Effects of Elevated Plasma Magnesium Versus Calcium on Cerebral Ischemic Injury in Rats

James L. Blair, DO, David S. Warner, MD, and Michael M. Todd, MD

Both Mg$^{2+}$ and Ca$^{2+}$ have been implicated as having roles in the pathomechanisms of cerebral ischemia. To further study the effects of these ions on postischemic histologic outcome, fasted rats were given one of three intravenous infusions: 5.0 mmol/kg MgCl$_2$, 5.0 mmol/kg MgCl$_2$+0.035 units/kg regular insulin, or 1.0 mmol/kg CaCl$_2$. This resulted in elevated plasma Mg$^{2+}$ or Ca$^{2+}$ concentrations in the corresponding groups. A fourth group received 0.9% NaCl (saline). Preinfusion plasma glucose concentration was similar for all groups and was unchanged after infusion in rats receiving either saline or MgCl$_2$+insulin. In contrast, postinfusion glucose concentration was increased in the MgCl$_2$ group ($p<0.001$) and decreased in the CaCl$_2$ group ($p<0.001$) relative to saline-treated rats. Following respective infusions, all rats underwent 10 minutes of reversible forebrain ischemia (bilateral carotid artery occlusion and systemic hypotension) followed by 7 days' recovery. Six of 12 CaCl$_2$-treated rats died 2–3 days after ischemia; all other rats remained neurologically indistinguishable, without gross neurologic deficits. Histologic injury in the neocortex and caudate was moderate in all groups. In the hippocampus, MgCl$_2$+insulin resulted in 66±6% (mean±SD) dead CA1 pyramidal cells, which was similar to the amount in saline-treated rats (68±10%). Injury was increased in the MgCl$_2$ group (79±4% dead cells), while in surviving CaCl$_2$-treated rats, injury was decreased (54±13%). We conclude that the increased injury in MgCl$_2$-treated rats and the decreased injury noted in surviving rats receiving CaCl$_2$ are due to the plasma glucose concentrations present prior to ischemia. When glucose concentration was controlled, no cerebral protective effect of MgCl$_2$ was evident. (Stroke 1989;20:507–512)

Because calcium has been implicated as a mediator of ischemically induced neuronal injury, investigators have attempted to ameliorate damage by using compounds known to block calcium entry. One such agent, inorganic magnesium ion (Mg$^{2+}$), has met with varying degrees of success. For example, Vacanti and Ames reported an improved neurologic outcome from a combination of mild hypothermia and intravenous MgCl$_2$ in a rabbit model of spinal cord ischemia. While the contribution from hypothermia may have been of greater significance, Mg$^{2+}$ has been shown to significantly lower spinal cord metabolic rate. By contrast, in a small sample of cardiac arrest patients, Ruiz et al failed to show cerebral protection from a treatment regimen including intravenously administered MgCl$_2$.

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More compelling evidence for the efficacy of Mg$^{2+}$ has been provided by in vitro studies. Using a model simulating rabbit retinal ischemia, Ames and Nesbett increased preischemic Mg$^{2+}$ concentration in the incubation medium to one that greatly reduced energy requirements and showed a significant improvement in postischemic recovery of 2-deoxyglucose uptake. Similarly, in a rat hippocampal slice preparation subjected to anoxia, Kass et al found a marked protective effect of MgCl$_2$ as evidenced by recovery of postanoxic orthodromically conducted evoked population spikes; no such effect could be found for nimodipine.

To our knowledge, no attempt has been made to confirm these potential benefits of Mg$^{2+}$ therapy with respect to improved histologic outcome in an in vivo animal model of cerebral ischemia. Therefore, we studied the potential for Mg$^{2+}$ to prevent neuronal death in the rat forebrain by preischemic intravenous loading with MgCl$_2$. Those areas of the brain with known selective vulnerability to ischemia were evaluated histologically 1 week after a reversible 10-minute near-complete ischemic insult and were compared with histologic injury observed in saline-treated control rats. To examine and con-
trast the effects of intravenous Ca\(^{2+}\) loading, CaCl\(_2\) was administered to another group of rats before ischemia to determine if elevated plasma calcium concentration could exacerbate cell death.

Materials and Methods

With Institutional Animal Care and Use Committee approval, we studied 40 fasted Sprague-Dawley rats (body wt 250–325 g; age range 9–10 weeks). Following anesthetic induction with 4% halothane in O\(_2\), each rat was endotracheally intubated and ventilated with a small animal respirator delivering 0.7% halothane in 30% O\(_2)/balance N\(_2\)O. Tidal volume and respiratory rate were adjusted to maintain normoxia and normocapnia. The tail artery was cannulated for blood pressure monitoring and sampling for blood gas analysis. A rectal temperature probe was placed, and body temperature was servo-regulated at 36.8–37.2° C by surface heating or cooling. Via a ventral neck incision, both carotid arteries were isolated and snared. The right internal jugular vein was cannulated, and 50 IU i.v. heparin was given. Muscle paralysis was provided by a 1.0-mg intravenous bolus of succinylcholine repeated as necessary. Bipolar electroencephalographic (EEG) activity was monitored from a pair of needle electrodes inserted into the temporalis muscle bilaterally. All surgical incisions were infiltrated with 0.5% lidocaine. Following surgery, halothane was discontinued. The rats continued to be ventilated mechanically, receiving 70% N\(_2\)O/30% O\(_2\), and a ventilation after termination of ischemia. A second group (n=8) received intravenous 0.9% NaCl. In all three groups, solutions were administered at the rate of 2 ml/hr. Five minutes after the respective infusions were completed, a 1-ml venous (postinfusion) blood sample was drawn for the same plasma determinations as the preinfusion samples.

After evaluation of histologic and plasma chemistry data from the first three groups, a fourth group of rats was added (n=6). This group was treated identically to the Mg\(^{2+}\) group except for the addition of 0.03–0.04 units/kg regular pork insulin (Eli Lilly and Co., Indianapolis, Indiana) to the MgCl\(_2\) infused to maintain preischemic plasma glucose values in the range of that observed for saline-treated rats.

All rats then underwent 10 minutes of reversible near-complete forebrain ischemia induced by a single intravenous bolus of 3.5 mg trimethaphan followed immediately by bilateral carotid artery occlusion and simultaneous central venous exsanguination, as required, to maintain MABP at 50±5 mm Hg throughout the ischemic interval. Onset and maintenance of ischemia was confirmed by continuously monitoring an isoelectric EEG. After 10 minutes, ischemia was reversed by simultaneous removal of the carotid artery snares and reinfusion of the previously shed blood. All rats then received 0.4 ml of 0.6 M NaHCO\(_3\) to counteract systemic acidosis as well as 2 ml of whole blood from a fasted donor rat to replace blood sampling losses.

MABP and EEG were recorded 2, 5, 10, 15, and 20 minutes after ischemia to evaluate hemodynamic stability and the return of forebrain electrical activity. Ten minutes after ischemia, PaO\(_2\), PaCO\(_2\), and arterial pH were measured. The vascular catheters were then removed, the vessels were ligated, and the incisions were closed. Thirty minutes after ischemia, N\(_2\)O was discontinued, and the rats were allowed to awaken. The rats soon became ambulatory and were housed in cages with free access to pellet food and water.

On Day 7 of the experiment, all rats were weighed again and anesthetized with 3.0% halothane in 30% O\(_2)/balance N\(_2\)O. Following tracheal intubation, the rats were ventilated by a small animal respirator delivering 0.7% halothane in 30% O\(_2)/balance N\(_2\)O. Via the ascending aorta, the brains were perfusion-fixed with formalin in situ. After being embedded in paraffin, the brains were subserially sectioned (8 μm) in the coronal plane and stained with celestin blue/acid fuchsin. Damage was assessed as the presence of necrotic, acidophilic (pink or red) neurons, which are considered to be irreversibly damaged since they have been consistently found to undergo cytolysis and removal from brain tissue. Hippocampal injury at two anatomically standardized levels was quantified by an experimenter blinded to treatment group by direct visual counting of acidophilic neurons at ×320 magnification. Injury in the cortex at the level of the subfornical organ and the caudate nucleus in the lateral and dorsal aspects at the level of the septal nuclei at their widest point was assessed using a crude damage index (CDI) as follows: 0, no damage; 1, rare to occasional acidophilic cells per field (approximately 10% damage); 2, moderate number of acidophilic cells per field (approximately 10–50% damage); or 3, frequent acidophilic cells per field (>50% damage).

Plasma osmolality was determined from a 20-μl sample using a micro-osmometer (Model 3MO,
TABLE 1. Physiologic Values for Rats Before, During, and After Reversible Near-Complete Forebrain Ischemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (n=8)</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>106±8</td>
</tr>
<tr>
<td>After</td>
<td>117±6</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>39.1±2.4</td>
</tr>
<tr>
<td>After</td>
<td>42.1±3.2</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.39±0.02</td>
</tr>
<tr>
<td>After</td>
<td>7.37±0.03</td>
</tr>
<tr>
<td>Body temperature (° C)</td>
<td></td>
</tr>
<tr>
<td>During</td>
<td>37.0±0.1</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>117±7</td>
</tr>
<tr>
<td>After</td>
<td>110±7</td>
</tr>
<tr>
<td>20 min</td>
<td>128±9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>283±24</td>
</tr>
<tr>
<td>After (7 days)</td>
<td>300±26</td>
</tr>
</tbody>
</table>

Values are mean±SD. Ischemia, 10 minutes of bilateral carotid artery occlusion combined with systemic hypotension (mean arterial blood pressure [MABP] of 50±5 mm Hg).

Results

Physiologic values are given in Table 1. All rats were normoxic, normocapnic, and normothermic before ischemia and during the immediate recirculation period. MABP was similar for all groups at all times. Plasma chemistry values are given in Table 2. Before infusion, no difference was observed between groups for plasma Mg²⁺ or Ca²⁺ concentrations or osmolalities. As expected, postinfusion plasma Mg²⁺ and Ca²⁺ concentrations were significantly elevated in appropriate groups. Plasma osmolality remained unchanged.

Plasma glucose concentration was similar between groups before the infusions. In contrast, postinfusion plasma glucose concentration varied markedly

TABLE 2. Plasma Chemistry Values for Rats Before and After Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (n=8)</td>
</tr>
<tr>
<td>Mg²⁺ (mg/dl)</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Before</td>
<td>1.6±0.2</td>
</tr>
<tr>
<td>After</td>
<td>9.3±0.6</td>
</tr>
<tr>
<td>Ca²⁺ (mg/dl)</td>
<td>8.7±0.7</td>
</tr>
<tr>
<td>Osmolality (mosm/l)</td>
<td>308±3</td>
</tr>
<tr>
<td>Before</td>
<td>310±2</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*tp<0.05, 0.01, respectively, different from saline group.
between groups (Figure 1). Postinfusion glucose concentration in the Mg$^{2+}$ group (221±29 mg/dl) was significantly elevated ($p<0.001$) compared with both the saline group (153±20 mg/dl) and the Ca$^{2+}$ group (112±14 mg/dl). Conversely, postinfusion glucose concentration in the Ca$^{2+}$ group was significantly decreased relative to the saline group ($p<0.001$). Postinfusion plasma glucose concentration for the Mg$^{2+}$+insulin group (167±10 mg/dl) was not significantly different from that of the saline group, per experimental design.

All rats resumed spontaneous ventilation 45 minutes after ischemia and were able to walk within 2 hours of reperfusion. However, a unique mortality (six of 12 rats) occurring 2–3 days after ischemia was observed in the Ca$^{2+}$ group; no mortality was seen in the remaining groups. All rats were moderately hyperexcitable to both sound and handling by 3–4 days after ischemia, but overt (and brief) seizures occurring approximately 24 hours before death were noted in only one Ca$^{2+}$ rat. Surviving rats in all groups exhibited return of feeding behavior identified by an increase in body weight over the 7 days (Table 1).

Histologic data was partially lost for one Ca$^{2+}$ rat in the caudate nucleus and for two Mg$^{2+}$ rats in the hippocampus due to processing or sectioning errors. Within the hippocampus, the extent of injury quantified as percent acidophilic neurons varied between treatment groups (Table 3). Injury was most severe in the Mg$^{2+}$ group, with 79±4% of the pyramidal cells in CA1 being acidophilic (Figure 1); this compared with 68±13% cellular mortality in the saline group. Among surviving Ca$^{2+}$ rats, a further reduction in the number of acidophilic CA1 neurons was observed (54±13%) ($p<0.001$ vs. Mg$^{2+}$, $p<0.001$ vs. saline). The addition of insulin to MgCl$_2$ significantly reduced injury (66±6%) compared with that of rats receiving only MgCl$_2$ ($p<0.001$). Hippocampal CA1 injury in the Mg$^{2+}$+insulin group was nearly identical to that in the saline group. CA3 and CA4 sectors showed few acidophilic neurons, without differences among the groups. The dentate gyrus was spared from injury, except for a rare dead neuron occurring in all groups.

Within the neocortex, the CDIs were statistically indistinguishable among the four groups. With rare exception, a CDI of 2 was assigned to all rats, with no discernable pattern of deviation observed as a function of treatment. This was also the case for the caudate. Frank infarction was absent in all rats evaluated.

**Discussion**

The forebrain ischemia model developed by Smith et al. is appropriate for evaluating the potential protective benefits from MgCl$_2$; the insult is sufficiently mild to allow recovery of the rat, thus providing full histologic maturation of the injury, yet is severe enough to produce distinct pathologic changes. Furthermore, outcome can be pharmacologically modified, as shown in our study as well as in other work with the calcium entry blocker flunarizine. Based primarily upon in vitro evidence, we expected a benefit from MgCl$_2$; surprisingly, a worsened histologic injury was observed. By contrast, previous work associating ischemically induced Ca$^{2+}$ entry with worsened outcome led us to predict that an increase in plasma Ca$^{2+}$ concentration would either worsen outcome or have no effect (given the normal preexisting 10,000:1 ratio of extracellular to intracellular Ca$^{2+}$).

In our experiment, a nearly twofold increase in preischemic plasma Ca$^{2+}$ concentration resulted in two distinctly different types of outcome. That is, while a 50% delayed postischemic mortality occurred, surviving CaCl$_2$-treated rats were found to have consistently less hippocampal injury than saline-treated controls. Although brains from the dead rats were not evaluated, the relatively uniform decrease in histologic injury observed in surviving CaCl$_2$-treated rats suggests that the etiology was not cerebral, but perhaps of cardiac or respiratory origin.
Other than elevated plasma concentrations of Mg\(^{2+}\) and Ca\(^{2+}\) in groups so infused, the only measured parameter that was significantly affected by treatment was plasma glucose concentration. Bennett et al\(^9\) have shown significant antagonism of Ca\(^{2+}\) activity by Mg\(^{2+}\) in the perfused rat pancreas. At markedly elevated levels (as were obtained after infusion in the Ca\(^{2+}\) group), calcium, which is the stimulus–secretion coupler for insulin,\(^{18,19}\) causes a release of insulin and a moderate decrease in plasma glucose concentration. Hypermagnesemia produces the opposite effect by displacing Ca\(^{2+}\) at the islet cell membrane; plasma insulin levels are reduced and hyperglycemia ensues.\(^{20}\)

Preischemic plasma glucose concentrations have been established as a critical determinant of outcome from cerebral ischemia.\(^{21-24}\) Given this background, we recognized the importance of determining whether the observed intergroup differences in histopathologic outcome were due to the ions themselves or were secondary to changes in glucose concentrations. To examine this, the Mg\(^{2+}\)-insulin group was added to the study immediately after histologic information from the first three groups was obtained. When plasma glucose levels were held within the control range by the addition of insulin, no independent Mg\(^{2+}\)-mediated decrease in neuronal necrosis could be demonstrated.

There are potential explanations for our failure to demonstrate protection from MgCl\(_2\). First, Mg\(^{2+}\) may not have crossed the blood–brain barrier in meaningful concentrations. Some information exists concerning the effects of acute hypermagnesemia on brain Mg\(^{2+}\) concentration. Opelt et al\(^{25}\) observed a modest 21% increase in cerebrospinal fluid (CSF) Mg\(^{2+}\) concentration in dogs over 5.5 hours while plasma Mg\(^{2+}\) concentration was three to four times normal. Similarly, Chutkow\(^{26}\) found an increase in CSF Mg\(^{2+}\) concentration identifiable within minutes after an intraperitoneal injection of MgCl\(_2\) into hypermagnesemic rats; the CSF, however, was found not to reflect the brain parenchyma, which lagged several hours behind in showing an increase in Mg\(^{2+}\) concentration. Other studies have failed to identify an increase in CSF Mg\(^{2+}\) concentration during acute hypermagnesemia.\(^{27}\) Thus, it could be questioned if an acute and transient hypermagnesemia would significantly increase the bioavailability of Mg\(^{2+}\) at the neuronal level. This may in part explain the discrepancy between our results and those reported by Kass et al,\(^9\) who evaluated the efficacy of Mg\(^{2+}\) in protecting neurons from anoxia using the hippocampal slice preparation. In that case, Mg\(^{2+}\) was directly available to tissue by superfusion and thus was not dependent upon transport across the blood–brain barrier.

In addition, blockade of Ca\(^{2+}\) entry by Mg\(^{2+}\) may be more beneficial if provided 2–3 days after ischemic injury, when a significant increase in brain Ca\(^{2+}\) concentration becomes evident.\(^{28}\) Though Mg\(^{2+}\) may reduce Ca\(^{2+}\) influx, our study indicates that any terminal calcium-mediated events remain unaffected by preischemic Mg\(^{2+}\) loading. Elevated plasma Mg\(^{2+}\) concentration produces a brisk diuresis, and the rat kidney is capable of promptly restoring levels to normal.\(^{29}\) If Mg\(^{2+}\) is capable of ameliorating such injury, it would seem necessary to ensure an elevated Mg\(^{2+}\) concentration in the extracellular fluid beyond the acute insult.

In conclusion, a preischemic intravenous infusion of MgCl\(_2\) into rats increased plasma glucose concentration and worsened histologic outcome compared with saline-treated controls. When plasma glucose was held normal, MgCl\(_2\) had no effect on ischemic histologic outcome. Conversely, infusion of CaCl\(_2\) before ischemia significantly reduced plasma glucose concentration. A 50% postischemic mortality rate occurred, although a significant reduction in histologic injury was observed in survivors. Because the interactions of Mg\(^{2+}\), Ca\(^{2+}\), and plasma glucose concentration appear critical in predicting outcome, our results underline the importance of verifying in vitro results in an intact preparation.

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