Oration

PERINATAL NEUROPROTECTION : CURRENT CONCEPTS AND FUTURE PERSPECTIVES

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THE BURDEN OF PERINATAL ASPHYXIA

Perinatal asphyxia is a major cause of childhood morbidity and neonatal mortality. The incidence of Hypoxic ischemic Encephalopathy (HIE) associated with perinatal asphyxia is 1-2/1000 in high income countries and 20/1000 in low-middle income countries (LMIC)1. In LMICs one in three infants suffering from severe perinatal asphyxia will died with one in two surviving infants suffering from significant brain injury and lifelong disability [2].

Improvement of Maternal and Child Health services in Sri Lanka has led to a steady decrease in neonatal mortality achieving the lowest infant mortality in South Asia[3]. The three major causes of neonatal mortality in Sri Lanka are Prematurity/ low birth weight, congenital malformations and asphyxia[4]. Asphyxia accounts for 14% of neonatal mortality in the country. Prevention of perinatal asphyxia related morbidity and mortality stands as a major target for improving Maternal and Child Health in Sri Lanka. Introducing a standardized protocol for active hypothermia treatment for the management of infants following asphyxia is an important step hereby.

PERINATAL MANAGEMENT AND RISK ASSESSMENT TO PREVENT ASPHYXIA

Identifying pregnancies at high risk of asphyxia event is an important focus of prevention strategies aiming to stratify perinatal management practice to potentially reduce asphyxia and its devastating consequences. In a recent study [5] we have presented an antepartum clinical score to identify women with high risk for having a baby with HIE. Parity, polyhydramnios, pre-rupture of membranes, gender, concerns about foetal growth and prematurity were co-variants of score, being strongly associated with risk of HIE, still birth, resuscitation or neonatal death. In this study elective delivery via caesarean section at 37 weeks of gestation following antenatal risk assessment was estimated to prevent 14% of HIE cases.

SPECIFIC VULNERABILITY OF THE EARLY BRAIN ON STRUCTURAL AND FUNCTIONAL DEVELOPMENT

During the development of the human brain neuron proliferation and cell migration are prerequisite for the structural brain development starting early at week 8-10 of foetal development[6]. The periventricular germinal matrix is a major site for neuronal stem cells, neuron proliferation and cell migration in the immature brain. Developmental processes including cell migration, cell branching, synaptogenesis and glia cell proliferation are highly active from 22 weeks of gestation onwards.

In addition to the structural immaturity of the developing brain, it has been demonstrated that the immature brain demonstrates a vulnerability to inflammation triggered by bacterial lipopolysaccharide, hypoxia-ischemia[7] and oxidative stress. Bacterial endotoxin acting in a synergistic way, sensitizing the immature brain aggravating hypoxic-ischemic injury [8,9].

The neuropathology of perinatal brain injury is marked by i) white matter injury including focal or diffuse parenchymal lesions, apoptotic cell death of immature oligodendrocytes and marked hypo-myelinisation; ii) grey matter injury demonstrating neuronal loss and impaired neuronal guidance and iii) "functional brain injury" demonstrating impaired structural and functional connectivity and pattern of brain plasticity on neonatal MRI studies[10].

SPECIFIC PATTERN OF PERINATAL BRAIN INJURY GERMINAL MATRIX – INTRAVENTRICULAR HAEMORRHAGE (GM-IVH) AETIOLOGY / PATHOPHYSIOLOGY

GM-IVH is a well described pattern of perinatal brain injury. The incidence of GM-IVH is directly related to prematurity and rarely occurs beyond 32 weeks of gestation.

In addition to the immaturity of the brain being the major predisposing factor for the development of GM-IVH further factors have been identified contributing in the pathophysiology of GM-IVH: i) impaired brain perfusion (hypoxia/ischaemia), ii) infection (chorioamnionitis) , iii) oxidative stress (lack of anti-oxidants) and iv) vascular anatomy (venous drainage).

The pattern of injury involves haemorrhage in the periventricular germinal matrix with extending blood in the ventricles and associated parenchymal lesions due to venous infarction / obstruction and inflammation triggered periventricular white matter injury.

CONSEQUENCES OF GM-IVH

The extend of GM-IVH described on trans-fontanelle cranial ultrasound is directly related to the acute and long-term consequences on cognitive and motor function outcome. Large GM-IVH consists of a 3-5-fold increased risk of major neurological disability in extremely preterm born infants and might lead to progressive post-haemorrhagic dilatation of the ventricular system (PHVD) with subsequent development of hydrocephalus in 40% of infants with PHVD[11].

"RISK REDUCTION AND OUTCOME IN GM-IVH"

Published meta-analyses illustrate 4 variables significantly associated with a risk reduction in development of GM-IVH in preterm infants: i) #Antenatal steroid application[12], ii) #Prophylactic indomethacin treatment for closure of patent ductus arteriosus[13], iii) #Delayed cord clamping[14] and iv) *Maternal vitamin K[15] (# risk reduction for IVH; *risk reduction for severe IVH).

MANAGEMENT OF SEVERE GM-IVH /POST HAEMORRHAGIC VENTRICULAR DILATATION (PHVD)

A severe complication in GM-IVH is the development of PHVD in cases with extended IVH (grade III bilateral or grade III plus parenchymal

lesion). A recently published RCT[16,17] compared different treatment thresholds for intervention in preterm infants with PHVD (defined by cranial ultrasound Ventricle Index (VI) parameter). This study confirmed that ultrasound guided assessment and decision on threshold in treatment resulted in early intervention (day 10 versus day 15 of life) by lumbar tap followed by insertion for ventriculostomy access device and daily CSF drainage (10 ml/kg) led to lowest reported ventriculoperitoneal shunt incidence so far reported for both group (19% versus 23%) and composite outcome (Death, need for VP shunt) and neurodevelopmental assessment (Bayley scales) at 24 month of life were significantly in favour for the early intervention group. A recently published observational clinical study on the management of PHVD[18] comparing ultrasound guided "early" treatment approach (mean first intervention day 13) as described in the RCT by de Vries et al.[16] with an management following clinical criteria for intervention (mean first intervention at day 47) described significant differences in need for shunt placement (20% versus 92%) and cognitive performance at 2 years (Bayley III scales Mental Developmental Index: 95 versus 68). Both studies provide evidence that early recognition and active management of GM-IVH is an essential and timely intervention in PHVD being associated with improved cognitive outcome and low incidence of need for shunt placement / development of hydrocephalus.

HYPOXIC ISCHAEMIC ENCEPHALOPATHY (HIE) AETIOLOGY / PATHOPHYSIOLOGY

The timing in the pathophysiology of perinatal asphyxia is associated with distinct pattern of neonatal encephalopathy and its clinical course and neurological outcome[19] Acute global brain injury pattern in HIE is typically characterised by low APGAR score, severe acidosis and clinical symptoms of moderate to severe encephalopathy following an uneventful pregnancy with regular foetal growth. An acute global brain injury pattern (deep grey matter / basal ganglia lesion) is frequently associated with acute intrapartum events (placental abruption; umbilical cord pathology).

The chronic hypoxic ischemic brain injury pattern ("watershed" lesions / chronic cortical injury) demonstrates a high incidence of maternal and antenatal recognized problems (Intra uterine growth restriction, long and difficult labour). Infants developing a watershed" lesions / cortical injury pattern are likely less compromised at birth but presenting with meconium aspiration, symptomatic hypoglycaemia or infection.

Infants suffering from acute global brain Injury pattern have a higher mortality rate and in up to 50% suffer from cerebral palsy and long-term sequel [20].

The pathophysiology of an acute global HI event follows a time course that is well described by the changes in cerebral blood flow[20]. Initial cerebral ischaemia/reperfusion and resuscitation is followed by a prolonged phase of hypoperfusion (» latent phase) which lasts for 1-6 to 24h. It is followed by local hyper-perfusion and secondary cellular damage in the penumbra (» Secondary "energy failure" phase 6 to 24 h to days). The restorage of normal brain perfusion is called tertiary phase and lasts from weeks to years. MRI spectroscopy enables to study the underlying biochemistry of molecular injury cascades involved during the time course. Taken from experimental studies it has become apparent

that the major aim following resuscitation is the recovery of oxidative metabolism and recovery of mitochondrial injury / "Secondary energy" loss during the Latent phase [21].

ACTIVE HYPOTHERMIA TREATMENT

A positive effect of active hypothermia treatment in neonates suffering from severe asphyxia was first described in 1958 by B. Westin and colleagues [22] in a study including 10 severely asphyxiated infants immersed in 23-28° Celsius cold water presenting with intact survival of 9/10 at 16-24 months of life.

Following extensive experimental studies in the 1990th several RCTs comparing moderate hypothermia versus normothermia in infants with moderate to severe HIE demonstrated were performed. The metanalysis[23] including results of 3 of the 10 trials (n=767 infants) demonstrated significant benefit for active hypothermia treatment in neonates born from 36 weeks' gestation (starting less 6 h after birth and cooling at 33.5° Celsius) on neurological outcome and significant reduced mortality following hypothermia treatment analysing data from all included infants (n=1320 infants). Since, further studies have been performed in different clinical settings presenting differences in mortality rates and disability scores recognized.

Active hypothermia treatment trials performed in LMIC countries often demonstrate disappointing results compared to studies performed in high income countries (HICs)[24]. Simply translating results and strategies from hypothermia trials performed in HICs into management in LMICs have failed. To some degree, the differences observed might be explained by less availability of antenatal care and thereby recognition of time course of foetal distress, as well as by the difference in the incidence of unrecognized perinatal infections or other health conditions. Importantly, the incidence of Group B streptococcal infection in neonates with HIE is increased and significantly associated with mortality compared with HIE alone[25]. Perinatal infection / chorioamnionitis predisposes to direct injury of the developing brain. A study from Uganda[26] involving neonates with neonatal encephalopathy reported a high incidence of pathogenic bacterial species detected (8.9 %) in comparison to unwell neonates admitted for a different reason (2.0 %).

ACTIVE HYPOTHERMIA TREATMENT: CLINICAL SETTING

Active hypothermia treatment should be offered to neonates presenting with moderate to severe clinical sign of HIE. Currently it is advised to perform active hypothermia in infant born from 36 weeks' gestation onwards starting immediately after birth until less 6 h - "latent phase" - after birth targeting a body core temperature of 33.5° Celsius. Infants will particularly benefit, if hypothermia is started within first 3 hours of life [27].

Active hypothermia treatment should be applied using automated devices for whole body cooling. The use of active cooling achieves target temperature in a shorter period and maintains better temperature stability compared to passive cooling setting[28]. Manual low-technology cooling devices have been applied in low cost setting. However non-automated cooling settings require high degree of observation and care to perform cooling in a safe - constant temperature - clinical setting [24]. In addition to hypothermia treatment, it is important to address hypoglycaemia, seizures

and suspected infection appropriately in neonatal HIE [29]. In view of a high incidence of non- visible "electrical" seizure activity, it is advisable to include monitoring of brain activity using cerebral function monitors (Amplitude integrated EEG) for early recognition and course of impaired brain function (low voltage activity) under treatment and management of seizure activity.

THE ROLE OF NEUROIMAGING IN HIE

Structural neonatal MRI has been introduced to study the brain injury pattern in neonatal encephalopathy. The British Association for Perinatal Medicine[30] has published a framework for neonatal MRI studies in term infants with acquired brain injury, encephalopathy or seizures. MRI is useful in aiding prediction of neurological[19] and neurodevelopmental outcome in neonates with hypoxic-ischaemic encephalopathy (HIE). neonates with clinical signs of acquired brain injury, neonatal encephalopathy (NE) or seizures should undergo neuroimaging.

OUTLOOK ON MANAGEMENT OF HIE PHARMACOLOGICAL NEUROPROTECTION

Experimental work and clinical studies have been performed aiming to augment neuroprotection adding pharmacological intervention to active hypothermia treatment. Targets for pharmacological neuroprotection along the basic pathophysiological mechanisms are restorage of cellular energetics and mitochondrial function, counteracting pathophysiological cascades during reperfusion / re-oxygenation, anti-inflammation and cell death (apoptosis/necrosis) as well augmenting endogenous repair mechanism [31].

Drug targets for neuroprotection that so far have been studied in randomized clinical trials are: Melatonin[32,33], Erythropoietin[34] and Xenon[35] Currently studies are focussing of inclusion criteria for active hypothermia treatment in view of delayed start of hypothermia treatment > 6 hours of life and including infants born less 36 weeks of gestation and identifying the additional risk of perinatal infection (GBS) on the outcome.

REMOTE ISCHAEMIC PRECONDITIONING (RIC)

In 1993 it was first described by K. Przyklenk and colleagues that: "brief ischemia in vascular bed also protects remote, myocardium from subsequent sustained coronary artery occlusion" Since, RIC has been extensively studied in adult myocardial ischaemia and stroke. RIC is a simple applicable non-invasive technique for example inflating and deflating blood pressure cuff placed on the upper arm. An activation of afferent neuronal pathways is observed within the remote organ and blood-borne protective factor(s), appearing to recruit intracellular signalling pathways to promote immune modulatory effects, increase local blood flow and augmenting cellular repair mechanisms within the target organ[20]. In neurological hypoxic ischemic conditions, RIC has been shown to reduce stroke infarct size in the adult and in a neonatal model of HIE experimental studies, confirm the effect of RIC performed at onset of reperfusion preserving cell metabolism and reducing apoptosis in the white matter[37,38]. Further experimental work and clinical studies are needed to confirm a possible neuroprotective benefit in infants treated for HIE.

ACTIVE CELL TREATMENT AND EXTRACELLULAR VESICLES

Cell based therapy for perinatal brain injury are currently strongly looked at[38,39]. Clinical trials have been set up to study the potential of stem cell (SC) therapy in infants at risk of developing cerebral palsy following perinatal brain injury. These studies focus on the major caused of perinatal brain injury discussed earlier (GM-IVH[40] ; HIE[41]). Both conditions are associated with increased risk of development of long-term neurological sequel in particular cerebral palsy and cognitive disability.

The human umbilical cord blood contains of a variety of SC types (Mesenchymal stem cells, endothelial progenitor cells, haemopoietic SCs) that have been studied in view of active cell therapy[39]. Umbilical cord stem cells are easily to be obtained and ready availability in acute injury. Mesenchymal SC from umbilical cord have a low immunogenicity and exhibit a greater proliferative activity than mesenchymal SC from the bone marrow.

There are two goals for active cell therapy in perinatal brain injury: First replacement of lost cells and second supporting endogenous mechanism via cell based molecular signalling supporting cells in the injured areas to survive by regulating inflammation and active cell death, supporting neurogenesis and myelination. The effect of cell replacement is expected to be small (5-10%) compared to the expected effect of transplanted SCs from molecular (paracrine) signalling (90-95%). Experimental studies[42] have shown that mesenchymal stem cell derived extracellular nanoparticles containing gene encoding microRNA ameliorate inflammation induced preterm brain injury. It appears that a given neuroprotective effect of active cell treatment is less about the cell but more about the molecular signalling via extracellular vesicles orchestrating restoration and repair of brain structure, attenuating inflammation, reactive astrogliosis and promoting myelinisation following acute brain injury.

CONCLUSION

Antenatal recognition of perinatal risk, prevention of prematurity and excellent perinatal management are keys to neuroprotection in neonates. It is important to recognize that the immature brain demonstrates specific vulnerability during development. Perinatal inflammation or infection are important confounders in hypoxic-ischaemic perinatal brain injury. Post haemorrhagic hydrocephalus is a severe complication following germinal matrix-intraventricular haemorrhage in preterm born infants less 32 weeks requiring early recognition and active management. Active hypothermia treatment is the corner stone of current management of HIE. For the future specific pharmacological interventions and active cell therapy are under investigation whether to provide additional neuroprotective effect in infants with HIE.

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