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Therapeutic hypothermia for neonatal encephalopathy (NE) has successfully translated to standard care, confirming the fundamental validity of extensive preclinical research across multiple species and settings (1). The challenge now is to further improve neuroprotection, so that more babies survive NE without disability. Current protocols for therapeutic hypothermia appear to be near-optimal (2-4), so the obvious solution is to add other neuroprotective agents to hypothermia. Logically, we should follow the example of the development of anticancer drugs and systematically test different combinations in a stepwise fashion, starting with agents that are already clinically approved for other indications (5).

Excitotoxicity during hypoxia-ischemia

Early research on the mechanisms of delayed cell death after hypoxia-ischemia (HI) focused on the observation that excitatory amino acids ("excitotoxins") such as glutamate accumulate in the extra-cellular space during HI, and during intense seizures (6). Exposure to excitotoxins can facilitate excessive entry of calcium into the cell and so activate delayed cell death pathways (5). There was great excitement when specific antagonists seemed to dramatically reduce cell death (7). We now know that these excitatory channels are just one of many calcium channels that open during HI (8), and that the apparent benefit in small animal studies was confounded by drug-induced hypothermia (7). Consequently, excitotoxin antagonists failed to translate in adult clinical trials (9). Further, in preterm fetal sheep, infusion of the non-competitive glutamatergic antagonist dizocilpine after HI was associated with very limited improvement in neuronal survival, in only one hippocampal region, and combined dizocilpine infusion with hypothermia did not augment hypothermic neuroprotection (10).

Could increased serum magnesium augment hypothermic neuroprotection?

Magnesium is an endogenous, physiological anti-excitotoxic agent, acting by voltage dependent inhibition of glutaminergic channels (11, 12). There are multiple pragmatic features in its favor as a potential neuroprotectant, as well as a range of encouraging clinical evidence. It is very inexpensive, its physiological effects are relatively well understood (13, 14), including vasodilation at high concentrations, and it is widely used in clinical practice and so regulatory approval would be easily gained. Systematic meta-analysis of 5 randomized controlled trials of magnesium sulfate ($MgSO_4$) in NE suggested that it significantly reduced adverse short-term outcomes, with trends for improved long-term outcomes but greater mortality (15). Critically, meta-analysis of large randomized controlled trials of maternal $MgSO_4$ in preterm labor found that it was associated with significantly reduced risk of cerebral palsy (number needed to treat: 64) (16). The pathophysiological basis of this association is unclear, and the effects on the primary outcome of death or disability were mixed; a significant overall effect was seen only in the subset of trials designed to test for neuroprotection

The preclinical evidence for neuroprotection after HI with $MgSO_4$ is mixed. In rodents, initial studies of treatment after HI or ischemia were very promising (17). These studies did not control for the vasodilator effects of magnesium that can promote confounding hypothermia. Studies that included rigorous temperature control showed little benefit (17). A recent study showed dramatic protection with $MgSO_4$ given before HI in rats (18). However, $MgSO_4$ was given as large boluses before HI, and the evidence suggests that protection was mediated through preconditioning. This would not be practical after HI injury. In large animals, $MgSO_4$ by itself was not neuroprotective after HI in piglets (19, 20), or in near-term fetal sheep (21). Indeed, in preterm fetal sheep $MgSO_4$ infusion for 48 hours after acute HI was associated with impaired oligodendrocyte maturation (22).

Notwithstanding these results, in adult rodents, studies of combined $MgSO_4$+hypothermia after ischemia suggest significant additive benefit (23-25). Moreover, $MgSO_4$ has significant antiseizure properties. It has been used for many years to reduce the risk of maternal seizures during moderate to severe eclampsia, and may be more effective than anticonvulsants such as phenytoin (26). Consistent with this, in preterm fetal sheep, $MgSO_4$ infusion markedly reduced seizures after profound HI, with greater benefit seen in male fetuses (27). Seizures are associated with increased anaerobic stress in the brain in neonatal NE (28). In normothermic near-term fetal sheep, abolishing post-ischemic seizures with an infusion of dizocilpine reduced injury in more mildly affected regions, although not in the core infarct (29). Given that therapeutic hypothermia reduces but does not abolish seizures during NE (30, 31), it is plausible that combining hypothermia and Mg could further suppress seizures and associated excitotoxic stress, and so improve outcomes. This background strongly suggests that $MgSO_4$+hypothermia combination therapy is well worth testing despite its limitations as sole therapy. Given that safety trials of hypothermia plus $MgSO_4$ have already being undertaken (32-34), it was urgent to undertake rigorous preclinical studies in large animal, translational models.

Magnesium sulfate plus hypothermia in the piglet

In this issue, Lingam *et al* report that the combination of an infusion of $MgSO_4$ with hypothermia after HI in anesthetized male, term piglets was safe, and was associated with a small, incremental improvement in some but not all endpoints (35). Encouragingly, after 48 hours recovery, compared to hypothermia alone the combination was associated with a small reduction in the total number of dead brain cells, summed across all brain regions, and increased numbers of surviving oligodendrocytes in the white matter tracts, although myelination itself was not assessed. However, there was no significant improvement in cell death in any brain region taken in isolation. Further, there was no significant improvement in

recovery of amplitude integrate electrophysiological (aEEG) activity or in magnetic resonance spectroscopy (MRS) parameters that clinically are closely associated with neurodevelopmental outcomes at 18 months of age after NE (36). Although the authors report that there was a trend towards better aEEG (p=0.09), it is of concern that the authors also found an increase in activated caspase-3 (35). This raises the possibility that there was upregulation of cell death that might attenuate the apparent histological benefit during a longer period of recovery. Finally, the reader should note that treatment was started just 1 hour after HI. It is very challenging to start an experimental intervention this soon after birth. Given that essentially all neuroprotective treatments show dramatic loss of efficacy with greater delay after HI (1), it is likely that the results of the current study represent the best possible outcome for this approach.

Conclusion

Although it is not what was hoped for, the present findings from Lingam *et al* are highly valuable negative information. Critically, the lack of benefit on the clinically well validated MRS measures of outcome suggests that we should not proceed to a phase three clinical trial of add-on $MgSO_4$ for NE. This is an important saving of time, money and effort. In the longer term, given its excellent safety profile, it is just plausible that it might be considered for studies of multidrug "cocktails" for neuroprotection, but much more work is vital before such a complex study can be considered. Finally, these data provide further evidence that anti-excitotoxic therapy after HI has limited benefit for the developing brain.

Author contributions

RG, LB and AJG contributed to the conception and design of the manuscript, drafting the article and approving the final version.

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