A Model of Cerebral Palsy From Fetal Hypoxia-Ischemia

Matthew Derrick, MBBS; Alexander Drobyshevsky, PhD; Xinhai Ji, MD; Sidhartha Tan, MD

Abstract—Disorders of the maternal-placental-fetal unit often result in fetal brain injury, which in turn results in one of the highest burdens of disease, because of the lifelong consequences and cost to society. Investigating hypoxia-ischemia in the perinatal period requires the factoring of timing of the insult, determination of end-points, taking into account the innate development, plasticity, and enhanced recovery. Prenatal hypoxia-ischemia is believed to account for a majority of cerebral palsy cases. We have modeled sustained and repetitive hypoxia-ischemia in the pregnant rabbit in utero to mimic the insults of abruptio placent and labor, respectively. Rabbits have many advantages over other animal species; principally, their motor development is in the perinatal period, akin to humans. Sustained hypoxia-ischemia at 70% (E22) and 79% (E25) caused stillbirths and multiple deficits in the postnatal survivors. The deficits included impairment in multiple tests of spontaneous locomotion, reflex motor activity, motor responses to olfactory stimuli, and the coordination of suck and swallow. Hypertonia was observed in the E22 and E25 survivors and persisted for at least 11 days. Noninvasive imaging using MRI suggests that white matter injury in the internal capsule could explain some of the hypertonia. Further investigation is underway in other vulnerable regions such as the basal ganglia, thalamus and brain stem, and development of other noninvasive determinants of motor deficits. For the first time critical mechanistic pathways can be tested in a clinically relevant animal model of cerebral palsy. (Stroke. 2007;38[part 2]:731-735.)

Key Words: behavior ▪ brain ▪ dystonia ▪ fetal anoxia ▪ hypoxia ▪ infant ▪ ischemia ▪ magnetic resonance imaging ▪ newborn ▪ placental insufficiency ▪ premature ▪ uterus

Cerebral palsy is a nonprogressive disorder of the developing brain principally affecting the motor system. Cerebral palsy affects 2 to 3 per 1000 newborns, with a conservative estimate of its impact on society being ≈$5 billion per year. Cerebral palsy can be associated with epilepsy and abnormalities of speech, vision, and intellect. The impact of diseases affecting the newborn are much higher than diseases that affect the elderly because of the burden of disease when one considers mortality, years of life lost, and years of productive life lost. If one compares the economic impact of disease of an elderly person at the end of life compared with that of disease of a fetus or baby, the impact of the latter is far more. Lifetime costs for all persons of cerebral palsy are estimated to total $11.5 billion.1 These costs underscore the need to urgently develop preventive and secondary therapeutic measures for the fetus and newborn. Little progress has been made over the past few decades on the prevalence of cerebral palsy.2–4 The lack of development of new therapies has been partly because of the malpractice climate in United States.5 Progress is also slow because of the unique obstacles facing investigators studying brain injury in the perinatal period, principally related to the issue of timing.6 During the progression of neurological development, the timing of the insult and of end points becomes crucial in any study. The investigator has to take into account not only the plasticity of the stage of development but also the pace of enhanced recovery and the normal process of apoptosis that varies with different time periods of development. Another major obstacle has been the paucity of perinatal animal models manifesting neurobehavioral deficits.

Dynamic In Vivo Measurement of the Developing Brain Requires Noninvasive MRI

The developing brain undergoes rapid changes and the dynamic changes in brain structure and function is unique to this age. This necessitates the use of repeated noninvasive measurements. MRI is being increasingly used in premature newborn human infants and supplements the information obtained from the traditional mode of brain imaging, ultrasound. A specific technique about diffusion weighted imaging gives additional information about white matter development, myelination, and acute injury from H-I. This is also important for animal studies because these studies ultimately have to be clinically relevant. We used MRI as a noninvasive imaging tool to better characterize the dynamic changes after brain injury.

Methods

Recently, we described a model in preterm rabbits that resulted in hypertonia and neurobehavioral findings in postnatal day 1 rabbits (P1) mimicking those found in cerebral palsy.8,9 Briefly, in vivo uterine ischemia was induced in pregnant dams (Myrtle’s Rabbits; Thompson Station, TN) by inflation of an aortic balloon at a level.
proximal to the uterine arteries.8,10–12 This protocol resulted in global hypoxia to the fetus that was accompanied by immediate fetal bradycardia (from 180 to 80 bpm) and an immediate decrease in microvascular blood flow (laser Doppler measurements) to the fetal cerebral cortex which remained down for the duration of uterine ischemia. Preterm fetuses (21 and 22 days’ gestation) were subjected to sustained global hypoxia, and near-term fetuses (29 days’ gestation) to repetitive global hypoxia from uterine ischemia. The dams survived and gave birth spontaneously at term gestation (31.5 days).

Because severely affected kits were not able to survive if left with the rabbit dam, we hand-fed these kits by the orogastric route using a soft silicone catheter.8 We found the best survival in those rabbit kits fed rabbit milk.

The MRI methods and results have been described before.13 Briefly, rabbit kits at postnatal day 1 (10 days after uterine ischemia) and 5 were imaged under sedation with intramuscular injection of a mixture of ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (1.0 mg/kg). We used a 4.7 T Bruker Biospec system (Bruker), with custom-made surface coils used for excitation and reception, and inner diameter of 28 mm. Diffusion weighted imaging in 3 orthogonal directions (diffusion trace-weighted) were performed on 10 oblique coronal brain slices with diffusion weighted spin echo sequence, repetition time/echo time was 2000/35 ms, with b-values of 780 s/mm² and one reference image with b=0 s/mm²; matrix size was 128×64, and 8 averages taken. Slice thickness/in-plane resolution were 1/0.156 mm.13

Results
Cerebral Palsy Model in Rabbits
These results have been described extensively in previous publications.8,9 Newborn kits that survived (58%) the hypoxic-ischemic insult, at 22 days’ gestation (E22), displayed significant impairment in multiple tests of spontaneous locomotion, reflex motor activity, motor responses to olfactory stimuli, and the coordination of suck and swallow.8 Increased tone of the limbs at rest and with active flexion and extension were observed in the survivors of the preterm (Figure 1A and 1B) but not in the survivors of the near-term insult.

Surviving Rabbit Kits Continue to Show Hypertonia
The motor deficits remained unchanged over the first 11 days in the survivors (Figure 1C). The finding of persistent and unchanging hypertonia fulfills one of the requirements for the diagnosis of cerebral palsy.14

What Determines Hypertonia
The central features of cerebral palsy are hypertonia and postural changes. In our animal model we have attempted to explain why some kits became hypertonic and some did not.13 We hypothesized that white matter injury was responsible for the hypertonia. After in vivo global fetal hypoxia-ischemia in pregnant rabbits at E22 and subsequent birth at term, newborn kits at P1 with and without hypertonia were compared. The aim was to examine white matter injury by diffusion tensor MRI indices, including fractional anisotropy (FA).13

At P1, FA and area of white matter were significantly lower in corpus callosum, internal capsule and corona radiata of the hypertonic kits than controls, whereas nonhypertonic kits were not different from controls (Figure 2). The decrease in FA correlated with decrease in area only in hypertonic kits, whereas nonhypertonic and control kits showed no correlation. More importantly, values below a threshold of FA combined with area could identify a sub-population of kits that only contained hypertonic kits. (Figure 3)13 A reduction in volume and loss of phosphorylated neurofilaments in corpus callosum and internal capsule were observed, which suggests an axonal pathology as an explanation for the rabbit hypertonia.13 In humans, abnormal signal intensity in the internal capsule is an accurate predictor of neurodevelopmental outcome in term hypoxic-ischemic infants.15 Rabbit gray matter structures showed loss in area in most structures (Table), and the combination of gray and white matter lesions is consistent with human studies.16

Discussion
The development of an animal model that incorporates etiology and structural and functional features17 provides future

Figure 1. Muscle tone score of forelimbs (A) and hindlimbs (B) comparing controls (n=32) with kits after sustained hypoxia for 30 minutes at E22 (30 minutes, n=12) and 40 minutes at E22 (40 minutes, n=20). Data are shown as a box and whisker plot. Black circles, mean; white circles, 99th percentile; groups significantly different by Kruskal-Wallis test and 40-minute group significantly different compared with controls by Wilcoxon signed rank test. C, Percentage of survivors with hypertonia (black circles) is unchanged as all the hypertonic kits at P1 remain hypertonic at P11. The number of survivors falls (gray squares) with time even with intensive care.

Figure 2. Fractional anisotropy in select white matter structures is significantly decreased (P<0.05, ANOVA) in the hypertonia group (n=32) compared with controls (n=19), whereas the no hypertonia group (n=20) was not different. White matter injury is probably diffuse in nature.
can injure the fetal brain. Thus, the various animal models can be categorized according to the subunit of the maternal-placenta-fetal unit studied. For example, maternal and fetal models have been used in rats, mice, guinea pigs, and sheep. Examples of placental models can be found in rats, mice, sheep and our rabbit model. However, the most common animal models are postnatal in origin and use stroke pathophysiology; examples are found in rats, mice, pigs, sheep, and baboons. Carotid artery occlusion alone does not cause behavior changes in rodents. Combined with hypoxia, behavioral changes are elicited only with sophisticated tests,18–20 but no overt motor deficits21,22 or transient motor deficits23,24 are observed, even with bilateral carotid occlusion.25 There have been very few studies investigating motor and tone outcomes in higher animals, such as sheep, piglets or nonhuman primates.

In 50% to 80% of cases, cerebral palsy is antepartum in origin, and most of the cases of antenatal hypoxia-ischemia are considered idiopathic.26 One of the leading causes of cerebral palsy from antenatal hypoxia-ischemia is abruptio placentae.27 We have been interested in developing suitable animal models of hypoxia-ischemic brain injury that mimic clinical perinatal brain injury. Thus, we focused on a rabbit model of acute placental insufficiency to investigate hypoxia-ischemia-mediated brain injury.10–12

Advantages of Rabbit as an Animal Model
The principal advantage of using rabbits is that motor development and white matter development are close to that of humans. Rodents are postnatal brain developers, with most of myelin formation occurring after the first week.28 Almost all other mammals are prenatal brain developers. This is true even for primate species that are considered the closest to humans where most of the myelin development is over before

Figure 3. Scatter plot of FA (ordinate) versus area of internal capsule (abscissa), measured on the same MRI slice. In the hypertonia group (n = 32), both FA and area values were low and there was significant correlation (r = 0.51, P = 0.006) between FA and area, unlike in controls (n = 19) or nonhypertonic kits (n = 20). This would suggest that white matter injury is the primary event that leads to loss of both variables. A combination of FA values <0.35 and area <2.2 mm² (gray rectangle) identified kits that have 100% likelihood of hypertonia. The rectangle encompassed 54% of the hypertonic population.

maternal-placental-fetal and postnatal models

The clinically applicable models of perinatal hypoxia-ischemia have centered on the maternal-placental-fetal unit. Disorders of any of the components of the maternal-placental-fetal unit can injure the fetal brain. Thus, the various animal models can be categorized according to the subunit of the maternal-placenta-fetal unit studied. For example, maternal and fetal models have been used in rats, mice, guinea pigs, and sheep. Examples of placental models can be found in rats, mice, sheep and our rabbit model. However, the most common animal models are postnatal in origin and use stroke pathophysiology; examples are found in rats, mice, pigs, sheep, and baboons. Carotid artery occlusion alone does not cause behavior changes in rodents. Combined with hypoxia, behavioral changes are elicited only with sophisticated tests,18–20 but no overt motor deficits21,22 or transient motor deficits23,24 are observed, even with bilateral carotid occlusion.25 There have been very few studies investigating motor and tone outcomes in higher animals, such as sheep, piglets or nonhuman primates.

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Parameters of White and Gray Matter Structures Comparing Hypertonia and Nonhypertonia Newborn Rabbit Kits at P1 After 40 Minutes of Hypoxia-Ischemia at E22

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FA</th>
<th>ADC</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypoxia/No Hypertonia</td>
<td>Hypoxia/Hypertonia</td>
<td>Hypoxia/No Hypertonia</td>
</tr>
<tr>
<td>White matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior commissure</td>
<td>1.06 ± 0.07</td>
<td>0.93 ± 0.06</td>
<td>1.08 ± 0.04</td>
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<tr>
<td>Corona radiata</td>
<td>0.98 ± 0.05</td>
<td>0.84 ± 0.04*</td>
<td>1.03 ± 0.03</td>
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<tr>
<td>Corpus callosum</td>
<td>0.94 ± 0.03</td>
<td>0.89 ± 0.03*</td>
<td>1.05 ± 0.03</td>
</tr>
<tr>
<td>Fimbria hippocampi</td>
<td>1.02 ± 0.04</td>
<td>0.95 ± 0.04*</td>
<td>1.01 ± 0.04</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>0.92 ± 0.04</td>
<td>0.88 ± 0.04*</td>
<td>1.01 ± 0.03</td>
</tr>
<tr>
<td>Pedunculus thalami</td>
<td>1.01 ± 0.05</td>
<td>0.88 ± 0.04*</td>
<td>1.02 ± 0.03</td>
</tr>
<tr>
<td>Optic tract</td>
<td>0.98 ± 0.06</td>
<td>0.86 ± 0.08</td>
<td>0.92 ± 0.05</td>
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<tr>
<td>Gray matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.89 ± 0.07</td>
<td>0.85 ± 0.05</td>
<td>1.05 ± 0.04</td>
</tr>
<tr>
<td>Cerebral cortex</td>
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<td>0.83 ± 0.04</td>
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<td>Hippocampus</td>
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<td>0.85 ± 0.03</td>
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<tr>
<td>Putamen</td>
<td>0.91 ± 0.06</td>
<td>0.88 ± 0.04</td>
<td>1.04 ± 0.03</td>
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<tr>
<td>Red nucleus</td>
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<td>0.88 ± 0.07</td>
<td>1.45 ± 0.21</td>
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<tr>
<td>Thalamus</td>
<td>1.03 ± 0.09</td>
<td>0.93 ± 0.07</td>
<td>1.05 ± 0.05</td>
</tr>
</tbody>
</table>

Values are normalized to nonhypoxic controls. Bold font indicates significant difference from control group. *Significant difference between hypertonic and nonhypertonic kits with P < 0.05.
birth. This makes sense because these mammals have to have enough motor function at birth to escape predators. Rabbits, however, are burrow animals and are perinatal brain developers. Myelination begins soon after birth in our rabbits at approximately P5. Interestingly, humans, in their evolutionary leap from the rest of the primates, have become perinatal brain developers, with prolonged development of myelination. Myelination starts in the prenatal period, and that of some sensory and motor fibers begin at birth, but the process continues during the first year of life.

Another advantage of rabbits is that rabbits are similar to humans in that both have low circulating levels of xanthine oxidase, a free radical generator, and are unlike rodents that have high levels. If this enzyme is crucial in the pathophysiology of free radical-mediated injury in humans, then the rodent could be unsuitable for investigating this mechanism. Further advantages of our model are that it is a true fetal model, involves global hypoxia-ischemia, the dam is not significantly affected by the uterine ischemia, rabbits are relatively inexpensive with an average of 8 fetuses per dam, and dams fully recover from survival surgeries and can give birth normally.

**Survival of Postnatal Kits Is Directly Linked to Intensive Care**

The survival of postnatal kits after antenatal hypoxia-ischemia could be certainly improved with improvements in rabbit neonatal intensive care. In human hypoxia-ischemic encephalopathy, a high percentage of sick infants would also die if they did not have the benefit of neonatal intensive care that includes ventilator care, parenteral nutrition, and artificial feeds. We are trying to reach the same level of sophistication for postnatal rabbit kits as for humans, but this involves a lot of time and money.

**Conclusion**

In conclusion, a clinically relevant model was generated from antenatal H-I that mimics the insult of acute placental insufficiency in humans, and resulted in a cerebral palsy phenotype. This model strongly supports the concept that antenatal factors are predominantly related to the subsequent development of cerebral palsy. The behavioral end points have become accessible to study to investigators. It is our hope that this model will further the investigations of cellular and molecular mechanisms that precede cerebral palsy and to assess whether these deficits can be ameliorated by therapeutic interventions.

**Sources of Funding**

This study received grant support from NIH, NS43285, and March of Dimes Birth Defects Foundation 294.

**Disclosures**

None.

**References**


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*Stroke.* 2007;38:731-735
doi: 10.1161/01.STR.0000251445.94697.64
*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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