CEREBRAL PALSY

A Baby Step for Nano

Sidhartha Tan*

Nanomedicine treatment postnatally in an inflammatory model of cerebral palsy ameliorates motor deficits (Kannan *et al.*, this issue).

CEREBRAL PALSY: COST AND PREVALENCE

Cerebral palsy (CP) is a developmental disorder that has lifelong consequences with high burdens of disease, taking into account loss of productive years and socioeconomic impact on caregivers, parents, and siblings. CP describes a group of permanent disorders of movement and posture that limits activity and mobility. The origin of CP is from an insult to a growing, developing fetal or infant brain; it is usually first diagnosed at 18 to 24 months of age and clinically defined at 4 to 5 years. It is estimated that the lifetime cost for all patients with CP is $11.5 billion (*1*). The number of CP patients totals 800,000 in the United States [in one survey between 1997 and 2005, the prevalence was 4 children in 1000 that were ≥3 years old (*2*)]. Preventing one patient from developing CP would save almost $1 million (*1*), not factoring in increased productivity of the patient and his or her family. Yet, funding for CP research is a tiny fraction of that for other neurodegenerative diseases.

Advances in obstetric care over the past 40 years have not made a dent in the incidence of CP (*3*). Unfortunately, improved neonatal care has been counterbalanced by an increase in premature births over the past decade, as prematurity is an independent risk factor for CP. It is projected that despite these advances, the prevalence of CP will not decrease until new treatments for the disorder are found. In this issue of *Science Translational Medicine*, Kannan and colleagues have introduced the possibility of an exciting new nanotechnology-based neuroprotective strategy for CP that, when given at birth, releases the drug intracellularly in the brain and improves inflammatory response and motor performance in baby rabbits (kits) (*4*) (Fig. 1).

Department of Pediatrics, NorthShore University Health System and University of Chicago, Evanston, IL 60201, USA.
*Corresponding author. E-mail: sidtan@uchicago.edu

ANIMAL MODELS AND MOTOR DEVELOPMENT

The two pathognomonic findings of CP are hypertonia and postural deficits. Despite the nonprogressive and sometimes labeled as "permanent" nature of CP, the developing brain continues to develop, respond, and adapt to the ravages of the initial insult; thus, the final diagnosis of CP depends on a combination of motor delay, neurologic signs, and persistence of primitive reflexes. There are thought to be three broad pathophysiological mechanisms resulting in CP, often with overlap: (i) congenital predisposition and genetic causes, (ii) inflammation and infection [which is modeled in the study by Kannan *et al.* (*4*)], and (iii) a combination of hypoxia and ischemia. Unfortunately, the cause is often ascribed to the events around birth and delivery—akin to the proverbial straw that broke the camel's back, often forgetting the unobserved events in the rest of the pregnancy.

One of the perplexing questions is how the normal motor development of humans came about from an evolutionary perspective. Rodents start and complete their motor development after birth (postnatal type). Rabbits (used by Kannan and colleagues) start development before birth and complete it after birth (perinatal type). Most other mammals, including nonhuman primates, complete their motor development before birth (prenatal type). In the evolutionary leap from other primates, humans have returned to a perinatal type akin to rabbits. The difference is that motor development in humans is prolonged compared with the rabbit, with motor skills taking years to develop in the human.

What is distinctive about the study by Kannan *et al.* (*4*) is the choice of rabbits as the animal model. To develop a neuroinflammation rabbit model with newborn motor deficits similar to that found in human CP, the authors injected *Escherichia coli* endotoxin into the rabbit mother's uterus at various places in the myometrial layer, to induce an inflammatory reaction in fetuses at approximately 90% term gestation. This is meant to mimic the most common cause of neuroinflammation in human babies, which occurs from chorioamnionitis in the mother, often due to bacterial infection. A previous rabbit model used actual *E. coli* intracervical injection at 70% gestation, which would mimic the ascending cervix-to-uterine endometrium infection in humans but had high fetal and maternal mortality (*5*). A rat model also used *E. coli* injected via laparotomy to the uterine bifurcation at 77% gestation so as to mimic the ascending infection (*6*). Another rat model used lipopolysaccharide toxin given via intraperitoneal injection to pregnant rats at 81% gestation, but it is uncertain whether the toxin reached the fetuses by diffusion or through the maternal circulation (*7*). Unfortunately, rodent models do not manifest a neurobehavioral correlate of human CP, even if the brain injury is quite severe.

NANO TO THE RESCUE

Our understanding of CP is lacking because the etiological insult is often in the perinatal period, and there is a long gestation between initial insult and the final motor manifestations of CP. Coupled with a paucity of markers of fetal injury and inability to diagnose fetal brain injury, it has been hard to develop neuroprotective therapies for CP. There is no treatment targeting fetal brain injury in cases of maternal inflammation. The only two treatments that have impact on CP, albeit small, in the perinatal period are antenatal magnesium sulfate for premature birth and postnatal hypothermia for term perinatal asphyxia. A postnatal rescue strategy was used by Kannan *et al.* using a pro-drug approach with targeted delivery of *N*-acetyl-L-cysteine (NAC) nanoformulations to specific cell types involved in neuroinflammation (microglia) and scar formation (astrocytes) (*4*). NAC was linked to polyamidoamine dendrimers via a disulfide linker, which enabled NAC to cross the blood-brain barrier and reach microglia and astrocytes. This nanoformulation, called D-NAC, was administered intravenously postnatally to rabbit kits within 6 hours of birth (day 1), which is also the cutoff used for postnatal hypothermia. A distinctive feature of the approach by Kannan *et al.* is the comparison of cell-targeted nanotherapy with a whole-brain therapy (free NAC alone).

Animals were videotaped on days 1 and 5 to observe motor function and tone. The nanoformulation strategy improved the locomotor score almost to normal healthy levels by day 5, with a dose of D-NAC that was 10-fold less than the free drug and with a far superior neuroprotective effect than that of the free NAC. The locomotor improvement was also accompanied by an improvement in hypertonia, which is the distinctive feature of CP.

For translation to human newborns, drug dosage studies will be needed. A dose of D-NAC that was only 1% of the highest dose of free NAC showed a trend to be more effective at improving motor function in CP kits. This lowered drug dosage has the potential advantage of a decrease in nonspecific and off-target effects and is especially useful in the developing brain, where basic biochemical processes are involved in proliferation, differentiation, and normal cell death and apoptosis. One unanswered question is whether the relative incompetence of a broad-based strategy, such as free NAC, is because of the resulting competing mechanisms of action—one protective and one destructive—in different cells.

A REDOX MECHANISM

The strategy was to deliver the NAC nanoformulation intracellularly where high local concentrations of glutathione cleaved NAC from the dendrimer. NAC then acted by providing one thiol (–SH) group to the antioxidant pool. NAC is believed to provide an L-cysteine, which is incorporated into glutathione (GSH). Because there was no net gain of –SH bonds, the finding of an increase in GSH levels in brain after dendrimer-NAC administration is only a partial explanation for the neuroprotective effect. If GSH increases without a corresponding

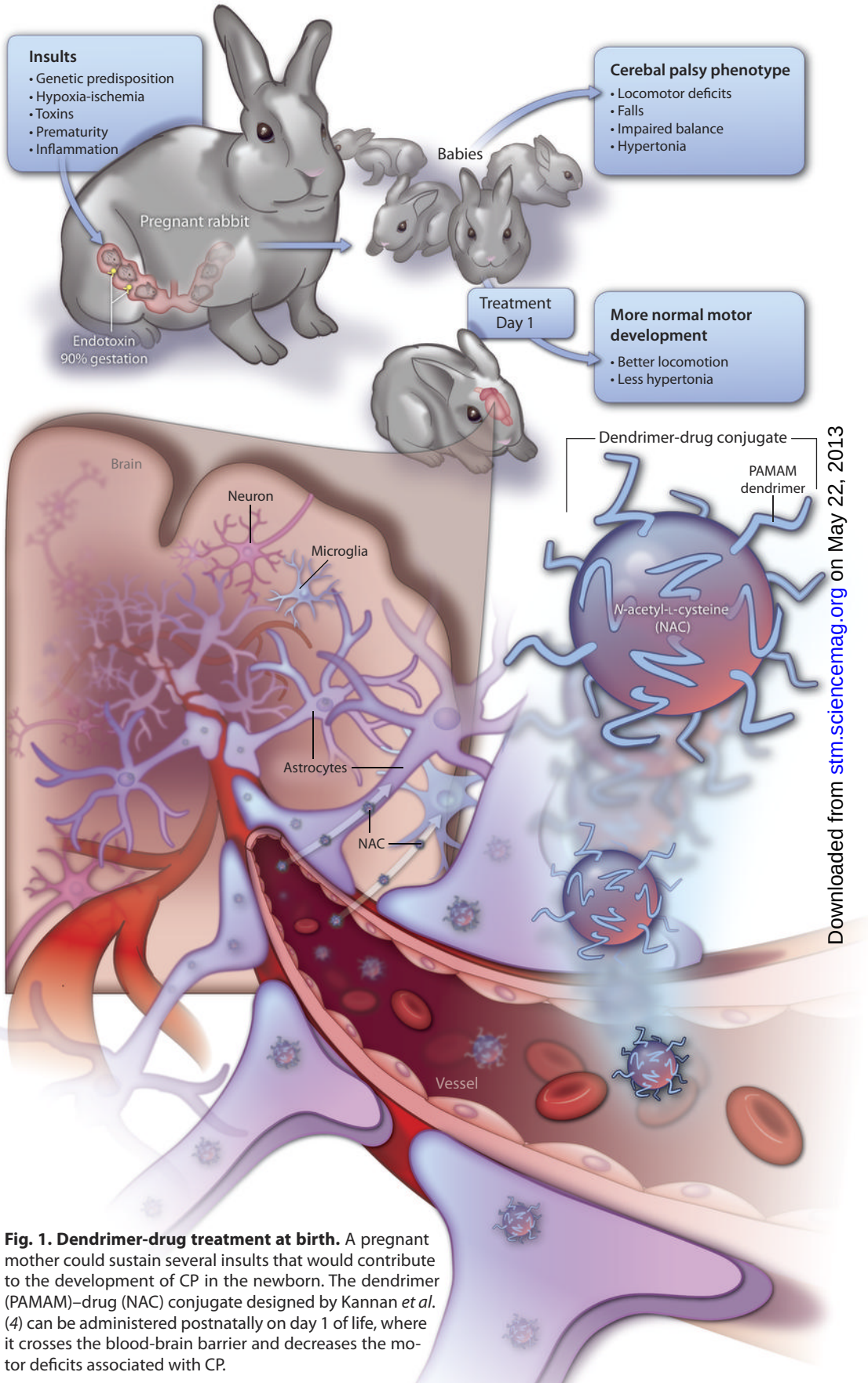

Fig. 1. Dendrimer-drug treatment at birth. A pregnant mother could sustain several insults that would contribute to the development of CP in the newborn. The dendrimer (PAMAM)–drug (NAC) conjugate designed by Kannan *et al.* (*4*) can be administered postnatally on day 1 of life, where it crosses the blood-brain barrier and decreases the motor deficits associated with CP.

CREDIT: B. STRAUCH/*SCIENCE TRANSLATIONAL MEDICINE*

increase in its oxidized form, glutathione disulfide (GSSG), then the redox status becomes more negative. The greatest negative redox state occurs during cellular proliferation, less so for differentiation, and least so for cell apoptosis (least negative redox equating to most oxidative stress). Proliferation, differentiation, and apoptosis can occur simultaneously in the developing brain.

There are three possible explanations for the neuroprotective effects described by Kannan *et al.* (*4*), which are worthy of further investigation because none of these concepts have been shown for perinatal brain. First, it is possible that the NAC released from the dendrimer in the intracellular compartments changes the cysteine/cystine redox pool rather than the GSH/GSSG pool. In human colonic epithelial cells (*8*) and plasma (*9*), the less negative redox status of the cysteine/cystine suggests that there is greater oxidation than GSH/GSSG because it is proximal to the oxidative events. The cysteine/cystine pool also influences signal transduction differently from the GSH/GSSG pool (*8*). Second, NAC could be acting through other mechanisms, such as the reduced form of nicotinamide adenine dinucleotide phosphate or thioredoxin pathways. Third, the decrease in nitrotyrosine described by Kannan and colleagues would suggest that the nitric oxide biology inside the microglia is affected. Microglia itself can be the source of reactive nitrogen species that results in such nitrotyrosine formation. The numerous direct and indirect biochemical pathways of decreased formation of reactive nitrogen species by intracellular NAC cannot be summarized here, but they would certainly explain the results of depressed tumor necrosis factor–α (TNFα) and nuclear factor κβ (NF-κB) reported by the authors (*4*). The study by Kannan *et al.* reaffirms the contention that not all oxidative stress is equal; that the time, place, and milieu of oxidative stress matter for onset of brain injury; and that redox pools behave differently in intracellular compared with extracellular compartments.

TRANSLATING TREATMENTS FOR THE DEVELOPING BRAIN

The use of nanomaterials in perinatal brain injury opens up new vistas in the realm of diagnostics, imaging, and drug delivery for newborn babies. Kannan *et al.* are the first to develop a nanotherapeutic approach for the perinatal period. A lot remains unknown about the developing brain; as such, every nanomaterial investigated must first be tested for systemic safety, as was done by Kannan and colleagues. Before such a dendrimer strategy reaches the clinical realm, more detailed toxicology studies need to be done to rule out potential side effects, such as the effect of protonated amine groups (on the dendrimer) in inhibiting membrane potassium ion channel function (*10*). Not all nanomaterials are created equal, and each nanomaterial selected will interact differently with the developing brain—hopefully in a way that improves therapy for the myriad development disorders that remain without a cure, including CP.

REFERENCES AND NOTES

1. Centers for Disease Control and Prevention (CDC), Economic costs associated with mental retardation, cerebral palsy, hearing loss, and vision impairment—United States, 2003. *MMWR Morb. Mortal. Wkly. Rep.* **53**, 57–59 (2004).
2. S. L. Boulet, C. A. Boyle, L. A. Schieve, Health care use and health and functional impact of developmental disabilities among US children, 1997-2005. *Arch. Pediatr. Adolesc. Med.* **163**, 19–26 (2009).
3. K. Himmelmann, G. Hagberg, P. Uvebrant, The changing panorama of cerebral palsy in Sweden. X. Prevalence and origin in the birth-year period 1999–2002. *Acta Paediatr.* **99**, 1337–1343 (2010).
4. S. Kannan, H. Dai, R. S. Navath, B. Balakrishnan, A. Jyoti, J. Janisse, R. Romero, R. M. Kannan, Dendrimer-based postnatal therapy for neuroinflammation and cerebral palsy in a rabbit model. *Sci. Transl. Med.* **4**, ra46 (2012).
5. B. H. Yoon, C. J. Kim, R. Romero, J. K. Jun, K. H. Park, S. T. Choi, J. G. Chi, Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. *Am. J. Obstet. Gynecol.* **177**, 797–802 (1997).
6. Y. Pang, S. Rodts-Palenik, Z. Cai, W. A. Bennett, P. G. Rhodes, Suppression of glial activation is involved in the protection of IL-10 on maternal *E. coli* induced neonatal white matter injury. *Brain Res. Dev. Brain Res.* **157**, 141–149 (2005).
7. M. K. Paintlia, A. S. Paintlia, E. Barbosa, I. Singh, A. K. Singh, *N*-acetylcysteine prevents endotoxin-induced degeneration of oligodendrocyte progenitors and hypomyelination in developing rat brain. *J. Neurosci. Res.* **78**, 347–361 (2004).
8. D. P. Jones, Y. M. Go, C. L. Anderson, T. R. Ziegler, J. M. Kinkade Jr., W. G. Kirlin, Cysteine/cystine couple is a newly recognized node in the circuitry for biologic redox signaling and control. *FASEB J.* **18**, 1246–1248 (2004).
9. D. P. Jones, V. C. Mody Jr., J. L. Carlson, M. J. Lynn, P. Sternberg Jr., Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. *Free Radic. Biol. Med.* **33**, 1290–1300 (2002).
10. G. Nyitrai, O. Kékesi, I. Pál, P. Keglevich, Z. Csíki, P. Fügedi, A. Simon, I. Fitos, K. Németh, J. Visy, G. Tárkányi, J. Kardos, Assessing toxicity of polyamidoamine dendrimers by neuronal signaling functions. *Nanotoxicology* 1–13 (2011).

10.1126/scitranslmed.3003934

Citation: S. Tan, A baby step for nano. *Sci. Transl. Med.* **4**, 130fs8 (2012).