In Search of Clinical Neuroprotection After Brain Ischemia
The Case for Mild Hypothermia (35°C) and Magnesium

Bruno P. Meloni, PhD; Kym Campbell, BVMS; Hongdong Zhu, PhD; Neville W. Knuckey, MD

Background and Purpose—Brain injury after stroke and other cerebral ischemic events is a leading cause of death and disability worldwide. Our purpose here is to argue in favor of combined mild hypothermia (35°C) and magnesium as an acute neuroprotective treatment to minimize ischemic brain injury.

Methods and Results—Drawing on our own experimental findings with mild hypothermia and magnesium, and in light of the moderate hypothermia trials in cardiac arrest/resuscitation and magnesium trials in ischemic stroke (IMAGES, FAST-Mag), we bring attention to the advantages of mild hypothermia compared with deeper levels of hypothermia, and highlight the existing evidence for its combination with magnesium to provide an effective, safe, economical, and widely applicable neuroprotective treatment after brain ischemia. With respect to effectiveness, our own laboratory has shown that combined mild hypothermia and magnesium treatment has synergistic neuroprotective effects and reduces brain injury when administered several hours after global and focal cerebral ischemia.

Conclusions—Even when delayed, combined treatment with mild hypothermia and magnesium has broad therapeutic potential as a practical neuroprotective strategy. It warrants further experimental investigation and presents a good case for assessment in clinical trials in treating human patients after brain ischemia. (Stroke. 2009;40:2236-2240.)

Key Words: stroke ▶ cerebral ischemia ▶ magnesium ▶ hypothermia ▶ combination therapy

Brain ischemia is a medical emergency with few specific treatments available to minimize the acute injury. Current therapies are limited to clot removal (tPA thrombolysis, intraarterial fibrinolysis, mechanical removal), aspirin, and decompressive hemicraniectomy for ischemic stroke,1 and moderate hypothermia (33°C) for cardiac arrest.2 Our attention here is on 2 interventions that, individually, have been incompletely investigated (mild hypothermia; Table 1) or found wanting at clinical trial (magnesium; Table 2). We contend that, when combined, these 2 interventions have the potential to be safe, practical, and broadly applicable as therapy for brain ischemia. For the purposes of this review we define mild hypothermia as body temperatures from 36.5 to >34.5 and moderate as 34.5 to 30.0°C.

Hypothermia and Neuroprotection
The broad principles of therapeutic hypothermia established from animal studies are that earlier induction is better than later, increased depth of hypothermia increases efficacy, longer durations (12 to 72 hours) are more effective than shorter (<12 hours), and slower rewarming is better than faster.17 Increasing the depth of hypothermia, however, comes at an escalating cost to some nonneurological body systems. Moderate hypothermia by surface cooling, as applied in comatose cardiac arrest and stroke patients, requires patients to be intubated, ventilated, and in intensive care and is associated with considerable morbidity, making it poorly amenable to routine use. While the development of intravascular cooling catheters has made moderate hypothermia achievable in awake patients,18 the process is invasive and requires specialized equipment and staff.

Mild hypothermia, by contrast, can be achieved in awake patients outside of intensive care units using intravenous cold saline and external cooling devices (helmets, neck, torso vests),19,20 and it largely avoids the deleterious effects of deeper hypothermia. Furthermore, mild hypothermia is likely to be less inhibitory of endogenous neurosurvival pathways and has been shown to optimize cerebral oxygen delivery and consumption in traumatic brain injured patients.19 Experimental evidence shows that short durations of intraschismic mild hypothermia are neuroprotective; more importantly, delayed induction of mild hypothermia, if prolonged (24 hours), appears to be as efficacious as moderate hypothermia.20,21

As yet, no large scale study has assessed the neurological outcome of induced mild hypothermia after ischemic stroke, though several pilot trials are complete, planned, or underway (Table 1). From these trials and from studies using healthy volunteers it has been established that mild hypothermia can be safely induced in waking patients for up to 24 hours.
provided that shivering and discomfort are medically controlled.22–24 With considerable safety and practical advantages, and proven experimental efficacy, prolonged therapy with mild hypothermia warrants more attention.

Magnesium and Neuroprotection
With several postulated mechanisms of action,14,25 magnesium salts have been shown to be neuroprotective in animal models of cerebral ischemia, seizure, subarachnoid hemorrhage (SAH), and traumatic brain injury (TBI). Clinical trials have already been conducted for ischemic stroke (Table 2), SAH, and TBI. The IMAGES trial (12-hour treatment window) found magnesium to be largely ineffective, with a benefit in lacunar strokes only.14 However, because of concerns that IMAGES applied an unrealistically long enrolment period, the FAST-Mag trial (2-hour treatment window) was established, and this trial is ongoing.16 The phase 2 SAH trial suggested that magnesium reduced the occurrence of delayed cerebral ischemic events and of poor outcomes.26 In contrast, a phase 3 TBI trial suggested that magnesium tended to worsen outcomes (though magnesium is still administered as standard of care to hypomagnesemic TBI patients).27

In consequence of the negative IMAGES report, we reviewed the preclinical studies evaluating magnesium treatments for global and focal cerebral ischemia.25 From this review it emerged that in many of the experimental ischemia studies, magnesium did not in fact show a significant neuroprotective effect. Crucially, in the majority of the positive studies, the animals’ postischemic body temperatures were not reported; thus it was not possible to rule out the confounding effects of spontaneous hypothermia. It remained to be proven, then, that magnesium has neuroprotective properties beyond those conferred by hypothermia.

Experimental Mild Hypothermia and Magnesium Studies
In investigating this point we have found that, in rats controlled to be normothermic, magnesium alone is not effective in reducing brain damage after either focal or global ischemia.28,29 However, in rats that do not have their body temperatures actively regulated after ischemia, the animals experience a 1- to 5-hour period of mild hypothermia; these animals do show neuroprotection, but only if magnesium-treated (that is, the hypothermia is necessary, but not sufficient to impart the observed neuroprotective effect).29,30 The effect is preserved, too, with delayed treatment. Commencing 2 hours after global ischemia, a 24-hour duration of controlled mild hypothermia (35°C) alone was neuroprotective, but when combined with an 48-hour intravenous infusion of magnesium the level of protection was almost doubled (improved from 43% to 76%; Figure 1).31

In a permanent focal ischemia model starting treatments 2 hours postischemia, we evaluated mild hypothermia (35°C for 24 hours), magnesium (24-hour i.v. infusion), and the two in combination.32 Under these conditions, only the combination treatment with magnesium and mild hypothermia significantly reduced infarct volumes when compared with saline controls (by 54%) (Figure 2A). The

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Table 1. Clinical Trials of Mild Hypothermia in Cardiac Arrest and Ischemic Stroke

<table>
<thead>
<tr>
<th>Study</th>
<th>Method of Cooling/Treatment</th>
<th>No. of Subjects: Hypothermic/Controls</th>
<th>Delay From Ischemic Episode</th>
<th>Temperature</th>
<th>Duration</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac arrest</td>
<td>Cold saline infusion; Patients comatose</td>
<td>63/62</td>
<td>In field; usually within 1 hour</td>
<td>34–35°C</td>
<td>Variable; up to 24 hours</td>
<td>Trend to improved outcomes</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>Surface cooling; Patients awake; pethidine treated</td>
<td>17/56</td>
<td>Within 12 hours</td>
<td>35.5°C</td>
<td>6–10 hours</td>
<td>No serious side effects &amp; treatment not associated with poor outcome</td>
</tr>
<tr>
<td>Controlled hypothermia in large infarction (CHILI)</td>
<td>Surface cooling; Patients awake; pethidine &amp; surface warming to control shivering</td>
<td>0/0</td>
<td>Within 72 hours</td>
<td>35°C</td>
<td>48 hours</td>
<td>Trial on hold</td>
</tr>
<tr>
<td>Cool brain-stroke trial</td>
<td>Surface cooling; Patients unconscious</td>
<td>8/6</td>
<td>Within 24 hours</td>
<td>33–35°C</td>
<td>48–72 hours</td>
<td>Passive re-warming. No serious side effects or rebound hyperthermia</td>
</tr>
<tr>
<td>Nordic cooling stroke study</td>
<td>Surface cooling; Patients awake; pethidine given to control shivering</td>
<td>37/0</td>
<td>Within 6 hours</td>
<td>35°C</td>
<td>9–12 hours</td>
<td>Trial terminated due to poor patient recruitment</td>
</tr>
<tr>
<td>Combined cytoprotection tPA stroke trial</td>
<td>Endovascular or surface cooling; caffeine + tPA; Patients awake; pethidine, buspirone &amp; surface warming used to control shivering</td>
<td>18/0</td>
<td>Within 5 hours</td>
<td>33–35°C</td>
<td>24 hours</td>
<td>Hypothermia when combined with caffeine is safe; Phase II trial planned</td>
</tr>
</tbody>
</table>

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Meloni et al. Clinical Neuroprotection After Brain Ischemia 2237
The effect was also seen when treatment was commenced 4 hours after ischemia (Figure 2B), but not 6 hours.

We conclude that the neuroprotective effects of magnesium are unmasked by and act synergistically with hypothermia.

### Clinical Implications

In light of the above, it is possible that the IMAGES trial was negative at least in part because stroke patient management did not involve active hypothermia induction, thus preventing the expression of magnesium mediated neuroprotection. On a more positive note, we suggest that with cardiac arrest patients, for whom therapeutic moderate hypothermia is already in use, there could well be potentiation of the existing neurological benefit by the inclusion of magnesium therapy (note that the evidence for magnesium therapy after cardiac arrest is currently equivocal33). Also, we suggest that in future magnesium or hypothermia clinical trials, consideration should be given to assessing the 2 treatments in combination.

Fortuitously, it has been shown that, at least in healthy individuals, intravenous magnesium reduces the shivering threshold, increases the cooling rate, and reduces discomfort during surface cooling to 34 to 35°C.24,34

To enhance the practicality of treatment, sequential magnesium and hypothermia therapy might be used. For example, early magnesium treatment by paramedical staff en route to hospital (as in the FAST-Mag trial) could be followed, after further patient assessment, by induction of hypothermia. Alternatively, hypothermia could be induced in the field with intravenous cold saline, followed later by controlled hypothermia and magnesium treatment. With regard to compatibility with existing treatment, it has already been shown that the enzymatic activity of tPA is not significantly impaired.

### Table 2. Clinical Trials of Magnesium in Stroke

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Subjects: Magnesium/Controls</th>
<th>Delay From Ischemic Episode</th>
<th>Magnesium Salt</th>
<th>Dose (Intravenous)</th>
<th>Comments/Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wester et al, 19848</td>
<td>14/0</td>
<td>Within 12 hours</td>
<td>MgSO₄</td>
<td>15 mmol bolus + 96 mmol over 24 hours for 5 days</td>
<td>Treatment increased serum magnesium levels to between 1.5 to 2.5 mmol/L</td>
</tr>
<tr>
<td>Muir and Lees, 1995⁹</td>
<td>30/30</td>
<td>Within 12 hours</td>
<td>MgSO₄</td>
<td>8 mmol bolus + 65 mmol over 24 hours</td>
<td>Magnesium well tolerated, with no significant side-effects</td>
</tr>
<tr>
<td>Muir and Lees, 1998¹⁰</td>
<td>6–7 for each dose/6</td>
<td>Within 24 hours</td>
<td>MgSO₄</td>
<td>8, 12, or 16 mmol bolus + 65 mmol over 24 hours</td>
<td>Magnesium well tolerated, with 16 mmol loading infusion chosen for further trials</td>
</tr>
<tr>
<td>Galeas et al, 1999¹¹</td>
<td>510/277</td>
<td>Unknown</td>
<td>Magnesium aspartate HCl</td>
<td>7.5 mmol daily</td>
<td>Magnesium treatment improved patient survival and condition at 30 and 90 days</td>
</tr>
<tr>
<td>Lampi et al, 2001¹²</td>
<td>21/20</td>
<td>Within 24 hours</td>
<td>MgSO₄</td>
<td>16 mmol over 15 minutes + 142 mmol over 24 hours for 5 days</td>
<td>Magnesium treatment appeared to have a positive effect on patient outcome</td>
</tr>
<tr>
<td>IMAGES pilot trial¹³</td>
<td>25/26</td>
<td>Within 12 hours</td>
<td>MgSO₄</td>
<td>16 mmol over 15 minutes + 65 mmol over 24 hours</td>
<td>Treatment protocol safe</td>
</tr>
<tr>
<td>IMAGES phase 3 trial¹⁴</td>
<td>1188/1198</td>
<td>Within 12 hours</td>
<td>MgSO₄</td>
<td>16 mmol over 15 minutes + 65 mmol over 24 hours</td>
<td>Did not reduce death or disability significantly, although it may be of benefit in lacunar stroke</td>
</tr>
<tr>
<td>FAST-MAG pilot trial¹⁵</td>
<td>20/25</td>
<td>Within 12 hours</td>
<td>MgSO₄</td>
<td>10 mmol over 10 minutes in field + 6 mmol on hospital arrival + 65 mmol over 24 hours</td>
<td>Treatment feasible and safe</td>
</tr>
<tr>
<td>FAST-MAG phase 3 trial¹⁶</td>
<td>618 enrolled as of 9/9/08</td>
<td>Within 2 hours</td>
<td>MgSO₄</td>
<td>16 mmol over 15 minutes + 65 mmol over 24 hours</td>
<td>Trial ongoing</td>
</tr>
</tbody>
</table>
either by high magnesium concentrations or by mildly reduced temperatures, or by mildly reduced temperatures, and in fact it is possible that the combined therapy could extend the therapeutic time window for IPA thrombolysis.

Conclusion
Clinical trials will ultimately be necessary to establish whether these experimental findings translate to efficacy in treating brain ischemia. In the meantime further studies are planned to confirm that the protection afforded by magnesium and mild hypothermia is long-lasting and functionally significant; to assess whether the combination of magnesium with moderate hypothermia is more efficacious than with mild hypothermia; to assess whether sequential administration of the 2 treatments has similar efficacy to concurrent treatment; to assess whether longer durations of mild hypothermia (36, 48 hours) provide better protection; and to assess magnesium and mild hypothermia in thrombo-embolic/TPA stroke models. Also, it is important that our findings be shown to be reproducible in other laboratories.

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Disclosures
None.

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