Assessment of cognitive function following magnesium therapy in the traumatically injured brain

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Abstract. Many studies have examined the preclinical efficacy of Mg2+ therapy in models of traumatic brain injury. However, more of these studies have examined sensorimotor and motor performance than cognitive performance following injury. The present paper reviews the use of Mg2+ therapy to facilitate cognitive recovery in several models of cortical injury in the rodent. The first study examined the ability of daily injections of MgCl2 (1 or 2 mmol) to impair acquisition of a reference memory task in the Morris Water Maze. Additional studies examined the ability of MgCl2 to improve cognitive function following bilateral anterior medial cortex ablations, bilateral frontal cortex contusions, and unilateral frontal contusions. The results from these studies indicate that MgCl2 therapy is biologically active and readily crosses the blood-brain barrier because daily injections of MgCl2 impaired learning of a reference memory task in intact rats. Mg2+ therapy for brain injury revealed that administration of post-injury MgCl2 effectively improved recovery of cognitive deficits following injury. These results suggest that Mg2+ therapy is effective in facilitating cognitive recovery of function following brain injury; however, there are task and dose-dependent aspects to this recovery.

Key words: recovery of function, brain damage, magnesium, cognitive performance, MWM

The purpose of the present paper is to provide a review of the literature using Mg2+ therapy to improve cognitive function following traumatic brain injury (TBI). There is ample evidence that Mg2+ plays an important role in the pathophysiological processes following TBI and that Mg2+ therapy is effective in promoting functional recovery in a variety of animal models [1-3]. However, there is still a gap in the research on Mg2+ therapies between cognitive and motor/sensorimotor assessments following injury. Specifically, this paper will focus on a series of studies conducted in our laboratory that have investigated the ability of Mg2+ therapy to improve cognitive recovery following focal and traumatic brain injuries in the rat.

The importance of Mg2+ in normal cellular functioning has been well documented, as has its importance in the pathophysiology following injury. Recently, several reviews have been written that address these issues [1-3] so only a brief synopsis will be provided in this paper. Mg2+ is involved in many critical cellular processes including: cellular respiration, protein synthesis, membrane stability and regulation of vascular tone. McIntosh and colleagues have shown that CNS injury disrupted Mg2+ homeostasis [4, 5]. Fluid percussion brain injury (FPI) produces a rapid and severe decline in intra- and extracellular Mg2+ levels, which correlates significantly with the severity of the behavioral deficits observed following injury [4, 5]. A recent paper has reviewed the use of Mg2+ therapy following global and focal ischemia and relates these preclinical studies to the IMAGES and FASTMAG clinical stroke trials [6]. These authors conclude that one possible factor in the unsuccessful IMAGES trial was the fact that most of the successful preclinical studies failed to control
for hypothermia. The use of Mg\(^{2+}\) therapy in acoustic trauma, which has many similar pathophysiological mechanisms to TBI, has also recently been reviewed [7]. These findings suggest that Mg\(^{2+}\) plays an important role in normal physiology and in the pathophysiological events that occur following injury to the nervous system and that Mg\(^{2+}\) therapies are still being applied to various disease/injury states.

**Effects of Mg\(^{2+}\) therapy on cognitive function following injury**

There are four models of brain injury that have been used to study the effects of Mg\(^{2+}\) therapy on functional outcome. Of these models three of them induce a traumatic injury; cortical contusion injury (CCI) [8], diffuse axonal injury (DAI) [9], and FPI [10, 11]. The other model involves focal damage to specific regions of the cerebral cortex [12-14]. With these injury models it is possible to examine the recovery of function of both sensorimotor and cognitive behaviors. Normally the cognitive/spatial testing commences between 7 and 15 days post-injury. The Morris water maze (MWM) is a standard task for measuring cognitive/spatial performance. This task uses a water filled tank and an invisible escape platform and many different aspects of memory can be assessed [15, 16]. Briefly, a reference memory trial consists of placing the rat into the water at one of 4 randomly chosen start locations. A computer-assisted video tracking system is used to measure the swim latency and distance to the submerged escape platform. On trials designed to measure working memory the escape platform is relocated to a new position in the tank everyday. Additionally, the Barnes spatial maze, a dry version of the MWM can also be used to assess cognitive/spatial performance [9]. Rats are placed on an elevated 1.2 m circular table top with 19 holes cut around the periphery. The rat learns the location of the hole that leads to an escape tunnel. The latency to find the correct hole is then analyzed.

Several studies have investigated the ability of Mg\(^{2+}\) to improve acute cognitive function in animal models of TBI. It has been shown that administration of MgCl\(_2\) following FPI improved cognitive outcome by reducing memory loss following injury [17]. However, administration of MgCl\(_2\) failed to improve the acquisition of a reference memory task in the MWM following injury [18]. It has also recently been shown that administration of MgSO\(_4\) (250 μmol/kg, i.v.), 30 min following DAI improved recovery of a spatial memory task on the Barnes maze [9]. In addition, administration of an intravenous solution of MgCl\(_2\) (150 μmol) prior to FPI prevented the occurrence of injury-induced impairments in working and reference memory in a radial maze [19].

There has been very little attempt to examine the ability of Mg\(^{2+}\) therapy to resolve long-term cognitive dysfunction. A recent study has shown severe cognitive deficits in the acquisition of a reference memory task in the MWM, 8 months post-FPI [20]. The administration of MgSO\(_4\) (125 mmol, i.v.) or NPS 1506 (NMDA antagonist) failed to improve the acquisition of the reference memory task compared to vehicle controls. However, it was found that MgSO\(_4\) did reduce the amount of ipsilateral hippocampal cell loss; thus, preservation of the hippocampus failed to result in significant cognitive improvement [20]. Given the limited number of studies and their mixed results, it is important to further investigate the effect of Mg\(^{2+}\) therapy on the recovery of cognitive function following injury.

**Recent Laboratory Data**

**Mg\(^{2+}\) therapy and learning**

From a purely pharmacological standpoint, administration of Mg\(^{2+}\) prior to acquisition of learning should have a detrimental effect, given the NMDA antagonistic properties of Mg\(^{2+}\). The NMDA antagonists MK-801 and PCP have been shown to disrupt spatial learning in rodents [21-23]; however, in some cases a facilitative effect can be shown [24]. In order to examine the biological activity of Mg\(^{2+}\) administration on the acquisition of learning, we administered MgCl\(_2\) (1 μmol/kg or 2 μmol/kg, i.p.) prior to the acquisition of learning a reference memory task in the MWM. Intact rats were injected daily 30 min prior to running in the MWM, and tested for 5 consecutive days, 4 trials per day with an intertrial interval (ITI) of 15 min. In the reference memory version of the MWM the submerged escape platform stays in the same location for each trial. As can be seen in figure 1, the vehicle-treated (0.9% saline, i.p.) group showed steady acquisition of the task. In comparison, the MgCl\(_2\)-treated animals showed a more varied response. The initial acquisition of the 2 μmol group was slightly lengthened on day 1 (albeit not significantly) compared to the other groups. On day 4, both groups of MgCl\(_2\)-treated animals started to show a lengthening of their escape latencies compared to vehicle-treated animals. On days 4 and 5 the comparison of swim latencies between the 2 μmol and saline group was significantly different (p < 0.01).

Thus, it appears that daily dosing with the higher dose of MgCl\(_2\) produced a significant degree of
amnesia after 3 days. Unfortunately, testing was terminated after 5 days so it is unknown if this effect would have persisted or worsened with additional testing. It should be noted that, in general, the NMDA antagonists that work at the PCP site on the NMDA receptor (i.e., MK-801 and PCP) seem to have a greater amnesic effect than MgCl₂. However, this may raise some concerns for continued dosing regimens of Mg²⁺ therapy lasting more than a couple days. Thus, given these behavioral results it is clear that systemic injections of MgCl₂ do indeed exert behavioral effects in uninjured animals with an intact blood-brain barrier (BBB), and therefore give support to the ability of MgCl₂ to cross the BBB. This finding supports earlier research on this [25] and is an important conclusion, given the earlier view that Mg²⁺ may not actively cross the BBB because systemic elevations in serum Mg²⁺ levels did not correlate with elevations of cerebrospinal fluid or brain levels [26]. A recent clinical study found that 24 hours of induced hypermagnesemia (MgSO₄, i.v.) only marginally, but significantly, increased total and ionized Mg²⁺ levels in cerebrospinal fluid [27]. These authors concluded that cerebrospinal fluid levels of Mg²⁺ were maintained following acute brain injury and thus limited the bioavailability of the MgSO₄ treatments [27].

**Examination of Mg²⁺ therapy following focal injury**

The initial studies from our laboratory investigated the ability of Mg²⁺ therapy to improve functional recovery of sensorimotor behavior using an electrolytic lesion model of cortical injury [12, 13, 28, 29]. However, we were also interested in examining cognitive function and used a bilateral focal ablation model to produce cognitive impairments. Rats were given small (4 mm²) electrolytic lesions aimed at the bilateral anterior medial cortex (bAMC) of the frontal lobe [14]. Administration of Mg²⁺ therapy occurred 15 min following injury with rats receiving either injections of MgCl₂ (1 or 2 mmol/kg, i.p.) or saline (1 mL/kg). This regimen was repeated again.
24 and 72 h later, so that each rat received 3 injections within the first 72 h following injury. Behavioral testing began 5 days after injury and included the assessment of cognitive function. The MWM was used to investigate the acquisition of reference and working memory. In addition, the MWM tank was also used to examine spatial ability using a delayed matching-to-sample (DMTS), a very sensitive measure of spatial working memory.

As can be seen in figure 2, the results of the behavioral testing indicated that bAMC lesions produced severe deficits in cognitive function on both the reference and working memory tasks in the MWM. Mg2+ therapy with either the 1 or 2 mmol dose did not significantly facilitate the acquisition of reference memory in the MWM; however, the mean swim latencies for both MgCl2 groups were greatly improved compared to saline in the last 2 blocks of testing. Although Mg2+ therapy did not have any statistically significant improvement on reference memory in the present study, it did improve working memory performance in the MWM. Administration of the 2 mmol dose of MgCl2 significantly reduced the number of trials needed to reach a criterion of 80% correct choices compared to saline-treated rats, while the 1 mmol dose of MgCl2 did not, although the number of trials was greatly reduced.

Given these significant results, it was concluded that bAMC lesions produced severe impairments in reference and working memory in the MWM. Mg2+ therapy had a dose-dependent effect on cognitive recovery following bAMC lesions. Administration of the 2 mmol/kg, high dose, resulted in the most facilitative effects. The low dose, 1 mmol/kg, also improved working memory, just not to the same degree as the high dose. Thus, Mg2+ therapy was effective in a task and dose-dependent manner.

Mg2+ therapy in the traumatically injured brain

Most of the previous work investigating the ability of Mg2+ therapy to improve cognitive function utilized the FPI or DAI models of TBI. To examine the ability of Mg2+ therapy following TBI, groups of rats were...
prepared with CCI or sham surgeries and then assigned to either MgCl₂ (1.0 mmol/kg, i.p.) or saline treatment conditions [8]. Mg²⁺ therapy was administered 15 min and 24 hr following injury. Following injury, rats were examined for cognitive/spatial performance in the MWM, investigating the acquisition of reference and working memory. Administration of MgCl₂ following CCI significantly reduced some of the behavioral impairments observed following injury (figure 3). The acquisition of reference memory in the MWM was significantly improved compared to saline-treated rats. In contrast, MgCl₂ did not improve working memory performance. Following the completion of testing the lesion analysis revealed that administration of MgCl₂ did not significantly reduce lesion size compared to saline-treatment.

In a second TBI study, we examined the ability of Mg²⁺ therapy to improve cognitive/spatial performance following unilateral CCI. Groups of rats were given unilateral CCIs or sham surgeries of the left sensorimotor motor/frontal cortex. One hr following injury rats were administered 1 mmol/kg MgCl₂ or saline. Rats were tested for their ability to acquire a reference memory task in the MWM on 4 consecutive days (4 trials/day) starting 11 days after CCI. Their working memory performance was measured on days 16 and 17. It was found that the single 1 mmol/kg dose of MgCl₂ effectively facilitated the acquisition of the reference memory task compared to treatment with saline (figure 4). In a similar manner, the working memory performance was greatly enhanced following CCI in the Mg²⁺-treated rats compared to the saline-treated rats. In fact, the working memory performance of the Mg²⁺-treated rats could not be distinguished from the sham controls on either day of working memory testing (see working memory graph in figure 4). Mg²⁺ treatment appeared to have prevented the occurrence of the working memory deficit following unilateral frontal injury. Although this is a striking effect, we have also seen a similar effect with nicotinamide treatment following TBI [30, 31].

**Discussion**

The results of these studies have demonstrated a wide range of activities for Mg²⁺ therapy and cogni-
tive function. It was first shown that daily injections of MgCl₂ prior to the acquisition of a learning task blocked the acquisition of a reference memory task. This study showed the biological activity that Mg²⁺ therapy has following systemic administration. Mg²⁺ therapy incorporating MgCl₂ in several models of cortical ablation and TBI was shown to have positive effects on cognitive recovery. However, this effect occurred in a task and dose-dependent manner. Following focal ablation of the bAMC, MgCl₂ improved working memory performance on several measures and slightly improved reference memory performance. Our CCI studies have shown that MgCl₂ administration following injury improved cognitive performance in a task-dependent manner.

Previous studies that have examined the ability of Mg²⁺ therapy to improve cognitive performance following injury have shown mixed results. For example, administration of MgCl₂ following FPI has been shown to improve cognitive outcome by reducing memory loss in the MWM [17], but administration of MgCl₂ failed to improve the acquisition of a reference memory task in the Morris water maze (MWM) following injury [18]. Thus, in a similar manner the current series of studies has shown similar mixed results. That is, significant effects were seen in some cases and non-significant effects in others. However, in general we saw significant improvements in cognitive function by MgCl₂ in each of our studies. The discrepant results mainly varied based on dose and task-dependent properties of the studies.

Currently, the efficacy of Mg²⁺ treatment following TBI has been established in a variety of animal models [4, 10, 12, 13, 17, 29, 32–38]. Although the efficacy of acute preclinical Mg²⁺ administration is no longer in question there is evidence that long-term improvement may be difficult to obtain [20]. This is a critical issue that needs more experimental investigation especially in light of the recently failed double-blind MgSO₄ clinical trial in TBI patients [39].

From a mechanistic standpoint, Mg²⁺ therapy has multiple routes by which it can disrupt the pathophysiological processes that occur following injury. In addition to offsetting injury-induced Mg²⁺ depletion [4, 5] and preventing excitotoxic neuronal death [40] mediated by the NMDA receptor, Mg²⁺ has been shown to have several other effects. For instance, administration of MgSO₄ has been shown to limit the generation of injury-induced edema following closed-head injury [32]. Administration of MgCl₂ has also been shown to reduce the expression of p53 mRNA, a gene associated with the induction of cell death, following lateral FPI [41]. In this study it was found that 750 μmol/kg of MgCl₂ reduced the expres-
sion of p53 mRNA in the injured cortex compared to saline-treated controls [41]. High concentrations of Mg2+ (~3 mM) have been shown to inhibit lipid peroxidation [42]. Recently it has been shown that MgSO4 (30 mg/kg) reduced aquaporin-4 immunoreactivity, thus contributing to edema reduction following injury [43]. Regardless of the mechanisms of action, the data presented in this review has shown that MgCl2 has strong biological activity, appears to cross the BBB, and can improve cognitive performance following cortical ablation or TBI.

Conclusion

The studies presented in this current review have demonstrated a wide range of activities for Mg2+ therapy in relationship to cognitive function in the rat. Daily injections of MgCl2 prior to the acquisition of a learning task blocked the acquisition of a reference memory task. Following focal ablation of the bAMC, Mg2+ therapy improved working memory performance on several measures. In our CCI studies it has been shown that MgCl2 administration improved performance on some tasks, but not on others. Although MgCl2 had some dose and task dependent properties, in general, it did significantly improve cognitive outcome following injury.

References


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