The Oxidative Injury Theory and Treatments of Alzheimer Disease: Our Winding Road

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Anti-dementia Drugs

In the early 1980s, I developed tacrine as the first pragmatic treatment for Alzheimer disease (AD), together with my colleagues at the University of California at Los Angeles (UCLA) and the University of Pittsburgh.[1-3] Tacrine was synthesized by Adrian Albert as part of the Australian World War II effort to find an intravenous antiseptic.[3] Use of tacrine to treat AD was unanticipated by the scientific community, and there was considerable controversy.[4,5] Yet 7 years later, in 1993, tacrine (Cognex®) was the first FDA-approved treatment for AD.[6]

The theory behind tacrine was the cholinergic hypothesis.[7,8] According to this theory, drugs that enhanced cholinergic neuronal function would improve memory. This might be achieved by acetylcholinesterase inhibitors (ACIs), stimulation of the nicotinic receptor, or enhancement of acetylcholine production.

The intent of tacrine was symptomatic treatment: to assist failing cholinergic neurons. Prevention or reversal of the disease process was never intended with ACI therapy. It was understood that the deficit of the cholinergic system seen in AD was a result of the disease, not the cause.

There was skepticism and doubt about our results.[4,5] Our data was investigated for 3 years by the U.S. Food and Drug Administration (FDA) and also by UCLA. Neither investigation showed wrongdoing or improper data use. Yet, a shadow hung over the work because I had bothered to submit a patent on tacrine based the new usefulness as a treatment for AD.[8] This patent came after presentation of the first five cases in 1985 to the Monsanto Company, acquired last year by Bayer. Monsanto informed me that they could not patent tacrine, but I could patent it as a "Use Patent." To have commercial value, tacrine had to have a patent. "If you did that, come back and see us," Monsanto said.

Despite differences in protocols, research conducted by the federal government and a major pharmaceutical company confirmed that tacrine was safe and effective. On Mar 28, 1989, the U.S. patent on tacrine was issued. The drug now had commercial value and could go to market like other drugs.

On Sep 9, 1993, the FDA approved tacrine for the treatment of AD as a "Type 1—New Molecular Entity."[6] Since then, three other drugs have been approved for AD therapy. The last ACI to be approved was rivastigmine in 2000. The average time from FDA application to approval of drugs is 12 years, and the estimated average cost of taking a new drug from concept to market exceeds $1 billion.[9] The fourth anti-dementia drug, memantine (Namenda) was approved in 2003 as the first in a novel class of AD medications acting on the glutaminergic system by reversibly blocking NMDA glutamate receptors.

Theories of AD Etiology

Since 1990, tens of millions of dollars have been invested in AD research, and numerous phase III candidates have failed. There have been no further anti-dementia drugs.[10,11]

Why?

The majority of therapeutic trials for AD are based on the amyloid hypotheses (aH) (Figure 1) or the T tau hypothesis (tH) (Figure 2). By these theories, the microscopic hallmarks of AD, namely amyloid plaques and neurofibrillary tangles, were toxic and caused the disease. The new therapies were designed to prevent or clear amyloid plaque or tangles.

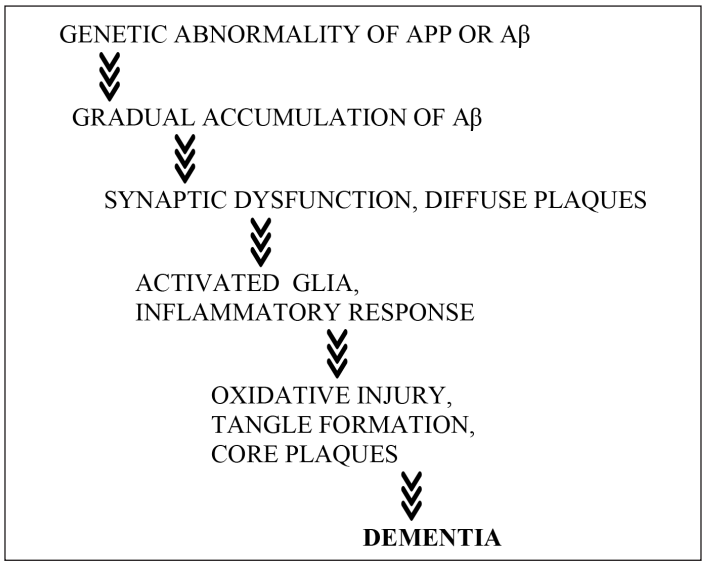

Figure 1. Amyloid Beta Hypothesis (aH)
APP = amyloid precursor protein
Aβ = amyloid beta

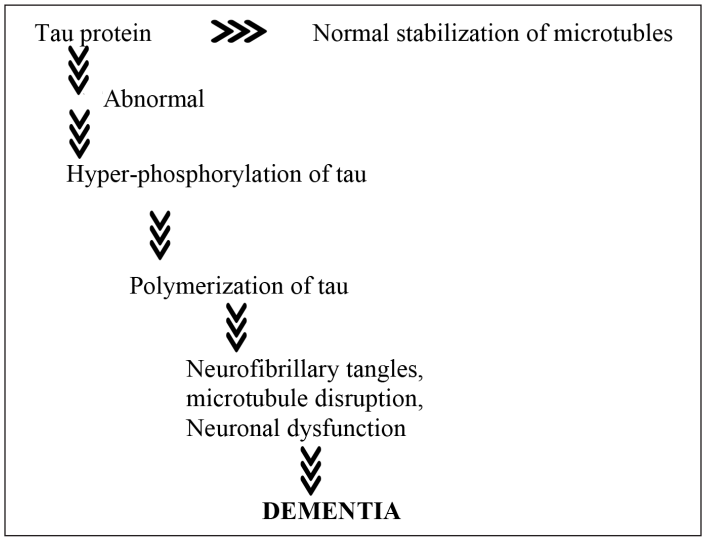

Figure 2. Tau Protein Hypothesis (tH)

Our view was that amyloid plaques and tangles represented "tombstones" that marked the location of dead neurons.[11,12] True, tombstones can cause death, but their principal function is to mark the location of contained localized CNS inflammation.[13] The proper model for an amyloid plaque relationship to AD is similar to the relationship of tuberculosis to Ghon complexes. Ghon complexes seen in the lungs isolate the tuberculosis bacillus, thus preventing widespread disease. The function of amyloid beta is apparently similar. Amyloid beta will absorb the heavy metals released locally from neurons undergoing apoptosis.[14]

Oxidative Injury Theory

Looking at other possibilities, the Free Radical or Oxidative Injury Theory (OiT) of AD was more consistent with the literature. As a general theory, aging may be viewed as a gradual, inevitable process, with accumulation of certain oxidative lesions with the ultimate destination of AD for 100 percent of people who live long enough. Oxidative injury can damage DNA, lipids, proteins, and sugars within cells.[15] Imbalance between intracellular production of free radicals/reactive oxygen species (ROS) and the antioxidant defense mechanism results in oxidative stress. Neurons are especially subject to redox imbalance, so minor cellular stresses can cause irreversible injury, leading to neurodegenerative diseases.

A molecule with an unpaired electron is extremely reactive and eager to acquire an electron in any way possible. These are called free radicals, and they will attach to other nearby molecules to modify them biochemically. The new combination can squelch the unstable electron, or create another free radical in a chain reaction. Free radicals can damage virtually any biomolecular structure, including nucleic acids, membrane lipids, proteins, glycoproteins, sugars, and small molecules.

Physically, where would the oxidative injury occur in the brain? It would not be restricted to a single location, such as the mitochondria. The answer is clearly that it would occur in numerous subcellular locations.[11] These would include nuclear proteins, nuclear membranes, endoplasmic reticulum, mitochondria, Golgi apparatus, neuronal cytoplasm, neuronal cell membrane, intracellular space, and blood-brain barrier.

The common free radicals are superoxide, hydroxyl, alkoxyl, peroxyl, and nitric oxide radicals.[15] Non-free radical molecules include singlet oxygen, hydrogen peroxide, and hypochlorous acid. These are classified as reactive oxidative species (ROS). These are the offense. ROS have extremely short half-lives ranging from nanoseconds to seconds. They are created by metabolism, such as the oxidation of fatty acids and the actions of cytochrome P450 enzymes and chronic inflammatory cells. Various common molecules such as catecholamines and hemoglobin are ROS.

On defense are the body's antioxidants. Enzymatic antioxidant systems and cellular molecules protect cells against ROS. Three principle enzymes are superoxide dismutase (SOD), catalase, and glutathione peroxidase, but there a number of others. Endogenous cellular molecules that are antioxidants include glutathione, vitamin C, vitamin E, vitamin A, uric acid, bilirubin, reduced coenzyme Q10, and many others. There are five classes of exogenous antioxidants: vitamins, amino acids, minerals, fatty acids, and herbals.

Given this complexity, the more rational view of the OiT requires a specific initial insult to the brain, which creates a self-perpetuating focal inflammation.[11,16] There are many potential initial brain insults.[16] The localized inflammation in the central nervous system would smolder and move, like rheumatoid arthritis, to other areas of the brain. The key to this "modified OiT" is the initiating injury to the brain. A partial list of suspected initiating injuries are: infections, anoxic episodes, traumatic brain injuries, toxins and heavy metals, autoimmune vasculitis, metabolic events such as hypoglycemia, etc. So, in the end, there are numerous "causes" of AD, but they go down a final common pathway to create the disease.

If AD is the product of these localized brain injuries followed by localized inflammations, the OiT would explain: (1) why traumatic brain injury-related dementia under the microscope looks similar to AD;[17] (2) why viral infections, such as herpes virus, are associated with AD;[18] (3) the presence of amyloid beta as an effort by the brain to maintain homeostasis in a zone of neuro-inflammation and amyloid plaques as tombstones that mark where neurons died and not as a cause of the neuronal death; and (4) recent data on nonsteroidal antiinflammatories and aspirin showing a delay in the onset of AD.[19]

Until recently, antioxidant therapies have not shown promise in memory-impaired humans.[20,21] Perhaps this is because oxidative injury occurs at many cellular levels, and prior antioxidant trials utilized only a single or small number of intracellular targets.

AntiOx Formula

In 2000 we filed a patent application on a health supplement intended to prevent or improve neurocognitive disorders such as AD.[22] The patent application rested on the fact that antioxidants have five forms. These are: (1) vitamins, (2) certain amino acids, (3) certain minerals in defined doses, (4) herbals that contain complex, often overlapping antioxidants, and (5) certain lipids. Antioxidant vitamins include: beta carotene, vitamin A, coenzyme Q10, vitamin B12, folic acid, vitamin B3, vitamin B5, vitamin B6, vitamin C, and vitamin E. Antioxidant amino acids include: L-glutathione, L-lysine, L-methionine, and taurine. Antioxidant minerals include: boron, iodine, manganese, magnesium, selenium, and zinc. Antioxidant herbals include: curcumin, ginkgo biloba, ginseng, gota kola, and blueberry (*Vaccinium angustifolium*). Antioxidant lipids include: lipoic acid, phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine. Not all lipids are antioxidants.

Our antiOx formula based on this patent has been marketed since 2000.[23] It is important to point out that unlike most pharmaceutical products, the antiOx formula does NOT have a single or small number of secret active agents. All 34 components are important. All 34 components had evidence in the literature, before 2000, that they were absorbed from the bowel and did cross the blood-brain barrier. In the literature of the late 20th century, synergy between these components was occasionally documented.[24]

Because of our prior experience with the regulatory process, we elected to market our AntiOx formula as a health

supplement, not as a pharmaceutical treatment, or a "health food." FDA classifies and regulates human treatments in various classes. Pharmaceuticals are highly regulated. As noted above, the average time from FDA application to approval of drugs is 12 years, and the estimated average cost of taking a new drug from concept to market exceeds $1-2 billion.[9]

In 2000, the health supplement alternative allowed the formula to immediately go to market. Health supplements are regulated as food. In exchange for going directly to market, each bottle must be labeled, "These statements have not been evaluated by the Food and Drug Administration. THIS PRODUCT IS NOT INTENDED TO DIAGNOSE, TREAT, CURE, OR PREVENT ANY DISEASE" [stress added]. The research was based on "normal" seniors, yet this is the pool of people in whom AD will develop.[25]

On May 11, 2004, the U.S. Patent Office granted our patent, but our antiOx formula had been marketed primarily to our patients for four years.[22] Early use of the formulation was in a variety of settings: acute in-hospital settings, wound-care medicine, nursing homes, and outpatient internal medicine and psychiatric practices. The intent was to discover adverse effects from the formulation, or perhaps drug-supplement adverse interactions. No serious adverse effects were seen in several thousand patients. The theory behind the patent was published in 2004, and research on effectiveness began.[16]

AntiOx Formula Research

In 2010 we published a double-blind study of 113 "normal" subjects between ages 50 and 75 who had no evidence of dementia.[26] Fifty-four subjects received placebo and 59 received our antiOx formula (active agent). Memory testing was with a 50-part paired association test putatively looking at function of the right temporal lobe and higher cortical centers.[26,27] The Names Learning Test (NLT_{50}) presents 50 general categories such as "color" paired to a specific color, such as "purple." After giving the whole list, the subject is cued with "What was the name of the color?" These results are seen in Figure 3. A second memory test was the 20-word immediate recall test ($_{20}$WR), which looked at hippocampal competence (Figure 4). The $_{20}$WR rapidly presents 20 non-related one-to-two-syllable words, then pauses for a minute before asking the subject "How many can you remember?" The subject is given two minutes to respond, with no penalty for wrong guesses. The study ran 4 months with testing at baseline, 1 month, and 4 months (Figures 3 and 4). For NLT_{50}, an adjusted linear regression modeling of the change in NLT_{50} scores from four months to baseline revealed a statistically significant 5.2 point higher change in the active group compared to the placebo group (p = 0.015). For $_{20}$WR, an adjusted linear regression modeling of the change in $_{20}$WR from 4 months to baseline revealed a statistically significant 1.7 point higher change in the active group compared to the placebo group (p =0.005).[26] A secondary outcome was to test reduction of serum homocysteine, a marker for cardiovascular and AD risk. Twenty-five antiOx subjects and 17 placebo subjects voluntarily gave samples at baseline and 4 months. A two-sample t-test for the mean change in homocysteine in the

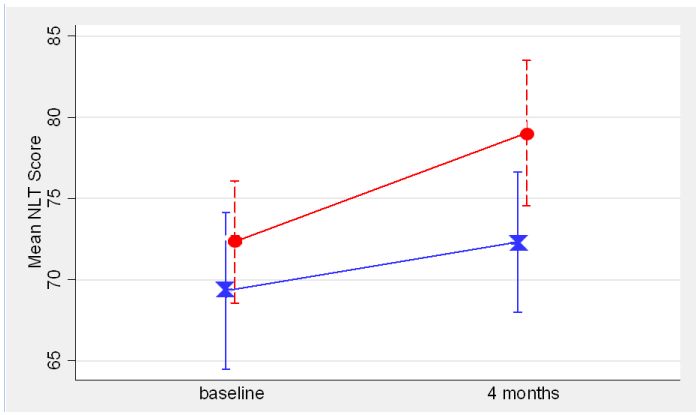

Figure 3. Names Learning Test with 50 Pairs. Double-blind 4-month study

Active Group *Placebo Group*

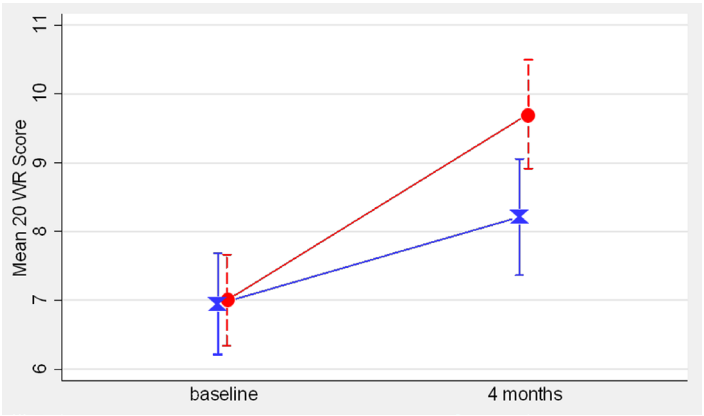

Figure 4. 20-Word Immediate Recall Test. Double-blind 4-month study

Active Group *Placebo Group*

active versus the placebo group did reveal a statistically significant 1.57 µmol/liter decrease after 4 months (95% CI = −2.72, −0.42, two-sided p = 0.009).[26]

In 2007 Kamat et al. reviewed 20 years of antioxidant use in humans and animal studies.[20] They concluded that the theory of antioxidant causation of AD and related disorders was sound, but that the implementation of antioxidant therapies in humans "must be flawed."

We concurred. There appeared to be three reasons for this failure. First, prior work used massive amounts of single antioxidants or simple combinations. Second, the testing instruments were crude and insensitive. Third, the negative human results were "epic multi-center, multi-nation projects." Consistency of data is very difficult to obtain with multiple researchers spread over vast distances administering remarkably complex protocols. Such protocols would drift toward non-significance. Wojsiat et al. added the possibility that high-dose single antioxidants upset balance and could be detrimental.[21]

In 2018 we published a randomized single-blind study comparing our antiOx formula to the most popular one-a-day multivitamin over six months.[28] Again the subject population was "normal" seniors aged 50 – 75 years. Initial analysis of the data was initiated halfway through the study. Because significance was reached, the study was terminated after 63

subjects were enrolled. Thirty-three subjects were assigned to the multivitamin group (mV) and 30 subjects were in the antiOx group. The memory test battery was administered at baseline, 1 month, 3 months, and 6 months. The Names Learning Test was expanded to 100 paired associated items (NLT_{100}), which demonstrated little "ceiling" or "floor" effect and could detect subtle memory deficits. Normal was 65/100 (SD = 4.2). The antiOx group demonstrated improvement in declarative memory by and beyond 30 days (Figure 5). This continued over the 6-month study. The one-a-day group showed no improvement. Working memory was measured by the $_{20}$WR. The antiOx group demonstrated highly significant improvement by 30 days (Figure 6).

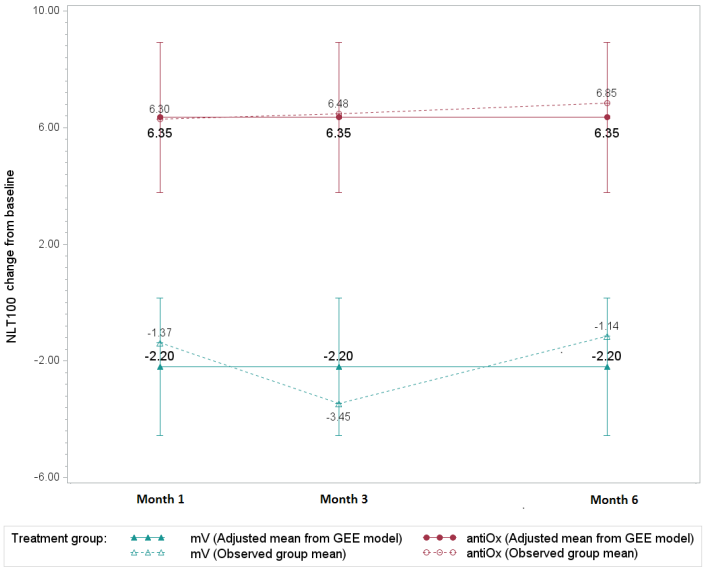

Figure 5. Change Score from Baseline Names Learning Test with 100 Pairs (NLT_{100}). Single-blind double-active agent: the generalized estimating equation (GEE) model adjusted for the NLT_{100} baseline score.

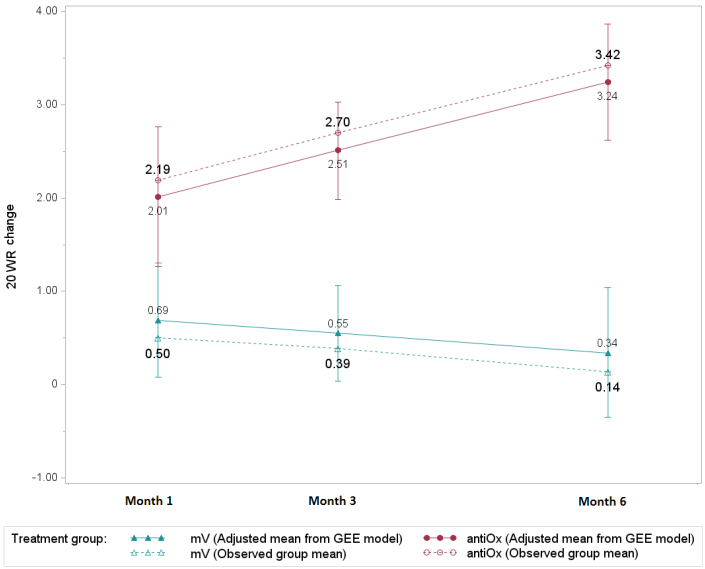

Figure 6. Change Score from Baseline 20-Word Immediate Recall Test ($_{20}$WR). Single-blind double-active agent: the GEE model adjusted for the baseline $_{20}$WR score, time, and the interaction between the time and treatment.

When comparing the "Change Score" of $_{20}$WR for each group at time periods 1 month, 3 months, and 6 months to the baseline $_{20}$WR scores using the generalized estimating equation (GEE) method, the improvements in the 90-days-from baseline scores compared with the scores at 30 days from baseline were highly significant. Additionally, there was significant improvement in the 180-days-to-baseline data versus the 60-days-to-baseline data (Figure 6). We speculate that this would imply that the antiOx formula actually stimulates neurogenesis in the hippocampus. Indeed, several of the components of our antiOx formula have independently demonstrated an ability to induce neurogenesis.

The Continuum from Normal to AD

It could be argued that improving the memory of "normal" seniors is not relevant to AD. Yet it is generally agreed that the development of AD is a subtle continuum from "normal" to mild cognitive impairment to diagnosable AD. Our antiOx formula is thought to work by squelching free radicals and promoting cellular repair, especially in neurons. It logically follows that our formula prevents and/or treats the actual disease process of AD.

Recently, a number of alternative treatments, many depending on antioxidant properties, have been tested on AD patients. Chan et al. reported improvement in early AD patients with a six-component complex antioxidant.[29] Over one year their AD subjects improved as measured by the dementia rating scale, clock-drawing tests, neuropsychiatric inventory, and activities of daily living. Improvements seen by this complex antioxidant were equivalent to the improvement seen in studies of AD pharmaceuticals donepezil and galantamine. They later demonstrated the same antioxidant-improved memory and cognitive performance in community-dwelling adults without dementia.[30] Maccioni and colleagues have put combined Shilajit (BrainUp®), a natural antioxidant substance containing fulvic acid and B vitamins, in a clinical treatment trial of mild AD subjects.[31,32]

Single Case Report

Both AD and Parkinson disease became known through a single case report. Perhaps a single case would be instructive here.

Mrs. AC was diagnosed with AD in 2000 at age 80 with a Mini-Mental State Exam (MMSE) score of 18, and was started on our antiOx formula. The course of illness ended 17 years later at age 97. Also, at age 80, she had a simple left mastectomy for breast cancer. Three months after initiation of our antiOx formula, the MMSE improved to 28. She began to play golf again and bridge. Her husband died one year after diagnosis. Three years later she remarried at age 83. At age 84 and again at 85, she won a major golf tournament for golfers 80 and older. At age 87 she had a right knee replacement but did not regain full function of the right leg. At age 88 she began to have a progressive right hemiparesis. Evaluation

revealed a C4-C7 angioma. This was surgically removed in a six-hour procedure. Recovery was nearly complete, but she used a walker for safety thereafter. At age 90 she suffered paroxysmal onset of complete heart block and was treated with a pacemaker. The MMSE in this episode dropped to 19. She was placed on amantadine and rivastigmine. She began having anxiety and agitation. This was managed in the final 7 years of her life with propranolol 40-60 mg by mouth in the morning and at dinner.[33] At age 92 she developed bowel motility issues (presbycolon) resulting in several ER visits for disimpaction. This was treated with linaclotide three times a week. At age 94 she entered an assisted-living facility with a MMSE of 22. She was placed on methylphenidate 20 morning and noon for motivation, focus, and energy. She did well. She left the assisted living for social functions three or four days per week. Her recall of these events was limited. At age 97 she suffered a sudden deterioration of function and had several falls. She had several infections over the 3 months before her death. Examination of blister packs revealed that the assisted living staff had inexplicably withheld the methylphenidate for the final 3 months of her life. After treatment of the third infection in 6 weeks, she suffered sudden death. She was 97 years and 10 months old at time of death. Figure 8 is a photo taken two weeks before AC's death. The course of AD was in this patient on our antiOx formula, which was taken for 17 years, was quite exceptional.

Current and Future Treatment of AD

The OiT gives hope for future effective treatments for AD. Other complex potent antioxidant formulations might be more effective. But knowing that apoptosis and focal-diffuse micro-inflammation are part of the process opens other avenues to therapy. It is known that some medications promote neurogenesis. This can be further defined and developed. Antiinflammatory medications, such as aspirin and nonsteroidal antiinflammatory drugs, delay AD.[19] But agents that down-modulate CNS inflammation with better safety can be sought out. Montelukast (Singulair) may be one of these agents.[34] Strangely, tacrine, our original compound has both antioxidant and antiviral properties.[2,15] There are potent antiinflammatory and/or antioxidant agents used in treatment of rheumatoid arthritis that can be explored for use in advanced AD. Still another possibility is vaccination for prevention of some infectious agents that may result in dementia, such as herpes viruses or Lyme disease.[18] Acute hypothermia treatment for traumatic brain injury (TBI) is another approach to dementia prevention.[17,35] Agents that positively effect cytokines such as IL-3 may prove helpful.[36] There are also others that are beyond the scope of this discussion.

At present, my treatment of AD patients consists of (1) removal of all sedatives, which often increase confusion; (2) control of agitation by use of propranolol titrated to need; (3) antioxidant therapy (our antiOx formula); (4) drugs that promote neurogenesis—frequently antidepressants fill this function; (5) a safe-for-long-term antiinflammatory agent such as montelukast;[37] and if needed an anticholinesterase inhibitor.

Figure 7. Photograph of Amy C. at age 97 years, 8 months, 2 weeks before her demise.

Conclusion

The future for treatment of AD and related disorders looks very positive if drug development is re-focused on agents that affect pathogenesis and enhance damage repair.

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