Potential stroke treatment may extend time to prevent brain damage

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A naturally occurring substance shrank the size of stroke-induced lesions in the brains of experimental mice — even when administered as much as 12 hours after the event, Stanford University School of Medicine researchers have shown. The substance, alpha-B-crystallin, acts as a brake on the immune system, lowering levels of inflammatory molecules whose actions are responsible for substantial brain damage above and beyond that caused by the initial oxygen deprivation of a stroke.

The finding, which will be published online July 25 in *Proceedings of the National Academy of Sciences*, is of great potential significance. Every year brings nearly 800,000 new stroke patients in North America. "That's one every 40 seconds," said Gary Steinberg, MD, PhD, director of Stanford's Institute for Neuro-Innovation and Translational Neurosciences and one of the study's two senior authors. Steinberg is also the Bernard and Ronni Lacroute-William Randolph Hearst Professor of Neurosurgery and the Neurosciences, and chair of neurosurgery at the medical school.

The largest single cause of severe neurological disability and the third-leading cause of death in the United States, stroke accounts for an estimated $74 billion annually in related costs, including treatment and additional assistance for the three of every four stroke patients whose ability to perform the activities of daily life is impaired. Strokes are caused by a sudden drop in the flow of blood to the brain resulting from a clot or, less often, bleeding. One of every three stroke patients is under the age of 65. In all, there are 5.4 million stroke survivors in the United

States and 15 million worldwide.

The only currently approved drug for stroke — tissue plasminogen activator, or tPA — dissolves clots that keep oxygenated blood from reaching brain tissue. To be effective, tPA must be administered within about 4.5 hours after the stroke. But patients' brains must first be scanned to rule out the possibility that the stroke was caused by bleeding, which tPA would exacerbate, rather than by blockage.

Moreover, tPA does nothing to counter the stroke's insidious inflammatory aftershock: a flood of noxious chemicals secreted by angry immune cells that rush in to the affected area, causing significant further damage.

Alpha-B-crystallin appears to act as a sponge, sopping up those bad actors and stopping inflammation from making a bad situation worse.

Alpha-B-crystallin is a major structural protein in the eye's lens. It is also constantly made in the heart. In other tissues, including the brain, its production can be triggered by stressful events, such as oxygen deprivation or excessive heat or cold. Growing evidence suggests that alpha-B-crystallin can help curb inflammatory activity in the brain.

"The brain doesn't roll over and play dead when it's under attack," said Lawrence Steinman, MD, the other senior author of the new study, who is the George A. Zimmermann Professor of Neurology and Neurological Sciences and Pediatrics as well as chair of Stanford's interdepartmental program in immunology.

In an earlier study, published in Nature in 2007, Steinman and his colleagues found that the presence of alpha-B-crystallin could help reduce the severity of brain damage caused by multiple sclerosis, a chronic, debilitating autoimmune disease of the brain. Other studies

published this year by his group have shown that alpha-B-crystallin limits the damage caused by blood-supply cutoffs to heart tissue and the retina.

It seemed logical to see if this protein could mitigate the effects of a stroke. "We made a jump from its relevance in inflammatory diseases such as multiple sclerosis," Steinberg said. "To my knowledge, nobody had looked at concentrations of alpha-B-crystallin after a stroke, either in people or in an experimental animal model before."

So, along with first authors Ahmet Arac, MD, a postdoctoral scholar in Steinberg's lab, and Steinman's former graduate student Sarah Brownell, PhD, Steinberg and Steinman turned to a standard animal model: the laboratory mouse. They found that, in mice bioengineered to lack alpha-B-crystallin, experimentally induced stroke lesions were more massive than those induced in otherwise genetically similar mice whose cells were capable of making the protein. The alpha-B-crystallin-deficient mice had worse neurological function after the stroke than did the normal mice.

The researchers also found that supplying synthetic alpha-B-crystallin to the deficient mice reduced brain-lesion sizes after a stroke, even when the substance was administered 12 hours after the stroke was induced. And they saw elevated alpha-B-crystallin levels in blood plasma from both human patients and mice after a stroke. (The human samples were obtained from study co-author Gregory Albers, MD, the Coy Foundation Professor of Neurology and Neurological Sciences and the director of the Stanford Stroke Center).

"In younger patients, the larger the stroke, the higher the concentration of alpha-B-crystallin," said Steinberg. Interestingly, increased alpha-B-crystallin levels were not detected in plasma from patients over the age of 80, whose strokes typically have worse consequences than those affecting younger patients.

Finally, the investigators demonstrated that alpha-B-crystallin-treated mice produce fewer inflammatory immune-signaling molecules and more anti-inflammatory ones than untreated mice.

At the doses given to the mice in this study, alpha-B-crystallin appeared to be nontoxic. "This is a naturally occurring molecule the body is already producing, although maybe just not enough of it," said Steinberg. "We're just supplementing it." If further studies by other labs and in other models confirm and extend the findings, alpha-B-crystallin may be an excellent candidate for clinical trials in stroke, Steinman and Steinberg both said.

"This is the first demonstration of an efficacious brain-protecting agent that targets the inflammatory aspect of stroke in a novel way, and it can be given at quite a delay," said Thomas Carmichael, MD, PhD, professor and vice chair of neurology at the David Geffen School of Medicine at UCLA. Carmichael, a stroke expert, did not participate in the study but is familiar with its methodology and results. "Tissue plasminogen activator has a fairly narrow risk-to-benefit ratio. The longer you wait, the more likely it is to stimulate a hemorrhage."

Provided by Stanford University Medical Center