Magnesium Sulfate Therapy Is of Mild Benefit to Young Rats with Kaolin-Induced Hydrocephalus

OSAAMA H. KHAN, TERRY ENNO, AND MARC R. DEL BIGIO

Department of Pathology, University of Manitoba [M.R.D.B.] and Manitoba Institute for Child Health [O.K., T.E.], Winnipeg, Manitoba, Canada

ABSTRACT

Hydrocephalus causes damage to periventricular white matter at least in part through chronic ischemia. Magnesium sulfate (MgSO_4) has been shown to be protective in various models of neurologic injury. We hypothesized that this agent would ameliorate the effects of experimental childhood-onset hydrocephalus. Hydrocephalus was induced in 3- and 4-wk-old rats by injection of kaolin into the cisterna magna. Tests of cognitive and motor function were performed on a weekly basis. In a blinded and randomized manner, MgSO_4 was administered in two separate experiments (s.c. injection 0.85, 4.1, or 8.2 mM/kg/d), supplemented by osmotic minipump infusion (0.03 mM/d) to prevent low trough levels for 2 wk, beginning 2 wk after induction of hydrocephalus. The brains were then subjected to histopathological and biochemical analyses. With the 4.1 mM/kg/d dose, serum Mg^{++} levels were elevated transiently from 1.3 to ~7 mM/L. We observed statistically significant improvement in gait performance and reduced astroglial reaction. There was also a trend to improved memory performance, but no evidence

of increased myelin or synaptic protein content. The 8.2 mM/kg/d dose was associated with sedation and there was no evidence of improvement in any parameter. We conclude that MgSO_4 might be mildly protective in experimental hydrocephalus. (*Pediatr Res* 53: 970–976, 2003)

Abbreviations

Ca^{++} , calcium
CGaIT, ceramide galactosyltransferase
CSF, cerebrospinal fluid
GFAP, glial fibrillary protein
 Mg^{++} , magnesium ion
 MgSO_4 , magnesium sulfate
MR, magnetic resonance
MBP, myelin basic protein
NMDA, N-methyl-D-aspartate
PNPP, *p*-nitrophenylphosphorylcholine phosphocholine phosphodiesterase

Hydrocephalus is a common neurologic condition characterized by pathologic dilation of the cerebral ventricles. It is usually caused by obstruction of cerebrospinal fluid (CSF) flow. Axon damage in the periventricular white matter is one of the earliest pathologic consequences of ventricular dilation in humans and animals (1). The pathophysiology of hydrocephalus-induced brain damage is multifactorial, with contributions made by gradual physical stretching, compression, ischemia and possible accumulation of metabolic waste products (2). It has been postulated that physical trauma and ischemic injury to axons combine to alter membrane permeability leading to local influx of calcium and activation of calpains, which can cause proteolytic damage to the axonal cytoskeleton (3).

Magnesium is a calcium antagonist that blocks NMDA channel receptors in a voltage dependent manner, as well as voltage and receptor-operated calcium channels (4, 5). Magnesium sulfate therapy reduces lipid peroxidation after experimental spinal cord injury (6), long-term hypoxic-ischemic brain damage in 7-d-old rat (7), and the volume of traumatic brain lesions (8). It also facilitates recovery of function following electrolytic cortical injury (9). Magnesium may act as a cerebral vasodilator thereby improving the cerebral circulation (10) and preventing cell damage. Clinically, magnesium sulfate is used for seizure prophylaxis in the management of pregnant women with eclampsia. Some reports suggest that this treatment is associated with reduced incidence of cerebral palsy in low birth weight infants (11).

In an experimental model of hydrocephalus induced by injection of kaolin into the cisterna magna of immature rats we have previously demonstrated the presence of abnormal quantities of soluble ionic calcium and activated calpains in the periventricular white matter (3). Diminished blood flow has been demonstrated in the white matter of hydrocephalic rats (12). We hypothesized that administration of magnesium sul-

Received July 4, 2002; accepted January 8, 2003.

Correspondence: Marc R. Del Bigio, M.D., Ph.D., FRCPC, Department of Pathology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg MB R3E 0W3, Canada; e-mail delbigio@cc.umanitoba.ca

This work was funded by a grant from the Canadian Institutes of Health Research. Dr. Del Bigio has a salary award from the Manitoba Medical Services Foundation (MMSF Clinical Research Professorship). Mr. Khan holds a summer studentship award from the Children's Hospital Foundation.

DOI: 10.1203/01.PDR.0000061561.42921.5B

fate to young rats could ameliorate the abnormalities associated with moderate to severe early onset hydrocephalus.

METHODS

All animals were treated in accordance with guidelines set forth by the Canadian Council on Animal Care. The experiments were approved by the local animal use committee. All efforts were made to minimize suffering and the number of animals used. The experimental protocol is summarized in Fig. 1 and detailed below.

Animal preparation. Sprague Dawley rats were bred locally and 62 males were delivered after weaning at age 3 wk (weight 39–63 g). In the 1st experiment behavior testing was done before hydrocephalus induction. In the 2nd experiment rats were injected with kaolin immediately upon arrival, effectively 1 wk earlier than in the first experiment. Anesthesia was induced by intramuscular injection of ketamine/xylazine (90/5 mg/kg). The neck was shaved and, under aseptic conditions, a 27-gauge needle was inserted percutaneously into the cisterna magna. Sterile kaolin suspension (0.04 mL; 250 mg/mL in 0.9% saline) was injected slowly to induce hydrocephalus. In response to this quantity of kaolin, young rats experience gross enlargement of the cerebral ventricles and head and typically die within 6 wk. Controls received a sham injection with

needle insertion only. Blood samples were taken from the tip of the rat's tail under anesthesia before kaolin injection to obtain a baseline serum magnesium level (using Hitachi 737 Clinical Chemistry Autoanalyzer). Two to three rats were housed in standard cages and provided with a normal 12-h day/night lighting schedule with free access to water and pelleted food. As the rats became impaired, moistened food and water were provided on the cage floors.

Magnetic resonance imaging and assignment to treatment group. Magnetic resonance (MR) studies were performed using a Bruker Biospec/3 MR scanner equipped with a 21-cm bore magnet operating at a field of 7 T (Karlsruhe, Germany) to obtain T_2 -weighted images of the brain in the coronal plane. The widths of the lateral ventricles and cerebrum were measured in the rostral cerebrum immediately anterior to the third ventricle. Frontal horn size was expressed as a ratio determined by dividing the total width of the ventricles by the width of the cerebrum. These methods have been previously described in detail (13, 14). Rats underwent MR imaging no more than 24 h before commencement of drug treatments. After imaging (Fig. 2), the rats were stratified according to ventricle size index and assigned alternatively to drug treatment or control groups. Rats underwent MR imaging 2 wk later, no more than 24 h before sacrifice.

Figure 1. Line diagrams showing experimental design for $MgSO_4$ treatment of hydrocephalic rats. The age of the rats is shown below the line and the drug dose is shown above the upper arrow. In experiment 1, weekly behavior testing (Test) was started before kaolin injection (Kaolin) whereas in experiment 2 kaolin was injected soon after weaning. Magnetic resonance imaging (MRI) was done before implantation of infusion pumps (Pump) and treatment onset. The final MRI was done no more than 24 h before sacrifice (Kill).

Figure 2. A typical magnetic resonance image (T2-weighted) through the frontal cerebrum (coronal slice) of a hydrocephalic rat at the end of experiment 2. Moderately severe ventricular enlargement is apparent.

Preparation and administration of $MgSO_4$. In previously published work, short-term administration of 2.2–2.5 mM/kg/d $MgSO_4$ benefited young rats with hypoxic-ischemic brain injury (7, 15) and 2.2–5.0 mM/kg/d benefited adult rats with quinolinate or hypoglycemia-induced brain injury (16–18). Magnesium sulfate has a short half-life after bolus administration therefore we wanted to prevent low trough levels by continuous administration *via* osmotic minipumps. Sterile solutions of high (2.4 mM, saturated) and low dose (0.5 mM) $MgSO_4$ and saline control (2.4 mM) were prepared. Osmotic minipumps (Alzet model 2002, rated at $12.5 \mu\text{L/d} \times 14 \text{ d}$; DURECT Corp., Cupertino CA, U.S.A.) delivered the high dose solution at 0.03 mM/d. Assuming body weights of $\sim 160\text{g}$ at the onset of treatment, this equals 0.2 mM/kg/d. Osmotic minipumps were preloaded by the senior author and provided to the animal handlers in a blinded manner. The rats were anesthetized with ketamine/xylazine and pumps were implanted s.c. on the back under aseptic conditions. The treatment code was not broken until all behavioral, structural, and biochemical analyses were complete. To achieve doses comparable to those therapeutic in other studies, we needed to add supplemental boluses daily. Using the same solutions, syringes were loaded in a blinded manner and 0.85 or 4.1 mM/kg/d was injected s.c. every morning. Tail blood was obtained various times after injection at the time of sacrifice to determine roughly the kinetics of magnesium in blood. In experiment 1 a single daily bolus did not keep serum magnesium trough levels elevated. Because the results showed a potential dose-dependent benefit, a second experiment was performed with two groups; high dose infusion (2.4 mM) combined with 2 daily injections of $MgSO_4$ (total dose 8.2 mM/kg/d) compared with saline infusion/bolus control. The rats in this experiment were injected with kaolin 1 wk earlier (average weight 52.7g) than in the first experiment.

Behavioral testing. The outcome measures and testing protocols are the same as those described in prior drug treatments

of young rats with hydrocephalus (13). All testing was done in a blinded manner. The rats were weighed twice per week. On a weekly basis, beginning before kaolin injection in experiment 1, or after kaolin injection in experiment 2, several specific behavioral tests were performed. The rats were always tested in the same order and a given rat was tested at the same time of the day from week to week.

On the first testing day of each week, swimming speed was tested in a 20-cm-deep 15-cm-wide water trough by measuring the time to swim 150 cm. Memory was then assessed in a modified water maze test using a 90-cm pool (filled with 22°C opacified water) that contained a 13-cm-round hidden platform 1 cm below the water surface as previously described (14, 19). Testing took place in a dimly lit room with a single wall illuminated to provide directional cues. The rats were placed in the center of the pool and allowed to swim until they found the platform. A trial consisted of four attempts to find the platform, each attempt beginning with the rat facing a different quadrant. If the rat failed to complete the task in 60 s, it was given a 30-s rest period before the next attempt. Three trials were performed during the course of the day; each was separated by a 3-h interval. The times of the 4 attempts were averaged for each trial.

On the second testing day of each week, the rats were observed in an open field situation for a period of 1 min to assess arousal, grooming, and gait by using a previously validated set of parameters (20). Quantitative monitoring of spontaneous activity was then performed for 10 min in a square enclosure ($43 \times 43 \text{ cm}$) with 15 infrared beams (spaced every 3 cm) along the floor in each of two horizontal directions and a third set of similarly spaced beams 8.5 cm above the floor (Opto-Varimex; Columbus Instruments, Columbus OH, U.S.A.). Vertical, total, and ambulatory beam breaks were counted, the latter being defined as an interruption in a series of adjacent beams. Finally, gait agility was assessed using a rotating cylinder (7-cm diameter) (Economex, Columbus Instruments) in 2 separate trials. First, endurance at a constant speed of 5 rpm was assessed for a maximum of 2 min. Second, we measured the ability to stay on the cylinder, which accelerated beginning at 2.5 rpm and increasing at a rate of 0.1 rpm every second for up to 2 min. The time was recorded from the moment the rat was placed on the rotating cylinder until it fell off. The rotating cylinder test is complex and involves proprioceptive, tactile, vestibular, and motor functions.

Histopathological and biochemical studies of brains following drug treatments. At the end of the 2-wk drug treatment period and following the final MR imaging session, the rats were given an overdose of ketamine/xylazine (90:5 mg/kg), blood was drawn for magnesium level analysis, the vascular system cleared by transcardiac perfusion with ice-cold 0.1M PBS, and the brains were removed quickly. The cerebral hemispheres were spread apart to facilitate dissection of the corpus callosum from one side. Samples of the corpus callosum, parietal and frontal cerebrum, hippocampus, and cerebellum were dissected and frozen in liquid nitrogen, after which they were stored at -80°C . The remaining pieces were immersion fixed in 10% buffered formalin. The anterior cerebrum, cut coronally at the level of the optic chiasm, was

embedded in paraffin. Sections (6 μm thickness) were stained with solochrome cyanine for visualization of myelin. Corpus callosum thickness was measured in the midline and adjacent to the cingulum. As described previously in detail (21, 22), frontal cerebrum homogenates were used to quantify glial fibrillary acidic protein (GFAP), synaptophysin, and myelin basic protein (MBP) by using Western blots. Corpus callosum homogenates were used to assay the myelin related enzyme *p*-nitrophenylphosphorylcholine phosphocholine phosphodiesterase (PNPP), and the oligodendrocyte-related enzyme ceramide galactosyltransferase (CGaLT) by biochemical means. Two or three replicated studies were performed to ensure reproducibility and allow meaningful statistical analysis.

Statistical analysis. Data are presented as mean $\pm$ SEM. For Western blots, the densitometric values were normalized to those of control values and percent changes relative to control are indicated. They were analyzed by nonparametric methods, either Mann-Whitney test or Kruskal-Wallis test as appropriate for the number of groups. Quantitative behavioral data were analyzed to confirm a normal distribution. Statistical analysis then consisted of two-tailed *t* test or ANOVA with posthoc Fisher least square difference calculations for inter-group comparisons, as appropriate. To take into consideration multiple tests, statistical significance was defined as $p < 0.03$. Software used was StatView 5 (SAS Institute; Cary NC, U.S.A.). Power was calculated using the online Power Calculator tool (<http://ebook.stat.ucla.edu/calculators/powercalc/>).

RESULTS

Experiment 1. For the final analysis 24 rats were used (8 saline, 8 low dose, 8 high dose). Excluded from the analysis were 1 rat that died during kaolin injection, 5 that died of uncertain cause, and 1 that was euthanized during drug treatment because of severe neurologic deficits. There was no obvious adverse effect of the MgSO_4 in these rats. The average pretreatment serum Mg^{++} level was 1.13 ± 0.01 mM/L. Samples taken the day of sacrifice showed that Mg^{++} levels were high (>7 mM/L) for only the first 120 min (Fig. 3).

Morphologic and biochemical analysis of brains from hydrocephalic rats at the end of the experiment revealed no statistically significant difference between the groups for body weight, ventricle size, PNPP, CGaLT, MBP, and synaptophysin (Table 1). The high dose group (4.1 mM/kg/d) performed better on the accelerating rotating cylinder ($p = 0.0248$) and tended to perform better on the continuous speed cylinder and in the water maze test (Figs. 4 and 5). Corpus callosum thickness showed a trend in improvement. The power of these tests is in the range of 0.25–0.30; to increase the power to 0.80 would require sample size of ~ 35 per group. There was a statistically significant reduction in reactive gliosis as indicated by GFAP content in the frontal cerebrum ($p < 0.02$).

Experiment 2. We conducted the second experiment to determine whether higher MgSO_4 doses (8.2 mM/kg/d) could protect rats more effectively. For the final analysis 21 rats were used (4 nonhydrocephalic control, 10 saline, 7 high dose). Excluded from this experiment were 2 rats that died during kaolin injections, 1 that died of unknown cause during drug

treatment, 3 that were euthanized before drug treatment, and 3 that were euthanized during drug treatment due to severe neurologic deficits. Also, 2 rats that we thought were likely to die due to severe early neurologic deficits were treated in an unblinded manner with high dose MgSO_4 . One of these rats survived until the end of the experiment, however, neither are included in the final analysis. The high dose MgSO_4 injections were associated with profound lethargy and sedation for 1–2 h after the boluses. In contrast to experiment 1, serum Mg^{++} levels were significantly elevated ($p < 0.02$) in the treated group in the early trough phase, 3–6 h after the bolus.

Morphologic and biochemical analysis of brains from hydrocephalic rats at the end of the experiment revealed no statistically significant difference between the groups for any of the parameters tested (Table 2). The treated group had a tendency to perform worse on the rotating cylinder; the sedative effect of the MgSO_4 likely played a role. The GFAP protein quantity tended to decrease in the high dose MgSO_4 group.

DISCUSSION

These experiments using young rats with kaolin-induced hydrocephalus show that MgSO_4 treatment, in a dose-dependent manner, has a mild protective effect, but that high doses are associated with adverse effects, particularly sedation. The serum concentrations of Mg^{++} achieved here have previously been shown to be associated with increased levels of Mg^{++} in brain tissue (23). MgSO_4 treatment can inhibit the intracellular Ca^{++} increase following experimental head trauma (24) and can protect the traumatized brain when administered as late as 24 h after the injury (25, 26). We have previously shown that calcium-activated proteolysis (activated calpain) is associated with the axonal injury in experimental hydrocephalus (3). Therefore Mg^{++} might prevent activation of calpains by antagonizing Ca^{++} movements into damaged axons. Alternately, like nimodipine, which is protective to a greater degree than MgSO_4 in this experiment (13), Mg^{++} might enhance cerebral blood flow (10), thereby reducing the

Figure 3. Scatter plot showing serum Mg^{++} levels taken at different times after last injection of MgSO_4 or vehicle control in experiment 1. Mg^{++} levels were substantially elevated for 120 min, and returned to normal levels by 230 min.

Table 1. Results of magnesium sulfate treatment in hydrocephalic rats (experiment 1)

	Control saline (4.1 mM/kg/day)	Low MgSO ₄ (0.85 mM/kg/day)	High MgSO ₄ (4.1 mM/kg/day)
Sample size	8	8	8
Ventricle area index (pre-treatment)	0.215 ± 0.045	0.229 ± 0.044	0.195 ± 0.016
Ventricle area index (post-treatment)	0.344 ± 0.062	0.302 ± 0.074	0.291 ± 0.042
Body weight (g) (pre-treatment)	156 ± 10	179 ± 9	174 ± 8
Body weight (g) (post-treatment)	240 ± 13	269 ± 13	256 ± 8
Swim time (s) 150 cm (post-treatment)	15.3 ± 1.5	13.3 ± 2.7	10.2 ± 1.2
Modified water maze test (post-treatment, 1st trial) (s)	25.9 ± 9.6	18.9 ± 10.6	11.9 ± 7.9
Modified water maze test (post-treatment, 3rd trial) (s)	15.6 ± 6.7	18.8 ± 10.7	5.43 ± 3.2
Ambulatory activity (post-treatment) (beam breaks in 15 min)	1670 ± 464	1472 ± 429	1022 ± 238
Endurance on roller (continuous speed, post-treatment) (s)	48.7 ± 16.5	76.7 ± 21.2	75.6 ± 21.4
Endurance on roller (accelerating speed, post-treatment) (s)	13.6 ± 7.7	28.6 ± 9.2	51.6 ± 14.8*
Corpus callosum thickness (μm)	134 ± 39	222 ± 49	225 ± 54
CGaIT activity corpus callosum (nM/mg protein/h)	1.25 ± 0.27	1.16 ± 0.11	1.41 ± .27
PNPP activity corpus callosum (nM/mg protein/h)	82.1 ± 9.0	76.9 ± 5.0	99.3 ± 17.8
MBP frontal cerebrum (arbitrary densitometric units)	1.00 ± 0.14	1.01 ± 0.15	0.84 ± 0.13
Synaptophysin frontal cerebrum (arbitrary densitometric units)	1.00 ± 0.02	1.09 ± 0.06	1.06 ± 0.088
GFAP frontal cerebrum (arbitrary densitometric units)	1.00 ± 0.08	0.76 ± 0.09	0.69 ± 0.10†

All data are expressed as mean ± standard error of the mean.

* $p = 0.0248$ high dose vs. control, ANOVA with post hoc Fisher least square difference test for intergroup comparisons.

† $p = 0.0213$ high dose vs. control hydrocephalic, Kruskal-Wallis test for nonparametric data.

Pre-treatment—data obtained immediately before starting drug treatment, 2 weeks after kaolin injection ~ 6 weeks age.

Post-treatment—data obtained immediately before starting drug treatments, 4 weeks after kaolin injection at ~ 8 weeks age.

Figure 4. Line graph showing latency times (in seconds) to finding the hidden platform in the water maze test on the first (morning) trial of each weekly assessment in experiment 1. Week 2 and week 3 correspond to the period of hydrocephalus onset. Drug treatment was administered during week 4 and week 5. At the end of week 5, the high dose MgSO₄ group tended to perform better than the untreated hydrocephalic controls ($p = 0.3065$).

Figure 5. Line graph showing time (in seconds) spent on the accelerating rotating cylinder of each weekly assessment in experiment 1. In the final week the high dose MgSO₄ group performed better than untreated hydrocephalic controls ($p = 0.0248$).

Table 2. Results of magnesium sulfate treatment on hydrocephalic rats (experiment 2)

	Control saline (4.1 mM/kg twice/day)	MgSO ₄ (4.1 mM/kg twice/day)	Nonhydrocephalic controls
Sample size	10	7	4
Ventricle area index (pre-treatment)	0.199 ± 0.029	0.206 ± 0.023	—
Ventricle area index (post-treatment)	0.213 ± 0.023	0.198 ± 0.022	0.001 ± 0.0001
Body weight (g) (pre-treatment)	127 ± 4	131 ± 5	148 ± 11
Body weight (g) (post-treatment)	221 ± 9	231 ± 11	281 ± 16
Swim time (s) 150 cm (post-treatment)	15.9 ± 1.7	19.0 ± 3.2	14.0 ± 2.0
Modified water maze test (post-treatment, 1st trial) (s)	5.3 ± 1.3	8.7 ± 3.6	4.9 ± 1.1
Modified water maze test (post-treatment, 3rd trial) (s)	2.8 ± 0.4	3.4 ± 0.4	2.1 ± 0.1
Ambulatory activity (post-treatment) (beam breaks in 15 min)	922 ± 117	592 ± 240	1128 ± 300
Endurance on roller (continuous speed, post-treatment) (s)	120 ± 0.0	67.1 ± 18.9	119.3 ± 0.75
Endurance on roller (accelerating speed, post-treatment) (s)	75.3 ± 11.3	55.8 ± 18.7	90.3 ± 13.2
Corpus callosum thickness (μm)	313 ± 28	296 ± 25	390 ± 74
CGaIT activity corpus callosum (nM/mg protein/h)	2.77 ± 0.14	2.90 ± 0.12	2.52 ± 0.13
PNPP activity corpus callosum (nM/mg protein/h)	69.3 ± 4.1	80.6 ± 4.3	83.9 ± 1.1
MBP frontal cerebrum (arbitrary densitometric units)	1.00 ± 0.12	0.85 ± 0.22	1.07 ± 0.16
Synaptophysin frontal cerebrum (arbitrary densitometric units)	1.00 ± 0.07	0.94 ± 0.08	0.93 ± 0.08
GFAP frontal cerebrum (arbitrary densitometric units)	1.00 ± 0.11	0.75 ± 0.09	0.59 ± 0.10
Serum magnesium (mM/L)†	1.3 ± 0.2	1.6 ± 0.06‡	1.2 ± 0.3

All data are expressed as mean ± standard error of the mean.

* $p < 0.0001$ normal rats vs. both hydrocephalic groups, ANOVA with post hoc Fisher least square difference test for intergroup comparisons.

† 300 min following last bolus.

‡ $p < 0.02$ high dose MgSO₄ vs. control hydrocephalic, Student t test.

white matter damage caused by hydrocephalus. In both trials, MgSO₄ treated hydrocephalic rats had reduced GFAP content in the cerebrum, suggesting that there was less reactive astroglial change due to hydrocephalus (1). It is possible that this is due to a reduced axon injury stimulus, or perhaps it reflects a direct effect of Mg⁺⁺ on astrocytes; in culture Mg⁺⁺ causes increased proliferation of glial precursors but not mature astrocytes (27), and Mg⁺⁺ can modulate amino acid fluxes in astrocytes (28).

An obvious shortcoming of this experiment was that MgSO₄ at high doses caused sedation in the rats. This may have obscured a protective effect on behavior. However, we did not observe structural or biochemical protection therefore this dose was likely without benefit. We do not know if the protection conferred by MgSO₄ is permanent. It would be useful to determine the effect by extending the experiment following discontinuation of the drug; however, because hydrocephalus is progressive new damage would be incurred. The reverse experiment, in which hydrocephalic rats are deprived of dietary Mg⁺⁺ would also be of use for understanding interactions with Ca⁺⁺. Direct measurements of brain Mg⁺⁺ would help interpretation of all these experiments.

In conclusion, we find that MgSO₄ provides mild protection against brain damage in a rat model of childhood-onset hydrocephalus. However, we cannot recommend this for therapy in humans at this time. It should be noted that a recent study showed antenatal MgSO₄ in preterm labor was associated with adverse neurologic outcome, in contrast to expectations (29). We will continue to explore pharmacological approaches that might be used as a supplement to shunt treatment of hydrocephalus.

Acknowledgment. We thank Dr. R. Buist for assistance in the MR imaging.

REFERENCES

1. Del Bigio MR 1993 Neuropathological changes caused by hydrocephalus. *Acta Neuropathol (Berl)* 85:573–585
2. McAllister JP, II, Chovan P 1998 Neonatal hydrocephalus. Mechanisms and consequences. *Neurosurg Clin N Am* 9:73–93
3. Del Bigio MR 2000 Calcium-mediated proteolytic damage in white matter of hydrocephalic rats? *J Neuropathol Exp Neurol* 59:946–954
4. Simpson JJ, Eide TR, Schiff GA, Clagnaz JF, Hossain I, Tverskoy A, Koski G 1994 Intrathecal magnesium sulfate protects the spinal cord from ischemic injury during thoracic aortic cross-clamping. *Anesthesiology* 81:1493–1499
5. Marinov MB, Harbaugh KS, Hoopes PJ, Pikus HJ, Harbaugh RE 1996 Neuroprotective effects of preischemia intraarterial magnesium sulfate in reversible focal cerebral ischemia. *J Neurosurg* 85:117–124
6. Suzer T, Coskun E, Islekel H, Tahta K 1999 Neuroprotective effect of magnesium on lipid peroxidation and axonal function after experimental spinal cord injury. *Spinal Cord* 37:480–484
7. Sameshima H, Ikenoue T 2001 Long-term magnesium sulfate treatment as protection against hypoxic-ischemic brain injury in seven-day-old rats. *Am J Obstet Gynecol* 184:185–190
8. Vink R, Nimmo AJ, Cernak I 2001 An overview of new and novel pharmacotherapies for use in traumatic brain injury. *Clin Exp Pharmacol Physiol* 28:919–921
9. Hoane MR, Barth TM 2001 The behavioral and anatomical effects of MgCl₂ therapy in an electrolytic lesion model of cortical injury in the rat. *Magn Res* 14:51–63
10. Brewer RP, Parra A, Lynch J, Chilukuri V, Borel CO 2001 Cerebral blood flow velocity response to magnesium sulfate in patients after subarachnoid hemorrhage. *J Neurosurg Anesthesiol* 13:202–206
11. Nelson KB, Grether JK 1995 Can magnesium sulfate reduce the risk of cerebral palsy in very low birthweight infants? *Pediatrics* 95:263–269
12. Massicotte EM, Buist R, Del Bigio MR 2000 Altered diffusion and perfusion in hydrocephalic rat brain: a magnetic resonance imaging analysis. *J Neurosurg* 92:442–447
13. Del Bigio MR, Massicotte EM 2001 Protective effect of nimodipine on behavior and white matter of rats with hydrocephalus. *J Neurosurg* 94:788–794
14. Del Bigio MR, Crook CR, Buist R 1997 Magnetic resonance imaging and behavioral analysis of immature rats with kaolin-induced hydrocephalus: pre- and postshunting observations. *Exp Neurol* 148:256–264
15. Galvin KA, Oorschot DE 1998 Postinjury magnesium sulfate treatment is not markedly neuroprotective for striatal medium spiny neurons after perinatal hypoxia/ischemia in the rat. *Pediatr Res* 44:740–745
16. Hallak M, Berman RF, Irtrenkauf SM, Janusz CA, Cotton DB 1994 Magnesium sulfate treatment decreases N-methyl-D-aspartate receptor binding in the rat brain: an autoradiographic study. *J Soc Gynecol Invest* 1:25–30
17. Kaya M, Kucuk M, Kalayci RB, Cimen V, Gurses C, Elmas I, Arican N 2001 Magnesium sulfate attenuates increased blood-brain barrier permeability during insulin-induced hypoglycemia in rats. *Can J Physiol Pharmacol* 79:793–798
18. Wolf G, Keilhoff G, Fischer S, Hass P 1990 Subcutaneously applied magnesium protects reliably against quinolinat- induced N-methyl-D-aspartate (NMDA)-

- mediated neurodegeneration and convulsions in rats: are there therapeutical implications. *Neurosci Lett* 117:207–211
19. Kraemer PJ, Brown RW, Baldwin SA, Scheff SW 1996 Validation of a single-day Morris water maze procedure used to assess cognitive deficits associated with brain damage. *Brain Res Bull* 39:17–22
 20. Moser VC, Tilson HA, MacPhail RC, Becking GC, Cuomo V, Frantik E, Kulig BM, Winke G 1997 The IPCS Collaborative Study on Neurobehavioral Screening Methods: II. Protocol design and testing procedures. *NeuroToxicology* 18:929–938
 21. Del Bigio MR, Kanfer JN, Zhang YW 1997 Myelination delay in the cerebral white matter of immature rats with kaolin-induced hydrocephalus is reversible. *J Neuropathol Exp Neurol* 56:1053–1066
 22. Del Bigio MR, Zhang YW 1998 Cell death, axonal damage, and cell birth in the immature rat brain following induction of hydrocephalus. *Exp Neurol* 154:157–169
 23. Hallak M, Berman RF, Irtenkauf SM, Evans MI, Cotton DB 1992 Peripheral magnesium sulfate enters the brain and increases the threshold for hippocampal seizures in rats. *Am J Obstet Gynecol* 167:1605–1610
 24. Ustun ME, Duman A, Ogun CO, Vatansev H, Ak A 2001 Effects of nimodipine and magnesium sulfate on endogenous antioxidant levels in brain tissue after experimental head trauma. *J Neurosurg Anesthesiol* 13:227–232
 25. Heath DL, Vink R 1999 Optimization of magnesium therapy after severe diffuse axonal brain injury in rats. *J Pharmacol Exp Ther* 288:1311–1316
 26. Heath DL, Vink R 1999 Improved motor outcome in response to magnesium therapy received up to 24 hours after traumatic diffuse axonal brain injury in rats. *J Neurosurg* 90:504–509
 27. Krueger RC Jr, Santore MT, Dawson G, Schwartz NB 2001 Increased extracellular magnesium modulates proliferation in fetal neural cells in culture. *Dev Brain Res* 127:99–109
 28. Philibert RA, Rogers KL, Allen AJ, Dutton GR 1988 Dose-dependent, K⁺-stimulated efflux of endogenous taurine from primary astrocyte cultures is Ca²⁺-dependent. *J Neurochem* 51:122–126
 29. Mittendorf R, Dambrosia J, Pryde PG, Lee KS, Gianopoulos JG, Besinger RE, Tomich PG 2002 Association between the use of antenatal magnesium sulfate in preterm labor and adverse health outcomes in infants. *Am J Obstet Gynecol* 186:1111–1118