion. Animals treated with the loading dose and the 0.24 mmol/kg/h infusion also had increased neuronal survival (20%), but it did not reach statistical significance, while CA1 survival in saline treated control animals was 5%. Taken together, the results from this study indicate that magnesium is only neuroprotective when combined with post-ischaemic hypothermia.

To address this issue further, our subsequent study [27] first assessed the efficacy of an intravenous magnesium sulphate loading dose at 0.36 mmol/kg administered immediately before ischaemia followed by an infusion of magnesium at 0.12 mmol/kg/h over 48 hours in rats. Immediately post-ischaemia one group of rats had their body temperature lowered to 35°C for 6 hours, while a second group had their body temperature maintained at 37°C. Animals receiving a 6 hour period of hypothermia demonstrated 9.4% CA1 neuronal survival, whereas animals treated with magnesium alone or magnesium and 6 hours of hypothermia demonstrated 5.1% and 37.9% CA1 neuronal survival respectively. These results are in line with our previous study [24] showing that for magnesium to be effective following global ischaemia it must be associated with post-ischaemic hypothermia.

We next assessed if the same magnesium/hypothermia treatment protocol would still be effective if administration was commenced 2 hours after global cerebral ischaemia. In these experiments rats made hypertensive showed 6.1% CA1 neuronal survival and rats receiving magnesium and hypothermia showed 8.1% CA1 survival. Based on this finding we decided to extend the duration of hypothermia and evaluated the efficacy of magnesium treatment combined with a 12 or 24 hour duration of mild hypothermia (35°C) commencing 2 hours post-ischaemia. Rats receiving 12 or 24 hours of hypothermia alone showed 5% and 43% CA1 neuronal survival respectively. Rats receiving the combination of magnesium and 12 or 24 hours of hypothermia showed 9% and 76% CA1 survival, respectively. In summary, these results show that for magnesium to be effective post-ischaemia it must be combined with a prolonged duration of hypothermia. It should also be noted that while prolonged hypothermia alone (24 hour) was neuroprotective the combination of magnesium and hypothermia was significantly more effective.

### Efficacy of magnesium in focal cerebral ischaemia animal models

Results obtained from fourteen studies with magnesium in models of focal cerebral ischaemia are summarised in Table 2 and will be discussed below. As in the global ischaemia studies, great variability in study design was encountered, and hence it is difficult to compare outcomes. Eight focal ischaemia studies have reported significant neuroprotective activity with magnesium, while six have not (Table 2).

The first study to evaluate magnesium in a focal cerebral ischaemia model was by Izumi et al. [28]. A rat permanent middle cerebral artery occlusion (MCAO) model was utilized and magnesium chloride was administered intraperitoneally (1 mmol/kg) immediately before and 1 hour after MCAO. In a separate group of animals the initial magnesium dose was also administered with insulin. Total infarct volumes in magnesium (122 mm³) and magnesium/insulin (92 mm³) treated rats were significantly reduced compared with saline treated controls (165 mm³). The reduction in infarct volume in rats treated with magnesium and insulin (44%) compared with magnesium alone (26%) is most likely attributable to the neuroprotective effect of insulin. Importantly, the authors measured rectal temperature at 1.5, 4, 24 and 48 hours after MCAO and measurements ranged from 36.7-37.7°C during this period.

A second study, published in the form of a conference abstract, Roffe et al. [29] compared magnesium chloride treatment in mice when administered intra-
## Table 2. Summary of studies using magnesium following focal cerebral ischaemia.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal model</th>
<th>Magnesium salt</th>
<th>Route</th>
<th>Magnesium dose</th>
<th>Dose in mg/kg</th>
<th>Time of treatment</th>
<th>Post-ischaemic temperature monitoring</th>
<th>Neuroprotection and assessment method</th>
</tr>
</thead>
<tbody>
<tr>
<td>[28] Izumi et al. (1991)</td>
<td>Permanent MCAO, rat</td>
<td>MgCl₂</td>
<td>IP</td>
<td>1 mmol/kg x 2</td>
<td>95 mg/kg x 2</td>
<td>Immediately after and 1 h after ischaemia</td>
<td>Measured 1.5, 4, 24 &amp; 48 h after ischaemia</td>
<td>Yes; reduced infarct volume at 48 h (TTC)</td>
</tr>
<tr>
<td>[29] Roffe et al. (1996)</td>
<td>Permanent MCAO, mouse</td>
<td>MgCl₂</td>
<td>IP</td>
<td>1 mmol/kg x 2</td>
<td>95 mg/kg x 2</td>
<td>Immediately after and 1 h after ischaemia</td>
<td>No</td>
<td>No; based on infarct volume at 24 h (tetrazolium blue)</td>
</tr>
<tr>
<td>[30] Marinov et al. (1996)</td>
<td>2 or 1.5 h transient MCAO, rat</td>
<td>MgSO₄</td>
<td>IA</td>
<td>0.75 mmol/kg or 0.25 mmol/kg</td>
<td>90 mg/kg or 30 mg/kg</td>
<td>Immediately before ischaemia</td>
<td>Monitored for 45 min after ischaemia</td>
<td>Yes; reduced infarct volume at 24 h (TTC)</td>
</tr>
<tr>
<td>[31] Schmid-Elsaesser et al. (1999)</td>
<td>1.5 h transient MCAO, rat</td>
<td>MgCl₂</td>
<td>IV</td>
<td>1 mmol/kg x 2</td>
<td>95 mg/kg x 2</td>
<td>Before and immediately after ischaemia</td>
<td>Monitored for 1 h after ischaemia &amp; animals housed in warm cages for 8 h</td>
<td>No; (only 25% reduction) based on infarct volume at 7 days (H&amp;E)</td>
</tr>
<tr>
<td>[32] Lee et al. (1999)</td>
<td>Permanent MCAO, rat</td>
<td>MgSO₄</td>
<td>IA</td>
<td>0.75 mmol/kg</td>
<td>90 mg/kg</td>
<td>10 min before ischaemia</td>
<td>No</td>
<td>Yes; reduced infarct volume at 24 h (TTC)</td>
</tr>
<tr>
<td>[33] Yang et al. (2000)</td>
<td>Permanent MCAO, rat</td>
<td>MgSO₄</td>
<td>IV</td>
<td>0.75 mmol/kg</td>
<td>90 mg/kg</td>
<td>2, 6 or 8 h after ischaemia</td>
<td>No</td>
<td>Yes; reduced infarct volume at 72 h (TTC)</td>
</tr>
<tr>
<td>[34] Kinoshita et al. (2001)</td>
<td>2 h transient MCAO, rat</td>
<td>MgSO₄</td>
<td>IV</td>
<td>0.21 mmol/kg</td>
<td>25 mg/kg</td>
<td>During MCA occlusion period</td>
<td>No</td>
<td>Yes; reduced infarct volume at 24 h (TTC)</td>
</tr>
<tr>
<td>[35] Lin et al. (2002)</td>
<td>1 h transient MCAO, gerbil</td>
<td>MgSO₄</td>
<td>IP</td>
<td>0.75 mmol/kg</td>
<td>90 mg/kg</td>
<td>30 min before ischaemia</td>
<td>No</td>
<td>Yes; reduced infarct volume at 24 h (TTC)</td>
</tr>
<tr>
<td>[36] Westermaier et al. (2003)</td>
<td>1.5 h transient MCAO, rat</td>
<td>MgSO₄</td>
<td>IV &amp; IA</td>
<td>0.75 mmol/kg</td>
<td>90 mg/kg</td>
<td>Immediately before ischaemia</td>
<td>As for [31]</td>
<td>No; (25% reduction) based on infarct volume at 7 days (H&amp;E)</td>
</tr>
<tr>
<td>[37] Chung et al. (2004)</td>
<td>Permanent MCAO, gerbil</td>
<td>MgSO₄</td>
<td>IP</td>
<td>0.75 mmol/kg</td>
<td>90 mg/kg</td>
<td>10 min before ischaemia</td>
<td>No</td>
<td>Yes; reduced infarct volume at 24 h (TTC)</td>
</tr>
<tr>
<td>Reference</td>
<td>Animal model</td>
<td>Magnesium salt</td>
<td>Route</td>
<td>Magnesium dose</td>
<td>Dose in mg/kg</td>
<td>Time of treatment</td>
<td>Post-ischaemic temperature monitoring</td>
<td>Neuroprotection and assessment method</td>
</tr>
<tr>
<td>-----------------</td>
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</tr>
<tr>
<td>[26] Zhu et al. (2004)</td>
<td>Study 1. 45 min transient MCAO, rat</td>
<td>MgSO$_4·7$H$_2$O</td>
<td>IV</td>
<td>0.18 mmol/kg or 0.26 mmol/kg or 0.72 mmol/kg</td>
<td>44 mg/kg or 88 mg/kg or 177 mg/kg</td>
<td>Immediately before ischaemia</td>
<td>Monitored &amp; maintained normothermic for 6h</td>
<td>No; based on infarct volume at 24 h (TTC)</td>
</tr>
<tr>
<td></td>
<td>Study 2. 2 h transient MCAO, rat</td>
<td></td>
<td>IA</td>
<td>0.37 mmol/kg or 0.74 mmol/kg</td>
<td>45 mg/kg or 90 mg/kg</td>
<td>Immediately before ischaemia</td>
<td>No</td>
<td>No; based on infarct volume at 72 h (TTC)</td>
</tr>
<tr>
<td>[16] IMAGES (2004)</td>
<td>Stroke human clinical trial</td>
<td>MgSO$_4$</td>
<td>IV</td>
<td>0.2 mmol/kg + 0.034 mmol/kg/h for 24 h</td>
<td>24 mg/kg + 4.1 mg/kg/h</td>
<td>Within 12 h of stroke onset</td>
<td>Normal patient monitoring</td>
<td>No; based on neurological outcome</td>
</tr>
<tr>
<td>[38] Westermaier et al. (2005)</td>
<td>Permanent MCAO, rat</td>
<td>MgSO$_4$</td>
<td>IV</td>
<td>0.75 mmol/kg or 1 mmol/kg x 2 or 1 mmol/kg + 0.5 mmol/kg/h for $\approx2.5$ h</td>
<td>90 mg/kg or 120 mg/kg x 2 or 120 mg/kg or 60 mg/kg/h</td>
<td>Immediately before ischaemia</td>
<td>As for [31]</td>
<td>Yes; based on infarct volume at 7 days (H&amp;E). Note: 31% reduction for 0.75 mmol/kg dose was not significant</td>
</tr>
</tbody>
</table>

* IMAGES trial has been included for reference purposes.  
* Authors reported that same dose administered IV was ineffective.
peritonely (1 mmol/kg) immediately after permanent MCAO and again at 1 hour. A second group of mice received magnesium and insulin and controls received saline. After 24 hours infract volumes did not significantly differ, although there was a trend for larger infarcts in magnesium only treated mice. In addition, magnesium treatment increased oedema in infarcted hemispheres, which was not evident in animals treated with magnesium and insulin.

Marinov et al. [30] compared two doses of magnesium sulphate (0.75 mmol/kg and 0.25 mmol/kg) administered intra-arterially (carotid artery) before MCAO in rats. Two periods of reversible focal ischaemia were used (1.5 or 2 hours) and infarct assessment was at 24 hours. Following 1.5 hours of MCAO the 0.75% and 0.25 mmol/kg magnesium doses reduced infarct volume from 24% to 9% and 17%, respectively. The 0.75 mmol/kg dose also significantly reduced brain edema. Following 2 hours of MCAO, only treatment with the 0.25 mmol/kg magnesium dose was assessed, with cortical infarct volume being reduced from 24% to 19%. Animal body temperature was not monitored during the 24 hour post-ischaemic period.

The next study by Schmid-Elsaesser et al. [31] compared the efficacy of magnesium alone and in combination with the anti-oxidant tirilazad following 1.5 hours of transient MCAO in rats. Magnesium chloride was administered intravenously (1 mmol/kg) before and immediately after ischaemia, and animals were allowed to survive for 7 days. Magnesium treatment reduced total infarct volume by 25%, but this was not statistically significant. However this treatment did significantly reduce cortical infarct volume by 37%. Tirilazad reduced total infarct volume by 48% and the combination of magnesium and tirilazad reduced total infarct volume by 59%. Generally, improved neurological functional outcomes reflected reductions in infarct volume for the different treatments. Animal body temperature was monitored for 1 hour after reperfusion and animals were kept in a warm environment for the first eight post-operative hours (Robert Schmid-Elsaesser personal communication).

Lee et al. [32] compared the efficacy of magnesium and the sodium channel blocker mexiletine, alone and in combination, in a permanent MCAO rat model. Intra-arterial administration of magnesium sulphate (0.75 mmol/kg) 10 minutes prior to MCAO significantly reduced infarct volume. Pre- and early (0.5 hour) post-MCAO intraperitoneal treatment with mexiletine also significantly reduced infarct volume, however combined pre-treatment with magnesium and mexiletine did not. The additive adverse effects on cardio-pulmonary function of combined magnesium/mexiletine treatment was suggested as a reason for a lack of neuroprotection. Animal body temperature was not monitored over the 22-24 hour post-ischaemic period. Interestingly, the authors of this study commented that in preliminary experiments the same magnesium treatment protocol administered intravenously did not reduce infarct volume.

Next, Yang et al. [33] assessed the neuroprotective efficacy of intravenously administered magnesium (0.75 mmol/kg) 2, 6 or 8 hours post MCA embolization in rats. Rat survival significantly increased only in animals treated 2 hours after ischaemia, but improvement in neurological outcome was observed in all magnesium treated groups. Magnesium treatment administered 2 and 6 hours, but not 8 hours post-ischaemia significantly reduced infarct volume. Animal body temperature was not monitored during the 72 hour post-ischaemic period.

Kinoshita et al. [34] administered an intravenous infusion of magnesium sulphate (0.21 mmol/kg) continuously for 2 hours between the onset of MCAO and reperfusion. Twenty-four hours after ischaemia magnesium treated rats exhibited significantly reduced infarct volume. Molecular markers associated with neuronal injury were also generally reduced in the brains of rats treated with magnesium. Animal body temperature was not monitored during the post-ischaemic period.

A gerbil transient focal cerebral ischaemia model was used to assess the effect of magnesium sulphate (0.36 mmol/kg) administered intraperitoneally 30 minutes prior to 60 minutes of ischaemia [35]. Infarct volume was reduced by 38% when measured 24 hours after cerebral ischaemia. Magnesium treated animals also showed better preservation of brain glucose, lactate, pyruvate and glutamate levels compared with untreated controls. In this study post-ischaemic body temperature was not monitored.

Westermayer et al. [36] compared the efficacy of intra-arterial and intravenous magnesium sulphate in a rat model of transient focal ischaemia. Rats were subjected to 90 minutes of MCAO and magnesium sulphate (0.75 mmol/kg) was infused immediately before ischaemia. Both intra-arterial and intravenous treatment improved neurological recovery and although total infarct volume was reduced by ≈25% at 7 days post-ischaemia it was not statistically significant. Animal management post-ischaemia was the same as in the Schmid-Elsaesser et al. study [31].
In a study by Chung et al. [37] the efficacy of magnesium sulphate (0.75 mmol/kg) alone and in combination with FK506 was assessed when administered intraperitoneally 30 minutes prior to permanent focal cerebral ischaemia in the gerbil. Magnesium reduced infarct volume by 25%, FK506 by 41% and the combination of magnesium and FK506 by 50% when measured 24 hours post-ischaemia. Animal body temperature was not monitored in the post-ischaemic period.

In 2004, we were involved in two studies that independently re-evaluated the efficacy of magnesium sulphate when administered immediately prior to transient focal cerebral ischaemia in rats [26]. In the Perth study, MCAO was for 45 minutes and body temperature was controlled during and after ischaemia. In the Canberra study, MCAO was for 2 hours and body temperature was only controlled during ischaemia. Three different doses (0.18, 0.36 or 0.72 mmol/kg) of magnesium were administered intravenously in the Perth study and 2 different doses (0.37 or 0.74 mmol/kg) of magnesium were administered intra-arterially in the Canberra study. No significant differences in total, cortical and striatal infarct volumes between saline and magnesium treated animals were observed when measured 24 or 72 hours after ischaemia in either study. In a subsequent experiment from the Perth laboratory rats were administered with the 0.36 mmol/kg magnesium sulphate when administered immediately prior to transient focal cerebral ischaemia in the gerbil. Magnesium reduced infarct volume by 25%, FK506 by 41% and the combination of magnesium and FK506 by 50% when measured 24 hours post-ischaemia. Animal body temperature was not monitored in the post-ischaemic period.

In a recent study, Westermaier et al. [38] compared the efficacy of different doses of intravenously administered magnesium sulphate following transient focal ischaemia in the rat. Infarct volume was assessed 7 days after ischaemia. Treatments consisted of a single dose (0.75 mmol/kg) immediately before ischaemia, a dual dose before ischaemia (1 mmol/kg) and before reperfusion (1 mmol/kg) and a dose before ischaemia (1 mmol/kg) followed immediately by an infusion (0.5 mmol/kg/h) for 150 minutes. In line with their earlier studies [31, 36], the single magnesium dose, which reduced infarct volume by 31%, was not statistically significant. The dual dose and combined loading and infusion doses of magnesium significantly reduced infarct volume by 32% and 41%, respectively. Animal management post-ischaemia was the same as in the Schmid-Elsaesser et al. study [31].

### Magnesium and stroke clinical trials

Several pilot trials of magnesium in acute stroke have been completed. In an early study [39] to assess serum magnesium levels, an intravenous bolus dose of magnesium sulphate (15 mmol) followed by a 4 mmol per hour infusion for 5 days resulted in increased serum magnesium concentrations to between 1.5 and 2.5 mmol/L (normal range 0.75 - 1.0 mmol/L).

Muir and Lees [40] examined the safety and tolerability of magnesium in 60 patients given magnesium sulphate (8 mmol over 15 minutes followed by 65 mmol over 24 hours or 2.7 mmol/h) intravenously within 12 hours of clinically diagnosed acute stroke. Serum magnesium level rose from 0.76 mmol/L to 1.42 mmol/L over 24 hours and remained significantly higher than in the saline placebo group at 48 hours. No differences in blood pressure or adverse events between the magnesium- and placebo-treated patients were observed. They concluded that magnesium is a safe and feasible potential therapy in acute stroke.

In a subsequent trial [41], to optimise the dosing regimen for a multi-center trial, intravenous magnesium as a loading dose of 8, 12 or 16 mmol, followed by a 65 mmol infusion over 24 hours was administered to 25 patients within 24 hours of the onset of clinically diagnosed stroke. There were no obvious effects of magnesium on heart rate, blood pressure or blood glucose. The 16 mmol loading infusion achieved target serum concentrations (1.49 mmol/L) most rapidly. Survival curve analysis found a trend in favour of magnesium, though no significant differences in outcome measures were observed in magnesium and placebo-treated groups.

Lampl et al. [42] performed a placebo-controlled, double-blind study using a magnesium sulphate intravenous loading dose (16mmol) and infusion (6 mmol/h for 5 days), administered within 24 hours of stroke onset. Twenty-one patients received magnesium and 20 patients received placebo (saline); patients were followed for 1 month. Several outcome scales (Orgogozo, Mathew, Rankin) indicated that magnesium treatment had a significant positive effect on patient outcome. Based on their positive results the authors indicated that a larger trial was needed to confirm their findings.

The two earlier clinical studies [40, 41] demonstrated that intravenous administration of magnesium was tolerated well in stroke patients. Due to the sample sizes however, these studies were not powerful enough to detect any favourable clinical outcome. As a result two large multi-centre clinical trials
(IMAGES and FAST-MAG) were recommended: the IMAGES trial [16], which is now complete, and the phase III FAST-MAG trial [17], which commenced in 2005.

The IMAGES stroke trial [16] consisted of a randomised trial comprising 2589 patients who each received 16 mmol magnesium intravenously over 15 minutes followed by 65 mmol over 24 hours, or matching placebo, within 12 hours of acute stroke. No significant differences in mortality and disability were found between patients treated with magnesium and placebo. Furthermore, magnesium was found to be largely ineffective with benefit only observed in patients suffering lacunar strokes. The FAST-MAG pilot trial [17, 43] showed that paramedic initiation of magnesium treatment can be achieved within 2 hours in 75% of patients, without serious adverse effects. Clinical outcomes were encouraging, but the full potential of early magnesium administration as an acute stroke treatment will need to wait until completion of the phase III FAST-MAG trial.

Why have studies using magnesium following cerebral events produced conflicting results?

Before we discuss possible reasons for conflicting findings in magnesium studies it is important to point out that few of the studies are directly comparable. This is due to differences in ischaemic model, animal species and strain, temperature monitoring, and dosage, route and time of magnesium administration. Despite the differences in experimental studies we propose several possible explanations that may account for the conflicting data.

Confounding effects of post-ischaemic hypothermia

Despite the established fact that both intra-ischaemic and post-ischaemic hypothermia can be neuroprotective following cerebral ischaemia [44-50], most studies only monitored and maintained normal body temperatures during the ischaemic period. In the magnesium studies described above (tables 1 and 2), body temperatures were maintained at normothermic levels during ischaemia, but in most there is no mention of body temperature monitoring or maintenance during recovery. This is an important factor because it is well documented that post-surgery hypothermia commonly occurs in animal models of cerebral ischaemia and it has been shown that post-ischaemic hypothermia has confounded the results of previous animal studies [50-57]. Therefore, in the magnesium studies that did not monitor animal body temperature and maintain animals at normothermic levels post-ischaemia the confounding effects of hypothermia cannot be ruled out.

Besides our own studies [24, 26], only four focal and one global cerebral ischaemia study have paid consideration to post-ischaemic animal body temperature. In a focal ischaemia study by Izumi et al. [28], while no measures were implemented to maintain animal body temperature, recordings were made at 1.5, 4, 24 and 48 hours after ischaemia. Magnesium treated animals experienced a 0.5°C drop in rectal temperature at the 1.5 and 4 hour time measurements and a 26% reduction in total infarct volume after 48 hours. This level of infarct volume reduction is similar to what was obtained in our laboratory when animal body temperature was not actively controlled (unpublished observation and see below). In focal ischaemia experiments originating from Schmid-Elsaesser’s laboratory [31, 36, 38], animals were monitored and maintained normothermic for 1 hour after reperfusion and housed in a warm environment for eight post-operative hours. Continuous monitoring of post-operative body temperature was not performed. Findings from Schmid-Elsaesser’s laboratory have revealed that, depending on the treatment schedule, magnesium administration can result in significant and non-significant reductions in infarct volume. In the global study [22], in order to maintain rat body temperature between 37 and 38°C in the first few hours after ischaemia, rats were housed, when necessary at 30°C. In this study, no reduction in CA1 and subiculum injury was observed.

The possibility of post-ischaemic hypothermia confounding experimental studies with magnesium is further supported by findings obtained in our laboratory [24, 25]. In an initial study evaluating the neuroprotective efficacy of magnesium following global ischaemia we did not monitor or control post-ischaemic body temperature and we observed a neuroprotective effect with magnesium when administered in pre- and post-ischaemia treatment protocols [23]. However, in subsequent studies we demonstrated that magnesium treatment is not neuroprotective following both global and focal cerebral ischaemia under controlled post-ischaemic normothermic conditions. Furthermore, following global ischaemia we observed that if body temperature was not controlled animals became mildly hypothermic during surgical recovery and magnesium treatment significantly reduced CA1 neuronal death. Similarly, we have observed that if body temperature is not
controlled following focal ischaemia, infarct volume is reduced by 25%. While not statistically significant, this finding indicates that the presence of hypothermia does have an effect on magnesium’s ability to reduce brain injury.

Additional support for hypothermia confounding magnesium’s neuroprotective effect comes from recent data from our laboratory [27]. When we compared magnesium efficacy in normothermic animals and in animals rendered mildly hypothermic (35°C) for 6 hours immediately post-ischaemia we observed that treatment with magnesium and mild hypothermia together increased CA1 neuronal survival from 5% to 38%. Importantly no neuroprotection was observed in normothermic animals receiving magnesium or in animals rendered hypothermic for 6 hours post-ischaemia. These findings illustrate two important points with respect to hypothermia confounding experimental data. One is that ischaemic control animals, which experience some level of hypothermia, may not show any sign of neuroprotection. The second is that the combination of magnesium and hypothermia has unmasked a neuroprotective effect. To this end, as in other studies [58, 59], we have shown that mild hypothermia (34-35°C) must be prolonged (24 hours) when commenced post-ischaemia to produce a protective effect and that post-ischaemic treatment with magnesium and mild hypothermia is more effective than either treatment alone [27]. Taken together these findings further highlight the need to maintain post-ischaemic animal body temperature in drug evaluation studies and, since in the majority of magnesium/cerebral ischaemia studies this was not done, questions must be raised regarding the validity of the data generated.

Dosage, route and time of magnesium administration

Given the fact that the confounding effects of hypothermia cannot be ruled out in the majority of magnesium studies it is even more difficult to speculate on whether magnesium dosage and route of administration is another contributing factor for the conflicting findings. This is further compounded by the fact that the optimal dose of magnesium remains unknown. It should be highlighted that loading doses of magnesium used in global ischaemia studies have ranged from 0.36-5.0, 2.5-7.5 and 16.6 mmol/kg for intravenous, subcutaneous and intraperitoneal administration, respectively. In the focal studies, intravenous/intraarterial doses have ranged from 0.18-0.75 mmol/kg and intraperitoneal doses from 0.75-1 mmol/kg. In some cases multiple dosing has been performed. Only four studies have used an administration protocol similar to that used in the clinical stroke trials consisting of an initial intravenous loading dose plus an intravenous infusion dose. Okawa [20] used a 0.664 mmol/kg loading dose and an infusion dose of 0.332 mmol/kg/h for 3 hours followed by 0.083 mmol/kg/h for 45 hours in dogs following global ischemia. Three studies from our laboratory have used a 0.36 mmol/kg loading dose and infusion doses at 0.06, 0.12, 0.24 or 0.48 mmol/kg/h for 48 hours in rats following global ischemia. Although it is not possible to determine if one particular route and dose of magnesium administration is superior over another, in our own laboratory we have observed that a loading dose of 0.36 mmol/kg and a 0.12 mmol/kg/h infusion dose provided the greatest level of CA1 survival following global ischemia when animals self-regulate their body temperature post-ischaemia.

The time of administration is also likely to influence any neuroprotective effects afforded by magnesium. Most studies have administered magnesium before or immediately after cerebral ischaemia, hence in these studies it is likely that increased magnesium levels were present in the brain at the time of ischaemia. Post-ischaemia treatments with magnesium following focal and global cerebral ischaemia have produced positive outcomes, but as mentioned above, the contribution of hypothermia in these studies was not determined. However, even if hypothermia has confounded treatment outcomes, it is encouraging to note that delayed treatment with magnesium can be effective.

Why was IMAGES trial unsuccessful?

Several reasons have been proposed for the lack of significant treatment effect with magnesium in the IMAGES trial [16]. These include: 1) delayed treatment with magnesium (median time 7 hours after stroke) reduced the likelihood of a positive outcome; 2) sample size was insufficient to reveal a small but clinically relevant effect; 3) magnesium was detrimental in some patients obscuring a beneficial effect in others and; 4) magnesium may not be an effective neuroprotective agent in human stroke patients. On this point, the trial investigators raised concerns regarding the validity of animal cerebral ischaemia models, specifically the focal model, since the preclinical data provided the basis for the human clinical trail. However, a review of the animal studies implicating post-ischaemic hypothermia as a contributing factor to the neuroprotective effect of mag-
MAGNESIUM AND CEREBRAL ISCHAEMIA

Magnesium may provide a plausible explanation as to why some animal experiments have produced positive outcomes. Moreover, since our own data has shown magnesium to be ineffective following cerebral ischaemia in normothermic animals, this provides a further explanation as to why the IMAGES trial was unsuccessful, as current patient management does not involve hypothermia induction. Therefore, it remains likely that magnesium treatment following stroke, if combined with mild hypothermia, would be beneficial.

The phase III FAST-MAG trial [17], which is currently underway will assess whether field administration of magnesium within 2 hours of stroke onset improves clinical outcomes. Importantly, the FAST-MAG trial will address whether the delayed magnesium treatment that occurred in the IMAGES trial was a reason for its ineffectiveness. However, based on our own assessment of magnesium under normothermic conditions we predict that if FAST-MAG patients are maintained normothermic this trial will also show little benefit. We believe that for magnesium to produce a positive outcome after stroke it needs to be combined with mild hypothermia. Hypothermia induction may only require a reduction in body temperature of 1-2°C involving basic cooling measures in conscious patients. Experiments in our laboratory are currently assessing the neuroprotective effect of combined magnesium/mild hypothermia (35°C) treatments when applied several hours after both global and focal cerebral ischaemia. It is anticipated that our experimental findings will enable better design of future clinical trials to test the efficacy of combined magnesium/mild hypothermia to improve patient outcome following cerebral ischaemia/stroke.

Conclusion

The rationale for trials of magnesium as a neuroprotective agent following stroke was based on its known cellular actions that counteract damaging ischaemic processes and on positive experimental data obtained from rodent models of cerebral ischaemia. However, closer scrutiny of the animal data shows that approximately 40% have not shown a neuroprotective effect and that the majority of positive studies are potentially confounded by post-ischaemic hypothermia. In addition, animal experimental design has not always been appropriate with respect to the clinical setting due to magnesium dosage, and to the time and route of magnesium administration. Moreover, recent animal studies under controlled post-ischaemic conditions indicate that magnesium is only neuroprotective when combined with hypothermia. Finally, additional information regarding the efficacy of magnesium as a stroke treatment will be available on completion of the FAST-Mag trial, but in the meantime the neuroprotective potential of magnesium should be explored when combined with post-ischaemic hypothermia in cerebral ischaemia models.

References


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