Magnesium as a Neuroprotective Agent: A Review of Its Use in the Fetus, Term Infant with Neonatal Encephalopathy, and the Adult Stroke Patient

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Keywords

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Abstract

Magnesium is an intracellular cation essential for many enzymatic processes and cellular functions. Magnesium sulfate acts as an endogenous calcium channel antagonist at neuronal synapses, thought to prevent excessive activation of N-methyl-D-aspartate receptors by excitatory amino acids, such as glutamate, and by downregulation of proinflammatory pathways. Early intervention is essential in the prevention of the secondary phase of neuronal injury. The immature brain is particularly prone to excitotoxicity, and inflammation has been strongly implicated in the pathogenesis of cerebral palsy. This article explores the current status of magnesium being used as an adjunct to hypothermia in term neonatal encephalopathy (NE) against a background of its use in other populations. There is some evidence for magnesium sulfate as a neuroprotective agent, however animal studies of NE at term equivalent age have been confounded by concomitant hypothermia induced by magnesium itself. Nevertheless, the combination of magnesium and cooling has been shown to be more effective than either treatment

alone in adult rodents. In the preterm baby, magnesium sulfate given antenatally in threatened preterm labor has demonstrated a significant reduction in the risk of cerebral palsy at 2 years of age, though the benefit is not clear at school age. In adult clinical studies of ischemic and hemorrhagic stroke, there have been disappointing results for magnesium sulfate as a neuroprotective strategy. Importantly, clinical neurological scores may be affected by the increased hypotonia observed. We suggest that magnesium sulfate should be carefully re-evaluated as a neuroprotective agent given its favorable safety profile, relative low cost, and widespread availability.

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Introduction

Magnesium is an ionized mineral essential to hundreds of enzymatic processes, including hormone receptor binding, energy metabolism, muscle contractility as well as neuronal and neurotransmitter function [1]. It is primarily an intracellular cation, and stores are distributed between bone (53%), muscle (27%), and soft tissue (19%). Serum magnesium levels are tightly controlled (0.65–1.05 mmol/L), and homeostasis is maintained through intesti-

Fig. 1. The N-methyl-D-aspartate receptor. Magnesium atoms block the ion channel, preventing sodium and calcium entry into the postsynaptic neuron. Magnesium is displaced by partial depolarization of the neuron.

nal absorption, storage in bones, and renal excretion [1, 2]. Magnesium has an inhibitory effect at neuronal synapses, leading to its use as an anticonvulsant, particularly in eclamptic seizures [3]. We discuss the potential use of magnesium sulfate as an adjunct with hypothermia for term neonatal encephalopathy (NE), studies of the antenatal use of magnesium sulfate for threatened preterm delivery and the use of magnesium sulfate in adult brain injuries. In each patient population we explore the neuroprotective potential of magnesium, its mechanism of action and efficacy in preclinical and clinical trials.

Role of Magnesium in Cellular Metabolism

Magnesium is an important cofactor in over 300 enzymatic reactions and is essential to normal cellular function. Magnesium acts as a counterion for ATP and stabilizes many ATP-dependent processes, including glucose utilization, protein, and nucleic acid synthesis [4]. It contributes to the structural integrity of nucleic acids, proteins, and mitochondria [5].

As an endogenous calcium antagonist, magnesium serves a number of regulatory roles at neuronal and neuromuscular synapses. It blocks calcium entry at the pre-synaptic junction, preventing excessive acetylcholine release and stimulation at the neuromuscular junction. It also has a depressant effect at the postsynaptic membrane through the voltage-dependent block of N-methyl-D-aspartate (NMDA) receptors (Fig. 1) [1]. This action as an NMDA receptor antagonist underpins one of the main proposed mechanisms of magnesium neuroprotection.

Excitotoxicity

The precise mechanism by which magnesium provides neuroprotection has not been well established. One of the most commonly held theories is that magnesium prevents excitotoxic damage through NMDA receptor blockade. This postsynaptic receptor normally strengthens synaptic connections when repeatedly activated (long-term potentiation) and plays a crucial role in memory function [6]. Activation of the NMDA receptor by excitatory neurotransmitters permits the influx of calcium ions, serving as secondary messenger for physiological cell processes, e.g. regulation of transcription factors and DNA replication [7, 8].

Neurons exposed to hypoxic stress are unable to maintain normal glutamate homeostasis, resulting in excessive stimulation of NMDA receptors. This results in a cascade of “excitotoxic” events causing acute cell swelling and delayed cell degeneration [9]. This delayed neuronal injury is mediated by excessive calcium influx into the cell, triggering catabolic enzymes (e.g., proteases, phospholipases, endonucleases) and free radical production (Fig. 2). Glutamate excitotoxicity and the loss of intracellular calcium homeostasis also triggers cellular “suicide” programs, leading to apoptosis [9].

The NMDA receptor itself is composed of 4 subunits (heterotetramer), similar to a hemoglobin molecule. Receptor subunits containing NR2B have a high permeability to calcium [10] and are particularly abundant in preterm white matter [11]. While this may serve an important role during the rapid growth and myelination in early neuronal development, it may also confer particular vulnerability to preterm white matter. This may explain in part the different patterns of injury between preterm and term hypoxia-ischemia [8, 12].

Magnesium is an endogenous calcium antagonist and provides a voltage-dependent blockade of the NMDA receptor. Through inhibiting the rapid influx of calcium, magnesium may prevent the secondary cascade of injury that leads to cell death [1]. This theory is supported by preclinical data, both in vitro and in vivo. Magnesium has been shown to reduce excitotoxic damage induced in mice by ibotenate, a glutamatergic agonist [13]. Extracellular levels of glutamate are reduced in magnesium-treated gerbils following focal cerebral ischemia [14]. Furthermore, incubation of primary oligodendrocyte precursor cells with magnesium appears to improve cell survival following oxygen glucose deprivation [15].

The extent of injury secondary to excessive NMDA receptor activation however remains controversial. Alternative NMDA receptor antagonists have shown limited

Fig. 2. Mechanisms of excitotoxic-mediated injury.

improvement in neuronal survival and in less injured regions after HI [16, 17] and in the absence of thermoregulation, improved neuronal survival has been attributed to drug-induced hypothermia [18–20]. A recent clinical trial of xenon, an NMDA receptor antagonist in combination with cooling was also disappointing; though a delay of up to 10 h after birth in initiating therapy may have contributed to the lack of efficacy [21].

Magnesium and Inflammation

Inflammation and infection have been implicated in neuronal injury. Magnesium sulfate may confer neuroprotection through downregulation of the inflammatory

cascade. Magnesium significantly decreased the frequency of maternal and neonatal monocytes producing $\text{TNF-}\alpha$ and IL-6 when exposed to LPS in vitro [22]. Preclinical data have also demonstrated that magnesium reduces levels of proinflammatory cytokines (IL-6, $\text{TNF-}\alpha$) [23] in LPS-treated pregnant rodents as well as improves the offspring's learning ability at 3 months [24].

A potential anti-inflammatory mechanism is the inhibition of the nuclear factor- κB (NF- κB) signal pathway. NF- κB is a transcription factor present in the cell cytoplasm and rapidly activated by inflammatory or immunological stimuli. On activation, NF- κB enters the nucleus and initiates transcription of multiple genes to produce

proinflammatory cytokines, cell adhesion molecules as well as regulators of apoptosis [25]. Gao et al. [26] demonstrated that magnesium sulfate significantly reduces NF- κ B activity by inhibiting its translocation into the nucleus in LPS-sensitized adult rodent microglia.

In the preterm infants, inflammation may be an important etiological factor of brain injury. The risk of cerebral palsy in preterm infants increases in the presence of chorioamnionitis (OR 4.2, CI 1.4–12), prolonged rupture of membranes (OR 2.3, CI 1.2–4.2), and maternal infection (OR 2.3, CI 1.2–4.5) [27]. Preterm labor itself may have an underlying infective origin as demonstrated by raised proinflammatory cytokines in cord blood (IL-1, IL-6, IL-8, and TNF- α). Maternal infection also increases the risk of cerebral palsy in term infants (OR 9.3, CI 3.7–23), especially if combined with perinatal hypoxia-ischemia [28].

The theory that magnesium attenuates infective or inflammatory processes however has yet to be borne out in clinical trials. Subgroup analysis of the NICHD cohort receiving antenatal magnesium for the prevention of cerebral palsy demonstrated no benefit among infants exposed to chorioamnionitis [29].

Animal Models of Neuroprotection

Animal models of hypoxia-ischemia have been used to assess the neuroprotective potential of novel therapeutic strategies. The Rice-Vannucci rodent is one of the most commonly used animal models of hypoxia-ischemia, combining unilateral carotid artery ligation with moderate hypoxia to generate cerebral injury [30]. Most studies using this method measure infarct area or volume and histological assessment of neuronal apoptosis to measure outcomes. Magnesium sulfate efficacy trials from term equivalent animals (postnatal day 7) have generated conflicting results [18]. Studies demonstrating neuroprotection were confounded by coexisting hypothermia, and those that maintained normothermia failed to show benefit.

Large animal models provide an opportunity for more translational and clinically relevant outcomes. Magnesium sulfate failed to demonstrate a reduction in the level of secondary energy failure on magnetic resonance imaging [31] or severity of tissue damage in a piglet model of hypoxia-ischemia [32]. In addition, magnesium sulfate has not demonstrated improvement of EEG or neuronal loss in fetal sheep undergoing umbilical cord occlusion at human term equivalent age (0.85 gestational age) [33].

Magnesium sulfate has also been evaluated in adult preclinical models of traumatic brain injury. Animals in-

jured by fluid percussion to exposed dura (parasagittal) were treated with magnesium sulfate. Although there was no benefit observed in posttraumatic learning, there was a significant reduction in tissue loss in the hippocampus [34]. Similarly, magnesium sulfate significantly improved motor outcomes in rodents following diffuse axonal brain injury [35].

Animal studies of magnesium sulfate in fetal neuroprotection are limited compared to models of neonatal hypoxia-ischemia. Timed-pregnant rodents have been used as a model of maternal infection to evaluate the role of magnesium in modulating inflammation to improve developmental outcomes in offspring [23, 24].

Temperature-controlled studies by Galinsky et al. [36, 37] assessed the efficacy of magnesium sulfate given 24 h prior to umbilical cord occlusion and maintained the infusion for a further 24 h after insult in preterm fetal sheep at 104 days gestation (term is 147 days). Magnesium sulfate did not affect the cardiovascular response (degree of hypotension) during umbilical cord occlusion and thus did not alter insult severity. Although magnesium sulfate significantly reduced the frequency of seizures after asphyxia, it did not improve EEG recovery or survival of subcortical neurons [36]. Magnesium sulfate was in fact associated with a reduction in mature (olig-2-positive) oligodendrocytes in the intragryal and periventricular white matter and immature (CNPase-positive) oligodendrocytes in the intragryal region. The mechanism of this loss is unclear. The authors postulate that prolonged magnesium NMDA blockade may interrupt neuronal-oligodendrocyte signaling and thus hinder oligodendrocyte differentiation and axonal myelination. Microglial infiltration did not differ between magnesium sulfate and control groups, suggesting that magnesium sulfate did not suppress inflammation in the 72 h following hypoxia-ischemia [36].

Clinical Neuroprotection Studies

Neonatal Encephalopathy in Term Infants

Therapeutic hypothermia has been successfully implemented as a neuroprotective strategy in 2010 (National Institute of Clinical Excellence) [38]; however, in spite of this, 50% of newborns with moderate to severe hypoxic-ischemic encephalopathy (HIE) will die or suffer long-term disabilities such as cerebral palsy [39]. Therefore there is an urgent need to continue to develop new strategies to improve the care of this vulnerable population.

Magnesium inhibition of excessive NMDA receptor activation provides a biologically plausible mechanism to limit the delayed “secondary” phase of neuronal cell death following perinatal hypoxia-ischemia. Interestingly, low magnesium levels at birth have been observed in infants with severe HIE (0.64 mmol/L, 95% CI 0.47–0.87) compared to mild or no HIE (0.81 mmol/L, 95% CI 0.75–0.87) and controls (0.72 mmol/L, 95% CI 0.69–0.76) [40]. It remains unclear whether low magnesium at birth is a result of severe hypoxia or whether it confers vulnerability rendering the infant susceptible to greater injury.

A pharmacokinetic study of magnesium sulfate by Levene et al. [41] demonstrated doses of 250 mg/kg magnesium sulfate (MgSO_4) were not associated with significant hypotension or bradycardias in term infants following perinatal hypoxia-ischemia. The subsequent Randomized Asphyxia Trial (RAST) however was suspended following incidences of significant bradycardia, which transpired to be the result of infants inadvertently receiving almost twice the intended trial dose. The pharmacokinetic study had used a 12.5% solution of magnesium sulfate, based upon the heptahydrated magnesium salt ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$). The pharmaceutical company, commissioned to supply the RAST with a 12.5% trial medication, however provided a 12.5% solution based on the anhydrous salt (MgSO_4); this solution was effectively double the intended concentration, and therefore almost double the dose of magnesium was administered [42]. The RAST recruited 50 patients prior to suspension (25% of the planned cohort), and no significant differences were found in mortality between groups. There was a trend towards higher mortality in infants given magnesium, although there was a disproportionately high number of infants with severe HIE in that group [unpubl. data; communication with trial investigator D. Evans].

There have since been 6 randomized placebo-controlled trials assessing the use of magnesium sulfate in term hypoxia-ischemia, 5 of which were conducted prior to the introduction of therapeutic hypothermia. These trials included infants born with at least 35 weeks gestation with signs of moderate to severe encephalopathy NE (Table 1). There was however significant heterogeneity between trials in drug dosing and timing as well as outcome measures. All trials reported giving magnesium within 24 h of birth; however, only 3 stated this was within 6 h [43, 44]. One study protocol gave a single 250 mg/kg dose of MgSO_4 [45] while others opted for an initial dose of 250 mg/kg followed by repeat doses of either 125 mg/kg [33, 44] or 250 mg/kg [43, 46, 47] at 24 and 48 h. Bhat et al. [43] and Ichiba et al. [46] reported favorable-

term composite outcomes, defined by a normal neurological exam at discharge, normal CT brain and normal oral feeding by 2 weeks. These findings did however not translate to significant neurodevelopmental improvement at 6 months [44] and 2 years [33].

Kashaba et al. [45] adopted a novel approach, assessing the levels of excitatory amino acids (glutamate, aspartate) in the CSF at birth and after 72 h. They noted higher levels of glutamate and aspartate in infants with severe hypoxia-ischemia, supporting the theory that secondary energy failure was the result of excitotoxic damage. Magnesium sulfate therapy however did not alter the levels of these amino acids.

Rahman et al. [47] evaluated the safety and efficacy of magnesium sulfate combined with cooling following supportive evidence from adult rodent studies [48–50]. They reported a favorable safety profile of magnesium sulfate administered during therapeutic hypothermia with no significant difference in death or hypotension between treatment groups. The study however had several methodological limitations: hypotension was defined as either mild-moderate (single inotrope) or severe (multiple inotropes) rather than specifying inotrope doses or mean arterial blood pressure values; inclusion criteria varied between centers depending on the availability of amplitude-integrated EEG, and 5/60 infants included in the analysis underwent selective head cooling rather than total body hypothermia. Long-term outcomes for this study have yet to be published.

A comprehensive meta-analysis by Tagin et al. [51] demonstrated a significant reduction in short-term composite of “unfavorable” outcomes, defined by abnormal neurology, amplitude-integrated EEG or neuroimaging (RR 0.48, 95% CI 0.30–0.77). Ichiba et al. [52] repeated their study in 30 newborns with moderate to severe HIE (based on Sarnat criteria) and administered magnesium sulfate within 6 h of birth. They reported normal neurodevelopmental outcomes in 73% of infants at 18 months, though the study was limited by the absence of a control arm. There may be some benefit in the use of magnesium in term infants with HIE; however, studies are limited by small numbers, trial heterogeneity and an absence of long-term outcome data.

Fetal Neuroprotection

Magnesium sulfate is a familiar drug in obstetrics and has been used in the management of eclamptic seizures since the early 1900s. Randomized controlled trials have since demonstrated its superiority over other anticonvulsants, and it is currently recommended in the treatment

Table 1. Summary of clinical trials: magnesium for neonatal encephalopathy

Study	Type of study	Dose and timing of MgSO ₄	Inclusion criteria	Outcome measure	Summary of findings
Bhat et al. [43], 2009	Single-center RCT <i>n</i> = 40	250 mg/kg/dose daily for 3 days <i>1st dose given within 3 h of birth</i>	Age <6 h Gestation >37 weeks Moderate-severe HIE Evidence of perinatal asphyxia (3 out of 4): – Signs of fetal distress – IPPV required >2 min – pH <7, BE >15 in 1st hour – 5 min Apgar <6	Time to oral feeds Neurological exam at discharge CT of the brain (day 14) EEG within 72 h and on day 14	2 patients had apneas during 2nd dose of MgSO ₄ needing ventilation Composite “good” outcome (normal neurology, CT scan, sucking) better in magnesium group (OR 5.5, CI 1.2–23.6)
Gathwala et al. [44], 2010	Single-center RCT <i>n</i> = 40	250 mg/kg loading dose 125 mg/kg at 24 and 48 h <i>1st dose given within 30 min of birth</i>	Apgar at 5 min <6 Gestation >37 weeks	CT brain, EEG, neurology at discharge Development at 6 months (Denver II)	Neurology of infants at randomization not stated No significant difference in EEG, CT findings; no difference in development
Groenendaal et al. [33], 2002	Single-center RCT <i>n</i> = 22	250 mg/kg loading dose 125 mg/kg at 24 and 48 h <i>Time of 1st dose not stated</i>	Gestation >37 weeks At least 3 of: – Sign of fetal distress – cord pH <7.10 – Need for resuscitation at birth – Apgar at 10 min <5 – Need for IPPV at 10 min	aEEG scoring – Before infusion – First 3 h – 12 h – 24 h Follow-up at 24 months (Griffiths')	Study terminated following the death of 2 infants receiving high magnesium doses No significant effect on aEEG or long-term neurodevelopment
Ichiba et al. [46], 2002	Multicenter RCT <i>n</i> = 33	250 mg/kg/dose daily for 3 days Dopamine given to magnesium group (5 µg/kg/min) <i>1st dose given within 24 h of birth</i>	All of the criteria below: – Gestation >37 weeks – Apgar <7 at 5 min – Need for IPPV at 10 min – Seizures within 24 h	EEG (day 14) CT (day 14) Establishing oral feeds (day 14) Composite “good” outcome (normal EEG, CT, and feeding)	Mean time of 1st dose 10.1 h Composite short-term good outcome was significantly improved in magnesium group (<i>p</i> < 0.04) No difference between individual outcome measures
Khashaba et al. [45], 2006	Single-center RCT <i>n</i> = 47	250 mg/kg/dose, 1 dose only <i>Dose given within 24 h of birth</i>	Gestation >37 weeks Apgar at 5 min <3 and/or 1st gasp >10 min after birth Severity of HIE based on neurological exam	CSF glutamate and aspartate at admission and 72 h	Overall raised glutamate and aspartate with severity of HIE Excitatory amino acids increased after HI and decreased by 72 h MgSO ₄ did not alter levels of amino acids in CSF
Rahman et al. [47], 2015	Multicenter RCT <i>n</i> = 60	250 mg/kg/dose daily for 3 days <i>Dose given within 6 h of birth</i>	Gestation >35 weeks Criterion A (one of the following): – Apgar at 10 min <5 – Need for IPPV at 10 min – pH <7 or BE >–16 within 1 h of life Criterion B: Clinical encephalopathy or seizures	Short-term outcomes only (mortality, seizures, hypotension, IVH, NEC, pulmonary hemorrhage, pulmonary hypertension, and pneumothorax)	No significant difference in short-term adverse outcomes Hypotension defined as mild-moderate or severe Inotrope dosage and BP not reported

MgSO₄, magnesium sulfate; RCT, randomized controlled trial; HIE, hypoxic-ischemic encephalopathy; IPPV, intermittent positive pressure ventilation; BE, base excess; CT, computer tomography; aEEG, amplitude-integrated electroencephalography; OR, odds ratio; CI confidence interval; CSF, cerebrospinal fluid; HI, hypoxia-ischemia; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; BP, blood pressure.

Table 2. Summary of clinical trials: fetal neuroprotection

Trial	Inclusion criteria	Exclusions	Dose and timing of MgSO ₄	Outcome measure	Summary of findings
BEAM Rouse et al. [61], 2008	GA 24–31 weeks High risk of preterm delivery within 2–24 h (PPROM, cervical dilation 4–8 cm) <i>n</i> = 2,241	Delivery likely within 2 h or dilatation >8 cm, PPRM before 22 weeks, fetal anomalies, any maternal contraindication to MgSO ₄	Bolus: 6 g Infusion: 2 g/h	Composite (death by 1 year or moderate-severe CP by 2 years)	No difference in primary composite outcome (RR 0.97, CI 0.77–1.23) Reduction in moderate to severe CP by 2 years (RR 0.55, CI 0.32–0.95)
MagNET Mittendorf et al. [58], 2002	Preterm labor GA 24–34 weeks <i>Tocolysis arm</i> Active premature labor (dilatation <4 cm) <i>n</i> = 92 <i>Neuroprotection arm</i> Active premature labor (dilatation >4 cm) <i>n</i> = 57	Clinical evidence of pre-eclampsia or infection	<i>Tocolysis</i> Bolus: 4 g Infusion: 2–3 g/h <i>Neuroprotection</i> Bolus: 4 g Infusion: none	Cranial US during admission Diagnosis of CP at 18 months	Analysis of both groups combined highlighted increased composite adverse outcomes with magnesium (IVH, PVL, CP, death) (OR 2.0, CI 0.99–4.1)
PREMAG Marret et al. [60], 2007	GA <33 weeks (no lower limit set) Expected to deliver within 24 h <i>n</i> = 573	Fetal malformations, cardiovascular instability, renal insufficiency, pregnancy-associated vascular disease, indication for LSCS, recent ingestion of calcium blocker, digitalis, indomethacin, received steroids, aminoglycosides, betamimetics in last 1 h	Bolus: 4 g Infusion: none	Overall neonatal mortality before discharge Severe white matter injury on cranial US	No significant benefit in mortality or white matter injury
ACTOMgSO ₄ Crowther et al. [59], 2003	GA <30 weeks (no lower limit set) Birth expected within 24 h <i>n</i> = 1,062	Already in 2nd stage of labor, received MgSO ₄ in this pregnancy, any contraindications to MgSO ₄ (RR <16/min, absent patella reflex, UO <100 mL/4 h)	Bolus: 4 g Infusion: 1 g/h	Mortality up to 2 years, CP at 2 years, combined outcome	No significant difference in mortality (RR 0.83, CI 0.64–1.09), cerebral palsy (RR 0.83, CI 0.66–1.03) or combined outcome (RR 0.75, CI 0.59–0.96)
Magpie Duley [62], 2002	Not delivered or within 24 h postpartum Pre-eclampsia <i>n</i> = 1,544 (<37 weeks)	Hypersensitivity to magnesium, renal impairment, myasthenia gravis	MgSO ₄ 4-g bolus with infusion 1 g/h	Eclampsia Mortality at discharge	No clear difference in neonatal mortality (RR 1.02, 99% CI 0.92–1.14)

MgSO₄, magnesium sulfate; GA, gestational age; PPRM, premature rupture of membranes; CP, cerebral palsy; RR, relative risk; CI, confidence interval; US, ultrasound; IVH, intraventricular hemorrhage; PVL, periventricular leukomalacia; LSCS, lower segment Caesarean section; UO, urine output.

of eclamptic seizures as well as seizure prophylaxis [3]. The neuroprotective properties of magnesium in preterm infants was first observed by Nelson and Grether [53], who observed that in utero exposure to magnesium sulfate for pre-eclampsia or tocolysis was lower in very-low-birth-weight infants (<1,500 g) with cerebral palsy compared to controls (7.1 vs. 36%). While this promising finding was corroborated by some [54], the results proved controversial with other reports failing to show benefit [55, 56] as well as concerns of increased mortality in extreme preterm infants exposed to magnesium tocolysis [57].

Over the last decade, a number of large prospective randomized controlled trials have been conducted to assess the safety and efficacy of magnesium sulfate as a fetal neuroprotective agent (Table 2).

In the Magnesium Endpoint Trial (MagNET 2002) [58], women in preterm labor were recruited between 24 and 34 weeks gestational age. They were stratified into 2 groups, those suitable for tocolysis (cervical dilatation <4 cm) and those who did not meet tocolysis criteria. The “tolcolysis” group was randomized to receive magnesium sulfate (4-g bolus and 2–3 g/h infusion) or alternative therapy as deemed by the obstetrician. The other “neuro-

protective” group was randomized to magnesium sulfate bolus (4 g) or 0.9% saline. The study was however stopped prematurely due to concerns of a higher neonatal mortality rate in the magnesium group. Combined analysis of the trial arms did not demonstrate any reduction in cerebral palsy.

Two subsequent trials, the Australasian Collaborative Trial of Magnesium Sulfate (ACTOMgSO₄) [59] study in 2003 and the French PREMAG [60] Study in 2007 did not demonstrate an increased mortality with magnesium use. Neither trial however yielded significant improvements in rates of cerebral palsy at 2 years. The ACTOMgSO₄ trial did report a reduced rate of substantial motor dysfunction, as defined by a Gross Motor Function Classification (GMFCS) level of 2 or worse.

The Beneficial Effects of Antenatal Magnesium Sulfate (BEAM) [61] study in 2008 was one of the largest randomized controlled trials of magnesium sulfate involving 2,241 women (singletons or twins) at 24–31 weeks gestation. This study demonstrated a significant reduction in moderate to severe (GMFCS 2–4) cerebral palsy as well as cerebral palsy overall. In addition to these 4 trials, the MAGPIE trial (2002) [62] was designed to assess whether magnesium sulfate prevented eclampsia in women with pre-eclampsia. Many of the participating centers were in developing countries and reported a comparatively higher pediatric mortality compared to other studies. There was no significant reduction in the rates of cerebral palsy associated with antenatal magnesium sulfate exposure.

A comprehensive meta-analysis of these 5 trials demonstrated antenatal magnesium sulfate reduced both the risk of cerebral palsy (RR 0.69, CI 0.54–0.87) and substantial gross motor dysfunction (RR 0.61, CI 0.44–0.85). The number of women needed to treat to prevent 1 infant developing cerebral palsy was 63 [63].

Outcome data at school age (6–11 years) however were disappointing. The ACTOMgSO₄ trial followed up 77% of their cohort and found no significant difference in cognitive, academic, attention or behavioral outcomes. The earlier finding of reduced gross motor dysfunction did not translate to an overall reduction in the severity of cerebral palsy at school age [64]. Long-term follow-up data from the PREMAG cohort (7–14 years) also reported no significant difference in neuromotor, cognitive, or language ability. They did observe fewer incidences of grade repetition, specific educational needs, and overall better parental perception of child health [65].

To date, there have been at least 5 meta-analyses [63, 66–69] and an evaluation of cost-effectiveness [70] that

all support the use of antenatal magnesium sulfate as a neuroprotective agent. Clinical adoption of this intervention was initially slow with concerns raised over the lack of a statistical difference in primary outcomes as well as safety data raised in 1 trial [71]. The American College of Obstetricians has supported the use of magnesium sulfate in preterm neuroprotection, however encourages clinicians to develop guidelines locally [72]. Both Australia [73] and Canada [74] issued guidelines detailing the use of magnesium sulfate as neuroprotection of fetuses born less than 30 weeks and 32 weeks, respectively. The National Institute of Clinical Excellence have recently recommended using magnesium sulfate in mothers in preterm labor at gestational ages 24–29 + 6 weeks and considering it in those at gestational age between 30 and 33 + 6 weeks [75].

Although the long-term follow-up data from ACTOMgSO₄ and PREMAG are disappointing, the findings do not negate the reduction in cerebral palsy at 2 years seen across the 5 trials included in the meta-analysis. They do however highlight the need for ongoing long-term evaluation of this intervention.

NE in Preterm Infants

NE seen in term infants represents a distinct clinical entity to the more chronic evolving cerebral white matter injury associated with prematurity. The preterm brain is particularly vulnerable to injury due to highly active dendritic and axonal growth as well as the exaggerated inflammatory response of an immature immune system. Although hypoxic-ischemic events may complicate preterm delivery, there is limited evidence that interventions trialed in term infants can be directly translated to the preterm population. A small pilot study of selective head cooling in infants between 32 and 35 weeks gestation was associated with significant adverse effects [76]. Designing a randomized control trial to evaluate neuroprotective strategies in preterm infants with NE is challenging due to the relatively low incidence and difficulties in accurately identifying signs of encephalopathy.

Adult Neuroprotection

In addition to the preterm and term infant populations, magnesium sulfate has been evaluated as a rescue therapy in adult neurological injuries. The proposed mechanism of benefit includes NMDA blockade as well as dilatation of penetrating cerebral arterioles.

The Intravenous Magnesium Efficacy in Stroke [77] (IMAGES) trial was a large double-blind randomized

controlled trial assessing the benefit of magnesium sulfate in acute ischemic strokes. The trial recruited 2,368 participants with a clinical diagnosis of stroke, aiming to start magnesium sulfate or placebo within 12 h from the onset of symptoms. Disappointingly, magnesium sulfate did not affect the primary outcome of death or disability 90 days after the event. There was however a significant improvement in a subgroup of patients with lacunar infarcts, mostly secondary to small cortical emboli.

The lack of efficacy in the IMAGES trial was thought to be a result of delayed magnesium therapy as only 3% of individuals received the drug within 3 h of symptoms. This led to the novel approach of prehospital initiation of therapy pioneered in the Field Administration of Stroke Therapy–Magnesium (FAST-MAG) trial in 2004 [78]. Saver et al. [79] subsequently enrolled 1,700 patients to receive magnesium sulfate or placebo within 2 h of symptom onset. Patients received a loading dose by paramedics and were started on a 24-h magnesium sulfate infusion on arrival to hospital. Magnesium sulfate was however not shown to reduce death or level of disability at 90 days. The trial primarily involved acute ischemic strokes (73%) rather than intracranial hemorrhage (23%). Subgroup analysis of stroke type did not show any alteration of treatment effect.

Trials of hemorrhagic strokes have mostly focused on the use of magnesium sulfate in aneurysmal subarachnoid hemorrhage. Approximately a third of survivors deteriorate 3–14 days after hemorrhage as a result of delayed cerebral ischemia. The underlying etiology of this process is likely multifactorial, including oxidative stress, vasoconstriction, inflammation and cortical spreading depression [80]. Magnesium sulfate was not found to improve clinical outcomes after aneurysmal subarachnoid hemorrhage in a large randomized controlled trial [81] and meta-analysis [82].

Limitations of Studies

Although the use of magnesium sulfate in fetal neuroprotection has shown promise in human clinical trials, results from neonatal and adult neurological injuries have been disappointing. There are a number of factors that may be contributing to this apparent lack of efficacy.

Magnesium levels in trials are usually measured in serum, which represent less than 1% of the total body content and do not accurately reflect intracellular levels [4]. Using serum levels alone to define a neuroprotective concentration may be insufficient if the neuroprotective mechanism

Fig. 3. Mean serum and CSF magnesium levels at baseline and after infusion in piglets – unpublished data (error bars represent standard deviation).

is through intracellular anti-inflammatory mechanisms in addition to synaptic NMDA receptor blockade.

Preclinical rodent data suggest a neuroprotective “target serum level” of approximately 2–3 mmol/L [83, 84], noting cardiodepressive effects at higher concentrations [84]. However, in vitro studies on rodent hippocampal neurons have suggested magnesium concentrations 2–4 times normal serum levels may be necessary to achieve benefit [85, 86]. Achieving at least double serum magnesium levels in the CSF may provide a challenge given the limited CSF penetration with peripherally infused magnesium. Pharmacokinetic data from adult neurosurgical studies demonstrated doubling plasma magnesium resulted in only a modest 11–21% increase in CSF levels [87]. We have demonstrated similar findings in a piglet model of NE (Fig. 3). Furthermore, CSF and serum magnesium levels do not correlate well following peripheral infusion. Levels in the serum rapidly rise within 30 min and then fall, whereas it takes 90 min before a significant rise is detected in the CSF [88].

The adage “time is brain” is a key principle underpinning successful neuroprotective strategies. Developing a delivery mechanism to achieve a “neuroprotective” magnesium concentration in the CSF whilst avoiding the toxicity associated with high serum levels represents a major challenge.

Conclusion

Magnesium sulfate could be considered as an adjunct to hypothermia with its inherent advantages of widespread availability, low cost and good safety profile. It has

been extensively evaluated in a number of different neurological disorders across all age groups from the preterm to the elderly subject. Evidence of benefit appears most convincing in fetal neuroprotection, possibly due to the increased susceptibility of the immature brain to excitotoxicity and increased infective and inflammatory risks associated with prematurity.

The use of magnesium sulfate in term NE however remains controversial. Early trials of magnesium sulfate in term infants with perinatal asphyxia were limited by small numbers, methodological heterogeneity and mostly predated the widespread implementation of therapeutic hypothermia. In the postcooling era, neuroprotective interventions are likely to take the form of adjuncts to incrementally improve outcomes beyond those achievable by hypothermia alone. Magnesium sulfate has been shown

to augment therapeutic hypothermia in adult rodent models; however, caution is warranted given possible adverse effects on neuronal cell architecture. Further preclinical evaluation is essential to ensure safety and efficacy of magnesium sulfate neuroprotection prior to further human clinical trials.

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