Researchers discover drug target for stimulating recovery from stroke

March 21 2012

Investigators at the Stanford University School of Medicine have shown that removing a matched set of molecules that typically help to regulate the brain's capacity for forming and eliminating connections between nerve cells could substantially aid recovery from stroke even days after the event. In experiments with mice, the scientists demonstrated that when these molecules are not present, the mice's ability to recover from induced strokes improved significantly.

Importantly, these beneficial effects grew over the course of a full week post-stroke, suggesting that, in the future, treatments such as drugs designed to reproduce the effects in humans might work even if given as much as several days after a stroke occurs. The only currently available stroke treatment — tissue plasminogen activator, or tPA — must be given within a few hours of a stroke to be effective, and patients' brains must first be scanned to determine whether this treatment is appropriate. Moreover, while tPA limits the initial damage caused by a stroke, it doesn't help the brain restore or replace lost connections between nerve cells, which is essential to recovery.

The mice in the study had been genetically bioengineered to lack certain molecules that one of the Stanford researchers had previously shown to play a major role in modulating the ease with which key nerve-cell connections are made, strengthened, weakened or destroyed in the brain. The molecules in question include "K" and "D," two of the 50 or so members of the so-called MHC class-1 complex, which plays a key role in the function of the immune system. Alternatively, when a receptor

called PirB, which binds to these MHC molecules, is not present, the same improved outcome from stroke happens — significant, because receptors make good drug targets.

It was only a few years ago that Carla Shatz, PhD, professor of neurobiology and of biology, and her colleagues surprised the neuroscience and immunology communities by showing that these molecules "moonlight" in the brain. Here, their job appears to involve inhibiting the readiness of connections among nerve cells (known as synapses) to grow stronger or weaker in response to experience.

Learning and memory require the constant, coordinated strengthening of some synaptic connections and weakening of others. But this very flexibility, if it becomes excessive, is thought to put the brain at risk for conditions such as epilepsy or schizophrenia. The molecules Shatz has been exploring can be seen as providing a measure of stabilizing ballast.

However, in order to re-establish brain functions that have been lost in the massive nerve-cell die-off that follows an extraordinary event such a stroke, it's necessary to restore lost synapses and form new ones at a rapid pace. It's also important to retrain surviving circuits to take over functions formerly served by lost circuits — this is the basis of rehabilitation therapy.

Under such circumstances, one might ask, might it be a good idea to ease up on the brake pedal?

"Nobody had ever thought any of these molecules had anything to do with stroke," said Shatz, who is the Sapp Family Provostial Professor and also is the director of Bio-X, Stanford's interdisciplinary biosciences research consortium. "But our lab had shown in 2009 that mice bioengineered to lack them performed like Olympians on motor-learning tasks."

A couple of years ago, Shatz and her colleague, Rona Giffard, MD, PhD, professor of anesthesia and a veteran stroke researcher, grew bored during a scientific meeting and began whispering about their work "to cheer ourselves up," Shatz said. It occurred to them that teaming Shatz's molecules with Giffard's animal-research expertise could provide answers to this question.

The results, which will be published Mar. 22 in *Neuron*, were unequivocal and potentially quite clinically significant: Mice genetically engineered to lack either K and D or PirB, a major cell-surface receptor for these molecules, experienced markedly better recovery in their motor performance after a stroke than did normal mice. Giffard and Shatz are the senior authors of the Neuron study.

"This is the very first time anyone has looked for a role of these molecules in stroke, or in recovery from stroke," said Giffard. "Targeting recovery, as opposed to just halting the damage, would have the widest possible chance to help patients after stroke, and could help patients who cannot receive tPA."

The collaboration was accelerated by the fact that Jamie Adelson, a PhD candidate in Shatz's laboratory who shared first authorship of the study with postdoctoral researcher George Barreto, PhD, of Giffard's lab, had worked in the Giffard lab before joining Shatz's group.

Tests indicated that concentrations of K and D — which in a healthy brain are already abundant at synapses — rose dramatically in normal mice's brains after an induced stroke, perhaps inhibiting recovery.

The researchers trained their mice in certain athletic activities, such as balancing on a spinning horizontal rod at gradually increasing rotation speeds, or traversing the rungs of a small "ladder" suspended horizontally just a half-inch or so above a board. Then the researchers induced

strokes in the trained animals by cutting off blood supply to a region of the brain that is involved in motor performance. One week later, the animals lacking K and D had recovered their athletic skills substantially better than the normal animals had. Moreover, lab tests showed that the stroke-affected area of the K/D-deficient mice was considerably smaller than was the case for the controls.

Observations showed that sprouting of new nerve fibers in stroke-affected areas was more abundant in the K/D-deficient mice, too.

Next, the team turned to K and D's counterpart receptor PirB, which binds to K and D as well as to many similar MHC molecules. Shatz has previously shown that this molecule, well-known to immunologists, abounds in the brain. Receptors make excellent targets for therapeutic intervention, as small molecules that bind and block them can often be designed by pharmaceutical and biotech companies and academic pharmacologists. In addition, PirB is the key receptor not only for K and D, but also for all of the roughly 50 different but related MHC molecules that have been identified so far. So blocking PirB could, in theory, be more efficient than attempting to block each of the 50-plus MHC molecules independently or impairing only one or a few of them.

So the Stanford researchers performed similar tests on mice genetically engineered to lack PirB. Just like the mice lacking K and D, PirB-deficient mice recovered their athletic ability better than normal mice did. Not only did PirB-lacking mice's brains show smaller areas affected by the induced stroke than the normal mice's brains did, but these areas also were noticeably smaller one week after the stroke than they were a day afterward, suggesting that restoration of damaged tissue may have occurred. In a dish, glucose- and oxygen-deprived hippocampal slices from the PirB-lacking mice suffered less nerve-cell death than slices from normal mice.

One drug-development expert, Corey Goodman, PhD, called the findings "exciting." Goodman is chair of the board of four biotechnology companies, a board member of two others and co-founder and managing director of venBio LLC, a venture-capital firm. Goodman, who is familiar with the study but was not involved in it, spent 25 years as an academic neuroscientist at Stanford and the University of California-Berkeley before moving into commercial drug development. He noted that, as is typical in early research, what works in a mouse doesn't necessarily translate into human benefits. He said he'd also like to see the results of experiments with normal mice in which MHC/PirB binding could be blocked conditionally (for example, with a small molecule that could enter the brain and bind to PirB), starting at different time points after an induced stroke. "To take this into human trials, you'd need a molecule that could get into the brain and that's very specific to PirB" or MHC molecules, he said.

Nevertheless, Goodman pronounced the results "encouraging."

"There is, today, very little we can do for stroke patients, and it has to be done almost immediately," he said. "What makes me optimistic is that this mechanism has to do with growth and repair that takes place days later. This approach has the look of one that might be able to work as long as days and weeks after a stroke."

Provided by Stanford University Medical Center