Researchers develop DNA 'patch' for canine form of muscular dystrophy

March 16 2009

Using a novel genetic technology that covers up genetic errors, researchers funded in part by the National Institutes of Health have developed a successful treatment for dogs with the canine version of Duchenne muscular dystrophy, a paralyzing, and ultimately fatal, muscle disease.

The technology, known as "exon skipping" uses tailor-made snippets of DNA-like molecules as molecular "patches." These patches cover up mutant DNA sequences that code for making an important muscle protein. The mutant sequences occur in portions of the gene known as exons, which contain the information needed to make the muscle protein. By covering up the mutant regions, the DNA patches allowed the dogs to make an imperfect—but functional—version of the protein, and significantly improve their muscle functioning.

Earlier studies showed that it was possible to inject the patches into the bloodstream of mice and deliver them throughout the animals' bodies. The current finding shows that the DNA patches could be delivered by injection throughout the entire body in a much larger animal than a mouse, raising the possibility that they might be successfully delivered throughout the body to human muscles as well. Moreover, the current study represents an advance over the earlier efforts in that it was able to use several different kinds of DNA patches. A combination of different patches, known as a cocktail, would be needed to treat most of the human cases of the disease, which can involve many different exons.

The canine version of Duchenne muscular dystrophy occurs naturally in dogs, and affects the same gene that is affected in the human form of the disease.

"This is a promising finding," said Duane Alexander, M.D., director of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), one of the NIH institutes that provided partial funding for the study. "It's an important step toward realizing the goal of developing a treatment that could alleviate the symptoms of this disorder."

Funding was provided through the NIH Muscular Dystrophy Consortium, which conducts research on muscular dystrophy. In addition to the NICHD, other institutes supporting the consortium are the National Institute of Arthritis and Musculoskeletal and Skin Diseases and the National Institute of Neurological Disorders and Stroke. The NICHD provided additional funding for the study through its National Center for Medical Rehabilitation Research. Other funding was provided by the Foundation to Eradicate Duchenne, Inc., the Department of Defense CDMRP program, the Jain Foundation, Inc., the Crystal Ball Event of Hampton Roads and the Muscular Dystrophy Association, and the Ministry of Health, Labour, and Welfare of Japan.

Muscular dystrophies are a group of disorders causing muscle deterioration and weakness. Duchenne muscular dystrophy occurs almost exclusively in males, affecting 1 in every 3,500. Symptoms begin at about 3 years of age, with muscle weakness resulting in difficulty walking and talking. Most boys with the condition lose the ability to walk by age 12, and death usually occurs by the early 20s, from heart and respiratory failure.

The study was published on line in the *Annals of Neurology* and conducted by Toshifumi Yokota, Ph.D., and Eric Hoffman, Ph.D., of

Children's National Medical Center, Washington, D.C., and Shin'ichi Takeda, M.D., Ph.D., of the National Center of Neurology and Psychiatry, Ogawa-Higashi, Kodaira, Tokyo, Japan, as well as other researchers at Children's, Carolinas Medical Center, Charlotte, N.C. and the National Center in Japan

Duchenne muscular dystrophy results from errors in the gene for dystrophin, a key component of muscles. Dr. Hoffman explained that individuals with Duchenne muscular dystrophy vary in the locations and kinds of the mutations occurring in the gene. Also, many boys with Duchenne muscular dystrophy have mutations that affect multiple exons, and so would require more than one kind of patch. The dogs in the study carried a mutation affecting multiple exons. The researchers used a cocktail containing multiple DNA patches to bypass the affected exons.

The patches, DNA-like molecules called morpholinos, are manufactured in a laboratory. Injections of the morpholino cocktail directly into the dogs' bloodstream curbed deterioration of the animals' skeletal muscles and improved muscle functioning. The injections resulted in widespread production of dystrophin-like protein, at about 26 percent of normal levels. However, the treatment was unable to prevent deterioration of the animals' hearts. The researchers theorized that the muscles of the heart are less porous than the skeletal muscles, and did not absorb sufficient quantities of the morpholinos to curb the deterioration.

The researchers said that other means of delivering the morpholinos to the heart would need to be explored.

They added, however, that the study results showed that it would be possible to use a cocktail of morpholinos to patch the multiple mutations that occur in the human form of the disorder.

Source: NIH/National Institute of Child Health and Human

Development