Dendrimers get cerebral

By Tim Fulmer, Senior Writer

U.S. researchers have developed a dendrimer-based therapy that reduced brain inflammation and improved the motor function of newborn rabbits with cerebral palsy.[1] The team now needs to determine the therapy's treatment window and its efficacy in the multiple types of cerebral palsy.

Cerebral palsy encompasses a group of disorders characterized by motor impairments that result from damage to the fetal or infant brain. The most common causes of the disease are intrauterine infection, prenatal hypoxia, trauma during delivery and premature birth.

Compared with the other causes, intrauterine infection is often associated with an exaggerated inflammatory response in which activation of microglia and astrocytes in the neonatal brain leads to very high levels of proinflammatory cytokines that cause brain injury before and after birth.[2,3]

The challenge is figuring out how to design an anti-inflammatory therapy that crosses the blood brain barrier (BBB) in sufficient quantities and localizes in activated microglia and astrocytes.

To tackle the task, the team turned to dendrimers, which are highly branched polymers that can be linked to small molecules and used as drug delivery vehicles. In prior work, the team showed that direct injection of dendrimers into the neonatal rabbit brain led to accumulation in activated microglia and astrocytes.[4]

The researchers wanted a systemic therapy, and thus they decided to link the dendrimer to a therapeutic and shuttle it across the BBB to treat disease following an i.v. injection.

The linker was a disulfide molecule, which is rapidly cleaved by glutathione in microglia and astrocytes. For the therapeutic, the researchers chose N-acetylcysteine (NAC), a generic anti-inflammatory marketed to treat acetaminophen-induced liver injury.

Intraperitoneal delivery of NAC has shown anti-inflammatory and neuroprotective effects in rodent models of perinatal brain injury, suggesting the compound can cross the BBB by itself.[5,6]

The open question was whether attaching NAC to a dendrimer would result in significantly higher efficacy compared with unconjugated NAC in the brain of a mammal with cerebral palsy.

Indeed, in a rabbit model of prenatal endotoxin-induced cerebral palsy,[7,8] i.v. delivery of the dendrimer-NAC (D-NAC) conjugate within six hours of birth significantly increased motor function ($p<0.001$) and hind limb muscle tone ($p<0.001$) compared with injection of NAC or vehicle control.

In the brains of those rabbits, D-NAC significantly decreased the levels of molecular markers of oxidative injury ($p<0.01$), the activity of proinflammatory microglia ($p<0.01$) and neuronal cell loss ($p<0.01$) and increased myelination levels ($p<0.05$) compared with NAC or vehicle control.

In five-day-old brain sections stained for the proinflammatory marker complement receptor 3 (Cr3; Cd11b), D-NAC significantly lowered total microglia activation compared with NAC or vehicle ($p<0.01$).

The study was led by Rangaramanujam Kannan, professor of ophthalmology at **The Johns Hopkins University School of Medicine**, and Roberto Romero, chief of the Perinatology Research Branch and program director for Perinatal Research and Obstetrics at the **National Institute of Child Health & Human Development**.

Results were published in *Science Translational Medicine*.

"The effectiveness of the D-NAC treatment, administered in the postnatal period for a prenatal insult, suggests a new window of opportunity for the treatment of CP [cerebral palsy] after birth in humans," the authors wrote.

Looking long term

"Longer-term studies with significantly greater numbers of animals will be needed to work out the safety of D-NAC in the developing brain and over the lifetime of the animal," said Dorothea Jenkins.

Jenkins, an associate professor of pediatrics at the **Medical University of South Carolina**, told *SciBX* she has just completed a Phase I trial of unconjugated NAC to reduce brain injury in infants exposed to chorioamnionitis (intrauterine inflammation).

In that trial, i.v. NAC was delivered before birth to pregnant women diagnosed with chorioamnionitis and to their infants after birth. NAC "readily crosses the placenta and gets into the fetal bloodstream," Jenkins noted.

There, the molecule can protect the fetus from "even mild hypoxia-ischemia associated with fairly normal labor, which could otherwise be a second hit to the fetal brain following chorioamnionitis," she said.

Initial safety data from the trial were presented last month at the **American Pediatric Society and Society for Pediatric Research** meeting in Boston. Unconjugated NAC was safe and well tolerated in 25 mothers and their infants, said Jenkins.

A potential advantage of linking NAC to a dendrimer is that it "may make NAC more effective at lower doses," said Jenkins. "Though it may also be an expensive modification. So a cost-benefit analysis will be important."

Kannan said the dendrimer-based approach "may also work for other causes of cerebral palsy such as hypoxia-ischemia, where inflammation may have a secondary role in perpetuating the injury."

He also noted that dendrimers could be used to deliver many compounds besides NAC. "We will explore other drugs and combination therapies that could help treat cerebral palsy on multiple fronts," he said.

Kannan said it will be "very important to see if the improvement in motor function can be sustained up to adulthood in the animal studies."

The findings are covered by pending patents and are available for licensing.

Fulmer, T. *SciBX* 5(19); doi:10.1038/scibx.2012.486
Published online May 10, 2012

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COMPANIES AND INSTITUTIONS MENTIONED

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