

ALZHEIMER'S DISEASE: AN UPDATE

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Alzheimer's disease begins with the loss of short-term memory, followed by progressive dementia which involves memory loss, impairment of intellectual functioning, judgement and decision making. It is the most common cause of dementia in adults in north America and Europe. At present there is no effective treatment to prevent or ameliorate the progression of Alzheimer's disease, which is the commonest cause of dementia in the elderly. The characteristic neuropathological hallmarks of Alzheimer's disease are β A4-containing plaques, neurofibrillary tangles and amyloid infiltration of cerebrovascular walls. Understanding of the biological basis of cognitive disorders is undergoing rapid advancement. The research progress and hypothesis about the brain abnormalities underlying Alzheimer's disease such as amyloid cascade hypothesis, cholinergic theories and excitotoxic hypothesis are discussed in the present article. In spite of this, various treatments for Alzheimer's disease such as cognitive enhancers, metabolic enhancers, vitamins and hormones, neuropeptides, psychostimulants etc. including other current approaches viz. growth factors, transplantation of neuronal tissue and immunisation with amyloid- β peptide are also discussed. However, novel methods for delivering these molecules into the brain requires to be developed before launching their clinical trails.

Keywords: Alzheimer's disease; Cognitive enhancers; Transplantation; Immunisation

Introduction

Alzheimer's disease (AD) is a dementing illness that affects over 10% of persons over the age of 65 residing in the community, and over 50% of residents of psychiatric nursing homes. It is the fourth leading cause of death in industrialised nations of USA and Europe (after heart disease, cancer, and stroke). Currently, about 3 million Americans, or approximately 8% of the elderly population in the United States, suffer from AD. The burden and prevalence of dementia are shown in tables 1 and 2.

Dementia is a clinical diagnosis and is often made based upon clinical features meeting the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) for the various dementias listed there including dementia of the Alzheimer's type, vascular dementia (formerly multi-infarct dementia), dementia due to other general medical conditions, substance-induced persisting dementia, dementia due to multiple etiologies, and dementia not otherwise specified. In addition,

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Table 1. Burden of dementia in Disability Adjusted Life Years (DALY) by sex and geographic region (hundreds of thousands of DALY).

	India	Sub-Saharan Africa	China	Middle Eastern Crescent	Other Asia	Latin America	Formerly Socialist Economies	Established Market Economies	Demographically Developing Group	World
Males	9.8	4.1	12.6	4.6	6.8	4.0	5.1	16.7	41.9	63.7
Females	9.7	4.3	13.6	4.9	7.3	4.7	8.7	23.2	44.5	76.4

Table 2. Prevalence of dementia in people over 65 years of age.

Geographical location	Prevalence (%)
Lund (Sweden)	3.4
Framingham (USA)*	4.1
Baltimore (USA)	4.5
Shanghai (China)	4.6
Zaragoza (Spain)	5.5
Rochester (USA)	5.7
Cambridge (UK)	6.0
Appignano (Italy)*	6.2
Hisayama (Jama)	6.7

>60 years

there is DSM-IV diagnostic criteria for amnesic disorders of various types. On the other hand, AD – a specific type of dementia – is defined by pathological features, so a definitive diagnosis can only be made at postmortem autopsy. Nevertheless, diagnostic criteria have been developed for *possible* AD and for *probable* AD based upon clinical features. These criteria are called the National Institute of Neurological and Communicative Disorders and Stroke AD and Related Disorders Association (NINCDS/ADRDA) criteria for the clinical diagnosis of AD and are the best clinicians can do to make a diagnosis of and prior to autopsy or brain biopsy.

The clinical hallmark of AD is dementia, comprised of impairment in short-and long-term memory, abstract thinking, judgment and higher cortical

functioning such as language or motor function. However, other features often accompany AD and add significantly to the overall impairment of the patient, including disordered behaviour such as agitation and aggressiveness, psychosis, and anxiety.

Another important aspect to AD is its progressive nature. Mild AD, the earlier stage, at which a diagnosis can be made with confidence, is associated with decreased capacity for complex occupational and social tasks and often lasts about 2 years. Moderate AD generally causes interference with independent community survival, problems of choosing clothing, inability to drive a car, difficulty in preparing simple beverages such as coffee or tea, and inability to recall the current year. Moderate AD can progress to moderately severe AD in about 18 months.

At the stage of moderately severe AD, patients begin to require assistance with dressing and bathing, the mechanics of toileting, and may have difficulty counting backward from 10 and in participating in sports with slower steps when walking. The magnitude of cognitive and functional decline in moderately severe AD is often combined with disturbed behavior and makes care giving especially burdensome to spouses at this stage. This stage often lasts about 2½ years.

When patients become incontinent of urine and feces, begin to scream or cry

out frequently, and have speech limited to a few words, they have developed severe AD, which lasts a mean of 1½ years, ending frequently in death from pneumonia.

AD was originally defined as presenile dementia, but it now appears that the same pathology underlies the dementia irrespective of the age of onset. The term dementia of the Alzheimer type (DAT) thus denotes all dementias that do not have an obvious organic cause, such as stroke, brain damage or alcohol. Its prevalence rises sharply from the age of about 60 years, and reaches 90% or more

by the age of 95. Until recently, age-related dementia was considered to result from the steady and unavoidable loss of neurons that goes on throughout life, possibly accelerated by a failing blood supply associated with atherosclerosis. It is now clear that DAT is associated with quite specific biochemical abnormalities, which raises the hope – not yet realised – of being able to inhibit the neurodegenerative process by drug treatment. The biochemical mechanisms are well reviewed(1-4) and pathogenesis of AD can be understood from the figure.

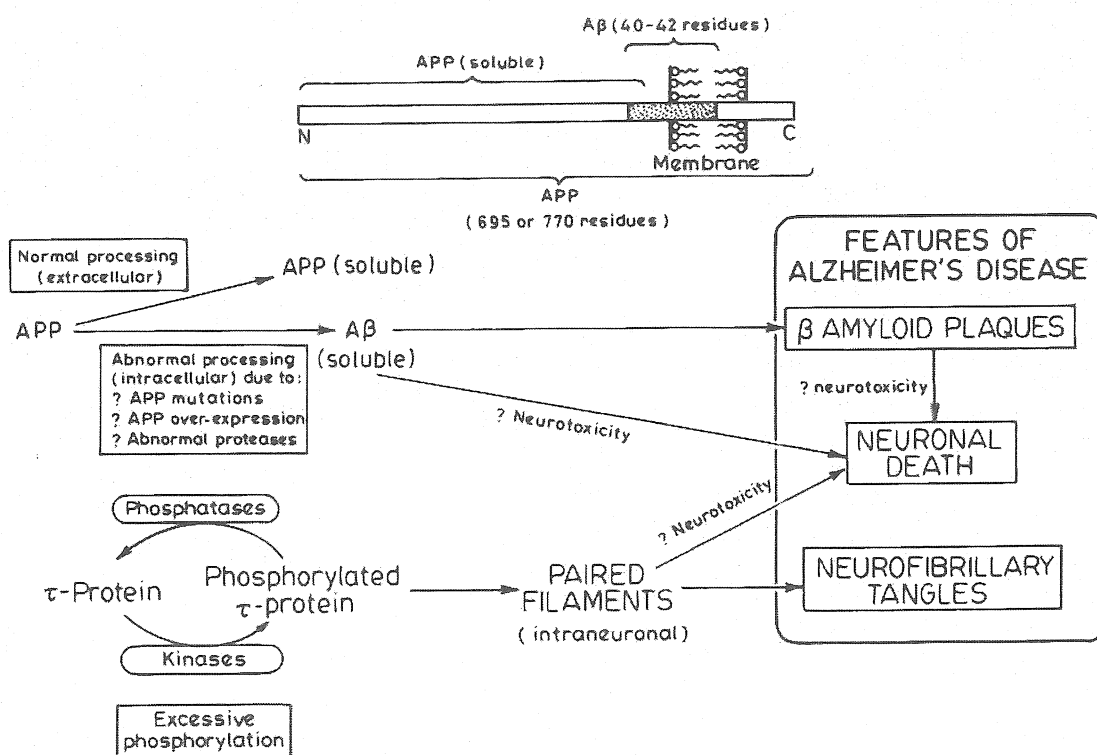


Fig. Pathogenesis of Alzheimer's disease. The amyloid precursor protein (APP) is normally processed by extracellular cleavage to produce the secreted (soluble) form of the protein, which is not associated with neuronal damage. Abnormal processing by cleavage at different sites results in formation of the soluble Aβ fragment, which causes neurodegeneration and the deposition of amyloid plaques. The microtubule-associated τ-protein is excessively phosphorylated in Alzheimer's disease and aggregates as intracellular paired helical filaments, which may be toxic to the cell, later giving rise to extracellular neurofibrillary tangles.

Short historical account of Alzheimer's life & professional career:

Any description of senile dementia will be incomplete without a brief description of the life and time of its pioneer. Aloysius (Alois) Alzheimer was born in the small Bavarian town of Marktbreit, in 1864. His father was a royal notary who moved with his family to the city of Aschaffenburg in the vicinity of Frankfurt two years after young Aloy's birth. However, it was only in the 1990s, when worldwide interest began to focus on AD, that the city elders of Marktbreit became aware of their famous son. In order to accommodate the increasing numbers of mostly Japanese and American visitors who began to roam the streets of Marktbreit in search of Alzheimer's birthplace, the city elders decided to designate one of the little stone houses as such. It is quite possible that Alzheimer was in fact born in a less attractive house near the more western margin of town.

For a period of 14 years after his graduation, Alzheimer worked as an intern, resident, and chief resident in the 'lunatic asylum' managed by the city of Frankfurt. Most of the patients of that institution at that time suffered from so-called paralytic disease, a manifestation of late stage syphilis. It was during his Frankfurt years that Alzheimer's interest in neuropathology was kindled by his close friend and colleague Franz Nissl (who later became a well-known neuropathologist). A sad blow to his personal life was the early loss of his wife Cäcilia, who died in 1901 after only seven years of marriage. After Cäcilia's death, he asked his sister to manage his household and care for his three children. He was only too happy to leave the Frankfurt area when Nissl asked him to join the Department of Psychiatry at the University of Heidelberg. The chairman of the Heidelberg department was the

godfather of German psychiatry, Emil Kraepelin. He moved to Munich shortly after Alzheimer's arrival in Heidelberg. Alzheimer accepted Kraepelin's offer to move with him to Munich, even though he had to work there for several months without pay. Kraepelin became very fond of Alzheimer and provided him with a well-equipped anatomical laboratory on the third floor of the famous Munich Department. He jokingly referred to Alzheimer as the 'psychiatrist with a microscope'. But Kraepelin was also worried about Alzheimer's heavy work-load, which comprised clinical duties, research, teaching, and (since 1906) a good deal of administration as the acting vice-chairman of that busy department. In 1904 Alzheimer fulfilled the requirements to become a university professor by submitting and defending a thesis entitled, 'Histological studies on the differential diagnosis of progressive paralysis'. In this 'Habilitationsschrift,' Alzheimer for the first time mentioned changes in senile brains ('miliary plaque formation') which clearly differ from those found in neurosyphilis. But it was his very short presentation at the 1906 convention of the southwest German psychiatrists in Tübingen that described the first clinical case of senile dementia exhibiting the new classical neuropathological changes. His mentor Kraepelin soon thereafter referred to these changes in the brains of demented older patients as 'AD'.

Alzheimer himself could not be credited with the first description of the senile plaque. This was provided by the neuroanatomist Redlich in 1898, whose work, however, was appropriately cited by Alzheimer in his 1904 thesis. Independent of Alzheimer, Mijake of Vienna and Oskar Fischer of Prague published early accounts of the senile plaques (in 1906 and 1907, respectively)

but contrary to Alzheimer, Fischer believed that these changes in senile brains (which he called 'drusal necrosis') might be the result of bacterial infection. Both in his 1898 paper 'The colloidal degeneration of the brain' and in his 1906 patient report, Alzheimer referred to deposits of a 'peculiar substance' that proved resistant to staining with most conventional dyes. Was it amyloid that Alzheimer recognized as being different from the other 'colloids'?

During his Munich years, Alzheimer was totally devoted to his work, believing that many so-called psychic diseases would show evidence of neuropathological changes if one were only to perform careful and extensive histopathological examinations of the brain in each case. Together with Kraepelin, Alzheimer is thus clearly considered to be the founding father of biologically oriented psychiatry. In his few hours of spare time, he retreated to his home to be with his family, avoiding most social contacts with the Munich medical and social establishment. For his children, he bought a vacation home on a nearby lake, which is still owned by one of his granddaughters. His professional goal was to write a text book on the 'Histopathology of Psychotic Disease', but due to his failing health, this never materialized. Alzheimer, in the meantime, had acquired an eminent position in German academic life of medicine. He was one of the four Editors of the prestigious *Zeitschrift für die gesamte Neurologie und Psychiatrie*, the leading journal of these fields at that time. Like many of his contemporaries, he was worried about the rising numbers of inpatients that populated the 'insane asylums'. In a most influential editorial in the *Zeitschrift*, he supported the addition of a division of psychiatry to the National Health Agency. He had the foresight to

conclude that only research by such a high-powered and independent institution could settle the question of whether the increase of insane patients resulted from genetic 'degeneration' or from environmental and social causes. However, in contrast to his colleague, Hoche, who in 1922 published the infamous book advocating euthanasia, Alzheimer made no public statements that could be misconstrued as outright support for the Eugenics Movement.

Against the advice of Kraepelin, who was concerned about his health, Alzheimer accepted, in 1912, the chairmanship of the Department of Psychiatry at the University of Breslau in then Upper Silesia (now Poland). During his train ride to Breslau he contracted what, in retrospect, might have been streptococcal angina, since his heart as well as his kidneys became affected. Alzheimer never completely recovered and, after three painful and ailing years, he died in Breslau in 1915, barely 52 years old(5).

Recognition of Alzheimer's disease as a major public health problem:

Following Alzheimer's description in 1907(6) of the typical pathological changes-the brain atrophy, the neuritic plaque, the neurofibrillary tangle in the case of a woman who died in her fifties following a several-year course of progressive memory loss, having the symptoms of difficulty in naming and delusions, the existence of this disorder as a presenile dementia was immediately recognized and the eponym was widely accepted. A number of cases of elderly individuals with so-called senility who had the same pathologic changes as described by Alzheimer in his presenile case were reported. But there was not unanimous agreement about the relationship of Alzheimer pathology and

'senile dementia'. In particular, other authors emphasized the occurrence of multiple strokes, particularly small strokes, in brains of older individuals who died at state hospitals in a senile condition(7), although as early as 1948. Newton(8) had noted that the clinical course of AD and that of senile dementia was quite similar, but not much attention was paid to this prescient report.

The modern scientific approaches to AD can be dated to the early 1960s when two laboratories(9,10) first described the ultrastructure of the plaques and tangles that Alzheimer observed using silver stains. Both Terry(9) and Kidd(10) noted the unusual twisted fibrils within the neurofibrillary tangles which are now termed 'paired helical filaments' and the amyloid structure of the protein that accumulated in the center of the neuritic plaque. The periodic structure of the paired helical filament in the neurofibrillary tangle and presence of degenerating swollen neurites surrounding an amyloid core within the neuritic plaque was found in both 'presenile' and 'senile' cases. In 1968, Blessed *et al.*(11) carried out a prospective study in a group of very elderly in nursing home and a chronic disease hospital, measuring cognition and function during life and comparing the scores obtained with the *number* of plaques and tangles and the *volume* of cerebrovascular infarcts present in the brains at postmortem examination. This seminal work established that, in the elderly population, the majority of cases showing dementia during life had pathological changes characteristic of AD, with the number of neuritic plaques showing a reasonable correlation with mental status and functional scores during life. Among those with major cerebral infarcts caused by cerebrovascular disease, the volume of the cerebral infarcts appeared related to the presence

of dementia. It thus became recognized that on a pathological basis, a majority of individuals in late life with dementia did, in fact, have essentially identical pathology to that of a typical presenile Alzheimer brain. It became apparent that the separation on the basis of age of onset was quite arbitrary and that, in fact, most patients with 'senility' or 'senile dementia' were not suffering from normal aging, but instead, had AD(12).

In 1976 and 1977, the interest in AD was intensified by a major research breakthrough when three laboratories in Great Britain reported a major loss of choline acetyltransferase, the marker of cholinergic terminals, in neocortex of the brains of Alzheimer patients(13-15). This intrigued clinicians because it was initially hoped that replacement therapy for acetylcholine would have the same usefulness in AD as replacement therapy with L-DOPA had in Parkinson's disease.

These advances in understanding the importance of AD and that it might be approached through biomedical research led to the convening of a workshop conference on AD that was sponsored jointly by three of the National Institutes of Health of USA– the National Institute on Neurological Diseases, the National Institute of Mental Health, and the newly formed National Institute on Aging – a workshop that was most successful in its attempt to interest a variety of neuroscientists and others in the problem of AD(16).

Biological basis of cognitive disorders and their treatments:

Understanding of the biological basis of cognitive disorder is undergoing rapid advancement. The research progress and hypothesis about the brain abnormalities

underlying AD can be seen under following points:-

Amyloid cascade hypothesis of AD : AD is essentially a disease in which the abnormal deposition of beta amyloid gets to the point that it destroys neurons. Thus, AD may be essentially a problem of too much formation of beta amyloid or too little removal of it. One idea is that neurons in some patients destined to have AD have an abnormality in the DNA that codes for a protein called amyloid precursor protein (APP). The abnormal DNA starts a lethal chemical cascade in neurons, ultimately resulting in AD. Specifically, the abnormal DNA causes the formation of an altered APP, which instead of being removed from the neuron, causes the formation of beta amyloid deposits. The beta amyloid deposits and fragments go on to form plaques and tangles. The presence of plaques and tangles signals cell damage and cell death. Sufficient cell damage and cell death gives rise to the formation of the symptoms in AD(17).

Another aspect of the amyloid cascade hypothesis is the possibility that something is wrong with a protein that binds to amyloid and removes it. This protein is called apolipoprotein E (APO-E). In case of 'good' APO-E, it binds to beta amyloid and removes it, preventing the formation of AD. In case of 'bad' APO-E, a genetic abnormality in the formation of APO-E causes it to be ineffective in how it binds beta amyloid. Ultimately, this causes beta amyloid to be deposited in neurons, which goes on to damage the neurons and causes AD.

Current therapeutics are aimed at the possibility that, altering the synthesis of APP or APO-E might change the deposition of beta amyloid and prevent the progressive course of AD. Another possibility is to inhibit the synthesis of beta amyloid, much the same way that

lipid lowering agents act to inhibit the biosynthesis of cholesterol in order to prevent atherosclerosis.

Cholinergic Theories of AD : One leading theory for the neurochemical basis of AD is a 'Cholinergic' deficiency hypothesis, which proposes that the memory disturbance in AD is caused by loss of cholinergic neuronal functioning. Perhaps this is because beta amyloid is deposited in cholinergic neurons and thus destroys them. Centrally acting antimuscarinic drugs such as scopolamine cause transient impairment of memory and learning both in the animals and humans(18,19). Moreover surgical and chemical lesioning of cerebral cholinergic system induces a cognitive deficit in animals(19-21). Brain autopsies of patients died from AD show a significant decrease in the activity of cortical and hippocampal choline acetyl transferase (ChAT) and a deficiency of cholinergic neurons in septum and nucleus basalis meganocellularis(22-24). Decreased activity of acetylcholine esterase (AChE) has a correlation with the degree of cognitive impairment(25). The earliest attempts to boost cholinergic functioning in AD were made using rather rudimentary cholinergic agents, namely the precursors for ACh synthesis and lecithin (phosphatidyl choline). This was based upon an analogy the Parkinson's disease where neurodegeneration of dopaminergic neurons causes symptoms that can be successfully treated by administering the precursor of dopamine, viz. L-DOPA. The hope was that giving the ACh precursors choline and lecithin would enhance ACh synthesis and thereby cause successful treatment of the symptoms of AD. However, results of the 17 clinical trials employing these agents in the treatment of mild to severe degree of AD did not show any

improvement in neurophysiological status or the cognitive performance(26). The lack of efficacy of cholinergic precursors is probably due to loss of presynaptic cholinergic neurons and deficiency of ChAT in AD patients, both of which are required for synthesis of acetylcholine(27). Moreover, large doses of these agents (choline>16 g/d, lecithin>20-100 g/d) are required and such doses are often difficult for patients to tolerate. On the other hand, long term administration of lecithin delays the deterioration of cognitive function(28,29). 2-Glycerophosphorylcholine is a recently developed cholinergic precursor that is metabolised to phosphorylcholine in the body(30). Administration of 2-glycerophosphorylcholine (800 mg qid) for six months leads to a modest improvement in cognitive status of the patients of AD(31). However this trial was neither blind nor placebo controlled. Many studies have also attempted to show the efficacy of cholinergic precursors in the AD, but this approach has unfortunately not proven to cause clinically significant results. Multiple studies of cholinergic precursors have led to essentially negative results that do not offer meaningful hope for improvements in patients with AD.

Currently, there are several approaches to boosting ACh functioning that are under investigation and that are attempting to improve memory functioning in AD. The most powerful and successful to date is to inhibit ACh destruction by inhibiting the enzyme acetylcholinesterase. This causes the buildup of ACh, which is no longer destroyed by acetylcholinesterase. This approach has led to the only therapy approved for treatment of AD in the United States, namely tetrahydroamino acridine (THA) or tacrine. THA also enhances monoaminergic neurotra-

nmission in addition to cholinergic neurotransmission by inhibiting MAO-A and MAO-B activities(32). The drug also blocks K⁺ channels, thereby may enhance the presynaptic release of ACh(33). Moreover, the drug directly binds to cholinergic receptors and high concentration of drug in brain suggests that all these effects may contribute to the beneficial effects of THA at therapeutic concentrations(34). Tacrine at 1.5 mg/kg dose in AD patients improved some symptoms of senile dementia(35). Result of most of the studies implicate that co-administration of lecithin does not offer any additional beneficial effect(36-38). In addition there have been 6 parallel, randomised, double blind, placebo controlled trials of tacrine without the concomitant use of lecithin(39). A recent multicentric study of longer duration with THA shows a significant dose dependent deceleration of cognitive deterioration in patients(40).

Suronacrine (HP-128) is a newer synthetic THA derivative. The drug is a less potent AChE inhibitor as compared to THA. Additionally, it has norepinephrine and dopamine uptake inhibiting and cholinergic receptor blocking properties. The drug has been demonstrated to be well tolerated in patients of AD(41). However, its therapeutic efficacy remains to be established. Velnacrine maleate (HP-029) is the main metabolite of tacrine and suronacrine in man and is a reversible AChE inhibitor. Due to its frequent side effects such as dizziness, fainting, diarrhoea, hepatotoxicity and neutropenia its development has been discontinued in 1994. Galanthamine is the only other drug besides tacrine to be approved by FDA in the treatment of AD. Oral administration of galanthamine (30 mg/day) for 6-10 weeks leads to an improvement in cognitive function

comparable to that achieved with tacrine(42). In spite of this, physostigmine, a centrally acting acetylcholinesterase inhibitor, has also been extensively studied in the treatment of AD. When administered iv to patients with AD, physostigmine improved retrieval capacity in all the five clinical trials(43). Similarly, 6 out of the 8 clinical trials conducted using orally administered physostigmine also demonstrated an improvement in the cognitive status of AD patients(43,44). Despite such encouraging results, the drug had failed to find a routine clinical use because of its short half life and limiting peripheral side effects. Heptylphysostigmine (L-693487) is a more lipophilic and longer acting carbamate derivative of physostigmine. The drug has been shown to facilitate learning and memory in animals(45,46) and was well tolerated in patients of AD(47). However, clinical trials of the drug had to be abandoned because of the incidence of neutropenia. As already discussed, the relentless march of the destructive process underlying AD is not halted by above mentioned drugs, meaning that the improvements in those patients who experience improvements is only transient and not sustained.

Another approach that has only met limited success regards to cholinergic agonists. Various agonists are under investigation, especially agonists for the M_1 cholinergic receptors. Cholinergic receptor agonists act by directly stimulating both the muscarinic and nicotinic postsynaptic receptors in central nervous system. Therefore, in contrast to cholinergic precursors, these agents do not require the presence of intact presynaptic neurons. Acetyl-L-carnitine, being a weak cholinergic agonist or because of its participation in the formation of acetyl coenzyme A, may exert a beneficial effect in cognitive disorders(48). Acetyl-L-

carnitine reverses amnesia, oedema and electrolyte imbalance following brain ischaemia in animals(49). Clinical studies employing acetyl-L-carnitine (2-3 g, po) daily for 6-12 months demonstrate an improvement and a delay in the rate of deterioration of cognitive status in AD patients(50,51). However, clinical trials of longer duration and involving more number of patients have been disappointing(52). Bethanechol, a short acting, selective muscarinic receptor agonist has to be administered by intracerebroventricle (icv) route because of its poor blood brain permeability. Beneficial effects of bethanechol on learning and memory in AD patients are modest(53-55). On the other hand, RS-86 is a long acting muscarinic agonist that effectively crosses blood brain barrier and is more potent at M_1 as compared to M_2 muscarinic receptors. However, oral administration of RS-86 produces a slight or no improvement in cognitive function, mood and social behaviour in patients of AD(56-60). Therefore, the drug is no longer being investigated for the treatment of AD. Arecoline is a cholinergic agonist with nicotinic and muscarinic receptor agonistic properties. Multiple administration or acute continuous infusion of arecoline did not have any significant improvement in memory in AD patients(61-65). On the other hand, continuous iv infusion for 5 days produces a significant improvement on verbal memory test(65). Short half life of arecoline due to its rapid hydrolysis may account for the negative results of the former reports. These reports suggest that the beneficial effects of muscarinic agonists in AD are equivocal. Moreover, density of muscarinic receptors is not reduced in the brains of AD patients as compared to age matched control individuals(66-68). Inhibition of ACh

release through stimulation of M_2 presynaptic receptors may explain non-effectiveness of cholinergic receptor agonists in AD. Nicotinic agonists are also being tested. A close association between the reduced number of nicotinic receptors and degree of cognitive impairment has been reported(68,69). Moreover, nicotine and nicotinic agonists were reported to improve cognition in animals(70,71). The possible advantage of stimulating nicotinic receptors is suggested by several epidemiological studies finding lower risks for AD among smokers. In addition, central nicotinic receptors are reduced in the brains of Alzheimer's patients. To date, no such agent has been licensed for the treatment of AD.

Yet another possibility is to develop an agent that can release ACh, perhaps through blocking potassium channels. Depolarisation of neuron can be inhibited *via* influx of potassium through both the voltage activated and calcium activated potassium channels. *In vitro* incubation of cultured hippocampal neurons with β -amyloid precursor protein led to activation of potassium channels and suppression of neuronal activity(72). Potassium channel blockers, fampridine (4-AP) and linopridine (DuP-996), induce neuronal depolarisation thereby augmenting the release of depolarisation. This depolarisation triggers the augmentation of ACh release as well as some other neurotransmitters such as dopamine and serotonin. Administration of fampridine for 6 weeks improved cognitive function of AD patients(73). In a more recent study no such improvement was noted, however the drug was administered for four days only(74). Linopridine protects against hypoxia induced memory deficit and facilitates learning in experimental animals(75,76). This approach is heavily dependent, however, on the presence of intact remaining presynaptic cholinergic

nerve terminals and may therefore only be effective in the early stages of the disease.

Excitotoxic hypothesis of Alzheimer's disease: The excitotoxic hypothesis of AD proposes that neurons degenerate because of excessive excitatory neurotransmission at glutamate neurons and the process is known as 'excitotoxicity'. Excitotoxicity is not only a hypothesis to explain neurodegeneration of AD but has also been invoked as an explanation for neurodegeneration in any number of neurological and psychiatric conditions, including the negative symptoms of schizophrenia, Parkinson's disease, ALS and even stroke. If neurodegeneration is caused by too much glutamate, then antagonists of glutamate could theoretically halt such a neurodegenerative process. The N-methyl-D-aspartate (NMDA) subtype of glutamate receptor is thought to mediate both normal excitatory neurotransmission as well as neurodegenerative excitotoxicity in the glutamate excitation spectrum.

Long-term potentiation (LTP) is the phenomenon of increased synaptic efficacy mediated through repetitive high frequency neuronal stimulation and is thought to be the physiological phenomenon involved in learning and development of memory. Stimulation of NMDA receptors, a subtype of excitatory amino acid (EAA) receptors is essential for LTP formation. BMY-21502, a pyrrolidinone derivative, known to produce LTP formation is undergoing phase III clinical trials for the treatment of cognitive dysfunction associated with AD(77). Overactivation of EAA receptors may lead to delayed neurodegeneration as a result of increased influx of calcium ions. Increased calcium may mediate cell

death by several mechanisms including activation of protein kinases, phospholipases, nitric oxide synthase, proteases, generation of free radicals, inhibition of protein synthesis and mitochondrial damage(78). Recent animal studies with mitochondrial nimodipine(79), a calcium channel blocker, may attenuate neurotoxicity by preventing calcium entry into the neuron. In clinical studies of AD patients, nimodipine(80) prevented further deterioration of cognitive dysfunctions.

Antagonists for any of the various modulatory sites around the NMDA – calcium channel complex would possibly restrict the flow of calcium and close the channel and therefore be candidates for neuroprotective agents. Such antagonists are being developed and tested in various disorders hypothesised to be mediated by an excitotoxic mechanism. This therapeutic approach follows in part clinical observations that AD and other neurodegenerative disorders are not static disorders, but are progressive, suggesting an active and ongoing neurobiological process underlying such disorders.

Excitotoxicity is thus a major current hypothesis for explaining a neuropathological mechanism that could mediate the final common pathway of any number of neurological and psychiatric disorders characterised by a neurodegenerative course. The basic idea is that the normal process of excitatory neurotransmission runs amuck, and instead of normal excitatory neurotransmission, things go out of hand, and the neuron is literally excited to death.

Various rudimentary "neuroprotective" agents and antioxidant/anti-free radical therapies have already begun clinical testings. Trials of vitamin E, which has some antioxidant/free radical scavenging activity, have already begun in Parkinson's disease, tardive dyskinesia and even AD,

but without definitive results to date. Vitamin E is not a very potent neuroprotective agent and may therefore not exhibit sufficient efficacy to show neuroprotective properties in short-term trials.

L-Deprenyl (selegiline) is an irreversible inhibitor of monoamine oxidase (MAO), specifically of the B form. It is currently used in the treatment of Parkinson's disease to boost the effectiveness of dopaminergic agents, and possible to delay disease progression. It is now being studied in AD, since it has certain "antioxidant" effects that may prove to be neuroprotective. Some studies suggest that L-deprenyl may have additive effects to those of tacrine, if only short term(17).

Nimodipine is a calcium channel antagonist, now marketed for cerebrovascular disease, that may normalize cellular calcium levels or possibly affect another mechanism such as calcium-activated enzymes involved in cognition. Nimodipine is in clinical trials to determine its efficacy in improving global measures of memory function. Calcium channel antagonists are already used as possible neuroprotective and/or cognitive enhancing agents, especially in Japan and in some European countries(17).

Sabeluzole is an agent with some neuroprotective activity in animal models of hypoxia and also enhances axonal transport and neurite outgrowth in neuronal cells in culture by an uncertain mechanism. It is currently undergoing clinical testing in AD(17).

Cognitive enhancers and early agents of unproven or limited efficacy:

Cerebral vasodilators: Investigation of cerebral vasodilators was originally based upon the hypothesis that dementia

is caused by atherosclerosis of cerebral vessels. Over the years, a wide variety of treatment modalities have been proposed to improve cerebrovascular circulation, including carbon dioxide, carbonic anhydrase inhibitors, anticoagulants, nicotinic acid (a vitamin B₆ derivative), pyritinol, meclofenate, vitamin E, hyperbaric oxygen and vasodilators such as papaverine, cyclandelate, isoxsuprine, vincamine, and cinnarizine. All these modalities were aimed at improving oxygen delivery to the brain. None of these strategies has proven to be effective, however, and the hypothesis that faulty circulation in the dementia process is no longer tenable.

Metabolic enhancers: During the era when AD and dementia in general were believed to result from cerebrovascular insufficiency, many drugs with vasodilator activity were originally tested in AD. Hydergine is the brand name of a mixture of ergot alkaloids, and was the first us FDA approved drug for the treatment of dementia, although not specifically for memory disturbances in AD. Hydergine was marketed as a 'cerebral vasodilator' due to its putative but fairly weak alpha adrenergic antagonist actions which might be expected to cause dilation of blood vessels. Subsequently, the drug was reclassified as 'metabolic enhancer(81)' because of its ability to change second-messenger cyclic adenosine monophosphate (cAMP) levels and because of the possibility that it acted as a partial agonist at dopamine, serotonin and norepinephrine receptors. Several studies of higher doses of Hydergine have shown some beneficial effects in dementia especially when cognitive impairment was mild(82) but more recent well designed trials have questioned its utility for moderately to severely ill patients(83,84).

Vitamins and hormones: Although vitamin B₁₂ and zinc abnormalities have been described in AD, most studies of replacement therapy with these agents have been negative. Thiamine and estrogen replacement therapies also have equivocal effects upon global assessment of cognitive functioning in trials of AD, leading to the testing of angiotensin-converting enzyme (ACE) inhibitors such as captopril, which may have memory-enhancing effects in animals. However, data from clinical studies remains uncertain. 4-aminopyridine enhances calcium influx into neurons with possible procholinergic activity and has been tested in AD with equivocal results.

Chelation: Speculation regarding the role of aluminum in AD has prompted clinical and experimental use of chelation therapy to remove aluminum. Trials with chelating agents such as desferrioxamine, however, have been negative and the potential efficacy of future chelation therapy is uncertain. Chelation therapy is now largely considered to be an expensive and elaborate placebo for the treatment of AD.

Nootropics: Nootropic drugs are a class of psychotropic drugs that enhance learning acquisition and reverse learning impairments in experimental animals. The term nootropics was introduced by Giurgea in 1972 to describe a novel class of compounds which have the ability to improve the integrative brain functions. In addition to the ability to enhance memory and learning feature, the other hypothesised actions are facilitation of the flow of information between the cerebral hemispheres, enhancement of the resistance of the brain to physical and chemical assault and lack of sedative, analgesic, or neuroleptic activity. These agents improve cerebral

metabolism by augmenting protein synthesis and ATP formation which may account for the ameliorative effect of these agents on mental performance(85,86). Piracetam is a prototype of this class which increases regional metabolism as measured by positron emission tomography(86).

Although piracetam administration is reported to improve behaviour, mood and performance in neurophysiological tests, there is no consistent evidence for a beneficial effect of this drug on cognitive function in patients of AD(87,88). Moreover, nootropics combined with various cholinergic precursors like lecithin and choline have proved to be ineffective in AD(89,90). Similarly, piracetam and oxiracetam do not alter the course of cognitive impairment due to AD(91,92). In USA, oxiracetam has been withdrawn from phase II clinical trials due to its lack of efficacy(93). However, aniracetam, a newer derivative of piracetam has been shown to be superior to placebo when administered orally at a dose of 1500 mg daily(94).

Neuropeptides: Several neuropeptide neurotransmitter systems are known to be distributed in AD, including somatostatin, corticotrophin-releasing factors, neuropeptide γ and substance P. Substantial evidence suggests the involvement of vasopressin and adrenocorticotrophic hormone (ACTH) in learning and memory in animals(95). The synthetic analogues of vasopressin, des-9-glycinamide-arginine VP and ACTH such as ACTH₄₋₁₀ do not have a notable beneficial effect in AD patients(96-98). Somatostatin concentration was also found to be reduced at cerebrum in patients of AD(99). Somatostatin analogues such as L 363, L 586 and octreotide do not improve cognition in AD patients(100,101).

Psychostimulants and neurotransmitter replacement therapies: Psychosti-

mulants such as methylphenidate may improve mood in depressed demented patients, but do not enhance cognition and knowledge to increase cerebral catecholamine turnover have proven to be of little value in AD(102,103). An early double blind placebo controlled study demonstrated an improvement in cognitive tests of patients of AD treated with L-DOPA and benserazide(104). However, the patients included in the study were relatively young and diagnostic criteria were poorly defined. Subsequent well-designed placebo controlled studies ruled out any beneficial effect of L-DOPA in AD(105).

Although the strategies to augment cholinergic neurotransmission have received utmost attention, the lack of success in reversing all cognitive deficits by targeting this neurotransmitter has prompted the researchers to investigate alternative neurotransmitter systems. Post-mortem brain analysis of AD patients have inconsistently shown low concentrations of norepinephrine, dopamine and dopamine β -hydroxylase(106). Moreover, neuronal cell loss and β -amyloid deposition has been demonstrated in the locus ceruleus(107). Therefore, several agents modulating the cerebral concentration of these monoamines have been tested for their possible therapeutic benefit in AD. Although, clonidine and guanfacine, α_2 adrenergic agonists, improve learning and memory in aged monkey(108) they do not show any beneficial effect in double blind placebo controlled clinical studies in patients with AD(109,110).

On the other hand, selegiline hydrochloride, a MAO-B inhibitor, administered in a dose of 10 mg/day demonstrated mild but significant improvement in cognitive tasks in a double blind placebo controlled trial. In

addition to its capacity to increase brain concentration of norepinephrine and dopamine, its antioxidant property may also contribute to its beneficial effect(111-113).

Miscellaneous: Substantial experimental evidences suggest the involvement of immune and inflammatory mechanism in AD. Activated microglia have been shown to be present near or within the affected neurons in AD(114). These cells produce complementary proteins(115), cytokines, reactive oxygen species(116) and nitric oxide(117). Activity of cytokines such as IL-1 β , IL-6 and TNF- α was found to be elevated in brains of patients of AD(118-120) and expression of β -amyloid precursor protein was influenced by these cytokines(121). Moreover, dystrophic neurites containing extracellular deposits of β -amyloid have been shown to possess C5b-C9 complement protein complex in their neuronal membrane. On the other hand, no such complement complex was present in age matched control neurons containing extracellular deposits of β -amyloid(122). These findings were further supported by epidemiologic data demonstrating that patients who take anti-inflammatory drugs or suffer from inflammatory disorders have a reduced risk of developing AD(123-125). Similarly, incidence of AD was reported to be much less in leprosy patients taking dapsone that has anti-inflammatory properties(126). More recently, this inverse relationship between AD and prior use of non-steroidal anti-inflammatory drugs has been confirmed in many prospective case control studies as well(127,128). These observations warrant the use of anti-inflammatory drugs in the palliation or prevention of AD. However, larger clinical trials of longer duration with these agents are currently underway and will help to

clarify the status of these interventions in AD. Colchicine and hydroxychloroquin are other anti-inflammatory drugs that can possibly be evaluated in AD. However, colchicine is used in animal experimental models for the production of permanent amnesia. Colchicine is believed to produce dose-dependent biphasic response, viz, facilitatory and deterioratory. Its facilitatory effect is yet to be clearly established.

In the past few years, involvement of free radicals is increasingly being implicated in neurodegenerative diseases like AD. β -amyloid has been demonstrated to augment the production of superoxide anion in endothelial cells(129), H₂O₂ in nerve cells(130) and NO₂ from microglial cells(131). Oxidative stress has been demonstrated to induce aggregation of soluble β -amyloid into insoluble plaques(132). Moreover, products of lipid peroxidation are elevated in cortical region of brains with AD(133). These observations point towards the possible beneficial effect of free radical scavengers in AD. Antioxidants, vitamin E and idebenone have been demonstrated to prevent cell death caused by β -amyloid protein and are currently being evaluated in the treatment of AD(134).

The therapeutic approaches in dementia can be summarized as in table 3.

Other current research approaches:

Growth factors: Neural regeneration or increased resistance to destructive process may be achievable with selected neurotrophic factors. Nerve growth is the prototype with particular potential to synergise with cholinergic therapy, because its receptors are primarily

Table 3. Therapeutic approaches in dementia.

Approach	Mechanism	Drugs and therapies	Status
Available or in development			
Improved blood flow/ Psychostimulation	Vasodilation Effects on monoamine receptors	Dihydroergotoxine Pentoxifylline	Many trials of vasodilators, psychostimulants, antidepressants etc. Efficacy not proven.
Nootropic agents	Not known	Piracetam	Improved learning and memory in animal models. Efficacy not proven.
Cholinergic replacement therapy	Cholinesterase inhibitors	Physostigmine Tacrine	Modest efficacy claimed in some trials. Hepatotoxicity reported with tacrine.
	Muscarinic agonists	Arecoline Pilocarpine New compounds in development	Some efficacy in animal models. Peripheral side effects not yet tested.
Hypothetical Improved neuronal survival Halting disease process	Neurotrophic factors	Nerve growth factors (NGF) and other growth factors	Improved forebrain cholinergic function in animal models. Special delivery systems needed for human use.
	Inhibition of amyloid formation	APP protease inhibitors, Inhibitors of APP phosphorylation	Not yet developed.
	Inhibition of τ -protein deposition	Inhibitors of phosphorylation	Not yet developed.
	Inhibition of excitotoxicity	Glutamate antagonists, Ca-channel blockers, Protease inhibitors, etc.	Being developed mainly in other indications (e.g. stroke).

localised on cholinergic neurons and it is present in relatively high levels in the basal forebrain where cholinergic neurons

degenerate in AD. Moreover, these changes are prevented by exogenous administration of nerve growth factor

(NGF)(135). Cerebrolysins, brain derived neurotrophic factor (BDNF), epidermal growth factor (EGF) and NGF are agents with marked neurotrophic activity. They attenuate degradation of jeopardised neurons and induce the sprouting of remaining healthy neuronal cells. Intracerebrovascular infusion of NGF has been shown to improve survival of cholinergic neurons and prevent the decrease in AChE, ChAT activity and was reported to enhance cognitive performance in fimbria-forebrain transected rats(136,137). Similarly, neocortical implantation of genetically modified fibroblasts which synthesise and release NGF, improves mnemonic deficit and improves cholinergic neuron survival in these animals(138). These encouraging results of animal studies have led to the possibility of employing trophic factors in the therapy of AD. In a single clinical study conducted so far, NGF (icv) has led to a slight improvement in verbal episodic memory(135). Future developments of newer strategies such as grafting of genetically modified cells, liposomal delivery or direct transfection of neurotrophic gene may overcome the obstacle of icv administration.

Another growth factor like substance is GM₁ ganglioside. Gangliosides in the brain are complex lipids associated with developing synapses. GM₁ ganglioside is capable of preventing neuronal degeneration in several animal models, and may also prevent retrograde degeneration of cholinergic neurons in the rat basal forebrain resulting from damage to the cerebral cortex. Although these strategies are in the very earliest stages of development, they represent concrete examples in animal models where endogenous trophic molecules potentially could be used to treat degenerative diseases such as AD.

Transplantation: The hypothesis that implanting healthy neuronal tissues may promote regeneration and return of function in the defected brain originates from animal experiments using tissues from fetal CNS, peripheral nerve, paraneural tissue and cultured cells. When transplanted into the brain, these tissues may exert therapeutic effects *via* a variety of mechanisms, like they may act as a chemical generator (e.g., of growth factors), or as a generator of glial cells, which in turn promote neuronal function. They may also provide the brain with regenerating axons in the transplant material that may innervate other neurons from the diseased brain. At present, this is a highly theoretical area of research without current clinical applications.

Immunisation with amyloid- β peptide: People suffering from AD develop a progressive dementia in adulthood accompanied by three main structural changes in the brain. They are diffuse loss of neurons in the hippocampus and neocortex, accumulation of intracellular protein deposits termed neurofibrillary tangles and accumulation of extracellular protein deposits termed amyloid or senile plaques, surrounded by misshapen nerve terminals (dystrophic neurites)(139). A main constituent of these amyloid plaques is the amyloid- β peptide (A β), a 40-42 amino acid protein that is produced through cleavage of the β -amyloid precursor protein (APP). A β seems to have a central role in the neuropathology of AD(140). Familial forms of the diseases have been linked to mutations in the APP and the presenilin genes(141,142). Disease linked mutations in these genes resulted in increased production of the 42-amino acid form of the peptide (A β ₄₂)(143-147), which is the predominant form found in the amyloid plaques of

AD(148,149). The PDAPP transgenic mouse, which overexpresses mutant APP (in which the amino acid at position 717 is phenylalanine instead of the normal valine), progressively develops many of the neuropathological hallmarks of AD in an age- and brain-region dependent manner(150,151). In a recent study, transgenic animals were immunised with A β ₄₂, either before the onset of AD-type neuropathologies (at 6 weeks of age) or at an older age (11 months), when amyloid- β deposition and several of the subsequent neuropathological changes were well established(152). The results of the above study showed that immunisation of the young animals essentially prevented the development of β -amyloid plaques formation, neuritic dystrophy and astrogliosis. Further, treatment of the older animals also markedly reduced the extent and progression of these AD-like neuropathologies.

These results raised the possibility of vaccination with A β against human AD. But before this can be seriously entertained, several questions must be answered as; Can injection with human A β induce enough of the antibody? Will immune tolerance frustrate this, as it has been often done with attempts to target cancers with antibodies? The most critical question is whether depletion of the amyloid plaques is accompanied by an improvement in the behavioural/neurophysiological impairments and a reduction in the nerve cell death of AD? In other words, does immunisation of the A β simply clear a neuropathological by-product or can it cure the disease?

Future combination chemotherapies for disorders associated with cognitive disturbance and memory loss:

There are tremendous economic incentives for developing the 'cure' and

treatment of choice for AD. Thus, it is not difficult to understand why most drug development activities for the dementias target a single disease mechanism, just as they do for virtually every other psychiatric disorder. The goal of most ongoing drug development programs in psychopharmacology is to find the single, best and only therapy for that disorder. In reality, however, it seems overly simplistic to conceptualise disorders with cognitive disturbances as the product of a single disease mechanism. Diseases such as AD not only have features of cognitive and memory disturbance but may also have various psychotic, mood, and behavioral deficiencies plus a certain neurodegenerative component. Also, it takes a leap of faith to believe that such complex disorders could ever be satisfactorily treated with a single entity acting by a single therapeutic mechanism.

Indeed, how realistic is it to ask a single therapeutic agent for AD to treat the memory and cognitive symptoms of dementia; to treat the often associated disorganized symptoms of AD such as psychosis, agitation, and poor impulse control; and to prevent further neurodegeneration.

Thus, AD treatments of the future may combine one treatment for cognitive and memory symptoms (perhaps some sort of procholinergic agent such as a cholinesterase inhibitor), with another treatment for disturbed, agitated, and violent symptoms of poor impulse control (possibly an atypical neuroleptic, or an agent working by a combination of actions on dopamine and serotonin receptors), with a neuroprotective agent such as glutamate antagonist. In the long run, some sort of molecular-based therapy to prevent genetically programmed disease progression will

also form part of the portfolio of treatment for AD.

The reason for this combined medication approach to AD is that it does not appear to be sufficient merely to replace the deficiencies in acetylcholine activity by drugs that enhance cholinergic function. This approach can boost memory function measurably in some patients, only to have this enhancement eroded by further disease progression within a few months in most patients. The replacement of cholinergic deficiencies by various pharmacological strategies may help memory; correct other neurotransmitter imbalances (e.g., of dopamine and serotonin); may treat behavioral disorders; neuroprotective and genetic strategies may halt the degenerative process and replace the functions of degenerated nerves.

Clinical trials with multiple therapeutic agents working by several mechanisms can be quite difficult to undertake, but as there is a clinical trials methodology that exists in the cancer chemotherapy literature, it may be an approach that should be applied for complex neurodegenerative disorders with multiple underlying disease mechanisms.

Conclusion

Recent studies have changed our understanding of the molecular pathology of AD. An improved understanding of the molecular basis of AD has simplified the concept of its underlying cause and raises exciting possibilities for approaches to new and specific treatment of AD. The amyloid cascade hypothesis is a leading theory to explain the neurodegeneration of AD, perhaps by deposition of neurotoxic amyloid protein. It is now emphasised to develop proteinase inhibitors and phosphorylation inhibitors of tau protein as future drugs for AD. Due to the fact that no single drug has proven to be

beneficial, emphasis is now being put on combined drug therapy for the treatment of AD. Moreover current research is now focused on transplantation of neuronal tissues and immunisation with amyloid- β peptide to treat AD patients.

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