Tasha awoke early, unable to feel the right side of her face: it was numb. At first, she thought she was still dreaming, but she heard her husband downstairs in the kitchen, and knew that she was awake. She got herself up and checked the mirror for marks on the side of her face from sleeping in an awkward position. She saw nothing. When she put her hand to her face, she suddenly felt a profound weakness in her right arm. Through habit and an act of sheer will, Tasha washed and dressed, intending to make her way to work, where she served as the executive assistant to a bank vice president. Tasha had a full day ahead of her, and whatever was going on would have to be dealt with later. A 52 year-old African American woman, Tasha was always there for everybody—her growing children, her husband, her boss, and her coworkers—which did not leave much time for herself. Things such as regular exercise and a healthy diet were luxuries she could not afford. She had steadily gained weight during the past two decades and felt the extra burden of her size as she tried to make her way down the stairs.

At the top of the stairs, she felt her right leg buckle; it also was numb and weak. Vision in Tasha's right eye became blurry, and the straight staircase looked like a spiral. Feeling dizzy, she sat down on the landing; her balance and coordination had left her. She called out in fear, and her husband rushed to her. But when he asked what was wrong, Tasha felt confused. She had the worst headache of her life. Tasha's husband called 911, and she was rushed to the hospital. Tasha had had a stroke.

Stroke—"brain attack"—continues to be a killer and advances in treatment have been disappointing. Several clues to its causes that have emerged in population studies are not fully understood, and their significance in developing effective treatments is not clear. Although stroke occurs with similar frequency in men and women, women have strokes at a younger age and die more frequently. African Americans are three to five times more likely to have strokes than are Caucasians. Risk factors include high fat diet, obesity, high blood pressure, diabetes, and smoking. Correcting these habits and conditions should reduce the incidence of stroke, but stroke is complex and represents a major scientific and medical challenge.

A STRATEGY TO PROTECT THE BRAIN

Developing a safe and effective therapy to protect the brain after a stroke, a process known as "neuroprotection," represents a major unsolved challenge for researchers. Current medications prescribed after stroke do not do this, and a great deal of research has focused on developing a safe and effective therapy for use after stroke. However, while a long list of neuroprotective compounds has shown encouraging results in experimental models in animals, most of these potential medications have resulted in disappointing outcomes after clinical trials in humans.[1-3]

We can identify many reasons for this failure. First and foremost, the onset of stroke triggers a complex process in the brain that includes multiple cellular and molecular events occurring at different times. As a result, the target at which any particular drug is aimed may not be a key factor in disease development. In an ischemic stroke—that is, one caused by a blood clot—the brain is very sensitive to the phase when the blood begins to flow again, what is called "reperfusion." Therefore, the time window during which a neuroprotectant might help is also important. In addition, the method of delivering of the drug to a stroke patient is critical, because a drug's concentration, at a specific cellular site in the brain, during a relatively prolonged period of time, must be appropriate. Moreover, the actual design of the clinical trial itself, including the timing for evaluating the outcomes, affects the results. Another challenge is that animal models of a disease such as stroke often show only part of the clinical picture seen in humans. Although animal experiments will continue to contribute new knowledge and significant breakthroughs, we must be careful about the design of those studies and not overinterpret the results.

Many efforts have been made to block the destructive processes that stroke triggers in the brain, and often emphasis is placed on understanding how brain cells die. In my laboratory, we have used a different approach: we try to gain insight into how the brain defends itself. That is, we aim to unravel the chemicals that the brain generates to survive. Chemicals of this general type, which make things happen in cells, are termed "signals" or "messengers." Our work has identified novel signals, or messengers, that promote brain cell survival, and we are studying these messengers to see how they can be used for neuroprotection in stroke. This is a step by step

process, first understanding how the brain defends itself against the consequences of stroke, then figuring out how to exploit those natural, endogenous mechanisms for protecting the brain. We have focused, in particular, on the significance of the essential fatty acid DHA (docosahexaenoic acid) that is present in high quantities in the cell membranes of the brain.

A distinguishing characteristic of the central nervous system is its highly networked organization of synapses and closely interacting cells that results in one of the largest membrane surface areas of all cells of the human body. To understand this, imagine the membrane of a neuron in the hippocampus, spread out on a flat surface. You will see, as part of the large surface area, the extensive branching and complexity of dendrites and dendrite spines (largely made up of postsynaptic membranes). During brain development, many complex proteins and other molecules organize and build these dendrites. The dendrites' shape and length dramatically change during both learning (strengthening) and aging (progressive decline). In stroke, a sudden loss of synapses occurs, while in Alzheimer's disease, the demise of synapses is slow and ongoing.

Other brain cells (astrocytes, oligodendrocytes, and microglia) also have very large plasma membrane surface areas. These cells interact with each other and with endothelial cells that line blood vessels in the brain. All of these cells also have extensive intracellular membranes.

Phospholipids (water insoluble substances, or "lipids," that contain phosphorous) are major structural constituents of these cell membranes, and their role has not been sufficiently appreciated. Small specific pools of membrane phospholipids are reservoirs of potent, biologically active lipids. When cell membranes are stimulated by cell signaling activity, enzymes (called phospholipases) free lipid messengers from these reservoirs. The lipid messengers then regulate and interact with other signaling cascades to contribute to the development, differentiation, function protection, and repair of the nervous system.[4]

This release of lipid messengers has put us on the track of one messenger with great promise in neuroprotection. Some of these messengers are derived from essential fatty acids contained in phospholipids. (Essential fatty acids are those that the human body is unable to make and that, as a consequence, we must obtain from what we eat.) One of these essential fatty acids, DHA (a member of the omega-3 family of essential fatty acids abundant in fish) is more plentiful in the human central nervous system than in any other part of the body.

DHA's neurobiological significance is just beginning to be clarified, but we know that it is continuously required for forming and maintaining the structural and functional integrity of membranes of neurons and photoreceptors. DHA is involved in learning, memory, and vision. The brain's supply of DHA is provided by the liver, where DHA is incorporated into the bloodstream's lipoproteins for delivery to the brain cells where it is needed.

Stroke fosters increased oxidative stress in the brain, a process triggered by toxic cell products formed as a result of excessive oxygen. Oxidative stress damages many brain cell components. For example, it impairs the mitochondria, the power house of the cell, and it disturbs the communication between neurons. Oxidative stress triggers chain reactions within the cell and initiates a process known as apoptosis, which, in essence, is a cell death from within, because the DNA of genes is chopped off in pieces and the cell falls apart. Since oxidative stress also triggers the release of DHA, our research team turned our attention to whether DHA could tell us how oxidative stress in stroke might be countered.

A NEWLY DISCOVERED MESSENGER IN THE BRAIN

Using sophisticated technology, we recently detected in the brain the synthesis of a messenger we call neuroprotectin D1 (NPD1)—a messenger made from DHA through oxygenation.[5] (In this instance, oxygen is good, because it is added in a well-regulated reaction, unlike excessive oxygen produced by oxidative stress.) We wanted to find out if NPD1 was a participant in the brain's response to stroke.

To find out, we used a procedure in experimental animals called "middle cerebral artery nylon suture occlusion," (MCA-O) to cut off blood flow in the artery for two hours, producing an infarct—an area of damaged tissue resulting from obstructed circulation—that resembles an ischemic stroke caused by blockage of the middle cerebral artery in humans. This experimental technique also allows a restoration of blood flow resembling reperfusion in human ischemic stroke. Animals that undergo this procedure develop large infarcts and, as a result, have severe neurological deficits.

We discovered that when stroke activates the pathway in the brain described above—by which enzymes free lipid messengers—DHA gives rise to NPD1, and NPD1, in turn, strongly inhibits some of the damage (infiltration of white blood cells and expression of the enzyme COX-2, both of which are part of the inflammatory process) that results from ischemia and reperfusion as well as decreasing the size of the stroke infarct as shown in Figure 1. In the side of the brain affected by the stroke, NPD1 peaks at 8 hours, and after 25 hours of restored blood flow, it is still elevated, reflecting the ability of the brain to form NPD1 from endogenous DHA.

In another experiment, NPD1 was infused using a minipump into the third ventricle of the brain of experimental animals undergoing 48 hours of reperfusion after 1 hour of artery occlusion (MCA-O) and neuroprotection took place. This result demonstrates directly the neuroprotective bioactivity of NPD1.[6]

Further experiments demonstrated that NPD1 acts within the brain cell, before damage to the mitochondria can occur, by serving as a brake for oxidative stress in the cascade of events that leads to cell death. This messenger enhances proteins that promote cell survival and decreases proteins that facilitate cell death, as shown in Figure 2. The cumulative outcome of the actions of NPD1 is protection of cell integrity and function.

Discovery of NPD1 has revealed some of the ways the brain modulates its response to inflammatory injury and provides potential targets for new drugs to treat neurological disorders, in addition to stroke, that have a neuroinflammatory component, such as traumatic brain injury, spinal cord injury, and degenerative diseases such as Parkinson's and Alzheimer's diseases.

BOLSTERING NPD1 IN THE BRAIN AFTER STROKE

Albumin, an important protein in blood plasma (fluid), is able to carry fatty acids in the blood stream by way of sites on the protein's surface where the fatty acids attach. Our research team decided to take advantage of this transport function to try delivering DHA to the brain after stroke. We reasoned that albumin carrying DHA, if injected intravenously in the peripheral circulatory system, would gain access to the damaged brain, since stroke disrupts the normal blood-brain-barrier that prevents many substances from entering the brain. If albumin-mediated delivery of DHA to the brain was successful, it would promote synthesis of NPD1 and hence neuroprotection.

To test this prediction, we used the MCA-O procedure in experimental animals. When we tested the animals 72 hours after treatment with DHA-albumin (administered intravenously two hours after the two-hour blockage), the animals' neurological test scores were better than those of animals treated with albumin alone. Infarcts in the cortex of the DHA-treated animals were 86 percent fewer than in animals injected with only a saline solution. Similarly, the size of infarcts in animals who received the DHA-albumin was 70 percent less than that in the saline-treated animals. Albumin alone did not significantly reduce infarcts in the part of the brain called the corpus striatum, but DHA-albumin reduced them by 50 percent. In addition, swelling of the brain in animals who received the DHA-albumin was reduced by 58 percent. When we analyzed the animals' brain chemistry 20 hours after the experimentally caused strokes, we discovered a large accumulation of NPD1 in the hemisphere on the same side as the experimental stroke in animals treated with DHA-albumin, showing a correlation between NPD1 and remarkable neuroprotection.[7]

A clinical trial sponsored by the National Institutes of Health is studying the use of albumin alone in human stroke patients. This trial was based on previous research in animals by Myron Ginsberg, M.D., at the University of

Miami showing that moderate- to high-dose human albumin therapy is neuroprotective in models of experimentally-induced cerebral ischemia.[8] Initial results of the clinical trial have demonstrated that human serum albumin injected into the blood stream does elicit neuroprotection. Shortcomings in the procedure were found, however, because high-dose albumin, while effective in protecting the brain, also thickens the blood and may, on occasion, precipitate congestive heart failure. As a consequence, using a lower concentration of albumin is desirable, but this lower concentration is not as effective as a higher one. Thus DHA combined with low dose albumin, as we have demonstrated, may be a safe and effective approach to protecting the brain after stroke.

HOPE FOR NEUROPROTECTION

The brain responds to any kind of injury with a plethora of signals, some of which are harmful and some of which are protective; the preponderance of signals in either direction influences the overall outcome (neuronal survival or death). Such is the case with inflammatory and counter-inflammatory lipid messengers. Therapeutic strategies targeting neuroprotection will mount the first truly effective defense against brain damage in stroke and very possibly prove to be the most effective approach to other central nervous system disorders with a neuroinflammatory component.

Stroke depletes discrete specific pools of DHA from the brain, first, because ischemia releases free DHA within the tissue and, second, because the surge of oxygen when blood flow is restored activates excessive oxidative stress and consumes more DHA. But lipids such as DHA are involved in maintaining the structural and functional integrity of brain cell membranes and, therefore, replenishment of DHA after a stroke may be required. DHA from what we eat is essential for this, but most diets do not contain adequate amounts of fish, which is rich in omega-3 fatty acids, including DHA. For reasons we do not yet fully understand, DHA cannot be forced into the brain during a short period of, for example, a diet based predominantly on fish. Therefore, a balanced diet with plenty of fish should be the norm throughout life.

As a therapy in acute stroke, delivery of DHA to the brain—for example by injecting albumin that carries DHA—will provide the brain with the precursor for NPD1 and enable it to rebuild critical phospholipid pools depleted during ischemia and reperfusion. Still to be defined are what signals turn on the formation of NPD1, and we must also learn more details about NPD1's mechanism of action in rescuing brain cells from oxidative stress. This information, in turn, may provide a template for developing new neuroprotective drugs. The brain, after all, knows how to defend itself, and drugs emulating this natural ability could be powerful weapons in the battle against the destruction caused by stroke.

Although research on neuroprotection mechanisms and their application, whether for stroke or for other devastating diseases such as Alzheimer's, has in many ways been disappointing so far, we are learning more each day about the cellular and molecular mechanisms involved in these diseases. At the same time, a new emphasis in translational research is bringing neuroscience discoveries made in the laboratory closer to the patient, such as Tasha with whose story we began.

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