Effects of Magnesium Treatment in a Model of Internal Capsule Lesion in Spontaneously Hypertensive Rats

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Background and Purpose—The study aim was to assess the effects of magnesium sulfate (MgSO₄) administration on white matter damage in vivo in spontaneously hypertensive rats.

Methods—The left internal capsule was lesioned by a local injection of endothelin-1 (ET-1; 200 pmol) in adult spontaneously hypertensive rats. MgSO₄ was administered (300 mg/kg SC) 30 minutes before injection of ET-1, plus 200 mg/kg every hour thereafter for 4 hours. Infarct size was measured by T2-weighted magnetic resonance imaging (day 2) and histology (day 11), and functional recovery was assessed on days 3 and 10 by the cylinder and walking-ladder tests.

Results—ET-1 application induced a small, localized lesion within the internal capsule. Despite reducing blood pressure, MgSO₄ did not significantly influence infarct volume (by magnetic resonance imaging: median, 2.1 mm³; interquartile range, 1.3 to 3.8, vs 1.6 mm³ and 1.2 to 2.1, for the vehicle-treated group; by histology: 0.3 mm³ and 0.2 to 0.9 vs 0.3 mm³ and 0.2 to 0.5, respectively). Significant forelimb and hindlimb motor deficits were evident in the vehicle-treated group as late as day 10. These impairments were significantly ameliorated by MgSO₄ in both cylinder (left forelimb use, \( P \leq 0.01 \) and both-forelimb use, \( P \leq 0.03 \) vs vehicle) and walking-ladder (right hindlimb score, \( P < 0.02 \) vs vehicle) tests.

Conclusions—ET-1–induced internal capsule ischemia in spontaneously hypertensive rats represents a good model of lacunar infarct with small lesion size, minimal adverse effects, and a measurable motor deficit. Despite inducing mild hypotension, MgSO₄ did not significantly influence infarct size but reduced motor deficits, supporting its potential utility for the treatment of lacunar infarct. (Stroke. 2008;39:448-454.)

Key Words: endothelin ▪ lacunar infarct ▪ magnesium ▪ functional recovery ▪ white matter

White matter constitutes a high proportion of brain tissue involved in stroke, particularly lacunar strokes (=25% of all ischemic strokes). Lacunar stroke produces small lesions normally located in subcortical regions, often involving white matter fiber tracts and usually resulting from the occlusion of a single, perforating artery. Axonal injury after cerebral ischemia has received little attention compared with the abundant literature on the pathophysiology of gray matter. Rodent models of white matter damage most commonly use neonates. Most studies of white matter damage in the adult rat use in vitro preparations of the rat optic nerve or spinal cord or in vivo models, such as chronic cerebral hypoperfusion or demyelinating lesions, to mimic allergic encephalomyelitis or multiple sclerosis. However, the pathophysiology involved in these conditions is not representative of acute stroke pathology. Therefore, more pertinent animal models, such as the recently described endothelin-1 (ET-1)–induced ischemia of the internal capsule (IC), are required to study the pathophysiology of lacunar infarct and the influence of neuroprotective drugs on white matter.

Despite demonstrating neuroprotective properties in several animal stroke models, a recent large clinical trial, the Intravenous Magnesium Efficacy in Stroke trial, found no significant effect of MgSO₄ given within 12 hours of symptom onset in stroke patients. However, preplanned subgroup analysis revealed significant benefit in patients with lacunar stroke syndromes. Post hoc analysis of Intravenous Magnesium Efficacy in Stroke trial data also suggested benefit in patients with higher blood pressures (BPs) at presentation.

Justification for the clinical trials of magnesium was based on animal studies reporting significant neuroprotection with...
MgSO₄ and MgCl₂ in rodent models of both reversible and permanent middle cerebral artery occlusion, with a reduction in infarct volume of up to 61% (reviewed in Muir⁸). In vitro permanent middle cerebral artery occlusion, with a reduction studies⁹,¹⁰ indicated a potential neuroprotective effect of temperature-controlled drill for placement of a 30-gauge needle into placed in a stereotaxic frame, and a craniectomy was made with a /H11006 0.5°C with a heating pad. Animals were body temper- and N₂O (30%:70%, vol/vol) delivered via face mask. Body temper-

ET-1 Injection Into the IC

The IC was chosen, first because it is one of the larger white matter tracts in the rodent brain and second because Frost et al⁶ recently reported that ET-induced lesions of the IC result in a measurable sensorimotor deficit in rats. Furthermore, a unilateral section of the pyramidal tract at the brainstem level results in deficits in voluntary motor functions and impair-

Materials and Methods

Animals

Experiments were performed under license from the UK Home Office and were subject to the Animals (Scientific Procedures) Act of 1986. Adult male SHR (mean±SD weight, 335±18 g; Charles River) were subjected to unilateral ET-1 injection into the left IC. Animals were randomly allocated to active (n=15) or control (n=15) treatment by an independent technician who prepared the injection materials accordingly and provided this in masked form to the researcher. Criteria were set to exclude animals that died before 11 days or that failed to demonstrate infarction within the IC territory. Analysis was thus conducted on a per-protocol basis. Investigators who measured infarct size with magnetic resonance imaging (MRI) and histology and scored behavioral data were blinded to treatment allocation. In a separate group of SHR, blood magnesium concentration and BP were determined after repeated injections of either vehicle (n=4) or magnesium (n=4). Magnesium levels were also determined in 2 adult Sprague-Dawley rats. A schematic illustration of the experimental protocol is shown in Figure 1A.

ET-1 Injection Into the IC

Animals were anesthetized with halothane (1.5%) in a mixture of O₂ and N₂O (30%:70%, vol/vol) delivered via face mask. Body temper-

Figure 1. Experimental protocol. A. Rats were handled and habituated before ET-1 lesioning. Cylinder and walking-ladder tests were performed before surgery (baseline) and on days 3 and 10 after ischemia. Quantification of ischemic damage was performed by MRI T2-weighted images (day 2) and histology (day 11). B. The stereotaxic injection was performed at an angle of 25° to avoid the track-

Magnesium Administration and Dose

The schedule of magnesium injections was determined from previ-

T2-Weighted MRI

MRI was carried out 2 days after ET-1 injection on a Bruker Biospec 7-T MRI system. Anesthesia was induced with 5% and maintained with 1.5% halothane (in 30% O₂:70% N₂O, vol/vol). Rats were intubated and mechanically ventilated at 60 beats/min. Heart and respiration rates were monitored throughout the procedure, and body temperature was maintained at 37°C. A rapid-acquisition relaxation enhancement, T2-weighted sequence was used to determine the precise lesion location: rapid-acquisition relaxation enhancement factor 16, repetition time 5086 ms, echo time 70.1 ms with an in-plane resolution of 250×250×250 μm, and 15 slices. A second T2-weighted image set was acquired throughout the lesion: rapid-

Behavioral Tests

Both cylinder⁴ and walking-ladder⁷ tests were performed before ischemia to obtain baseline values and then on days 3 and 10 after ischemia. To limit variability, all behavioral tests were performed at the same time (11 AM to 3 PM) by the same investigator. No habituation to the cylinder before the experiment was allowed. All sessions were video-recorded for subsequent analysis. This test was
Table. Plasma Levels of Mg²⁺ (mmol/L) After Repeated MgSO₄ Injections

<table>
<thead>
<tr>
<th>Time</th>
<th>SHR (n=4)</th>
<th>SD (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.54±0.06</td>
<td>0.62±0.02</td>
</tr>
<tr>
<td>1 Hour</td>
<td>1.75±0.02‡</td>
<td>1.72±0.00‡</td>
</tr>
<tr>
<td>2 Hours</td>
<td>1.68±0.06‡</td>
<td>1.71±0.13‡</td>
</tr>
<tr>
<td>3 Hours</td>
<td>1.99±0.02‡</td>
<td>1.75±0.07‡</td>
</tr>
<tr>
<td>4 Hours</td>
<td>1.85±0.30‡</td>
<td>1.77±0.01‡</td>
</tr>
<tr>
<td>5 Hours</td>
<td>2.03±0.25‡</td>
<td>1.67±0.17‡</td>
</tr>
<tr>
<td>6 Hours</td>
<td>1.21±0.12‡</td>
<td>1.29±0.05‡</td>
</tr>
<tr>
<td>7 Hours</td>
<td>0.92±0.08°</td>
<td>0.93±0.01*</td>
</tr>
</tbody>
</table>

Normal range is 0.66–0.95 mmol/L. Statistical differences for each strain compared with baseline: *P<0.05, †P<0.01, ‡P<0.001 (repeated-measures ANOVA, Fisher’s protected least significant difference test). There was no significant difference in plasma magnesium levels between Sprague-Dawley (SD) rats and SHR.

Walking-Ladder Test
This test was developed to assess fine motor deficits precisely and requires rats to cross an elevated horizontal ladder (length, 1 m) of irregularly spaced rungs (diameter, 1 mm). The irregular pattern limits the rat’s ability to learn the rung arrangement. All trials were recorded on video and visualized by image to determine each paw placement score. Scores were assessed as previously described: 0 = total miss, 1 = deep slip, 2 = slight slip, 3 = re-placement, 4 = correction, 5 = digits, and 6 = correct placement. An error was defined as a score of 0, 1, or 2 and a slight error, as a score of 3, 4, or 5. Animals were handled regularly before behavioral testing and trained to cross the ladder before test sessions. Each rat was tested on 5 different irregular rung patterns during each session.

Histology Analysis
Animals were euthanized and transcardially perfused with heparinized saline followed by 4% paraformaldehyde 11 days after ischemia. Brains were processed and embedded in paraffin wax, and 6-μm sections throughout the lesion were stained with Luxol Fast Blue for analysis of white matter damage. Ischemic damage was quantified microscopically on 13 sections throughout the lesion, with infarct volume calculated from infarct areas and the distance between the measured sections.

Statistical Analysis
Infarct volumes are expressed as median and interquartile range and compared between treatment groups by 2-sample t tests: †P<0.05 compared with baseline, and t tests: †P<0.05, ‡P<0.01, ™P<0.001 compared with the vehicle group at the same time point.

Results
Effect of MgSO₄ on Plasma Magnesium Level and BP
The dosing regimen maintained mean plasma magnesium levels >1.40 mmol/L over the course of the injection schedule (the Table) and induced a modest but significant hypotensive effect in SHR (Figure 2).

ET-1–Induced Ischemic Damage in the IC
ET-1–induced ischemic damage, measured on day 2 by T2-weighted MRI (Figure 3A), was not significantly different between the vehicle (median 1.6 mm³, interquartile range 1.2 to 2.1, n=15) and magnesium-treated (2.1 mm³, 1.3 to 3.8, n=13) groups (Figure 3B). Correct lesion localization in each animal was confirmed by histology on day 11 (Figure 3C). The lesion size as assessed by histology was not significantly different between groups but was significantly decreased compared with day 2 in both vehicle (0.3 mm³, 0.2 to 0.5, P<0.0001) and MgSO₄–treated (0.3 mm³, 0.2 to 0.9, P<0.001) groups (Figure 3B).

Cylinder Assessment of Forelimb Asymmetry
Before ischemia, forelimb use was ≈50% for “both” forelimbs simultaneously and 25% use for each individual forelimb (left or right forelimb alone) in both vehicle and magnesium-treated groups (Figure 3D).

Figure 2. Effect of MgSO₄ on mean arterial BP. MgSO₄ was injected at 300 mg/kg SC (arrow 1) and 200 mg/kg (arrows 2–5). Repeated-measures ANOVA, Fisher’s protected least significant difference test: *P<0.05 compared with baseline, and t tests: †P<0.05, ‡P<0.01, ‡‡P<0.001 compared with the vehicle group at the same time point.
magnesium groups (Figure 4A). ANOVA with repeated measures revealed a significant modification of forelimb use in the vehicle group after the lesion, with a decrease in “both” forelimb placements at day 3 ($P < 0.02$) and day 10 ($P < 0.003$) compared with baseline and an increase of left forelimb use at both time points ($P < 0.02$), whereas there was no modification of forelimb use after ischemia in the magnesium group (Figure 4A). Magnesium treatment provided a significant improvement of forelimb use after ischemia compared with the vehicle group on both the left forelimb ($P < 0.01$) and “both” forelimb ($P < 0.03$) use, as revealed by ANCOVA.

Figure 4B shows the variation in the total number of forelimb placements in the cylinder. IC ischemia induced a decrease in the total number of placements in the vehicle group ($-15\%$, $P < 0.03$), whereas there was no significant improvement of forelimb use after ischemia compared with the vehicle group on both the left forelimb ($P < 0.01$) and “both” forelimb ($P < 0.03$) use, as revealed by ANCOVA.

Figure 4. Forelimb asymmetry after ET-1 lesion induction and effect of MgSO4. A, Each type of forelimb contact on the cylinder wall was expressed as a percentage of the number of total placements. Data were analyzed by repeated-measures ANOVA, Fisher’s protected least significant difference test: *$P < 0.05$, **$P < 0.01$ compared with baseline. B, Data for each forelimb are expressed as a percentage of the total number of placements and were analyzed by repeated-measures ANOVA, Fisher’s protected least significant difference test: *$P < 0.05$, **$P < 0.01$ compared with baseline in the vehicle group; †$P < 0.05$, ‡$P < 0.001$ compared with day 3.
change in the magnesium group (≈ 8%) at day 3. After 10 days, both groups showed a significantly higher number of forelimb contacts in the cylinder compared with baseline ($P<0.05$).

**Walking-Ladder Test**

The walking-ladder test allows assessment of skilled walking by scoring each limb placement during a crossing of the ladder: at baseline, the most frequent score was 6 (correct placement) in both groups (72.5 ± 7% for the vehicle group, 73.7 ± 7% for the magnesium group). Ischemia in the left IC was significantly associated with walking ability of the right limbs, as revealed by the mean score for each limb. The mean score for the right forelimb was significantly decreased in both groups at day 3, and this persisted to day 10 compared with baseline (Figure 5). At day 3, IC ischemia induced an impairment of the right hindlimb in the vehicle group compared with baseline ($P<0.02$), whereas there was no impairment in the magnesium group. This difference between groups at day 3 was statistically significant ($P<0.03$). At day 10, the right hindlimb score was not different from baseline in the 2 groups. The specificity of the motor deficit induced by left IC ischemia was confirmed by the absence of left-side deficits, on either forelimb or hindlimb placement. The left hindlimb score was increased compared with baseline at day 3 in the vehicle group ($P<0.01$) and at day 10 in the magnesium group ($P<0.003$) (Figure 5).

**Discussion**

Specific white matter damage in the IC was induced by ET-1 injection in SHR. Early MRI assessment of infarct location and size was confirmed by late histologic evaluation and behavioral assessment revealing a measurable motor deficit. This model has subsequently been used to test the influence of MgSO$_4$ on white matter ischemia with these outcome measures.

ET-1 is one of the most potent vasoconstrictors, and its local administration produces ischemic injury by a prolonged but reversible reduction of local blood flow.$^{19}$ ET-1 has consequently been used as a tool to induce localized focal cerebral ischemia in rats.$^{20}$ Models have been developed with ET-1 to induce ischemia within a particular artery territory, (eg, stereotaxic administration adjacent to the middle cerebral artery$^{21}$ and topical administration onto the exposed middle cerebral artery$^{22}$), with the extent of the damage related to the concentration of ET-1 administered.$^{22}$ Frost et al.$^6$ subsequently administered ET-1 into the IC of Sprague-Dawley rats to produce a white matter lesion with effects on behavioral deficits in placing and sensorimotor tests, which we adapted for use in the SHR. However, this required the design of a new set of stereotaxic coordinates because SHR have enlarged ventricles, and adverse effects of ET-1 were encountered when the needle track passed through the cerebral ventricles. These new stereotaxic coordinates also avoided any needle track damage to the sensorimotor cortex.

With this approach, ET-1 induced a small but reproducible lesion that was quantified by early T2-weighted MRI. Because we included a vehicle control for the magnesium study but not a sham control, we cannot discount the possibility that mechanical damage, unrelated to ischemia, was responsible for or contributed to the lesion. However, in the study of Frost et al.$^6$ a surgery control group (1-$\mu$L vehicle injection into the IC) showed no histologic damage or behavioral deficits. Therefore, the lesion identified in the current study is more likely a consequence of ET-1-induced ischemia rather than mechanical damage induced by the injection needle or volume.

MRI did not provide sufficient resolution to determine whether the damage was located precisely within the white matter or whether any damage extended into the neighboring gray matter. Therefore, histologic staining, specific for white matter (Luxol Fast Blue), was used to confirm the localization of the lesion to the IC in both groups. Lesion volumes as assessed by histology were significantly smaller compared with those derived from MRI. This is most likely due to a combination of the resorption of acute brain edema by day 11 and brain shrinkage due to dehydration of the brain during histologic processing.

Our lesion model revealed significant motor deficits of both the forelimb and hindlimb as measured by specific motor tests. The cylinder test showed forelimb use impairments during the 10 days after lesion induction. The walking-ladder test provided evidence of hindlimb placement impairments during this same period.

Although magnesium decreased BP, it did not significantly influence lesion size but produced a beneficial effect on functional outcome on both the forelimbs and the right hindlimb compared with the vehicle group. The significant effect of magnesium was more pronounced in the cylinder test, suggesting that cylinder data are less dependent than walking-ladder data on minor variability in the placement of lesions, especially in view of the small lesion volume and density of axonal tracts in this region.
Several studies have demonstrated that magnesium crosses the blood-brain barrier in both animals and humans.\(^8\) Transport from the blood to cerebrospinal fluid is regulated by active transport that maintains cerebral concentrations higher than those in serum (1.1 mmol/L vs 0.8 mmol/L in the rat).\(^23\) Previous rodent studies have revealed that systemic administration of magnesium leads to an increase in the cerebrospinal concentration of up to 25% for 6 hours after treatment.\(^24\) Even if the cerebrospinal concentration increases only modestly, there is preferential uptake of magnesium by pathologic regions, including those affected by focal ischemia (eg, hippocampus and cortex), resulting in magnesium levels sufficient to inhibit \(N\)-methyl-\(d\)-aspartate (NMDA) receptors.\(^25\) Furthermore, intracellular free magnesium concentrations may be increased by dissociation from ATP after stroke.\(^26\) Taken together, even small elevations of extracellular magnesium concentration may lead to a protective effect.

The mechanisms involved in white matter damage and functional recovery are still inadequately understood, and therefore, we can only hypothesize regarding the possible mechanisms for the observed magnesium-associated preservation of motor function. Magnesium-induced neuroprotection could include both vascular and neuroglial mechanisms. In spinal cord injury models, magnesium-induced vasodilation may improve blood flow and reduce vasospasm.\(^27\) Several mechanisms for the vascular effects of magnesium have been proposed, including antioxidant actions, blockade of voltage-dependent calcium channels, release of \(NO\),\(^28\) and inhibition of ET-1 vasoconstriction.\(^29\) However, if the severity or duration of ischemia were reduced by magnesium, one would expect a reduction in infarct volume, and this was not apparent in our data.

Magnesium may provide cellular protection via a number of potential mechanisms. First, protection of oligodendrocytes, the main cell type constituting white matter, may occur via voltage-dependent blockage of ion flux through the glutamatergic NMDA receptor. Oligodendrocytes express several subunits of the NMDA receptor.\(^30,31\) Therefore, magnesium could contribute to white matter integrity by blocking NMDA receptors involved in excitotoxic damage. Second, magnesium inhibits the sodium–calcium ion exchanger, a major mediator of calcium influx in ischemic axons.\(^9\) Third, and more speculatively, magnesium may inhibit the transient receptor potential melastatin TRPM7 channels\(^32\) that are linked to delayed calcium influx in neurons and therefore excitotoxic damage independent of the early action of glutamate on NMDA receptors.\(^33\) However, this hypothesis requires further investigation to confirm the presence of transient receptor potential melastatin receptors in white matter and the inhibitory effect of magnesium on these receptors in vivo.

A beneficial effect of magnesium via an effect on gray matter could also be involved, because axotomy at the level of the IC induces death of nearly 50% of corticospinal neurons within the first week.\(^34\) In the brain, 80% of intracellular magnesium is bound to ATP, and magnesium is an essential cofactor in the activation of ATPases and is consequently involved in energy-dependent pathways and protein synthesis.\(^28\) Because magnesium is involved in ATP regeneration after ischemia, its cerebroprotective action may be attributed to maintaining ATP levels during periods of relative hypoxia.\(^35\) Therefore, the beneficial effect of magnesium could extend to an influence on neuronal survival via a direct effect on the neuronal cell body in the cortex.

In conclusion, stereotactic injection of ET-1 into the IC of SHR resulted in a small, reproducible white matter lesion and a measurable motor deficit in the contralateral forelimb and hindlimb. In this model of cardiovascular risk, systemic magnesium administration induced modest hypotension with no change in infarct size and an improvement in motor deficit, supporting further research on the potential utility of magnesium for the treatment of lacunar infarct.

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**Disclosures**

None.

**References**


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