Therapy slows onset and progression of Lou Gehrig's disease, study finds

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Studies of a therapy designed to treat amyotrophic lateral sclerosis (ALS) suggest that the treatment dramatically slows onset and progression of the deadly disease, one of the most common neuromuscular disorders in the world. The researchers, led by teams from The Research Institute at Nationwide Children's Hospital and the Ludwig Institute at the University of California, San Diego, found a survival increase of up to 39 percent in animal models with a one-time treatment, a crucial step toward moving the therapy into human clinical trials.

The therapy reduces expression of a gene called SOD1, which in some cases of familial ALS has a mutation that weakens and kills nerve cells called motor neurons that control muscle movement. While many drug studies involve only one type of animal model, this effort included analysis in two different models treated before and after disease onset. The in-depth study could vault the drug into human clinical trials, said Brian Kaspar, PhD, a principal investigator in the Center for Gene Therapy at Nationwide Children's and a senior author on the research, which was published online Sept. 6 in *Molecular Therapy*.

"We designed these rigorous studies using two different models of the disease with the experimenters blinded to the treatment and in two separate laboratories," said Dr. Kaspar, who collaborated on the study with a team led by Don Cleveland, PhD, at the University of California, San Diego. "We were very pleased with the results, and found that the delivery approach was successful in a larger species, enabling us to

initiate a clinical translational plan for this horrible disease."

There currently is no cure for ALS, also called Lou Gehrig's disease. The Centers for Disease Control and Prevention estimates there are about 5,000 new cases in the U.S. each year, mostly in people age 50 to 60. Although the exact cause of ALS is unknown, more than 170 mutations in the SOD1 gene have been found in many patients with familial ALS, which accounts for about 2 percent of all cases.

SOD1 provides instructions for making an enzyme called superoxide dismutase, which is found throughout the body and breaks down toxic molecules that can be damaging to cells. When mutated, the SOD1 gene yields a faulty version of the enzyme that is especially harmful to motor neurons. One of the mutations, which is found in about half of all familial ALS patients, is particularly devastating, with death usually coming within 18 months of diagnosis. SOD1 has also been implicated in other types of ALS, called sporadic ALS, which means the therapy could prove beneficial for larger numbers of patients suffering with this disease.

Earlier work by Dr. Kaspar and others found that they could halt production of the mutated enzyme by blocking SOD1 expression, which in turn, they suspected, would slow ALS progression. To test this hypothesis, the researchers would not only need to come up with an approach that would block the gene, but also figure out how to specifically target cells implicated in the disease, which include motor neurons and glial cells. What's more, the therapy would preferably be administered noninvasively instead of direct delivery via burr holes drilled into the skull.

Dr. Kaspar's team accomplished the second part of this challenge in 2009, when they discovered that adeno-associated virus serotype 9 (AAV9) could cross the blood-brain barrier, making it an ideal transport

system for delivering genes and RNA interference strategies designed to treat disease.

In this new work, funded by the National Institutes of Health, the researchers blocked human SOD1, using a technology known as short hairpin RNA, or shRNA. These single strands of RNA are designed in the lab to seek out specific sequences found in the human SOD1 gene, latch onto them and block gene expression.

In one of the mouse models used in the study, ALS develops earlier and advances more quickly. In the other, the disease develops later and progresses more slowly. All of the mice received a single injection of AAV9-SOD1-shRNA before or after disease onset.

Results showed that in the rapid-disease-progressing model, mice treated before disease onset saw a 39 percent increase in survival compared to control treated mice. Strikingly, in mice treated at 21 days of age, disease progression was slowed by 66 percent. Perhaps more surprising was the finding that even after symptoms surfaced in these models, treatment still resulted in a 23 percent increase in survival and a 36 percent reduction in disease progression. In the slower-disease-onset model, treatment extended survival by 22 percent and delayed disease progression by 38 percent.

"The extension of survival is fantastic, and the fact that we delayed disease progression in both models when treated at disease onset is what drives our excitement to advance this work to human clinical trials," said Kevin Foust, PhD, co-first author on the manuscript and an assistant professor in neurosciences at The Ohio State University College of Medicine.

In addition to the potential therapeutic benefit, the study also offers some interesting insights into the biological underpinnings of ALS. The

role of motor neurons in ALS has been well documented, but this study also highlighted another key player—astrocytes, the most abundant cell type in the human brain and supporters of neuronal function.

"Recent work from our collaborator Dr. Cleveland has demonstrated that astrocytes and other types of glia are as important if not more important in ALS, as they really drive disease progression," said Dr. Kaspar. "Indeed, in looking at data from mice, more than 50 percent of astrocytes were targeted throughout the spinal cord by this gene-delivery approach."

Ideally, a therapy would hit motor neurons and astrocytes equally hard. The best way to do that is to deliver the drug directly into the cerebrospinal fluid (CSF), which would reduce the amount of SOD1 suppression in cells outside the brain and reduce immune system exposure to AAV9—elements that would add weight to an argument for studying the drug in humans.

Injections directly into CSF cannot be done easily in mice, so the team took the study a crucial step further by injecting AAV9-SOD1-shRNA into the CSF of healthy nonhuman primates. The results were just as the team hoped—the amount of gene expression dropped by as much as 90 percent in motor neurons and nearly 70 percent in astrocytes and no side effects were reported, laying the groundwork towards moving to human clinical trials.

"We have a vast amount of work to do to move this toward a clinical trial, but we're encouraged by the results to date and our team at Nationwide Children's and our outstanding collaborators are fully committed to making a difference in this disease," Dr. Kaspar said.

The findings could impact other studies underway in Dr. Kaspar's lab, including research on Spinal Muscular Atrophy, an often fatal genetic

disease in infants and children that can cause profoundly weakened muscles in the arms and legs and respiratory failure.

"This research provides further proof of targeting motor neurons and glial cells throughout the entire spinal cord for treatment of Spinal Muscular Atrophy and other degenerative diseases of the brain and spinal cord, through a less invasive manner than direct injections," said Dr. Kaspar, who also is an associate professor of pediatrics and neurosciences at The Ohio State University College of Medicine.

More information: Foust KD, Salazar DL, Likhite S, Ferraiuolo L, Ditsworth D, Ilieva H, Meyer K, Schmelzer L, Braun L, Cleveland DW, Kaspar BK. Therapeutic AAV9-mediated Suppression of Mutant SOD1 Slows Disease Progression and Extends Survival in Models of Inherited ALS. *Molecular Therapy*. DOI: 10.1038/mt.2013.211 ; accepted article preview online 2013 Sept 6.

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