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Experimental treatments for hypoxic ischaemic encephalopathy

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ABSTRACT

Hypoxic ischaemic encephalopathy continues to be a significant cause of death and disability worldwide. In the last 1–2 years, therapeutic hypothermia has entered clinical practice in industrialized countries and neuroprotection of the newborn has become a reality. The benefits and safety of cooling under intensive care settings have been shown consistently in trials; therapeutic hypothermia reduces death and neurological impairment at 18 months with a number needed to treat of approximately nine. Unfortunately, around half the infants who receive therapeutic hypothermia still have abnormal outcomes. Recent experimental data suggest that the addition of another agent to cooling may enhance overall protection either additively or synergistically. This review discusses agents such as inhaled xenon, N-acetylcysteine, melatonin, erythropoietin and anticonvulsants. The role of biomarkers to speed up clinical translation is discussed, in particular, the use of the cerebral magnetic resonance spectroscopy lactate/N-acetyl aspartate peak area ratios to provide early prognostic information. Finally, potential future therapies such as regeneration/repair and postconditioning are discussed.

Contents

1. Introduction

Neonatal encephalopathy remains a significant problem worldwide. An estimated 4 million babies die every year during the neonatal period, and one quarter of these deaths are attributed to neonatal asphyxia [1]. Even in the developed world, neonatal encephalopathy is a common clinical condition affecting approximately 2 per 1000 neonates [2] and accounts for a substantial proportion of admissions to neonatal intensive care; 10–15% of cases will die in the neonatal unit, 10–15% will develop cerebral palsy and up to 40% will have other significant disabilities including blindness, deafness, autism, epilepsy, global developmental delay, and problems with cognition, memory, fine motor skills and behaviour [3–5].

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Within the last decade, therapeutic hypothermia for infants with hypoxic ischaemic encephalopathy has been studied in pre-clinical models [6] and several major randomized clinical trials in the developed world [7]. Despite the clinical heterogeneity of perinatal asphyxia and the use of different cooling methods there are consistent findings that hypothermia reduces the extent of neurological damage and improves survival without disability [8]. Therapeutic hypothermia is now widely offered to moderately and severely asphyxiated infants in countries and centers which participated in the trials [9].

Despite the promising outcome of these trials, the reduction in disability or death at 18 months with therapeutic hypothermia is modest — meta-analyses indicate that the composite adverse outcome reduces from 58% to 47% with cooling [5,8]. Thus approximately half the infants who receive therapeutic hypothermia still have an abnormal outcome and some infants with the most severe injuries may not be rescued [7]. Recent experimental data suggest that hypothermia extends the duration of the therapeutic window [10,11] and that certain drugs given during this time may augment neuroprotection [11–13]. Research is now being focused on pre-clinical studies of drugs, which act synergistically or additively with hypothermia with the hope that combination therapy might reduce the overall number of infants needed to treat to improve intact survival.

2. Biomarkers in hypoxic–ischaemic encephalopathy

2.1. Overview of biomarkers

Biomarkers are critically needed in neonatal encephalopathy because the timing of brain injury is heterogeneous and difficult to identify. Biomarkers may have several roles, including: (a) the identification of who is injured, (b) the extent of injury, (c) the timing of injury, (d) identification of the most likely outcomes with and without therapy and (e) speeding up clinical translation of interventions. In a recent systematic review of studies where urine, serum and cerebrospinal fluid biomarkers were taken within the first 96 h after birth in infants with neonatal encephalopathy, four markers were revealed as potentially predictive of abnormal outcomes in the meta-analysis: serum and CSF interleukin-1b, serum interleukin-6, and CSF neuron-specific enolase [14]. None of these biomarkers, however, had been studied extensively enough to warrant routine clinical use and further work is needed for their validation.

Speeding up clinical translation is an important aspect to consider in future trials; therapeutic hypothermia for hypoxic ischaemic encephalopathy took some 15 years to translate from pre-clinical large animal studies to an established therapy in the clinic [15]. During this time, experimental studies were performed showing consistent benefit of cooling [6,16] and clinical trials were based on 12–18 month neurodevelopmental outcome data. Thus clinical translation occurred only after these randomized controlled trial (RCT) results were known [8]. Although knowledge of the neurodevelopmental outcome of therapies is critically important, robust and sensitive quantitative biomarkers with properly validated outcome measures at age18 months or later can help with speeding up clinical translation.

2.2. MR biomarkers

In clinical practice, magnetic resonance imaging (MRI) is increasingly used in newborn infants after perinatal asphyxia where it is an effective biomarker for both disease and treatment effect [17]. To increase prognostic objectivity, quantitative cerebral biomarkers such as proton (^{1}H) magnetic resonance spectroscopy (MRS) have been utilized [18] and a recent meta-analysis of the prognostic accuracy of MR methods demonstrated that the thalamic ^{1}H MRS Lactate/N-acetyl aspartate (Lac/NAA) peak area ratio acquired between days 5–14 is a highly sensitive and specific biomarker of long term neurodevelopmental outcome in infants with neonatal encephalopathy [19] as well as in adult stroke [20]. The ^{1}H MRS lactate peak area metabolite ratios can be used in *in vivo* pre-clinical as well as clinical studies; Lac/NAA has been validated as a bridging biomarker and will be used as a surrogate endpoint in phase II clinical trials of neuroprotection.

In the UCL piglet perinatal asphyxia model we acquire phosphorus-31 (^{31}P) and ^{1}H MRS at baseline and serially during the 48 h following transient hypoxia–ischaemia; the increase in brain lactate and reduction in NAA over this time correlates with brain tissue injury as described below [21]. The biphasic decrease in nucleotide triphosphate (NTP)/exchangeable phosphate pool (EPP = Pi + PCr + NTP) and reciprocal increase in brain lactate during and following a transient hypoxic–ischaemic insult are shown in Fig. 1. In the late 1980s, studies in infants with birth asphyxia were performed using ^{31}P MRS and the characteristic pattern of evolving energy failure in the hours and days after birth in neonatal encephalopathy was described [22]. We have recently focused on ^{1}H MRS, in particular brain Lac/NAA. Brain lactate increases during hypoxia–ischaemia as mitochondria are unable to continue nucleotide triphosphate (NTP) synthesis at a rate sufficient to supply the brain. Glycolysis increases to provide additional NTP and lactate concentration increases. Acidosis increases as well as hypoxic depolarization of cells, cytotoxic oedema, excessive intracellular accumulation of calcium and extracellular accumulation of excitatory amino acids. Following reperfusion, clearance of lactate occurs over ~30 min [23]. A secondary increase in brain lactate may occur as early as 3 h after ischaemia [24,25] due to renewed production in tissues [26] and may persist for considerable periods of time [18,27]. The events leading to the secondary rise in brain lactate are thought to include failure of mitochondrial activity

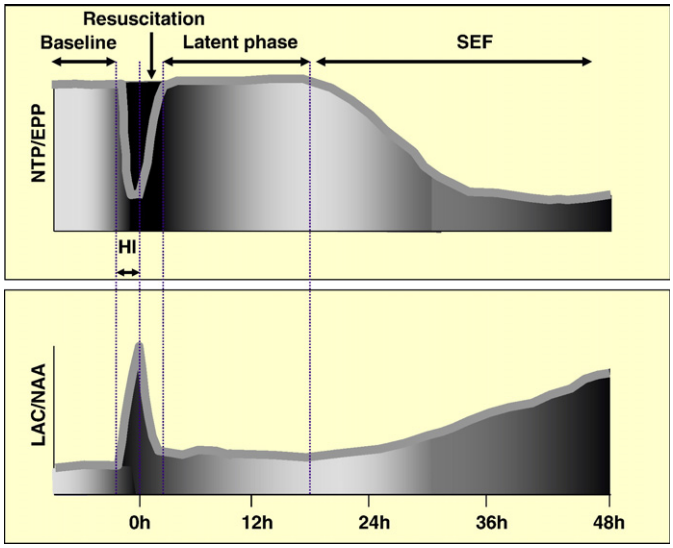

Fig. 1. Schematic diagram illustrating the biphasic pattern of energy failure associated with a transient hypoxic–ischemic insult visualized using 31P MRS (top) and 1H MRS (bottom) in the UCL piglet model. 31P MRS: nucleotide tri-phosphate (NTP)/exchangeable phosphate pool (EPP = Pi + PCr + NTP) is shown on the *y* axis. The change in NTP/EPP during transient hypoxia–ischaemia (HI), resuscitation, the latent phase (period between the recovery from acute HI and the evolution of secondary energy failure (SEF)) and SEF itself are shown. 1H MRS: lactate/N acetyl aspartate (NAA) is shown on the *y* axis. The change in Lac/NAA during transient HI, resuscitation, latent phase and SEF are shown. During the acute energy depletion, some cells undergo primary cell death, the magnitude of which will depend on the severity and duration of HI. Following perfusion, the initial hypoxia-induced cytotoxic edema and accumulation of excitatory amino acids typically resolve over 30–60 min with apparent recovery of cerebral oxidative metabolism (latent phase). It is thought that the neurotoxic cascade is largely inhibited during the latent phase and that this period provides a "therapeutic window" for therapies such as hypothermia and other agents. Cerebral oxidative metabolism may then secondarily deteriorate 6–15 h later (as SEF). This phase is marked by the onset of seizures, secondary cytotoxic edema, accumulation of cytokines and mitochondrial failure. Mitochondrial failure is a key step leading to delayed cell death.