Gene replacement treats copper deficiency disorder in mice

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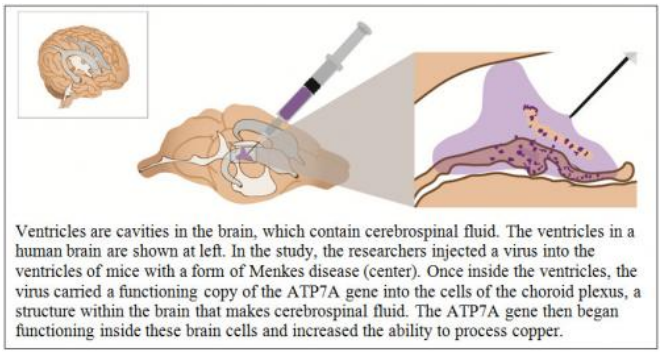

Ventricles are cavities in the brain, which contain cerebrospinal fluid. The ventricles in a human brain are shown at left. In the study, the researchers injected a virus into the ventricles of mice with a form of Menkes disease (center). Once inside the ventricles, the virus carried a functioning copy of the ATP7A gene into the cells of the choroid plexus, a structure within the brain that makes cerebrospinal fluid. The ATP7A gene then began functioning inside these brain cells and increased the ability to process copper.

(Medical Xpress) -- Gene therapy plus an injection of copper dramatically improved survival in mice with a condition that mimics the often fatal childhood disorder Menkes disease, according to a study by researchers at the National Institutes of Health.

Menkes disease results from a poorly functioning copy of the gene ATP7A. Ordinarily, the gene helps the body process the trace metal copper. In Menkes disease, the gene malfunctions. As a result, copper is not available for proper development. Although the disorder affects the entire body, it is most pronounced in the brain, which requires relatively larger amounts of copper during development than other organs and tissues. Copper is used in the formation of red blood cells, and for keeping nervous system tissue, bones, and the immune system healthy.

The syndrome affects 1 out of 50,000 to 100,000 newborns each year, the vast majority of them boys, according to the study's senior author, Stephen Kaler, M.D., head of the Unit on Human Copper Metabolism in the Molecular Medicine Program at the NIH's Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). The disorder varies in severity, depending on the degree to which the gene is disabled. Those with the most poorly functioning copy of the gene fare worst, often dying very early in life. Current treatment consists of daily copper injections until a child is 3 years old, Dr. Kaler explained.

Copper injections can be effective when Menkes disease is detected early and there is some ability to process copper, as in the case of an ATP7A gene with partial function. However, severe and advanced cases generally are not treatable. Most children with the disease die before they reach 3 years of age.

In their study, Dr. Kaler and his colleagues tested a treatment to add a normal copy of ATP7A to mice with a malfunctioning copy of the gene. Their results suggest that one day it might be possible to provide Menkes disease patients with a functioning ATP7A gene.

"Even with our best efforts to date, mortality from this disease remains high," Dr. Kaler said. "These pre-clinical findings in a mouse model of the disease suggest that gene therapy may be a way to help those human infants with Menkes disease who are the most difficult to treat at present."

The study's first author was Anthony Donsante, Ph.D., also from the Unit on Human Copper Metabolism. Other authors included researchers at the NIH's National Institute of Neurological Disorders and Stroke, the University of Minnesota Medical School in Duluth and the Armed Forces Institute of Pathology.

The study findings appear online in the journal *Molecular Therapy*.

The researchers conducted their study using a strain of mice with a largely disabled copy of ATP7A. In these mice, the ability to transport

copper is severely disrupted. The animals do not respond to copper injections alone and typically die within two weeks of birth. Functional ATP7A genes were inserted into the animals' brains via a harmless virus carrying the gene. Once injected, copies of the virus entered some of the cells in the animals' brains. The ATP7A gene then began functioning inside these brain cells and increased the ability to process copper.

The researchers divided the mice into three groups. They injected copper alone into the first group of mice. They injected the ATP7A-containing virus alone into a separate group of mice. Mice in both of these groups tended to live two to three days longer than did untreated mice. However, none survived beyond three weeks, the age at which mice typically are weaned from their mothers.

In a third group of mice, the researchers first injected the ATP7A-containing virus and then, a day later, a dose of copper. On average, these mice survived three times longer than did the untreated mice. Nearly 90 percent survived to weaning. Ten months after they were born, more than 20 percent were still alive.

On closer examination, the researchers learned that copper levels were much higher in the brains of mice receiving the treatment of ATP7A plus copper than were copper levels in the brains of the untreated mice. In addition, the brains of the treated mice showed far less damage than those of untreated mice.

Dr. Kaler explained that the study's success may have been due to the area of the brain where the ATP7A-carrying viruses were injected. The researchers injected the treatment inside the brain's ventricles-fluid-filled cavities inside the brain. This fluid is referred to as cerebrospinal fluid, because it bathes the brain and spinal cord. Cerebrospinal fluid is manufactured by specialized cells inside the ventricles, the choroid plexus epithelial cells. When the researchers examined brain tissue from the mice, they found that the injected ATP7A gene had been incorporated into these cells.

"Throughout life, choroid plexus cells are replaced very infrequently, which suggests that the beneficial effect of this treatment could persist indefinitely," Dr. Kaler explained.

Direct injections of the combined treatment into the brain ventricles were shown to be effective in mice. But direct injections into the brain carry a risk of swelling and infections. For patients with Menkes disease, it would be preferable to find other, less intrusive, ways to deliver the replacement gene. One potential strategy would be to deliver the treatment into the cerebrospinal fluid of the spinal cavity in the lower back, using a common injection procedure known as lumbar puncture.

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