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Neonatal brain injury as a consequence of insufficient cerebral oxygenation

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Abstract

Neonatal brain hypoxic-ischemic injury represents a serious health care and socio-economical problem since it is one of the most common causes of mortality and morbidity of newborns. Neonatal hypoxic-ischemic encephalopathy is often associated with signs of perinatal asphyxia, with an incidence of about 2-4 per 1,000 live births and mortality rate up to 20%. In about one half of survivors, cerebral hypoxic-ischemic insult may result in more or less pronounced neuropsychological sequelae of immediate or delayed nature, such as seizures, cerebral palsy or behavioural and learning disabilities, including attention-deficit hyperactivity disorder. Hypoxic-ischemic injury develops as a consequence of transient or permanent restriction of blood supply to the brain. Severity of hypoxic-ischemic encephalopathy varies depending on the intensity and duration of hypoxia-ischemia, on the type and size of the brain region affected, and on the maturity of the foetal/neonatal brain. Though a primary cause of hypoxic-ischemic injury is lack of oxygen in the neonatal brain, underlying mechanisms of subsequent events that are critical for developing hypoxic-ischemic encephalopathy are less understood. Their understanding is however necessary for elaborating effective management for newborns that underwent cerebral hypoxic-ischemic insult and thus are at risk of a negative outcome. The present paper summarizes current knowledge on cerebral hypoxic-ischemic injury of the neonate, fundamental processes involved in etiopathogenesis, with a special focus on cellular and molecular mechanisms and particular attention on certain controversial aspects of oxidative stress involvement.

REVIEW ARTICLE

Abbreviations:

ACOG	- The American Congress of Obstetricians and Gynecologists
AMPA	- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AAP	- American Academy of Pediatrics
ATP	- adenosine-5′-triphosphate
CA1	- cornus ammonis 1
CMACE	- Centre for Maternal and Child Enquiries
CT	- computed tomography
cUSG	- cranial ultrasonography
Epo	- erythropoietin
HIE	- hypoxic-ischemic encephalopathy
HII	- hypoxic-ischemic insult
ILCOR	- International Liaison Committee on Resuscitation
JSTx	- joro spider toxin
MK-8015	- methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5,10-imine
MRI	- magnetic resonance imaging
MRS	- magnetic resonance spectroscopy
NAC	- N-acetyl cysteine
NBQX	- 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline
NIRS	- infrared spectroscopy
NMDA	- N-methyl-D-aspartate
PCr	- phosphocreatine
PET	- positron emission tomography
ROS	- oxygen species

INTRODUCTION

Perinatal asphyxia and neonatal brain hypoxic-ischemic (HI) injury are of the most common causes of neonatal mortality and morbidity in newborns. The incidence of perinatal asphyxia is just about 3.0 per 1000 live births and for hypoxia-ischemia it is 1.5; 30% of cases of neonatal hypoxic-ischemic encephalopathy (HIE) in developed populations and 60% in developing populations present some evidence of intrapartum hypoxia-ischemia (Kurinczuk *et al.* 2010; Law *et al.* 2006; Pierrat *et al.* 2005). The consequences are however quite serious. A mortality rate (15–20%) and a risk of severe disabilities (25%) resulting from HI insult in the perinatal period are however quite high (Shankaran *et al.* 2005; Vannucci & Perlman 1997). Deaths from HIE are significantly related to gestational age (CMACE 2011).

Brain injury develops as a result of transient, intermittent or permanent restriction of cerebral blood supply. Severity of the injury varies depending on the intensity and duration of the HI insult, on the maturity of the foetal/neonatal brain, and on the type and size of the brain region insulted (Douglas-Escobar *et al.* 2015; Back *et al.* 2007; Fan, *et al.* 2005; Moustafa & Baron 2008; Riddle *et al.* 2006). Other aspects affecting the outcome include nutritional status of the brain, intrauterine growth restriction, infection, pre-existing brain pathology, developmental brain defects, and the frequency and severity of seizures at early postnatal age. Neonatal HIE refers to neurological abnormalities occurring during the early days of life of the newborn who has suffered from a hypoxic or ischemic brain insult.

It has to be mentioned that there is some terminological confusion concerning the topic. Until recently, the terms of perinatal asphyxia and neonatal HIE have been used rather loosely, as synonyms. In fact, each of the clinical signs of HIE were often, yet wrongfully, interpreted as a result of birth asphyxia. This led to a misconception of HIE as a marker of improper perinatal management. In fact, terms such as perinatal or birth asphyxia, respiratory distress syndrome, neonatal encephalopathy or HIE indicate a specific diagnosis (World health statistics 2011). The term birth asphyxia is not quite precise as it implies that intrapartum anoxia has occurred. So, instead of perinatal asphyxia, neonatal HIE, a more appropriate term, should be used. It refers to cerebral HI insult occurring in the perinatal period with resulting HIE in newborns. Nevertheless, to provide direct evidence on cause and consequence relationship between cerebral ischemia-hypoxia and neonatal brain injury is often difficult (Cowan *et al.* 2003).

Forms of HIE are closely related to the degree of brain injury ranging from early mortality, through severe, moderate and mild impairment. HIE may be transient or chronic and of immediate or delayed nature, and includes seizures, cerebral palsy, epilepsy, mental retardation, neurobehavioural and learning disabilities, etc. (Steinman *et al.* 2009; Sedlackova *et al.* 2014; Himmelman *et al.* 2007; Nagy *et al.* 2005; Cirulli *et al.* 2003; Barnett *et al.* 2002).

Timely diagnosis is crucial for effective treatment of cerebral HI injury. Yet, neurological examination by itself or even combined with routinely applied instrumental techniques, like cranial ultrasonography or computed tomography, are not sensitive enough in the early course of the disease (Khan *et al.* 2010). During the last few decades, enormous effort has been invested into the search for more effective diagnostic tools. Providing clear evidence of the evolving brain HI injury and timely assessment of cerebral haemodynamics and oxygenation are of particular importance. Techniques based on magnetic resonance fulfil these requirements. They have become the most reliable approaches for timely diagnosis of the HI insult and are increasingly being introduced into clinical practice and research endeavours. Furthermore, effective management of newborns with cerebral HI insult requires also understanding of key mechanisms that underlie the development of various forms of HIE.

Severe and lifelong sequelae of cerebral HI injury of the newborn represent a serious medical issue with high socio-economic significance as the measures concern both the health-care system and the afflicted families, and these are in an obvious need of permanent support from the whole society (Barkowich & Sargent 1995; Logitharajah *et al.* 2009).

The purpose of the present paper was to provide a brief overview of mechanisms of HI insult to the neonatal brain and to summarise principal findings of both clinical reports and experimental studies from animal models of cerebral hypoxia-ischemia. Particular attention was given to oxidative stress, generally considered

as one of the underlying mechanisms, and also to failures in providing effective treatment by antioxidant therapy.

CLINICAL MANIFESTATION DURING AND AFTER DELIVERY

Clinically, infants with HIE often exhibit respiratory and feeding difficulties, bradycardia, depression of tone and reflexes, subnormal consciousness, cyanotic skin colour, and seizures. In 1958, Apgar and colleagues reported that hypoxia and acidosis were among underlying processes affecting newborns with low neurological scores (Apgar *et al.* 1958). Risk factors for HIE are associated with the period before conception, prenatal and intrapartum period (Table 1).

Generally, HI injury develops as a consequence of decreased blood flow to the brain. In stroke, blood supply is usually restricted locally (Ramaswamy *et al.* 2004), e.g. due to the occlusion of a particular cerebral artery by an embolus or thrombus; this leads to focal ischemia. Global ischemia or hypoxia, on the other hand, is often resulting from low blood or oxygen supply to the foetal/neonatal brain, e.g. due to placental insufficiency or low blood oxygen saturation, respectively (Trollmann *et al.* 2010). Depending on the type of cerebral hypoxia-ischemia, the evolving injury represents a focal, multifocal or diffuse pattern (Triulzi *et al.* 2006). Hence, arterial or venous infarction usually results in focal damage, while HIE is a typical example of diffuse injury (Chau *et al.* 2009b).

There are actually two critical periods in life when the risk of cerebral HI is quite high – the perinatal period and that of advanced age. In both periods, the insult is associated with an increased risk of death or persisting neurological defects in survivors (Hurn *et al.* 2005). In fact, mothers and infants are during pregnancy and in the postpartum period especially vulnerable to thrombotic and thromboembolic complications, often resulting in stroke (Armstrong-Wells *et al.* 2009; Chalmers 2005; Nelson *et al.* 2007). Stroke is more common in the perinatal period than later in childhood or adulthood. Yet, unlike in older children or adults, there are hardly any clinical signs that would permit even a presumptive diagnosis of perinatal stroke in newborns (Ramaswamy *et al.* 2004; Wu *et al.* 2005). This does rather complicate early diagnosis of cerebral HI insult in neonates. Furthermore, some infants, whose neurological status did not indicate any concern in their neonatal period or who were born in facilities missing an appropriate neuroimaging technique, may be diagnosed with perinatal stroke later, approximately at the age of 4–5 months, based on the onset of seizures and/or failure to thrive. This is considered as the main reason why a vast majority of newborns may not be initially recognized as affected by perinatal stroke although they later evolve cerebral palsy (Golomb *et al.* 2001).

In general, susceptibility of newborns to HI insult depends on the state of brain development and maturation of its particular structures and processes, including the maturity of energy metabolism (Hummler *et al.* 2012; Juranek *et al.* 2009). Relative energy demands of various parts of the brain at the time of insult may actually determine their posthypoxic fate (Rutherford *et al.* 2010). Indeed, a profound HI insult results in diffuse injury in the hippocampus, basal ganglia, the thalamus, the perirolandic cortex and brain stem in full-term infants, while in preterm newborns it tends to affect the lower basal ganglia and brainstem and spare the cortex (Barkowich & Sargent 1995; Logitharajah *et al.* 2009). Less serious encephalopathy, on the other hand, was associated with parasagittal watershed territory infarcts in term neonates (Miller *et al.* 2005a), while preterm newborns usually suffer from intraventricular haemorrhage and periventricular white matter damage (Liauwa *et al.* 2008a; Nikas *et al.* 2008).

Tab. 1. Risk factors for mother, baby and labour (Badawi *et al.* 1998a,b; CMACE, 2011; Chau *et al.* 2009a; Ozturk *et al.* 2001).

mother	child	labour
socioeconomic status → malnutrition or obesity	premature or postmature infant	abnormal placenta (placental abruption and placenta previa)
hypoglycaemia, anaemia, thyroid disease	intrauterine growth restriction	occipitoposterior/breech position
intrauterine infections, maternal fever	chromosomal or congenital anomalies	tight nuchal or prolapsed cord
preeclampsia, bleeding in pregnancy	foetal coagulation disorders	medicated childbirth
family history of neurological disease		immediate umbilical cord clamping
multiple pregnancy		other acute intrapartum events
conception after infertility treatment		
assisted reproductive technology		

EARLY DIAGNOSIS AND PERINATAL MONITORING

As the hypoxic consequences may be very serious for the immature brain, HI insult has to be diagnosed as soon as possible. Indeed, early diagnosis of cerebral HI insult is inevitable for effective treatment. Foetal monitoring should contribute to timely detection of foetal brain hypoxia, and application of immediate measures may reduce perinatal morbidity and mortality. In fact, detection of an evolving foetal HI insult is the most common indication for pregnancy termination by caesarean section. Recent development of a number of prenatal tests has opened a window on the process. Furthermore, continuous monitoring of labour and immediate care when required are crucial for preventing adverse outcomes related to childbirth (Soni 2009). Intrapartum foetal monitoring is usually performed by cardiotocography (CTG), biophysical profile test, foetal pulse oxymetry, and by the ST segment analysis of foetal electrocardiogram.

Postnatal diagnosis includes evaluation of classical clinical parameters, such as Apgar score (1953), partogram (1954), Sarnat classification of HIE (1976), MacLennan's criteria of an acute intrapartum hypoxic event (1999), pH from umbilical artery, lactate levels in foetal blood, and base excess. In fact, umbilical cord blood gas and acid-base assessments are the most objective determinations of the foetal metabolic condition at the moment of birth (ACOG Committee Option No. 326. 2005). The overall postpartum condition of the foetus is evaluated by a neonatologist. However, newborn encephalopathies of varying aetiologies can overlap and thus make it difficult to identify causality through the use of neurological examination only (Handley-Derry *et al.* 1997; Nelson *et al.* 1996). Hence, a proper and timely diagnosis of cerebral HI injury depends solely on neuroimaging. Instrumental procedures may include computed tomography (CT), positron emission tomography (PET), cranial ultrasonography (cUSG), and near infrared spectroscopy (NIRS) or magnetic resonance (MR) examinations. To detect brain oedema, a dominant pathophysiological event, CT and MR techniques are frequently used (Schaefer *et al.* 2008).

However, the most widely used instrumental approaches – CT and cUSG, particularly the resistance index of the middle cerebral artery obtained with Doppler ultrasonography, often fail to reveal cerebral HI insult at its early stages (Eken *et al.* 1995; Rutherford 1994). Enormous effort has been therefore invested into finding a new non-invasive approach to assessment of cerebral haemodynamics and brain oxygenation. Actually, NIRS allows calculations of cerebral blood flow and blood volume in the brain and also provides information on brain oxygen consumption (Huang *et al.* 2004; Nicklin *et al.* 2003). Nowadays, other non-invasive, non-destructive and real-time-operating techniques, namely magnetic resonance imaging (MRI) and spectroscopy (MRS), are increasingly utilised in neurological and neonatology departments to confirm or refute cerebral HI injury (Fan *et al.* 2003; Juranek & Baciak 2011; Liauwa *et al.* 2008b). Compared to other neuroimaging approaches, these techniques are advantageous specifically in depicting the site and extent of cerebral HI insult very precisely, at its early stage (Chau *et al.* 2009a). They also enable better differentiation of the affected from the non-affected regions, i.e. distinguishing the core and penumbra of the HI damage (Boichot *et al.* 2006; Liauwa *et al.* 2008b; Moustafa & Baron 2008).

CELLULAR AND MOLECULAR MECHANISMS

Primarily, lack of oxygen reduces energy metabolism, which, if persisting, leads to a failure of cellular functions and finally may result in cell death. Yet animal experiments and clinical studies indicate that in addition to energy loss there are also other mechanisms accounting for neuronal damage. Even sublethal HI insult can set in motion a series of deleterious reactions that finish off affected neurons and deteriorate the adjacent ones that have not been damaged during the initial insult. Eventually, exposure to sublethal HI insult induces an endogenous protection (hypoxic preconditioning) of neuronal tissue against the subsequent more severe hypoxic insult (Cui *et al.* 2004). Thus, following global ischemia, neurons do not always die immediately or all at once. In some of them, damage develops hours or days after the insult. Some neurons undergo necrosis, while in other neurons HI insult triggers apoptosis. Hence under acute energy depletion, some cells undergo cell death leading to tissue injury, whose magnitude depends on the severity and duration of the HI episode (Northington *et al.* 2011).

CYTOSOLIC CALCIUM OVERLOAD

Energy depletion resulting in membrane depolarisation in both neurons and glia leads to subsequent increase in cytosolic calcium concentration via Ca^{2+} influx through specific voltage operated channels. In turn, calcium-dependent processes in various cell compartments may get upregulated (MacDonald *et al.* 2006) Thus activation of calcium-dependent degradative enzymes may substantially contribute to the pathogenesis of cerebral HI insult (Gavini *et al.* 2000; Juranek & Baciak 2009, 2011; Juranek & Bezek 2005; MacDonald *et al.* 2006). Due to cytosolic calcium overload, a massive release of excitatory amino acids, particularly glutamate, into the extracellular space is frequently observed. Resulting over-activation of glutamate receptors leads to an enhanced influx of ions, such as Ca^{2+}, Na^{+} and Cl^{-}, causing further intracellular overload with these ions, ultimately driving water molecules into the cell. This results in development of regional cytotoxic oedema,

which can affect surrounding regions by oppression and thus reduce their perfusion. Subsequent extravasation causes extracellular vasogenic oedema. The reduced perfusion may lead to secondary membrane depolarisation in the close vicinity of the primary insult and to the spreading of oedema within the brain. The overall process may then be aggravated by increasing intracranial pressure, with readily occurring compression of cerebral vessels, herniation, etc. (Beitzke *et al.* 2008; Moustafa & Baron 2008).

EXCITATORY NEUROTRANSMITTER TOXICITY (EXCITOTOXICITY)

A large body of evidence indicates that excitatory neurotransmitters, particularly amino acids such as glutamate, glycine and aspartate, are excessively released into the synaptic cleft during hypoxia-ischemia. In most cases, the most abundant glutamate exhibits neuronal toxicity. Hence, excitotoxicity is also involved in the neuronal damage observed during low-energy states. Glutamate mediated excitotoxicity and Na+/K+ ATPase failure lead to necrotic cell death. Diffusion of glutamate and K^+ within extracellular space can propagate a series of spreading waves of depolarisation. Water shifts to the intracellular space via osmotic gradients and cells begin to swell developing cytotoxic oedema.

Evidence linking excitotoxins to HI cell death include (i) synaptic activity typical for hypoxic cell death, (ii) specific glutamate antagonists preventing hypoxic cell death, (iii) glutamate exposure mimicking hypoxic cell death, (iv) glutamate accumulating extracellularly during hypoxia (due to both its decreased uptake and increased release), and (v) topography of neuronal damage due to HI insult imitating that of glutamate synapses. Regional distribution of glutamatergic neurons explains some of the injury patterns of HI injury (Menezes & Shaw 2006).

PRIMARY AND SECONDARY ENERGY FAILURE

Failure of energy metabolism is considered to be one of the most prominent aetiopathogenic events of brain HI insult (Williams *et al.* 1992a). The brain of mammals has very high energy demands. Energy is constantly required for maintaining membrane potential, for membrane repolarisation after each action potential firing, for synthesis and reuptake of neurotransmitters, etc. Energy is primarily produced via oxidative phosphorylation in brain mitochondria in the form of adenosine-5'-triphosphate (ATP). Another high-energy phosphate, phosphocreatine (PCr), is synthesised in creatine kinase catalysed reaction utilising excess of ATP and represents a short-run energy depot. With its high rate of oxidative phosphorylation, the brain relies solely on continuous blood supply of oxygen. Thus, resulting from to severe hypoxia or ischemia critically reduced cerebral oxygenation leads to a primary failure of energy metabolism in the brain. Indeed, reduction of oxygen tension leads to the inhibition of oxidative phosphorylation with concomitant depletion of the brain's energy reserves (Baciak *et al.* 2006; Cady *et al.* 2008; Hanrahan *et al.* 1996).

Following the phase of primary energy failure, cerebral metabolism may recover during reperfusion/reoxygenation period, and then deteriorate again in a secondary energy failure phase. Free radicals, lactic acid, cerebral oedema, and inflammation cannot develop in non-perfused, completely ischemic-hypoxic tissue. However, as the HI insult is often transient, it is followed by a reperfusion phase, which may normalise the intracellular pH and oxygen concentration and lead to a temporary restoration of energy metabolism (Berger *et al.* 1996). The reperfusion phase or "latent-phase" is the period between reestablishment of apparently normal cerebral metabolism after HI and the start of secondary energy failure. Reperfusion is necessary for the reversal of deleterious events leading to necrotic neuronal death during the primary phase of injury, yet it can simultaneously cause additional (delayed) injury by attracting monocytes and a subsequent inflammatory response into the site of the primary insult. Indeed, following successful resuscitation and restoration of cerebral blood flow, oxygen, and glucose delivery, the concentration of high-energy phosphates and intracellular pH normalise with a transient improvement of cytotoxic oedema. After 30–60 min of reperfusion, the initial HI insult characterised by cytotoxic oedema and accumulation of excitatory amino acids typically resolves along with an apparent recovery of cerebral oxidative metabolism (Wachtel & Hendricks-Munoz 2011).

Secondary neuronal damage, starting at about 6–15 hours after the primary one, can continue for several days (Robertson *et al.* 2011). It is characterised by mitochondrial dysfunction, and initiation of the apoptotic cascade. This phase is also called "delayed neuronal injury". Duration of the delayed phase varies, yet it appears to increase usually over the first 24–48 hours and starts to resolve thereafter. In the human infant, the duration of this phase is correlated with adverse neurodevelopmental outcomes at 1 year and 4 years after the insult (Roth *et al.* 1997). This phase is marked by the onset of clinical manifestations – seizures, secondary cytotoxic oedema, accumulation of cytokines, and mitochondrial failure. A secondary insult is characterised by another wave of glutamate release, reactive oxygen species, nitric oxide, inflammatory reactions, and apoptosis (Northington *et al.* 2011; Blomgren & Hagberg 2006).

Hence, due to cerebral reoxygenation, a temporary restoration of energy metabolism may occur along with transient recovery of ATP and PCr production (Lorek *et al.* 1994; Selman *et al.* 2004; Winter *et al.* 2009). Thereafter, a secondary energy failure may take place due to a delayed impairment of mitochondrial function,

whose cause is not lack of oxygen but rather an inability of mitochondria to utilise it. Complex biochemical mechanisms are involved, including an increased mitochondrial sequestration of calcium, swelling and blebbing of mitochondria and initiation of apoptosis (Cady *et al.* 2008; Puka-Sundvall *et al.* 2000).

Understanding key aspects of HIE pathogenesis, and of reperfusion injury in particular, may help to identify a unique window of opportunity for neuroprotective interventions. Specifically, the period between the primary and secondary energy failure represents a possible therapeutic window. Duration of the reperfusion phase is inversely related to insult severity; latent-phase brevity may explain an apparently less effective neuroprotection following severe cerebral HI insult (Iwata *et al.* 2007).

OXIDATIVE STRESS INVOLVEMENT

Newborns and particularly preterm infants are at high risk of oxidative stress and they are very susceptible to free radical oxidative damage (Saugstad *et al.* 19986). Indeed, there is evidence of an imbalance between antioxidant- and oxidant-generating systems which causes oxidative damage (Jacob 1995). Buonocore *et al.* (2002) noted that the evidence of oxidative stress on the seventh day of life exists not only in hypoxic but also in non-hypoxic preterm babies and concluded that preterm infants are at high risk for oxidative stress. In fact, the brain may be especially at risk of free radical-mediated injury because neuronal membranes are rich in polyunsaturated fatty acids and because the human newborn has a relative deficiency of brain superoxide dismutase and glutathione peroxidase. Due to its higher concentration of polyunsaturated fatty acids and the maturity of the N-methyl-D-aspartate receptor system the term brain is at higher risk of oxidative stress than that of the preterm foetus. Also, early in its differentiation, the oligodendrocyte may be vulnerable because of active acquisition of iron for differentiation at a time of relative delay in the development of certain key antioxidant defences in the brain. Thus, ROS produced by different mechanisms, such as ischemia-reperfusion, neutrophil and macrophage activation, Fenton chemistry, endothelial cell xanthine oxidase, free fatty acid and prostaglandin metabolism, may contribute to the pathogenesis of perinatal brain injury (for review see Buonocore *et al.* 2002).

Actually, oxidative stress and reactive oxygen species (ROS) in particular have been indicated to play a significant role in brain HI injury (Martin *et al.* 2000). On the other hand, ROS were found to participate in many physiological processes, including intra- and inter-cellular signalling, oxygen sensing, inflammatory reaction, immune response, apoptosis, cancer protection, etc. (Cai *et al.* 2007; Juranek & Bezek 2005; Juranek & Soltes 2012). Nonetheless, when the production of ROS overwhelms innate antioxidative capacity of the tissue, they may exacerbate evolving injury due to oxidative damage of lipid, protein, nucleic acid and other important macromolecules. In fact, production of ROS has been indicated to be involved in cerebral HI insult (Kumral *et al.* 2005) and therapeutic strategies using compounds with a combination of antioxidant and other, more specific, properties are on the way (Juranek *et al.* 2010; Robertson *et al.* 2012).

Cytosolic calcium overload activates also Ca^{2+}-dependent NOS, particularly nNOS. At high concentrations, $NO^{\bullet}$ reacts with superoxide ($O^{\bullet -}$) to produce peroxynitrite ($ONOO^{-}$), which in turn induces lipid peroxidation and mitochondrial nitrosylation. Toxic ROS may alter cell membrane milieu resulting in increasing the activity of Ca^{2+}-ATPase leading to further intracellular accumulation of Ca^{2+} (Gavini *et al.* 2000). Membrane depolarisation and mitochondrial dysfunction develop with a massive production of $O^{\bullet -}$ and decline in sulfhydryls, such as glutathione, as a consequence. Catabolic processes triggered by Ca^{2+} also contribute to ROS formation and HI injury via (i) irreversible proteolytic conversion of xanthine dehydrogenase to xanthine oxidase, producing significant amounts of $O^{\bullet -}$ and H_2O_2, (ii) degradation of cellular DNA by activation of endonucleases, and (iii) activation of cytosolic phospholipases, increasing eicosanoid release and inducing the formation of inflammatory mediators. Indeed catabolic enzymes and ROS are involved in the process of neuronal necrosis (Ankarcrona *et al.* 1995; Dirnagl *et al.* 1999; Johnston *et al.* 2001; Robertson *et al.* 2012; Siesjo & Bengtsson 1989).

PHARMACOLOGICAL AND NON-PHARMACOLOGICAL INTERVENTIONS

Possible ways of pharmacological influence of HI insult, of each particular pathogenic events aiming to avoid developing their respective consequences, are summarised in Table 2. Besides, there exist possibilities of non-pharmacological interventions, such as modulation of the oxygen concentration in the inspired gas mixture during the resuscitation of newborns suffering from acute HI insult. Other promising approach is reducing temperature, either globally or locally, i.e. via body or head cooling, respectively.

Pharmacological therapies

Potential neuroprotective pharmacological strategies target different pathways leading to neuronal cell death in response to HII are summarized in Table III. Potential neuroprotective therapies can be grouped by the mechanism of action/effect as follows: decreasing basal metabolism; glucose supplementation; calcium channel blockers and chelators; agents that inhibit glutamate release, uptake, or antagonists of glutamate receptors; blockade of free radical generation or removal; blockade of downstream effects and inhibitors of inflammatory effects; anti-apoptotic agents;

Tab. 2. Possible ways of pharmacological intervention during HI injury.

Pathogenic event	Consequence	Treatment
low brain oxygenation	↓ oxidative phosphorylation ↓ [ATP]	Erythropoietin, EPO-mimetics (also by decreasing basal metabolism, e.g. by hypothermia)
↓ [ATP]	membrane depolarisation	supporting anaerobic glycolysis (particularly by glucose supplementation)
membrane depolarisation	activation of voltage operated channels ↑ $[Ca^{2+}]i$	Ca^{2+} channel blockers
↑ $[Ca^{2+}]_i$	glutamate release activation of Ca^{2+} dependent processes (e.g. nNOS, xanthine oxidase)	cytosolic Ca2+ chelators Na^{+} channel modulator antagonists of glutamate receptors
glutamate release	glutamate receptors activation, excitotoxicity massive Ca^{2+} influx	antagonists of glutamate receptors
xanthine oxidase activation	ROS production, ↑ [ROS]	Allopurinol
↑ [ROS]	oxidative molecule alteration	Edaravone N-acetyl cysteine Melatonin Resveratrol and polyphenols Deferoxamine Vitamin C and E
massive Ca^{2+} influx	activation of degradative processes, cytosolic calcium overload , mitochondrial calcium overload, cytochrome c release, apoptosis	Anaesthetics Mg^{2+} Ca^{2+} channel blockers
apoptosis (in massive scale)	tissue injury	Melatonin, Dipyrone
inhibitor of programmed necrosis	tissue injury	Necrostatin

and growth factors to promote repair. These therapies include from magnesium and antioxidant enzymes, free radical inhibitors and free radical scavengers through xenon, melatonin, erythropoietin, glycine proline-modified analogue up to transplantation of neural stem/progenitor cells.

Erythropoietin

Erythropoietin (Epo) is a haematopoietic cytokine with erythropoietic effects. Epo mediates an adaptive tissue response to stress and tissue protection (Brines *et al.* 2000). Epo and its receptors were upregulated after brain injury, and the levels of Epo in cerebrospinal fluid were correlated positively with outcomes (Casals-Pascual *et al.* 2008). Many animal (Zhu *et al.* 2009; Keller *et al.* 2007; Kumral *et al.* 2005; Sun *et al.* 2005; Demers *et al.* 2005) and human (Ohlsson *et al.* 2015; Juul *et al.* 2008; Fauchere *et al.* 2008) studies proved its neuroprotective effects on brain injury, including perinatal HIE. Disadvantages of Epo are its adverse effects (polycythemia, thrombosis, haemangioma) (Haiden *et al.* 2005; Doggrell 2004; Leung 2000).

Non-haematopoietic Epo-mimetics

Novel potential Epo derivatives and mimetics have been developed to be used in case of HI injury. An Epo lysine obtained by Epo carbamylation, an asialo-Epo and a new peptide termed Epotris (Pankratova *et al.* 2010; Sturm *et al.* 2010; Siren *et al.* 2009; Kirkeby *et al.* 2008) fall within this group.

Glucose

The blood level of glucose plays an important role in the development of perinatal HIE (Hattori & Wasterlain 1990). Options on the level of glucose in relation to seriousness of HII are different. Voorhies *et al.* (1986) supplemented glucose to immature rats and subsequent hyperglycaemia did not increase the extent of HI brain damage, in contrast to adult rats. But increasing serum glucose during HI injury to the newborn piglet's brain worsened brain damage (LeBlanc *et al.* 1993). A study by Nadeem *et al.* (2011) observed blood glucose values within 72 hours of birth in 52 term infants with HIE. During the first 72 hours of life, the blood glucose profile in infants with HIE varies widely despite management protocol. Early hypoglycaemia (0-6 hours of life) was associated with severe HIE with adverse outcome. Zovein *et al.* (2004) concluded that HI brain injury is associated with transient compensatory changes targeted at protecting glucose delivery to fuel cellular energy metabolism.

Calcium channel blockers and cytosolic Ca^{2+} chelators

The elevation of cytosolic calcium during and following HII requires suitable therapy for its reduction. Alps *et al.* (1988) demonstrated the ability of nimodipine, nica-

rdipine, flunarizine and lidoflazine to reduce delayed neuronal death in the CA1 region of gerbil hippocampus following transient bilateral carotid artery occlusion. The results showed that overall protection was conferred on ischemic neurones by nicardipine, and to a lesser extent by flunarizine and lidoflazine, but not by nimodipine. In a pilot study, nicardipine was tested in four severely asphyxiated infants but the positive effect was counteracted by the adverse effects of significant haemodynamic disturbance (Levene *et al.* 1990). Disadvantage of Ca^{2+} channel blockers is significant adverse cardiovascular effects. In many studies chelating agents were studied for their potential protective properties in various neurodegenerative diseases, including HI injury (Bernardinelli *et al.* 2004; Armstrong *et al.* 2001; Spigelman *et al.* 1996). The compounds tested included e.g. 2-aminophenol-N,N,O-triacetate; 2-aminophenol-4-fluorophenol-N,N,O-triacetate; 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetra-acetate; ethylene-diamine-tetraacetic acid; ethyleneglycolbis(β-aminoethyl ether)-N,N,N′,N′-tetra-acetate; N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; and tetrakis (2-pyridylmethyl) ethylenediamine.

Na^+ channel blockers

Tetrodotoxin is a potent neurotoxin, specifically blocking voltage-gated sodium channels on the surface of nerve membranes. Tetrodotoxin treatment was found to prevent the delayed Ca^{2+} overload in rat hippocampal neurons (Randall & Thayer 1992).

Antagonists of glutamate receptors

The hippocampus and the cortex which receive high glutamatergic input and posses many excitatory amino acid receptors are particularly vulnerable to O_2 deprivation. Agents like D(–)-2-amino-5-phosphonopentanoate, a selective N-methyl-D-aspartate receptor (NMDA) antagonist; 5-methyl-10,11-dihydroxy-5H-dibenzo(a,d) cyclohepten-5,10-imine (MK-801), dextromethorphan, noncompetitive antagonists of excitatory amino acid neurotransmission; and a L-kynurenine – NMDA receptor inhibitor, all putatively reduced the amount of damage produced by hypoxia and unilateral carotid artery occlusion in neonatal rodents (Robotka *et al.* 2008; Sas *et al.* 2008; Keller *et al.* 2008; Hattori *et al.* 1989). If postponed for more than 1–2 h after HII, neuroprotection by NMDA antagonists was ineffective (Hagberg *et al.* 1994), it showed for the delayed expression of neurodegeneration that was seen during HIE (Johnston *et al.* 2001). MK-801 treatment was with benefit when initiated up to 75 min after the hypoxic episode (Hattori *et al.* 1989; McDonald *et al.* 1989) and improved disrupted mitochondrial function (Gilland *et al.* 1998). L-kynurenine is a metabolic precursor of kynurenic acid. In contrast with kynurenic acid, L-kynurenine is transported across the blood-brain barrier, and it may therefore come into consideration as a therapeutic agent in certain neurobiological disorders. Using fluorescent microscope and Fluoro-Jade, Robotka *et al.* (2008) and Sas *et al.* (2008) proved the neuroprotective effect of L-kynurenine (administered together with probenecid, an organic acid transporter inhibitor) on adult rat brain after global ischemia. A study by Comi *et al.* (2006) confirmed sex-dependent protective effect of dextromethorphan in 7-day postnatal mice. Only the male pups were protected while brain injury in the female pups was unchanged. In a study by Keller *et al.* (2008) dextromethorphan reduced inflammation-sensitised NMDA receptor-mediated excitotoxic brain damage in newborn mice, without triggering apoptotic degeneration. In 2010 Yuede *et al.* published a study about negative effects of NMDA glutamate antagonists, specifically phencyclidine. They concluded that a dissociative anaesthetic with phencyclidine properties caused neuroapoptosis depending on the developmental age at the time of exposure and on the number of phencyclidine applications. Other glutamate antagonists are AMPA/kainates. They include 6,7-Dinitroquinoxaline-2,3-dione (DNQX), 6-cyano-7-nitroquinoxaline-2,3-dione, non-selective [2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f] quinoxaline (NBQX), 6-cyano-7-nitroquinoxaline-2,3-dione], AMPA-preferring, kainate-preferring (γ-D-glutamylaminomethanesulfonic acid), or Ca^{2+}-permeable AMPA/kainate receptor antagonists (joro spider toxin, JSTx). Furthermore, NBQX or JSTx blocked oxygen-glucose deprivation-induced Ca^{2+} influx. Influence of AMPA/kainate receptors is one of the major mechanisms in acute HI injury to oligodendroglial precursor cells (Deng *et al.* 2003). In the study by Lobner & Lipton (1993) DNQX had no effect on the

Tab. 3. Using oxygen for newborn resuscitation.

1992	(ILCOR)	"100% O_2 should be used, it is not toxic - no reason to be concerned"
2000	(ILCOR/AAP)	"100% O_2 should be used, however if O_2 is not available use room air"
2005	(ILCOR/AAP)	"The optimal O_2 concentration is not known for newborn resuscitation. There is no reason to change the initial O_2 concentration."
2010	(ILCOR/AAP)	"... it is best to begin with air rather than 100% O_2 "
2011	(ILCOR/AAP)	"Resuscitate term infant with room air (21% O_2), for preterm infants it is not known; however, a concentration at either extreme (21% or 100%) may result in an O_2 saturation that is too low or too high."

Source: AAP – American Academy of Pediatrics; ILCOR - International Liaison Committee on Resuscitation

Ca^{2+} influx in *in vitro* ischemia, but in combination with MK-801 it prevented long-term synaptic transmission failure in the CA1 region of the rat hippocampal slice.

Allopurinol

Allopurinol is an antioxidant inhibiting conversion of xanthine dehydrogenase to xanthine oxidase, and this stops production of $O^{\bullet-}$ and H_2O_2. Allopurinol was administrated to pregnant women at term with suspected intrauterine hypoxia and reduced moderate HIE in infants (Kaandorp *et al.* 2010 and 2012), and was effective in immature rats (Williams *et al.* 1992b).

N-acetyl cysteine

N-acetyl cysteine (NAC) is a thiol, a mucolytic agent and a precursor of L-cysteine. NAC is a source of sulfhydryl groups in cells and scavenger of free radicals as it interacts with ROS such as hydroxyl radical and H_2O_2. Uses of NAC in different diseases including cystic and pulmonary fibrosis, cancer, parectamol-induced liver toxicity, cardiovascular diseases, human immunodeficiency virus infections have been reviewed previously (Zafarullah *et al.* 2007). NAC was found to be effective against the inflammation in the foetal brain induced in utero by bacterial lipolysaccharide. It prevented oxidative stress and loss of glutathione in the hippocampus, restored long-term recognition performance and improved learning deficits, reduced cerebral oxidative stress with improved cerebral oxygen delivery and reduced caspase-3, hydrogen peroxidase and lipid hydroperoxide concentrations in the cortex. It also improved neonatal reflexes and reduced both white and gray matter damage 4 weeks after HII exerted neuroprotective effect in 78% of brain injury and inhibition of apoptosis in neonatal rats, mice and piglets (Liu *et al.* 2010; Lante *et al.* 2008; Lante *et al.* 2007; Wang *et al.* 2007a; Jatana *et al.* 2006; Paintlia *et al.* 2004; Plaisant *et al.* 2003).

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine), a hormone of pineal gland, plays an important role in regulating circadian sleep-wake cycle and reproductive cycles. The ability of melatonin to act as an antioxidant and melatonin neuroprotective benefits have been shown by many researchers (Drury *et al.* 2014; Tan *et al.* 2007; Konecna *et al.* 2001). Marketed as a dietary supplement and used in the treatment of a number of diseases including insomnia, jet lag, epilepsy, ageing, even cancer, and improved immunity, yet the overall effects of melatonin on human health are still largely unknown (Bubenik & Konturek 2011; Reiter *et al.* 2010; Altun & Ugur-Altun 2007). The melatonin concentration in humans and rodents is not steady and varies depending on the time of day (Zawilska *et al.* 2000). Physiological levels of melatonin in the human blood are in a concentration range of 10–100 pM, but neurons and glia can accumulate melatonin periodically, to a level exceeding 50 times its plasma concentration (Cardinali *et al.* 1997; Pang *et al.* 1990). Melatonin in combination with cooling improved neuroprotection in newborn piglets with moderate and severe HIE (Robertson *et al.* 2013), and was effective in models of HIE in neonatal rat pups (Kaur *et al.* 2010; Olivier *et al.* 2009; Wang *et al.* 2007b). Neuroprotection was also seen in sheep foetuses with asphyxia (Miller *et al.* 2005b). After birth asphyxia in the spiny mouse, melatonin reduced signs of cerebral inflammation and apoptosis (Hutton *et al.* 2009). Melatonin was given with benefit to children with asphyxia and neonatal sepsis (Fulia *et al.* 2005; Gitto *et al.* 2005).

Edaravon

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186) is a free radical scavenger and inhibitor of ion-dependent lipid peroxidation, which has been approved in Japan for the use in patients with cerebral infarction due to thrombosis or embolism (Tabrizchi 2000; Otomo *et al.* 1998). Ikeda *et al.* (2002) confirmed its protective effect on HI brain damage in neonatal rats and Yasuoka *et al.* (2004) demonstrated decrease of necrotic and apoptotic cell death and inhibition of mitochondrial injury in a neonatal rat model.

Resveratrol and polyphenols

Resveratrol, a polyphenol with antioxidative effect, was studied by West *et al.* (2007) for its neuroprotection. The study showed that pomegranate polyphenols enriched extract and resveratrol reduced caspase-3 activation after neonatal HI injury. A similar result was found with the use of pomegranate juice alone (Loren *et al.* 2005). Resveratrol also affected nitric oxide synthase expression after perinatal HII (Seo *et al.* 2008).

Deferoxamine

Deferoxamine is commonly known as a chelator of non-protein-bound iron which can cross the blood-brain barrier (Hurn *et al.* 1995). In experimental animal models of lambs (Shadid *et al.* 1998), mice (Sarco *et al.* 2000) and rats (Palmer *et al.* 1994), it was positively effective in HI damage of the newborn brain. Papazisis *et al.* (2003) found a remarkable reduction of HI evoked neurons in the CA1 hippocampal region and decreased levels of glutamate and aspartate in hippocampal tissue after deferoxamine treatment.

Vitamin C and E

Vitamin C and dehydroascorbic acid can scavenge free radicals and penetrate the blood-brain barrier (Jackson *et al.* 1998). Acsorbic and dehydroascorbic acids reduced the infarct volume in reperfused cerebral ischemia (Huang *et al.* 2001). Only one randomized-controlled clinical study in asphyxiated infants has been performed, which found that the ascorbic acid and ibuprofen combination had no effect on outcome at 6 months of age (Aly *et al.* 2009).

A mitochondrial antioxidant, Mito vitamin E, showed no benefit in postnatal treatment of 7-day-old

rats (Covey *et al.* 2006), but vitamin E in combination of methylprednisolone reduced HI brain neonatal injury (Daneyemez *et al.* 1999).

Anaesthetics

Some anaesthetic agents used as analgesics at subanaesthetic concentrations may exert a neuroprotective effect; however, there are only few studies of prenatal treatment with anaesthetics. Neuroprotective properties of sevoflurane and xenon to reduce HI brain damage were tested in a model of intrauterine PA. Both agents tested reduced the number of apoptotic neurons and increased cell viability in the hippocampus at the seventh postnatal pup day (Azzopardi *et al.* 2013; Yang *et al.* 2012) and provided long-lasting neuroprotection (Luo *et al.* 2008). Xenon is a non-competitive antagonist of the NMDA aspartate subtype of the glutamate receptor (Franks *et al.* 1998). Its effect includs activation of K^+-channels (Gruss *et al.* 2004), inhibition of the Ca^{2+}-calmodulin-activated kinase II (Petzelt *et al.* 2001), activation of anti-apoptotic effectors (Ma *et al.* 2007). Pretreatment with 70% xenon 4 hours before HI injury reduced the infarction volume 7 days after and after 30 days it improved neurologic functions (Ma *et al.* 2006). Combined xenon and hypothermia was effective (Thoresen *et al.* 2009; Martin *et al.* 2007). Neuroprotective effects have been demonstrated in piglets (Faulkner *et al.* 2011; Chakkarapani *et al.* 2010), but xenon alone without cooling did not provide significant neuroprotection (Thoresen *et al.* 2009).

Magnesium sulphate

Magnesium sulphate is commonly known for its positive effect in the preterm population (Doyle *et al.* 2009), but its influence on term infants with HI injury, studied on pre-clinical animal models, was found controversial (de Haan *et al.* 2009; Penrice *et al.* 1997), as it exerted a harmful hypotensive effect in high doses (Levene *et al.* 1995).

Other potential neuroprotective avenues

Other avenues of potential neuroprotection include analgetic/antipyretic (Zhang *et al.* 2011); platelet-activating factor antagonists (Liu *et al.* 1996) ; adenosinergic agents (Halle *et al.* 1997); monosialoganglioside (Tan *et al.* 1994); growth factors, e.g., nerve growth factor (Holtzman *et al.* 1994) ; blocking the apoptotic pathways, i.e. minocycline (Arvin *et al.* 2002); stem/progenitor cells (Daadi *et al.* 2010; Sato *et al.* 2008; Keller *et al.* 2006) and inhibitor of programmed necrosis (Northington *et al.* 2011). Dipyrone is an analgesic and antipyretic drug. In one study by Zhang *et al.* (2011) dipyrone inhibited mitochondrial cell death cascades. An antibiotic, ceftriaxone, a semisynthetic analogue of cephalosporin, as pre-treatment prior to HI brain injury in neonatal rats, reduced the brain injury score, improved myelination, decreased hippocampal apoptotic cell death, restored learning and memory deficit, and had a prospective neuroprotective effect (Lai &Yang 2011). Another antibiotic, doxycycline, reduced long-term cerebral tissue loss and white matter damage after neonatal HI injury (Widerøe *et al.* 2012). An insulin-like growth factor-1 (IGF-1) is a potent neurotrophic factor (neurothrophin) and belongs to the family of proteins that induce survival, development and function of neurons (Reichardt 2006; Hempstead 2006); this hormone regulates age-related alterations in NMDA receptor subtypes (Sonntag *et al.* 2000). Guan *et al.* (2001) examined in rats the long-term effects of IGF-1 on late neuronal loss 20 days after HI injury and found improved neurobehavioural outcome, somatosensory function, by ongoing progressive neuronal death during brain recovery from HI injury. One study investigated the protective effects of glycyl-L-prolyl-L-glutamic acid analogue, glycine 2-methyl proline glutamate, on HI damage in the neonatal brain with good results (Harris & Brimble 2006). One study on lambs confirmed that low-dose IGF-1 therapy promoted neuronal rescue after HII in utero, but the effect was dose-dependent (Johnston *et al.* 1996). New strategy with Connexin hemichannel blockade had also neuroprotective effect (Davidson *et al.* 2014). The potential mechanisms of the neuroprotection were promotion of neovascularisation and astrogliosis and the normalisation of interleukin-6 levels (Svedin *et al.* 2007).

Modulation of the concentration of inspired oxygen

Optimal management of oxygen during neonatal resuscitation becomes particularly important because of the evidence that either insufficient or excessive oxygenation can be harmful to the newborn infant. There are concerns about the potential adverse effects of 100% oxygen on respiratory system and cerebral circulation and also the potential tissue damage from the increased ROS concentration. We intend to present how the idea of using reduced oxygen for resuscitation has developed. Table 3 shows that in 1998, the general guidelines indicated that one hundred percent oxygen should be used to treat perinatal asphyxia. In 2010, we see that term infants are resuscitated with air.

Studies Solas *et al.* (2001, 2004a,b), Huang *et al.* (1995) and Kutzsche *et al.* (1995) examining blood pressure, cerebral perfusion, and various biochemical measures of cell damage in asphyxiated animals resuscitated with 100% oxygen versus 21% oxygen (room air) have shown conflicting results. One study by Lundstrom *et al.* (1995) of preterm infants exposed to 80% oxygen found lower cerebral blood flow when compared with those stabilized using 21% oxygen. Some animal data indicated the opposite effect, i.e. reduced blood pressure and cerebral perfusion with 21% oxygen (room air) versus 100% oxygen (Solas *et al.* 2001).

In some studies, room air resuscitated infants recovered more quickly, with a reduction in mortality rate and no evidence of harm, than infants resuscitated with 100% oxygen, as assessed by Apgar scores, time to

first breath, and time to first cry (Tan *et al.* 2005; Davis *et al.* 2004; Saugstad *et al.* 1998). In the study by Solberg *et al.* (2012) supplementary oxygen used for the resuscitation of newborns led to an increase of lipid peroxidation in brain cortical neurons. Some clinicians recommend resuscitation with an oxygen concentration of less than 100% and some may start with no supplementary oxygen (i.e. room air). Saugstad (2011) suggests starting resuscitation with room air, being prepared to add oxygen in selected cases. Finan *et al.* (2011) recommended room air as the initial gas for all babies, with the exception of very preterm babies in whom supplemental oxygen (between 30% and 90%) should be given. It is not known whether supplemental oxygen during resuscitation affects the protection offered by subsequent therapeutic hypothermia (Dalen *et al.* 2012).

Temperature modulation

The protective effect of hypothermia as treatment of HIE has been considered for half a century (Apgar 1956). There is controversy in placing a newborn into a normo-thermic (37 °C) or hypothermic (33 to 35 °C) incubator. The study by Williams *et al.* (1992b) showed a decrease of ATP in the experimental rat model and its fast recovery at the temperature of 31 °C and 34 °C, while at 37 °C the production of ATP remained inhibited. A similar situation was for the ratio Pi by PCr.

Therapeutic hypothermia reducing brain injury in survivors after perinatal asphyxia (Edwards *et al.* 2010), is the most promising neuroprotective intervention to date for infants developing moderate to severe HIE. The protective mechanism of therapeutic hypothermia is multifactorial and is attributed to a broad inhibitory activity against several biological processes of brain injury. Experimental studies found that therapeutic hypothermia after HI insult affected the apoptotic process (Davidson *et al.* 2015; Jacobs *et al.* 2013; Carlsson *et al.* 2012; Bennet *et al.* 2007; Ohmura *et al.* 2005; Zhang *et al.* 2001), prevented vasogenic cerebral oedema, had a significant protective effect on neuronal loss and immature oligodendrocytes (Bennet *et al.* 2007; Gunn *et al.* 1997), decreased the inflammatory response by microglial activation (Fukui *et al.* 2006), decreased loss of high-energy phosphates, reduced oxygen consumption, nitric oxide, and glutamate, suppressed free radical activity and excitatory amino acid neurotransmitters and induced genes reducing neuronal death (Ferriero 2004, Jacobs *et al.* 2007, Wilkinson *et al.* 2007).

Term or near-term infants, with evolving moderate to severe HIE, should be treated with therapeutic hypothermia. The recommended temperature is between 33°C and 35°C. Hypothermia is usually induced by cooling the whole body with a blanket or mattress, cooling the head only with a purpose-made cap or sometimes in combination of selective head cooling with mild systemic hypothermia. Intracorporeal temperature is continuously monitored, using a rectal or nasopharyngeal thermometer, as a proxy for brain temperature. Treatment is started as soon as possible after diagnosis, usually within 6 hours of birth, and continued for approximately 72 hours. The infant is then slowly warmed to normal body temperature. The effects of different cooling methods have generally been similar (Sarkar *et al.* 2009), although a small study by Rutherford and colleagues (2005) reported a decrease in the incidence of severe cortical lesions on MRI in infants with encephalopathy treated with selective head cooling.

Systematic recent randomised controlled trials and smaller studies (more than 3500 infants in total) reported a lower risk of death in cooled infants (whole body or head) in the first 18 months of life than in infants treated by standard care (Azzopardi *et al.* 2009; Edwards *et al.* 2010; Gluckman *et al.* 2005; Jacobs *et al.* 2011; Lando et al., 2010; Lin *et al.* 2006; Rutherford *et al.* 2010; Shah 2010; Sarkar *et al.* 2009; Shankaran *et al.* 2005; Schulzke *et al.* 2007; Simbruner *et al.* 2010; Zhou *et al.* 2010). Rutherford *et al.* (2010) concluded that whole-body cooling significantly reduced cerebral lesions in basal ganglia, thalamus, and posterior limb of the internal capsule, cortex or white matter.

Although therapeutic hypothermia is a significant advance in the developed world and improves the outcome, Laptook *et al.* (2008) evaluated that the probability of death and disabilities in infants with moderate to severe HIE was increased fourfold for each 1°C increase in body temperature. Gluckman *et al.* (2005) concluded that head cooling had no effect in infants with the most severe amplitude-integrated electroencephalogram changes, but it was beneficial in infants with less severe ones. Therapeutic hypothermia may not be very effective in infants in danger of life or with severe disabilities (Azzopardi *et al.* 2009; Gluckman *et al.* 2005) and in newborns whose placentas had abnormalities (Wintermark *et al.* 2010). In preterm infants, there are considerable, unresolved safety concerns concerning cooling (Gunn & Bennet 2008).

According to these findings, therapeutic hypothermia looks like one of a few effective, safe and potentially promising treatment strategies for neonatal brain injury and gives hope to the possibility of influencing HIE after perinatal HI insult. Studies are needed to determine the efficacy of combined therapeutic strategies with hypothermia therapy to achieve maximal neuroprotective effect.

In our opinion, these two important examples of non-pharmacological intervention indicate that pharmacotherapy may not be required if a proper management of newborns with signs of perinatal asphyxia and thus at risk of HIE would be applied in neonatology units.

CONCLUDING REMARKS

Severe and lifelong consequences of cerebral HI injury in newborns represent a serious health-care problem with high socio-economic impact. Lack of effective therapy of newborns with signs of perinatal asphyxia and thus at a risk of HIE represents a serious issue. In the present paper, we attempted to cover essential mechanisms of HI insult to the neonatal brain. Good understanding of key pathophysiological processes at cellular and molecular level may serve as a basis for a development of efficient therapy and/or management of newborns suffering from HI insult. We also provided a brief overview of instrumental approaches to follow-up stages of the evolving HI injury and its possible resolution if effectively treated. The paper is focused on ROS and resulting oxidative stress, which is commonly considered as a crucial underlying pathogenic event. However, treatment of newborns with HI insult by exogenous antioxidants often does not have anticipated effects.

In fact, most of pharmacological approaches are usually only partially effective. On the other hand, proper management of newborns with HI insult may result in improving their overall status and turn to a positive prognosis. This was demonstrated e.g. by a better recovery of cerebral energy metabolism under a mild hypothermia observed by the non-invasive phosphorus (^{31}P) MRS technique. Also, in many cases, reducing oxygen in the inspired gas mixture often results in better recovering of the affected newborns. As for the antioxidant therapy, although resulting in an improvement of some particular free radical parameters, it is often ineffective in improving the overall status of newborns after HI insult. Indeed, in the survey of Robertson *et al.* (2012), use of antioxidants was not scored very effective in many neonatology units, suggesting that oxidative stress is not the main underlying pathogenic event.

Concluding, the observed signs of oxidative stress in neonatal HIE, resulting from ROS overproduction, which itself may be understood as a consequence of calcium overload, the most prominent mechanism in the cell death and tissue injury. ROS may serve as markers of ongoing injurious events and they are likely to be involved in signalling processes, which represent an ultimate effort to keep or recover tissue integrity and its function.

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