Chapter

NEONATAL ASPHYXIA AND STROKE: MORBIDITY, MODELS, CONSEQUENCES, AND TREATMENTS

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ABSTRACT

Neonatal and early childhood asphyxia is a major cause of morbidity and chronic cognitive dysfunction. In developed countries approximately 2.4 births per 1000 have asphyxia related complications including encephalopathy. In this chapter we will discuss the burden of neonatal asphysxia and stroke, and the neurological consequences to the individuals afflicted, including epilepsy, cerebral palsy, and cognitive impairment. At present, treatment is focused on managing the symptoms of perinatal asphyxia. As new treatments are clearly needed, various models have been developed to test putative neuroprotectants. We discuss the strength and limitations of each of the most commonly used animal models. We finish with a discussion existing treatments for acute care, including hypothermia, and the most promising pharmacological candidates for clinical use.

1. INTRODUCTION

1.1. Peri/Neo-Natal Hypoxia and Hypoxia Ischemia

Hypoxia is pathologically low blood oxygen levels (Bracci, Perrone, and Buonocore, 2006) and ischemia is disruption in blood flow. Ischemia due to the occlusion of a blood vessel in the brain results in a stroke; a cascade of neuronal death that is extremely common in aging adults (del Zoppo, 1998). Given that the damage caused by ischemia is the result of hypoxia, why are the two terms used to describe some types of ischemia but not others? Why is the designation “hypoxia ischemia” most often associated with neurological damage in newborns, either around the time of birth (perinatal) or recently after birth (neonatal)?

The answer is in the history of research into neonatal hypoxia, a condition in which ischemia causes not only local hypoxia but also global hypoxia throughout the fetus. The fetus relies on nutrients and oxygen supplied through blood vessels of the umbilical cord, which can become compressed during birthing complications and cause ischemia of the umbilical cord, resulting in global hypoxia of the fetus and damage to sensitive organs (Satas, et al., 2003) . The brain is injured significantly more than other organs and these effects are lasting with significant effects of quality of life (Gunn, Gunn, deHaan, Williams, and Gluckman, 1997; Hopkins-Golightly, Raz, and Sander, 2003). The pathology is very similar to that caused by local ischemia in the brain (stroke) (Comi, Weisz, Highet, Johnston, and Wilson, 2004). This unique combination of ischemia and global hypoxia gave rise to the field of hypoxic ischemia in the peri/neo-natal period of life. This chapter will discuss the properties of peri/neo-natal hypoxia ischemia with respect to neuro-pathology, the animal models used to investigate this pathology, and potential interventions and pharmacological treatments that are under development.

1.1.1. Hypoxia Ischemia Prevalence and Risk Factors

Peri/neo-natal hypoxia is a pathological drop in oxygen levels in an infant's blood, often caused by compression of the umbilical cord or asphyxia due to birthing complications or a choking incident (Lawn, Shibuya, and Stein, 2005). In 1997 it was estimated by the World Health Organisation (WHO) that 19% of the 5 million infant deaths that year were caused by hypoxia related condition (Shiffman, 2010). The most prevalent birthing complication that results in hypoxia ischemia is nuchal cord, this involves the umbilical cord forming a loop around the neck of the fetus, and has been linked to an increase in perinatal mortality. During nuchal cord the umbilical cord can become compressed, blocking the supply of oxygenated blood to the fetus. This can cause acidosis of the fetal blood and hypercarbia (Hankins, Snyder, Hauth, Gilstrap, and Hammond, 1987). Nuchal cord occurs in 8-34.5% of births, with around 5% of all births having a "tight" nuchal cord (Ogueh, et al., 2006). Due to advances in intrapartum care most of these cases are asymptomatic. A study by Ogueh et al. (2006) analyzed data from 57,873 births and found that tight nuchal cord incidents resulted in lower birth weights, longer birthing times, increased incidences of shoulder dystonia, and increased cesarean intervention. These are often associated with ischemia and encephalopathy (Bloch, 2005).

In the developed world the incidents of such complications have decreased dramatically over the last three decades. In Canada between 1991 and 2004 the rate of hypoxia ischemia related complications dropped from 43.8 to 2.4 cases in every 1000 births (Dzakpasu, et al., 2009). This decrease is due to improved care with a rise in the use of fetal heart monitoring systems (cardiotocography) and, induced labor and caesarian births (Smith, Wells, and Dodd, 2000). In the developing world such data is difficult to acquire as stillbirths (including hypoxia related deaths) are often not reported and no birth or death certificate is generated. However it is likely that the rate of asphyxia related complications is much higher than that of developed countries (Lawn, et al., 2005).

Stroke is a disruption in the blood flow within the brain. This can be caused by a thrombus (ischemic stroke) or a rupture of a cerebral blood vessel (hemorrhagic stroke). Stroke during the perinatal and neonatal period was previously thought to be a relatively rare pathology, but recent evidence has suggested a far greater prevalence. Peri/neo-natal stroke has gone unnoticed due to plasticity of the neonatal brain. Because of the recent emergence

of this field and because of the physiological similarity to the hypoxia ischemia, peri/neonatal stroke has come under the research umbrella of hypoxia ischemia, sharing many animal models and putative therapies. Lynch et al. (2002) reviewed perinatal stroke which they defined as the developmental period between 28 weeks gestation to 28 days postnatal age and calculated the incidence of symptomatic perinatal stroke to be 1 in every 4000 births in the developed world. However, the authors report that because of the difficulty of diagnosis due to difficulty in assessing newborns for neurological function, the incidence of symptomatic stroke may be higher still. Because stroke can occur in any region of the brain the symptoms are more varied than that of hypoxia ischemia, and include motor deficits, sensory deficits, impairment of memory and cognition, and epilepsy (Kolk, Ennok, Laugesaar, Kaldoja, and Talvik, 2010).

2. Cellular Processes of Neuronal Death during Ischemia

2.1. Excitotoxicity

The reduction in oxygen levels and glucose levels in the brain following HI causes disruption in the neurons ATP production. This causes the mechanisms which maintain the cell membrane potential such as Na^+/K^+ ATPase to cease functioning, causing the cell to depolarize and release its neurotransmitters including glutamate (reviewed in (Nishizawa, 2001; Rivers and Ashton, 2010; Rothman and Olney, 1987). Increasing concentrations of glutamate in the extracellular space causes excitation of other neurons, primarily through NMDA and AMPA receptors. This not only increases the energy demands of cells in an already energy deprived environment, but also results in pathological levels of Ca^{2+} flux into the neurons. This causes osmotic influx of water resulting in cellular edema that can result in necrosis as well as disruption in mitochondrial function, resulting in apoptosis (Wahlgren and Ahmed, 2004). As the neurons depolarize and undergo necrosis further glutamate is released resulting in an excitotoxic cascade throughout the brain. This process occurs in both the immature and mature brain. However, recent evidence suggests that NMDA receptors in the developing brain have enhanced function, and that this is crucial for plasticity during development. This increases susceptibility to excitotoxicity in the immature brain (Johnston, et al., 2009). The ability of astrocytes to absorb high levels of neurotransmitters is reduced in the immature brain, with cerebral spinal fluid (CSF) levels of glutamate shown to be heightened in HI affected infants up to 16 hours after the insult (Pu, et al., 2000).

2.2. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

ROS and RNS are highly reactive molecules that can react with cellular components including proteins, fats, and nucleic acids. In the case of proteins this causes a breakdown of cellular process including enzyme function, ion pump function and receptor activity, and can result in depolarization of the cell, potentially initiating the excitotoxic cascade. In the case of fats such as phospholipids, membranes can become permeable, also resulting in

depolarization of the cell and of the mitochondria, causing a loss of energy production. This reaction is especially damaging as the phospholipids auto-catalyze the oxidation of other phospholipids resulting in widespread membrane failure. This can lead to further cellular edema and excitotoxicity. Oxidative damage to nucleic acids can result in mutation and a loss in translational and transcriptional function (Ikonomidou and Kaindl, 2011).

ROS and RNS are continuously produced by cells but are normally in balance with endogenous antioxidant mechanism that safely catalyze the reactions of ROS and RNS to non-harmful compounds. For example, the highly reactive ROS superoxide reacts with water to form hydrogen peroxide, catalyzed by superoxide dismutase. Hydrogen peroxide is then reduced with oxygen to form water, catalyzed by catalase (Ikonomidou and Kaindl, 2011). A major site of ROS and RNS production is mitochondria. Electrons in the electron transport chain (ETC) normally contribute to a controlled series reaction, but can become dissociated from the ETC before reaching the critical molecule complex IV, and instead react with other nearby molecules. These uncontrolled reactions form ROS and RNS; for example electron donation to oxygen produces superoxide.

If the balance between ROS, RNS and endogenous antioxidant mechanisms is disrupted, ROS and RNS can react with mitochondrial membrane phospholipids and disrupt the integrity of the membrane. This causes depolarization and a catastrophic reduction in of ATP synthesis. This also causes cytochrome C to leak from the membrane into the cytosol, and act as signaling molecule for apoptosis. During HI, ROS and RNS levels rise beyond antioxidant capacity, and through this pathway cause neuronal apoptosis for days after injury.

In HI, low oxygen levels prevent the donation of the electrons of the ETC, this causes electrons to build up on the ETC and leak from the chain, generating ROS and RNS. Once HI is over, reperfusion produces high levels of oxygen which results in uncontrolled electron donation to oxygen, generating more superoxide and other ROS and RNS. Until recently 100% oxygen was given to infants that had undergone an HI incident. It is now recognized that 100% oxygen may exacerbate reperfusion injury and that normal levels of oxygen may be less harmful (Pu, et al., 2000).

The developing brain is more sensitive to increases in ROS and RNS than the mature brain. One reason for this is because the developing brain has lower levels of antioxidants. One study has show that fetal serum levels of the strong antioxidant tocopherol were 80% lower than that of an adult. Similar deficiencies have been found for other antioxidants such as selenium. The developing brain is also restricted to the extent that it can upregulate antioxidant synthesis or function; for example, glutathione peroxidase and glutathione. Contradicting this however, some evidence suggests that in healthy full term births the ratio of antioxidant to ROS/RNS is greater than that of an adult, perhaps because of adaptive mechanisms that help the newborn adapt to the high oxygen environment outside the womb (Parmigiani, Gianotti, Pezzoni, Corradi, and Bevilacqua, 2011). However, this may not be true for premature infants, which is particularly significant because of the common co-morbidity of premature birth with perinatal HI (Drury, Nycyk, Baines, and Cooke, 1998) .

2.3. Inflammation

Under healthy conditions the brain is an immune privileged organ. Specialized glia cells called microglia, which act as the resident immune cell for the CNS, become activated upon

the molecular recognition of tissue damage or the presence of pathogens. Once activated microglia secret proinflammatory cytokines which promote the migration and activation of more microglia and cause the endothelium to express adhesion molecules that allow blood-born immune cells to infiltrate into the CNS. Once at the site of injury, immune cells produce a cocktail of neurotoxic factors that includes ROS/RN, cytokines, and glutamate.

These diffuse from the immediate site of injury (infarct core) into the surrounding tissues, generating further neuronal death in the proximal healthy tissue (penumbra). Immune cells produce NO from the oxidation of L-arginine to citrulline via various nitric oxide synthase (NOS) enzymes. NO has both vasodilatatory and antimicrobial effects, but when produced at pathological levels can cause neuronal damage by the activaton of apoptosis pathways and competition for oxygen with cyctochrome c (Klegeris, Bissonnette, and McGeer, 2003).

The cytokines produces by microglia and systemic immune cells have been shown to be neurotoxic in many *in vitro* studies (Klegeris, Bissonnette, and McGeer, 2003; Venters, et al., 1999). Cytokines including TNFα and IL1β induce astrocytes to produce NO via inducible NOS (iNOS) and affect cell signaling pathways involved in neuronal survival and apoptosis (J. K. Lee, Tran, and Tansey, 2009; Venters, et al., 1999). Infections during pregnancy can produce HI-like processes, and evidence suggests that cytokines produced by the mother can have neurotoxic effects on the fetus (Bloch, 2005).

NO produced by activated immune cells and astrocytes induce both the release of glutamate by neurons via depolarization and the inhibition of the reuptake glutamate by astrocytes (Bal-Price and Brown, 2001; Chao, Hu, Ehrlich, and Peterson, 1995).

Clear evidence of the role of neuroinflammation in neuronal death after has been provided by the use of transgenic mice that lack key proinflammatory cytokines, interleukin 1β and interleukin 18. The transgenic mice were protected from hypoxic injury compared with wild type controls (Felderhoff-Mueser, et al., 2005).

However, clinical studies of neuroinflammatory diseases, such as meningitis, demonstrate that neuroinflammation can occur without neuronal death, so oxidative injury may be necessary to initiate inflammation that results in neuronal death.

3. Brain Regions Vulnerable to the Effects of Hypoxia Ischemia

3.1. Prenatal and Perinatal Hypoxia Ischemia

The regions and cell types that are most vulnerable to damage change with the development stage of the brain. Prenatal and perinatal HI is associated with white matter injury such as periventricular leukomalacia (PVL) and periventricular white matter injury (PWMI) (Scafidi and Gallo, 2008). The prevalence of PVL and PWMI decreases with increased birth weight and length of pregnancy, with a preterm prevalence of 60 PVL infants per 1000 births compared to a full term prevalence of 1.3/1000 (Bracci, et al., 2006). The particular stage of cell development and receptor types expressed determines sensitivity to injury.

In prenatal and perinatal HI the insult occurs during production of oligodendrocytes when oligodendrocytes progenitor cells (OPC's) are immature and highly active (Bracci, et

al., 2006). During this stage, OPC's express an intermediate form of the AMPA receptor that lacks the GluR2 subunit.

This facilitates higher levels of Ca^{2+} transport, and makes the developing OPC's more susceptible to excitotoxicity (Johnston, et al., 2009). There is also evidence that the OPC's have low levels of antioxidant enzymes (Ikonomidou and Kaindl, 2011).

3.2. Neonatal Hypoxia Ischemia

In a full term infant of normal birth weight, oligodendrocytes production is advanced, but myelination of neurons is still at an early stage. Hypoxia during this period has little effect on the white matter, but does have substantial effects on neurons in the basal ganglia (Rice and Barone, 2000). Neonatal HI causes damage predominately to the putamen and the thalamus, and this can result in cerebral palsy; general non-progressive brain damage that occurs during development and predominately affects motor function. MRI studies suggest that the susceptibility of motor centers is due to high levels of glutamatergic input (Johnston, Trescher, Ishida, and Nakajima, 2001). This is further supported by fMRI studies that show newborns that have HI induced seizures have hyperactive neurons in these regions (Johnston, et al., 2001).

The hippocampi are key structures involved in spatial memory, and are vulnerable during neonatal hypoxia (Bass, et al., 2004). Although studies in humans are limited, it appears that hippocampi are among the first regions to be affected during HI. MRI and functional studies show that children that have undergone HI during or near birth have near normal intelligence with only mild dyspraxia can have severely affected memory and hippocampal volume (Gadian, et al., 2000). Little is known about the processes that cause hippocampal sensitivity despite extensively studies using rodent models of HI, although there is some suggestion that the hippocampus may be a site of intense excitotoxicity (Clarkson, et al., 2005; Gurd, et al., 2002). Complicating matters, these regions may be especially plastic during early development, potentially masking damage from behavioural assessment (Johnston, et al., 2009).

Interestingly, mechanisms of cell death change with development, with a shift from programmed cell death early in development to necrosis in the mature brain (Liu, Siesjo, and Hu, 2004). This suggests that targeting apoptosis for pharmacological intervention may be more successful in the developing brain than in the mature brain (Cowper-Smith, Anger, Magal, Norman, and Robertson, 2008).

4. Animal Models of Hypoxia Ischemia

Many animal models have been used to study peri/neo-natal HI. The most common animal models use sheep, pig or rodents. A review in 1996 found that 26% of all animal studies on HI were done using rodent models, with sheep and pig models representing 23% and 22% of the research respectively (Roohey, Raju, and Moustogiannis, 1997). Critically, this review found that 71% of all studies assessed the outcomes of the HI only between 0 and 24 hours after the insult. Recent evidence suggests that disease progression is variable

and can last for weeks or months after the initial insult (Kolk, et al., 2010; Vannucci and Vannucci, 2005). This has historically been a shortcoming of animal studies and has only recently started to be addressed (Vannucci and Vannucci, 2005). This section will give a brief description of some of the common animal models, addressing the advantages and disadvantages of each.

4.1. Primate

Primate models have the advantage of close homology to humans. The genetic profiles of the animals are similar to humans as well as the gestational process and development. The complexity of the brain is the most closely related to humans out of any animal model (Roohey, et al., 1997). Primate models normally involve inducing injuries either prior to birth or at birth. This is because brain development is very similar to the human condition so unlike other animal models such as rodent models, the estimated equivalent developmental stage of the fetus at birth is the similar to humans. Two models dominate the primate field; the most common involves cesarean birth where the umbilical cord is occluded for 10 to 15 minutes (Beckstrom, Humston, Snyder, Synovec, and Juul; Juul, et al., 2007). The second model involves warm saline emersion after birth prior to the first breath of the infant (Roohey, et al., 1997). Both models result in very similar histological and behavioral pathological effects to that of the human condition such that putative therapeutics that have been found to be effective in these models should be strongly considered for human trial.

The limitation of primate models involves expense and accessibility. The cost of primate models and the long gestational periods of the animals constrains experimental numbers, thus the studies are often underpowered. This is a particular problem in animal models of hypoxia, where highly variable outcomes are the norm (Rivers, et al., 2011). Ethical issues arise for the same reason that makes non-human primates excellent modesl of human pathologies. It is likely that these animals have a greater similarity to humans in experiencing suffering and pain than other animals. For this reason experiments on primates are often the target political and social debate, limiting their use in some countries (McNeil, 2008).

4.2. Sheep and Pig

Pigs and sheep are also used in models of peri/neo-natal HI. Both pigs and sheep have similar sized newborns to humans, with comparable stages of brain development and size. These factors result in the oxygen and other metabolic demands of the brain that are similar to humans (Northington, 2006). There are several different methods of inducing HI in these animals. Hypoxia of the pregnant mother and umbilical clamping are common (Northington, 2006). Other methods used are new born asphyxia or carotid artery ligation. These methods have the advantage of not needing anesthesia of the pregnant mothers (Domoki, et al., 2010) . Sheep are more analogous to the human with respect to number of fetuses per pregnancy (1-2), whereas pig litter sizes number from 8-14. Although this is less analogous to humans, large litters reduce costs and therefore can result in more statistically powerful studies. Physiologically, these animals are a good intermediate between rodent studies and non-human primate studies.

There are many limitations of these large animal studies. High costs of care are an issue as well as long developmental and gestational periods. This makes large experiments difficult, and limits the statistical power of studies that involve these animals. Furthermore, the behavior of on these animals is poorly characterised compare with rodents or humans, and the animals develop more quickly than humans, making long term comparisons difficult. Despite this, these models are popular as there are fewer ethical issues compared with primates, and the animals are readily available due to agricultural production.

4.3. Rat and Mouse

Rat and mouse models are the most popular models used in HI. The use of these animals has several advantages. These animals have fast development, are small in size, and are cheap to purchase and maintain, these factors allow for high numbers in a study. This increases the power of the study allowing researchers to study putative protective therapies that may only produce a small improvement in the condition. Furthermore, researchers can study many potential therapies in a relatively short period.

The availability of genetic knockouts and specific phenotypic strains are a further advantage of rodent models. Genetic knockouts allow for the testing of the function of certain genes/proteins during and after HI, as well as facilitating the testing of the specificity of probes used to assay changes in proteins (Chen, et al., 2006). Specific phenotypes, such as spontaneously hypertensive rats, allow the study of co-morbidities which can be common in human conditions (Bloch, 2005).

Using histological analysis such as cellular layering, myelination and neurogenesis, it appears that the development stage that is equivalent to the human at birth is after birth in the rodent is approximately 7 days post birth in both mice and rats (Ten, Bradley-Moore, Gingrich, Stark, and Pinsky, 2003; Vannucci and Vannucci, 2005). Although age correlations differ depending on which histological, behavioral, or physiological aspect is compared (Rice and Barone, 2000). This means the injuries can be induced in pups, without complicated caesarian. Some umbilical clamping models are still used in rodent models, however, the most common models involved ligation of one or more of the blood vessels that supply brain 7 days after birth. Two main models exist: (i.) ligation of both common carotid arteries, leaving only the vertebral arteries to supply blood to the brain. This results in global bilateral damage to the cortex. (ii.) A more common method involves ligation of one of the common carotid arteries followed by a period of hypoxia normally facilitated through a chamber filled with 5-8% oxygen in nitrogen. 7 day old rats normally require 2-3 hours of hypoxia to acutely produce a large unilateral infarction of the cerebrum, mice and older rats require much less time to produce a similar infarct (40-70 mins) (Rivers, et al., 2011). The advantage of the latter model is that it leaves an intact hemisphere which allows for histological, biochemical and behavioral comparisons. However, recent evidence now suggests that the apparent healthy hemisphere may have low levels of damage (Huang, Liu, Cheung, and Chen, 2009).

The shortfalls of rodent model arise from the difference in anatomy and physiology compare with humans. The development of the rodent brain is faster, with adult-like behavioral phenotype reached 20 days after birth in rats. There is therefore a clear gap between these models and clinical applicability.

Criticism of preclinical research have increased steadily over the last two decades as therapies found to be effective in preclinical research are failing to produce similar effects in large and expensive phase three clinical trials (Wahlgren and Ahmed, 2004). Some of these criticism focus on the models themselves, questioning the use of models such as rodent ones and whether they produce similar pathological physiological states to those in human conditions (Wahlgren and Ahmed, 2004).

5. TREATMENTS

5.1. Current Clinical Treatments

5.1.1. Prevention: Pharmacological and Surgical Interventions

Advances in prenatal and birthing care and diagnostics has seen significant decreases in perinatal HI and improved outcomes after HI (Smith, et al., 2000). Detection of complications such as nuchal cord and maternal infections has increased with greater monitoring during pregnancy.

This monitoring includes the use of cardiotocography to monitor the heart rate of the fetus, thus detecting possible causes of global hypoxia such as defibrillation or pathological bradycardia. Also, the use of fetal imaging using color Doppler and ultrasound, can detect if a complication like a tight nuchal cord requires more intense monitoring and possible cesarean birth (Ogueh, et al., 2006).

Oxytocin is a hormone that causes labour induction, and can be administered as a drug to induce labour (Milson, et al., 2002). Inducing labour has lowered the prevalence of HI by inducing birth in cases where the fetus has an acute complication such as bradycardia. Also if the pregnancy has gone on beyond 42 weeks, the size of the fetus increases the risk of birthing complications that are associated with hypoxia during birth (Ogueh, et al., 2006).

5.1.2. Rehabilitiation: Physiotherapy

Neuroplasticity is the ability of the brain to remodel structurally and functionally. The primary mechanisms of neuroplasticity is changes in the processes of the neurons including dendritic and axonal branching, changes in synapse number, and the formation of new neurons through neurogenesis (Holt and Mikati, 2011). After HI there is a neuroplasticitic reorganisation process that redistributes function from the damaged areas of the brain to the healthy cortex. In peri/neo natal HI dendritic and axonal branching as well as synaptogenisis is most likely the most important mechanism of neuroplasticity, as in the developing brain a surplus of neurons already exists (Holt and Mikati, 2011).

Neuroplasticity is strongly influenced by activity of the cortex. Pathways that are used and therefore activated more frequently are strengthened and developed. The developing brain is particularly plastic and is therefore better able to recover from injury. Therapies have been successfully developed for after HI that assist the rewiring process that is needed to maintain normal function. Challenging the affected individual in cognitive and motor tasks is referred to as enrichment, and has been shown to assist in the rewiring process.

Therapies involving increasing the movement of the effected limbs have been shown to be successful in adult stroke, and there is growing evidence that this is also true for

peri/neo-natal HI. From stroke studies it appears that several methods are useful, these include the manual assisting of movement of the limbs by physiotherapist in severally disable individuals. Also, constraining the use of unaffected limbs in more able individuals encourages use of the disabled limb. In the only slightly impaired individuals with mild dyspraxia, challenging games can be used, including ball games and precision grip games, to improve daily motor function (Kolk, et al., 2010).

Transcranial magnetic stimulation (TMS) is a strong directional magnetic field applied to the surface of the skin above the target area, that induces depolarization of the neurons under the area. When applied at different intensities and frequencies the brain region can be either induced into a hyper-excitable or a hypo-excitable state. This is thought to occur through long term potentiation (LTP) and long term depression (LTD) respectively (Holt and Mikati, 2011; Thordstein, Hallbook, Lundgren, van Westen, and Elam, 2011). TMS can downregulate or upregulate the use of certain brain regions, and so might be used to increase the excitability of a damaged area, or temporarily arresting function in complementary undamaged brain regions (thereby forcing use of compromised brain regions) (Bernard, Goldenberg, Armstrong-Wells, Amlie-Lefond, and Fullerton, 2008).

5.1.3. Limiting Brain Damage: Pharmacological Intervention

There is no pharmacological intervention for use after peri/neo-natal HI that has completed stage 3 clinical trials (Scafidi and Gallo, 2008; Yager, Armstrong, and Black, 2009). Current pharmacological interventions involve tightly controlling the homeostasis of the infant. The two systems that are most crucial to the infant survival and that must be controlled are carbon dioxide and blood glucose homeostasis. Hypocapnia or low CO_2 levels of the blood are associated with worsened outcomes following HI. Low levels of CO_2 results in vasoconstriction, potentially further reducing oxygenated blood flow to the brain, and increasing brain damage. However, despite excellent preclinical evidence, no clinical evidence exists that maintaining high of CO_2 is neuroprotective. In spite of this it still seems prudent to maintain normal or high levels of CO_2 of ventilated infants following HI. With respect to blood glucose, hyperglycemia has been shown in animal models to be neuroprotective, yet no clinical data exists on glucose related interventions. Hypoglycemia has been associated with poorer outcomes following HI and thus glucose levels are tightly controlled.

Seizure activity is common in the first 72 hours after an HI incident, and rarely afterwards. These seizures are not controlled by anticonvulsants as the potential risks of administering anticonvulsants to the developing brain may outweigh the potential benefits. A meta analysis of 7 different placebo controlled clinical trials that use anticonvulsants showed no benefit to the clinical outcomes of the infants (Evans, Levene, and Tsakmakis, 2007). Furthermore, seizures may be a symptom of mass neuronal death and may not be involved in exacerbating the damage (Rivers, et al., 2011). However, seizure have been associated with an increase in body temperature that will worsening the pathology (Wirrell, Armstrong, Osman, and Yager, 2001). Conversely HI induced epilepsy that continues beyond infancy must be controlled with antiepileptic to limit continued damage and improve quality of life (Glass and Sullivan, 2009).

5.1.4. Limiting Brain Damage: Hypothermia

Hypothermia can be induced by placing the infant in cold water, or by use of a cold cap on the infants head. Both methods are used to lower the body temperature of the newborn by 3-4^{0}C. Many mechanisms of neuroprotection have been proposed.

Hypoxia reduces rates of reactions and thereby lowers the metabolic demands of cells within the brain; this allows them to operate on a low oxygen and glucose budget. Animal model studies have shown a complete preservation of ATP levels from hypothermia (Yager and Asselin, 1996). This would prevent the loss of function of crucial cellular functions such as Na^+/K^+ ATPase and thus maintain membrane potential, preventing excitotoxic cascades and ROS/RNS related damage. The second mechanism could be an immune cell modulation effect. Immune cell function and reactivity is lower in lower temperatures, this could prevent the pathological neuroinflammatory effect seen for days or weeks after HI (Maekawa, et al., 2002). This mechanism could also explain why hypothermia must be maintained in the clinic for the relatively large period of time of 72 hours in order to be effective.

5.2. Putative Pharmacological Treatments

Apart from hypothermia, physical rehabilitation, and preventative surgical and pharmacological intervention, there have been few if any drug treatments that have been proven to be effective at reducing the burden of disability due to neonatal hypoxic encephalopathy. Nonetheless the pharmacological management of peri/neonatal hypoxia is an area of intense research. Treatment strategies can be roughly divided into those that are intended to reduce brain damage either during or subsequent to hypoxia ischemia, and those that are aids to rehabilitation.

5.2.1. Limiting Brain Damage

The pharmacological strategies for reducing brain damage following hypoxia ischemia that have been most intensively investigated can be grouped according to the main elements of the pathological cascade described above; excitotoxicity, oxidative stress, and inflammation. These correspond to drugs that reduce CNS excitation, antioxidant therapies, and anti-inflammatories and other drugs that affect wound healing respectively.

5.2.1.1. Inhibitors of CNS Excitation

Excitotoxicity is fundamentally a property of excessive glutamate signaling, and so antagonists at glutamate receptors have been at the forefront of attempts to reduce brain damage. These include inhibitors at the glutamate NMDA and AMPA receptors. NMDA receptor inhibitors have been extremely successful in a variety of animal model of cerebral ischemia, often causing dramatic reductions in brain damage (Clarkson, et al., 2005; Fernandez-Lopez, et al., 2006). However, this success in preclinical research has not translated into successful treatments in the clinic (Hoyte, Kaur, and Buchan, 2004; Lo, 2008a).

The reasons for this continue to be debated, with quality of preclinical experiments and publication bias cited as a factor (O'Collins, et al., 2011; E. Sena, van der Worp, Howells, and Macleod, 2007; E. S. Sena, van der Worp, Bath, Howells, and Macleod, 2010) along with the inapplicability of animal models and the incompatibility of the time windows for therapeutic

intervention with those used in animal testing. The timing of intervention appears to be critically important; NMDA receptor antagonists are protective in the acute stages of injury, but interfere with the beneficial effects of NMDA receptor activation that occur after injury during recovery and repair processes (Lo, 2008b).

Other drugs such as cannabinoid CB1 receptor agonists have been tested as neuroprotectants, with mixed results. Although CB1 receptors expressed on glutamatergic neurons can play a protective role by inhibiting glutamate release, CB1 can also be expressed on GABAergic neurons, and have a excitatory effect by inhibiting GABA release. As a result, cannabinoid CB1 agonists have given mixed results for neuroprotection, with no drugs in development. Similarly, CB1 receptor antagonists are protective in some models, but translation to the clinic does not seem likely at the time of writing (Ashton and Glass, 2008).

Despite the narrow time window for beneficial results from the administration of glutamate receptor antagonists and the problems with delayed administration of NMDA receptor antagonists, other glutamate receptors may still be a target for pharmacological management of hypoxic encephalopathy. Neonatal hypoxia is associated with seizures, which may either be a cause or a consequence of encephalopathy. This has suggested suppression of neuronal excitation as a treatment to reduce the long term effects of neonatal hypoxia. There are many possible targets for intervention, including most of the traditional targets for anticonvulsant and antiepileptic therapy, including glutamate receptors, GABA receptors and voltage sensitive sodium channels. Rat studies have indicated that inhibition of excitatory amino acid neurotransmission may be better in this respect than agonists at inhibitory receptors, such as benzodiazapenes (Jensen, 1995).

Talampanel (GYKI53773) is a non-competitive AMPA receptor antagonist (Aujla, Fetell, and Jensen, 2009) which has been shown to reduce seizures in a dose-dependent manner in a rat model of neonatal hypoxia. Given that seizures are predictive of brain damage in hypoxia ischemia (Rivers, et al., 2011) then telampanel could mark a change in strategy for the pharmacological management of CNS excitation in neonatal hypoxia, shifting from neuroprotection in the acute phase to the reduction of seizure-induced injury in the delayed phase of damage. Talampanel is under trial as antiepileptic drug in children and so may be used in the near future in the treatment of seizures associated with neonatal hypoxic encephalopathy. There is also some evidence of neonatal seizures can be successfully treated with traditional CNS depressants such as short acting benzodiazapenes (Sirsi, Nangia, LaMothe, Kosofsky, and Solomon, 2008). As there is considerable overlap between management of epilepsy subsequent to hypoxic encephalopathy and the reduction of seizure induced brain damage, then despite their failure as neuroprotectants in the acute phase of injury CNS inhibitors are likely to play a significant role in the pharmacological management of hypoxia ischemia.

5.2.1.2. Antioxidants

Drugs that target oxidative stress by ROS or RNS, antioxidants, like NMDA receptor antagonists, provide significant neuroprotection in animal models (Clarkson, et al., 2004; Gitto, Pellegrino, Gitto, Barberi, and Reiter, 2009; Sutherland, et al., 2004). However, also like NMDA receptor antagonists, these drugs have failed to work in the clinic. To take one well discussed example, NXY-059 is a free radical spin trap that unexpectedly failed in clinical trial in the SAINT-2 trial (Shuaib, et al., 2007).

Aside from the issue of the applicability of animal models to humans, there are at least three major arguments for why antioxidants have failed in clinical trial. First, cells contain a battery of endogeneous antioxidant mechanisms, such that oxidative stress is the result of an imbalance between free radical and ROS production and endogenous protective mechanisms. Therefore, the concentration of exogenous antioxidants to the sites of injury has to provide at least as much protection as that provided by endogenous mechanisms to be of benefit. The delivery of antioxidant drugs to potential sites of action has been challenging, and has been the topic of a great deal of innovative and ingenious research. Despite this, there is a large experimental gap between the behaviour of antioxidants *in vitro* and their effects *in vivo*.

However, this does not explain why antioxidants have been successfully used in animal models, and so some researchers have argued that exogenous antioxidants do reach key sites of action in significant quantities, but have complex effects depending upon subtleties of timing, dose, and physiology.

Reactive oxygen and nitrogen species are not only causes of damage at high concentrations, but are also signaling molecules in various aspects of physiology and homeostasis; for example, the maintenance of blood-flow. Moreover, sustained antioxidant treatment might cause adaptive changes in endogenous protective mechanisms, with unpredictable effects. And in a similar way to NMDA antagonists, although blockade of oxidative stress may be protective in a short time window of acute injury, antioxidants may have biphasic effects such that delayed administration interferes with essential recovery and repair processes (Lo, 2008b).

A third argument maintains that antioxidant therapy may well remain a viable treatment strategy for neuroprotection, but preclinical research has been of insufficient quality to support the decisions that have been made to move drugs into clinical trial. Macleod et al. (2008) have argued persuasively that the pre-clinical evidence for NXY-059 did not warrant progress to clinical trial, and that close analysis of the preclinical studies reveals a paucity of evidence for the drug's efficacy, with effects overestimated due to a lack of rigor in experimental design. The authors argue that this could be corrected by the widespread adoption of publication standards for preclinical animal studies similar to those that are now standard for clinical trials.

5.2.1.3. Anti-Inflammatory Drugs

If it is true that putative neuroprotectants necessarily have biphasic or even multiphasic effects dependent upon timing of delivery with respect to the phase of injury and recovery (Lo, 2008b), then this applies equally to anti-inflammatories. But unlike NDMA receptor antagonists and antioxidants, anti-inflammatories tend to have beneficial actions in the delayed phase of injury, whereas delivery at the acute phase can interfere with vital triggers to endogenous protective and recovery mechanisms.

The two most commonly used classes of anti-inflammatory drugs are corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs). Neither of these has proven suitable for treatment of cerebral ischemic injury. NSAIDs have strong effects on haemostasis; traditional NSAIDs increase risk of hemorrhage, and selective COX-2 inhibitors increase the risk of thrombosis.

It is not clear as yet whether these limitations will apply to all drugs that target the arachidonic acid pathways such as leukotriene inhibitors. With respect to steroids, not only is there no evidence for any beneficial effect the acute phase of injury (De Reuck, et al.,

1988; Norris and Hachinski, 1986) but various lines of evidence suggest that high dose steroid treatment can cause microvascular injury and vasospasm (De Reuck, et al., 1988; Edlow, et al., 2010; Linn, Desilva, and Peeters-Asdourian, 2009). Because of this, testing of non-traditional anti-inflammatory drugs in animal models of cerebral ischemia is an active field of research. For example, minocycline is neuroprotective in a range of models, including hypoxia ischemia (Cai, Lin, Fan, Pang, and Rhodes, 2006; Familian, Boshuizen, Eikelenboom, and Veerhuis, 2006; Maier, et al., 2007).

Another example of a non-traditional anti-inflammatory target is the cannabinoid CB2 receptor (Rivers and Ashton, 2010). Agonists for the CB2 receptor have been shown to be potentially neuroprotective in cerebral ischemia (Zhang, et al., 2007) although it has not yet been determined whether this is due to an anti-inflammatory effect, and whether CB2 activation is neuroprotective in the secondary phase of cerebral ischemic injury.

5.2.2. Rehabilitation and Repair

Just as non-pharmacological interventions have focused both on limiting brain damage during injury and on enhancing recovery of function through rehabilitation programs, so pharmacological strategies include not only neuroprotective drugs but also drugs that may act in a way to facilitate the plasticity of neural and associated tissue that is central to repair and recovery.

5.2.2.1. Neural Plasticity

The emerging evidence that drugs that are neuroprotective in the acute phase of injury can be damaging in the secondary phase (and vice versa) highlights the importance of the recovery and repair processes that take place after acute injury. The secondary phase of injury involves not only ongoing cell death, but also changes in microvasculature due to angiogenesis, neuronal remodeling such as that due to axonal sprouting, complex patterns of neuronal arrest and recovery due to endogenous inhibitory mechanisms, and more controversially, the generation of new cell cells from neural progenitors. A highly orchestrated and complex inter-related series of events takes place in the hours, days and months following cerebral injury, and only recently has it become clear that processes that are damaging over the short term in one phase of injury may provide vital triggers for recovery processes that follow (Lo, 2008b). To give three examples: (i.) matrix metalloproteinase (MMP) inhibitors are protective in the acute phase of injury by stabilizing the microvasculature, but are vital for vascular remodeling and angiogenesis in the recovery phase (Lee, et al., 2006); (ii.) activated microglia and macrophages may be cytotoxic in one phase of injury, but through phagocytosis perform a vital role in preparing tissue for subsequent axonal sprouting and remodeling; (iii.) although over-activation of NMDA-receptors leads to excitoxicity in the acute phase of injury (above), functioning NMDA receptors appear to be vital for not only the activation of endogenous neuronal protective mechanisms (Papadia, Stevenson, Hardingham, Bading, and Hardingham, 2005) but are also a central element in the regulation of the synaptic plasticity that is critical for the remodeling of neural networks in the recovery phase.

Considerations about the timing of delivery of protective drugs aside, pharmacological strategies for enhanced neural plasticity are still in the conceptual stage. Identification of biochemical targets for increased plasticity is a goal, but to our knowledge not yet an established reality.

Neurotrophic factors, neurosteroids, and glutamate receptor modulators are just some examples. However, one promising approach has been suggested by recent research by Clarkson et al. (2010) who used an inhibitor of GABA transport to enhance motor function in mice following stroke. GABA transporter dysfunction is thought to be a contributing element to the tonic inhibition of neural activity in the penumbral regions of cerebral injury, such that substantial neural capacity may not be truly lost, but be simply in a state of arrest.

Disinhibition of these arrested neurons in the delayed phase of injury may not only provide some immediate recovery of function, but by restoring normal neural activity allow for ongoing neural plasticity. An optimal strategy for the use of such drugs would seem to be as adjuvant to physical therapy, with drug treatment allowing for the neural activity necessary for the plastic changes guided by a rehabilitation program. Ideally, drugs will be found to enhance neural plasticity such that the effects of physical rehabilitation may be even further accelerated and amplified.

5.2.2.2. Neurogenesis

Since the discovery that there are two areas of the brain that produce new neurons throughout adult life (the sub-granular layer in the hippocampus and the sub-ventricular layer in the striatum), there has been hope that this may be exploited to improve recovery from injury (Okano and Sawamoto, 2008). However, although it seems clear that neurogenesis contributes to neural plasticity in the hippocampus and the olfactory bulb in healthy adults, it has not yet been determined whether the production of new neurons in the pathological brain is part of a recovery process (Vert and Daval, 2006). Neurogenesis is upregulated in a variety of neural pathologies, including chronic neurodegenerative diseases such as Huntington's disease (Vazey and Connor, 2010) and following acute injury such as stroke (Kozorovitskiy and Gould, 2003). Whether this is part of an endogenous repair process or merely an epiphenomenon of disorganized cell division, remains to be determined (Minger, et al., 2007; Zhao, Deng, and Gage, 2008).

Cells produced at the sub-ventricular layer do migrate to sites of injury following cerebral ischemia, but it has been estimated that only a small percentage of these differentiate into neurons (Vazey and Connor, 2010). Of the new neurons produced, it is not known whether they integrate into functioning neural networks and thus contribute to recovery. Following asphyxia is rats, cell proliferation is increased in the hippocampus, but most of these cells are glia (Keilhoff, John, Langnaese, Schweizer, and Ebmeyer, 2010). Therefore, instead of using one or more of the numerous drugs that are known to increase neurogenesis (Jagasia, Song, Gage, and Lie, 2006), a more nuanced treatment strategy would be to alter the microenvironment of the injured regions of the brain in such a way that facilitates cell survival, differentiation, and integration. Due to the potential impact this could have in providing more extensive neural networks for adaptive recovery, this is another field of intense research activity.

By contrast, although pharmacologically tractable, it is not yet known whether increasing the rate of cell division and migration from the neurogenic zones of the brain could be hazardous. For example, it is not known whether neurogenesis is an adaptive response to epilepsy, or whether it is a causal factor (Bernardino, Ferreira, Cristovao, Sales, and Malva, 2005). A challenge for neurogenesis research in hypoxia ischemia therefore is the identification of targets for improvements in adaptive and not maladaptive plasticity.

CONCLUSIONS

Hypoxia ischemia of the neonate is a common problem that reduces quality of life and can be fatal. HI causes a range of symptoms that include cognitive impairment and motor dysfunction, which can be debilitating for a lifetime. Following the period of low oxygen and low nutrients, regular cellular processes are impaired resulting in an excitotoxic and ROS/RNS cascade that is highly toxic to both neurons and oligodendrocytes progenitor cells. Subsequent damage is followed by a long period of neuroinflammation that lasts for weeks and evens months after the initial insults and causes further damage through cytokine signaling as well as excitotoxicity and RNS/ROS compounds. Despite increasing knowledge of the details of these processes, no pharmacological interventions are currently in clinical use, and hypothermia is currently the only intervention used to prevent brain damage. A variety of animal models have been developed to study this pathology but treatments found to be efficacious in these models have not translated into the clinic. This suggests that a change in the way animal models are used is needed. When putative treatments are tested, several different species and both sexes should be used, the treatment should be administered at a clinically relevant time after neurological insult, animals should be assessed over a prolonged period of time including behavioral tests, and co-morbidities should be addressed. The latter include preterm birth, hypocapnia, hypoglycemia and, possibly, maternal infections. These shifts may already be happening, and treatments may yet emerge from existing knowledge of mechanisms of neuroprotection. Strategies that should not yet be dismissed include anti-inflammatory treatment, inhibition of CNS excitation, antioxidant therapy, and facilitated neuroplasticity. The challenge now for researchers is to determine optimal times, doses, and regimes for maximizing the potential benefits of these treatments whilst minimizing the risks.

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