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BRAIN RESEARCH

Research Report

"Therapeutic time window" duration decreases with increasing severity of cerebral hypoxia–ischaemia under normothermia and delayed hypothermia in newborn piglets

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ARTICLE INFO

Article history:
Accepted 29 March 2007
Available online 1 April 2007

Keywords:
Neuroprotection
Hypothermia
Hypoxia–ischaemia
Therapeutic time window
Neuronal death
Latent-phase
Secondary energy failure

ABSTRACT

Objective: For optimal neuroprotection following transient perinatal hypoxia–ischaemia (HI), therapy should start before overt secondary energy failure and its irreversible neurotoxic cascade. Hypothermia is a promising neuroprotective intervention that also prolongs the therapeutic time window ("latent-phase"; the period between re-establishment of apparently normal cerebral metabolism after HI, and the start of secondary energy failure). The influences of HI severity on latent-phase duration and regional neuroprotection are unclear. Under normothermia and delayed whole-body cooling to 35 and 33 °C we aimed to assess relationships between HI severity and: (i) latent-phase duration; (ii) secondary-energy-failure severity; and (iii) neuronal injury 48 h following HI. ***Methods:*** Newborn piglets were randomized to: (i) HI-normothermia ($n=12$), (ii) HI-35 °C ($n=7$), and (iii) HI-33 °C ($n=10$). HI-35 °C and HI-33 °C piglets were cooled between 2 and 26 h after HI. Insult and secondary-energy-failure severity and latent-phase duration were evaluated using phosphorus magnetic resonance spectroscopy and compared with neuronal death in cortical-grey and deep-grey matter. ***Results:*** More severe HI was

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Abbreviations: ^{31}P, phosphorus-31; Pi, inorganic phosphate; PCr, phosphocreatine; NTP, nucleotide triphosphates; ATP, adenosine triphosphate; EPP, exchangeable high-energy phosphate pool; MRS, magnetic resonance spectroscopy; HI, hypoxia–ischaemia; F_iO_2, inspired oxygen fraction; T_{rectal}, rectal temperature; aEEG, amplitude-integrated electroencephalogram; SD, standard deviation; ADC, apparent diffusion coefficient

0006-8993/$ – see front matter
doi:10.1016/j.brainres.2007.03.083

associated with shorter latent-phase ($p=0.002$), worse secondary energy failure ($p=0.023$) and more cortical-grey-matter neuronal death ($p=0.016$). ***Conclusions:*** Latent-phase duration is inversely related to insult severity; latent-phase brevity may explain the apparently less effective neuroprotection following severe cerebral HI.

1. Introduction

Brain injury after transient hypoxia–ischaemia (HI) is an evolving process: transient severe HI and subsequent reperfusion/reoxygenation may lead to some immediate cell death but may additionally precipitate complex biochemical events which eventually lead to some additional delayed neuronal death (Taylor et al., 1999; Johnston et al., 2001; Northington et al., 2001; Orrenius et al., 2003; Brown and Bal-Price, 2003). In birth-asphyxiated infants, although phosphorus-31 (^{31}P) magnetic resonance spectroscopy (MRS) revealed apparently normal cerebral metabolism shortly after delivery (the "latent-phase"), despite adequate oxygenation and circulation, a secondary phase of impaired cerebral energy generation ensued 8 to 24 h after HI, characterised by progressive declines in phosphocreatine (PCr) and nucleotide triphosphates (NTP; mainly adenosine triphosphate (ATP)) and increased inorganic phosphate (Pi) (Azzopardi et al., 1989). This sequence of events has been modelled experimentally (Lorek et al., 1994) and termed "secondary energy failure".

Mild cerebral hypothermia initiated early in the latent-phase has been associated with long-lasting neuroprotection in both adult and perinatal species (Bona et al., 1998; Colbourne et al., 2000; Wagner et al., 2002; Agnew et al., 2003). The results of the first large multi-centre randomised trials of selective-head- and whole-body-cooling in neonatal encephalopathy suggest that mild hypothermia can improve intact survival at 18 months of age (Gluckman et al., 2005; Shankaran et al., 2005); however, these trials raise important questions about the optimal modality, timing and duration of cooling, which maximise neuroprotection. In addition, there is increasing evidence that inter- and intra-subject factors influence the neuroprotective potential of hypothermia. Cooling may be less protective in the most severe cerebral injuries (Haaland et al., 1997; Bona et al., 1998; Nedelcu et al., 2000; Gluckman et al., 2005) and the optimal temperature for neuroprotection may depend on brain region (Iwata et al., 2005). We have recently shown that cooling itself can prolong the latent-phase (O'Brien et al., 2006). Thus, if hypothermia delays the start of secondary energy failure, in addition to direct cerebroprotection, the therapeutic time window, during which additional treatments may provide further benefit, might also lengthen (Dietrich et al., 1995; Guan et al., 2000). However, the dependences of latent-phase duration and regional neuroprotection on insult severity have not been fully investigated. Using a newborn piglet model under normothermia and with delayed whole-body cooling to 35 and 33 °C, we aimed to assess relationships between HI severity and: (i) latent-phase duration; (ii) cerebral energy metabolism during secondary energy failure; and (iii) regional neuronal injury.

2. Results

In 7 animals experimentation terminated early: 2 due to NTP depletion persisting more than 2 h post-HI (1 HI-n and 1 HI-33); 2 due to equipment problems (2 HI-n); and 3 piglets died as a direct consequence of HI (2 HI-n at 18 and 28 h post-HI and 1 HI-33 at 20 h). All other piglets survived to 48 h after HI. Two brains were damaged during removal or histological processing (1 HI-n and 1 HI-33). Results from all these animals were not included in the analysis. Consequently the numbers of piglets analysed were: 6 HI-n; 7 HI-35; and 7 HI-33; in one HI-33 piglet MRS data were only available up to 36 h due to spectrometer failure.

Baseline physiology and biochemistry and temporal changes in systemic temperature during hypothermia have been reported elsewhere (Iwata et al., 2005; O'Brien et al., 2006); there were no significant weight, age, heart rate, blood pressure, or other differences between study groups before HI.

We aimed for HI insults of similar severity, however, amongst the piglets acute energy depletion ranged widely due to variations in the biological responses to both transient HI and resuscitation: mean acute energy depletions were 0.048 (0.021)h for HI-n; 0.070 (0.024)h for HI-35; and 0.086 (0.032)h for HI-33 but statistically similar ($p=0.116$).

2.1. *Dependences of latent-phase duration, secondary-energy-failure severity, and neuronal death on acute energy depletion and systemic temperature (multivariate analysis)*

The dependences on insult severity (the acute energy depletion – see Methods) of latent-phase duration, secondary-energy-failure severity (quantified as the minimum NTP/EPP 6–48 h post-HI where EPP is the exchangeable high-energy phosphate pool = $Pi + PCr + (\alpha+\beta+\gamma) - NTP$), and percentage neuronal death in the cortical grey matter and deep grey matter are shown in Figs. 1A–C. With acute energy depletion as covariate and compared with HI-n: HI-35 had a longer latent-phase ($p<0.001$), less severe secondary energy failure ($p=0.032$), and less neuronal death (cortical grey matter $p=0.001$, deep grey matter $p=0.020$); and HI-33 had a longer latent-phase ($p=0.014$), secondary-energy-failure severity similar to HI-n, and less neuronal death only in cortical grey matter ($p<0.001$) (Table 1). There was less cortical-grey-matter neuronal death in HI-33 than HI-35 ($p=0.022$), but latent-phase duration, secondary-energy-failure severity, and deep-grey-matter neuronal death were similar in both cooled groups. Multivariate analysis also showed that more severe HI (greater acute energy depletion) was associated with shorter latent-phase (Fig. 1A; $r^2=0.55$, $p=0.002$), more severe secondary energy failure (Fig. 1B; $r^2=0.44$, $p=0.023$) and greater neuronal death in cortical grey matter only (Fig. 1C; $r^2=0.75$, $p=0.016$).