

Amyloid Cascade in Alzheimer's Disease

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Introduction

Alzheimer's disease (AD), the most common cause of dementia, is a progressive and fatal neurodegenerative disorder characterized pathologically by atrophy of the cerebral cortex and hippocampus, with intraneuronal neurofibrillary tangles containing abnormally phosphorylated *tau* protein, extracellular amyloid plaques, and neuronal cell death, and clinically by gradual impairment of memory.¹ The patient gradually becomes progressively impaired in both cognitive and functional capacities. The loss of intellectual abilities is of sufficient severity to interfere with social and occupational functioning.

Memory destroying illness

The sensory experiences received by the human brain are processed and stored as memory. This information is recalled in an integrated fashion at an appropriate time. Memory fades in Alzheimer's disease and often it is compared to the erasure of a computer hard disk. Initially it involves failure to recall the recent events though the person is able to recollect the events that had taken place long ago. As the illness progresses, the old memory also gets disappeared and ultimately the patient fails to recognize the near and dear. This memory destroying illness is associated with loss of a lifetime memories that make up the identity of the person.

Pathological process

AD is associated with destruction of more than 100 billion neurons and their associated 100 trillion connections. There is progressive loss of cortical neurons and formation of amyloid plaques, intraneuronal neurofibrillary tangles and accumulation of a beta-amyloid in arterial walls of cerebral blood vessels

(amyloid angiopathy). Beta-amyloid is the major component of the plaques, whereas hyperphosphorylated *tau* protein is the major constituent of the neurofibrillary tangles. The pathological process of atrophy begins in the hippocampus and spreads to involve diffuse areas of temporal, parietal and frontal lobes of the cerebral cortex. There is symmetric enlargement of the third and fourth ventricles. The loss of neurons, especially in the nucleus *basilis* causes a relative deficiency of acetylcholine to result in different clinical manifestations.

Cholinesterase inhibitors

The neurotransmitter acetylcholine is necessary for clear thinking. It gets destroyed by the enzyme acetylcholinesterase (ChE). Since acetylcholine deficiency has been observed in AD, ChE inhibitors have been used to block the action of ChE so as to increase the cerebral concentration of acetylcholine essential for synaptic transmission. Donepezil, rivastigmine and galantamine (ChE inhibitors) facilitate an increase in the level of acetylcholine.² These agents show improvement in global function and reduce cognitive disturbances. There is reduction in behavioural disturbances and temporary stabilization of activities of daily living.³ As the destruction of the neurons proceeds relentlessly, the medications become ineffective after some time.

Mementine

The symptoms of AD are thought to be due to persistent activation of central nervous system N-methyl-D-aspartate (NMDA) receptors by the amino acid glutamate. Glutamate acts as the main excitatory neurotransmitter substance. Memantine, an NMDA receptor antagonist acts either by interfering with glutamate excitotoxicity or by providing symptomatic improvement through effects on functions of hippocampal neurons.⁴ Though it slows the cognitive decline in mild-to moderate AD, its effects also do not last long.

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New approaches to therapy

Efforts are being made to find treatment to slow or halt the memory destroying disease following better understanding of the molecular events that appear to trigger this disorder. It has kindled the hope of effectively slowing or stopping the gradual loss of neurons in the brain, and ultimately to stop the progression of the disease. Many drugs are under various stages of clinical trials and there are some promising preliminary results.⁵

Amyloid plaques and tangles

A cascade of events pertaining to amyloid, underlie development of AD.⁶ Amyloid cascade hypothesis is based on the fact that plaques and tangles of proteins in the cerebral cortex and limbic system deleteriously affect the higher functions of the brain. The plaques are deposited outside the neurons and are composed of a small protein called amyloid beta (A-beta). The tangles are found inside neurons, and their branching axons and dendrites. They are made up of filaments of proteins called *tau*. The plaques and tangles are responsible for the degeneration of the neurons. Amyloid-beta triggers the disruption and death of the neurons.

This hypothesis has led to the efforts of developing drugs to inhibit the production of A-beta and *tau*, and thus stop the harmful effects of these on the neurons.

A-beta is a short peptide. It is derived from the amyloid precursor protein (APP) with a part of the protein lying inside the cells and a part outside, sticking out of the cellular membrane. Two protease enzymes-beta secretase and gamma secretase are able to carve out A-beta from APP. This is a normal process occurring in all cells in the body.

In AD, there is an excess accumulation of A-beta. Initially beta secretase cuts APP found outside the cellular membrane with the help of aspartic acids. Then the presenilin protein, a component of the gamma-secretase enzyme cuts the remaining portion of APP found inside the membrane and releases A-beta into the aqueous environment outside the membrane, and gets attached to one another as small soluble assemblies (plaques). They are toxic to the neurons. Experimentally it has been shown that high concentrations of A-beta molecules in a test tube can assemble into fibrillary structures similar to those found in the plaques of AD. They have been shown to be toxic to neurons cultured

in petridishes.

The step wise process of oxidation and lipid peroxidation of cell membranes, glutamatergic excitotoxicity, beta-amyloid aggregation, inflammation and *tau* hyperphosphorylation, cause neurotoxicity, neuronal cell death and neurotransmitter deficit.

Neuritic plaques have a central core of insoluble deposit of amyloid beta-peptide surrounded by astrocytes, microglia and dystrophic neuritis consisting of paired helical filaments.⁷ Neurofibrillary tangles are made up of paired helical filaments of abnormally filled and phosphorylated *tau* protein in the neuron and its dendrites. More *tau* tangles are seen in the brain as the disease advances. There is also reduction in synaptic density, loss of neurons and degeneration in hippocampal neurons. There is a specific degeneration of neurons concerned with maintenance of specific transmitter cysteines and result in deficits of acetylcholine, nor-epinephrine, and serotonin.⁸ Though plaque formation arrests, plaque formation of tangles continues. It correlates with the progress and severity of dementia.

Genetic predisposition: Members of the families having a high risk of getting AD at a relatively young age, carry rare genetic mutations that encode APP specifically affecting the areas of the protein in and around the A-beta region. Genetic predisposition appears to be inherited as an autosomal dominant trait with relatively complete penetrance. Four different genes have been identified to be involved in the heritable form of the disease. The presence of the apolipoprotein E4 allele found on the long arm of chromosome 19 increases the likelihood of development of AD.⁹ Apolipoprotein E4 genotype appears to enhance A-beta peptide aggregation or decrease its cleavage. This makes them susceptible to develop the disease at a relatively young age. It has been shown persons with Down's syndrome (trisomy 21) exhibit much higher incidence of AD in middle age. This is due to the fact chromosome 21 contains APP gene. There is an increased production of A-beta from birth, and consequently an increased amyloid deposit beginning from a young age.

Mutations in two related genes called presenilin 1 and 2 lead to occurrence of severe form of AD very early in life. The mutations increase amount of A-beta that is prone to clumping. Mutations of presenilin-1 gene located on chromosome 14 which may lead to an early

onset autosomal dominant AD.¹⁰ Rarely the mutations of the presenilin-2 gene on chromosome 1 may cause autosomal dominant AD with an earlier onset of the disease and a shorter, more rapidly progressive course. The proteins encoded by the presenilin genes are part of the gamma secretase enzyme that help in the synthesis of the harmful peptides.

Protease inhibitors: It is not clear how A-beta destroys the neurons. Aggregates of A-beta found outside the neuron can initiate a cascade of events that can bring about an alteration of the *tau* protein inside the cell. A-beta aggregates are likely to bring about changes in the kinases that add phosphates onto proteins. There is likelihood of addition of an excess amount of phosphates to *tau*, resulting in formation of twisted filaments. The altered *tau* proteins are likely to act deleteriously by disrupting the microtubules carrying proteins along the axons and dendrites, and kill neurons. Thus A-beta plays the pivotal role in the initiation of AD. In this background, drugs are being produced targeting the proteases (protease inhibitors) that produce A-beta, and to inhibit their activity.

The proteases use aspartic acids to catalyze protein cutting reactions. Small-sized beta-secretase inhibitors are yet to be developed that can effectively pass through the blood brain barrier. Gamma secretase is the other enzyme involved in the formation of A-beta by cutting the remaining portion of APP inside the cell following the cleavage by beta secretase. Studies in mice have shown deletion of presenilin-1 gene genetically decreases the cutting of APP by gamma secretase. It has been proved that the protein encoded by the gene is essential for the function of the enzyme. Inhibitors of aspartyl proteases could block gamma-secretase cleavage of APP in cells. Gamma secretase also contains a pair of aspartic acids as in beta-secretase and are essential for catalyzing the protein cutting reaction.¹¹ Presenilin protein acts like an unusual aspartyl protease in the cell membranes. The inhibitors of gamma secretase are relatively small molecules that can penetrate blood brain barrier.

Inhibitors of aspartyl proteases could block gamma-secretase cleavage of APP in cells. Gamma secretases like beta secretases contain a pair of aspartic acids essential for catalyzing the protein cutting reaction. Presenilin protein appears to be an unusual aspartyl protease attached to the cell membrane. Two aspartic acids in presenilin lie within the membrane. They are very essential to the gamma secretase cleavage to

produce A-beta. Inhibitors of gamma secretase bind directly to presenlin. Gamma secretase enzyme plays an important role in maintenance of undifferentiated precursor cells in different parts of the body. Gamma secretase cuts a cell surface protein called Notch receptor. High doses of gamma secretase inhibitors cause toxic effects in mice by disrupting the Notch signal. Molecules have been identified that modulate gamma secretases so that A-beta production is blocked without affecting cleavage of Notch.¹¹ Attempts have been made to produce inhibitors that can curtail the creation of A-beta or create a shorter peptide that does not clump easily. Such a preparation called, Flurizan has shown promising results.

Immunization: The second strategy is to clear the brain of toxic assemblies of A-beta after its production by active immunization. It involves recruiting the patients own immune system to attack A-beta. Injection of A-beta into mice genetically engineered to develop amyloid plaques stimulated an immune response that prevented the plaques from forming in the brain of young mice and cleared plaques already present in older mice.¹¹

The mice produced antibodies that recognize A-beta and the antibodies made the microglia in brain to attack aggregates of the peptide. It improved learning and memory. However studies in humans, has lead to development of encephalitis probably through the action of T cells. However, immunization produced antibodies against A-beta and there was some improvement in memory and concentration. Passive immunization by injecting the antibodies into patients aims to clear the peptides. These antibodies produced in mouse cells and genetically engineered to prevent rejection in humans are unlikely to evoke occurrence of encephalitis as they do not trigger T cell response in the brain. The procedure was able to remove A-beta from the brain.

Immunization with selected parts of A-beta instead of entire peptide can stimulate the antibody producing B cell of the immune system without triggering T cells involved in the occurrence of encephalitis.

Non-immunological strategy: Non-immunological strategy to stop aggregation of A-beta compounds has been attempted. The compounds interact directly with A-beta to keep the peptide dissolved in the fluid outside brain neurons preventing formation of harmful clumps. Alzhemed, a small molecule apparently mimicking

heparin binds to A-beta and reduces peptide aggregation and shows some improvement in cognitive functions of patients with mild AD.

Targetting Tau: The *tau* filaments cause neuronal tangles, and they are a promising target to prevent degeneration of neurons. Inhibitors could block the kinases that place an excessive amount of phosphates onto *tau*, which is an essential step in filament formation. No success has been seen in production of such a drug. It is hoped such drugs might work synergistically with those targeting A-beta.

Reduction in production of APP: Cholesterol lowering agents (statins) used to cut risk of heart disease could become a treatment for AD. Epidemiologic studies have shown people taking statins have a lower risk of acquiring AD. By lowering cholesterol these drugs may reduce production of APP or perhaps affect the creation of A-beta by inhibiting activity of the responsible secretases. Attempts are made to prevent AD by using statins.

Cell-based therapy: Cell therapy is another approach in the treatment. The gene encoding a large protein such as nerve growth factor (NGF) was inserted into the skin biopsies obtained from patients with mild forms of AD. Such genetically modified cells were implanted surgically into the forebrain of the patients, with a view that the implanted cells would produce and secrete NGF, thus preventing the loss of acetylcholine producing neurons and improve memory. The treatment was associated with slowing down of cognitive decline.

Medical fraternity is eagerly looking forward for a break through in the research to provide a drug that could effectively slow or stop the gradual loss of

neurons. The treatment targeting A-beta may halt the occurrence or retard progress of Alzheimer's disease.

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