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Animal Models and Treatments for Cerebral Palsy

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Abstract
Cerebral Palsy (CP) is the most common pediatric neurodevelopmental physical disability with a prevalence of 2-5 cases for every 1000 live births. CP results from injury to the developing brain that occurs at the time surrounding birth. Although the severity and extent of CP symptoms vary among patients, all develop motor and posture disturbances which limit their activity. There is no known cure for CP and the discovery of safe and effective therapies remains an unmet goal that requires testing in animal models that best recapitulate the human disease. Due to the many developmental and functional differences between the neonatal brain and the adult brain, the ability to extrapolate adult data to the neonatal condition is very limited. For this reason, it is incumbent on scientists in the field of neonatal brain injury to address the questions of therapeutic efficacy of an array of potential therapies in a developmentally appropriate model. Toward that end, several of the established CP models have been adapted to different species and different ages. More recently a number of new models have also been introduced. Some of these models have been critical in identifying treatments that alleviate symptoms. This review gives a brief background on CP and goes on to describe animal models and potential treatments for the disease.

ABBREVIATIONS
BoNT-A: Botulinum Toxin A; CP: Cerebral Palsy; HI: Hypoxic-Ischemic; NMES: Bilateral Masseter and Temporalis Neromuscular Electrical stimulation; EMG: Surface Electromyography; PSG: Polysonomography

INTRODUCTION
Cerebral Palsy (CP) is the most common pediatric neurodevelopmental physical disability with the prevalence being reported as 2-5 cases for every 1000 live births. CP refers to a group of neurological disorders characterized by abnormal motor development and coordination. CP symptoms fall on a continuum of motor dysfunction, ranging from slight clumsiness at the mild end of the spectrum to impairments so severe that they render coordinated movement virtually impossible at the other end of the spectrum. Early signs can be detected before a child reaches 3 years of age. There are several CP sub-types based on the presentation of major symptoms. Ataxic CP is assigned to patients with poor coordination whereas spastic CP is used to describe patients with stiff or tight muscles and exaggerated reflexes. Athetoid CP refers to patients with continuous movement of their extremities or contortions of their body. In addition to motor impairments CP patients often present with cognitive and speech dysfunction. Patients may also display epilepsy, hearing or vision loss, reduced intellectual function, deficits in communication, and behavioral problems. Patients may also experience secondary musculoskeletal pain, and chewing and sleep disorders. The secondary alterations progress with age and include a reduced range of motion, increased joint stiffness and muscle weakness. Spastic CP is the most common form of the disease [1-5].

Although there are many interacting pathways, as well as genetics, that play a role in the disease, CP often results from injury to the developing brain that occurs either antenatal, perinatal or postnatal. Risk factors include preterm birth, infections during pregnancy, exposure to certain chemicals during pregnancy, and head trauma during birth. The most common causes of CP have been identified as hypoxia/ischemia (HI) and inflammation and consequently these two factors have been the basis for developing animal models of CP [1-5].

Animal Models of CP
Animal models are commonly used to investigate the various pathophysiological mechanisms underlying the development of diseases. Although models do not definitely recapitulate all aspects of the human disease they are critical for pre-clinical testing of potential therapies prior to translation to humans. Animal models of CP have been developed in a variety of species including mice, rats, rabbits, sheep and non-human primates. The most commonly used models include models of hypoperfusion,
and models using either infectious agents, bacterial products, or excitotoxic insults. A critical aspect for the modeling of CP is the timing of the brain insult as there are species differences in the timing of key brain maturation events. It has been reported that in rodents the period between embryonic day 17 (E17) and postnatal day 7-10 (P7-10) replicates many features seen in the third trimester in humans with P7-10 being roughly equivalent to a term human infant [1,6,7]. Developmental variations in neuroanatomy, cell proliferation, synaptogenesis, myelination and immune responses can affect the mechanism of injury as well as treatment effect [see [6] for an extensive review of brain maturation stages in the context of hypoxia-ischemia].

Animal models of stroke show many features of CP and have been used to study the disease. These models have reproduced the hypoxic-ischemic (HI) conditions, the apoptotic-necrotic pattern of white and gray matter damage and the neuromotor impairments. In the Rice-Vannucci HI model, P7 rats undergo unilateral ligation of the carotid artery followed by exposure to 8% oxygen hypoxic air. Although these animals are a model of neonatal stroke, they have also been used to study CP since the animals show brain damage, hypoxia/ischemia, inflammation and motor and cognitive deficits. This model can also be used to develop potential treatments for stroke and CP [1,5].

Many modifications of the Rice-Vannucci HI model have been made to try to get a model that better recapitulates features of human CP. Some of these modifications included varying the day of the animals used and the example of hypoxic conditions. For instance, one study used P1 rats since this time period is more like the human last trimester of gestation. There has also been research on which strains are more susceptible to this model. Other studies used P5 Wistar rats with no hypoxic conditions. The aim was to produce mostly white matter lesions which was accomplished in some studies but not others. In the Rice-Vannucci model, there is extensive damage to the gray matter. Another model reduced the hypoxic conditions to 6% oxygen in an attempt to produce less severe brain damage. A rodent model used LPS 4 hours before unilateral occlusion to study interactions between HI and infection/inflammation. More severe brain injury was noted [5,8].

There have also been mouse models produced using hypoxia-ischemia that have similar features to the rats – namely, both neurological and behavioral deficits that replicates many features of CP. When post-natal day 3 (P3) mice were used and analyzed 8 weeks after HI, neurobehavioral deficits were documented on the Morris water maze test which measures spatial learning and memory and the open field test which measures gross motor skills and cerebral integrity. There was also damage to the sensorimotor cortex, the striatum and the hippocampus as documented by MRI and histopathological analyses [7].

There are several larger animal models besides mice and rats that might be more applicable to humans. For instance, fetal sheep have shown to have advantages over rodent models. Repeated measurements can be performed in utero and they exhibit similarities to the third trimester in humans that can be accurately replicated. In rabbits, in vivo uterine ischemia by inflation of an aortic balloon at a level proximal to uterine arteries resulted in hypertonia and neurobehavioral findings in P1 rabbits mimicking those found in CP [9]. In pigtailed macaques CP was modeled using an acute perinatal asphyxia model. Occlusion of the umbilical cord for 12-18 minutes before birth induced hypoxic-ischemic conditions resulting in neurodevelopmental and long term physical and cognitive deficits associated with CP [10,11]. Overall, though, these models are important to develop early treatments and to determine if there are factors that worsen the condition [1].

Another way to try to replicate human CP is to introduce inflammatory agents in animal models since these agents play a role in brain injury. However, it has been found that only some aspects of the human condition are replicated by this approach. Additionally, these studies were performed at any early point in the gestation of the animal models and do not correlate with the timing of the development of CP. It has also been found in the animal models that gestational stress worsens the condition [1].

Treatments

There is no present approach to totally prevent or cure CP but many treatments have been developed that can help reduce its impact on the patient’s quality of life. As with many diseases, it is best that treatments are initiated early in the course of the disease. This is especially true with a progressive disease such as CP with limited windows for interventions. Current treatments include physical and occupational therapy and device or equipment to help with mobility and coordination, medications to alleviate motor symptoms and manage pain, and surgery to correct bone and joint deformities and improve posture and walking quality. Medications are also prescribed to control epilepsy, sleep disturbances, and other secondary symptoms such as emotional issues, and eating difficulties.

A number of treatment options aim to improve hand movement of children with the disease. These include a variety of activity based interventions. However, the effectiveness of these activities are unclear [4]. Another study examined whether adolescents with CP display deficits in locomotor adaptation. They used a split-belt treadmill where each leg is exposed to a different speed. The patient must then use locomotor adaptation to change the velocity of each leg. Following training, the CP patients were able to improve both their variability and adaptation indicating that they are able to learn this task. It is also suggests that repetitive training during rehabilitation could lead to a reduction in variability and enhanced motor skill learning [12].

One major motor deficit found in CP is abnormal gait [13,14]. Ways to improve these deficiencies are orthopedic surgery, spasticity management, physical therapy and orthotics. The choice of therapy or the correct combination is individualized based on the condition of the child. One study presenting a protocol for analyzing the effectiveness of instrumented gait analysis combined with traditional therapies compared with the traditional therapies alone. The results from these tests could then be used to alter the therapies that the patients receive [13]. There is some discussion of whether assessing gait deficiencies with a treadmill correlates with normal daily walking abilities. One study found that in both CP adolescents and in typical developing adolescents, matched on age, height and body mass, there were similar gait changes but that the CP adolescents had
a reduced adaptive capacity to absorb and decelerate speed. The treadmill does offer advantages, though, in that it is safe and the same speeds can be repeated. One goal of therapy is to try to increase the independence of people with CP by improving their walking and treadmill rehabilitation is frequently used [14].

Botulinum toxin A (BoNT-A) has been tested in children with CP. Although past studies questioned the safety of using this toxin, more current studies show that when the toxin was delivered intramuscularly, there were no adverse effects in those children receiving the toxin compared to those that received a sham injection. These injections are used to decrease spasticity and pain in the children since the botulinum toxin interferes with cholinergic transmission at the neuromuscular junction [15]. Other studies showed that when BoNT-A was injected into overactive muscles of the upper extremities of children with CP, there were improvements in the quality and range of movements which could then enhance the effects of other upper extremity therapies and activity performance [4,16].

Since chewing functions and sleep have been reported to be deficient in CP and these deficiencies can affect overall health, one study looked at ways to improve these deficits. Bilateral masseter and temporalis neuromuscular stimulation (NMES) was performed on patients with CP. This therapy acts to induce action potentials in motor nerves. The effectiveness was evaluated using surface electromyography (EMG) to detect muscle function and polysomnography (PSG) to detect sleep patterns. The results showed an improvement in both tests [2].

One group of investigators published a protocol for a combined approach using multidisciplinary care alone or in combination with rigid upper limb orthoses whose goal would be to maintain the muscles of the wrists, fingers and thumbs [4]. Another group of investigators reported on a protocol combining resistance training with functional anaerobic training. The resistance training focused on exercises involving the calf muscles which a muscle is commonly affected in CP. Similar tests have been previously reported but there have been questions about the lasting benefits of these tests. The goal of the anaerobic training is to for the neuromuscular system to perform maximum work in a short period of time. The hypothesis for this combined approach is that there will be gains in both strength of muscles and functional performance [3]. Another therapy that might be used as part of a combination therapy is whole body vibration which uses oscillatory motion. A META analysis study was performed to compare CP patients treated with whole body vibration to either those who received exercise as treatments or a control group. The cumulative data from 6 studies with 176 patients showed the CP patients who received the whole body vibration showed an improvement in gait speed, gross motor function and femur bone density [18].

Prevention and early rescue interventions for high risks subjects have also been introduced to reduce the possibility and severity of CP. Many of these therapies were first tested in animal models. When babies are born prematurely, they are at an increased risk of developing neurologic impairments, including CP, compared to babies who were born at full term. Preventive measures are recommended to the pregnant mother to decrease the risk of premature delivery and increase the ability of the fetus to tolerate injury. Common measures include a battery of prenatal care such as reducing exposure to infection or other toxic agents and improving nutrition. When preterm labor is inevitable prenatal steroids and magnesium sulfate are often prescribed and can help protect babies from the brain injury that leads to CP [25]. Previous studies on the effectiveness of using magnesium sulfate to prevent CP have been inconclusive. In addition, these studies raised some concerns about the safety of the drug for the mother. A META analysis was performed to integrate past findings on the neuroprotective effects of magnesium sulfate and the safety of the drug on both the fetus and mother. The conclusion was that when magnesium sulfate was administered to mothers who were at high risk for having a preterm baby, there was a reduced risk of having an infant with moderate to severe CP. There were no side effects of the drug on the infant. Although there were some for the mother, they could be reduced by lowering the dose of the drug [17]. Due to recent medical advances, premature babies have been able to live longer and hence require more postnatal and developmental care.

Rescue therapies have been developed to use during the immediate window following injury. For example, in both rodents and non-human primates, the effect of HI can be mitigated by mild hypothermia [19-22]. Cooling is currently used to treat newborn babies who have suffered a brain injury due to lack of oxygen around the time of birth [23]. In rats, postnatal daily intraperitoneal administration of the neuroprotective agent, erythropoietin, improved GABA A receptor subunit dysregulation and motor deficits induced by HI [24]. In a rhesus macaque HI model, a combined therapy approach using erythropoietin and hypothermia resulted in either erythropoietin or hypothermia alone [21]. Hypothermia is now standard care for HI neonate and clinical trials of erythropoietin are ongoing [25,26].

Stem cell treatment should be considered a potential therapy for CP since it may influence inflammation and lead to the development of new oligodendrocytes [1,5]. It has been used with some success in spinal cord injury models and in a demyelinated animal model. However, similar to the use of this therapy with other diseases, many factors need to be optimized including safety, timing and dose. As has also been seen with other diseases, an effective treatment for CP most probably will need to be a combined approach as one difficulty noted as to potential success of the therapies is that CP is a very heterogeneous disease [5].

Other therapies that have been shown to have limited success are upper limb surgery and removable orthoses [4], glutamate antagonists (blocks NMDA receptor channels), and vasoactive intestinal polypeptide and melatonin which both act by influencing secondary messenger systems [1].

**DISCUSSION AND CONCLUSION**

Despite the many therapies available to alleviate symptoms of CP there remains an unmet need for better preventive and early rescue therapies. Various animal models are being developed that mimic some aspects of the disease. Small animal models are best at reproducing the molecular events and large animals might be closer anatomically to model the human form of the disease.

Investigators should keep in mind, though, that there are
limitations in using these animal models. Most prominently is the difference in the size and complexity of the brains of these animals compared to humans and the rate of maturation. Because the size of the cerebral cortex is greatly different in humans compared to the animal models, there is a big difference in the period of neurogenesis. There are also sex differences in both human and animal models. There are differences in the corticospinal anatomy between the animals and humans which are important when analyzing motor impairments which is prominent in the disease. The animal models also have less subcortical white matter and analyze motor impairments which is prominent in the disease. The animal models also have less subcortical white matter and analyzing motor impairments which is prominent in the disease.

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