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Evidence for Therapeutic Intervention in the Prevention of Cerebral Palsy: Hope from Animal Model Research

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Knowledge translation, as defined by the Canadian Institute of Health Research, is defined as the exchange, synthesis, and ethically sound application of knowledge—within a complex system of interactions among researchers and users—to accelerate the capture of the benefits of research through improved health, more effective services and products, and a strengthened healthcare system. The requirement for this to occur lies in the ability to continue to determine mechanistic actions at the molecular level, to understand how they fit at the in vitro and in vivo levels, and for disease states, to determine their safety, efficacy, and long-term potential at the preclinical animal model level. In this regard, particularly as it relates to long-term disabilities such as cerebral palsy that begin in utero, but only express their full effect in adulthood, animal models must be used to understand and rapidly evaluate mechanisms of injury and therapeutic interventions. In this review, we hope to provide the reader with a background of animal data upon which therapeutic interventions for the prevention and treatment of cerebral palsy, benefit this community, and increasingly do so in the future.
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Cerebral palsy (CP) is defined as a group of permanent disorders of the development of movement and posture, causing activity limitations, attributed to non-progressive disturbances that occurred in the developing fetal or infant brain…often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour—epilepsy and musculoskeletal problems.[1] CP occurs in 2-3 of 1000 term births, increasing dramatically by up to 10-fold to 22 of 1000 in infants born prematurely.[2-4] In a population-based Canadian study conducted on 243 children with CP, Shevell et al.[5,6] showed that half of the children are affected with either spastic quadriplegia (35%) or diplegia (21%), with as many as 31% displaying spastic hemiplegia, and the remaining being expressed as dyskinetic (7%), ataxic-hypotonic (4%), and other subtypes (2%). In addition to these motoric disabilities, it is now clearly evident that more than 45% of these children, particularly in the more severely affected groups have comorbidities including intellectual disability, behavioral abnormalities, sensory deficits, and seizures or epilepsy.[5,6] Estimates regarding the cost per child affected with CP exceed $1 million and clearly do not include the emotional, physical, and economic burden placed on families.[7] In the United States, the total amount required for children with CP was $11.5 billion in 2003.[8]

Animal research in the area of ischemia or hypoxia-ischemia has been ongoing in an aggressive fashion for a number of decades, but it has faced challenges when being translated from the animal model to the human.[9-11] Many pitfalls have been identified, particularly in the adult models of stroke, that include, but are not exclusive to, the following: whether the animal model truly reflects human stroke, the complex molecular nature of the evolution of stroke or hypoxia-ischemia, the lack of long-term follow-up in stroke models, the simplicity of the species (rodent, pig, or sheep) compared with the human counterpart, and the statistical approach to animal modeling of stroke and stroke outcome. In the case of the human newborn, and the causes of CP, this paradigm becomes even more complex.

CP is caused by a host of etiologies that result in a pattern of developmental disabilities, arising from the time of birth. In the context of the current series of papers, we are largely referring to CP that arises as the final common pathway of a cardiovascular or cerebrovascular compromise to the fetus, before delivery. Literature in the last 20 years has shifted our thinking to show that a hypoxic-ischemic insult to the brain most often arises during the course of pregnancy and is the result of an antepartum insult.[12-14] Indeed, these studies suggest that in 80%-90% of children who have CP, as the result of a hypoxic-ischemic event, it is likely due to antepartum insults, or the

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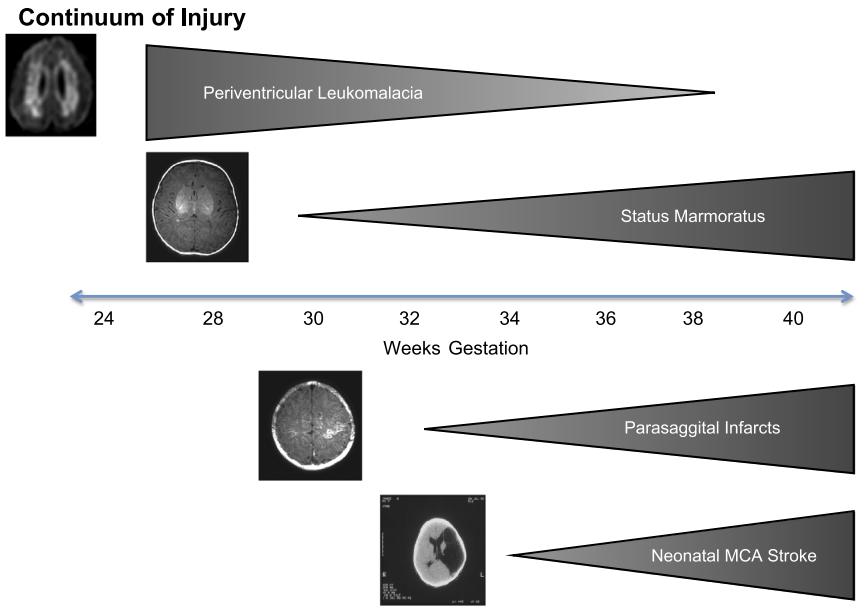

Figure 1 Evolution of brain injury based on gestational age. Periventricular leukomalacia during early third trimester 24-32 weeks). Status Marmoratus, parasagittal infarcts, and neonatal stroke occurring during late preterm and term gestation. (Color version of figure is available online.)

combination of an antepartum insult with a "secondary" intrapartum asphyxial challenge. Further complicating the therapeutic approach to CP is the fact that the immature brain is rapidly developing, such that vulnerable regions of the brain evolve, particularly during the last trimester to term birth. Hence, injuries in the premature baby, or those that appear to occur during the last trimester, are different from those that occur during the latter part of gestation (Fig. 1).

Patterns of brain injury in the immature brain, and hence the categorization of their CP, is dependent on the timing of injury during gestation, which in turn determines the intrinsic vulnerability of the vascular pattern, and the cell line affected. Therefore, insults that occur during the gestational ages of approximately 24-32 weeks result largely in 2 patterns of injury. The first is a perinatal stroke that occurs either due to a periventricular venous infarction or due to presumed perinatal ischemic stroke.[15-19] Both these latter insults are responsible for more than 90% of children with hemiplegic CP, which is in turn responsible for almost a third of all CP.[20] Most cases of spastic diplegia, quadriplegia, and those with dyskinetic CP arise predominantly from antepartum insults and are the result of a "global" insult to the fetal brain. Early studies in the rhesus monkey[21,22] as well as in the fetal sheep[23-25] showed that "near-complete" interruption of placental blood flow resulted in a deep gray matter or basal ganglia pattern of brain injury in the term newborn, whereas partial or incomplete repetitive ischemia caused lesions that were more cortical watershed in nature. These latter patterns have been clearly verified in humans by neuroimaging.[26,27,28]

In the premature infant, patterns of brain injury are dependent on both the paucity of vascular supply to the regions of the brain adjacent to the lateral ventricles (periventricular)[29] and the intrinsic vulnerability of the preoligodendrocyte.[30,31] Therefore, with the reduction in blood flow to the periventricular region, the immature oligodendrocyte appears targeted to the effects of the surrounding neurotoxins (glutamate) because of the inability of immature astrocytes to fully resorb the toxin.[32] In addition, the developing glial infrastructure does not yet have the capacity to fully provide antioxidant neuroprotection.[33-35] The pathophysiology relies on a combination of reduced cerebral blood flow whether during the preterm period, leading to white matter injury, or term infant, producing global injury, depletion of energy stores, cell-specific sensitivity such as in the preoligodendrocyte, and lastly, with or without the presence of an infection resulting in an inflammatory response. Thus, it is important to develop an animal model that is truly representative of the pathophysiology leading to CP. Ultimately, the goals for developing animal models of disease (CP) are for improving the outcomes and lives of the children and families we serve.

Given the variable patterns of brain injury that the fetal and neonatal brain are susceptible to and the complex interplay between the developing brain and its regional vulnerability to injury, it is not surprising that achieving therapeutic interventions is often seen as a moving target. In spite of this, at the cellular level, certain basic pathways are recognized as occurring, to greater or lesser degrees, in all types of the previously described injury subtypes. Hence, it is clearly recognized that with the curtailment of blood flow and the delivery of oxygen and glucose to the brain, cellular energy failure results secondary to the depletion of phosphate stores. This, in turn causes disruption of the Na^+K^+-ATPase pump, resulting in the release and accumulation of the excitatory amino acid glutamate into the synaptic cleft. The latter disrupts the cytoskeleton leading to a loss of membrane integrity and cellular necrosis. Furthermore, increases in glutamate and loss of the membrane pump result in an increase in

intracellular calcium, leading to a perpetuating cycle of free radical production and a prolonged inflammatory response. Ultimately, both damage the DNA and cell membrane integrity via lipid peroxidation and result in a protracted cell death by apoptosis.[36-38]

In addition to the aforementioned complexities, it has further come to light that fetal infection or inflammation contributes significantly to the process of fetal or newborn brain injury. Verma et al.[39] demonstrated a significant correlation between mothers with chorioamnionitis and low-birthweight babies affected with PVL. Wheater et al.[40] further demonstrated that the risk of development of CP was 4 times greater in infants who were exposed to mothers with an infection compared with those that were not. Furthermore, Wu and colleagues showed chorioamnionitis as an independent risk factor in both preterm and term infants leading to development of CP, supporting the role of inflammation as a significant risk factor associated with brain injury.[41,42] More recently, Miller's group demonstrated a close correlation between the clinical and biochemical signs of fetal inflammation and outcome, suggesting that the risk of CP increased with mounting evidence of infection.[43,44]

Despite the complexity and challenges in developing therapies for perinatal brain injury resulting from a hypoxic-ischemic insult, recent years have demonstrated significant success and therapeutic advancement. The role of animal models and the provision of preclinical evidence in this journey are substantial, with the future holding even greater promise.

Animal Models

Early studies utilized monkeys[45] and sheep[24,25,46] in the depiction of perinatal brain injury, in both the term and preterm fetus. These models helped to outline the pattern of injury and set the stage for understanding the differences between injury in the premature and mature infant. The rising expense of these models led to the development of smaller animal models, and the focus of most studies was on rodents. Vannucci and colleagues developed the immature rodent model as a modification of the Levine[47] adult stroke preparation.[48,49] In this model, a 7-postnatal day, nonprecocial, rat pup is used as a correlate to the late preterm and term infant (36-40 weeks gestation), though others have argued that a more accurate age would be at 10-postnatal days, based on the maturity of the brain amino acid and enzyme profiles.[50] The common carotid artery is ligated permanently, and the animal is exposed to hypoxia (usually 8%) for varying durations of 90-180 minutes. The result is a focal area of injury ranging from selective neuronal necrosis to frank infarction in the area of the middle cerebral artery, its severity is dependent on the duration of accompanying hypoxia. Although appearing as a perinatal ischemic stroke, the pathophysiology of injury has been well defined, and as such, has been recognized as being strongly representative of the cellular mechanisms underlying perinatal hypoxic-ischemic brain injury.[51-58] In recent years, several other models have been developed that more specifically depict a "global ischemic" insult and hence, presumably, are more representative of the diplegic and quadriplegic spastic cerebral palsies. In particular, Derrick et al. (2007) have developed a model in the rabbit kit that causes a global placental ischemic insult by occluding the descending aorta for a brief period and then allowing reperfusion. Phenotypically, the rabbit kits exhibit spastic fore and hind limb features. The challenges with this model are the precocial nature of the rabbit[59,60] and the inability to follow up these animals for long term. Hence the difficulty in evaluating therapeutic efficacy from a long-term recovery perspective.[61] Studies by the Lane group, and adapted by our own group, have recently utilized a model of bilateral uterine ligation thereby interrupting placental blood flow. The result is a model of placental insufficiency, resulting in growth retardation in the newborn rat pup and fetal brain abnormalities in myelination and hippocampal cell count. Behaviorally, the animals show signs of delays in early developmental reflexes as well as permanent abnormalities of motor and cognition.[62-64] Still others have developed models that have incorporated the role of fetal inflammation or chorioamnionitis as a significant risk factor in the development of CP, the treatment of which may provide a means of prevention.[65,66] Larger animal models developed for the purposes of investigating perinatal hypoxic-ischemic insults include the piglet and the sheep, both of which are actively utilized.[67,68] Not surprisingly, these latter models provide a tremendous window into the pathophysiology and mechanisms of brain injury, but are less well suited for long-term recovery. As in most animal models of human-related diseases, there is none that is ideal, and rather our knowledge and information must grow from the application of the right model for the circumstances.[69,70]

From the perspective of the utilization of animal models as a means of developing preclinical data to move forward with clinical trials, several criteria would need to ideally be met. Models of human disease should reflect the human with respect to the following: (1) development (anatomically and biochemically), (2) mechanisms of injury, and (3) pathologic and long-term behavioral phenotype. Few animal models are able to meet all of these criteria. Nonetheless, in the world of perinatal brain injury, and as it relates to the development of therapeutic interventions, the rodent model by Rice et al.[48] has provided the foundation for much of the preclinical data to date.

Evidence from Animal Models for Therapeutic Intervention

The approach to therapeutic interventions has largely focused on rescue therapies. That is, therapies that target damaged tissue in recovery and are administered in the time frame shortly after the insult has occurred. Moreover, these trials have further focused on the term infant with evidence of perinatal asphyxia, with little current work addressing the needs of the preterm. However, the newborn brain injury is often difficult to time, it most often happens before labor and delivery and without an identified etiology.[71] Hence, to address this

multifaceted potential for injury, the more recent thinking has broadened and is now inclusive of interventions that include the following: (1) prevention, (2) rescue, (3) rehabilitation, and (4) regeneration. Clearly, animal models play an important role in the development of all 4 of these approaches.

The following paragraphs address some key aspects of how evidence from animal model preclinical work has addressed, and in some cases moved forward to, successful clinical trials and alterations of standard of care. Recent publications have further outlined several important therapeutic advances that have the potential for advancement to clinical trial and we would recommend them as further review.[72,73] Though not meant to be an exhaustive review, we hope to highlight important progress that has been made in the field of animal model research and point out the tremendous and growing role this arena has to play in future endeavors regarding the treatment of perinatal brain injury.

Prevention

With the increasing incidence of premature birth and the recognition that most of the risk factors resulting in CP occur before labor and delivery, a new focus for therapeutic intervention has been treatment of the pregnant mother in an attempt to prevent injury from occurring. The purpose of these approaches is simple and similar to the approach taken by the use of folate for the prevention of spinal cord dysraphism. Hence, the concept is to enhance the endogenous capability of the fetus to withstand or circumvent an insult, often owing to the fact that the immature brain is relatively deficient in enzymes or enzyme systems that reduce injury secondary to inflammatory or oxidant-mediated cell death.

Tetrahydrobiopterin (BH_4) is a biogenic amine normally found in small concentrations in the fetal brain. It acts as a cofactor in the production of dopamine and nitric oxide synthase and is therefore believed to have a role in the prevention of oxidant stress.[74,75] Moreover, the addition of BH_4, or its precursor, enhanced survival in nigral slice cell cultures. Supplementing the diet of pregnant rabbits in late gestation with BH_4 or its precursor, sepiapterin, prevented the behavioral deficits seen in newborn rabbit kits following an insult causing prolonged placental insufficiency.[76]

Others have looked at the constituents of food. Resveratrol (RVT) is a polyphenol found in a number of foods, including red wine, peanuts, grapes, and others. Karalis et al. (2011) utilized the Vannucci 7-day rat pup model of unilateral common carotid artery ligation and treated their experimental group with RVT. They showed a significant reduction in behavioral deficits, as well as neuropathologic damage of both gray and white matter structures in the treatment group compared with controls.[77] Holtzman and colleagues showed that high and medium doses of RVT provided by intraperitoneal injections into rat pups before a hypoxic-ischemic injury reduced the brain damage associated with that injury, but that RVT injection after the injury had no beneficial effect.[78,79] Others have determined the effects of grape seed extract on the functional and pathologic outcomes of a hypoxic-ischemic insult in the 7-postnatal-day rat pup. Feng et al.[80] intraperitoneally injected 50 mg/kg of grape seed extract before induction of hypoxia and then provided additional doses following hypoxia in a multidose regime. In a second study, the authors looked at the effects of dosing at 1 and 3 hours following the hypoxic-ischemic insult.[81] In both study paradigms, the authors found significant improvement in outcome, behaviorally and pathologically. Moreover, the use of grape seed extract in this manner reduced evidence of lipid peroxidation and hence, likely also decreased oxidant stress. Pomegranate juice, another polyphenol, has also been examined as a preventive antepartum agent in the newborn rodent model. In a series of studies, pomegranate juice was added to the water of dams of rat pups who then were fed by the dam. At the 7-postnatal day, a typical hypoxia-ischemia injury was induced in the rat pups and neuropathologic assessment was done approximately 2 weeks later. As with other studies, the pomegranate juice provided before the injury, conferred neuroprotection and there was a reduction in apoptotic cell death, as determined by measurement of caspase-3 and calpain.

Our own laboratory has also determined the effects of natural health products in the form of broccoli sprouts. In early studies, pregnant rodent dams were fed broccoli sprouts at a dose of 200 mg/kg per day during the last trimester of pregnancy and throughout the period of suckling to postnatal day 14. Rodent dams underwent bilateral uterine artery ligation (BUAL) on E20, with spontaneous vaginal delivery occurring on E23. BUAL in this fashion produces placental insufficiency and fetal growth restriction resulting in injury to the hippocampus and white matter as shown in our lab (unpublished data) and by Lane and colleagues.[63,64] During the course of development, from PD3-PD21, early reflex behaviors were determined, after which the pups were sacrificed and assessed neuropathologically for brain injury. The data clearly indicate that the dietary supplementation of pregnant dams with broccoli sprouts prevents injury and improves functional outcome. Others have determined that the presumed mechanism of this beneficial effect is through the enhancement of phase II enzymes, the promotion of oxidant scavengers, and a powerful anti-inflammatory effect.[82-84]

The BUAL model has also been utilized by the Watanbe group of investigators to determine the potential benefit of melatonin as a prophylactic supplement given during pregnancy.[85] Melatonin is a naturally occurring pituitary hormone that, in previous experiments from this lab, has shown to be a potent antioxidant. Pregnant dams were fed a solution of 20 ug/ml of melatonin dissolved in water throughout their pregnancy. On E16, the uterine arteries were ligated for 30 minutes and then reperfusion was allowed to occur. The pups were delivered vaginally and killed on PD1. Examination of mitochondrial respiration, hippocampal cell death, and Thiobarbituric-Acid Reaction, as an indication of lipid peroxidation, revealed a significant beneficial effect of the melatonin supplementation. Unfortunately, long-term studies have not been accomplished to determine the permanence of this benefit.

Rescue

Rescue therapy refers to the implementation of an intervention in the immediate aftermath of an injury. Regarding this, one cannot discuss rescue therapy without the recognition that mild to moderate hypothermia has become the standard of care in the intensive care nurseries of the developed world. A number of systematic reviews on the subject of hypothermia have consistently shown a beneficial effect[86,87] under the following conditions and eligibility: (1) A diagnosis of acute perinatal asphyxia as defined clinically and physiologically by the presence of a neonatal encephalopathy and the presence of a cord pH <7.1; (2) term infant of 36 weeks gestation or greater; (3) hypothermia to 33.5°C within 6 hours of birth and generally lasting for 72 hours, and (4) the absence of complicating features such as congenital anomalies, sepsis, and cerebral malformations. Both whole-body cooling and selective head cooling have been shown to be effective, though controversy remains regarding whether there may be some benefit of one over the other, depending on the region of injury. Hence, some speculation exists that whole-body cooling may provide greater benefit for an acute near-total asphyxia event, resulting in basal ganglia injury, whereas selective head cooling may provide greater protection to newborns exposed to a prolonged partial insult, with a predominance of cortical injury. Results have further indicated that the benefit is larger in, though not exclusively confined to, infants with a moderate degree of encephalopathy, whereas those with a severe encephalopathy tend to show less benefit. In a study on childhood outcomes after hypothermia, it was shown that at longer-term follow-up at 6-7 years of age of the 97 children in the hypothermia group and the 93 children in the control group, death or an IQ score below 70 was observed in 46 (47%) and 58 (62%) children, respectively ($P = 0.06$); death was observed in 27 (28%) and 41 (44%), respectively ($P = 0.04$); and death or severe disability was observed in 38 (41%) and 53 (60%), respectively ($P = 0.03$). Other outcome data indicated that moderate or severe disability was observed in 35% and 38%, respectively ($P = 0.87$). Attention-executive dysfunction occurred in 4% and 13%, respectively, of children receiving hypothermia and those receiving usual care ($P = 0.19$), and visuospatial dysfunction occurred in 4% and 3%, respectively ($P = 0.80$). They concluded that the rate of the combined end point of death or an IQ score of less than 70 at 6-7 years of age was lower among children undergoing whole-body hypothermia than among those undergoing usual care, but the differences were not significant. However, hypothermia resulted in lower death rates and did not increase rates of severe disability among survivors.[88]

Preclinical animal work was at the heart of the rapid translation of postischemic hypothermia as a neuroprotectant to clinical trial and subsequently to standard of care in most neonatal intensive care units (NICUs). Work done by Richard S. Young was perhaps the first to show the benefits of mild hypothermia in the newborn rat pup.[89] Later studies in adult and newborn stroke models of intraischemic hypothermia showed the powerful effect even a 2°C-3°C decrease in body temperature had on preserving the brain.[90,91] Later studies were of course extended to postischemic hypothermia, and these too confirmed its benefit. [92,93] Subsequent clinical trials developed rapidly, throughout N. America, Europe, and Australia/New Zealand, which led to the implementation of this therapy as standard.

Current studies in the field of rescue therapy must include hypothermia as a control given its prevalence in the clinical world. Ongoing animal studies have now focused on questions related to how long one can delay hypothermia and still maintain a beneficial effect, and the concept of hypothermia plus the addition of pharmaceutical additives to enhance current benefits. With respect to the former, Sirimanne et al.[94] using their instrumented sheep model showed that delaying hypothermia for up to 5.5 hours following umbilical cord compression was only partially effective in protecting the brain from injury and that there may be some regional variability in this regard. Delaying hypothermia until after seizures occurred was not at all protective when compared with normothermic controls. In the Vannucci rat model of postischemic hypothermia, delayed cooling for up to 2 hours, but not up to 5 hours, was effective in providing neuroprotection, as evidenced by the pathologic scoring.[95] These findings were essentially confirmed by Thoresen et al.[96] in the piglet and rat models.

A number of studies have recently provided preclinical evidence for the combination of hypothermia with other pharmaceutical interventions as additive therapy. Several anticonvulsant medications have been studied. When phenobarbital was given intraperitoneally to 7-day PD rat pups, 15 minutes after a hypoxic-ischemic insult, and followed by postischemic hypothermia, the combined effects of phenobarbital and hypothermia were greater than either one alone, in both short- and long-term functional and pathologic outcomes.[97] A retrospective study of newborns who had experienced a perinatal asphyxia event and received both phenobarbital and hypothermia during the course of their recovery did not, however, confirm the additive beneficial effects of phenobarbital.[98] This same group also studied the role of topiramate in conjunction with hypothermia. Neither delayed postischemic hypothermia for 3 hours, nor was topiramate alone neuroprotective. The combination, however, improved pathologic and behavioral outcomes when compared with control.[99] Whether the effect of the medication is because of an effect on the underlying pathogenetic mechanisms of injury in combination with hypothermia or simply the effect of treating seizures remains a question. Our laboratory has shown that seizures complicating a hypoxic-ischemic injury can exacerbate that injury.[100] We have also shown that these seizures are associated with relative hyperthermia, and preventing this increase in temperature, by cooling, results in an improved long-term pathologic outcome.[101] The findings certainly suggest a role for seizure control and substantiate the logic of combining anticonvulsant therapy with hypothermia. The question remains however, as to whether, clinically, there will be an additive effect.

Other medications have been added to postischemic hypothermia in preclinical animal models, all using the 7-day

postnatal Vannucci model.[102] N-Acetylcysteine, a potent anti-inflammatory agent, has been shown to be protective of the newborn brain, and in combination with hypothermia, it reduces infarct volume and damage to white matter as evidenced by quantitative measurement of myelin basic protein. Unfortunately, this study only looked at the outcome at 48 hours and therefore is not long term enough to support clinical trials.[103] Most prominent among those is the work of Thoresen's group and the combination of Xenon with hypothermia for additional neuroprotection.[104] In their studies, both immediate or delayed use of Xenon gas, as an anesthetic, in combination with hypothermia for 3 hours, following a hypoxic-ischemic insult in the 7-day rat pup showed significantly improved outcomes over either treatment alone. Xenon has been shown to be a potent NMDA blocker and may also have additional beneficial effects, but is quite expensive and requires the presence of an anesthetist under current standards. Nonetheless, studies by this group have shown that short term use of Xenon is safe, and therefore may obviate these potential obstructions. Clinical trials are currently underway in Europe (personal communication).

Finally, there has been very strong preclinical evidence regarding the neuroprotective effects of erythropoietin (EPO) in the newborn. EPO stimulates red blood cell production and has been used for this purpose in many NICUs. Moreover, animal studies in the previously described rodent models of hypoxia-ischemia have shown its potential as an agent that can reduce brain damage. Unfortunately, the recent publications by 2 laboratories, which combined hypothermia with EPO, did not show benefit.[105,106]

Rehabilitation

Constraint-induced therapy is increasingly being utilized following injury for the benefit of improved upper limb mobility and has been reviewed by Andersen et al.[111] Using the unilateral ligation model in mice, Rha et al. (2011) subjected the animals to either control recovery, environmental-enriched recovery, or a combination of constraint and environmental enrichment. These authors found improved functional outcome and neurogenesis in the subplate neurons, 4 weeks after recovery in the group receiving the combination therapy, compared with either therapy alone. This is one of the only studies to have shown an improvement using constraint in an animal model of newborn stroke.[107]

Yang et al.[112] have further elucidated on the possibilities for rehabilitation of lower limb function. In our own laboratory, we have shown that environmental enrichment (animal physiotherapy) does provide good preclinical evidence for long-term, sustainable beneficial outcomes. Standard and enriched environments and their effects on brain recovery have been studied. Pathologically, enriched housing has been shown to significantly reduce damage in the thalamus of rats undergoing hypoxic-ischemic insults compared with controls. Interestingly, this reduction in thalamic damage is only evident in postnatal day-10 rat pups, equivalent to a full-term infant. Postnatal day-63 and -180 rats, equivalent to juveniles and adults, do not show a difference in brain injury, specifically within the thalamus, between standard and enriched housing. These results suggest that age is a variable that needs to be considered when finding an optimal therapeutic window for rehabilitation therapies.[108,109] The pathologic findings also correlated with improved functional outcomes. However, regarding this, we found that environmental enrichment or rehabilitation had a greater beneficial effect on males than on females. The findings certainly suggest that rehabilitation is a significant tool in recovery from hypoxia-ischemia and that gender is a variable that should be taken into account as a potential confounder.

Conclusion

Innovative therapies in the prevention and treatment of CP are increasingly becoming a possibility. Indeed, postischemic hypothermia has rapidly translated from the bench to the bedside in the last decade and has significantly improved the outcome of many children who may have evolved to develop CP. So much so that it is now the mainstay of therapy in most tertiary care NICUs in America, Europe, and Australia/New Zealand. Indeed, work is being done to expand the utilization of this transformative therapy by producing inexpensive methodologies for the induction of hypothermia in less-developed countries.[110] The ability to translate therapies to clinical trial and to transfer to practice is reliant on the production of preclinical evidence in the animal model of efficacy, safety, and permanence of effect. Particularly when it comes to therapies in children, the use of animal models are essential for the rapid turn around and determination of therapies that in the human child would often take decades to determine and may be seen as unethical in the absence of such preclinical data.

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