

## SPECIAL REVIEWS

# Treatment of hypoxic-ischaemic brain damage by moderate hypothermia

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To many people, especially writers of science fiction interested in preserving brains for narrative purposes, it seems self evident that cooling the brain protects it against hypoxic-ischaemic damage. Indeed, every day, cardiac surgeons and anaesthetists cool the brains of children during surgery to protect them against the effects of cardiac arrest or cardiopulmonary bypass.

However there has long been a hope that cooling the brain *after* hypoxia-ischaemia might lessen cerebral injury. Observational data in support of this were collected by Westin and colleagues 40 years ago,<sup>1</sup> but experimental studies in animal models at that time failed to support the hypothesis and it fell from favour.<sup>2</sup> Now the belief is gaining ground again among basic researchers that moderate brain cooling to around 32°C is one of several interventions which can be applied after hypoxia-ischaemia to modify the process of brain cell death and so lessen cerebral damage.

## Delayed cerebral damage after hypoxia-ischaemia

A cornerstone of this growing consensus was the realisation that not only do some cells die during hypoxia-ischaemia, but many more may die hours or days later.<sup>3</sup> In the 1980s this delayed cerebral injury was shown in infants with birth asphyxia using <sup>31</sup>P magnetic resonance spectroscopy (MRS): asphyxiated infants were usually found to have normal cerebral energy metabolism soon after resuscitation, but oxidative phosphorylation became impaired 9 to 24 hours later and remained low for many days.<sup>4</sup> The delayed impairment of energy metabolism was not contingent on continued hypoxia-ischaemia, nor was it associated with intracellular acidosis,<sup>5</sup> but its magnitude was linearly related to the severity of later neurodevelopmental impairment and reduced brain growth.<sup>6</sup>

This was clear evidence that the effects of birth asphyxia might become manifest only some days after resuscitation, and indeed more recent data suggest that abnormal cerebral energy metabolism and cell death can persist for weeks and months.<sup>7,8</sup> The mechanisms of this delayed cell death are complex, but the recognition that apoptosis (genetically controlled cell suicide which requires a biochemical pathway to be completed for death to occur)

was an important aspect of hypoxic-ischaemic cerebral injury made it easier to understand why cells should die late after injury.<sup>9,10</sup>

These results suggested that it might be possible to intervene before delayed cell death occurred and so lessen cerebral damage. The hypothesis was supported by experiments in animals which showed that a large number of treatments, ranging from administration of glutamate receptor antagonists to injection of growth factors, would reduce brain injury if given soon after the insult.<sup>11</sup> Thus rescue treatment of hypoxia-ischaemia after resuscitation was confirmed as a real possibility, and in some cases treatment could still be effective even if delayed for a considerable time after the hypoxic-ischaemic insult.<sup>12</sup>

## Neural rescue treatment using post-insult hypothermia

Many recent studies, mainly in adult animals, have shown that cooling of the brain by 3–4°C after experimental hypoxia-ischaemia reduces the severity of brain injury.<sup>13</sup> The more drastic temperature reductions used in older experiments do not seem to provide any additional benefit and indeed may be less effective as they have been associated with systemic toxicity.<sup>14</sup> Consequently most current research focuses on moderate rather than deep hypothermia.

Moderate hypothermia is one of the most robust and effective techniques available for reducing hypoxic-ischaemic cerebral damage. However, many experiments have used short-term histopathological examination to assess neural cell loss, which raised concern that hypothermia may not prevent injury, merely delay it. But a major study of adult gerbils cooled after hypoxia-ischaemia, found that they performed better than controls in neuropsychological tests six months later, showing that a persistent beneficial effect of cerebral function could be produced.<sup>15</sup> Some researchers now believe that in adult animals at least, a short period of hypothermia lasting a few hours will delay cell death while more prolonged cooling for a day or two permanently prevents cerebral injury.<sup>16</sup>

## Neural rescue using moderate hypothermia in the developing brain

Several groups have shown that post-insult hypothermia can reduce cerebral injury in the

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developing brain. In 7 day old rat pups hypothermia for a period as short as 3 hours after hypoxia–ischaemia had some neuroprotective effect,<sup>17</sup> and histological differences between treatment and control brains could still be detected 6 weeks later.<sup>18</sup> In 21 day old rat pups 72 hours of hypothermia was highly protective, although a delay of 6 hours before starting treatment significantly reduced the benefit.<sup>19</sup>

One study of 7 day old rats failed to show any benefit, but this may be explained by the severe nature of the hypoxic–ischaemic insult combined with a relatively brief period of 3 hours cooling after the insult.<sup>20</sup> In a study of newborn piglets 3 hours of cooling exerted only a modest neuroprotective effect and there was no protection at all in more severely injured animals.<sup>21</sup> By contrast, 12 hours of cooling by 4°C in newborn piglets produced a major reduction of both the delayed impairment in cerebral energy metabolism, and histological injury.<sup>22–23</sup>

All these studies have examined the effect of whole body cooling, but investigations of fetal sheep subjected to total cerebral ischaemia found that a substantial protective effect could be produced by a cooling device positioned around the fetal head.<sup>24</sup> In this model cooling was maintained for 72 hours, but clinically significant protection was achieved even if treatment was delayed for 5 hours after ischaemia.<sup>25</sup> Focal brain cooling was also effective in 7 day old rat pups.<sup>26</sup> Taken together these results suggest that optimal benefit is obtained by extending the duration of moderate hypothermia for 12 hours or longer, although they leave unanswered the question of whether whole body or selective head cooling is better.

#### How cooling works

Hypothermia applied *during* hypoxia–ischaemia is thought to be protective by preventing the decline in high energy phosphates that seem to initiate both apoptotic and necrotic cell death.<sup>27–28</sup> The mechanisms by which cooling *after* hypoxia–ischaemia prevent cell death are less clear. Hypothermia prevents the delayed decline in phosphocreatine and adenosine triphosphate, as well as the simultaneous increase in cerebral lactate concentration, seen 8–12 hours after hypoxia–ischaemia in newborn piglets.<sup>22–29</sup> However, it is not clear if preservation of energy metabolism in the delayed phase of injury is the primary mechanism by which hypothermia operates, or whether it represents an indicator of cellular protection mediated by other pathways.

Additional effects are probably involved. Hypoxia–ischaemia leads to high concentrations of glutamate in the synaptic cleft which induce excitotoxic neuronal death.<sup>30</sup> In both newborn piglets<sup>31</sup> and adult rats<sup>32</sup> hypothermia reduces the delayed increased in extracellular glutamate seen after hypoxia–ischaemia, and in a cell culture model of hypoxia–ischaemia mild cooling decreased the impairment in glutamate re-uptake that is an important factor in acute injury.<sup>33</sup> Hypothermia also reduced production of a downstream mediator of the excitotoxic

process: nitric oxide.<sup>31–34</sup> However, these mechanisms may not be a sufficient explanation, as in at least one model where hypothermia is effective there is no increase in nitric oxide production after hypoxia–ischaemia (Edwards *et al*, unpublished data), and the role of impaired glutamate re-uptake in delayed cell death is unclear.<sup>30</sup>

Hypothermia reduces the number of ischaemic depolarisations in injured but viable tissue,<sup>35</sup> and prevents the increase in cerebral impedance (which reflects impaired membrane function) during delayed cerebral injury in fetal lambs.<sup>24</sup> Reduced body temperature may also increase catecholamine secretion,<sup>36</sup> and as stress also improves neuropathological outcome in developing rats,<sup>37</sup> sympathetic stimulation may be another mechanism of protection.

Hypothermia reduces the number of apoptotic cells seen in the newborn piglet brain after hypoxia–ischaemia without affecting the number of necrotic cells, suggesting that it may specifically inhibit the apoptotic pathway.<sup>23</sup> However, comparable hypothermia delays rather than prevents apoptosis in cell culture systems,<sup>38</sup> and an attractive hypothesis is therefore that hypothermia delays commitment to apoptosis for long enough to enable endogenous protective mechanisms, including the production of growth factors,<sup>39–40</sup> to be induced. If this is correct it suggests that hypothermia may have a role in prolonging the therapeutic window during which other treatments might be applied.

Studies of adult animals have suggested other mechanisms through which hypothermia may act including: suppression of free radical action; prevention of ischaemia induced protein kinase C inhibition; or activation of transcription factors.<sup>41</sup> It remains to be seen whether these mechanisms are important in the developing brain. However, the success of hypothermic treatment probably depends on it, affecting several of the many mechanisms of damage which are activated by hypoxia–ischaemia.

#### Hyperthermia after hypoxia–ischaemia

There is a developing body of experimental evidence to suggest that increased brain temperature after hypoxia–ischaemia may worsen cerebral damage. Observational data from adults who have had a stroke<sup>42</sup> are supplemented by experiments which have shown increased glutamate release in hyperthermic animals, and worse histological outcome even if the period of hyperthermia is delayed for 24 hours after the period of hypoxia–ischaemia.<sup>43–44</sup> Of particular interest are epidemiological data showing a ninefold increase in the incidence of cerebral palsy in infants weighing more than 2500 g who were born to mothers with a fever exceeding 38°C.<sup>45</sup>

#### Adverse effects of hypothermia

Profound hypothermia to less than 30°C affects many physiological functions. It decreases perfusion and oxygenation by: impairing myocardial contractility; reducing cardiac output; and making myocardial muscle more prone to dysrhythmia<sup>46–47</sup>; as well as causing peripheral vasoconstriction; increasing blood viscosity<sup>48</sup>;

and shifting the oxygen dissociation curve of blood to the left.<sup>49</sup> This deranged circulation can lead to renal failure, pulmonary oedema, metabolic acidosis and inadequate cerebral blood flow.<sup>46</sup> Cooling also impairs clotting,<sup>50</sup> depresses the immune system,<sup>51</sup> disrupts serum potassium homeostasis,<sup>52</sup> alters acid base balance,<sup>53</sup> and is associated with hypoglycaemia.<sup>54</sup> Hypothermia may cause gastrointestinal lesions in the developing animal, although the data are somewhat contradictory.<sup>55</sup> These and other changes can significantly worsen the outcome of experimental subjects,<sup>14</sup> and early observational studies of newborn infants showed a number of these adverse events, of which pulmonary haemorrhage (probably due to raised left atrial pressure) was the most severe.<sup>57</sup>

However, although moderate hypothermia to around 32°C has been less well studied, adverse effects seem to be less severe. Indeed, newborn animals physiologically induce moderate hypothermia in response to hypoxia.<sup>58</sup> Recent studies of newborn piglets subjected to moderate whole body cooling for 12 hours as a treatment for either cerebral hypoxia-ischaemia or 3 hours for whole body hypoxia, showed little evidence of circulatory or metabolic disruption during cooling<sup>22</sup> or systemic pathology at necropsy.<sup>59 60</sup>

Nevertheless, systematic studies of the potentially toxic effects of moderate hypothermia maintained for longer periods are required, as the risk of adverse effects from moderate hypothermia seems likely to be increased if hypothermic treatment is prolonged to maximise its neuroprotective effect. There is likely to be a complex trade-off between optimising protection and minimising side effects which will involve decisions not only about the length and depth of hypothermia but also about the appropriate balance between selective head and whole body cooling.

### Conclusion

Cerebral hypoxia-ischaemia leads to a delayed period of cell death that begins some hours after adequate oxygenation is restored to the brain. Cooling the brain to around 32°C for between 12 and 72 hours, beginning after resuscitation, significantly reduces cerebral damage and long term sequelae in experimental models. This moderate hypothermia may be associated with relatively few adverse systemic effects, and although the mechanisms of cerebral protection are incompletely understood, interest is growing in the possibility of clinical neuroprotection by reduction in brain temperature.

- 1 Westin B, Miller JA, Nyberg R, Wedenberg E. Neonatal asphyxia pallida treated with hypothermia alone or with hypothermia and transfusion of oxygenated blood. *Surgery* 1959;45:868-79.
- 2 Oates RK, Harvey D. Failure of hypothermia as treatment for asphyxiated newborn rabbits. *Arch Dis Child* 1976;51:512-16.
- 3 Kirino T. Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Res* 1997;239:57-69.
- 4 Wyatt JS, Edwards AD, Azzopardi D, Reynolds EOR. Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury. *Arch Dis Child* 1989;64:953-63.
- 5 Mies G, Paschen W, Hossman K-A. Cerebral blood flow, glucose utilisation, regional glucose, and ATP content

- during the maturation period of delayed ischaemic injury in gerbil brain. *J Cereb Blood Flow Metab* 1990;10:638-45.
- 6 Roth SC, Edwards AD, Cady EB, et al. Relation between cerebral oxidative metabolism following birth asphyxia and neurodevelopmental outcome and brain growth at one year. *Dev Med Child Neurol* 1992;34:285-95.
- 7 Du C, Hu R, Csernansky CA, Hsu CY, Choi DW. Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? *J Cereb Blood Flow Metab* 1996;16:195-201.
- 8 Robertson NJ, Cox IJ, Counsell S, Cowan F, Azzopardi D, Edwards AD. Persistent lactate following perinatal hypoxic-ischaemic encephalopathy and its relationship to energy failure studied by magnetic resonance spectroscopy. *Early Hum Devel* (in press).
- 9 Edwards AD, Mehmet H. Hypoxia, ischaemia and apoptosis. *Arch Dis Child* 1996;75:F73-F5.
- 10 Edwards AD, Yue X, Cox P, et al. Apoptosis in the brains of infants suffering intrauterine cerebral injury. *Pediatr Res* 1997;42:684-9.
- 11 Gluckman PD, Williams CE. When and why do brain cells die? *Dev Med Child Neurol* 1992;34:1010-14.
- 12 Green EJ, Pazos AJ, Dietrich WD, et al. Combined postischemic hypothermia and delayed MK-801 treatment attenuates neurobehavioral deficits associated with transient global ischemia in rats. *Brain Res* 1995;702:145-52.
- 13 Ginsberg MD, Sternau LL, Globus MY, Dietrich WD, Busto R. Therapeutic modulation of brain temperature: relevance to ischaemic brain injury. *Cerebrovasc Brain Metab Rev* 1993;4:189-225.
- 14 Steen PA, Soule EH, Michenfelder JD. Deterioration of prolonged hypothermia in cats and monkeys with and without regional cerebral ischemia. *Stroke* 1979;10:522-9.
- 15 Colbourne F, Corbett D. Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. *J Neurosci* 1995;15:7250-60.
- 16 Ginsberg MD. Hypothermic neural protection in cerebral ischaemia. In: Welch KMA, Caplan LR, Reis DJ, Siesjo BK, Weir B, eds. *Primer on Cerebrovascular Diseases*. San Diego: Academic Press, 1997;272-6.
- 17 Thoresen M, Bagenholm R, Loberg EM, Apricena F, Kjellmer I. Posthypoxic cooling of neonatal rats provides protection against brain injury. *Arch Dis Child* 1996;74:F3-9.
- 18 Bona E, Loberg E, Bagenholm R, Hagberg H, Thoresen M. Protective effects of moderate hypothermia after hypoxia-ischaemia in a neonatal rat model: short and long-term outcome. *J Cereb Blood Flow Metab* 1997;17:S857.
- 19 Sirimanne E, Blumberg RM, Bossano D, et al. The effect of prolonged modification of cerebral temperature on outcome following hypoxic-ischaemic brain injury in the infant rat. *Pediatr Res* 1996;39:591-7.
- 20 Yager JY, Towfighi J, Vannucci RC. Influence of mild hypothermia on hypoxic ischaemic brain damage in the immature rat. *Pediatr Res* 1993;34:525-9.
- 21 Haaland K, Loberg EM, Steen PA, Thoresen M. Posthypoxic hypothermia in newborn piglets. *Pediatr Res* 1997;41:505-12.
- 22 Thoresen M, Penrice J, Lorek A, et al. Mild hypothermia following severe transient hypoxia-ischaemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatr Res* 1995;37:667-70.
- 23 Edwards AD, Yue X, Squier MV, et al. Specific inhibition of apoptosis after cerebral hypoxia-ischaemia by moderate post-insult hypothermia. *Biochem Biophys Res Commun* 1995;217:1193-9.
- 24 Gunn AJ, Gunn TR, De Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest* 1997;99:248-56.
- 25 Gunn TR, Gunn AJ, Gluckman PD. Substantial neuronal rescue with prolonged selective head cooling begun 5.5 hours after cerebral ischaemia in fetal sheep. *Pediatr Res* 1997;41:152.
- 26 Towfighi J, Housman C, Heitjan DF, Vannucci RC, Yager JY. The effect of focal cerebral cooling on perinatal hypoxic-ischemic brain damage. *Acta Neuropathol Berl* 1994;87:598-604.
- 27 Williams GD, Dardzinski BJ, Buckalew AR, Smith MB. Modest hypothermia preserves cerebral energy metabolism during hypoxia-ischemia and correlates with brain damage: A <sup>31</sup>P nuclear magnetic resonance study in unanesthetized neonatal rats. *Pediatr Res* 1997;42:700-8.
- 28 Yager JY, Brucklacher RM, Mujsce DJ, Vannucci RC. Cerebral oxidative metabolism during hypothermia and circulatory arrest in newborn dogs. *Pediatr Res* 1992;32:547-52.
- 29 Amess PN, Penrice J, Lorek A, et al. Mild hypothermia after transient hypoxia-ischemia reduces the delayed rise in cerebral lactate in the newborn piglet. *Pediatr Res* 1997;41:803-8.
- 30 Szatkowski M, Attwell D. Triggering and execution of neuronal death in brain ischaemia: two phases of glutamate release by different mechanisms. *Trends Neurosci* 1994;17:359-65.
- 31 Thoresen M, Satas S, Puka-Sundvall M, et al. Post-hypoxic hypothermia reduces cerebrocortical release of NO and excitotoxins. *Neuroreport* 1997;8:3359-62.
- 32 Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 1989;20:904-10.
- 33 Huang R, Shuaib A, Hertz L. Glutamate uptake and glutamate content in primary cultures of mouse astrocytes dur-

- ing anoxia, substrate deprivation and simulated ischemia under normothermic and hypothermic conditions. *Brain Res* 1993;618:346-51.
- 34 Tan WK, Williams CE, During MJ, *et al.* Accumulation of cytotoxins during the development of seizures and edema after hypoxic-ischaemic injury in late gestation fetal sheep. *Pediatr Res* 1996;39:791-7.
- 35 Chen Q, Chopp M, Bodzin G, Chen H. Temperature modulation of cerebral depolarization during focal cerebral ischemia in rats: correlation with ischemic injury. *J Cereb Blood Flow Metab* 1993;13:389-94.
- 36 Frank SM, Higgins MS, Breslow MJ, *et al.* The catecholamine, cortisol, and hemodynamic responses to mild perioperative hypothermia. A randomized clinical trial. *Anesthesiology* 1995;82:83-93.
- 37 Thoresen M, Bagenholm R, Loberg E, Apricena F. The stress of being restrained reduces brain damage after a hypoxic-ischaemic insult in the seven day old rat. *Neuroreport* 1997;7:481-3.
- 38 Kozma M, Ravirajan G, Edwards AD, Mehmet H. Moderate hypothermia reduces apoptosis in cultured neuronal cells deprived of nerve growth factor. *Pediatr Res* 1995;38:441.
- 39 Guan J, Williams CE, Skinner SJ, Mallard EC, Gluckman PD. The effects of insulin-like growth factor (IGF)-1, IGF-2, and des-IGF-1 on neuronal loss after hypoxic-ischemic brain injury in adult rats: evidence for a role for IGF binding proteins. *Endocrinology* 1996;137:893-8.
- 40 Klempt ND, Sirmann E, Gunn AJ, *et al.* Hypoxia-ischemia induces transforming growth factor beta 1 mRNA in the infant rat brain. *Brain Res Mol Brain Res* 1992;13:93-101.
- 41 Dietrich WD, Busto R, Globus MY, Ginsberg MD. Brain damage and temperature: cellular and molecular mechanisms. *Adv Neurol* 1996;71:177-94.
- 42 Reith J, Jorgensen HS, Pedersen PM, *et al.* Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996;347:422-5.
- 43 Baena RC, Busto R, Dietrich WD, Globus MY, Ginsberg MD. Hyperthermia delayed by 24 hours aggravates neuronal damage in rat hippocampus following global ischemia. *Neurology* 1997;48:768-73.
- 44 Takagi K, Ginsberg MD, Globus MY, Martinez E, Busto R. Effect of hyperthermia on glutamate release in ischemic penumbra after middle cerebral artery occlusion in rats. *Am J Physiol* 1994;267:H1770-6.
- 45 Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. *JAMA* 1997;278:207-11.
- 46 Steen PA, Milde JH, Michenfelder JD. The detrimental effects of prolonged hypothermia and rewarming in the dog. *Anesthesiology* 1980;52:224-30.
- 47 Bjornstad H, Tandem PM, Lathrop DA, Refsum H. Effects of temperature on cycle length dependent changes and restitution of action potential duration in guinea pig ventricular muscle. *Cardiovasc Res* 1993;27:946-50.
- 48 Poulos ND, Mollitt DL. The nature and reversibility of hypothermia-induced alterations of blood viscosity. *J Trauma* 1991;31:996-8.
- 49 Barcroft J, King WOR. The effect of temperature on the oxygen dissociation curve of blood. *J Physiol Lond* 1909;39:374-84.
- 50 Rohrer MJ, Natale AM. Effect of hypothermia on the coagulation cascade. *Crit Care Med* 1992;20:1402-5.
- 51 van Rijen EA, Ward JJ, Parry E, Little RA. Reticuloendothelial function after hemorrhage and hypothermia. *Shock* 1997;7:300-3.
- 52 Sprung J, Cheng EY, Gamulin S, Kampine JP, Bosnjak ZJ. Effects of acute hypothermia and beta-adrenergic receptor blockade on serum potassium concentration in rats. *Crit Care Med* 1991;19:1545-51.
- 53 Alfaro V, Palacios L. Acute mild hypothermia in awake unrestrained rats induces a mixed acid-base disorder. *J Appl Physiol* 1996;80:2143-50.
- 54 Tashima CK. Hypoglycemia in hypothermia. *N Engl J Med* 1973;289:920-1.
- 55 Blumenfeld J, Lifshitz F, Teichberg S, Wapnir RA. Experimental acute hypothermia and intestinal cellular integrity. *Res Commun Chem Pathol Pharmacol* 1978;20:605-8.
- 56 Schneider PA, Hamilton SR, Dudgeon DL. Intestinal ischemic injury following mild hypothermic stress in the neonatal piglet. *Pediatr Res* 1987;21:422-5.
- 57 Mann TP, Elliott RIK. Neonatal cold injury due to accidental exposure to cold. *Lancet* 1957;ii:229-34.
- 58 Wood SC, Gonzales R. Hypothermia in hypoxic animals: mechanisms, mediators, and functional significance. *Comp Biochem Physiol B Biochem Mol Biol* 1996;113:37-43.
- 59 Amess P, Howard S, Penrice J, *et al.* Systemic effects of hypothermia, a neuronal rescue therapy in newborn piglets. *Biol Neonate* 1997;73:40-6.
- 60 Haarland K, Loberg EM, Steen PA, Satas S, Thoresen M. The effect of mild posthypoxic hypothermia on organ pathology in a piglet survival model of global hypoxia. *Prenatal Neonatal Med* 1997;2:329-37.

## Hypothermic neural rescue treatment: from laboratory to cotside?

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The apparent simplicity of mildly cooling the brain to lessen the devastating effects of birth asphyxia has immediate appeal to clinicians faced with the hopelessness of a severely affected infant. Many view the results of recent experimental work as encouraging. However, while researchers may spend years in the laboratory collecting results which suggest that a particular treatment *should* be useful, it cannot be taken for granted that it will be. The scientific tools needed to demonstrate a useful biological effect are very different from those needed for accurate clinical evaluation, and investigators often need to change their focus considerably as a concept draws closer to the clinical arena.<sup>1</sup>

Enthusiasts who would like to use moderate hypothermia to lessen brain damage after hypoxic-ischaemic or traumatic cerebral insults must now face this difficult move from laboratory to cotside. The experimental evidence is strong, but will hypothermia work in practice?

### Interpretation of experimental studies

Many neural rescue treatments that were effective in studies of mature animals have not shown benefit in clinical trials of adult stroke patients. There is no room for complacency and laboratory results need to be interpreted with circumspection.

First, data acquired from studies of mature animals must be treated cautiously as brain injury probably proceeds differently in the newborn and there are relatively fewer studies of hypothermic neural rescue in newborn animals. Secondly, in most experiments the delay between hypoxic-ischaemic insult and initiation of cooling has been short, and in every case clearly defined, which may not accurately reproduce the clinical situation. Furthermore, the rate of cerebral injury in the experimental control groups has been high, often 100%; it will be difficult for a clinical trial to select control groups with such high rates of severe injury, and this will make demonstration of a clinical effect more difficult. Finally,

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although a variety of cooling temperatures and protocols have been tested in animal experiments, most studies have cooled the whole body so that relatively few data are available on selective head cooling, which seems to be the preferred option of many clinical researchers.

#### **Preliminary studies of moderate hypothermia in clinical practice**

Trials of cooling in adult patients with head trauma found that hypothermia was associated with quicker recovery and less severe brain injury than conventional neurointensive care, despite the fact that in one study the median time of establishing effective cooling was 10 hours after injury.<sup>2,3</sup> These important results support observational data showing a significant relation between better survival after stroke and lower body temperature in the 24 hours after onset of symptoms.<sup>4</sup>

A preliminary study of selective head cooling after birth asphyxia has been undertaken by Gluckman and colleagues in New Zealand, and analogous studies of whole body cooling have also been taking place at the Hammersmith Hospital in London. Thus far there is nothing to imply that moderate hypothermia is unsafe, and the results suggest a beneficial effect sufficient to justify further trials (Gluckman P D, personal communication).

#### **Establishing a clinical trial**

There is considerable interest worldwide in establishing trials of moderate hypothermia for treatment of cerebral hypoxic-ischaemic injury. However, a clinical trial of hypothermic neural rescue treatment after birth asphyxia faces specific difficulties.

##### **ENTRY CRITERIA**

Most clinical trials begin confident that the patient group under investigation has the disease being studied. This is not always the case in trials of birth asphyxia. The correlation between simple measures of intrauterine asphyxia and neurodevelopmental outcome is poor, and assignment of prognosis by clinical criteria in the first few hours after birth is difficult.<sup>5</sup> Consequently, if variables such as the Apgar score are used to enrol subjects there is a danger that large numbers of infants without an adverse prognosis may be entered into trials. At best, this will add experimental noise and increase the numbers needed for a statistically valid outcome; at worst, it may subject a large number of normal infants to potentially hazardous intervention.

A great many techniques for improving the assignment of prognosis after birth asphyxia have been investigated. Several provide a useful guide to neurodevelopmental outcome but there is no method which will allow certain determination of prognosis to be made within a few hours of birth.<sup>5</sup> However, recent studies have reported the value of continuous EEG monitoring in the first few hours of life, using single channel amplitude integrated EEG recordings (aEEG, often termed the "cerebral function monitor").<sup>6-8</sup> These techniques have a high predictive value for neurological

impairment.<sup>6,8</sup> We have found that electrophysiological techniques could be used effectively in non-specialist centres,<sup>9</sup> and our initial experience suggests that unlike deep hypothermia, moderate cooling has little effect on EEG amplitude, which opens the possibility of using it to determine prognosis even if hypothermia is started immediately after delivery. Further investigation is required, but aEEG may offer a method for selecting infants for trials of neural rescue treatment.

##### **SAMPLE SIZE**

The entry criteria and the estimated size of the treatment effect will determine the number of infants that need to be recruited to achieve a statistically valid study. A 25% reduction in adverse outcome might be a reasonable aim based on the results of animal studies. If infants were selected on aEEG criteria (which probably allow for a positive predictive value for bad outcome of about 90%), the calculated sample size for a trial with power = 0.8 and alpha = 0.5 would be about 50 infants in each group. However, if selection were based on simpler criteria such as the 5 minute Apgar score, where the positive predictive value is around 15%,<sup>10</sup> about 1000 infants would need to be randomised.

##### **EXIT CRITERIA**

A successful neural rescue treatment might alter the outcome of death to survival with severe impairment. Although data from animals and from adult head injury studies suggest that hypothermia has little effect on the most severe form of injury,<sup>3,11</sup> clinicians may feel that appropriate criteria need to be built into clinical trials to prevent this type of result.

Unfortunately it is not clear what exit criteria could be included to allow this to be achieved. The EEG may help identify those infants who are unlikely to benefit from further intervention: an isoelectric EEG or a burst-suppression pattern almost always indicates a very severe injury,<sup>6</sup> while seizures beginning immediately after birth may suggest that hypoxia-ischaemia occurred several hours beforehand and that it is too late for successful intervention.<sup>12</sup>

##### **OUTCOME MEASURES**

Surrogate measures of outcome are valuable when they provide a biological measure of brain injury that is independent of later social factors. Several potential surrogate measures could be applied at the end of the first week of life. Magnetic resonance imaging (MRI) or spectroscopy (MRS) offer useful surrogate outcomes. For example, absence of the normal MRI signal seen in the posterior limb of the internal capsule at the end of the first week after birth has a 100% positive predictive value for neurodevelopmental abnormality at 1 year of age.<sup>13</sup>

None the less the best outcome measure is a precise assessment of neurodevelopmental progress. Many studies have examined children at 1 year of age, but it is generally agreed that cerebral palsy cannot be accurately diagnosed until 3-5 years of age. Consequently follow up of any patient enrolled in a trial needs to be

prolonged, and the method of assessment clearly defined to allow a difference in outcome to be detected.

#### INTERVENTION

If hypothermia is regarded as analogous to a drug treatment several problems immediately become apparent: first, the dose and administration regimen remain inadequately defined. Animal studies have provided compelling evidence that hypothermia has a protective action, but systematic data on an optimal dose or duration are incomplete. The maximum permitted time delay before treatment is ineffectual is still unclear, although studies are in progress to define this interval. A delay of 10 hours did not prevent benefit in adults cooled after head trauma,<sup>3</sup> but some animal studies suggest that a delay of 6 hours significantly reduces the cerebroprotective effect.<sup>14</sup> Equally, it is unclear how long to continue with cooling. Protection has been seen in studies of developing animals with cooling periods varying between 3 and 72 hours.<sup>15 16</sup> While the possible adverse effects may be more significant over longer time periods, many researchers feel that longer cooling must be better. Indeed, evidence suggesting that after birth asphyxia cerebral energy metabolism remains abnormal for many months, together with studies of adult rats showing that cells die by apoptosis for a similar period after hypoxia–ischemia may suggest that even 72 hours is much too short.<sup>17 18</sup> More work is needed in this area.

Second, it is currently difficult to monitor the dose of hypothermia being administered, especially to the deep brain structures. In studies of animals the deep brain cools significantly less than cortex, especially when selective head cooling is used,<sup>19 20</sup> and common drugs such as barbiturates can selectively increase deep brain temperature.<sup>21</sup> MRI studies have shown that infants with basal ganglia injury have far worse neurological impairment than those with cortical damage alone.<sup>22</sup> Therefore, measurement of deep brain temperature will be important, and methods that record only cortical temperature may be inadequate. It has been suggested that a major reason for the failure of neural rescue treatments in adult stroke has been the poor permeation of drugs into the brain,<sup>23</sup> and it will be important to know that any therapeutic cooling regimen adequately penetrates deep brain structures. Without these, data interpretation and comparison of trial results will be difficult.

Non-invasive measurement of deep brain temperature can be achieved using magnetic resonance techniques,<sup>24 25</sup> but these methods are too cumbersome for routine, repeated clinical use. Measurements based on the zero-flux method have been attempted but not validated.<sup>26 27</sup> A portable non-invasive deep brain thermometer based on microwave technology is under development but is not yet available.<sup>28</sup>

Third, it is not clear whether total body hypothermia or selective head cooling is better. Our preliminary experience suggests that a simple convective device permits rapid induc-

tion and maintenance of total body hypothermia at 33°C. Head cooling devices incorporating cool water flow systems have also been found practical (Gluckman P D, personal communication). Fortunately, the infants' thermal inertia is such that either form of cooling system can be removed for a short period without clinically significant changes in body temperature, allowing medical and nursing procedures to be carried out without hindrance.

Further data are required to confirm whether deep brain cooling can be achieved in practice by topical cooling to the head alone, or if some measure of systemic hypothermia is necessary, although this information will be difficult to acquire until non-invasive deep brain temperature monitoring is available. A few results are available from animal experiments. These suggest that head cooling alone may be successful, but in some cases temperature gradients were significant and quite extreme surface cooling was required.<sup>16 19 20</sup> However, until more is known about the effects of selective head cooling it would be wise to assume that deep brain temperature may be higher than expected, and to plan trials accordingly.

Fourth, profound whole body cooling is associated with toxicity such as disturbed coagulation, vasoconstriction, ventilation–perfusion mismatch, left shift of the oxygen dissociation curve, reduced myocardial contractility, increased blood viscosity, reduced tissue perfusion and increased risk of sepsis.<sup>29 30</sup> Although current experience in infants has found little evidence of these effects with moderate hypothermia, systematic studies need to confirm that the treatment does not significantly increase the risk of these adverse events. Assessment of toxicity will affect decisions concerning the length of treatment and methods of cooling. Early clinical trials will probably need to make some compromise between selective and whole body cooling to achieve maximum brain cooling with minimum toxicity.

Fifth, attention has focused on physical cooling of the brain, largely because of its apparent simplicity. However, as the true complexity of the situation is revealed it may be worth reconsidering pharmacological agents that alter body temperature, either alone or together with physical cooling. In adults paracetamol was the single most effective method of cooling the brain,<sup>21</sup> and cannabinoids have also been suggested for this purpose.

#### Speed of intervention and the need for specialist centres

Intervention should probably begin as soon as possible after the insult. In practice there will be a delay while intensive care is started and the severity of the hypoxic–ischaemic insult is assessed. Further delay will occur if the infant has to be transported to a specialist centre. In our view these delays can be minimised by prior training and planning. Techniques such as the aEEG can be rapidly applied and analysed by staff with a relatively small amount

of training. Selection and recruitment of infants and induction of hypothermia within 4 hours of birth is feasible. If infants have to be transferred to specialist centres whole body hypothermia can be successfully maintained during transfer by simple means, although the precise control of brain temperature required for prolonged treatment requires specialist equipment.

### Perspective

The pharmaceutical industry has shown little interest in developing treatments for birth asphyxia. There are 146 new medicines currently being developed for children, eight for neurological disease—all for epilepsy.<sup>31</sup> No pharmaceutical treatment for hypoxic-ischaemic injury in infants is being developed, in contrast to 20 or 30 agents planned or in trial for adults. Candidate neuroprotective drugs for infants thus have to be taken from current pharmacopoeia, and to date trials have been disappointing: allopurinol showed no beneficial effect<sup>32</sup>; while the cerebroprotective effect of magnesium sulphate is currently the subject of two major trials, one of which has been terminated prematurely because of excess deaths in the treatment group.<sup>33</sup>

The experimental data supporting trials of moderate hypothermia as a neural rescue treatment after hypoxia-ischaemia are strong. They offer a genuine cause for optimism, and multi-centre clinical trials are being planned which will attempt to overcome the many difficulties detailed above.

- 1 Sackett DL, Richardson WS, Rosenberg W, Haynes RB. *Evidence-based Medicine*. New York: Churchill Livingstone, 1997.
- 2 Bernard SA, Jones BM, Horne MK. Clinical trial of induced hypothermia in comatose survivors of out-of-hospital cardiac arrest. *Ann Emerg Med* 1997;30:146-53.
- 3 Marion DW, Penrod LE, Kelsey SF, *et al.* Treatment of traumatic brain injury with moderate hypothermia. *N Engl J Med* 1997;336:540-6.
- 4 Reith J, Jorgensen HS, Pedersen PM, *et al.* Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996;347:422-5.
- 5 Patel J, Edwards AD. Prediction of neurological outcome after perinatal asphyxia. *Curr Opin Pediatr* 1997;9:128-32.
- 6 Hellström Westas L, Rosen I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. *Arch Dis Child* 1995;72:F34-F8.
- 7 Thornberg E, Ekstrom Jodal B. Cerebral function monitoring: a method of predicting outcome in term neonates after severe perinatal asphyxia. *Acta Paediatr* 1994;83:596-601.
- 8 Eken P, Toet MC, Groenendaal F, de Vries LS. Predictive value of early neuroimaging, pulsed Doppler and neurophysiology in full term infants with hypoxic-ischaemic encephalopathy. *Arch Dis Child* 1995;73:F75-F80.
- 9 Guarino Y, Brayshaw C, Edwards AD, Acolet D, Azzopardi D. Prediction of hypoxic-ischaemic encephalopathy by continuous EEG in asphyxiated full-term infants. *Pediatr Res* 1996;40:531.
- 10 Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. *BMJ* 1988;297:24-7.
- 11 Haaland K, Loberg EM, Steen PA, Thoresen M. Posthypoxic hypothermia in newborn piglets. *Pediatr Res* 1997;41:505-12.
- 12 Williams C, Gunn A, Mallard C, Gluckman P. Outcome after ischemia in developing sheep brain: an electroencephalographic and histological study. *Ann Neurol* 1992;31:14-21.
- 13 Rutherford MA, Pennock J, Counsell S, *et al.* Magnetic resonance imaging of the posterior limb of the internal capsule and neurodevelopmental impairment after hypoxic-ischaemic encephalopathy. *Pediatrics* (in press).
- 14 Sirimanne E, Blumberg RM, Bossano D, *et al.* The effect of prolonged modification of cerebral temperature on outcome following hypoxic-ischaemic brain injury in the infant rat. *Pediatr Res* 1996;39:591-7.
- 15 Thoresen M, Bagenholm R, Loberg EM, Apricena F, Kjellmer I. Posthypoxic cooling of neonatal rats provides protection against brain injury. *Arch Dis Child* 1996;74:F3-9.
- 16 Gunn AJ, Gunn TR, De Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest* 1997;99:248-56.
- 17 Du C, Hu R, Csernansky CA, Hsu CY, Choi DW. Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? *J Cereb Blood Flow Metab* 1996;16:195-201.
- 18 Hanrahan D, Azzopardi D, Bryant D, *et al.* Persistence of cerebral lactate after birth asphyxia. *Pediatr Res* 1995;38:436.
- 19 Towfighi J, Housman C, Heitjan DF, Vannucci RC, Yager JY. The effect of focal cerebral cooling on perinatal hypoxic-ischemic brain damage. *Acta Neuropathol Berl* 1994;87:598-604.
- 20 Gelman B, Schleien CL, Lohe A, Kuluz JW. Selective brain cooling in infant piglets after cardiac arrest and resuscitation. *Crit Care Med* 1996;24:1009-17.
- 21 Møllergaard P. Changes in human intracerebral temperature in response to different methods of brain cooling. *Neurosurgery* 1992;31:671-7.
- 22 Rutherford MA, Pennock JM, Schweiso J, Cowan F, Dubowitz L. Hypoxic-ischaemic encephalopathy: early and late magnetic resonance imaging findings in relation to outcome. *Arch Dis Child* 1996;75:F145-F51.
- 23 Pardridge WM. Drug delivery to the brain. *J Cereb Blood Flow Metab* 1997;17:713-31.
- 24 Corbett RJ, Laptook AR, Tollefsbol G, Kim B. Validation of a noninvasive method to measure brain temperature in vivo using <sup>1</sup>H NMR spectroscopy. *J Neurochem* 1995;64:1224-30.
- 25 Cady EB, D'Souza PC, Penrice J, Lorek A. The estimation of local brain temperature by in vivo <sup>1</sup>H magnetic resonance spectroscopy. *Magnet Reson Med* 1995;33:862-7.
- 26 Gunn AJ, Gunn TR. Effect of radiant heat on head temperature gradient in term infants. *Arch Dis Child* 1996;74:F200-3.
- 27 Simbruner G, Nanz S, Fleischhacker E, Derganc M. Brain temperature discriminates between neonates with damaged, hypoperfused, and normal brains. *Am J Perinatol* 1994;11:137-43.
- 28 Roberts I, Azzopardi D, Hand J, Edwards AD. Discrimination of deep from surface temperature with emitted microwave radiation in a brain-specific phantom. *Early Hum Dev* (in press).
- 29 Edwards AD, Wyatt JS, Thoresen M. Treatment of hypoxic-ischaemic brain damage by moderate hypothermia. *Arch Dis Child* 1998;78.
- 30 Lazar HL. The treatment of hypothermia. *N Engl J Med* 1997;337:1545-7.
- 31 Lucey JF. Neuroprotection and perinatal brain care. The field of the future, currently going nowhere. *Pediatrics* (in press).
- 32 Russell GA, Cooke RW. Randomised controlled trial of allopurinol prophylaxis in very preterm infants. *Arch Dis Child* 1995;73:F27-31.
- 33 Bennet P, Edwards AD. Use of magnesium sulphate in obstetrics. *Lancet* 1997;350:1491.



## Treatment of hypoxic-ischaemic brain damage by moderate hypothermia

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