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A new study shows that combining the supplement creatine and the antibiotic minocycline significantly slows disease progression and prolongs survival in a mouse model of amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease. The combined treatment was significantly more effective than either compound administered alone. Both creatine and minocycline have previously been shown to improve outcomes in a mouse model of this disabling neurological disease, but this study is the first to test a combination of the two.

"Given that these two compounds each work in ALS mouse models, we thought combining them might have an even stronger effect on symptoms and disease progression," says lead author Robert Friedlander, M.D., M.A., associate professor of neurosurgery at Brigham and Women's Hospital and Harvard Medical School in Boston. "Our results show that this kind of cocktail approach might be a new potential strategy for treating ALS." The study was funded in part by the DHHS's National Institute of Neurological Disorders and Stroke (NINDS) and appears in the February 2003 issue of *Annals of Neurology*. [1]

ALS is a progressive and fatal neurological disease affecting nerve cells that control movement. More than 5,000 Americans are diagnosed with ALS each year, and most patients die within 3 to 5 years after symptoms begin.

Creatine is an amino acid that is found naturally in meats and fish, and is also marketed as a dietary supplement. In 1999, researchers showed that creatine was better at prolonging survival in a mouse model for ALS than the prescription drug riluzole, which is already used to treat people with ALS. Diets supplemented with creatine extended the lives of ALS mice by about 18 percent compared to unsupplemented diets, while riluzole extended survival in mice by about 9 percent.

Researchers do not know how creatine works to slow the progression of ALS in mice, but Dr. Friedlander says there are several different hypotheses. "We are working very hard to understand the mechanism of creatine in mice with ALS," he says, adding that the most widely accepted hypothesis is that creatine may improve neurons' energy supply, making them more resistant to degeneration.

Minocycline is an antibiotic that has been used for about 30 years to treat a variety of infections, as well as acne and rheumatoid arthritis. In May 2002, Dr. Friedlander and colleagues published a study demonstrating neuroprotective effects of minocycline in an ALS mouse model. [2] Previous studies have also shown that minocycline protects neurons from dying in animal models of Huntington's disease, Parkinson's disease, stroke, traumatic brain injury, and a variety of other disorders.

Minocycline has at least two possible mechanisms of action for treating ALS. Recent studies have shown that problems in mitochondria -- tiny rod-shaped compartments within cells that break down food and produce energy - lead to the death of nerve cells controlling movement in ALS mice. Dr. Friedlander and colleagues recently published findings demonstrating that minocycline makes the mitochondria more resistant to changes that may trigger cell death. ALS also causes certain immune cells in the brain, called microglia, to release toxic compounds, Dr. Friedlander says. Minocycline appears to block this reactivity of microglia.

In the new study, Dr. Friedlander and colleagues studied mice with a mutation in the human SOD1 gene, which is found in about 20 percent of patients with familial ALS. Mice with this mutation develop nerve damage and neurological symptoms that mimic those of ALS in humans.

The researchers fed one group of 10 mice a diet supplemented with 2 percent creatine beginning at 3 weeks of age. Once the mice reached 4 weeks of age, the researchers also gave them injections of minocycline once daily until they died or were too sick to be tested further. For comparison, three other groups of mice received a creatine-supplemented diet plus saline (salt water) injections, minocycline injections alone, or saline injections alone.

The researchers assessed each mouse's motor strength and coordination on a weekly basis, starting at 10 weeks, by observing its ability to remain standing on a rod rotating at 5 and 15 revolutions per minute (rpm) for up to 7 minutes. Disease onset was defined as the first day a mouse could not remain on the rod for 7 minutes at 15 rpm.

The minocycline injections and creatine supplements delayed disease onset to 113 and 111 days, respectively, compared with 94 days in the untreated group. The two drugs delayed mortality to 142 and 141 days, respectively, compared to 126 days in the control group - a 13 and 12 percent improvement in survival. However, mice in the minocycline-creatine combination group did significantly better than mice receiving either creatine or minocycline alone. They did not show disease onset until 122 days and they survived for an average of 157 days - a 25 percent improvement in survival compared to untreated mice.

Dr. Friedlander says that this particular combination is especially promising because the mechanisms of action of the two compounds are different. "To block a disease pathway, you want to target different steps within the pathway. Although we don't fully understand the mechanism of creatine-mediated neuroprotection, we do know that it is not the same as the minocycline mechanism."

Researchers are currently testing the safety and efficacy of both creatine and minocycline in clinical trials for ALS. So far, there have been no significant negative side effects for either compound, although extremely high doses of creatine can cause kidney problems, says Dr. Friedlander. Both compounds also cross the blood-brain barrier and are effective when taken orally, making them good candidates for human clinical trials.

Before human trials of minocycline plus creatine can be conducted, researchers need to do further studies to better understand the disease process of ALS and to understand the mechanism, safety and appropriate doses of the combination treatment. Researchers are also working to develop new drug combinations for treating ALS in animal models.

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The NINDS is a component of the National Institutes of Health, within the U.S. Department of Health and Human Services, and is the nation's primary supporter of biomedical research on the brain and nervous system.

Reference:

[1] Zhang W, Narayanan M, Friedlander RM. "Additive Neuroprotective Effects of Minocycline with Creatine in a Transgenic Mouse Model of ALS," *Annals of Neurology,* February 2003, Vol. 53, Issue 2, pp. 267-270.

[2] Zhu S, Stavrovskaya IG, Drozda M, Kim BYS, Ona V, Li M, Sarang S, Liu AS, Hartley DM, Wu DC, Gullans S, Ferrante RJ, Przedborski S, Kristal BS, Friedlander RM. "Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice." *Nature,* May 2, 2002, Vol. 417, No. 6884, pp.74-78.