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Magnesium protects against neurological deficit after brain injury

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The biochemical factors that mediate secondary or delayed damage to the central nervous system (CNS) remain speculative. We have recently demonstrated that brain injury in rats causes a rapid decline in brain intracellular free magnesium (Mg^{2+}) and total magnesium concentrations that is significantly correlated with the severity of injury. In order to further investigate the relationship between Mg^{2+} and brain injury, we examined the effect of Mg^{2+} treatment on posttraumatic neurological outcome following fluid-percussion brain injury (2.0 atm) in rats. Since administration of ATP-$MgCl_2$ has been shown to be beneficial in a variety of models of organ ischemia, we also examined the efficacy of ATP-$MgCl_2$ or ATP alone in the treatment of experimental brain injury. Animals treated with low (12.5 μmol) or high (125 μmol) dose $MgCl_2$ at 30 min postinjury showed a significant dose-dependent improvement in neurological function when compared to saline-treated controls. Treatment with ATP-$MgCl_2$ (12.5 μmol) or ATP alone (12.5 μmol) caused no significant improvement in chronic neurological outcome. $MgCl_2$-treated animals showed no change in postinjury mean arterial blood pressure (MAP), whereas animals treated with either ATP-$MgCl_2$ or ATP alone showed a transient but significant fall in MAP ($P < 0.01$) during the drug-infusion period. Our results suggest that postinjury treatment with $MgCl_2$ is effective in limiting the extent of neurological dysfunction following experimental traumatic brain injury in the rat.

INTRODUCTION

Neuronal damage resulting from traumatic injury to the central nervous system (CNS) often involves perturbations of cellular energy metabolism[38,40]. Alterations in mitochondrial metabolism have been documented following brain injury[33], particularly under conditions where cerebral blood flow (CBF) is reduced below critical threshold levels. Using phosphorous (^{31}P) nuclear magnetic resonance spectroscopy (MRS), we have shown that a reduction in mitochondrial oxidative capacity occurs following fluid-percussion traumatic brain injury in the rat, as indicated by a reduced posttraumatic phosphocreatine/inorganic phosphate (P_{Cr}/P_i) ratio[38]. At this level of injury, CBF does not decline sufficiently to induce permanent ischemic changes[42]. Furthermore, in these studies, a prolonged (up to 8 h postinjury) fall in P_{Cr}/P_i ratio occurred in the absence of ATP depletion, acidosis, hypoxia or ischemia[38], suggesting that posttraumatic abnormalities of cerebral energy metabolism and mitochondrial dysfunction may be involved in the development of irreversible tissue injury.

One factor that may be responsible for the alterations in cerebral energy metabolism and inhibition of mitochondrial function following traumatic brain injury is the magnesium ion (Mg^{2+}). Using [^{31}P]MRS, we have demonstrated that intracellular free and total magnesium levels fall markedly after fluid-percussion brain injury in rats[39]. Moreover, this decline in brain magnesium was significantly correlated with the severity of tissue injury as reflected by neurobehavioral measures. Mg^{2+} is essential for membrane integrity, ATPase function and the synthesis of cofactors such as CoA and thiamine pyrophosphate[1] in addition to serving as an important regulator of glycolysis and the Krebs cycle[11,14]. Because the fall in intracellular magnesium after brain injury may be dele-

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terious to these basic cell functions and bioenergetic processes, thereby contributing to secondary injury, we speculated that postinjury treatment with magnesium might improve outcome after brain injury. In the present study, we have investigated whether treatment with $MgCl_2$ following traumatic brain injury in rats improves posttraumatic neurological outcome. Furthermore, since Chaudry and colleagues have shown that administration of ATP-$MgCl_2$ is beneficial in the treatment of hepatic[6,25] and renal ischemia[36], we also compared the therapeutic effectiveness of $MgCl_2$ vs ATP-$MgCl_2$ treatment following experimental brain injury.

MATERIALS AND METHODS

Surgical preparation

Sixty male, Sprague–Dawley rats (400–500 g) were initially anesthetized with sodium pentobarbital (60 mg/kg, i.p.). During surgical preparation and throughout the experiment, all wounds were infused with lidocaine hydrochloride, 2.0%. Femoral venous catheters (for drug administration) were inserted. A caudal arterial catheter was inserted for blood pressure monitoring. With the animal in a stereotaxic frame, the scalp and temporal muscle were reflected, and a 2.0-mm hollow female Leur-Loc fitting (used to induce trauma) was rigidly fixed with dental cement to the animal's skull in a craniotomy centered over the left parietal cortex, 5 mm anterior to lambda, 5 mm posterior to bregma, and 4 mm lateral to the sagittal suture. The dura was left intact at this opening. Stainless steel screw electrodes were inserted into the skull over the right and left parietal cortices (recording electrodes) and the anterior nasal bone (reference electrode) to record electroencephalographic (EEG) tracings.

Fluid-percussion injury (FP)

The fluid-percussion device used to produce experimental brain injury has been described by us in greater detail elsewhere[20]. Briefly, the device is a Plexiglas cylindrical reservoir, 60 cm long and 4.5 cm in diameter, bounded at one end by a Plexiglas, cork-covered piston mounted on O-rings. The opposite end of the reservoir is fitted with a 2-cm long metal housing on which a transducer (Gould, Inc.) is mounted and connected to a 5-mm tube (2-mm inner diameter) that terminates with a male Leur-Loc fitting. At the time of injury, the tube was connected to the female Leur-Loc fitting that had been chronically implanted over the exposed left parietal cortex of the rat. After the entire system was filled with 37 °C isotonic saline, injury was induced by a metal pendulum that struck the piston of the device from a predetermined height. The device produces a pulse of increased intracranial pressure (ICP) of 21–23 ms duration by injecting varying volumes of saline into the closed cranial cavity. The magnitude of injury is regulated by varying the height of the pendulum, which results in corresponding variations in ICP pulses expressed in atmospheres (atm). These pressure pulses are measured extracranially by a transducer housed in the injury device, recorded on a storage oscilloscope and photographed with a Polaroid camera.

Physiological monitoring

Systolic, diastolic and mean arterial pressure (MAP) were recorded continuously via the caudal arterial catheter. Pressures were monitored by strain gauge transducers, the output of which was recorded on a Narco Biotrace-40 physiograph. The EEG recording electrodes were connected to a Siegen Neuroscope Computerized Spectral EEG Analyzer (Siegen, Palo Alto, CA) in order to obtain continuous pre- and postinjury fast Fourier-transformed (FFT) spectral EEG recordings, including compressed spectral array (CSA), spectral histogram in the σ (0–4 Hz), θ (4–8 Hz), α (8–13 Hz) and β (13–30 Hz) range, and frequency/amplitude (power band) analysis.

Experimental protocol

Immediately following surgical preparation (1.5 h following initial injection of pentobarbital), a constant i.v. infusion of sodium pentobarbital (15 mg/kg/h) was begun. All animals were endotracheally intubated using an angiocath stylet and ventilated with room air using a Harvard rodent ventilator (Harvard, Braintree, MA). During a 2-h baseline period, MAP and EEG were continuously recorded. At the end of the 2-h baseline period, animals were attached to the FP device and injured at an injury level of 2.0–2.2 atm. At $t = 30$ min postinjury, each animal was randomly assigned to treatment with infusion of either (1) magnesium chloride (12.5 μmol = low

dose, n = 10 animals), (2) magnesium chloride (125 μmol = high dose, n = 10 animals), (3) ATP-$MgCl_2$ complex (12.5 μmol of each, n = 10 animals), (4) ATP alone (12.5 μmol, n = 10 animals) or (5) equal volume saline (n = 10 animals). The doses of $MgCl_2$ and ATP-$MgCl_2$ were chosen based on previous work by Chaudry and colleagues, demonstrating efficacy in various models of shock[4], and hepatic and renal ischemia[6,25,36]. Since bolus or rapid injection of magnesium salts or magnesium-ATP is known to have profound cardiodepressant effects, constant infusions of all drugs were performed over a 15-min interval at a rate of 0.25 ml/15 min (see ref. 4). MAP and EEG were monitored continuously for 2 h postinjury. At t = 2 h, i.v. pentobarbital infusion was turned off, animals were weaned off the ventilator, extubated, returned to their home cages and allowed to recover for chronic (4-week) neurological scoring. Ten additional animals were identically prepared but were not injured and served as sham controls.

Neurological evaluation

Chronic postinjury neurological scoring of motor function was performed daily for 4 weeks in all animals beginning at 24 h after injury using a previously described protocol[20]. Neurologic function was evaluated by a trained observer who was unaware of each animal's treatment, using an ordinal scale. Animals were graded from 4 (normal) to 0 (severely impaired) for each of the following 6 indices: (a) forelimb flexion upon suspension by the tail; (b) decreased resistance to lateral pulsion; (c) circling behavior upon spontaneous ambulation; (d) ability to stand on an inclined plane with the maximal angle at which the animal can stand for 5 s recorded (angle board) where 45–50° = 4, 40–45° = 3, 35–40° = 2, 30–35° = 1, 0–29° = 0; (e) the ability to remain on a narrow wooden balance beam (2-cm wide) using 1–4 paws, tail or paws plus tail within a 30-s time period ('grip' test) where 30 s = 4, 25–30 s = 3, 20–25 s = 2, 10–20 s = 1 and 0–10 s = 0; and (f) exploratory behavior in a computerized Opto-Varimex activity chamber (Columbus Instruments, Columbus, OH). A total composite functional neurologic score (0–24) was obtained by combining the scores for the several tests of motor function so that 24 = normal, 20–23 = slightly impaired, 10–20 = moderately impaired, 0–10 = severely impaired. These neurologic scores have been previously shown to be directly correlated with the severity of brain injury[20].

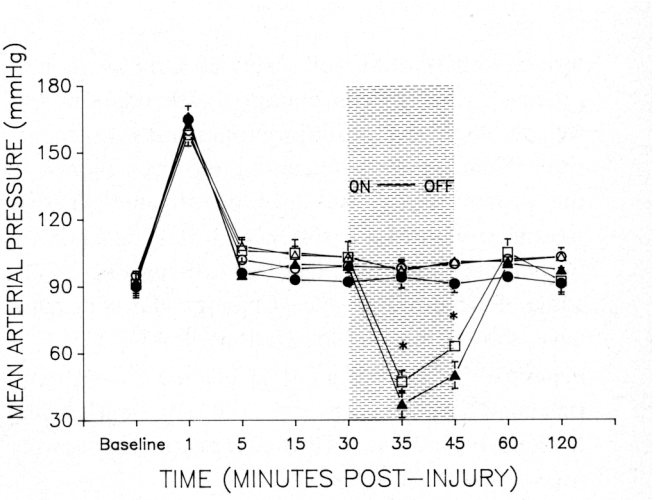

Fig. 1. Mean arterial blood pressure (MAP) following fluid-percussion brain injury and treatment at 30 min postinjury with either $MgCl_2$ (○, 12.5 μmol, n = 10); $MgCl_2$ (△, 125 μmol, n = 10); ATP-$MgCl_2$ (▲, 12.5 μmol, n = 10); ATP (□, 12.5 μmol, n = 10) or saline (●, equal volume, n = 10). *, $P < 0.05$ when compared to other treatment groups and predrug values.

Data analysis

Continuous variables compared across groups were examined utilizing analysis of variance (ANOVA) followed by Student-Newman-Keuls tests. Continuous variables subjected to repeated measurements over time (e.g. cardiovascular measurements) were subjected to repeated measurements ANOVA followed by Dunnett's tests at each time point. Ordinal measurements (neurological scores) were evaluated utilizing the non-parametric Kruskall–Wallis ANOVA and individual Mann–Whitney U-tests. Survival differences were compared using Fisher's Exact Probability Test. A P value <0.05 was considered statistically significant. All data are shown as mean ± 1 S.E.M.

RESULTS

Cardiovascular variables

Fluid-percussion brain injury produced a significant hypertensive response in all animals (from baseline of 96 ± 3 to 161 ± 3 mm Hg at 1 min postinjury; $P < 0.05$); blood pressure returned to baseline by 5 min (Fig. 1). By 30 min postinjury (immediately prior to drug administration), MAP values for all animals

were identical to baseline MAP values (mean = 97 ± 4 mm Hg). The infusion of $MgCl_2$ (at either dose) had no significant effect on MAP during the 15 min of constant infusion (Fig. 1). However, infusion of either ATP-$MgCl_2$ or ATP alone caused a significant fall in MAP during the infusion period (see Fig. 1) when compared to the other treatment groups. MAP returned to normal levels within 5 min following cessation of constant infusion of ATP-$MgCl_2$ or ATP alone. By 2 h postinjury, animals treated with both low- and high-dose magnesium showed a small but significant elevation in MAP ($P < 0.05$). ATP-$MgCl_2$ or ATP-treated animals had MAP values that were identical to those of controls over the remaining experimental period (Fig. 1).

Electrophysiological variables

In saline-treated animals, EEG amplitude (as measured by total power band analysis) in the injured hemisphere fell precipitously by 1 min postin-

TABLE I

EEG amplitude (power band analysis) following fluid-percussion brain injury and treatment at 30 min with saline, $MgCl_2$, ATP-$MgCl_2$ or ATP (n = 5/group)

Values are expressed as percentage of baseline values ($\mu V^2 \times 1/10$).

Treatment	*Hemisphere*	*Time (min) post-injury*					
		5	*10*	*15*	*30*	*60*	*120*
Saline	Left	8%	19%	30%	33%	37%	41%
	Right	20%	39%	48%	55%	58%	85%
$MgCl_2$ (low)	Left	10%	30%	34%	33%	49%	59%
	Right	26%	51%	52%	54%	68%	87%
$MgCl_2$ (high)	Left	21%	28%	32%	34%	51%	48%
	Right	32%	42%	47%	46%	56%	76%
ATP-$MgCl_2$	Left	19%	30%	33%	39%	62%	71%
	Right	20%	40%	49%	59%	75%	71%
ATP	Left	20%	27%	34%	35%	52%	63%
	Right	29%	48%	51%	57%	69%	83%

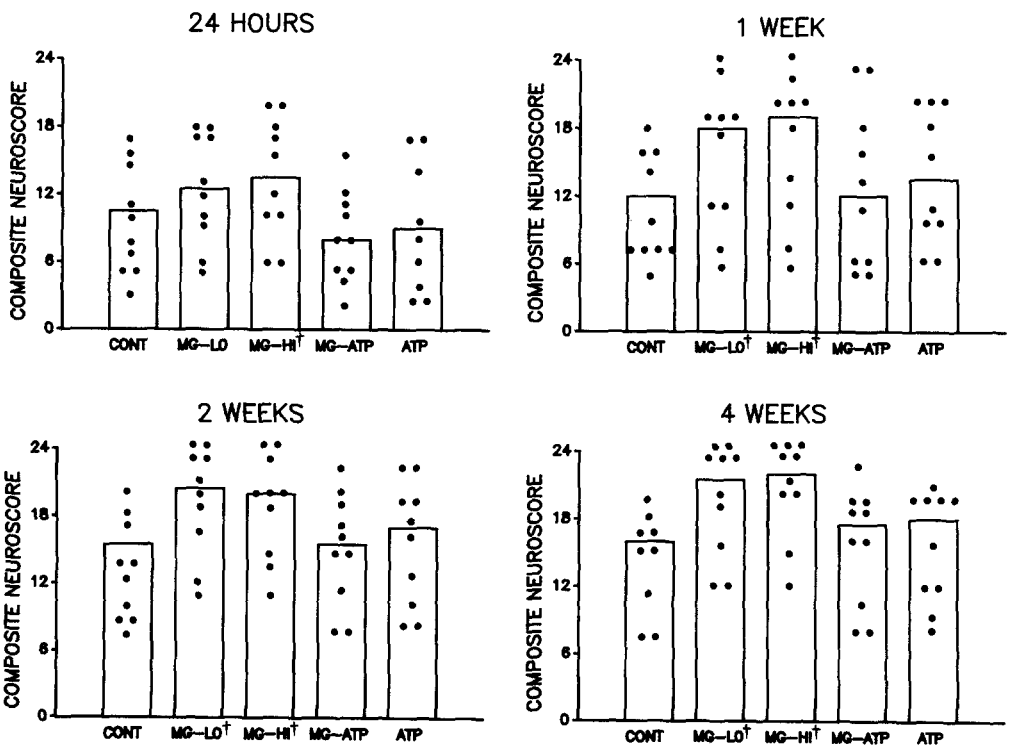

Fig. 2. Individual composite functional neurological scores obtained at 24 h–4 weeks following fluid-percussion brain injury, and treatment at 30 min postinjury with various pharmacological regimens. Cont = saline-treated control animals ($n = 10$); MG-LO = $MgCl_2$ (12.5 μmol, $n = 10$); MG-HI = $MgCl_2$ (125 μmol, $n = 10$); MG-ATP = ATP-$MgCl_2$ (12.5 μmol, $n = 10$); ATP = ATP (12.5 μmol, $n = 10$). †, $P < 0.05$ when compared to neurological scores from saline-treated controls.

jury to reach a nadir by 5 min (8% baseline; $P < 0.001$, Table I). Thereafter, EEG amplitude increased slowly but by 2 h postinjury had returned to only 41% baseline. EEG amplitude recorded from the contralateral hemisphere also fell significantly after injury but by 2 h had returned to 85% baseline. Treatment with $MgCl_2$, $MgCl_2$-ATP or ATP alone resulted in a slight but non-significant improvement in EEG amplitude in both injured and contralateral (uninjured) hemispheres by 30 min posttreatment (Table I). By 2 h postinjury, no significant differences were observed between treatment groups in EEG amplitude of the injured hemisphere.

Neurological outcome

At 24 h and 1 week postinjury, saline-treated animals exhibited a severe neurological impairment (median score = 10, Fig. 2). By 2 weeks postinjury, saline-treated animals showed some neurological improvement, but still exhibited a moderately severe neurological deficit (median score = 15.5) which persisted up to 4 weeks after injury. In saline-treated animals, both angle board recovery and open field activity ability showed the most marked impairment over the 4-week study period (Figs. 3 and 4). In contrast, postinjury treatment with $MgCl_2$ had a beneficial effect on neurological outcome. At 24 h postinjury, animals treated with low-dose $MgCl_2$ showed a small but non-significant improvement in composite neurological score (median score = 12.5), whereas animals treated with high-dose $MgCl_2$ showed a significant improvement in composite neurological score when compared to saline-treated controls (median score = 14 vs 10 for saline-treated animals; $P < 0.05$). By 1 week postinjury, animals treated with either low- or high-dose $MgCl_2$ showed significantly better neurological scores (median score = 18 and 19, respectively) than saline-treated animals (median score = 12) (Fig. 2). At 2 weeks postinjury, $MgCl_2$-treated animals manifested only slight neurological dysfunction; these animals remained only mildly impaired for the duration of the 4-week study. When

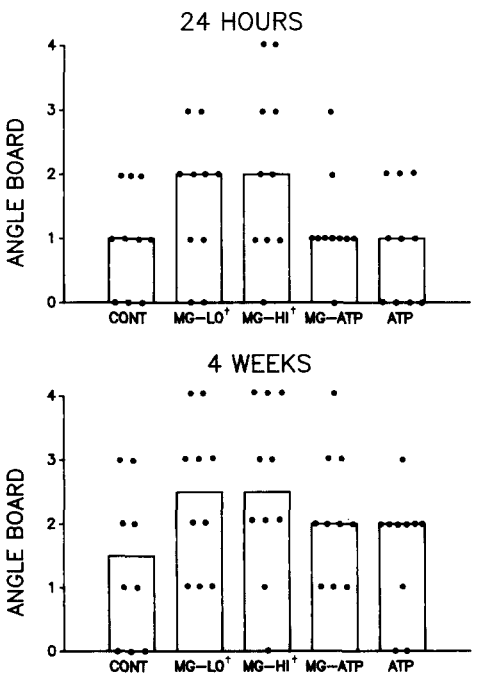

Fig. 3. Individual angle board (inclined plane) scores obtained at 24 h at 4 weeks following fluid-percussion brain injury and treatment at 30 min postinjury with various pharmacological regimens. Cont = saline-treated control animals ($n = 10$); MG-LO = $MgCl_2$ (12.5 μmol, $n = 10$); MG-HI = $MgCl_2$ (125 μmol, $n = 10$); MG-ATP = ATP-$MgCl_2$ (12.5 μmol, $n = 10$); ATP = ATP (12.5 μmol, $n = 10$). †, $P < 0.05$ when compared to neurological scores from saline-treated controls.

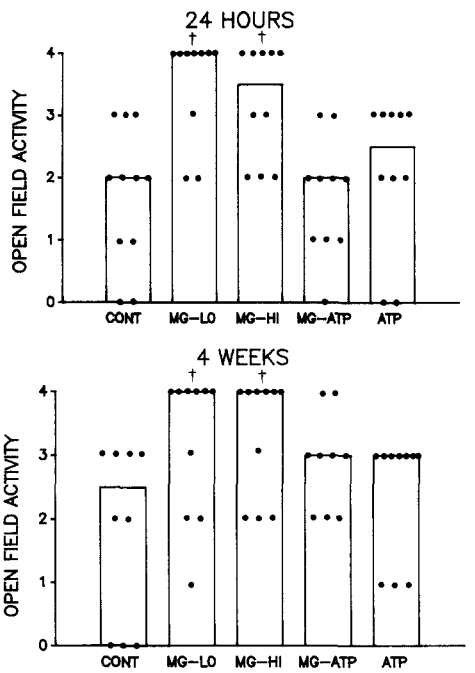

Fig. 4. Individual open-field activity scores measured at 24 h and 4 weeks following fluid-percussion brain injury and treatment. Cont = saline-treated control animals ($n = 10$); MG-LO = $MgCl_2$ (12.5 μmol, $n = 10$); MG-HI = $MgCl_2$ (12.5 μmol, $n = 10$); MG-ATP = ATP-$MgCl_2$ (12.5 μmol, $n = 10$); ATP = ATP (12.5 μmol, $n = 10$). †, $P < 0.05$ when compared to neurological scores from saline-treated controls.

neuroscore tests were analyzed individually, treatment with $MgCl_2$ at both doses had the greatest effect on improving angle board recovery and open-field activity scores (Figs. 3 and 4). $MgCl_2$ treatment caused a significant improvement in both angle board and open-field activity scores at 24 h postinjury ($P < 0.05$) that persisted over the 4-week study period.

Animals treated with ATP-$MgCl_2$ or ATP alone showed a slight improvement of neurological recovery beginning 2 weeks postinjury (Fig. 2). However, these neurological scores were not significantly better than those exhibited by saline-treated animals. When neuroscore tests were analyzed individually, treatment with ATP-$MgCl_2$ or ATP was found only to affect open-field activity: both ATP-$MgCl_2$ and ATP-treated animals showed significantly better activity scores ($P < 0.05$) at 1 week postinjury when compared to saline-treated controls; however, these differences had disappeared by 2 weeks.

Mortality

No animals died in any of the treatment groups following fluid-percussion brain injury over the 4-week postinjury observation period.

DISCUSSION

In the present study, treatment with $MgCl_2$ at 30 min following fluid-percussion traumatic brain injury significantly improved long-term neurological outcome. This finding complements previous observations that (a) a fall in cerebral intracellular free and total Mg^{2+} occurs following traumatic brain injury the extent of which is correlated with subsequent neurological deficit[39], (b) Mg^{2+}-deficiency will exacerbate posttraumatic neurological dysfunction and increase mortality in rats subjected to fluid-percussion injury[18], (c) prophylactic treatment with $MgCl_2$ can prevent the fall in intracellular free magnesium and improve neurologic recovery[19,39]. Although an acute decrease in both intracellular free and total magnesium could be due secondarily to a posttraumatic increase in both extracellular and intracellular water (edema), we have failed to observe any significant changes in tissue sodium or water content (wet wt./dry wt.) in the brain any time within the first 24 h postinjury in our fluid-percussion model of brain injury[10].

Treatment with $MgCl_2$ may improve neurological outcome following experimental brain injury for a variety of reasons. The magnesium ion is essential for a number of important cellular processes, including glycolysis[14], oxidative phosphorylation[11], protein synthesis, DNA and RNA aggregation[30], and the maintenance of plasma membrane integrity[1]. Since intracellular Mg^{2+} falls markedly after traumatic brain injury in the rat[39], postinjury treatment with $MgCl_2$ may serve to restore the functional ability of the cell to perform these processes. We have, in fact, found that pretreatment with $MgCl_2$ can prevent the decline of free intracellular Mg^{2+} observed after trauma[19,39].

The pathophysiological response to fluid-percussion mechanical brain injury is similar, in many ways, to that observed in a number of models of cerebral ischemia. We have observed both global and focal changes in cerebral blood flow in the acute postinjury period after fluid-percussion brain injury[42]. Alterations in localized pial circulation have also been observed and related to generation of free oxygen radicals associated with increased prostaglandin synthesis[41]. Our studies have also shown alterations in free fatty acids[10], lipid metabolism[10], and histopathological changes[8] that are reminiscent of those observed in models of focal cerebral ischemia.

Interestingly, increased calcium flux has been proposed as a final common pathway leading to cell death following both ischemic and traumatic injury to the CNS[27]. Since Mg^{2+} plays an important regulatory role in calcium transport and accumulation[1,11], alterations in intracellular Mg^{2+} could potentially exacerbate calcium-mediated neurotoxicity. In addition, previous studies have demonstrated that Mg^{2+} pretreatment protects against irreversible damage following spinal ischemia in rabbits[27,37] and will prevent cell death in cultured hippocampal neurons exposed to cyanide or anoxia[28]. These studies have suggested that Mg^{2+} may compete with calcium to prevent its uptake into cells, thereby preventing cell death from calcium entry. It is unlikely, however, that the effect of Mg^{2+} supplementation in the present study is due primarily to a blockade of calcium entry (rather than replacement of decreased endogenous levels) since: (1) calcium channel blockers do not appear to be effective in the treatment of brain injury[27]; and (2) brain-injured animals treated with Mg^{2+} still exhibit

abnormal neurons at the injury site that stain with Alazarin red, a histochemical marker for calcium accumulation (Noble and McIntosh, unpublished results).

Mg^{2+} also appears to play an important regulatory role in the gating of excitatory amino acid neurotransmitter channels within the brain[23]. This may be important since an increasing body of evidence suggests that excitatory amino acid neurotransmitters like glutamate and aspartate participate in tissue damage caused by cerebral ischemia[7,29,34] and brain injury[21]. Recent studies have demonstrated that excitatory amino acid A_1 receptors are modulated by local Mg^{2+} concentrations[12], and high concentrations of magnesium may inhibit presynaptic excitatory amino acid release[28]. Since excessive activation of excitatory amino acid receptors has been implicated in the onset of neuronal damage associated with cerebral ischemia[7,28], and treatment with excitatory amino acid receptor blockers has been shown to limit damage and improve outcome after cerebral ischemia[34], spinal injury[13] and brain injury[21], postinjury treatment with $MgCl_2$ may function to limit excitotoxin-induced secondary neuronal damage.

Both endogenous opioids and lipid hydrolysis/eicosanoid release have also recently been implicated in the pathogenesis of secondary tissue damage following traumatic brain injury[17,41]. Physiological concentrations of Mg^{2+} may affect opiate receptor binding in vivo[31]. Additionally, alterations in Mg^{2+} (Mg^{2+}-deficiency) has been shown to directly affect metabolism of arachidonic acid and prostanoid synthesis via a Ca^{2+}-dependent mechanism[24]. Therefore treatment with $MgCl_2$ after brain injury may also limit posttraumatic delayed injury via these mechanisms.

In addition to its role as biochemical modulator of cellular respiration, protein synthesis and neurotransmitter function, the magnesium ion plays an important role in the regulation of cerebral vascular tone[2]. Artificially lowering the Mg^{2+} content of cerebral blood vessels induces a rapid calcium-mediated contractile or vasospastic response in a variety of species (including rats, rabbits, dogs, pigs and humans), while raising Mg^{2+} titers will reduce spontaneous vascular tone in cerebral arteries and veins[2,32]. We have previously observed that regional CBF is markedly reduced in the area of greatest tissue injury up to 2 h following fluid-percussion brain injury in rats[42]. It is possible that in the present study, Mg^{2+} treatment would reduce cerebral vascular tone and prevent vasoconstriction or vasospasm that may occur as a result of an acute fall in cerebral Mg^{2+} concentrations following brain injury.

It is not clear to what extent postinjury treatment with $MgCl_2$ will elevate intracellular Mg^{2+} levels within the brain. Under normal conditions, the maintenance of constant CSF Mg^{2+} concentrations is exceedingly effective, since significant elevations in plasma Mg^{2+} result in CSF changes that are scarcely detectable[3,22]. Recent studies have suggested that the choroid plexus is involved in maintaining the constancy of CSF Mg^{2+} concentrations[26]. It is not known, however, to what extent an increase in plasma Mg^{2+} is reflected by increases in Mg^{2+} levels at other sites such as the walls of CNS vessels or interstitial fluid of brain parenchyma. Preliminary data from our laboratory suggests that postinjury treatment with $MgCl_2$ will elevate both plasma and brain intracellular free Mg^{2+} concentrations (Vink, unpublished data). A profound but brief disruption of the blood–brain barrier has been observed over the first several hours following fluid-percussion brain injury in rats[8,20]. This vascular disruption may allow enhanced Mg^{2+} and/or ATP-$MgCl_2$ entry into the CNS. Additionally, treatment of anoxic cells with ATP-$MgCl_2$ during reoxygenation may increase intracellular Mg^{2+} concentrations[15]. Finally, it has been demonstrated that although the plasma membrane is relatively impermeable to divalent cations such as Mg^{2+}, during anoxia or hypoxia the membrane will become increasingly permeable to these ions after reoxygenation[15].

In the present study, the efficacy of $MgCl_2$ was somewhat attenuated by addition of equimolar ATP. This effect may have been due to chelation of Mg^{2+} by the ATP and/or an inability of the ATP-$MgCl_2$ complex to cross the cell membrane. The similarity in effects of ATP and ATP-$MgCl_2$ treatments on outcome supports this notion. Alternatively, the severe hypotension induced by the systemic administration of the nucleotide may in itself potentiate the injury process. A decrease in blood pressure was not observed following $MgCl_2$ administration because of the slow infusion rate employed.

Chaudry and colleagues have shown that administration of combined ATP-$MgCl_2$ in vivo improves the

electrolyte balance and microcirculation of post-ischemic liver[25], improves cardiovascular function after myocardial ischemia[16], enhances postischemic renal function[36] and accelerates recovery of postanoxic cellular electrolyte homeostasis in isolated hepatocytes[15]. Other authors have also suggested that post-ischemic infusion of ATP-$MgCl_2$ complex enhances the recovery of cellular ATP levels by providing precursors for the resynthesis of cellular ATP[35]. However, it should be noted that in our model of brain injury at 2.0 atm, there is no depletion of intracellular ATP reserves[38]. Consequently, while ATP-$MgCl_2$ may be effective in conditions of energy depletion such as ischemia, it is unlikely that addition of ATP to magnesium treatment would have pronounced beneficial effects in our model of brain injury at the present level of injury. The present study suggests that post-injury treatment with magnesium salts alone is effective in limiting the extent of neurological motor dysfunction following brain injury. Although the effect of Mg^{2+}-infusion appears to be mediated by an increase in brain Mg^{2+} concentrations, future studies will address the mechanism of action of Mg^{2+} treatment in CNS injury.

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REFERENCES

1 Aikawa, J.K., *Magnesium: Its Biologic Significance,* CRC Press, Boca Raton, FL, 1981, pp. 21–29.
2 Altura, B.M., Altura, B.T., Gebrewold, A., Ising, H. and Gunther, T., Magnesium-deficient diets and microcirculatory changes in situ, *Science,* 223 (1984) 1315–1317.
3 Bradbury, M., *The Concept of a Blood-Brain-Barrier,* Wiley, New York, 1979, pp. 214–259.
4 Chaudry, I.H., Preparation of ATP-$MgCl_2$ and precautions for its use in the study and treatment of shock and ischemia, *Am. J. Physiol.,* 43 (1982) R604–R605.
5 Chaudry, I.H., Cellular mechanisms in shock and ischemia and their correction, *Am. J. Physiol.,* 245 (1983) R117–R134.
6 Chaudry, I.H., Clemens, M.G., Ohkawa, M., Schleck, S. and Baue, A., Restoration of hepatocellular function and blood flow following hepatic ischemia with ATP-$MgCl_2$, *Adv. Shock Res.,* 8 (1982) 177–186.
7 Choi, D.E., Ionic dependence of glutamate neurotoxicity, *J. Neurosci.,* 7 (1987) 369–379.
8 Cortez, S.C., McIntosh, T.K. and Noble, L.J., Experimental fluid-percussion brain injury: vascular disruption and neuronal and glial alterations, *Brain Research,* 482 (1989) 271–282.
9 Cserr, H.G., Physiology of the choroid plexus, *Physiol. Rev.,* 51 (1971) 273–311.
10 Demediuk, P., Faden, A.I., Romhanyi, R., Vink, R. and McIntosh, T.K., Traumatic brain injury in the rat: effects on lipid metabolism, tissue magnesium and water content, *J. Neurotrauma,* in press.
11 Ebel, H. and Gunther, T., Magnesium metabolism: a review, *J. Clin. Chem. Clin. Biochem.,* 18 (1980) 257–270.
12 Fagg, G., L-glutamate, excitatory amino acid receptors and brain function, *Trends Neurosci.,* (1985) 207–210.
13 Faden, A.I. and Simon, R.P., A potential role for excitotoxins in the pathophysiology of spinal cord injury, *Ann. Neurol.,* 23 (1988) 623–626.
14 Garfinkel, L. and Garfinkel, D., Magnesium regulation of the glycolytic pathway and the enzymes involved, *Magnesium,* 14 (1985) 60–72.
15 Hayashi, H., Chaudry, I.H., Clemens, M.G., Hall, M.J. and Baue, A.E., Reoxygenation injury in isolated hepatocytes: effect of extracellular ATP on cation homeostasis, *Am. J. Physiol.,* 63 (1986) R573–R579.
16 McDonough, P.F., Laks, H., Chaudry, I.H. and Baue, A.E., Improved mitochondrial recovery from ischemia with low dose ATP-$MgCl_2$, *Arch. Surg.,* 119 (1984) 1379–1389.
17 McIntosh, T.K., Hayes, R.L., DeWitt, D.S., Agura, V. and Faden, A.I., Endogenous opioids may mediate secondary damage after experimental brain injury, *Am. J. Physiol.,* 253 (1987) E347–E357.
18 McIntosh, T.K., Vink, R., Yamakami, I. and Faden, A.I., Changes in brain intracellular free magnesium influence neurological outcome after experimental brain injury, *Soc. Neurosci. Abstr.,* 13 (1987) 1501.
19 McIntosh, T.K., Vink, R., Weiner, M. and Faden, A., Alterations in free magnesium, high-energy phosphates and lactate following traumatic brain injury: assessment by nuclear magnetic resonance spectroscopy, *J. Cereb. Blood Flow Metab.,* 1 Suppl. (1987) S620.
20 McIntosh, T.K., Vink, R., Noble, L., Yamakami, I., Fernyak, S. and Faden, A.I., Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model, *Neuroscience,* in press.
21 McIntosh, T.K., Soares, H., Hayes, R. and Simon, R., The NMDA receptor antagonist MK-801 prevents edema and restores magnesium homeostasis after traumatic brain injury in rats. In J. Lehman (Ed.), *Recent Advances in Excitatory Amino Acid Research,* Alan R. Liss, New York, 1988.
22 Neuwelt, E., *Implications of the Blood-Brain-Barrier and*

its Manipulation, Plenum, New York, 1988, pp. 223–260.
23 Nowak, L., Bregestovski, P., Ascher, P., Herbelt, A. and Prochiantz, A., Magnesium gates glutamate-activated channels in mouse central neurones, *Nature (Lond.),* 307 (1984) 462–465.
24 Nigam, S., Averdunk, R. and Gunther, T., Alteration of prostanoid metabolism in rats with magnesium deficiency, *Prostag. Leukotr. Med.,* 23 (1986) 1–10.
25 Ohkawa, M., Clemens, M.G. and Chaudry, I.H., Studies in the mechanism of beneficial effects of ATP-$MgCl_2$ following hepatic ischemia, *Am. J. Physiol.,* 244 (1983) R695–R702.
26 Reed, D.J. and Yen, M.-H., The role of the cat choroid plexus in regulating cerebrospinal fluid magnesium, *J. Physiol. (Lond.),* 281 (1978) 477–485.
27 Robertson, C.S., Foltz, R., Grossman, R.G. and Goodman, J.C., Protection against experimental ischemic spinal cord injury, *J. Neurosurg.,* 64 (1986) 633–642.
28 Rothman, S., Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death, *J. Neurosci.,* 4 (1984) 1884–1891.
29 Rothman, S.M. and Olney, J.W., Glutamate and the pathophysiology of hypoxic-ischemic brain damage, *Ann. Neurol.,* 19 (1986) 105–111.
30 Rubin, H., Magnesium deprivation reproduces the co-ordinate effects of serum removal or cortisol addition on transport and metabolism in chick embryo fibroblasts, *J. Cell. Physiol.,* 89 (1976) 613–626.
31 Sadee, W., Pfeiffer, A. and Herz, A., Opiate receptors: multiple effects of metal ions, *J. Neurochem.,* 39 (1982) 659–667.
32 Seelig, J.M., Wei, E.P., Kontos, H.A., Choi, S.C. and Becker, D.P., Effect of changes in magnesium ion concentration on cat cerebral arterioles, *Am. J. Physiol.,* 43 (1983) H22–H26.
33 Siesjo, B.K. and Wieloch, T., Brain injury: neurochemical aspects. In: *Central Nervous System Trauma Status Report,* NINCDS publication, William Byrd Press, 1985, p. 260.
34 Simon, R.P., Swan, J.H., Griffiths, T. and Meldrum, B.S., Blockade of *N*-methyl-D-aspartate receptors may protect against ischemic damage in the brain, *Science,* 226 (1984) 850–852.
35 Stranski, M.E., Cooper, K., Thulin, G., Avison, M.J., Gaudio, K.M., Shulman, R.G. and Siegel, N.J., Postischemic ATP-$MgCl_2$ provides precursors for resynthesis of cellular ATP in rats, *Am. J. Physiol.,* 18 (1986) F834–F837.
36 Sumpio, B.E., Chaudry, I.H., Clemens, M.G. and Baue, A.E., Accelerated functional recovery of isolated rat kidney with ATP-$MgCl_2$ after warm ischemia, *Am. J. Physiol.,* 16 (1984) R1047–R1053.
37 Vacanti, F.X. and Ames, A., Mild hypotension and Mg^{++} protect against irreversible damage during CNS ischemia, *Stroke,* 15 (1984) 695–698.
38 Vink, R., McIntosh, T.K., Weiner, M.W. and Faden, A.I., Effects of traumatic brain injury on cerebral high-energy phosphates and pH: a ^{31}P NMR study, *J. Cereb. Blood Flow Metab.,* 7 (1987) 563–571.
39 Vink, R., McIntosh, T.K., Demediuk, P., Weiner, M. and Faden, A.I., Decline in intracellular free Mg^{2+} is associated with irreversible tissue injury following brain trauma, *J. Biol. Chem.,* 263 (1988) 757–761.
40 Wagner, K.R., Turnheim, P.A. and Eichhold, M.K., Acute changes in regional cerebral metabolite values following experimental rodent head injury, *J. Neurosurg.,* 63 (1985) 88–96.
41 Wei, E.P., Lamb, R.G. and Kontos, H.A., Increased phospholipase C activity after experimental brain injury, *J. Neurosurg.,* 56 (1982) 695–698.
42 Yamakami, I. and McIntosh, T., Effects of traumatic brain injury on regional cerebral blood flow in rats measured with multiple injections of radiolabeled microspheres, *J. Cereb. Blood Flow Metab.,* (1988), in press.