



# SOLUBLE OLIGOMERS IN ALZHEIMER'S DISEASE

## Criminal or Innocent Bystander?

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From the time of the first cases diagnosed, Alzheimer's Disease has long been a devastating illness for many aging individuals. Early Alzheimer's Disease cases were diagnosed using behavioral tests. However, since the early 1900s, scientists and physicians have found that biochemical examination and analysis of the brain, often after death of the patient, have been a better method of confirming that Alzheimer's Disease was the true cause of the memory loss. German psychiatrist Alois Alzheimer was one of the first scientists to offer a biochemical means of identifying Alzheimer's Disease in patients.

In 1906, Dr. Alzheimer first identified shrunken clusters of dead cells and proteins, known as plaques, in the brains of Alzheimer's Disease patients.<sup>1</sup> Since then, our understanding of the many biochemical players in Alzheimer's Disease increases at an astounding pace each year.<sup>2</sup> Much of this understanding has led scientists to propose different medical strategies to prevent or cure Alzheimer's.<sup>3,4</sup> However, despite these possibilities, real treatments for Alzheimer's have yet to become available. Current drugs appear to only briefly delay Alzheimer's in its early stages and do not halt or reverse the disease symptoms.<sup>3-5</sup>

This lack of progress in medical treatments results from

past, and possibly present, misunderstandings about what actually causes Alzheimer's Disease.<sup>6</sup> This idea is best highlighted by the classic difference between correlation and causation. Just because event A happens every time event B occurs does not mean event A causes event B. Perhaps, a yet unknown prior event C causes both events A and B. Many elegant scientific hypotheses which make perfectly good sense with limited data are later proved to be untrue as additional data is collected.

As an example, let us imagine that a naive scientist wishes to identify what is the legal profession of persons most likely to commit a crime. Initially, this scientist collects the professions of persons near crime scenes. From this data, we find that police officers are more often found near a crime scene than persons of most other professions. Therefore, a high correlation between crime and police officers may lead our naive scientist to incorrectly conclude that police officers cause crimes. To test this hypothesis, this scientist may suggest abolishing the police force. By doing so, this scientist would quickly realize that the hypothesis was wrong.

Discerning correlation from causation has posed a difficulty with identifying the main cause of Alzheimer's Disease. Scientists can only study what they can measure. It is therefore natural for scientists to support the variables which they find to correlate with Alzheimer's Disease symptoms as likely causes of the disease itself. Many early studies of Alzheimer's were focused on possible causes of the disease which turned out to be only correlation. However, recent medical successes in clinical trials reveal that recent ideas about the cause of Alzheimer's Disease may be getting close to the truth.

Free radicals and inflammation have been observed in the brain cells of Alzheimer's Disease patients throughout years of study.<sup>7,8</sup> This has led many scientists to believe that Alzheimer's Disease is caused primarily by external chemical or physical damage to the brain. A number of therapies have been attempted involving antioxidants and anti-inflammatory drugs, all of which have not proved effective at curing the dis-

ease.<sup>4</sup> Thus, while inflammation and free radicals may contribute to the disease, it is believed that the causative event of Alzheimer's Disease occurs prior to inflammation from the immune system or production of these free radicals.<sup>9-11</sup>

Another important observation in Alzheimer's Disease patients was the many plaques found throughout the brain.<sup>3</sup> Over many years of study, it was found that these plaques were mostly made of fibers comprised of a single protein called A $\beta$ .<sup>12</sup> The fibers which formed these plaques did not form inside the brain cells but are found on the outside of the cells. Since these plaques (and fibers) were so numerous in the brain of Alzheimer's Disease patients, it was believed that the fibers caused Alzheimer's Disease.<sup>12</sup> The A $\beta$  protein starts off as part of a larger protein, called the amyloid precursor protein (APP), and is chopped off by a protein called  $\gamma$ -secretase.<sup>3</sup> When part of APP, A $\beta$  does not cause problems. When enough A $\beta$  pieces are chopped off of different APP proteins, these "free" A $\beta$  proteins stack like rungs on a ladder and form large fibers. Then fibers then cluster together to produce the plaques.<sup>3</sup>

At first, the hypothesis that A $\beta$  fibers, known as amyloid fibers, caused Alzheimer's Disease appeared to be correct by a number of lines of evidence. First, amyloid fibers were found in patients with a variety of other neurological diseases such as Parkinson's Disease, Lou Gerhig's Disease, and Mad Cow Disease.<sup>13</sup> Also, studies in the lab showed that the A $\beta$  fibers kill brain cells, consistent with the idea that A $\beta$  fibers cause Alzheimer's Disease.<sup>14</sup> In addition, genetic mutations in the A $\beta$  gene lead to massive overproduction of A $\beta$  amyloid fibers in a small subset of Alzheimer's Disease patients who contract Alzheimer's Disease at an early age.<sup>3</sup>

Even recently, experiments with animals and clinical trials with Alzheimer's Disease patients helped make a strong case that A $\beta$  fibers cause Alzheimer's Disease. The drug Alzhemed makes the fibers less stable and appears to help transgenic mice and Alzheimer's Disease patients in early clinical trials.<sup>9</sup> Another drug, Flurizan, blocks the ability of  $\gamma$ -secretase to

chop A $\beta$  off of APP.<sup>15</sup> As with Alzhemed, Flurizan demonstrated a neuroprotective effect in mice and also in early clinical trials with patients. Finally, an interesting approach has been used where A $\beta$  fibers are used to immunize patients, through a procedure similar to a flu shot.<sup>10,11</sup>

The body's own immune system then helps remove the A $\beta$  fibers. A recent clinical trial using Ab fiber immunization had to be halted since some patients showed brain swelling.<sup>11</sup> However, follow-up studies of the patients showed halting and even reversal of Alzheimer's Disease symptoms in some cases.<sup>11</sup> In current clinical trials, a modified version of the immunization procedure is being used to avoid brain swelling in patients. Unlike previous ineffective drugs which target other possible causes of Alzheimer's Disease, drugs which target A $\beta$  fibers really do appear to make a difference against the progression in the disease.<sup>11</sup> However, the applicability and safety of these drugs with the general public remain unclear.

With all this evidence, it would appear that A $\beta$  amyloid fibers were indeed the true culprit in Alzheimer's Disease. The meaning of this finding is that our full research resources should be aimed at eliminating A $\beta$  amyloid fibers from patients. However, a number of nagging doubts persisted regarding whether these fibers were actually the true cause of Alzheimer's Disease.<sup>16,17</sup>

One of the earliest objections to the hypothesis that amyloid fibers cause disease, known as the "amyloid hypothesis," was that no correlation existed between amyloid plaques and regions of the brain deteriorated by Alzheimer's Disease.<sup>17</sup> For example, there were just as many plaques in the cerebral cortex, which is generally unaffected by Alzheimer's Disease, as in the long-term memory storage region of the hippocampus, which is severely impacted.<sup>17</sup> In fact, autopsies of deceased elderly individuals without Alzheimer's Disease often show a massive number of plaques with amyloid fibers in the cerebral cortex.<sup>3</sup> This lack of correlation has always proved to be a thorn in the side of those who promoted the amyloid hypothesis. In order to support the amyloid hypothesis, the explanation was

always given that hippocampus cells do not protect themselves as well as other brain cells from the deadly effects of the A $\beta$  fibers within the plaques. However, other evidence has come to light that perhaps smaller clusters of A $\beta$  proteins made of 10–20 A $\beta$  proteins, called “soluble oligomers”, are perhaps a more important cause of Alzheimer’s Disease than A $\beta$  fibers, made of thousands of A $\beta$  proteins.<sup>3</sup>

Just what is an A $\beta$  soluble oligomer? And what is the relationship between soluble oligomers and the large A $\beta$  amyloid fibers? A good way to think about soluble oligomers is to think about how people grow up. A human starts his/her life as a small infant, but grows into a larger adolescent, and finally matures into an even larger adult. By the same token, A $\beta$  proteins start off as small single “infant” molecules. About 10–20 of these A $\beta$  protein molecules stick together to form “adolescent” soluble oligomers, which are small but are still larger than proteins in the previous infant stage.<sup>3</sup> Many more A $\beta$  proteins stack onto the soluble oligomers and increase the size so that the soluble oligomer grows into a large “adult” amyloid fiber.<sup>12</sup>

Just as human teenagers behave differently from adults, so the immature soluble oligomers have different chemical characteristics from the mature amyloid fibers. Many scientists believe that the unique behaviors of the smaller soluble oligomers make them better candidates for the true cause of Alzheimer’s Disease.

First, A $\beta$  soluble oligomers will dissolve in water (soluble) while the amyloid fibers will not (insoluble). Therefore, A $\beta$  soluble oligomers can freely float through different regions of the brain while A $\beta$  fibers remain stuck where they have formed. This permits the A $\beta$  soluble oligomers to travel and directly interact with different membranes and protein receptors in brain cells than stationary amyloid fibers.

Second, unlike the plaques containing A $\beta$  amyloid fibers, the concentration of soluble oligomers between brain cells does correlate well with regions of the brain which experience deterioration in Alzheimer’s. This has been determined

through the recent use of antibody technology which can detect the amount of soluble oligomers in brain cells.<sup>18</sup>

Third, like the large A $\beta$  amyloid fibers, A $\beta$  soluble oligomers are also highly toxic to brain cells. In fact, studies with other proteins have shown that the soluble oligomers are more toxic than the amyloid fibers.<sup>19</sup> However, the results of these toxicity studies are highly dependent on the conditions of the experiments and are therefore somewhat controversial. Current studies are attempting to determine whether A $\beta$  soluble oligomers are more toxic than the A $\beta$  amyloid fibers.

If soluble oligomers, not amyloid fibers, are the true cause of Alzheimer's Disease, then why does anti-A $\beta$  therapies and vaccination help the patients in the clinical trials? It is likely that the vaccination sample of A $\beta$  injected into the patients contains a mixture of soluble oligomers AND amyloid fibers. Thus, antibodies will be produced against both the soluble oligomers along with the amyloid fibers. Thus, the curative effects of the vaccine may be due to the removal of the soluble oligomers, not the amyloid fibers.

How can A $\beta$  soluble oligomers kill brain cells? While this is hotly debated, three hypotheses have emerged as possible candidates. The first is that the soluble oligomers can bind to certain cell receptors on the outside of brain cells, which lowers their ability to function and also may continually elevate the immune response to an unhealthy level.<sup>3</sup> A second possibility is that the soluble oligomers stick to proteins which are necessary to maintain proper cell function or communication, effectively knocking these proteins out of action.<sup>20</sup> Based on recent experimental evidence, a new hypothesis has suggested that soluble oligomers insert themselves into cell membranes, form pores, and allow the "guts" of the cell to bleed out.<sup>21</sup> Currently, no consensus has been arrived at in regards to these hypotheses.

Furthermore, different studies have reported different shapes of A $\beta$  soluble oligomers. Some describe them as round clusters while others as ring shaped conglomerates of the A $\beta$  proteins.<sup>21</sup> Still other scientists found soluble oligomers, long

string-like shapes that appear to be miniature versions of the larger amyloid fibers.<sup>12</sup> Therefore, there is still much to learn about which of these shapes are deadly and which do not pose problems in Alzheimer's Disease.

Current lines of evidence appear to point to the small soluble oligomers as the main culprit that leads to Alzheimer's Disease. However, this is not to say that the larger amyloid fibers do not also contribute to Alzheimer's Disease. While they may not be as toxic as the soluble oligomers, A $\beta$  amyloid fibers may still kill brain cells. Also, the large amyloid fibers, while not as toxic as soluble oligomers, may act as storage reservoirs for the soluble oligomers. Over time, the soluble oligomers may continue to leak out, causing continual damage to brain cells.

The distinction between soluble oligomers and amyloid fibers is interesting to biochemists. But at the end of the day, the average citizen is interested in a cure for Alzheimer's Disease. So how does our understanding of A $\beta$  soluble oligomers help us find this cure? If we could find better ways to arrest these soluble oligomers in their adolescent state, we can use robotic technologies to screen thousands of different drugs against these soluble oligomers. Drugs which attach to and destroy the soluble oligomers may prove to be effective medical therapies for Alzheimer's Disease.

However, before the full arsenal of the pharmaceutical industry is focused on these soluble oligomers, basic researchers at academic institutions must continue to work to demonstrate that the soluble oligomers are truly worthy of this effort. The current scientific evidence does appear to point to soluble oligomers as a highly toxic agent both in the lab and in the brains of Alzheimer's patients. However, these observations, like the prior ideas of inflammation and amyloid fibers, may simply reflect correlation with and not causation of the disease. To decide whether soluble oligomers are truly the cause of Alzheimer's Disease or just the "flavor of the month" hypothesis, further studies of soluble oligomers are required. Perhaps something new will better provide an underlying rea-

son why the brain cells responsible for long-term memory selectively die in Alzheimer's Disease.

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