Magnesium Is Not Consistently Neuroprotective for Perinatal Hypoxia-Ischemia in Term-Equivalent Models in Preclinical Studies: A Systematic Review

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Key Words
Asphyxia · Brain injury · Cerebral palsy · Hypoxic-ischemic encephalopathy · Magnesium sulfate · Neuroprotection

Abstract
There is an important unmet need to further improve the outcome of neonatal encephalopathy in term infants. Meta-analyses of large controlled trials now suggest that maternal magnesium sulfate (MgSO\textsubscript{4}) therapy is associated with a reduced risk of cerebral palsy and gross motor dysfunction after premature birth, but that it has no effect on death or disability. Because of this inconsistency, it remains controversial whether MgSO\textsubscript{4} is clinically neuroprotective and, thus, it is unclear whether it would be appropriate to test MgSO\textsubscript{4} for treatment of encephalopathy in term infants. We therefore systematically reviewed the preclinical evidence for neuroprotection with MgSO\textsubscript{4} before or after hypoxic-ischemic encephalopathy (HIE) in term-equivalent perinatal and adult animals. The outcomes were highly inconsistent between studies. Although there were differences in dose and timing of administration, there was evidence that beneficial effects of MgSO\textsubscript{4} were associated with confounding mild hypothermia and, strikingly, the studies that included rigorous maintenance of environmental temperature or body temperature consistently suggested a lack of effect. On balance, these preclinical studies suggest that peripherally administered MgSO\textsubscript{4} is unlikely to be neuroprotective. Rigorous testing in translational animal models of perinatal HIE is needed before MgSO\textsubscript{4} should be considered in clinical trials for encephalopathy in term infants.

Introduction
Hypoxic-ischemic encephalopathy (HIE) around term remains a major cause of neonatal mortality and morbidity with lifelong chronic disabilities. Although therapeutic hypothermia for neonatal HIE improves survival without disability, with current protocols, nearly half of infants still die or survive with disability [1]. Thus, there is a crucial need to identify adjunct therapies to reduce the incidence and severity of HIE in near-term/term infants.
[2]. There has been considerable interest in magnesium sulfate (MgSO\(_4\)) because magnesium alleviates excitotoxic damage in vitro by binding to the magnesium site on the NMDA (N-methyl-D-aspartate) glutamate channel [3]; there is some evidence that it may also reduce secondary inflammation and associated injury [4], stabilize cell membranes [5], inhibit free radical production [5] and improve cardiovascular stability [6].

Supporting this, there is now evidence from meta-analyses of randomized controlled trials that antenatal administration of MgSO\(_4\) is associated with a small but significant reduction in the risk of cerebral palsy and gross motor dysfunction in early childhood after preterm birth [7, 8]. These meta-analyses included women in labor or at high risk of labor at \(\leq 33\) weeks gestation that received loading doses of 4–6 \(g\) of MgSO\(_4\) i.v. and/or a maintenance dose of 1–3 \(g/h\) or 5 \(g\) every 4 \(h\) i.m. However, although there was no significant change in the risk of death, there was a small trend in some of the trials, such that there was no net effect on overall death and disability [9, 10]. Thus, there has been controversy whether these findings truly demonstrate a direct neuroprotective effect, and the exact mechanisms how MgSO\(_4\) may confer neurological benefit remain controversial. Further, there is no strong clinical evidence that MgSO\(_4\) is neuroprotective for near-term/term infants with HIE after maternal administration [11] or neonatal administration [12–15].

The purpose of this review was to systematically evaluate preclinical studies on magnesium for perinatal neuroprotection and identify knowledge gaps that need to be addressed before magnesium-induced neuroprotection could be considered for pragmatic trials at term.

**Analysis Strategy**

In this review, an international group of researchers focusing on perinatal neuroprotection have examined the preclinical evidence for the therapeutic potential of MgSO\(_4\) for HIE. The studies were grouped into those that demonstrated improved or unaffected neural outcomes based on histological and/or behavioral assessments. Studies were further subdivided by species, age (perinatal vs. adult), type of insult used to induce HIE, treatment regime, timing of initiation of treatment (before or after the insult), extent of temperature monitoring (brain, core, body surface, ambient and none), main study outcomes (histological and/or behavioral) and survival time after treatment.

Studies were searched for on PubMed and MEDLINE (OvidSP) using the following terms: (brain injury OR hypoxia-ischemia OR cerebral ischemia) AND (magnesium OR MgSO\(_4\)). Other sources used to identify studies included relevant original manuscripts and reviews. We did not set a date limit to the search. Studies were deemed eligible if they presented clear histological and/or behavioral outcomes from in vivo experiments that investigated magnesium for neuroprotection. Abstracts were initially identified and screened by an unbiased investigator from our study group and duplicated by another investigator. Selection of relevant studies was by group consensus.

Inclusion criteria for the perinatal studies were in vivo studies of rodents on postnatal day 6 (P6) to P21, sheep at 0.85 gestation and term piglets, as these developmental ages broadly reflect cortical maturity of term infants. We excluded in vitro studies and those that did not meet these age criteria. For the adult studies, inclusion criteria were in vivo studies of ischemic brain disease, i.e. global ischemia or permanent and temporary focal ischemia, as these are well-established experimental paradigms of cardiac arrest and stroke/subarachnoid hemorrhage with distinct and well-defined patterns of regional brain injury. We excluded studies that did not assess the effects of magnesium independent of adjuvant therapy.

The methodological quality of the studies was assessed according to whether temperature was controlled, treatment was randomized and investigators were blinded to the intervention during histological and behavioral assessments. The results are summarized in tables 1 and 2.

**Perinatal Studies of Magnesium for Term HIE**

We identified and screened a total of 66 studies. Thirty-eight were excluded due to inappropriate developmental age, ex vivo studies or lack of assessment of the effects of magnesium independent of adjuvant therapy (adult studies only). Thus, 28 studies were surveyed in this study. Studies were then stratified by treatment regime, i.e. treatment before or after the insult, outcome and combination with other therapies.

Most of the neonatal and adult rodent studies measured infarct area or volume and performed semiquantitative assessments of neuronal apoptosis to examine neuronal loss/survival/severity of the insult. One neonatal rat study combined semiquantitative (graded) assessment of neuronal loss with the incidence of cyst formation and one study measured cerebral hemisphere weight as a broad indicator for improved neurodevelopmental outcome. In large animal studies, assessments of seizures in...
### Table 1. Perinatal studies of magnesium for HIE in at-term or near-term equivalents

| First author | Species | Insult | Regime | Timing | Temperature | (Histopathological) Outcome | Behavioral outcome | Surviv-
al |
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<tbody>
<tr>
<td><strong>Neuroprotection with magnesium</strong></td>
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<tr>
<td>Sameshima [20]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>270 mg/kg MgSO₄ i.p.</td>
<td>pre Tx: ~30 min</td>
<td>ambient t° 33°C during and after HI</td>
<td>i moderate and severe neuronal loss, p = 0.04</td>
<td>7 days</td>
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<tr>
<td>Turkylmaz [21]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>200 mg/kg MgSO₄ i.p. (n = 9) vs. vehicle (n = 9)</td>
<td>pre Tx: immediately before HI</td>
<td>no</td>
<td>i neuronal apoptosis index: MgSO₄ + HI: 36.6±22.1 vs. HI + vehicle: 61.0±16.0, p &lt; 0.05</td>
<td>3 days</td>
<td></td>
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<tr>
<td>Cetinkaya [22]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>3 × 275 mg/kg MgSO₄ i.p. (n = 6) ± melatonin 20 mg/kg i.p. vs. vehicle (n = 6)</td>
<td>pre ± post Tx: immediately before and at 0 and 24 h</td>
<td>no</td>
<td>i infarct volume by 23.7±5.1%: 28.9±1.9 fewer cortical apoptotic cells/unit area after MgSO₄ + HI vs. HI + vehicle, p &lt; 0.05 combination not different to monotherapy</td>
<td>3 days</td>
<td></td>
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<tr>
<td>Sameshima [23]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>loading dose 270 mg/kg MgSO₄ i.p. + maintenance dose 72 mg/kg/h MgSO₄ s.c. for 72 h (n = 23), maintenance only (n = 26) vs. vehicle (n = 46)</td>
<td>post Tx: 1.5 h</td>
<td>ambient t° 32°C during and after HI</td>
<td>i cyst formation by 31 and 37% in HI + MgSO₄ maintenance and loading + maintenance groups, respectively</td>
<td>7 days</td>
<td></td>
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<tr>
<td>Spandou [24]</td>
<td>P7 rat</td>
<td>HI: 1 h 8% O₂</td>
<td>4 × 500 mg/kg MgSO₄ i.p. (n = 20) vs. vehicle (n = 10)</td>
<td>post Tx: 0–16 h</td>
<td>no</td>
<td>i severity of neuronal loss 80% HI + MgSO₄ showed mild injury vs. 70% HI + vehicle showed moderate to severe injury, p &lt; 0.05</td>
<td>5 days</td>
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<tr>
<td>Pazaiti [25]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>4 × 500 mg/kg MgSO₄ i.p. (n = 29) vs. vehicle (n = 41)</td>
<td>post Tx: 0–16 h</td>
<td>no</td>
<td>i severity of neuronal loss 55% HI + MgSO₄ showed mild injury vs. 70% HI + vehicle showed moderate/severe injury, p &lt; 0.05</td>
<td>35 days</td>
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<tr>
<td>Thordstein [27]</td>
<td>P8 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>300 mg/kg MgSO₄ + FRS i.p. (n = 34) vs. vehicle (n = 34)</td>
<td>post Tx: 0 h</td>
<td>ambient t° 37°C during insult and 22°C after the insult</td>
<td>i loss of cerebral hemisphere weight; HI + MgSO₄ + FRS: 0.7% loss vs. HI + vehicle: 8.9% loss, relative to contralateral hemisphere, p &lt; 0.05 no monotherapy group</td>
<td>13 days</td>
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<tr>
<td>Goni-de-Cerio [26]</td>
<td>fetal sheep (0.85 g.a.)</td>
<td>partial UCO</td>
<td>400 mg/kg MgSO₄ i.v. (n = 10) vs. vehicle (n = 10)</td>
<td>post Tx: 20 min</td>
<td>core t° 37–39°C</td>
<td>85% i in cortical reactive oxygen species, 74% i in apoptotic cells, 29% i in intracellular Ca²⁺ after UCO + MgSO₄ vs. UCO + vehicle, p &lt; 0.05</td>
<td>2.5 h</td>
<td></td>
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<tr>
<td><strong>No neuroprotection with magnesium</strong></td>
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<tr>
<td>Gunn [28]</td>
<td>P21 rat</td>
<td>HI: 15 min 8% O₂ (n=15) vs. vehicle (n=15)</td>
<td>450 mg/kg MgSO₄ i.p.</td>
<td>post Tx: 1 h</td>
<td>ambient t° 34°C during and for 6 h after HI</td>
<td>no change in hippocampal neuronal loss HI + MgSO₄: 42±5% vs. HI + vehicle: 40±6% or infarction</td>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td>Galvin [29]</td>
<td>P7 rat</td>
<td>HI: 1.5 h 8% O₂ (n = 11) vs. vehicle (n = 11)</td>
<td>300 mg/kg MgSO₄ s.c.</td>
<td>post Tx: 0 h + daily to P13</td>
<td>ambient t° 37°C during HI</td>
<td>no improvement in striatal neuronal survival; HI + MgSO₄: 43% vs. HI + vehicle: 45%</td>
<td>11 days</td>
<td></td>
</tr>
<tr>
<td>Sameshima [20]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>270 mg/kg MgSO₄ i.p. (n = 24) vs. vehicle (n = 26)</td>
<td>post Tx: 30 min</td>
<td>ambient t° 33°C before, during and after HI</td>
<td>i cyst formation by 55%, p = 0.001 and 1 severity of neuronal loss, p = 0.01, after HI + MgSO₄ vs. HI + vehicle</td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td>Turkylmaz [21]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>200 mg/kg MgSO₄ i.p. (n = 9) vs. vehicle (n = 9)</td>
<td>post Tx: 0 h</td>
<td>no</td>
<td>no reduction in hippocampal neuronal apoptosis index in HI + MgSO₄: 46.9±8.6 vs. HI + vehicle: 60.0±14.0</td>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td>Spandou [24]</td>
<td>P7 rat</td>
<td>HI: 2 h 8% O₂</td>
<td>4 × 500 mg/kg MgSO₄ i.p. (n = 20) vs. vehicle (n = 10)</td>
<td>post Tx: 0, 2, 14 and 16 h</td>
<td>no</td>
<td>no reduction in neuronal loss in all studied regions in HI + MgSO₄ vs. HI + vehicle</td>
<td>7 days</td>
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</table>
fetal sheep using electroencephalography and brain energy metabolism in piglets using magnetic resonance spectroscopy were combined with histopathological assessments of brain injury. The majority of the studies surveyed presented data as continuous variables, however, 5 perinatal studies presented data using graded assessment.

Fifteen studies examined the effectiveness of MgSO₄ for neuroprotection in term or near-term HIE. The great majority of the studies (12/15) were performed in near-term fetal lambs, MgSO₄ was infused from 2 h before umbilical cord occlusion and similarly showed no benefit compared with 3/5 that found no effect [21, 24, 28, 29]. Most of these studies used relatively short survival times of <1–5 days (4/9) or 7–13 days (4/9); just 1 study examined outcomes after 35 days of recovery [25]; this study was also the only one to report improved sensorimotor function.

Seven perinatal studies reported no improvement with MgSO₄ after HI, all after short to medium survival times (2–11 days) [20, 21, 24, 28–32]. All of the rodent studies and 1 piglet study showing no benefit involved treatment after the insult [21, 24, 28, 29, 31, 32]. In the study in near-term fetal lambs, MgSO₄ was infused from 2 h before umbilical cord occlusion and similarly showed no benefit [30].

In rodent studies, 2/7 that reported neuroprotection controlled the environmental temperature during HI [20, 23], compared with 3/5 that found no effect [20, 28, 29]. None of the rodent studies directly measured brain or body temperature. The piglet study reported that rectal temperature was controlled [31, 32]. Although details were not provided in the fetal sheep study [30], other stud-

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**Table 1 (continued)**

<table>
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<tr>
<th>First author</th>
<th>Species</th>
<th>Insult</th>
<th>Regime</th>
<th>Timing</th>
<th>Temperature</th>
<th>(Histopathological) Outcome</th>
<th>Behavioral outcome</th>
<th>Survival</th>
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<tbody>
<tr>
<td>de Haan [30]</td>
<td>fetal sheep (0.85 g.a.)</td>
<td>4 × 5 min UCO</td>
<td>load: 300 mg MgSO₄ i.v., maintenance: 100 mg/h i.v. over 4.75 h (n = 9) vs. vehicle (n = 11)</td>
<td>pre + post Tx: 2–1 h after the last UCO</td>
<td>no change in core t°</td>
<td>no effect on EEG or neuronal loss in all regions studied after MgSO₄ + UCO vs. vehicle + UCO</td>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td>Penrice [31], Greenwood [32]</td>
<td>P1 piglets</td>
<td>bilateral CAO + 12% FeO₂</td>
<td>load: 1 × 400 mg/kg MgSO₄ i.v. + 2 × 200 mg/kg i.v. (n = 6) vs. vehicle (n = 6)</td>
<td>post Tx: 1, 12 and 24 h after ischemia</td>
<td>no change in rectal and tympanic t°</td>
<td>no 1 in secondary energy failure measured on MRS or gray and white matter injury in all regions studied in HI + MgSO₄ vs. HI + vehicle</td>
<td>2 days</td>
<td></td>
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</table>

CAO = Carotid artery occlusion; EEG = electroencephalography; FRS = free radical scavengers; g.a. = gestational age; MRS = magnetic resonance spectroscopy; t° = temperature; Tx = treatment; UCO = umbilical cord occlusion. Unless specified, data are means ± SD.
Table 2. Adult studies of magnesium for HI injury

<table>
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<tr>
<th>First author</th>
<th>Species</th>
<th>Insult</th>
<th>Regime</th>
<th>Timing</th>
<th>Temperature</th>
<th>(Histological) Outcome</th>
<th>Behavioral outcome</th>
<th>Survivial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marinov [35]</td>
<td>adult rat</td>
<td>temporary</td>
<td>MCAO</td>
<td>30 (n = 24) or 90 mg/kg MgSO4 (n = 18) i.a. vs. vehicle (n = 23)</td>
<td>pre Tx: immediately before occlusion</td>
<td>single core t¹ value 45 min post occlusion</td>
<td>28 and 60% ↓ in infarct volume in 30 and 90 mg/kg MgSO4 + MCAO groups vs. vehicle + MCAO, respectively, p &lt; 0.001</td>
<td>1 day</td>
</tr>
<tr>
<td>Sirin [36]</td>
<td>adult rat</td>
<td>GCI</td>
<td>(600 mg/kg; n = 12) s.c. vs. vehicle (n = 12)</td>
<td>pre Tx: 48 h before ischemia</td>
<td>data not presented</td>
<td>↓ hippocampal neuronal loss in MgSO4 + GCI = 36 ± 9% vs. vehicle + GCI = 47 ± 12%, p &lt; 0.05</td>
<td>4 days</td>
<td></td>
</tr>
<tr>
<td>Lee [37]</td>
<td>adult rat</td>
<td>permanent</td>
<td>MCAO</td>
<td>90 mg/kg MgSO4 i.a. (n = 9) vs. vehicle (n = 11)</td>
<td>pre Tx: 10 min before occlusion</td>
<td>data not presented</td>
<td>~35% ↓ in total brain infarct volume in MgSO4 + MCAO vs. vehicle + MCAO, p &lt; 0.05</td>
<td>1 day</td>
</tr>
<tr>
<td>Westermaier [38]</td>
<td>adult rat</td>
<td>temporary</td>
<td>MCAO</td>
<td>0.75 mmol/kg (100 mg/kg; MgSO4) i.a. (n = 10) or i.v. (n = 10) vs. vehicle (n = 10)</td>
<td>pre Tx: 15 min before occlusion</td>
<td>data not presented</td>
<td>no effect on infarct volume</td>
<td>Improved neurological deficit score in i.a. and i.v. MgSO4 + MCAO vs. vehicle + MCAO, p &lt; 0.05</td>
</tr>
<tr>
<td>Zhou [39]</td>
<td>adult gerbil</td>
<td>GCI</td>
<td>16 mmol/kg MgSO4 i.p. (1.9 g/kg; n = 12) vs. vehicle (n = 12)</td>
<td>pre Tx: 30 min before ischemia</td>
<td>no</td>
<td>↓ hippocampal neuronal apoptosis; average apoptotic neurons in MgSO4 + GCI = 35.6 ± 5.8 vs. vehicle + GCI = 60.3 ± 6.8, p &lt; 0.01</td>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td>Chung [40]</td>
<td>adult gerbil</td>
<td>permanent</td>
<td>MCAO</td>
<td>90 mg/kg MgSO4 i.v. (n = 7) vs. vehicle (n = 8)</td>
<td>pre Tx: 30 min before occlusion</td>
<td>no</td>
<td>25% ↓ in total infarct volume in MgSO4 + MCAO vs. MgSO4 + vehicle, p &lt; 0.05</td>
<td>1 day</td>
</tr>
<tr>
<td>Izumi [41]</td>
<td>adult rat</td>
<td>permanent</td>
<td>MCAO</td>
<td>2 × 1 mmol/kg MgCl2 i.p. (2 × 120 mg/kg; n = 16) vs. vehicle (n = 16)</td>
<td>post Tx: immediately after occlusion and 1 h after occlusion</td>
<td>intermittent core t¹ monitoring after occlusion</td>
<td>26% ↓ in total brain infarct volume in MgCl2 + MCAO vs. vehicle + MCAO, p &lt; 0.05</td>
<td>2 days</td>
</tr>
<tr>
<td>Yang [42]</td>
<td>adult rat</td>
<td>permanent</td>
<td>MCAO</td>
<td>90 mg/kg i.v. (n = 20) vs. vehicle (n = 10)</td>
<td>post Tx: 2, 6 or 8 h after occlusion</td>
<td>data not presented</td>
<td>60% and 48% ↓ in total brain infarct volume in the 2- and 4-hour injury groups, respectively, vs. vehicle + MCAO, p &lt; 0.05</td>
<td>3 days</td>
</tr>
<tr>
<td>Zhu [45]</td>
<td>adult rat</td>
<td>GCI</td>
<td>load: 40 mg/kg MgSO4 i.v. + 6 h hypothermia (n = 6) or mainte- nance: 10 mg/kg/h MgSO4 i.v. over 48 h + 24 h hypothermia (n = 6) vs. vehicle (n = 6)</td>
<td>post and pre Tx: loading dose: before insult, maintenance dose: 2 h after the insult, hypothermia started 2 h after the insult</td>
<td>core t¹ 37°C during and after the insult or 35°C after the insult (hypothermia groups)</td>
<td>38% and 76% ↑ in neuronal survival in MgSO4 + GCI loading dose + 6-hour hypothermia and maintenance dose + 24-hour hypothermia groups, respectively, vs. vehicle + GCI (n = 6), p &lt; 0.05 no effect of single MgSO4 therapy</td>
<td>7 days</td>
<td></td>
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<tr>
<td>Campbell [46]</td>
<td>adult rat</td>
<td>permanent</td>
<td>MCAO</td>
<td>load: 360 μmol/kg i.v. + maintenance: 120 μmol/kg/h i.v. over 25 h (n = 9) + hypothermia: t° 35°C over 25 h (n = 17) vs. vehicle (n = 17)</td>
<td>post Tx: 2 or 4 h after insult</td>
<td>core t¹ 37–37.5°C during and after the insult vs. 35°C after the insult</td>
<td>48% and 39% ↓ in total infarct volume with 2- and 4-hour combination therapy, respectively, vs. vehicle + MCAO, p &lt; 0.05 no effect of MgSO4 monotherapy</td>
<td>2 days</td>
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<tr>
<td>Song [47]</td>
<td>adult rat</td>
<td>temporary</td>
<td>MCAO</td>
<td>cold (15°C) i.a. MgCl2 120 mg/kg over 20 min (n = 12) or saline (n = 12) vs. vehicle (n = 12)</td>
<td>post Tx: 3 h after insult</td>
<td>local brain t° 37°C during and after the insult vs. 33–34°C after the insult</td>
<td>~64% ↓ in total infarct volume with combination therapy and improved neurological deficit score vs. vehicle + MCAO, p &lt; 0.01 no effect of MgSO4 monotherapy</td>
<td>2 days</td>
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**No neuroprotection with magnesium**

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<tr>
<th>First author</th>
<th>Species</th>
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<th>(Histological) Outcome</th>
<th>Behavioral outcome</th>
<th>Survivial</th>
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<tbody>
<tr>
<td>Blair [43]</td>
<td>adult rat</td>
<td>GCI</td>
<td>MgCl2 5 mmol/kg i.v. (600 mg/kg; n = 6) vs. vehicle (n = 8)</td>
<td>pre Tx: 10 min before ischemia</td>
<td>core t¹ 37°C</td>
<td>1% hippocampal neuronal injury in MgCl2 + GCI = 79 ± 4% vs. vehicle + GCI = 68 ± 10%, p &lt; 0.05</td>
<td>7 days</td>
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Magnesium for Hypoxic-Ischemic Brain Injury

DOI: 10.1159/000362206
ies have shown no significant effect of ischemia or asphyxia or drug treatments on cortical temperatures of fetal sheep [33, 34].

**Adult Studies of Magnesium for Cerebral Ischemia**

All of the adult studies in rodent models of cerebral ischemia used either (1) global ischemia induced by bilateral carotid artery occlusion or (2) focal ischemia using middle cerebral artery occlusion. Eight studies reported improved histological outcome for MgSO₄ alone [35–42], of which only 2/8 documented temperature at limited times after ischemia [35, 41]. Seven studies reported no improvement in histological outcome with MgSO₄ or MgCl₂ [43–48]. In all of these studies, postischemic temperature was monitored continuously and normothermia was maintained. Finally, 3 studies reported improved histological outcome when MgSO₄ was combined with hypothermia, all of which included maintenance of the target temperatures [45–47]. Survival times varied from 1 to 7 days. No study in either group reported long-term outcome, and only 2 reported any behavioral outcome [38, 47].

### Discussion

This systematic review shows that the effect of MgSO₄ treatment before or shortly after acute hypoxia-ischemia (HI) at term or near-term equivalent was highly inconsistent between studies. Of considerable concern, none of the perinatal rodent studies suggesting benefit directly controlled brain or body temperature, and most did not adequately control environmental temperatures. Similarly, none of the studies suggesting benefit in adult rodents closely monitored core or brain temperature, and only 2 studies included limited/intermittent measurements after ischemia. Conversely, it is striking that the majority of studies that did control environmental or body temperature did not find significant protection. Many studies in adult rodents reported that beneficial effects of MgSO₄ were associated with confounding mild hypothermia and that maintenance of normothermia abolished neuroprotection. Supporting this concern, large animal, ‘translational models’ of perinatal injury in which body temperature was either directly controlled or

<table>
<thead>
<tr>
<th>First author</th>
<th>Species</th>
<th>Insult</th>
<th>Regime</th>
<th>Timing</th>
<th>Temperature</th>
<th>(Histological) Outcome</th>
<th>Behavioral outcome</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu [44]</td>
<td>adult rat</td>
<td>GCI</td>
<td></td>
<td>pre and post Tx: loading dose: immediately before ischemia, maintenance dose: 2 h after ischemia</td>
<td>core t° 37°C after ischemia</td>
<td>no improvement in neuronal survival</td>
<td>7 days</td>
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<tr>
<td>Zhu [45]</td>
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<td>pre and post Tx: loading dose: immediately before ischemia, maintenance dose: 2 h after ischemia</td>
<td>core t° 37°C after ischemia</td>
<td>no improvement in neuronal survival</td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td>Campbell [46]</td>
<td>adult rat</td>
<td>permanent MCAO</td>
<td></td>
<td>post Tx: 3 h after occlusion</td>
<td>core t° 37°C after ischemia</td>
<td>no improvement in infarct volume at 48 h</td>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td>Song [47]</td>
<td>adult rat</td>
<td>temporary MCAO</td>
<td></td>
<td>post Tx: 3 h after occlusion</td>
<td>core t° 37°C after ischemia</td>
<td>no improvement in infarct volume, edema or neurological severity score</td>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td>Milani [48]</td>
<td>adult rat</td>
<td>GCI</td>
<td></td>
<td>post Tx: 2 h after ischemia or 1, 2, 24 and 48 h after ischemia</td>
<td>no differences in rectal t° during and 3.5 h after ischemia</td>
<td>no improvement in CA1 neuronal loss</td>
<td>7 days</td>
<td></td>
</tr>
</tbody>
</table>

GCI = Global cerebral ischemia; MCAO = middle cerebral artery occlusion; t° = temperature; Tx = treatment. Unless specified, data are means ± SD.
known to be highly stable suggested lack of effect after 2 or 3 days of recovery [30–32].

**Bioavailability**

One of the great unknowns associated with MgSO₄ for perinatal neuroprotection is whether direct or maternal administration can raise brain and cerebrospinal fluid magnesium concentrations to the level required for neuroprotection in the fetus or neonate. In culture, high concentrations of MgSO₄ are required to reduce NMDA channel activity [3]. In this setting, increased extracellular magnesium is associated with recovery of high-energy phosphate stores, improved rates of protein synthesis and neuronal preservation in ischemic hippocampal neurons from fetal and adult rodents [49, 50] and after global ischemia in adults when magnesium was given by direct intrahippocampal injection [51]. However, benefit required a 2- to 4-fold increase in extracellular magnesium concentrations. It is not clear whether it is possible to achieve such concentrations after peripheral administration. While in vivo studies have used measures of brain and cerebrospinal fluid excitotoxins, namely glutamate, to indirectly measure the local effect of systemically administered MgSO₄, these measurements are relatively crude, have produced conflicting data and are confounded by a lack of body temperature monitoring [24, 52]. Brain and cerebrospinal fluid magnesium concentrations are tightly controlled, such that in healthy dogs and rats, hypermagnesemia (3–4 times increased) was associated with modest changes in cerebrospinal fluid and tissue magnesium concentrations [53, 54]. Prolonged systemic magnesium administration was associated with a much smaller increase in magnesium levels in brain parenchyma (from approximately 0.30 to 0.47 mmol/l) than the increase in the systemic circulation (from approximately 0.8 to 2.5 mmol/l) [55].

**Vascular Effects**

Alternatively, it is plausible that MgSO₄ neuroprotection in preclinical studies may reflect vasodilation of cerebral microvessels through binding to calcium receptors in vascular smooth muscle [56]. In goats, both intravenously and after direct injection into the cerebral circulation, MgSO₄ was associated with increased cerebral perfusion and reduced vascular resistance [57]. Thus, it is possible that pretreatment with MgSO₄ in models of carotid ligation may promote an essentially artifactual improvement in perfusion during hypoxia [36, 39, 45].

Further, magnesium promotes vasodilation of peripheral vascular beds, including the mesenteric, renal, skeletal muscle and skin in rodents and larger animals [59]. Increased cutaneous perfusion is likely to increase heat loss through radiation; this may be considerable in small animals with a relatively large surface area and low thermal stability [60]. None of the perinatal studies assessed brain temperature; this may be important since in small animals, with relatively flat skulls, brain temperature can vary independently of body temperature [60]. Given that a mild reduction in body temperature can be neuroprotective [18, 60] and that the majority of studies suggesting neuroprotection did not rigorously control brain or body temperature (tables 1, 2), it is plausible that magnesium may be associated with iatrogenic neuroprotection through hypothermia. Consistent with this, adult rodents treated with magnesium and allowed to self-regulate body temperature after global cerebral ischemia had mildly lower core temperatures and better hippocampal neuronal survival than controls [44]. Conversely, maintenance of normothermia after ischemia abolished the apparent neuroprotection.

**Potential for Adjunct Therapy**

No studies of combined hypothermia and magnesium therapy in perinatal animals were identified. In adult rats, pretreatment with MgSO₄ before global cerebral ischemia [44], combined with mild spontaneous hypothermia after ischemia, reduced hippocampal neuronal loss more than MgSO₄ or hypothermia alone [45]. Further, combined treatment with MgSO₄ and mild hypothermia after permanent middle cerebral artery occlusion showed additive protection when administration of MgSO₄ was delayed by 2 or 4 h [46]. Similarly, intracarotid infusion of cold MgSO₄ (33–34°C) after focal brain ischemia was associated with a reduction in infarct volume, cerebral edema and neurological severity scores compared to saline or normothermic MgSO₄ (37°C) infusion alone [47]. Thus, combination therapy is promising but it has not yet been tested for neonatal HIE. One trial of hypothermia with MgSO₄ (Mag Cool) is currently listed on ClinicalTrials.Gov.

**Survival Time after Treatment**

The other major limitation of the perinatal studies identified in this review is that many used relatively short survival times, from 2.5 h to 5 days, and only 1 study reported improved neurobehavioral outcome. These shorter survival times provide important information about acute histological outcomes, however, brain injury
can evolve for many weeks [61], and neurodevelopmental outcome can be discordant from histological outcomes [60]. In adult rodents treated with MgSO₄ for cerebral injury, studies of survival times ≥7 days have rarely reported significant neuroprotection [44, 45, 48, 62], and 9% of the adult studies surveyed in this review showed neuroprotective effects of magnesium per se after 7 days. Similarly, 43% of perinatal studies identified in the present review demonstrated no improvement in neurodevelopmental outcome after survival times of 7–11 days.

While we were not able to specifically assess publication bias, most of the studies randomized treatment and or used blinded assessors for histological and behavioral examinations. However, 2 perinatal studies (1 from each category) and 3 adult studies (2 showed improved outcomes and 1 showed no improvement) did not document randomization or binding as part of their experimental protocol. Further, based on the major methodological differences in studies surveyed and lack of a common, specific outcome measure, we could not perform a meta-analysis of the studies surveyed. We found that all rodent studies that associated magnesium with neuroprotection did not perform adequate brain and/or body temperature monitoring, allowing for neuroprotection to be associated with confounding mild hypothermia rather than magnesium per se. Collectively, these methodological flaws indicate that further studies are required before attempting a meta-analysis of preclinical data for the neuroprotective benefit of magnesium.

Conclusion

This review of preclinical studies of MgSO₄ for near-term HIE highlights the highly inconsistent histopathological impact of treatment both before and after the insult. These inconsistencies are likely related to a lack of temperature control during and after HI, along with variability in the dose, timing of treatment and survival time after the insult. These findings strongly suggest that clinical trials of MgSO₄ for encephalopathy at term would be premature. Finally, despite very promising results from studies of global or focal ischemia in adult rodents, we were unable to identify any studies of MgSO₄ combination therapy in the developing brain. Taken collectively, these observations suggest a crucial need for further testing in translational animal models of HIE before magnesium should be considered for pragmatic trials as a potential adjunct therapy with hypothermia.

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