Cell Signaling Defect Is Associated with Neonatal Hydrocephalus

Prenatal Lithium Can Treat It

BY TOM VALEO

ARTICLE IN BRIEF

A research team, working with a mouse model of Bardet-Biedl syndrome (BBS), found that a form of neonatal hydrocephalus thought to be caused by impaired motile cilia that fail to move cerebral spinal fluid normally through the ventricles and spinal cord actually results from a cell signaling defect that prevents normal proliferation of neural progenitor cells, and also promotes apoptosis, resulting in reduced brain volume.

A form of neonatal hydrocephalus thought to be caused by impaired motile cilia that fail to move cerebral spinal fluid normally through the ventricles and spinal cord actually results from a cell signaling defect that prevents normal proliferation of neural progenitor cells, and also promotes apoptosis, resulting in reduced brain volume. This devastating condition, however, can be treated by administering lithium prenatally, investigators reported in the Dec. 6, 2012, edition of *Nature Medicine*.

The research team, working with a mouse model of Bardet-Biedl syndrome (BBS), determined that ventricle enlargement was apparent from the day of birth, even though the ependymal motile cilia don't begin to mature until several days later. Furthermore, blue dye injected into the mice showed no evidence of obstructive hydrocephalus, and there was no evidence of excess CSF production.

BBS, a rare disorder that causes hydrocephalus, pigmentary retinopathy, kidney problems, polydactyly, obesity and other symptoms, is considered a ciliopathy because many of the symptoms can be traced to dysfunctional cilia. For example, photoreceptors in the eye have modified primary cilia in the outer segment of the rods and cones, and are believed to contribute to the blindness characteristic of BBS.

DR. VAL C. SHEFFIELD and colleagues were able to show that neonatal hydrocephalus could be caused by cellular signaling defects that could potentially be ameliorated by administering lithium.

"When we started to investigate how BBS causes hydrocephalus, we expected a trivial answer," said Val C. Sheffield, MD, PhD, professor of pediatrics and director of the Division of Medical Genetics at the University of Iowa Roy J. and Lucille A. Carver College of Medicine, and a Howard Hughes Medical Institute investigator. "The ependymal cells that line the cerebral ventricles are populated with motile cilia thought to move the CSF. If the cilia were abnormal, that would lead to a lack of mobility of CSF, which would allow CSF to accumulate and cause hydrocephalus."

Instead, the motile cilia were not significantly abnormal, so the researchers turned their attention to the primary cilia. Almost every cell in the body has a primary cilium, which projects from the cell body and senses signaling molecules, including those that control cell growth.

"So we surmised that maybe some cells in the brain have primary cilia, and they're abnormal," said Dr. Sheffield, who has been studying BBS in his lab for 20 years.

Knowing that humans with BBS have reduced white and gray matter volume in their periventricular regions, first author Calvin Carter, a graduate student in Dr. Sheffield's lab, along with Timothy Vogel, MD, a neurosurgeon, looked for evidence of apoptosis and inadequate cell proliferation in the same region of their mouse model of BBS. They found twice as much apoptosis as normal, and half as much cell proliferation.

Continued on page 20

DR. JOSEPH G. GLEESON: "This paper is a breakthrough in how we think about the causes of hydrocephalus. The authors challenge the assumption that most causes of hydrocephalus result from obstruction of the flow of CSF through the ventricles. They demonstrate that at least some causes are probably caused by the underproduction of neuronal cells that take up space in the brain."

Dantrolene, Duchenne Muscular Dystrophy

Continued from page 17

drugs that each can give an incremental effect, and see if they can synergize, there is the potential for tremendous value."

EXPERT COMMENTARY

"These data, with an FDA approved drug, raise the possibility of using lower doses of drug, or getting more efficacy, either of which would probably be of great benefit in the field," said Dr. Flanigan of Ohio State University. "We have no idea how it does this, but the data are quite compelling. Being able to dose with less drug, and at less cost, is one of the promising findings."

He also noted that the results were not dependent on the backbone chemistry of the individual agents, or the sequence of the gene segment targeted. "Those are two critical findings," he said, since they broaden the likely applicability of the therapy. Both current antisense agents target exon 51, since it stands to benefit the most patients, but there are a large handful of other mutations that could potentially benefit from exon-skipping therapy.

Kathryn Wagner, MD, PhD, associate professor of neurology and neuroscience at Johns Hopkins University and director of the Center for Genetic Muscle Disorders at Kennedy Krieger Institute, concurred that the results are promising. "We may be nearing a meaningful therapy," she said. She noted that the decision to undertake this study while the exon-skipping clinical trials were still in the planning stages "showed incredible forethought." The result may be that combination clinical trials can be undertaken rapidly, especially since dantrolene has been used in the past in boys with DMD. "It is an extraordinarily exciting time for the Duchenne muscular dystrophy field," she said.

The study was funded from grants from the NIH, the Department of Defense, and the Foundation to Eradicate Duchenne, as well as through NIH funding for the UCLA Muscular Dystrophy Core Center. •

FOR FURTHER READING:

- Kendall GC, Mokhonova EI, Miceli MC, et al. Dantrolene enhances antisense-mediated exon skipping in human and mouse models of Duchenne muscular dystrophy. *Sci Transl Med*2012;4(164):164ra160. doi: 10.1126/scitranslmed.3005054.
- National Library of Medicine Resource on Duchenne muscular dystrophy: **http://1.usa.gov/iHUr40**.
- *Neurology Today* archive on Duchenne muscular dystrophy: **http://bit.ly/XUrxiq**

Neonatal Hydrocephalus

Continued from page 18

BRAIN SLICES: WHAT THEY FOUND

Closer examination of mouse brain slices revealed that the cells subject to excessive apoptosis and insufficient proliferation in the mutant mice expressed neuron-glial antigen 2 (NG2) and platelet-derived growth factor-alpha (PDGFR-a), markers of oligodendrocyte precursor cells. The mutant mice had about half as many cells expressing NG2 and PDGFR-a in the subventricular zone as wild-type mice. Neural progenitor cells in that area expressing NG2 and PDGFR-a displayed increased apoptosis and reduced proliferation, leading to a reduced number of oligodendrocyte progenitor cells.

"These were specific neural progenitor cells that were dying and not proliferating as fast," Dr. Sheffield said. "To prove this we needed conditional knockout mice that only expressed the BBS mutation in this class of cells and not in ependymal cells or others cell types. When we developed such mice, they also had hydrocephalus. The ependymal cilia were completely normal, but these mice had defects in BBS gene function in oligodendrocyte progenitor cells, and they developed hydrocephalus."

THE EFFECT OF LITHIUM

Further investigation implicated the AKT pathway. Since lithium is known to affect this kinase pathway, the researchers added lithium to the water of pregnant mice whose pups carried the BBS mutation. When the pups were born, the size of their cerebral ventricles was almost normal.

"Lithium modulates some signaling pathways in the brain, including the excitability of glutamate neurons," said Dr. Sheffield.

Although not all human BBS patients have overt hydrocephalus, volumetric MRI scans of 21 BBS patients found increased CSF volume, but normal CSF pressure, in all, according to a 2011 paper in *BMC Medical Genetics*.

And some of the same authors contributed to a 2011 paper in the *New England Journal of Medicine* that linked the same AKT pathway to Proteus syndrome, the genetic disorder better known as Elephant Man disease, which causes extreme overgrowth of skin, bone, connective tissue, brain, and other tissues.

"It reinforces the findings in the Sheffield paper," said lead author Leslie G. Biesecker, MD, chief of the Genetic Disease Research Branch at the NIH's National Human Genome Research Institute. "Here's a disorder with AKT overstimulation that causes brain overgrowth, and Sheffield has found a deficiency in the AKT pathway that causes brain undergrowth, which is perfectly consistent."

DR. LESLIE BIESECKER said he considers the work by Dr. Sheffield and his colleagues very exciting "because it moves hydrocephalus out of realm of a plumbing problem and into the realm of a cell biology problem, which is so much more sophisticated. It gets into neural cellular function and shows these patients are missing neural progenitors. It's not just that they have excess fluid; they have excess CSF volume because they have reduce cellular compartments."

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The paper also suggests that lithium might alleviate this form of neonatal hydrocephalus, a fairly common defect that occurs in one to three out of every 1,000 live births. "But lithium has been associated with heart defects, and patients with BBS can have heart defects," Dr. Biesecker said. "They might be more vulnerable than normal to lithium. A lot of work would need to be done before you'd go down that path."

The findings of the Iowa researchers have drawn praise from others involved in similar research.

NEW THINKING ABOUT CAUSES OF HYDROCEPHALUS

"This paper is a breakthrough in how we think about the causes of hydrocephalus," said Joseph G. Gleeson, MD,

Continued on page 21

Neonatal Hydrocephalus

Continued from page 20

a professor in the department of neurosciences and pediatrics at the University of California, San Diego, and a Howard Hughes Medical Institute investigator.

"The authors challenge the assumption that most causes of hydrocephalus result from obstruction of the flow of CSF through the ventricles," said Dr. Gleeson, whose lab studies the genetic factors of brain development. "They demonstrate that at least some causes are probably caused by the underproduction of neuronal cells that take up space in the brain. I think the most provocative aspect of the paper is the finding that the cilia are abnormal in this model, but yet not playing a role in moving CSF, as previously thought. Rather they are important for sensing growth factor cues that mediate neurogenesis."

Still, several questions remain, as Dr. Gleeson mentioned in a commentary in the same issue of *Nature Medicine* written with Bethany N. Sotak, a graduate student in his lab. Are the mechanisms that cause hydrocephalus with or without increased CSF pressure the same? How do motile and non-motile cilia contribute to the etiology of hydrocephalus? And can PDGF signaling explain the penetrance of hydrocephalus in the mouse model of BBS, which is much higher than in humans?

Joseph J. Volpe, MD, neurologist-in-chief emeritus at Boston Children's Hospital and the Bronson Crothers distinguished professor of neurology at Harvard Medical School, questioned the use of the term "hydrocephalus" in the paper, claiming that the condition described doesn't involve an increase in production of CSF or an obstruction in its flow. Nor is it a problem of absorption, as in communication hydrocephalus.

"What they are describing is a dilation of the ventricles that's caused by a disturbance in the development of cerebral white and gray matter, so they're smaller than they should be," said Dr. Volpe.

However, other papers describing mouse models of hydrocephalus do not include intracranial pressure as part of the definition, according to Dr. Sheffield. "BBS1 and other BBS models fit the phenotype for hydrocephalus as defined by mouse models in the scientific literature," he said. "It is clear that BBS mice do not have aqueductal stenosis or other forms of obstructive hydrocephalus, but I am sure that the mechanism we describe in the manuscript is causing the phenotype in BBS mice, and that some humans with communicating hydrocephalus likely have the same mechanism in play."

Despite his reservations, Dr. Volpe found the *Nature Medicine* paper interesting in the way it links a molecular defect in non-motile cilia associated with progenitor cells to a disturbance in their proliferation and survival. "As a result, there is an impairment in the development of white and gray matter, so the cerebrum is too small, and the ventricles are larger," he said. "I think this paper relates to potential disturbances involving the primary cilia and the proliferation and survival of progenitor cells, which could impair neuronal and glia growth. It is an interesting issue." •

FOR FURTHER READING:

- Carter CS, Vogel TW, Sheffield VC, et al. Abnormal development of NG2(+) PDGFR-α(+) neural progenitor cells leads to neonatal hydrocephalus in a ciliopathy mouse model. *Nature Med* 2012;18(12):1797-1804.
- Keppler-Noreuil KM, Blumhorst C, Biesecker LG, et al. Brain tissue- and region-specific abnormalities on volumetric MRI scans in 21 patients with Bardet-Biedl syndrome (BBS). *BMC Med Genet* 2011;12:101.
- Lindhurst MJ, Sapp JC, Biesecker LG, et al. A mosaic activating mutation in AKT1 associated with the Proteus syndrome. *N Engl J Med* 2011;365(7):611-619.
- Sotak BN, Gleeson JG. Can't get there from here: Cilia and hydrocephalus. *Nature Med* 2012;18(12):1742-1743.