- 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, Sirimanne 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, Tande 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. *Perinatal 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.¹

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,

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.^{2,3} 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.⁴

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.⁵ 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.⁵ 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").⁶⁻⁸ These techniques have a high predictive value for neurological

impairment.^{6,8} We have found that electrophysiological techniques could be used effectively in non-specialist centres,⁹ 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%,¹⁰ 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,^{3,11} 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,⁶ while seizures beginning immediately after birth may suggest that hypoxia-ischaemia occurred several hours beforehand and that it is too late for successful intervention.¹²

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.¹³

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,³ but some animal studies suggest that a delay of 6 hours significantly reduces the cerebroprotective effect.¹⁴ 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.^{15 16} 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.^{17 18} 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,^{19 20} and common drugs such as barbiturates can selectively increase deep brain temperature.²¹ MRI studies have shown that infants with basal ganglia injury have far worse neurological impairment than those with cortical damage alone.²² 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,²³ 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,^{24 25} but these methods are too cumbersome for routine, repeated clinical use. Measurements based on the zero-flux method have been attempted but not validated.^{26 27} A portable non-invasive deep brain thermometer based on microwave technology is under development but is not yet available.²⁸

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.^{16 19 20} 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.^{29 30} 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,²¹ 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.³¹ 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 effect³²; 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.³³

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 ¹H 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 ¹H 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.