HYPOXIC-ISCHEMIC BRAIN INJURY IN THE NEONATAL PERIOD – CURRENT CONCEPTS, NOVEL DIAGNOSTIC APPROACHES AND NEUROPROTECTIVE STRATEGIES*

HIPOKSIČNO-ISHEMIČNA OKVARA MOŽGANOV V NEONATALNEM OBDOBJU – SODOBNI POGLEDI, NOVE DIAGNOSTIČNE METODE IN NEVROPROTEKTIVNI UKREPI

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Abstract *In the presenting paper, we describe mechanisms of brain injury following a hypoxic-ischemic event in the neonatal period. Neuronal death occurs in two major phases, the primary neuronal cell loss at the time of the insult and the delayed neuronal cell loss, occurring about 6 hours – 4 days after the injury. We describe different cellular mechanisms responsible for the neuronal death. The main patterns of brain injury that can be readily recognized with the newer neuroimaging techniques are dependent on the gestational age of the newborn. In order to apply novel neuroprotective treatments to the newborns with hypoxic-ischemic encephalopathy (HIE), the newborns at risk have to be identified as early as possible. Among the most useful diagnostic methods are amplitude-integrated EEG, new markers of brain lesions and different modalities of magnetic-resonance imaging. During resuscitation of neonates with HIE the importance of prevention of hyperoxia, and, during intensive care, of hypocapnia and hyperglycemia is stressed. In the treatment of newborns with HIE hypothermia, by means of both selective head cooling or whole body hypothermia, reduced the risk of death and disability according to three multicenter randomized controlled studies. It is therefore recommended for treatment of HIE in the newborn. One of the potentially beneficial effects of therapeutic hypothermia is also widening of the therapeutic window for intervention with other neuroprotective regimens. Among these, treatments with erythropoietin or minocycline seem to be clinically promising.*

Key words *hypoxia-ischemia; newborn; neuroprotection; hypothermia; amplitude-integrated electroencephalography*

Izvleček *V pričujočem prispevku sva opisala mehanizme možganske okvare po hipoksično-ishemičnemu dogodku v neonatalnem obdobju. Odmiranje nevronov se odvija v dveh korakih, kot prvotna izguba nevronov v času hipoksično-ishemičnega dogodka in kot zakasnela izguba nevronov, ki sledi 6 ur – 4 dni po prvotnemu dogodku. Opisala sva različne celične mehanizme, ki so povezani z odmiranjem nevronov. Glavni vzorci možganske okvare, ki jih lahko spremljamo s sodobnimi slikovnimi preiskavami, so odvisni od gestacijske starosti novorojenčkov. Da bi lahko novorojenčkom s hipoksično-ishemično encefalopatijo (HIE) pomagali z nevroprotektivnimi zdravljenji, je potrebno ogrožene novorojenčke odkrivati čim bolj zgodaj. Med najuporabnejšimi diagnostičnimi metodami, ki jih danes uporabljamo v ta namen, sodijo amplitudno-povprečena elektroencefalografija,*

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* The authors acknowledge the financial support from the state budget by the Slovenian Research Agency (project No. Z3-9060-0312-06).

novi biokemični kazalci možganske okvare in različne tehnike magnetno-resonančnega slikanje glave. Opisala sva pomen preprečevanja hiperoksije med oživljanjem novorojenčkov s HIE in preprečevanja hipokapnije ter hiperglikemije v obdobju intenzivnega zdravljenja le-teh. Tri multicentrične raziskave kažejo, da je zdravljenje novrojenčkov s HIE s hipotermijo, tako s selektivnim ohlajanjem glave, kot z ohlajanjem celega telesa, povezano z manjšim tveganjem za smrt in možgansko okvaro, zato je takšno zdravljenje priporočljivo. Eden od možnih koristnih učinkov terapevtske hipotermije je tudi podaljšanje časovnega okna, v katerem je mogoče novorjenčke zdraviti še z drugimi nevroprotektivnimi ukrepi. Med temi klinično največ obetata zdravljenji z eritropoetinom in minociklinom.

Ključne besede *hipoksija-ishemija; novorojenec; nevroprotekcija; hipotermija; amplitudno-povprečena elektroencefalografija*

Introduction

Hypoxic-ischemic cerebral injury that occurs during the perinatal period is one of the most commonly recognized causes of severe, long-term neurological deficits in children.[1] Hypoxic-ischemic encephalopathy (HIE) of the newborn occurs with the incidence of 1–4/1000.[2] Between 20 % and 50 % of newborn infants affected by perinatal brain injury die during the newborn period, and 25–60 % of the survivors suffer from permanent neurodevelopmental handicaps, including cerebral palsy, seizures, mental retardation, and learning disabilities.[2–4] Hypoxic-ischemic brain injury occurs at or near the time of birth and may be amenable to post-natal neuroprotective interventions.[5] The aim of this article is to review current concepts of perinatal hypoxic-ischemic brain injury, novel diagnostic methods and neuroprotective strategies.

Mechanisms of brain injury following hypoxic-ischemic event

Following a hypoxic-ischemic insult, it appears that neuronal death occurs in two major phases, the primary neuronal cell loss at the time of the insult and the delayed neuronal cell loss.[6] Neuroimaging studies have shown, that brain injury following a hypoxic-ischemic event evolves over days, if not weeks.[7]

Primary cell loss is related to cellular hypoxia, which leads to exhaustion of high-energy metabolism (primary energy failure) and cellular depolarization. During primary energy failure, studies suggest that there are three closely interrelated mechanisms involved in the death of neurons. Firstly, depolarization due to hypoxia causes an influx of sodium and a lesser efflux of potassium with passive chloride entry along the electrochemical gradient. This leads to cell swelling and, if sufficiently severe, cell lysis.[8] Secondly, intracellular calcium accumulation occurs due to both excessive entry of calcium due to failure of ion channels and of calcium removal by the sodium-calcium pump.[9] Thirdly, extracellular glutamate accumulation (excitotoxicity) due to failure of energy-dependent re-uptake and excessive release is also a key mechanism stimulating intracellular calcium accumulation through the N-methyl-D-aspartate (NMDA)-receptor-channel complex.[10] Further cell membrane damage may occur due to the action of free radicals in the immediate reperfusion phase.[11] However, many neurons do not die during the primary phase of neuronal death. Rather, a cascade of pathologic processes is triggered and leads to further loss of neurons, starting some hours later and extending over several days.

This secondary loss of neurons is termed secondary or delayed neuronal death. This phase may be associated with hyperexcitability and cytotoxic edema from about 6 hours – 4 days after the injury, as found in a study on fetal sheep.[12] In this study, 15 chronically instrumented fetal sheep following transient cerebral ischemia were studied to estimate changes in extracellular space. The peak of the secondary edema was found at 28 ± 6 hours after the insult. In the human infant, the severity of the secondary energy failure is correlated with adverse neurodevelopmental outcome at 1 and 4 years.[13] The mechanisms involved in delayed neuronal death include excitotoxicity,[14] cytotoxic actions of activated microglia, mitochondrial failure,[15] NO synthesis,[16] exposure to free radicals,[17] inflammation,[18] and apoptosis.[19] Recent data suggest that apoptosis plays a prominent role in the evolution of hypoxic-ischemic injury in the neonatal brain and may be more important than necrosis after injury.[20] A prominent degree of neuronal injury has been also associated with the development of seizures and changes in cerebral blood volume and flow in the near-term fetal sheep model of asphyctic brain injury.[15, 16] Clinically, it is this delayed phase of neuronal injury that is amenable to potential intervention(s). Factors such as the severity, pattern and type of insult, as well as the gestational age and metabolic status, including temperature, of the infant are crucial determinants of the neuropathology of hypoxic-ischemic brain injury in the newborn.[4] Advanced methods of neuroimaging, such as magnetic resonance imaging (MRI), magnetic resonance spectroscopy, and diffusion-weighted MRI, have identified patterns of damage after ischemic insult to the newborn brain. In a study of 104 children with evidence of bilateral hypoxic-ischemic brain damage, at least three different patterns were observed with the use of MRI.[21] These patterns are dependent on the gestational age of the infant, because certain neuron groups exhibit age-specific vulnerability. Periventricular leukomalacia was observed in premature infants with a history of subacute or chronic hypoxia and ischemia. Lesions in the basal ganglia and thalamus occurred in full-term babies who had profound asphyxia. Multicystic changes were seen in a minority of infants who had severe encephalopathy but only a mild hypoxic-ischemic event; this group may include babies who had underlying fetal infections or metabolic disorders that had eluded diagnosis. These data suggest that injury is related to the gestational age at the time of the insult, although the severity or chronicity of the insult may be a better indicator of eventual outcome.

Similar insults to the neonatal brain will manifest themselves differently in different babies in terms of the injury, as observed on imaging studies such as MRI, and in terms of neurodevelopmental outcome. Such variability has also been observed in animal models and appears to be genetically based.[22] Certain polymorphisms may increase the risk for many complex diseases.[23, 24] However, susceptibility factors for neonatal brain injury have yet to be identified clearly.

Until the last decade, management strategies have largely been supportive and not targeted toward the processes of ongoing injury.[25] However, novel exciting strategies aimed at preventing ongoing injury are being clinically evaluated and offer an opportunity for neuroprotection, if brain injury is diagnosed and treated early enough.

Novel diagnostic approaches to newborns with hypoxic-ischemic encephalopathy

Amplitude-integrated electroencephalography

Among newer diagnostic methods used in newborns with HIE is amplitude-integrated electroencephalography (aEEG). It was designed in the late 1960s by Maynard, but not until it was used to study newborns with HIE in 1980s did it gain widespread clinical use.[26, 27] The aEEG has been also shown to be of use for selection of newborns for neuroprotective therapies, such as hypothermia, within the first hours after birth.[28]

Depending on the severity of the HIE, different background patterns can be observed on aEEG tracings: continuous normal voltage (CNV), discontinuous normal voltage (DNV), burst suppression (BS), continuous low voltage (CLV), and isoelectric or flat trace (FT). Epileptiform activity can also be detected with aEEG as a single seizure (SS), repetitive seizures (RS), or status epilepticus (SE). The presence or absence of sleep-wake cycles can also be determined with the use of aEEG.[29, 30] The correlation between the background pattern on aEEG and the grade of HIE was found to be consistent.[31, 32] The trends of background patterns on aEEG tracings and the time interval between birth and appearance of sleep-wake cycles have been found to be of predictive value.[33, 34] With the use of aEEG it is possible to detect and treat clinically silent epileptiform discharges, which significantly improves the outcome of these newborns.[35] Monitoring of newborns with aEEG has been found to be of clinical value also in newborns with non-neurological life-threatening conditions demanding intensive treatment with extracorporeal membrane oxygenation (ECMO).[36] In the study of Pappas et al., all of the newborns who showed continuous normal voltage on aEEG tracings throughout the treatment had a normal neurological outcome.

In the Department of Pediatric Surgery and Intensive Care, University Medical Centre Ljubljana, we have been using aEEG for monitoring of newborns with HIE since 2001.[30] Our findings are in accordance with the findings of other authors. We have seen good correlation between aEEG and standard EEG recordings when comparing background activity, while the correlation in assessing epileptiform activity was less obvious. In full-term newborns without severe hypoxic-ischemic encephalopathy who had other neurological conditions we have found aEEG to be of value for detection of epileptiform activity.

Newer digital aEEG monitors, as opposed to the single channel analog aEEG devices (such as Lectromed cerebral function monitor), offer 2-channel aEEG tracings (one aEEG channel for each hemisphere) with simultaneous standard EEG tracings (in our department we use the BrainZ monitor). In a recent study using such devices, good predictive value has been found for aEEG findings combined with clinical examination and magnetic resonance investigation of the head.[37]

Biochemical markers of hypoxic-ischemic brain injury of the newborn

Among the earliest biochemical markers of brain hypoxia in the CSF and/or plasma studied were lactate which accumulates in hypoxic cells due to glycolysis and hypoxanthine which is formed by ATP breakdown.[38, 39] Although several studies found a correlation between the degree of HIE and concentrations of both markers, their long term prognostic value was poor.[40] Among enzymes released from the cells during brain hypoxia, the first studied were LDH with isoenzymes, creatine-kinase (particularly its brain-specific isoenzyme CK-BB) and adenylate kinase.[41] Again, their concentrations were correlated with early clinical signs of HIE, but only CK-BB in the CSF had a prognostic value for further development in some studies while others could not confirm it.[42]

Other markers of brain hypoxia in CSF and/or plasma were as follows: monoamine neurotransmitters norepinephrine and dopamine;[43] neuron-specific enolase;[44] components of damaged glial cells, such as acidic glial protein[45] and protein S-100;[46] hydroperoxide and advanced oxidation protein products;[47] nucleated red blood cells and non-protein bound iron,[48] and, most recently, activin, a glycoprotein expressed in the central nervous system.[49] Again, most of these markers correlated with early clinical signs of HIE whereas they did not have long-term prognostic value.[50]

Newer imaging techniques

Bedside ultrasound (US) serial imaging performed with the newer advanced US machines remains an important imaging technique in newborns with HIE.[51] Although no study has been performed in which US imaging would be compared with MRI, it is clear from other studies that MRI is invaluable for precise diagnostic evaluation of brain injury.

Several studies performed in the latter years have shown importance of MRI in early diagnosis as well as in follow-up studies of newborns with HIE.[21, 52, 53] Perinatal lesions are best detected between the first and second week of life. Very early MRI, performed within the first three days of life is of clinical importance when deciding whether to continue with treatment or not in the artificially ventilated newborns, but the MRI changes are subtle.[54] Standard T1 weighted MRI performed with a 1–1.5 T or stronger machine is best for evaluation of basal ganglia and posterior end of the capsula interna, while T2 weighted MRI imaging is better for early detection of ischemic lesions and imaging differentiating between white and grey matter.[55] Diffusion weighted imaging is best for detection of ischemic lesions within the white matter.[54] With the use of MRI in newborns with HIE, it is possible to predict the pattern of the later neurodevelopmental deficit. Venous contrast imaging and arterial angiography can contribute additional diagnostic and prognostic data. Diffusion tensor imaging has an even greater sensitivity for detection of brain lesions. Fractional anisotropy is considerably lowered in white matter after moderate to severe hypoxic-ischemic brain injury. Although neonatal MRI is a field experiencing rapid development in some centers, for many neonatologists this imaging technique is still difficult to perform due to lack of MR machine availability and a relatively complicated procedure for preparation of a newborn undergoing MRI (absence of ferromagnetic material in incubators, respirators, monitors, etc.).

Positron emission tomography (PET) has been used for imaging of brain metabolism in animals and humans with HIE. In a study performed on fetal lambs, global cerebral metabolic rate was significantly lower in lambs subjected to cord occlusion than in controls.[56] Although PET has been used in evaluation of adult patients with stroke,[57] it's clinical value in the neonatal period has not been evaluated.

Treatment

Resuscitation of newborns with room air instead of with 100 % oxygen

In the last fifteen years an increasing number of studies have shown that resuscitation with room air instead of with 100 % oxygen poses a considerably smaller oxidative stress on newborns.[58] Newborns resuscitated with room air presented spontaneous respirations sooner,[59] and those who did not experience hyperoxemia and hypocapnia had a better psychophysical development afterwards.[60] Two meta-analysis have been published confirming a smaller mortality of newborns who were not resuscitated with 100 % oxygen.[61] Minimizing oxidative stress is not only expected to protect newborn brain, but also other vital organs. On the basis of the findings from animal and human studies, some authors recommend routine use of resuscitation with room air or at least supplementation of oxygen in lower concentrations.[62]

The importance of normocapnia and normoglycemia

In 2006 Perlman wrote an extensive overview of different procedures and drugs which can be potentially used in treating newborns with HIE.[63] Most newborns with moderate to severe brain injury have to be artificially ventilated. Normocapnia or slight hypercapnia have to be maintained, while hypocapnia has to be avoided because additional ischemic damage due to vasoconstriction can be expected. The results of animal experiments have shown major morphological changes following hypocapnia during resuscitation, mild hypercapnia has been found to be protective, whereas severe hypercapnia was also found to be related to a poor outcome, possibly because of detrimental effects on circulation.[64] Arterial blood pressure should be maintained within normal limits, while hydration should be on the lower limit. However, in a recent meta-analysis, the authors could not find any randomized study to support fluid restriction in HIE of the newborn.[65] In newborns with HIE normoglycemia has to be maintained at all times, because hypoglycemia and hyperglycemia both aggravate the brain injury.[66, 67] One of the possible mechanisms of cell damage of hyperglycemia is the stimulation of inducible nitric oxide synthase (iNOS) leading to higher concentrations of iNOS, mitochondrial damage, and intracellular edema.

Drugs with potential neuroprotective effects

Among the first potential drug candidates in the treatment of newborns with HIE was a calcium blocker, nicardipine. Because this drug was related to severe drop in blood pressure, its use was discontinued in clinical studies.[68]

Studies on barbiturates were giving contradictory results: while thiopental was not shown to have any effect on the later neurological disability, high doses of phenobarbital (40 mg/kg) immediately after birth were related to a better neurodevelopmental outcome of newborns with HIE.[69] However, in another study early administration of phenobarbital immediately after hypoxic-ischemic injury resulted in higher morbidity and mortality of asphyxiated newborns.[70]

In spite of promising first results with the use of magnesium sulphate (antagonist of glutamate receptors) in treatment of newborns with HIE,[71] larger studies did not confirm the clinical benefits of its use.[72] Magnesium sulphate treatment did also not show any improvement on aEEG background pattern.[73]

Animal studies using allopurinol, a xanthine oxidase inhibitor, showed that allopurinol reduces free radical production, preserves the cerebral energy state, reduces brain edema, and improves the brain electrical activity when given before reperfusion. However, a recent clinical study on newborns with HIE did not show any benefit of allopurinol administration, because treatment started postnatally was too late to reduce the early reperfusion induced free radical surge.[74] The authors speculated that allopurinol administration to the fetus with (imminent) hypoxia via the mother during labor might be more effective in reducing free radical induced post-asphyxial brain damage.

Erythropoietin (EPO) is another potential candidate for neuroprotective treatment of newborns with HIE. Its neuroprotective effects are attributed to the fact that EPO lowers intracellular levels of calcium and diminishes glutamate toxicity, apoptosis, inflammation, and has an antioxidative effect.[75] Although many animal studies have proven the beneficial effect of EPO administration, clinical data on newborns are still scarce. Besides neuroprotective role, EPO shows also neurogenetic properties, similarly to vascular endothelial growth factor (VEGF): they both promote regeneration after hypoxic-ischemic brain injury.[76]

Animal studies have shown that melatonin is a potent free oxygen radical scavenger.[77] Among other potential neuroprotective substances are 2-iminobiotin, estradiol, minocycline, and xenon. In a recent randomized clinical study in adults with ischemic stroke minocycline significantly improved outcome compared to placebo.[78]

Therapeutic hypothermia

The most acclaimed neuroprotective treatment of newborns with HIE to date is early therapeutic hypothermia, starting within 6 hours after hypoxic-ischemic event. Several animal studies have shown beneficial effects of therapeutic hypothermia: lowering of metabolic rate, minimizing the extent of apoptosis, formation of NO and free oxygen radicals, epileptiform activity, cerebral edema, and maintenance of the hematoencephalic barrier.[79]

Currently there are two approaches to therapeutic hypothermia: head cooling with a specially designed cap (Cool Cap), accompanied with mild hypothermia of the whole body (rectal temperature 34–35 °C), and whole body cooling with a cooling blanket, accompanied by moderate hypothermia of the whole body (rectal temperature 33–34 °C). After early diagnosis of HIE, usually by means of aEEG and clinical signs, hypothermia is initiated within 6 hours after hypoxic-ischemic event and is maintained for the next 48–72 hours. During hypothermia, several parameters have to be strictly monitored: heart rate and function, blood pressure, electrolytes, blood gases, blood glucose, and coagulation factors. Rewarming of the patient has to be graduate and slow, using a warming blanket or warm air.

Several studies have investigated the possible side effects of hypothermia. The results of these studies have shown no major side effects if rectal temperature was maintained between 33–35 °C.[80–83] In the year 2005 the results of a smaller multicenter hypothermia study on newborns with severe HIE and of gestational age > 35 weeks were published.[84] Sixty-five newborns were randomized within 6 hours after birth and their rectal temperature was maintained at 33.5 °C with a cooling blanket for 48 hours. At one year follow up, 52 % of newborns treated with hypothermia had severe motor disability as opposed to 84 % of newborns that were not cooled, a difference found to be significant. The first large multicentre study published was the »Cool Cap« study,[85] which included 234 newborns of gestational age > 36 weeks with acidosis, Apgar scores < 5 at 10 minutes, and clinical and pathological changes on aEEG tracings. Hypothermia was initiated within first 6 hours after birth using a cooling cap, reaching rectal temperatures of 34.5 °C, and was continued for the next 72 hours. At 18 months the treated and non treated groups were compared, but no significant difference in outcome was found. If the results were stratified, a significant difference was found for the less severely asphyxiated newborns in favor of treatment with hypothermia. Severely abnormal motor scores were recorded in 24 % of hypothermia and 64 % of normothermia patients. In the third multicentre study of Shankaran and coworkers,[86] 208 newborns with gestational age of > 36 weeks, clinical signs and history of HIE were included. Whole body hypothermia was employed for 72 hours, with the rectal temperature being set to 33.5 °C. At 18 months follow-up, their findings demonstrated the safety and effectiveness of whole-body cooling in reducing the risk of death or disability among infants with moderate or severe encephalopathy.

Expert groups for neonatal hypothermia support the use of therapeutic hypothermia, but under strict conditions of protocols, that were used in larger studies, extensive follow-up of patients, and contributing data to hypothermia registries.[87, 88] In a recent study, not more than 6.4 % of the neonatal units in the United States were found to use therapeutic hypothermia.[89] In Slovenia we take part of the nEuro Network Neonatal Hypothermia protocol and registry, founded by Prof. Simbruner in 2001. This protocol was approved by Slovenian Ethical Committee of the Ministry of Health of Republic of Slovenia. The protocol suggests the parents of the patients to be informed, but their informed consent is not mandatory for the initiation of treatment.

Marianne Thoresen, one of the pioneers of therapeutic hypothermia in newborns, suggests that hypothermia should be started immediately after resuscitation (without intermittent warming of the patient) and combining hypothermia with other treatment modalities might prove to be of even greater value. To date, it is also not clear which cooling method, whole body or head cooling is more efficient. In a recent MRI study of newborns with HIE, the methods did not differ in basal ganglia lesions, but there were significantly less cortical lesions in newborns cooled with the cooling cap.[90]

Conclusions

Until last decade, management strategies of newborns with HIE have been mainly supportive. Three multicentre studies have approved routine clinical use of therapeutic hypothermia, giving the possibility to actively modify the natural history of perinatal brain injury in a favorable way. However, the study protocols have to be strictly followed, the findings should be monitored with registers, and the children should have rigorous follow-up in order to provide best possible care. Other neuroprotective strategies are being investigated, but their clinical value remains to be proven by the future studies.

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Accepted 2008-02-16