Improved motor outcome in response to magnesium therapy received up to 24 hours after traumatic diffuse axonal brain injury in rats

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Object. The goal of this study was to establish the therapeutic window during which delayed therapy with MgSO_4_ improves neurological motor outcome in rats that have suffered severe traumatic axonal brain injury.

Methods. Severe brain injury was induced in male Sprague–Dawley rats by using the impact–acceleration model of severe traumatic diffuse axonal brain injury. Injured animals were subsequently treated with MgSO_4_ (750 μmol/kg) infused intramuscularly at 30 minutes or at 8, 12, or 24 hours after trauma and were tested for neurological motor outcome during the following week by using the rotarod test. Injured untreated (control) animals demonstrated highly significant (p < 0.001) neurological motor deficits that were sustained over the 1-week assessment period. Animals treated with MgSO_4_ at 30 minutes or at 8 or 12 hours postinjury demonstrated significantly improved motor outcomes compared with untreated control animals at all time points (0.001 < p < 0.05). Animals treated with MgSO_4_ at 24 hours had motor scores that were similar to those of untreated control animals early in the week, but demonstrated a significantly more rapid recovery in function and, by the end of the assessment period, they demonstrated significantly improved motor scores (p < 0.01). Repeated administration of MgSO_4_ over the 1-week observation period did not further improve outcome.

Conclusions. The present results demonstrate that Mg^{2+} plays a neuroprotective role following severe diffuse traumatic axonal brain injury. Moreover, Mg^{2+} therapy significantly improved motor outcome when administered up to 24 hours after injury, with early treatments providing the most significant benefit. Repeated administration beyond 24 hours postinjury did not provide additional neuroprotection.

Key Words • neurotrauma • magnesium sulfate • brain injury • treatment evaluation • therapeutic window • rat

TRAUMATIC injury to the central nervous system is known to result in neurological deficits through both primary and secondary injury mechanisms. Primary mechanisms include those mechanical events that occur at the time of the traumatic insult such as axonal shearing, tearing, and stretching, whereas secondary mechanisms are those that occur at later time points and include biochemical and physiological events such as neurotransmitter release, ion changes, lactic acidosis, energy failure, and edema formation, among others. Although many of these injury mechanisms have been characterized using focal models of experimental brain injury, recent studies in which experimental models of diffuse axonal brain injury^14,22_ have been used have confirmed that these mechanisms are also common to the process of diffuse axonal injury (DAI). Indeed, DAI in itself is thought to be one of the secondary injury factors that result in irreversible tissue damage with consequent neurological deficits.8,31

Diffuse axonal injury occurs in more than 80% of all cases of brain injury as a result of motor vehicle accidents.1 When considering all forms of traumatic brain injury (TBI), 60% of all severe brain injuries and 30% of all moderate brain injuries demonstrate significant DAI.28 Moreover, there is a clear association between the severity of the DAI and high incidences of morbidity, mortality, and development of a persistent vegetative state following TBI.10,23,31 Despite this, few investigators have characterized the metabolic events associated with development of DAI or developed therapies that have a demonstrated potential to improve outcome following brain trauma that is primarily composed of DAI. In part, this is because of the absence of a suitable experimental model of DAI. However, the recent development of a rodent impact–acceleration model of DAI has now permitted such studies to occur.8,14,23

Metabolic, histological, and neurological characterizations of impact–acceleration brain injury in rats have shown that this form of DAI has many features in common with the focal models of brain injury.8,14,23 In particular, there is a significant and sustained decrease in free-Mg^{2+} concentration in the brain for up to 1 week after injury and the degree of deficit recorded on a daily basis is linearly correlated with the degree of neurological
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motor deficit. Moreover, treatment with either MgCl2 or MgSO4 has recently been shown to improve this outcome in a manner similar to that observed in previous studies in which focal injury models were used. The efficacy of Mg2+ as a neuroprotective agent has also been shown in other conditions such as spinal ischemia, brain ischemia, anoxia, and subarachnoid hemorrhage. Furthermore, with recent clinical evidence of Mg2+’s neuroprotection in stroke and, possibly, cerebral palsy, the agent has been entered into clinical trials for these conditions.

What has been lacking, however, in all of these studies, is the establishment of the therapeutic window for Mg2+ treatment. Given the critical nature of this limitation in any pharmacological intervention, such information is of vital importance not only for further studies of magnesium therapy in trauma, but also for the ongoing trials in stroke. Using the rodent impact–acceleration model of diffuse axonal brain injury, the present study uses the rotarod test of motor function to characterize the efficacy of MgSO4 administered at different time points postinjury. In addition, to determine whether sustained therapy would result in any further improvement in outcome over a single bolus injection, an additional group of animals was administered repeated doses of MgSO4 based on the concentration of the free-Mg2+ ion in blood posttrauma.

Materials and Methods

All experimental protocols were approved by the James Cook University Experimental Ethics committee according to guidelines established for the care and use of animals in experimental research, as outlined by the Australian National Health and Medical Research Council.

Animal Preparation and Induction of Injury

Injury was induced using the closed-head injury model of DAI, which has been described in detail elsewhere. Briefly, male Sprague–Dawley rats, weighing 350 to 450 g each, were given access to food and water ad libitum before anesthesia was induced by intraperitoneal administration of 60 mg/kg sodium pentobarbital. An arterial catheter was inserted into each rat tail and was used to monitor blood pressure and obtain blood samples. The animals were intubated and ventilated on room air (Harvard Rodent Ventilator; Harvard Apparatus, S. Natick, MA). Body temperature was maintained throughout the procedure with a thermostatically controlled heating pad set at a rectal temperature of 37°C.

After exposing the skull via a midline incision, a stainless steel disk measuring 10 mm in diameter and 3 mm in depth was cemented centrally along the coronal suture between the lambda and bregma by using a polyacrylamide adhesive. The animals were secured in the prone position on a 10-cm-deep foam bed and severe DAI was induced by dropping a 450-g brass weight from a distance of 2 m. The mechanics of the injury and the resultant pathophysiological changes have been described in detail elsewhere. The mortality rate associated with the use of this model was previously reported to be 18%. In the current study, the mortality rate was 16% and death generally occurred within the 1st hour after impact. These animals were eliminated from the study. Once the surviving animals were stabilized and the skin overlying the exposed skull was sutured, they were randomized into their treatment groups and, thereafter, returned to their cages and allowed to recover for 1 week of neurological motor testing.

Treatment Protocol

The animals were randomly assigned to four groups (six rats each) to receive an intramuscular bolus of MgSO4 (750 μmol/kg) at 30 minutes or 8, 12, or 24 hours after induction of severe TBI. The dose and route of administration of the MgSO4 were chosen on the basis of dose–response studies performed following TBI. A fifth group of six animals served as nontreated controls. In addition to characterizing the therapeutic window based on the effectiveness of delayed therapy, the study examined the effects of repeated administration of MgSO4 in a sixth group of six animals that received an initial 30-minute bolus of MgSO4 (750 μmol/kg) followed by additional doses every 12 hours after injury. Determination of the frequency of this repeated administration regimen was based on the serum free-Mg++ measurements described in Results.

Blood Ionized Magnesium

The aim of the repeated administration of MgSO4 was to maintain the blood free-Mg++ concentration at a higher level than preinjury values for a prolonged period of time. Accordingly, a free-Mg++ analyzer (model 98814; AVL Medical Instruments, Zurich, Switzerland) was used, as previously described, to determine blood free-Mg++ levels in both untreated animals and animals treated with MgSO4. The ion-selective Mg++ electrode is constructed using a neutral carrier with poor selectivity for CA++, such that any minor CA++ interference can be corrected for by a patented mathematical algorithm. The duration between the time at which the original bolus injection of MgSO4 was given and the point at which the blood free-Mg++ concentration had returned to preinjury levels was chosen as the interval to be used in the repeated administration regimen for MgSO4.

Neurological Assessment

The animals were assessed for neurological motor outcome using the rotarod test, as previously described in detail elsewhere. This test was found to be the most sensitive for detection of motor deficits following impact–acceleration-induced TBI. Briefly, scores were based on the animals’ performances on a rotarod device, which consisted of a motorized rotating assembly of 18 rods (1 mm in diameter) on which the animals were placed. To walk as the rods rotated beneath them, the animals were required to grip a rod, thus introducing a grip test component to the assessment. The rotational speed of the device was increased from 0 to 30 rpm in intervals of 3 rpm for 10-second periods. The duration in seconds and the revolutions-per-minute score were recorded at the point at which the animal either completed the 2-minute task, fell from the rods, or gripped the rods and spun for two consecutive revolutions rather than actively walking on the rungs. Animals were pretrained on the device for 1 week (two training sessions per day) before injury was induced. The mean value in seconds during this period was used as a preinjury baseline.

Statistical Analysis

All data are expressed as the mean ± standard error of the mean. Significance in rotarod scores was determined using repeated-measures analysis of variance followed by individual Student-Neuman-Keuls tests. A probability value less than 0.05 was considered significant.

Results

Following impact–acceleration-induced trauma, the control animals demonstrated a significant neurological motor deficit as assessed by the rotarod paradigm (Fig. 1). Before injury, the animals achieved a score of 108 ± 3 seconds. At 24 hours after trauma, these control animals achieved a rotarod score of 51 ± 4 seconds (p < 0.001), which, according to previously published results, is indicative of severe injury. Consistent with previous results, there was a tendency for the animals’ rotarod performances to improve over time following trauma. Nonetheless, this trend was not a statistically significant improvement and the posttraumatic rotarod score was al-
ways significantly less than the preinjury score throughout the entire assessment period.

In contrast, animals treated with MgSO₄ at 30 minutes after injury had a 24-hour rotarod score of 78 ± 5 seconds, which was significantly better than the scores recorded in the injured control rats (p < 0.01). Moreover, these MgSO₄-treated animals demonstrated a significant improvement in rotarod scores over the assessment period, such that by 4 days after injury, there was no significant difference between the postinjury (108 ± 4 seconds) and preinjury (113 ± 3 seconds) scores within this group (Fig. 1). At all time points after injury, there was a significant difference in rotarod scores between MgSO₄-treated and control animals. Similarly, in rats that received delayed treatment with MgSO₄ at 8 or 12 hours postrauma, rotarod scores were significantly better than those recorded in the control animals, although recovery to preinjury levels was not as rapid as that in the 30-minute MgSO₄ treatment group (Fig. 1). Differences in outcome could not be attributed to the cardiovascular effects of MgSO₄ administration. The mean arterial blood pressure in all animals had returned to approximately preinjury levels within 15 minutes after injury (103 ± 3 mm Hg), which is consistent with previous reports in this model. A subsequent intramuscular injection of MgSO₄ never resulted in more than an insignificant 5% change in this value.

The animals that were treated with MgSO₄ at 24 hours after severe impact–acceleration brain injury demonstrated a similar 24-hour rotarod score to that of control animals (52 ± 8 seconds). However, in contrast with the animals in the control group, the MgSO₄-treated animals demonstrated a more rapid recovery in rotarod performance during the 1-week assessment period than the control animals (Fig. 1). Indeed, by 7 days postinjury, the rotarod performance in the animals treated with MgSO₄ at 24 hours postinjury was significantly better (p < 0.01) than their performance at 24 hours, an improvement that was not observed in the control animals.

Clearly, early treatment with MgSO₄ resulted in a significant improvement in posttraumatic outcome following impact–acceleration-induced DAI. However, it was still unknown whether repeated administration of MgSO₄ would result in any further improvement in outcome when compared with a single bolus administration. To ascertain the treatment interval for such a repeated administration protocol, we measured blood free-Mg⁺⁺ concentration following injury (Fig. 2). Consistent with previous results, severe brain injury resulted in a small but significant decline (p < 0.05) from a normal preinjury level of 0.46 ± 0.03 mM to 0.35 ± 0.03 mM by 1 hour after trauma. This decline persisted for 2 days posttrauma. In contrast, animals administered an intramuscular bolus of MgSO₄ demonstrated an elevated blood free-Mg⁺⁺ concentration reaching a maximum of 1.1 mM at 2.5 hours after drug administration (Fig. 2). Thereafter, the blood free-Mg⁺⁺ level declined to preinjury levels by 12 hours posttrauma. Accordingly, we chose a 12-hour protocol for repeated administration of MgSO₄.

Following administration of the MgSO₄ dose given 30 minutes after trauma, intramuscular administration of MgSO₄ at 12-hour intervals over the 1-week posttraumatic assessment did not improve neurological motor outcome in rats when compared with the single bolus (30-minute)–treated animals (Fig. 3). Indeed, there was a tendency for the animals that received repeated treatment to perform slightly less effectively (approximately 7%) than their single-treatment counterparts. However, this trend was not statistically significant and there was still a highly significant improvement in outcome when compared with the control animals.

**Discussion**

The present study has demonstrated that administration
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Administration of MgSO4 at 8 and 12 hours after brain injury resulted in the most significant improvement marked the improvement. Administration at 30 minutes after trauma resulted in the most significant improvement in outcome compared with the control animals (p < 0.001), although the improvements demonstrated with administration at later time points. However, this is unlikely to be the case, given that Fuchs-Buder, et al.,9 have demonstrated at a delayed but sustained increase in Mg++ in cerebrospinal fluid Mg++ remained elevated despite the fact that the serum free-Mg++ returned to normal levels. Furthermore, in our own laboratory we have shown, by phosphorus magnetic resonance spectroscopy, that a single bolus injection of Mg++ enters the brain and restores brain free-Mg++ concentration for at least 24 hours after trauma.14 Thus, it appears that MgSO4 has no difficulty penetrating the blood-brain barrier after brain injury.

The fact that Mg++ treatment initiated up to 12 hours postrauma is the most effective for recovery from DAI is supported by recent results obtained in Mg++ treatment studies in both clinical stroke and myocardial infarction. In a small randomized pilot trial of MgSO4, in acute stroke, Muir and Lees20 noted that a doubling of serum Mg++ levels within the first 12 hours after stroke resulted in a decrease in the number of early deaths. The efficacy of the drug is now being investigated in the current Intravenous Magnesium Efficacy in Stroke (IMAGES) clinical trial coordinated by Muir and Lees at the Western Infirmary, Glasgow, Scotland. Similarly, early clinical results have suggested that Mg++ may lower the rates of both early and late mortality after myocardial infarction,20 a finding supported by the second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2), which found that doubling the serum Mg++ concentration by administering MgSO4 led to an improvement in patient survival.40 Our experimental results in trauma are consistent with these clinical findings. The dose used in the present study doubles the serum free-Mg++ concentration. Furthermore, this dose of Mg++ salt had to be given within 12 hours after the injury to achieve a statistically significant improvement when compared to control animals.

A number of previous experimental trauma studies have shown that Mg++ salts are neuroprotective following TBI,15,27,38,41 in some instances proving to be equal to, or better than, the N-methyl-d-aspartate (NMDA) antagonists.34,42 However, neuroprotective effects of Mg++ have also been demonstrated in a variety of other trauma-related pathological entities, both in vivo and in vitro. After ischemia, an increase in extracellular Mg++ concentration enhances recovery of hippocampal neuronal high energy phosphates6 and reduces infarction size to a degree that is at least equivalent to that of NMDA antagonists.25 Even when administered at 24 hours after forebrain ischemia, Mg++ has been shown to reduce CA1 hippocampal necrosis,26 Magnesium administered after subarachnoid hemorrhage has been shown to prevent cerebrovasospasm,32 and in rats with treatment with Mg++ before lesioning of the sensorimotor cortex resulted in a reduction in the initial magnitude of contralateral deficits as well as facilitated sensorimotor outcome.17 Moreover, such treatment prevented subcortical atrophy in the ipsilateral posterior striatum. Finally, Mg++ protects against anoxic damage in rat hippocampal slices.20 Thus, Mg++ may be effective not only in reducing deficits caused by traumatic axonal injury, but also other pathophysiological events associated with trauma such as anoxia, hemorrhage, ischemia, or focal lesions.

Although the mechanisms by which Mg++ is protective are unknown, several possibilities have been proposed. In addition to its well-known vasodilation and antithrombotic effects,2 there are a number of postulated cytoprotective mechanisms. These include effects on metabolism, partic-
ularly phosphorylation reactions that generate adenosine triphosphate, the functioning of Na+/K+ adenosine triphosphatase with subsequent effects on edema, membrane integrity and permeability, and effects on neurotransmitter release. However, it is the effect of the Mg++ ion on Ca++ ion flux that has received considerable attention in recent years. The Mg++ ion is known to be a natural antagonist of Ca++ channels in general and, in particular, to act as a voltage-dependent blocker of the NMDA channel. Indeed, a number of experimental TBI studies have demonstrated that postinjury treatment with NMDA channel blockers, including Mg++, limits excitotoxin-induced secondary neuronal damage. These authors demonstrate in vitro that the Mg++ block of the NMDA channel is reduced after neural injury and that this reduction may be linked to either a decline in Mg++ levels, or a change in the structure of the NMDA channel. Moreover, increasing Mg++ levels inhibits presynaptic excitatory amino acid release and provides neuroprotection.

Conclusions
The present in vivo findings support a neuroprotective role for Mg++ following severe diffuse TBI. In addition, the results have demonstrated for the first time that Mg++ therapy can be effective even when given several hours after the initial injury and that repeated administration beyond 24 hours postinjury does not necessarily provide additional neuroprotection.

References
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