

REPAIRING

the **Brain**

Saving lives by saving brain cells: Researchers at the LSU Neuroscience Center in New Orleans are learning how to help the brain repair itself



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The highway is nearly empty as a blue minivan drives through the night across the Atchafalaya. Suddenly, a torrential Louisiana rainstorm begins. The driver, a father of two, is blinded as a tractor trailer barrels past him. Losing control, he careens into the concrete divider and suffers a traumatic blow to his head. The trauma produces cellular disruptions, cutting off the blood supplying precious oxygen to his brain. Within minutes, neurons in the man's brain begin to die. Brain damage, leading to disability or death, is imminent. Two little girls waiting for him at home are about to face life without a father.

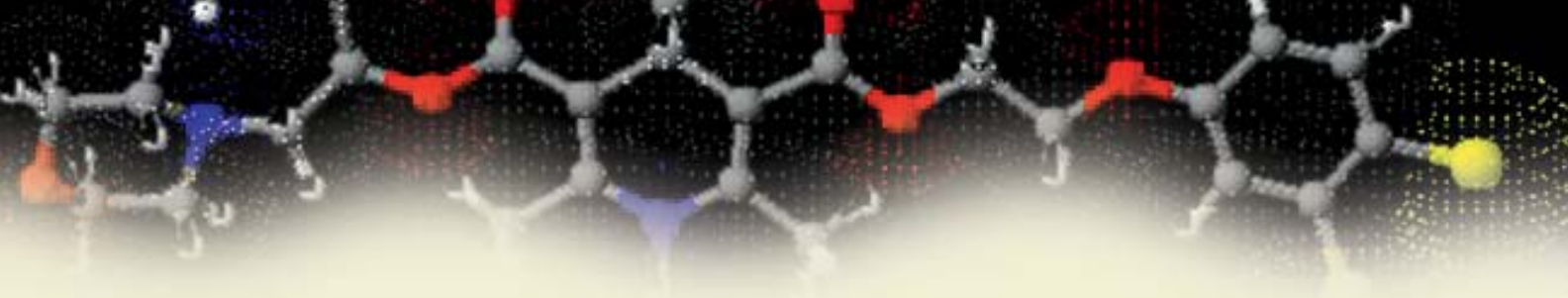
This is a typical scenario for accidents involving head trauma today, but tomorrow's scenarios may be different due to the discovery of neuroprotective approaches by Dr. Nicolas Bazan and his team at the LSU Neuroscience Center of Excellence, a research arm of the LSU Health Sciences Center in New Orleans. In the hands of first-responders, such neuroprotective approaches could save that father's brain before the damage becomes irreversible.

Brain cells (neurons) are vulnerable to lack of oxygen, trauma, oxidative stress (a form of chemical cascade that triggers cell damage and death), and seizures that occur in brain damage, stroke, epilepsy, Alzheimer's and other neurodegenerative diseases. Medical researchers have been investigating for years the biological principles that govern when and how this vulnerability ensues and whether neurons actual-

ly have built-in defenses to promote survival in the face of so many challenges. Bazan's goal has been to elucidate these principles; his dream has been to devise treatments that could activate those built-in defenses to promote survival of injured neurons.

Bazan and his team have recently taken a giant step closer to the dream, with their discovery of chemical signals made in the brain that promote survival of the brain and retina. The retina, at the back of the eye, is also comprised of neurons and operates according to many of the same principles as the brain. Their deciphering of these signals has resulted in the creation of new experimental drugs that have the potential to protect the eyes and brains of people who suffer a stroke or trauma, blinding diseases of the retina such as age-related macular degeneration, and other neurodegenerative diseases.





Nearly 35 years ago, Bazan discovered that brain ischemia or seizures cause a rapid rise in the free form of fatty acids known as arachidonic acid (AA) and docosahexaenoic acid (DHA, an omega-3 fatty acid). He postulated that this rise in AA and DHA caused by seizures and ischemia is related to the activation of an enzyme at the synapse (the area that communicates information, signals, and instructions from one neuron to the next). But Bazan and others in the scientific community have sought an explanation for this phenomenon ever since.

WHAT IS THE SIGNIFICANCE OF DHA?

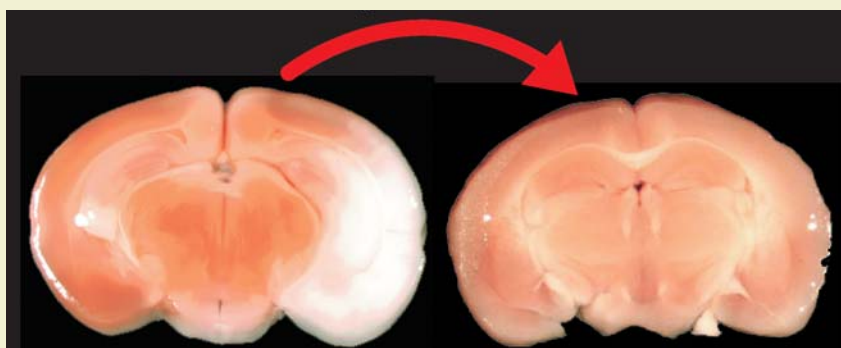
The highest concentrations of the DHA fatty acid are in the brain and retina. DHA must be available in the food we eat: our bodies do not synthesize this essential fatty acid. Several years ago, a specific route from the liver that supplies DHA to the retina and the brain was identified by Bazan. Scientists have determined that these tissues tenaciously conserve the fatty acid through prolonged periods of dietary deprivation, and it is known that long-term DHA deficiencies impair learning and memory. And in the case of blinding retinal degenerations, DHA deficiencies correlate with loss of photoreceptors of the retina and consequent loss of vision.

Bazan found that DHA is converted by enzymes into products that have wide-ranging actions as “messengers” in cells. Several experiments performed in his laboratory served as the basis for the idea that DHA products may promote brain and retina survival. In the 1980s, he and his colleagues first isolated DHA metabolites in the retina and called them “docosanoids.” AA metabolites are collectively known as “eicosanoids” from the Greek root for “twenty,” referring to the number of carbon atoms in the AA molecular backbone; “docosa” is the Greek root for “twenty-two,” the number of carbon atoms in the DHA backbone.

A major technical problem that Bazan ran into in the course of his investigations was that the lipid messengers he was trying to uncover are present in minuscule quantities in the brain and retina. He needed to develop powerful analytical instruments to study them. In response to these challenges, he built facilities at the Neuroscience Center devoted to the nascent science of lipidomics: the study of lipids and their biological activities.

At the heart of the Center’s lipidomics core is a unique instrument—the tandem liquid chromatography-photodiode array-electrospray ionization mass spectrometer—that is capable of assisting in the discovery of previously unknown brain chemicals by working out their structure and measuring them. This instrument distinguishes how individual hydrogen atoms or hydroxyl groups are arranged in a three-dimensional space. By allowing Bazan to test his hypotheses directly, the tandem mass spectrometer technology has moved his research forward exponentially.

Just last year, in a collaboration studying mice undergoing experimental stroke, Bazan and Dr. Charles Serhan from Harvard Medical School discovered the docosatriene




Sections of the brain two days after experimental stroke in mice. The brain on the left displays damage (white area). The red arrow indicates administration of Neuroprotectin D1 drug. After treatment, the damaged (white) area decreases, indicating neuroprotection.

messengers that arise from enzymatic reactions on free-DHA in injured brains. The tandem mass spectrometer tool allowed the collaboration between LSU and Harvard to identify several intermediate molecules that result from the action of the enzymes on DHA released by ischemic injury. The team also identified a second pathway that is activated by aspirin. Since aspirin is used prophylactically in cerebrovascular diseases, Bazan is now molecularly dissecting this action because it has the potential to lead to the discovery of additional brain regenerative mechanisms.

NEUROPROTECTION

When one of these DHA metabolites, 10,17S-docosatriene, was administered to the mice immediately after the experimental stroke, the amount of brain tissue that was stroke-damaged was reduced by approximately one half. Additional experiments showed that 10,17S-docosatriene, in the stroke-affected side of the brain, also had two other significant effects: 1) it reduced the activity of intracellular signaling molecules that drive certain genes to express proteins that stimulate inflammatory



responses, and 2) it decreased the number of brain-invading, injury-producing, polymorphonuclear (PMN) leukocytes. PMNs gather around oxygen-deprived brain tissue following stroke and increase the amount of the damaged tissue.

When a neuron is damaged, a chain of events is set in motion that drives the cell toward a self-destructive death known as apoptosis. During apoptosis, a series of cascades of enzymatic action cut the cell's DNA into fragments. Since a cell's ability to maintain its integrity depends upon energy production through the mitochondria and appropriate gene expression through the DNA, apoptosis renders the cell incapable of maintaining life and the cell (in this case, a neuron) falls apart.

Very recent studies at the Neuroscience Center demonstrate that 10,17S-docosatriene fine-tunes protein expression in the cell to such a degree that it blocks apoptosis and the cell survives. To make these cutting-edge discoveries Bazan's team used a human retinal pigment epithelial cell culture. In these retinal cells, 10,17S-docosatriene protected the cells against DNA cleavage; it increased the expression of anti-apoptotic proteins while decreasing that of pro-apoptotic proteins. Due to its neuroprotective ability, Bazan proposed to name the compound Neuroprotectin D-1: science's first identified neuroprotective docosanoid.

CURING BLINDING EYE DISEASES

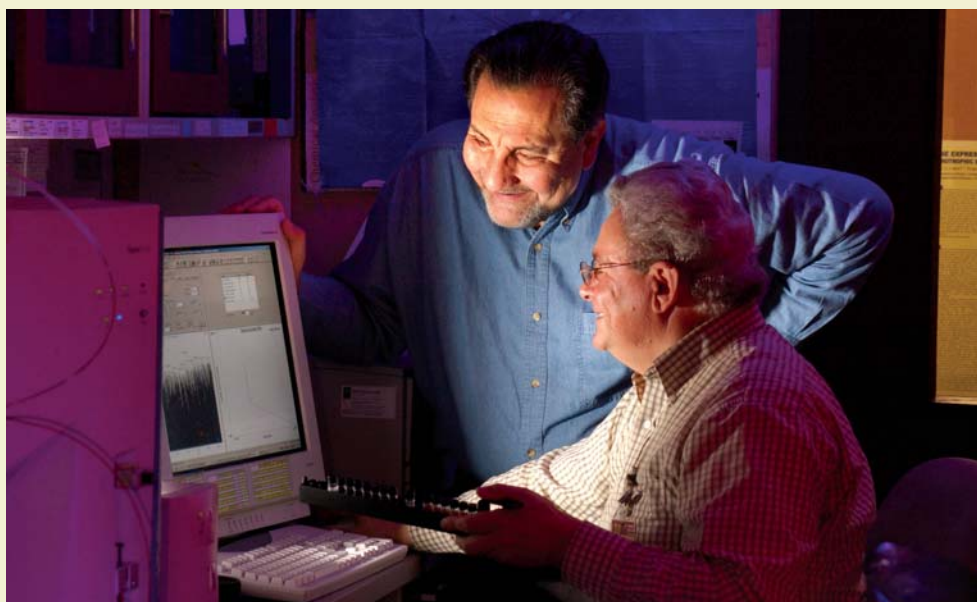
Another critical branch of the cellular and molecular mechanisms that Bazan's group investigated is the DNA damage that results from apoptosis in the retina's pho-

toceptors. It is important to note that the retinal pigment epithelial cell and the photoreceptors have an intimate relationship that results in blindness when broken. Bazan's discoveries also uncovered the critical nature of this intimate relation.

In the wake of these exciting findings, Bazan and his collaborators are currently designing neuroprotective drugs based on Neuroprotectin D-1. Not only does Bazan see the potential for life-saving drugs used by emergency-room physicians on stroke victims, but he also sees their use as essential for first-responders at the scene of car accidents, sports injuries, and other head trauma situations. While the patient's vital functions are being stabilized by first-responders, and typically during the first several hours of emergency medical intervention, head trauma often leads to irreversible brain damage. If neuroprotective drugs are given at the earliest possible moment—at the side of the road by paramedics, for example—catastrophic brain damage could be avoided or limited.

Bazan envisions a future that is even more exciting—one in which these neuroprotective compounds could be attached to nanoparticles or other molecules to freely circulate through the blood to the retina, where they would be released to heal damaged photoreceptors. Similar drug delivery strategies may be used to reach the brain and prevent damage.

It's possible that the innovative approaches being developed at LSU's Neuroscience Center today could one day save a lot of neurons—and save a lot of eyes, lives and families in the process.



Dr. Victor Marcheselli, research assistant professor with the LSU Neuroscience Center, School of Medicine (right), and Bazan review neuroprotection results using lipidomic analysis by tandem mass spectrometry.