Toxic Breakups: The Deadly Relationship Between Alzheimer's Disease and Nitric Oxide Gas

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Summary

Mitochondrial fragments lead to cell death and are found in brains of patients with Alzheimer's disease, but there is no known link between mitochondrial fragmentation and Alzheimer's. Gas produced from a biomarker of Alzheimer's may possibly lead to the fragmentation of mitochondria, thus leading to cell death.

Introduction

Every 70 seconds, someone is diagnosed with Alzheimer's disease, which is a 100% fatal disease[1]. Currently, 5.3 million U.S. citizens suffer from this form of dementia, 2794 of them died in 2006, and it is the seventh leading cause of death[1]. There is no cure, and treatment options are limited, as there are also no definitive tests for this disease. The most distinctive characteristics of Alzheimer's disease (AD) are the tau tangles and amyloid-beta (Aβ) plaques. These biomarkers are only found once a patient has passed away, when an autopsy of the brain is conducted.

Scientists are constantly looking for more biomarkers for the disease, and the search for a living biomarker is of utmost importance because the sooner the diagnosis is made, the sooner treatment can begin. One of the current biomarkers, amyloid-beta (also known as Aβ), is known to release nitric oxide, which causes mitochondria to fragment[2]. However, the exact process is unclear. As mitochondrial fragments are found in brains of Alzheimer's patients, understanding the process of the breakdown of mitochondria and its connection to AD could lead to a possible living biomarker for this devastating disease. In an article from *Science* magazine, researchers Cho and his colleagues clearly display the relationship between mitochondrial fragmentation, nitric oxide from Aβ, and an inconspicuous protein called dynamin related protein, or Drp1[3].

Drp1 is known to be a regulating protein that induces mitochondrial fission (fragmentation), which plays a part in apoptosis (programmed cell death)[4,5,6,7]. In general, apoptosis is a healthy occurrence, just like chocolate is healthy in moderation. However, when Drp1 is mutated, it becomes dysfunctional and is no longer conducting its functions in moderation. This results in an increase in apoptosis and mitochondria fragments. Exactly how this mutation is connected to AD was unknown. This was the question that the researchers wanted to answer. Cho and his associates hypothesized that the dysfunction results from the connection between Drp1 and nitric oxide[3].

In order to verify their hypothesis, the researchers attempted to determine if Drp1 is affected by nitric oxide. They took brain cells and, by transfection, tagged them with a mitochondrial marker. This marker acts like glow-in-the-dark paint, and it would illuminate when viewed under fluorescent microscopy, allowing them to monitor any changes in the mitochondria. Their results showed that when the neurons were introduced to a nitric oxide donor, S-nitrocystein (SNOC), within one hour the mitochondria clearly displayed filament fragmentation[3]. To verify that it was truly SNOC causing the fragmentation, they conducted further tests to see if changes in dosage of SNOC would result in changes in fragmentation. They found that as SNOC dosage increased, so did the mitochondrial fragmentation in neurons. These findings concur with previous research that the fragmentation of the mitochondria can result from the introduction of nitric oxide[7]. The next step was to determine exactly how the nitric oxide was causing the fragmentation. They hypothesized that it had something to do with the protein's shape.

As with all proteins, shape is the end-all-be-all. If you change the shape of a protein, the result is a change in function. For instance, if you change the square peg to a round one, it no longer works in the square hole. The researchers had verified that the fragmentation resulted from the blending of Drp1 and SNOC3. Now they would attempt to verify the process by which SNOC is changing Drp1's shape, and thereby its function. Cho and his associates determined that SNOC caused the S-nitrosylation of Drp1, which is the process in which a nitric oxide compound is covalently bonded to the protein[6]. This resulted in the mutant protein, SNO-Drp1. They also compared levels of SNOC, and the changed protein, SNO-Drp1. They found that in cultures, SNOC expressed the mutant protein, SNO-Drp13. The results correspond with previous research that Drp1 can be S-nitrosylated, and that the nitrosylation of Drp1 changes its ability to initiate mitochondrial fission [2,8].

After the mutation of Drp1 had been verified, the researchers wanted to link it to Alzheimer's disease. Specifically, they hypothesized that the mutant Drp1, SNO-Drp1, was actually induced by Aβ. If you will remember, Aβ (amyloid beta) composes the plaques found in brains of Alzheimer's patients. To verify that the fragmentation from SNO-Drp1 was due to contact with nitric oxide from amyloid beta, Cho and his associates performed a biotin-switch assay to compare the levels of SNO-Drp1 in cells that expressed both SNOC, APP, and Aβ. APP, or amyloid precursor protein, is the protein responsible for the Aβ plaques. If the SNO-Drp1 was found in significant quantities with cells that had APP, it would link the protein to Aβ. This was exactly what the researchers found[3]. In the assay, the cells that did not express APP, SNOC, or Aβ did not express SNO-Drp1. However, cells that had any of these three characteristics showed significant signs of SNO-Drp1, indicating a correlation between SNO-Drp1 and Aβ. They further extended these studies to humans by examining human brains. They found increased levels of SNO-Drp1 in 17 of 17 AD brains. Most interesting is the fact that they did not find increased levels of the mutated Drp1 in either brains of Parkinson's patients, or non-CNS diseased patients. This data shows that SNO-Drp1 is a unique biomarker to Alzheimer's disease.

While they found that SNO-Drp1 increased fragmentation in AD brains, Cho and his associates also determined that it was a specific mutation to the protein that

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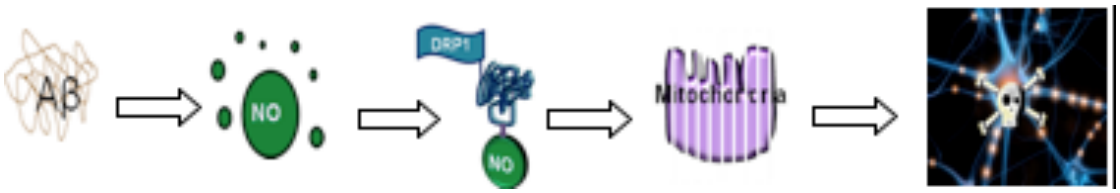

Figure 1: A deadly relationship. Researchers found that amyloid-beta produced nitric oxide, which attaches to a fission and apoptosis inducing protein called Drp1. The attachment of the nitric oxide resulted in an increase of mitochondrial fission, which ultimately resulted in an increase in cell death.

could be the cause of untold damage[3]. Therefore, the researchers performed nine mutations at the cysteine level, and found that only one of the mutations saw a clear decrease in SNO-Drp1; the mutation called C644A. C644A is a mutation conducted at the 644th codon where the amino acid cysteine is replaced with alanine.

Once the mutation was identified, they compared the amount of cell death when a neuronal cell was introduced to the wild type of SNO-Drp1 and the mutated C644 version of SNO-Drp1. The results confirmed their hypothesis[3]. The wild type of SNO-Drp1 had increased levels of cell death and the mutated version showed a lower level of cell death by slightly less than half. This indicated that cysteine in the 644th position was essential for SNO-Drp1 formation[3]. This was the last piece of the puzzle. With all the data gathered, they were able to clearly show that Aβ produced nitric oxide which resulted in a change to the protein Drp1, called SNO-Drp1. This change, which was the attachment of NO to the protein, assisted by cysteine at the 644th position, caused an increase in mitochondrial fragmentation and cell death in Alzheimer's brains[3].

One of the most fascinating parts of this research was the use of human tissue. Oftentimes, researchers use mouse or other models for their cellular base. This method is often very successful in the laboratory, but can result in an inability to apply the findings to humans. By collecting this data in human cells, there was no denying that this process is specific to human neural mitochondria. This has provided hope for a living biomarker to use for Alzheimer's testing. Previous research has also shown that CDK1, a cyclin-dependent kinase that is involved in cell death, can phosphorylate Drp1 and stimulate mitochondrial fragmentation[6]. This could be another avenue of research, where the relationship between the phosphorylation and nitrosylation of Drp1 could be examined.

Even so, this research is not a cure for Alzheimer's disease. Patients will still die from this disease, and there will still be an increasing number of people diagnosed with it. However, Cho and his associates have now provided both a possible avenue for treatment, and a possible testing mechanism. If they are able to mutate the Drp1 protein in Alzheimer's patients, they may be able to slow the progression of neuron degeneration, and in the end, slow the progression of this disastrous disease by breaking up the relationship between Drp1 and nitric oxide.

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