CHAPTER- 1

Medicinal Chemistry of Arecoline Derivatives as M1 Receptor Agonist in Alzheimer’s Disease Research
1.0 History of Alzheimer's Disease (AD) Research

Kraeplin, a German scientist, who worked in Munich Psychiatric Clinic, made important contributions to the knowledge of different forms of dementia, especially those associated with psychosis, cerebral arteriosclerosis and senile dementia. Another scientist, Alois Alzheimer, working in the same clinic discovered that dementia was caused by abnormalities of tissues, cells and biochemicals.

On 3 November 1906, Alzheimer attended a meeting of South Western German Psychiatrists in Tubingen, where he presented a new and famous case history that led to the pioneering work on AD. A year later, he published the findings in a paper entitled “On a peculiar disease of the cerebral coaster” (Alzheimer 1907). The presentation involved the case history of a 51 year old female Auguste D, who was reportedly jealous of her husband, showed signs of paranoia, suffered from loss of memory, and her symptoms were observed to be progressive.

The case study included cognitive impairment, hallucinations, and focal symptoms also the post mortem analysis of her brain with extensive senile plaque and neurofibrillary angles. Microscopic examination by Silver Staining (Biels Chowsky staining) showed arteriosclerotic lesions and miliary foci (plaques) in cerebral cortex. The presentation revealed clinical distinction between presenile and senile dementia. Alzheimer expressed doubt as whether to put all this collective spectrum of clinical conditions could be placed under one disease. In later years, Kraeplin published the edition of his famous handbook of psychiatry in 1910, which involved several case studies of brains of presenile dementia and plaques. In this handbook Kraeplin used the term ‘Alzheimer's disease’ to refer to a particular type of dementia, which had been described by Alzheimer. Although Kraeplin gave the same name to refer to the presenile forms of dementia characterized by the pathology described by Alzheimer, it appears that he considered this to be essentially the same as senile dementia, and that it differed from the senile form mainly in so far as its age of onset was concerned. This has led to arguments as to whether presenile dementia is simply an early onset form of senile dementia; the controversy lasted from then till recently. At present, it is generally accepted that the two forms (presenile and senile dementia) are pathologically identical and, both types are referred to as Alzheimer’s disease. Many arguments have been put
forward to explain why Kraeplin so eagerly named AD on the basis of studies of only a handful of brains. It has also been argued that he intended to strengthen the case for the organic cause of psychiatric illness in order to counter Freud’s-theories. Torack (1978) even suggests that in reality, neither Alzheimer nor Kraeplin discovered AD; it was Freud who did so. Alzheimer published a study of another cause of the disease in 1911. But this time he did not find neurofibrillary tangles. By 1912, nearly 50 articles had been published on plaques and tangles in dementia, and it was recognized that tangles and senile plaques were not significantly elevated in syphilis, affective disorders and arteriosclerotic dementia

For most of the twentieth century, the diagram of AD was reserved for individuals between the ages of 45-65 who developed symptoms of presenile dementia. During this time senile dementia itself (as a set of symptoms) was considered to be a more or less a normal outcome of the aging process, and was thought to be caused by age related brain arterial “hardening.” In the 1970s and early 1980s as the symptoms and brain pathology were identical for Alzheimer victims older and younger than age 65, the name “Alzheimer disease” began to be used, within and outside the medical profession, during 1970s and early 1980s both for afflicted individuals of all ages. During this period the term senile dementia the Alzheimer type (SDAT) was often used to distinguish those who were over 65 and did not fit the classical age criterion. Eventually, the term Alzheimer’s disease was adopted formally in the psychiatric and neurological nomenclature to describe individuals of all ages with the characteristic common symptoms pattern, disease course and neuropathology

Morphometric studies done in 1970s and 1980s showed that the atrophy of AD brain, causing the cortical thinning and enlargement of the later cerebral ventricles, was the result of a massive neuronal loss particularly, in the temporal, parietal and entorntinal cortex, the hippocampus and amydala. The gross atrophy in advanced AD can now be used as an aid diagnosis by the use of brain imaging technique. Such aids to clinical diagnosis are of the utmost importance because, although differential diagnosis has greatly improved in the last three decades, in reality the definition of AD is still a clinco-pathological one requiring the presentation in life of dementia and the demonstration of Alzheimer neuropathology post-mortem. Currently, assessment of most demented patients relies largely on psychometric testing. Although the accuracy of diagnosis of
elderly patients is now in the order of 80-90%, it is still essentially a diagnosis of exclusion with an outcome of probable AD. Psychometric testing includes brain imaging consists of computerized tomography (CT) and magnetic resonance imaging (MRT) which are the most commonly used.

Animal experimentation in the 1960s provided much of the evidence to establish a link between short-term memory loss and a reduction in cholinergic function. In 1976 David Bowen's group showed a dramatic decrease in the cortical activity of the enzyme acetyltransferase in AD patients, which was later confirmed by reports of a loss of magnocellular neurons of the basal forebrain cholinergic nucleus.

The biochemical nature of senile plaques goes back to earlier period than the discovery of plaque in human brains. When senile plaques were first discovered, many scientists saw similarities between them and other deposits known generally as 'amyloid' from which the nature of the peptide deposited in senile plaques derives its name. Such deposits were first detected by Rikotansky in 1842, who used iodine staining of tissues and in the 1850s Vischow coined the name 'amyloid' to describe these apparently starch like deposits. The diseases in which these deposits formed were generally named the amyloidosis. In 1859, Freidrich and Kekule obtained evidence to prove that 'amyloid' deposits, far from being starchy, were mainly proteinaceous.

By the early 1910s plaques had been isolated from brains of AD patients and had been subjected to biochemical analysis. Nikaido et al., for example, showed that this major constituent was proteinaceous. Amino acid analysis of which demonstrated that isolated plaques were rich in glutamate, glycine, leucine and alanine, while plaque cores were rich in glycine, glutamate, aspartate, serine and leucine (Nikardo et al., 1970, 1971). These and other studies were the beginnings of serious biochemical investigations of Alzheimer plaques and they coincided with important improvements in biochemical methods.

1.1 Clinical Symptoms in AD

In comparison to late-onset AD, early-onset AD has been reported to be more often familial and is defined clinically by the presence of greater aphasia and apraxia.
Traditionally, the age of 65 has been used as a cut off line to separate late-onset senile dementia of the Alzheimer type from early-onset on presenile-onset AD, though no empirical justification for this cutoff line has been presented. Although early-onset cases were previously noted to have a more malignant downhill course 6 more recent reports have failed to demonstrate that such cases progress more rapidly. 8-13 Variables except agitation. The variables with significant betas were depression, language, and orientation. Language impairment and higher depression scores (Table 1) were associated with early onset, whereas greater difficulties with orientation were associated with later onset of AD (Table 2). Square root transformation of skewed variables and replacement of the Blessed Information-Memory-Concentration Test score by the AD Assessment Scale cognitive subscale score in the calculation of rate of progression did not substantially affect the results. The principal findings in this study are that early onset is associated with the presence of greater language and praxis difficulties and that early onset predicts the development of greater behavioral disturbance, notably depression, during the course of the illness. The association between prominent language and praxis difficulties and early onset is entirely consistent with the existing literature. 6,7,14,15 Prominent language and praxis disturbance in early onset cases may reflect varying neurochemical deficit profiles, 16 differential hemispheric involvement 15 or contrasting regional distribution of neuropathological changes in presenile-onset and senile-onset cases. Neurochemical and neuropathological data indicate that early-onset patients have more widespread and more severe losses in various biochemical markers than the late-onset patients 16-19

Furthermore, positron emission tomographic studies, showing right hemisphere hypometabolism, indicating prominent visuospatial impairment, in early-onset cases have been reported. 19 There is little empirical support for the validity of the cutoff line between <65 and _65 years, and the only postmortem validation for defining distinct neurochemical bases for early- and late-onset AD used ages of <79 and _79 years. 16 Although age at onset may be more likely to be associated with particular clinical symptoms, it does not appear to predict rate of disease progression. 13 Considerable confusion remains regarding the correct definition of early- and late-onset cases. Further research replicating and extending research finding must include more patients in the <55 age group to clarify trends and the >70 age group to more accurately reflect the community population with this disorder.
Common early symptoms of AD are,

1. Confusion
2. Disturbances in short-term memory
3. Problems with attention and spatial orientation
4. Personality changes
5. Language difficulties
6. Unexplained mood swings

It is important to understand that AD does not affect every patient in the same way. The stages listed below represent the general progression of the disease.²⁰

**Stage 1:** Early in the illness, Alzheimer’s patients tend to have less energy and spontaneity, though often this goes unnoticed. They exhibit minor memory loss and mood swings, and are slow to learn and react. After a while they start to shy away from...
anything new and prefer the familiar. Memory loss begins to affect job performance. The patient is confused, gets lost easily, and exercises poor judgment.

**Stage 2:** In this stage, the Alzheimer’s victim can still perform tasks independently, but may need assistance for more complicated activities. Speech and understanding become slower, and patients often lose their train of thought in mid-sentence. They may also get lost while traveling or forget to pay bills. As Alzheimer’s victims become aware of this loss of control, they may become depressed, irritable and restless. The individual is found to clearly become disabled. The distant past may be recalled, while recent events are difficult to remember. Advancing AD affects the victims’ ability to comprehend where they are, the day and the time. Caregivers must give clear instructions and repeat them often. As the Alzheimer’s victims mind continues to slip away, the patient may invent words and not recognize familiar faces.

**Stage 3:** During the final stage, patients lose the ability to chew and swallow. The very essence of the person is vanishing. Memory is now very poor and no one is recognizable. Patients lose bowel and bladder control, and eventually need constant care. They become vulnerable to pneumonia, infection and other illnesses. Respiratory problems worsen, particularly when the patient becomes bedridden. This terminal stage eventually leads to the tragic death.

The usual first symptom noticed is memory loss which progresses from seemingly simple and often fluctuating forgetfulness (with which the disease should not be confused) to a more pervasive loss of recent memory, then to that of familiar and well-known skills or objects or persons. Aphasia, disorientation and disinhibition usually accompany the loss of memory. AD may also include behavioural changes, such as outbursts of violence or excessive passivity in people who have no previous history of such behaviour. In the later stages, deterioration of musculature and mobility, leading to bed fastness, inability to feed, and incontinence will be seen if death from some external cause (e.g. heart attack or pneumonia) does not intervene. Average duration of the disease is approximately 7-10 years, although cases in which the occurrence of the final stage has been found varying from 4-5 years to 25 years.
1.2 The Medical Diagnosis of AD

A medical diagnosis of Alzheimer’s relies on a number of factors. Alzheimer’s symptoms must be documented and family members interviewed. A complete physical examination and medical history must be taken, which must include blood tests to rule out other causes of dementia. Brain imaging tools may be used to help make a medical diagnosis.

The only sure way to diagnose Alzheimer’s is to examine brain tissue after death. A medical diagnosis of AD in a living patient is divided into two categories: possible and probable. A diagnosis of “possible” Alzheimer’s indicates that while symptoms may be caused by the disease, the diagnosis is unable to rule out alternative causes. A “probable” medical diagnosis indicates that all other alternatives have been discounted.

Improving the methods early diagnosis of Alzheimer’s is a primary goal of much research. Drug treatments currently available, and those, which are in development, work best when the disease is treated in the earliest stages, before symptoms become severe and disruptive to day-to-day functioning.

The earlier an accurate diagnosis of Alzheimer’s is made, the better. This holds true for everyone involved, for individuals with the disease, their families, clinicians and researchers. For people with Alzheimer’s and their families, an early and definitive diagnosis provides an opportunity to plan and to pursue options of treatment and care while the patient can still make his own decisions. Clinicians increasingly will need effective tools for identifying people in the early stages of the disease, as new interventions are developed to stop or slow progression of symptoms. In research, earlier and more accurate diagnosis will simplify and improve the process of recruitment for clinical trials meant to test new preventive drugs.

AD pathology may begin to develop in the brain 10 to 20 years or more before clinical symptoms yield a diagnosis. Scientists have been actively looking for ways to diagnose Alzheimer’s in its pre-symptomatic or pre-clinical stages, and enormous progress has been made in this area. Recent advances in imaging and clinical assessment will help to identify patients in very early stages of the disease. Eventually, combinations
of specific imaging strategies with genetic, clinical and cognitive assessments may become the key to identifying people with an high risk of developing AD.

1.2.1 Describing Symptoms and Memory Loss

A list of symptoms is compiled during diagnosis, including memory loss, language difficulties, and personality changes. People suffering from memory impairment and other symptoms of Alzheimer's often underplay the severity of their symptoms. Family members should meet with the examining doctor and report any symptoms or signs of memory loss noticed by them.

1.2.2 Testing Memory

Clinical tests, such as the Mini-Mental State Examination (MMSE), are used to evaluate memory and cognitive function. While such tests are helpful and provide information about the severity of symptoms, they alone cannot be used alone to make a medical diagnosis.

1.2.3 Blood Tests: Kidney and Liver Function

Blood tests are used to rule out secondary causes of dementia symptoms. Kidney function, liver function, and hormone imbalances may all be detected by blood tests. Blood tests are also used to check for electrolyte imbalances, diabetes, and vitamin deficiencies, which may also produce in Alzheimer's symptoms.

1.2.4 Medical Imaging and Scans

Imaging tools, such as CT, MRI and PET scans are used to rule out physical causes of dementia symptoms. Scans may reveal blood clots, brain tumors, and evidence of strokes.

1.2.5 Magnetic Resonance Imaging (MRI)

Another study used MRI which is a much more common technique than PET imaging, to determine whether persons in a very early phase of developing Alzheimer's could be identified prior to a clinical diagnosis. The participants in the study received MRI scans at the start of and then were closely observed for three years to determine who
subsequently developed changes that met clinical criteria for Alzheimer's. The researchers found that they could identify people who would develop the disease over time with an high rate of accuracy, based on significantly smaller baseline volume measurements for specific brain regions, which likely reflects loss of brain cells in these areas.

A variation on this technique, called dynamic MRI, is now undergoing testing at the University of California, Los Angeles. Scientists at this university made comparative study of MRI scans of the brains of people with Alzheimer's with that of those who do not have it, filming the progression of damage to different brain areas over time. People with early AD showed damage deep within the brain. As the disease progressed, damage spread to the sides and then overwhelmed other areas of brain.

These and related studies indicate specific brain areas in the underlying early pathology of Alzheimer's and suggest that, by focusing on these areas, it may be possible to use existing imaging techniques to better identify people at greatest risk of developing the disease. These promising MRI techniques will need further research, refinement, and validation before MRI can become a part of standard clinical practice.

CT scan uses multiple x-rays to take pictures of the brain. Occasionally, brain tissue deterioration can be observed by a simple CT. However, this usually indicates that the disease is in an advanced stage.

1.2.6 Positron Emission Tomography (PET) Imaging

The gene APOE-e 4 has been associated with increased risk of Alzheimer's. Scientists have been increasingly interested in knowing whether the brain and brain function of people who carry one or more copies of APOE-e 4 are different from those who do not carry the gene and finding out if AD-like symptoms can be identified before the disease is diagnosed clinically. PET imaging can provide information on the metabolism of glucose (which the brain uses to fuel cellular processes) in specific brain regions, which is used to measure brain activity in those regions.

Recent studies using PET show that, despite similarities in age, gender, education, family history of dementia, and baseline performance on memory and other cognitive tasks, individuals with the APOE-e 4 gene(s) have reduced glucose metabolism in several
areas of their brains compared to people who do not have the gene(s). The differences in metabolism were found to be even greater two years after initial evaluation. Lower metabolism at the start of the study predicted a greater cognitive decline in subjects at genetic risk for Alzheimer's. Though longer follow-up studies are needed to determine how many of the APOE-e 4 carriers actually develop AD, these findings suggest that a combination of metabolic rate in the brain (as measured by PET) and genetic risk factors may be one way to detect AD before symptoms are evident.

A PET scan may provide a method of making an earlier Alzheimer's diagnosis. A PET scan works much like a CT scan, but radiolabelled metabolites are injected into the bloodstream and actually show up on the images. This allows the radiologist to see how well the brain is working. Active areas of the brain are highlighted on a PET scan, while areas of degeneration are dark. An EEG may reveal brain waves slower than normal, which is a possible indication of AD.

1.2.7 Spinal Taps and Screening Research

Spinal taps may reveal the presence of tau or beta amyloid proteins in spinal fluid. Both proteins are associated with AD. Clinical trials are exploring the possibility of using the presence of these proteins in spinal fluid may be used to make a definitive medical diagnosis. If they succeed, it may become possible to diagnosis Alzheimer's in its earliest stages, allowing for earlier medical interventions.\(^{21,22}\)

1.2.8 Cognitive Markers

Components of a standardized clinical assessment instrument appear to predict which individuals with very mild symptoms of impairment (called "mild cognitive impairment", or MCI) or "questionable" Alzheimer's have a high likelihood of converting to the disease over time. The assessment instrument, the clinical dementia rating (CDR), is a clinical interview which stages Alzheimer's from normal to severe, based on six categories of function. In one important study, the likelihood of progressing to Alzheimer's during a three-year follow-up period was related to the sum of the scores in the six CDR categories. This score, combined with selected clinical interview questions, identified 89% of those questionable individuals who subsequently converted to Alzheimer's in the study. These findings provide guidelines for using a clinical
assessment to identify patients most likely to convert from questionable AD to AD, improving the possibility of earlier diagnosis and earlier implementation of available interventions.

1.2.9 Normal Age-Related Cognitive Change

Improved characterization of normal cognitive function and underlying brain changes over the life course will help in distinguishing and understanding normal from abnormal changes in memory, learning, and attention with age. Such understanding will help both to confirm and hopefully, even to alleviate the anxiety of many older Americans and their families, who may observe modest but perceptible changes in their own cognitive function in themselves or in that of a loved one and fear that such changes are signs of a decline into Alzheimer’s or dementia. While some experts believe that virtually all cases of so-called mild cognitive impairment (MCI) will eventually convert to AD in some people the process may progress so slowly that the person dies of other causes before full-blown Alzheimer’s symptoms become evident. Understanding why the process is slower in some people, and finding ways to intervene so that Alzheimer’s does not develop, continues to be an area of considerable research.

1.2.10 Biological Markers Detecting Amyloid Plaques

Scientists have been searching for a marker to be used in living patients to identify amyloid plaques that may have been present in the brain prior to the clinical diagnosis of the disease. A new molecular test has recently been developed that identifies plaques in Alzheimer’s brain tissue examined after death, and has been shown to accurately identify plaques throughout the brain in laboratory animals as well. This test is a prototype for ones that might eventually be used to identify plaques in the brains of living people, which would permit monitoring of the development and progression of Alzheimer’s as well as the clearance of plaques in response to anti-amyloid therapies. This research is, however, preliminary and ongoing.\(^{23}\)
1.3 Basic Types of AD

1.3.1 Familial Alzheimer’s Disease (FAD)

Familial AD (FAD) is a rare form of AD, affecting less than 10 percent of AD patients. It is associated with gene mutations on chromosomes 1, 14, and 21. In 1992, researchers at the University of Washington at Alzheimer’s disease Center (ADC) in Seattle, supported by NIA and the National Institute of Neurological Disorders and Stroke (NINDS), discovered a link between FAD cases and genes in a particular region of chromosome 14. They were then able to identify the defective gene which they named presenilin 1. Many cases of FAD are caused by presenilin 1. In FAD, all offspring in the same generation have a 50/50 chance of developing AD if one of their parents had it. FAD occurs in younger people (usually before age 60) than sporadic AD (SAD) does. Almost all FAD cases are known so far have been found to have an early onset and tend to progress more quickly than the late-onset form of AD.

1.3.2 Sporadic Alzheimer’s Disease (SAD)

Sporadic AD (SAD) is far more common than FAD. It generally occurs later in life and appears to be related to the apoE gene found on chromosome 19. ApoE comes in several different forms or alleles, but three occur most frequently. People inherit one allele (apoE2, apoE3, or apoE4) of the apoE gene from each parent. Having one or two copies of the E4 allele increases a person’s risk of getting AD. Having one or two E4 alleles of the apoE gene maximize a person’s risk of AD, but not to 100 percent. AD researchers are studying people who inherit different forms of this gene to learn more about risk factors of AD. Scientists are yet to determine the exact degree of risk of AD for any given person based on apoE status.

1.4 Epidemiology of AD

There are an estimated 24 million people with dementia worldwide. By 2040, it is projected that this figure will have increased to 81 million. Much of the increase will be in developing countries. Already more than 60% of people with dementia live in developing countries, but by 2040 this will rise to 71%. The fastest growth in the elderly
population is taking place in China, India, and their south Asian and western Pacific neighbors.

To estimate the number of cases of dementia, we apply prevalence rates from studies to population figures. Very little is known about the prevalence of dementia outside the more developed countries (Asia, Europe, North America, Australia and Japan), so it is difficult to estimate the number of cases of dementia worldwide. AD supports the 10/66 Dementia Research Group, which aims to quantify prevalence and incidence rates in developing countries, so that we can make better estimates in those regions.

- Approximately four million Americans have AD. In a 1993 national survey, 19 million Americans said they had a family member with AD, and 37 million said they know someone with AD.

- One in ten persons over 65 and nearly half of those over 85 have AD. A small percentage of people as in their 30s and 40s get the disease.

- A person with AD will live an average of eight years and as many as 20 years or more from the onset of symptoms.

- More than seven of ten people with AD live at home. Family and friends provide almost 75 percent of the home care. The remainder is “paid” care, costing an average of $12,500 per year. Families pay almost all of that out-of-pocket.

- Half of all nursing home residents suffer from AD or a related disorder. The average cost for nursing home care is $42,000 per year, but can exceed $70,000 per year in some areas of the country.

- The average lifetime cost per patient is $174,000.

- The federal government estimates show that approximately $349.2 million for AD research was spent in 1998. This represents $1 for every $287 the disease now costs society. The federal investment in heart disease, cancer and AIDS is four to seven times higher.
An estimated 4 million Americans, about 140,000 of them in Massachusetts, have AD or a related disorder. Some 19 million people have a family member who suffers from dementia.

1 in 10 people over 65 and nearly half of those over 85 have Alzheimer’s. A small percentage of the people in their 40s, 50s and early 60s also get the disease. All people with Down syndrome show the neuropathology of AD by about age 35.

14 million Americans, 350,000 of them in Massachusetts, will have the disease by mid-century unless a cure or a means of prevention is found.

Alzheimer’s is the 4th leading cause of death among adults. Typical life expectancy is 8-10 years from the onset of symptoms, although some people have lived with the disease for 20 years or more.

Over 70% of people with Alzheimer’s live at home. Almost 75% of their care is provided by families and friends. Most of the rest is paid for out-of-pocket by these families at an average annual cost of $12,500.

The typical primary family caregiver is in her 70s and has two chronic health problems. The stress of care giving affects health the third one of these family caregivers will die before the person with Alzheimer’s for whom they are caring.

Alzheimer’s is the 3rd most expensive disease in the nation, after heart disease and cancer, with an average lifetime cost per patient of $174,000.

Over half of all nursing home residents have AD or a related disorder. The average cost of their care is $45,000 per year. In Massachusetts, it can exceed $70,000.

In 1997, the federal government spent about $320 million on Alzheimer’s research, or about $1 for every $319 the disease now costs society. Federal investment in heart disease, cancer and AIDS is 3-5 times higher.

Finding a treatment that could delay onset by five years could reduce the number of individuals with AD by nearly 50 percent after 50 years. Increasing age is the
greatest risk factor for Alzheimer's. One in 10 individuals over 65 and nearly half of those over 85 are affected.25

- Rare, inherited forms of AD can strike individuals as early as their 30s and 40s.26 A person with AD will live an average of eight years and as many as 20 years or more from the onset of symptoms as estimated by relatives. 27 From the time of diagnosis, people with AD survive about half as long as those of similar age without dementia. Average survival time is affected by age at diagnosis and severity of other medical conditions. 28 National direct and indirect annual costs of caring for individuals with AD are at least $100 billion, according to estimates used by the Alzheimer's Association and the National Institute on Aging 29,30.

- AD costs American business $61 billion a year, according to a report commissioned by the Alzheimer’s Association. Of that figure, $24.6 billion covers Alzheimer health care and $36.5 billion covers costs related to caregivers of individuals with Alzheimer’s, including lost productivity, absenteeism and worker replacement. 31 More than 7 out of 10 people with AD live at home, where almost 75 percent of their care is provided by family and friends.27 The remainder is “paid” care costing an average of $19,000 per year. Families pay almost all of that out of pocket. 32 Half of all nursing home residents have AD or a related disorder.33

- The average cost for nursing home care is $42,000 per year. 34 but can exceed $70,000 per year in some areas of the country.

- The average lifetime cost of care for an individual with Alzheimer’s is $174,000.29

- Medicare costs for beneficiaries with Alzheimer’s are expected to increase 75 percent, from $91 billion in 2005 to $160 billion in 2010; Medicaid expenditures on residential dementia care will increase 14 percent, from $21 billion in 2005 to $24 billion in 2010, according to a report commissioned by the Alzheimer’s Association. 35 The Alzheimer’s Association has awarded more than $200 million in research grants since 1982, according to our audited annual financial statements.
The federal government estimates spending approximately $647 million for AD research in fiscal year 2005.

1.4.1 Prevalence

- One out of eight people aged 65 and are older has Alzheimer’s and nearly one out of two over age 85 has it.

- Age is the greatest risk factor for Alzheimer’s. A small percentage of Alzheimer cases is caused by rare, genetic variations found in a few hundred families worldwide.

- Only 19 percent of people with Alzheimer’s and other dementias actually have the diagnosis recorded in their medical records.

- Seventy (70) percent of people with Alzheimer’s and other dementias live at home, cared for by family and friends.

- Seventy (70) percent of nursing home residents have some degree of cognitive impairment; 47 percent of all nursing home residents have a diagnosis of Alzheimer’s or another form of dementia in their medical records.

1.4.2 Mortality

- From 2000 to 2004, deaths from AD increased by 32.8 percent. Deaths from heart disease decreased by 8 percent, breast cancer deaths decreased by 2.6 percent, prostate cancer deaths decreased by 6.3 percent, and stroke deaths decreased by 10.4 percent.

- In fact, the number of deaths caused by Alzheimer’s may be under-reported because persons with the disease usually have one or more serious co-existing conditions, such as heart disease or stroke, which end up being cited on death certificates.

- People with Alzheimer’s in general have decreased survival in the general population. One study noted that people with Alzheimer’s survive about half as long as those of similar age who didn’t have Alzheimer’s. Survival time was four
to six years after diagnosis, but survival time can be as long as 20 years from the detection of the first symptoms.

1.4.3 Cost of AD

- Direct and indirect costs of Alzheimer’s and other dementias amount to more than $148 billion annually.

- In 2005, Medicare spent $91 billion on beneficiaries with Alzheimer’s and other dementias and that number is projected to more than double to $189 billion by 2015.

- The medical costs of those with AD and other dementias are more than double the amount of those without when one or more other chronic conditions, such as coronary heart disease and diabetes, is present.

- Almost 10 million Americans are caring for a person with Alzheimer’s or another dementia; approximately one out of three of these caregivers is 60 years or older.

- In 2005, it is estimated that unpaid caregivers of people with AD and other dementias provided 8.5 billion hours of care valued at almost $83 billion dollars.

1.5 Etiopathology of AD

AD is marked by a breakdown in communication among nerve cells. This breakdown results in a loss of function in the neurons and eventually cell death. Cell death is predominant in the region of the brain that is responsible for controlling memory. This region has a structure known as the hippocampus. When nerve cells die in the hippocampus short-term memory fails and a decline is witnessed in an individual’s ability to perform familiar tasks. Even greater damage is witnessed in the areas of the cerebral cortex that control reason and language. Gradually language skills and judgment become impaired and personality changes, with the advent of emotional outbursts, wandering, agitation, and other disturbing behaviors.

AD is an irreversible, progressive brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes and a decline in thinking ability. These losses are related to the death of brain cells and a breakdown of connection
between them. The destruction caused by AD ultimately makes these nerve cells to stop functioning. A major question that concerns the researcher is whether or not AD is hereditary. Genetic studies of AD of late have been very productive, promising to provide an important insight into the deadly events that afflict the brains of patients it strikes. Of all cases of the illness, a significant fraction is familial Alzheimer’s disease (FAD) with an early onset (developing in patients younger than 60) caused by inheritance of defective genes\(^{36}\) (Figure 1).

Figure 1: Biochemical pathway linked APP mutation and neuronal death in FAD

1.5.1 Related Risk Factors

The several risk factors that influence the cause of Alzheimer disease include aging, a family history of AD, a history of head trauma, oxidative factors, inflammatory factors or chemicals, glutamate, virus, bacteria, electromagnetic fields, childhood malnutrition, possibly educational experiences and environmental factors (e.g. aluminum, zinc, copper, iron and toxins). Aging increases every individual’s risk of developing AD. At age 65, the risk of developing AD is about 12%. By age 80, the risk goes up to approximately 50%. It has been hypothesized that everyone would eventually develop AD, if they lived long enough to reach the 130.\(^{37}\) Children of a parent with AD may run a 3-6 times greater risk of developing the disease than others. It is also common among elderly people, in whose family there are two close relatives with the disease.\(^{38}\)
trauma could result in loss of consciousness.\textsuperscript{38-39} The higher the education of people, the lower the risk of developing AD. A possible explanation for this relationship is that though some highly educated people may actually be at equal risk of developing the disease as those with less education, the symptoms of AD may not manifest in people with more education, as they are capable of hiding the symptoms much longer. Conversely, people with lower educational qualification may be more exposed to factors that are deleterious (harmful) to the brain over the course of their lives.\textsuperscript{40} Exposure to an excessive amount of environmental toxins, heavy metals like aluminum or electromagnetic radiation causes AD. A hereditary genetic predisposition toward AD, exposure to any one of the risk factors or factors in combination or their determine the age and the extent of developing AD.\textsuperscript{41}
Table 3: Locus of mutated genes and their genetic mechanism in late and early onset AD.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic Mechanism</th>
<th>Onset</th>
<th>Population effected</th>
<th>Chromosome</th>
<th>Locus/Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenilin 1</td>
<td>Causative</td>
<td>Early</td>
<td>&lt; 2%</td>
<td>14</td>
<td>AD$_3$</td>
</tr>
<tr>
<td>Amyloid precursor protein</td>
<td>Causative</td>
<td>Early</td>
<td>20 families</td>
<td>21</td>
<td>AD$_1$</td>
</tr>
<tr>
<td>Presenilin 2</td>
<td>Causative</td>
<td>Early</td>
<td>2 families</td>
<td>1</td>
<td>AD$_4$</td>
</tr>
<tr>
<td>Unknown</td>
<td>Causative</td>
<td>Early</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>APOE 4</td>
<td>Susceptibility</td>
<td>Late</td>
<td>-40%</td>
<td>19</td>
<td>AD$_2$</td>
</tr>
<tr>
<td>Unknown</td>
<td>Susceptibility</td>
<td>Late</td>
<td>Unknown</td>
<td>12 P11-q13</td>
<td>AD$_5$</td>
</tr>
<tr>
<td>Unknown</td>
<td>Susceptibility</td>
<td>Late</td>
<td>Unknown</td>
<td>9</td>
<td>AD$_7$</td>
</tr>
<tr>
<td>Unknown</td>
<td>Susceptibility</td>
<td>Late</td>
<td>Unknown</td>
<td>10q 24</td>
<td>AD$_6$</td>
</tr>
<tr>
<td>Choline acetyl transferase</td>
<td>Susceptibility</td>
<td>Late</td>
<td>Unknown</td>
<td>10q 11.23</td>
<td>Unknown</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>Susceptibility</td>
<td>Late</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Vascular Endothelial Growth factor</td>
<td>Susceptibility</td>
<td>Late</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Intracellular Adhesion Molecule-1 Gene</td>
<td>Susceptibility</td>
<td>Late</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Above-mentioned gene mutation (Table 3) and/or non-genetic risk factors may lead to aggregates of Ab$_{42}$ (neurotoxin). Amyloid is deposited in cerebral blood vessels as cognophilic angioopathy and within the neuropil as diffuse extracellular deposits, often with an associated neuritic reaction. Amyloid deposits in brain tissue consist primarily of aggregates of 40-43-residue peptides termed Aβ. Aβ is derived by proteolytic cleavage
of transmembrane glycoprotein family known as amyloid precursor protein (APP). This explains the pathogenesis of not only the inherited forms of this disease, but also that of the idiopathic variety. AD can loosely be thought of as a progressive form of dementia, where neuronal loss results in cognitive impairment. Pathologically it has various characteristics, not all of which are necessarily present in every case. Macroscopically the brain shows widened cerebral sulci, cortical atrophy and compensatory increased ventricular size due to parenchymal loss. Microscopically, the major features are neurofibrillary tangles (Figure 2) and senile plaques.

Figure 2: Neurofibrillary tangles in blood vessel
Neurofibrillary tangles are mainly composed of hyperphosphorylated Tau protein (Figure 3), which is an axonal protein involved in microtubule assembly – which are made up of β-amyloid and abnormally phosphorylated Tau protein. Amyloid is a glycoprotein resembling starch, which is deposited in the internal organs during amyloidosis.

Figure 4: Amyloid deposition viewed with fluorescence microscopy
pathogenesis of AD is an area still under intensive investigation. Despite advances in the understanding of its pathological basis there are still disagreements about its fundamental mechanisms and new theories are in circulation about its cause.

Most theories center around fibrillar β amyloid. Scientists have identified two proteases that they believe cause the formation of amyloid plaques, a common microscopic abnormality found in AD. Deposition of amyloid depends on the proteolytic cleavage of amyloid precursor protein (APP) by two proteases, namely β secretase and γ secretase. It is thought that over-expression of the gene that directs cells to make β secretase causes an increase in the amount of β secretase cleavage products and ultimately an increase in the amount of amyloid deposition. However, despite its undeniable presence in the brains of those with Alzheimer’s, findings do not seem to show it as the causative agent. It has been suggested that this is so because experimental mouse models are used and it is possible that AD is specifically a primate disease. On developing a primate model, using rhesus monkeys, findings showed amyloid to be not only present but also almost definitely the causative agent – there was neuronal loss circumferentially around the site of injection. Not only this but also the injection of fibrillar β amyloid induced hyperphosphorylated tau protein. This is the protein responsible for the neurofibrillary tangles – another characteristic microscopic abnormality commonly found in the brains of those with AD (Figure 5).

![Normal neuron](image1)

![Neuron with neurofibrillary tangles](image2)

**Figure 5:** Neurofibrillary tangles in microtubules of neuron
A more recent theory about the pathogenesis of AD is its possible cause by inflammatory processes associated with ageing, and not by amyloid plaque deposits as is most widely believed. Although β amyloid is still very much involved, the cause of the disease is thought to be the formation of toxic proteins, as opposed to a build up of amyloid plaques and neurofibrillary tangles (Figure 6) within brain nerve cells. Scientists continue to debate over the amyloid cascade hypothesis (the hypothesis that amyloid fibrils drive neurodegeneration). This is mainly due to a lack of evidence as amyloid often forms at sites distant from those of neuronal loss and can develop in people who have normal cognitive function and no evidence of local neuron damage. Researchers discovered evidence that soluble toxins are the responsible agent of disease progression. They suggested that a new form of amyloid — “amyloid β derived diffusible ligands” or ADDLs — form in the brain in the presence of certain inflammatory proteins. These ADDLs have different properties from β amyloid. Amyloid β fibrils kill a wide range of nerve cells including those that are not affected in AD, whereas ADDLs affect only those types of nerve cells that become atrophied in the disease. Also, ADDLs are soluble and can therefore diffuse throughout the brain whereas amyloid β fibrils are not and the locations at which they form do not always correlate with areas of disease.

Other scientists have agreed that a soluble toxic protein may be responsible for the disease. One suggestion is the possibility of organophosphorous (OP) molecules being the fundamental cause as there is evidence that OP molecules attach to Tau proteins.
and cause them to tangle. It is recognized that AD has progressed from a rare to a common disease in line with the use of OP molecules in insecticides, and that these insecticides may be persistent, as was DDT, as opposed to the environmentally friendly chemicals we are led to believe them to be.

Researchers have found oxidative damage to brain cells to be a potential indicator of Alzheimer disease activity. Oxidative damage to cells is a result of free radical activity, free radicals being released during normal cell processes. It is thought that this leads to oxidative stress, which causes tissue inflammation - a suspected cause of AD.

Yet another suggestion for the basis of AD is neuronal cholesterol turnover misregulation. Apparently “faulty brain cholesterol dynamics” can cause a change in both tau and β amyloid neurochemistry. It is thought that the essential role of cholesterol in synaptic plasticity and neurodegeneration may be a link to AD.

In summary AD Etiopathology (Figure 7) can be either familial or sporadic type, though most of the times, but operates through the specific genes present in the specific chromosome. Genetically AD is carried from parents to offspring only in 5% of the AD population (familial type) and rest of the AD population in which AD is caused by unknown factors (sporadic), it could be various risk factors that interact with susceptible genes in chromosomes, may trigger biochemical cascade leading to nerve cell death. The various genes including presenilin gene (chromosome 14), presenilin 3 gene (chromosome 1) APP gene (chromosome 21) and other susceptible genes (Amyloid expressing gene 4 (APOE4), bleomycin gene, vascular endothelial growth factor gene and unknown genes) interact with variety of risk factors independently or collectively, such as toxins, electromagnetic radiation, dietary factors, virus, bacteria, metals (Aluminium, Copper, Zinc and Iron) may trigger the activation of presenilin proteins, present on the cell membrane of neuronal cell, which is also co-regulated by Ach, the reduced acetylcholine at synapse, mainly attributed to down regulation of acetyl transferase gene, (reduced biosynthesis of Ach).
The reduced Ach causing down regulation M1 receptor, nicotinic receptor and up regulation of M2 receptor, cause activation of presenilin proteins. The upregulated presenilin proteins activate β-secretase instead of α-secretase (α-secretase is activated when presenilin proteins are down regulated). β-secretase so released, cleaves 770 amino acid containing APP peptide (attached transmembrane) at 670/671 and γ secretase further cleaves at 710/711 or at 712/713 of App to releases 40 or 42 fragment of β-amyloid (neurotoxic) and β-APP (670 fragment). The β-amyloid (40 or 42 fragment) released into extracellular space of neuron, has carboxylic acid terminal, which tends to aggregate each other. (α Secretase in normal or healthy brain neuronal cells, cleave App peptide at 687 and followed by cleavage of γ secretase at 710/711 or at 712/713 of App to release 23 or 25 fragment of α amyloid (neurotrophic or neuroprotective) and α APP, α amyloid has one end as amino terminal at the cleaved site). The β-amyloid released in AD brain cells at appropriate biological milieu in various phases (lag and growth) undergoes β-amyloid plaque formation (Figure 8), involving multiple steps as follows.

**Amyloid:** any fibril, plaque, seed, or aggregate that has the characteristic cross-β sheet structure.
**Amyloidogenic precursor**: a protein or peptide that upon incubation under appropriate conditions will form amyloid fibrils or plaques.

**Amyloid fibril**: long ribbons of amyloid ~10nm in diameter and >100nm in length. Most often observed *invitro*.

**Amyloid plaque**: the form of amyloid most often found *invivo* – often comprised of aggregated amyloid fibrils.

**Amyloid protofibril/filament**: a species of amyloid smaller in diameter (3-6nm) and length (<100nm) than typical for amyloid fibrils, thought to be a possible direct precursor to amyloid fibrils perhaps through lateral aggregation.

**Amyloid seed (or template)**: a species of a critical size or structure that rapidly elongates to form larger amyloid species possibly by providing a proper scaffold for amyloid assembly.

**Amyloidogenic oligomer**: A small aggregate of precursor that is smaller than the critical “seed” size but still may have some of the structural characteristics of amyloid.

**Amyloidogenic fold**: a structure of the precursor that must be accessed prior to amyloidogenic aggregation, thought to retain substantial secondary structure possibly including some of the native fold. It could be related to a misfolded or molten globule structure.

**Folded state**: The native (functional) state of the precursor.

**Folding intermediate** – A partially folded or misfolded structure of the precursor. These partially folded structures are potentially the same as or precursors to amyloidogenic folds.

**Denatured state**: The unfolded state of the precursor.

**Unstructured aggregate**: Completely or partially denatured proteins tend to aggregate non-specifically without forming a particular structural motif.
The plaque so joined has tendency to occupy the death receptor on the transmembrane of neuronal cell in brain (Figure 9), triggering sequential intracellular cascade (signal transduction) involving activation of various kinases (MAP kinase,
phosphokinase) causing hyperphosphorylation of tau proteins of microtubules. Hyperphosphorylated tau proteins tend to aggregate each other to form paired helical filaments, which further aggregate to form neurofibrillary tangles (at the intracellular space of neuron). The presence of two abnormal structures in the brain, amyloid plaques and neurofibrillary tangles, have long been markers of AD.

Neurofibrillary tangles consist of insoluble twisted fibers that are found inside the brain’s cells. They primarily consist of a protein called tau, which forms part of a structure called a microtubule. The microtubule helps transport nutrients and other important substances from one part of the nerve cell to another. In AD, however, the tau protein is abnormal and the microtubule structures collapse. Neurofibrillary tangles are generally found within the cell bodies of large pyramidal neurons, from where they extend into proximal apical dendrite, but not into the axonal hillock. As the tangle comes to occupy more of the cytoplasm, the nucleus is displaced to an eccentric position and dendritic arborization is pruned.

![Figure 10: How the Brain and Nerve Cells Change During AD](image)

The morphology of the tangle (Figure 10) conforms to the outlines of the pyramidal cell while it remains extracellular, but after cell death, the tangle cells dispersed in the extracellular space as it is invaded and progressively degraded by astrocytes. The loss of pyramidal cells is highly correlated with the accumulation of
neurofibrillary tangle (Borisdareff et al., 1993). Neurofibrillary tangles are first found in the pre α layer (Layer II) of the transentorhinal cortex and the entorhinal cortex (Stages I and II). By the time neurofibrillary tangles spread to layer I of Ammon’s horn in the hippocampus, extra cellular tangles will have appeared in the entorhinal cortical regions (Stage III). Next, tangles appear in the deeper pre- α layer (layer IV) of the entorhinal cortex and in CAI of the hippocampus (Stage IV). Pathology then spreads into sectors CA2, 3 and 4 of the hippocampus, the sabiculum, and into isocortical area, particularly the temporal and parietal cortices (Stage V). The final stage VI is characterized by more extensive spread in neocortical areas, with relative sparing of primary motor and sensory areas. The appearance of neurofibrillary tangles in the granule cells of the fascia denta. These entire neurofibrillary tangles spreading selectively kills the brain cells and brings about selective memory loss and learning impairment.

![Healthy brain vs. Alzheimer’s brain](image)

**Figure 11:** Normal Brain and AD Brain

An overall shrinkage of brain tissue is witnessed as AD progresses (Figure 11). In addition, the ventricles, or chambers within the brain that contain cerebrospinal fluid, are noticeably enlarged. In the early stages of AD, short-term memory begins to decline when the cells in the hippocampus, which is part of the limbic system, degenerate. The ability to perform routine tasks also declines. As AD spreads through the cerebral cortex (the outer layer of the brain), judgment declines, and emotional outbursts may occur and ability to use language is impaired. Progression of the disease leads to the death of more nerve cells and subsequent behavior changes, such as wandering and agitation. The ability to recognize faces and to communicate is completely lost in the final stages.
Patients lose bowel and bladder controls, and eventually need constant care. This stage of complete dependency may last for years before the patient dies. The average length of time from diagnosis to death is 4 to 8 years, although it can take 20 years or more for the disease to run its course.

Thus conventional tertiary amine drugs such as pilocarpine and arecoline, which demonstrate only modest cholinergic activity and are closed as partial agonists, have been the subjects of intense structure activity studies.

### 1.6 Currently Available Treatment Strategies for AD

Although a wide spectrum of pharmacological approaches to treating AD have been investigated in the past 30 years, none of them cure AD. Currently available medications after relatively small symptomatic benefit for some patients but do not slow disease progression. Various treatment strategies available are explained below:

#### 1.6.1 Acetylcholinesterase Inhibitors

Inhibition is thought to be important because there is a reduction in activity of the cholinergic neurons. AchE-inhibitors reduce the rate at which acetylcholine (Ach) is broken down and hence increase the concentration of Ach in the brain (combating the loss of Ach caused by the death of cholinergic neurons). Acetyl cholinesterase-inhibitors seem to modestly moderate symptoms but do not alter the course of the underlying dementing process. Different AchE inhibitors used include **Tacrine** (cognex): As the first medicine approved for AD, it came in the market with a great deal of hope but many patients are administered with tacroine, do not show any improvement, while those that do requiring only modest gains at high doses and further complicating its use by a short half life, that requires that it be taken four times a day. Its use sows multiple side effects of liver problems, nausea, facial flushing, diarrhea and more rarely weakness, vomiting and dizziness.

**Donazepil** (Aricept) It too is a cholinesterase inhibitor, but with a long half life that allows once a day dosage and much better patient compliance studies on AD patients have again demonstrated only modest improvement after 24 weeks of use. It is better tolerated without liver toxicity and does not cause cholinergic side effects until high doses are administered.
**Galantamine hydrobromide** (Reminyl): Received approval from the FDA in 2001. Given a twice a day to AD patients, it may lead to modest improvement in the condition of some patients.

**Rivastigmine tartrate** (Exelon) is also available for the treatment of mild to moderate AD. Its efficacy is similar to the other medications.

### 1.6.2 Choline and Lecithin Supplements

Studies on laboratory animals have shown that choline and lecithin (phosphatidyl choline) increases the production of brain Ach levels. This approach has proved unsuccessful in AD, which in retrospect is not surprising because of the lack of presynaptic cholinergic neurons, which such precursor loading strategies would require in order to increase production. The loss of cholinergic neurons in AD is probably too great; by the time symptoms develop for the reduction in Ach production to be remedied by increasing production in the remaining neurons.

### 1.6.3 NMDA Antagonists

The evidence of the involvement of glutamergic neuronal excitotoxicity in the etiology of AD led to the development and introduction of memantine. Memantine is a novel MMDA receptor antagonist and has been shown to be moderately clinically efficacious.

### 1.6.4 Anti-inflammatory agents

Regular use of non-steroidal anti-inflammatory drugs like Ibuprofen and aspirin reduces the chance of dementia, but the risks appear to outweigh the drugs benefit as a method of primary prevention.

### 1.6.5 Anti-oxidants

High doses of Vitamin E (in combination with vitamin C) seem to reduce AD risk in cross sectional studies, but not in a randomized trial and so are not currently a recommended preventive measure because of observed increases in overall mortality.\(^{43}\)
1.6.6 Cholesterol-lowering Drugs

Statins (e.g. mevastatin, simavastatin) reduce AD risk in observational studies, but so far not in randomized controlled trials.

1.6.7 Estrogen

Some studies have suggested that estrogen used by women to treat the symptoms of menopause also protects the brain. Experts also wondered whether using estrogen could reduce the risk of AD or slow the disease. Clinical trials to test estrogen, however, have not shown that estrogen can slow the progression of already diagnosed AD. A study has found that women over the age 65 who used estrogen with a progestin are at greater risk of dementia, including AD, and that older women using only estrogen could also increase their chance of developing dementia.

1.6.8 Calcium Channel Blockers

It has been suggested that disturbances of calcium homeostasis may have a role to play in neuronal deterioration of AD and this speculation has resulted in a number of trials of calcium channel antagonists, especially nimodipine. To date, most of the published studies, including one with a significant number of subjects (Fischhof, 1993), report encouraging trends. However, further information, including that from a large multicenteric study, is still awaited (Ban et al., 1990).

1.6.9 Neuropeptide Strategies

Vasopressin effects memory tasks in various animal models in which lesions are found to induce one or other type of amnesia or memory impairment. The level of vasopressin in the hippocampus is reduced in AD, although it is normal elsewhere, and this discovery has led to a number of studies of vasopressin and similar compounds in therapeutic trials, including one relatively large study of 115 subjects (Woltus et al., 1990). In none of these has there been convincing evidence of a significant benefit to sufferers. Other peptide-directed strategies have included the use of adrenocorticotropic hormone, corticotrophin-releasing factor and noloxone, with either marginal or negative results; similarly somatostatin analogs have been successful. Neuropeptides are unlikely to cross the blood-brain barrier (BBB) easily, are rapidly metabolized in the peripheral
circulation (consequence, they have short half life) and there is little of any indication of an appropriate dosing schedule.

1.6.10 Vitamin B12 Replacement Therapy

Vitamin B12 deficiency occurs more frequently in AD than in normal subjects. There is no evidence that B12 replacement therapy alters the natural history of this condition. Although if untreated it may well contribute to the cognitive decline.

1.6.11 Psychological Interventions, Occupational and Life Style Therapies

Cognitive and behavioral interventions and rehabilitation strategies may be used as an adjunct to pharmacological treatment, especially in the early to moderately advanced stages of disease. Treatment modalities include counseling, psychotherapy, reminiscence therapy, reality orientation therapy, and behavioral reinforcements as well as cognitive rehabilitation training. Intellectual stimulation (e.g. playing chess or doing a crossword) and regular physical exercise reduce the risk of AD.

1.7 Cholinergic System in AD

1. In 1974, Drachman and Leavitt demonstrated that the blockade of the cholinergic receptors in young healthy individuals produce a memory deficit, which is similar to that seen in AD patients. Subsequently, a severe loss (up to 95%) of cholinergic markers in the cerebral cortex in AD subjects was independently reported by two research groups. Later studies showed significant decreases (of varying extents, ranging between 15% and 95%) in the number of cholinergic neurons in the NbM of AD patients. Furthermore, the severity of the cholinergic deficits in AD was found to be positively correlated to the severity and duration of the AD. This encouraged the development and introduction of pharmacotherapies that would involve the cholinergic system modulating agents such as inhibitors of AChE (Orgogozo, 2003). However, the belief that cholinergic therapy may be used to eliminate memory and cognitive deficits in demented patients soon decreased. Clinical trials using these cholinergic drugs showed only modest improvements and could not restore cognitive function. There are several factors that could influence such an outcome. First, cholinergic degeneration is not apparent in cases with mild cognitive impairment.
2. These individuals are the main target group for the disease prevention. Moreover, there is no general brain cholinergic system lesion in AD. The cholinergic nuclei in the brainstem remain relatively intact in contrast to the basal forebrain cholinergic neurons co-expressing p75NTR. Finally, catecholaminergic neurons show even more prominent losses in activity at early stages of the disease than cholinergic cells. Therefore, the current treatment strategies that use cholinomimetics at preclinical or early stages of the disease might prove to be productive when combined with other therapeutic approaches than when used alone.

3. The cholinergic system is not the only neurotransmitter system that degenerates in AD. Other systems, such as the serotonergic and noradrenergic systems, are also affected by the disease (Mann 1983, Palmer et al. 1987). Indeed, the hallmarks of AD pathology, β amyloid plaques and neurofibrillary tangles, are associated with most neuron types independent of the transmitter. Therefore, the basis of the cholinergic therapy for AD is mainly symptomatic. However, some reports have suggested that the cholinergic therapy may also reduce amyloid accumulation. Nevertheless, since the cell loss becomes very substantial at the later stage of the disease, the beneficial symptomatic effects of cholinergic therapy are likely to be achieved only at the early stages of AD. Even though the effects of cholinergic therapy are modest so far, they are still important as even small improvements in the symptoms can significantly improve the quality of life and postpone the institutionalization. The basic mechanisms by which the cholinergic therapy affects cognition are still poorly understood. Therefore, this study was designed to give valuable information about the mechanisms of cholinergic system and cholinergic therapy in cognitive processes. A few studies on cholinergic receptors in human brain during aging have been summarized by Norderg and Winbald (1986a) in most studies, individuals over age 50 years are considered. Both decrease and no changes in muscarinic receptors number have been reported in cerebral cortex, hippocampus and caudate nucleus. Norderg and Winbald (1986a) reported a significant decrease in number of muscarinic binding sites for quinuclidinyl benzilate (3H QNB). Binding for human hippocampus from 44 controls (0-100 years). In the hippocampus, same authors for found a similar decrease with age nicotinic (H-tubacurarine receptors).
1.7.1 AD and Cholinergic Receptors

Numerous studies have been reported cholinergic deficits in the brains of patients with AD. The post Mortem AD brains contain reduced amount of binding sites for H3Acetylcholine. The decreases in nicotinic binding sites correlate closely with enzymatic choline acetyltransferase (ChAT) and acetylcholinesterase (AChE). Activity changes suggesting a possible presynaptic localization of lesion in AD.

1.7.2 Cholinergic Neurotransmission and APP Metabolism

Several lines of evidence suggest that modulating cholinergic neurotransmission can influence the metabolism of APP. First, stimulation of M1 and M3 receptors with muscarinic receptor agonists or ChEI greatly increases the production of secreted APP with concomitant decreases in Aβ generation. It was first reported by (Nitsch et al., 1992), using cells that were stably transfected with human mAChRs (M1, M2, M3, M4), that carbachol, a nonselective muscarinic receptor agonist, increased the amount of secreted APP released in cells expressing M1 and M3 receptors, but not in cells expressing the M2 or M4 subtypes. The increase of APP secretion could be blocked by the muscarinic antagonist atropine or by protein kinase C (PKC) inhibitors, suggesting that PKC mediates the receptor-controlled APP secretion. In addition, increased APP secretion from rat brain slices and cell cultures was also observed after indirect activation of muscarinic ACh receptors via the inhibition of AchE. Further, increased APP secretion has been demonstrated to be associated with a reduction of Aβ generation. Hung et al. (1993) have shown that in cell lines over expressing mAChR and huAPP with AD-associated mutations, the increase in APP secretion after mAChR stimulation is accompanied by a 50% reduction in the release of soluble Aβ and by an increase in the generation of the non-amyloidogenic p3 fragment. Based on these findings, it was postulated that the activation of M1/M3 mAChR-associated signaling pathways enhances α-secretase activity but decreases β-secretase processing of APP (Nitsch and Growdon, 1994). In addition, it should be noted that a few studies have reported that treatment with nicotine, both invitro and invivo, could also modify APP processing by increasing the release of secretary form of APP . Second, a reduction in cholinergic neurotransmission has been linked to enhanced APP expression/production, which may potentially lead to an increase in Aβ production. In rats, excitotoxic lesions of the cholinergic basal
forebrain or transaction of fimbria-fornix have been reported to increase the levels of APP mRNA or APP immuno reactivity in the cerebral cortex, or hippocampus.\textsuperscript{55} Similarly, selective cholinergic lesions of basal forebrain have also been shown to increase APP gene expression or protein level in rats and in marmosets. However, whether an enhanced APP expression/production would lead to increased production of Aβ, and further the deposition of Aβ has not yet been clarified due to a lack of invivo AD models (Beach et al., 2000; Boncristiano et al., 2002). On the other hand, it should be noted that several studies have reported contrasting results showing a decrease or no changes in the APP levels after lesion of the cholinergic basal forebrain.\textsuperscript{56} Together, these findings imply a role of cholinergic dysfunction in the promotion of Aβ production, and, further, support the argument that treatment with ChEIs may slow down the disease progression by modulating APP processing into the less amyloidogenic direction.\textsuperscript{57}

### 1.7.3 Cholinomimetic Therapy in AD

A prediction of the cholinergic hypothesis is that drugs that potentiate central cholinergic function should improve cognition and perhaps even some of the behavioral problems experienced with AD. There are a number of approaches to the treatment of the cholinergic deficit in AD, most of which have initially focused on the replacement of ACh precursors (choline or lecithin) but these agents failed to increase central cholinergic activity. Other studies have investigated the use of ChE inhibitors that reduce the hydrolysis of ACh (Figure 8) for example, physostigmine. More recent investigational compounds include specific M1 muscarinic or nicotinic agonists, M2 muscarinic antagonists, or improved “second generation” ChE inhibitors.

Additionally potential symptomatic therapeutic avenues relevant to the cholinergic hypothesis of AD have resulted from the rapid development in the understanding of the molecular pathology of the disease. For example, during the development of cholinergic neurons in the basal forebrain, they express functional nerve growth factor (NGF) receptors. In adult life, these neurons seem to remain responsive to NGF. Consequently, intraventricular administration of NGF has been shown to prevent the lesion induced loss of cholinergic neuronal cell bodies and to accelerate the recovery of behavioral deficits in learning.\textsuperscript{58} Another approach is the transplantation of ACh rich foetal tissue grafts, which has been shown to improve the cognitive performance of primates after excitotoxic
lesions of cholinergic nuclei. Thus, although such approaches may provide additional future possibilities for the palliative treatment for AD, the use of ChE inhibitors is the most well developed approach for the treatment as on date.

1.8 Muscarinic Receptors

Acetylcholine and carbamylcholine can bind to both muscarinic and nicotinic receptors, yet the responses elicited by activating each receptor differ in several ways. Muscarinic responses are slower, and produce excitation or inhibitions, which involve second messenger systems, rather than the direct opening of an ion channel. Muscarinic receptors are G protein-coupled receptors and mediate their responses by activating a cascade of intracellular pathways. Muscarine is the prototypical muscarinic agonist and derives from the fly agaric mushroom Amanita muscaria. Like acetylcholine, muscarine contains quaternary nitrogen important for action at the anionic site of the receptor (an aspartate residue in transmembrane domain III). Most muscarinic agonists obey the “rule of five” atoms from the quaternary ammonium moiety to the terminal atom.

Muscarinic receptors are found in the parasympathetic nervous system. Muscarinic receptors in smooth muscle regulate cardiac contractions, gut motility and bronchial constriction. Muscarinic receptors in exocrine glands stimulate gastric acid secretion, salivation and lacrimation. Muscarinic receptors are also found in the superior cervical ganglion where they can produce at least two physiologically distinct responses. In addition, muscarinic receptors are found throughout the brain, including the cerebral cortex, the striatum, the hippocampus, the thalamus and brainstem.

There are two different types of acetylcholine receptors — the nicotinic and muscarinic receptors. The muscarinic receptors are further classified into M1–M5 subtypes. The M1 receptors are widely distributed throughout the neurons of the central nervous system. Coupled to stimulatory G-proteins, M1 muscarinic acetylcholine receptors (Figure 12) have a stimulatory effect on neurotransmission when bound by an agonist. M3 and M5 receptor subtypes also have a stimulatory effect on the target tissue, whereas the M2 and M4 subtypes are inhibitory.
In general the classical muscarinic antagonists such as atropine recognize a single class of binding sites as determined in binding assays. In the 1980’s, several selective muscarinic antagonists were identified. Pirenzepine 1 was very useful in the characterization of M1 muscarinic receptors, while AF-DX 116 2 was used to identify M2 receptors in the heart. M3 receptors are found in smooth muscle and in both exocrine glands (e.g., lachrymal glands) and endocrine glands (e.g., pancreas). Muscarinic agonists bind heterogeneously to receptors in both the brain and peripheral nervous system.

In the late 1980’s, molecular cloning techniques identified five different subtypes of muscarinic receptors. Each receptor shares common features including specificity of...
binding for the agonists acetylcholine and carbamylcholine and the classical antagonists atropine and quinuclidinyl benzilate. Each receptor subtype couples to a second messenger system through an intervening G-protein. M1, M3 and M5 receptors stimulate phosphoinositide metabolism while M2 and M4 receptors inhibit adenylate cyclase. The tissue distribution differs for each subtype. M1 receptors are found in the forebrain, especially in the hippocampus and cerebral cortex. M2 receptors are found in the heart and brainstem while M3 receptors are found in smooth muscle, exocrine glands and the cerebral cortex. M4 receptors are found in the neostriatum and M5 receptor mRNA is found in the substantianigra, suggesting that M5 receptors may regulate dopamine release at terminals within the striatum. The structural requirements for activation of each subtype remain to be elucidated (Table 4).

Table 4: The following is the summary of muscarinic acetylcholine receptor

<table>
<thead>
<tr>
<th>Receptors</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Cortex, Hippocampus</td>
<td>Herat</td>
<td>Exocrine glands, GI tract</td>
<td>Neostriatum</td>
<td>Substantianigra</td>
</tr>
<tr>
<td>Antagonists</td>
<td>Pirenzepine</td>
<td>AF-DX116</td>
<td>pF-HHSiD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Agonists</td>
<td>Xanomeline, CDD-0097</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G protein</td>
<td>Gaq/11</td>
<td>Gai/0</td>
<td>Gaq/11</td>
<td>Gai/o</td>
<td>Gaq/11</td>
</tr>
<tr>
<td>Intracellular Response</td>
<td>Phospholipase Cβ</td>
<td>Adenyl cyclase inhibition</td>
<td>Phospholipase</td>
<td>Adenyl cyclase inhibition</td>
<td>Phospholipase Cβ</td>
</tr>
</tbody>
</table>

1.8.1 Muscarinic Receptor Agonist

A muscarinic receptor agonist is an agent that enhances the activity of the muscarinic acetylcholine receptor. The muscarinic receptor has different subtypes, labelled M1-M5, allowing for further differentiation.
In the form of pilocarpine, muscarinic receptor agonists have been used medically for a long time. A number of muscarinic agonists have been developed and are under investigation to treat AD. These agents are show promising as they are neurotrophic, are found to decrease amyloid depositions, and to improve damage due to oxidative stress. Tau-phosphorylation is decreased and cholinergic function enhanced. Notably several agents of the AF series of muscarinic agonists have become the focus of such research. AF102B, AF150(S), AF267B. In animal models that mimick the damage of AD, these agents appear promising.

The ability for the quaternary ammonium group to fit into an anionic site on muscarinic receptors may be an important factor for the binding of a ligand to muscarinic receptors. For an example of the requirement of the quaternary amine moiety, consider that dimethylaminoethylacetate (the tertiary form of acetylcholine) is 1000-fold less than acetylcholine, in part due to a lower affinity for the receptor.

The molecule of acetylcholine is flexible and may form an infinite number of conformations from the extended to the quasi-ring structure. The three-membered ring of acetoxyycyclopropyl-trimethylammonium iodide 3 demonstrates the concept that the extended form of acetylcholine exhibits the highest intrinsic activity. The trans isomer has much higher an activity than the cis isomer, which orients the ester and the quaternary amine together.

While the quaternary nitrogen is essential for eliciting full muscarinic responses with muscarinic agonists, there are a few potent muscarinic agents, which contain tertiary amines (e.g., arecoline, oxotremorine and pilocarpine). They are potent both peripherally and centrally, although they are of limited therapeutic value because of the wide range of
cholinergic responses that they elicit. Oxotremorine 5 is of interest because of its ability to produce tremors, thereby providing an early model for Parkinson's disease.

Simple tertiary amines do not show considerable potency for the receptor, but this can be counteracted if the rest of the molecule binds potently to the receptor (e.g., through an ester bioisostere). Oxotremorine fills this role with an amide group in a pyrrolidone ring as the nitrogen replaces oxygen in a hydrogen bond acceptor role. Arecoline (isolated originally from the betel nut) has a reversed ester acetylcholine profile, while pilocarpine 6 has its ester in the cyclic form of a lactam ring, which may increase the binding interaction. In general, it is important to have two sites for hydrogen bond acceptance in the ester isostere. The orientation of the ester isostere may be important for selective action as well.

The events associated with G protein-coupled receptor activation are as follows.

1. Agonist binds to the receptor, which has a high affinity for agonists at rest.
2. The binding of the agonist stabilizes a receptor conformation promoting receptor/G protein coupling and allows GTP to exchange for GDP on the G protein α subunit.
3. The binding of GTP leads to the dissociation of the G protein from the receptor, thereby lowering agonist affinity. The agonist then dissociates from the activated receptor.

4. The G protein consists of three subunits (α, β and γ) which also dissociate. The α subunit activates the appropriate second messenger system (e.g., phospholipase C for M1 receptors). The β and γ subunits can exert independent actions.

5. The α subunit is inactivated by the hydrolysis of GTP to form GDP by a GTPase intrinsic to the G protein (GTPase activity may be activated by other intracellular proteins called GTPase activating proteins [GAPs]).

6. The α subunit (with GDP bound) can then recombine with the β and γ subunits. The receptor is then in a high affinity state and ready for the binding of another agonist.

1.8.2 Cognitive Functions Mediated by Muscarinic Receptor

Muscarinic receptors in the central nervous system (CNS) are involved in the regulation of learning and memory functions. Originally, Drachman and Leavitt (Drachman and Leavitt, 1974) found that the muscarinic antagonist, scopolamine, produced amnesia in young volunteers similar to that observed in aged non-demented people (Bartus et al., 1982). In rodents, scopolamine is known to disrupt performance in spatial learning and memory. However, the cognitive effects mediated by muscarinic receptors are not specific to learning and memory, since muscarinic receptors also mediate processes that are needed in the regulation of attention and arousal. It is possible, that disruption of attentional and other non-cognitive processes in the CNS is the actual cause of the learning and memory deficits caused by muscarinic antagonists.

Classically, muscarinic receptors have been divided into M1 and M2 receptors, with high and low affinities to muscarinic receptor antagonist pirenzepine, respectively. Muscarinic receptor subtypes mediate different aspects of behavior, and there are reports indicating that postsynaptic M1 receptors mediate the performance-disrupting effects of scopolamine. For example, in the water maze and radial arm maze tasks, pirenzepine, a selective M1 antagonist, causes performance disturbance similar to that produced by scopolamine or atropine. In addition, pirenzepine has been reported to cause more specific effects on spatial short-term memory performance than scopolamine in the
delayed non-matching to position task (DNMTP). In this study, the result of pirenzepine administration was a delay-dependent disruption of performance, whereas scopolamine induced a non-specific and delay-independent disruption of all task parameters, including motivation and motor performance (Andrews et al., 1994). In the same study, an M2 receptor antagonist (AFDX 116) had no effect on DNMTP performance, suggesting that the M2 receptors do not mediate the disrupting effect of muscarinic antagonists on spatial short-term memory. In theory, it is possible that blockade of presynaptic M2 receptors enhances the function of ACh system by increasing the release of ACh, which may lead to beneficial effects on cognitive behavior.

1.8.3 Muscarinic Receptors in Ageing and AD

Spatial learning deficit in aged rats has been used as a model for the cognitive decline related to ageing and AD (Barnes, 1994). The anatomical and physiological changes occurring in the aged rodent brain resemble those occurring in AD patients. For example, the decrease in the ACh synthesizing enzyme ChAT during ageing may reflect degeneration of cholinergic cells in the basal forebrain, although the evidence supporting a ChAT decrease in the rodent brain is somewhat contradictory for both the cortex and HC. In addition, AChE activity is decreases in both AD (Namba et al., 2002) and rodent ageing. Aged rats, like young rats with hippocampus damage, show greater impairment in water maze learning than do young controls. Those aged rats that are most severely impaired in spatial tasks also have the greatest amount of degeneration in cholinergic cells in the basal forebrain, which further supports the view that the degeneration of the septohippocampal cholinergic system is linked to age-related learning impairment. Furthermore, the function of muscarinic receptors may be disrupted over the course of ageing, since both memory-impaired and memory-intact aged rats are more sensitive to the disrupting effects of scopolamine than young rats (Gallagher et al., 1994). The spatial learning deficit of aged rats can be used for investigating the efficacy of drugs in improving memory function, and the results from such studies may help in finding new treatments for the cognitive decline found in AD.

1.8.4 Muscarinic Receptor 1 (M1 receptor) and AD

M1 receptor is a G protein coupled receptor, located on outer surface of the cell membrane of neurons in the brain. It is a glycoprotein with molecular weight of
approximately 64 KD. Stimulation of the same will bring down the formation of 
neurotoxic β amyloid via secondary messengers. Amyloid formation is an early event in 
brain's of AD patients and defines much of the histopathology of AD. β amyloid is 
deposited in cerebral blood vessels, as they diffuse to extra cellular space may trigger 
neuritic reaction. The α β amyloid fragment deposited in AD brains is neurotoxic where 
as the N-terminal portion of APP may have neuroprotective and neurophilic effects 
formed by stimulation of M1 receptor.67

The hypothesis that cholinergic hypo function and its relation with β- amyloid 
formation leading to cognitive deficits in patients with AD has prompted the designing of 
novel treatment strategies to restore lost cholinergic function. Two major strategies have 
been used in this regard. (1) By inhibition of acetylcholinesterase or (2) by activation of 
muscarinic receptor-1, which is relatively unchanged, may serve as a target for rational 
drug design of cognitive disorders for the treatment of AD.

The therapeutic potential of M1 selective muscarinic agonist including AF102B, 
AF150 (S), AF267B (the AF series) is evaluated and compared, when possible with 
several FDA approved acetylcholinesterase inhibitors. These M1 agonists can elevate 
APPs, decrease tau protein phosphorylation/ hyperphosphorylation *invitro* and *invivo* 
studies, and restore cognitive impairments in several animal models in AD.

Combination therapy of M1 agonist with cognitive restoring neutraceuticals like 
choline from lecithin or by ache-Is are likely to be therapeutically beneficial in the 
treatment of AD.

The cholinergic restitutive strategies in view of the prominent cholinergic 
dysfunction in AD. The primary implication of the cholinergic hypothesis is the potential 
of central cholinergic function should improve the cognitive and behavioral impairment 
associated with AD. The rationale for the use of direct cholinergic agonists rests on the 
fact that postsynaptic (M1) cholinergic receptor is relatively intact in AD and that 
presynaptic (M2) cholinergic receptors which are decreased in AD, regulate acetyl 
choline release.
Arecoline had positive acute effects on some areas of cognition in two small studies. But its use is limited by its requirement for intravenous administration for the treatment, and also it is proved to be carcinogenic.

AD is an irreversible, progressive brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes and a decline in thinking abilities. These losses are related to the death of brain cells and the breakdown of the connections between them. The course of this disease varies from person to person, as does the rate of decline. On average, AD patients, live 8 to 10 years after they are diagnosed, however, the disease can last for up to 20 years. It is the most common form of dementing illness among middle and older adults, which accounts for nearly 70% of dementias.

AD is the most common cause of dementia among people age 65 and older. The prevalence of AD doubles every 5 years beyond age 65. Prevalence is the number of people in a population with a disease at a given time. In fact, some studies indicate that nearly half of all people aged 85 and older have symptoms of AD.

Amyloid β protein (β A₄) is a major component of senile plaques, a distinct neuropathological lesion observed in the brains of AD patients. Deposition of aggregated β A₄ amyloid deposits in plaques and neurotoxicity that is associated with such deposits, suggest an involvement in the etiology of AD. The β A₄ peptide is a proteolytic product of the amyloid precursor protein (APP), a membrane associated glycoprotein whose processing may be followed recreation of non-amyloidogenic App isoforms, such secreted App isoforms may participate in neuroprotective mechanism and has been proposed that APP secretion participates in NGF mediated neurite outgrowth. APP secretion involves proteolytic cleavage by an unidentified protease ("α secretase") at residue 687 to the fully mature APP₇₇₀, which corresponds to residue 16 of the β A₄.

Arecoline molecule lacks specificity to M1 receptor, it gets easily hydrolyzed in stomach(clinically tested through intravenous administration). Moreover, it is myloid formation is an early event in brains of AD patients and defines much of the histopathology of AD. Amyloid is deposited in cerebral blood vessels as cognophilic angiopathy and within the neuropil as diffuse extracellular deposits, often with an associated neuritic reaction. Amyloid deposits in brain tissue consists primarily of aggregates of 40-43 residue peptides termed as beta amyloid protein which is derived by
proteolytic cleavage of a transmembrane glycoprotein family known as amyloid precursor protein (APP). App has a long extracytoplasmic N-terminal domain followed by a single transmembrane segment and a short intracytoplasmic c-terminal tail. The αβ moiety begins 25 residues from the neuron of membrane in the extracytoplasmic portion, and extends 11 to 15 amino acid used, into the transmembrane portion. Under a variety of experimental conditions, the αβ amyloid fragment deposited in AD brains is neurotoxic whereas the N-terminal portion of APP may have neuroprotective and neurophilic effects.

**Figure 13:** Linkage between M1 receptor and processing of APP

Based on tissue culture data, several alternative proteolytic mechanism have been identified that metabolizes APP (Figure 13). This is an α-secretase pathway with cleavage within the αβ domain itself that generates two non amyloidogenic metabolites: A secreted N-terminal derivative and its cell associated C-terminal counterpart. Endosomal – lysosomal APP processing generates multiple C-terminal derivatives, some of which contain intact αβ sequences that are potentially amyloidogenic, αβ fragments can also be generated by others β-secretase and secretase 2 pathways.
1.8.5 The Linkage of M1 Receptor and β Amyloid and Tau Phosphorylation

In a normal synapse acetylcholine release presynaptically bind to M1 receptor and form the complex of acetylcholine receptor Go/11. α - APPs are increased following these interaction Via PLC- β dependent and MAPK dependent pathways tau protein hyperphosphorylation is increased by reduced PKC that activates the GSK 3β (and/or activating other kinase or upregulating phasphatase). These are vicious cycles that link the cholinergic hypofunction with β amyloid and tau phosphorylation. A cholinergic hypofunction in AD may lead to formation of β – amyloid, which might impair the coupling of M1 receptor with G proteins. This disruption in coupling may lead to formation of β amyloid transduction, to a reduction in levels of trophic amyloid precursor protein (αAPPs) and generation of more. β amyloid that can also suppress Ach synthesis and release further aggravating further cholinergic deficiency. Tau microtubule associated protein is neuronal specific and its expression is necessary for neurite outgrowth hyperphosphorylated tau proteins are the principal fibrous component of the neurofibrillary tissue tangle pathology in AD.

1.8.6 Biochemical and Pharmacological Effects of M1 Agonists

As therapeutic agents, M1 agonists in the short term may palliate symptoms of AD and improve memory function. In long term, M1 agonists have the potential to modify the underlying pathophysiology of AD, and can thereby prevent or retard the course of dementia. Several M1 agonists, including AF series were tested in various animal models. In this context, the M1 agonists from the AF series restored memory and learning deficits in several animal models that mimic cholinergic and / or other deficits reported in AD, without producing adverse central and peripheral side effects at effective doses and showed a relatively wide safety margin (>200-500 fold). The extensively accumulated preclinical database indicates that these compounds have a fewer adverse effects and a higher safety margin in animal studies compared with other compounds of the same class.

Given the plethora of beneficial effects described so far, one wonders why the use some of the tested muscarinic agonists has failed in clinical trials in AD patients. Is this a
unique case where either the side effects were too severe, the therapeutic potential too weak and/or the therapeutic concept was wrong?

In this context, ache-inhibitors (tacrine, donazepil and rivastigmine) are the approved FDA drugs that show beneficial, albeit limited, symptomatic effects on cognitive dysfunction's in AD patients. It is not reasonable to presume that when compared with M1 agonists, only the Ache-inhibitors should be effective in AD. Since,

a. In general, the effect of the Ache-inhibitors is mediated via increased synaptic concentration of ach, and that by activation of M1 mAchR enhances memory and learning processing.

b. Mechanistically, as M1 agonists induces the same effects as Ach via M1 mAchR.

c. Some muscarinic agonists have shown positive cognitive effects similar to those obtained with AChE-inhibitors.

It is also unlikely that beneficial cognitive effects of these Ache-inhibitors are mediated entirely, by receptors other than the mAchRS (that is only nicotinic receptors). Notably several nicotinic agonists are developed for AD treatment. However, nicotinic receptors are decreased in AD brains, hampering perhaps the effectiveness of this therapeutic strategy in AD.

On the other hand, one cannot ignore the possibility that better effects might be obtained with a combined treatment that stimulates both postsynaptic mAchR (eg: M1 mAChR) and brain nicotinic receptors.

Most studies with muscarinic agonists employed clinical protocols designed to show their use in a symptomatic treatment on cognition and behavior in AD patients. Unfortunately when studies failed in particularly in phase III. They were performed with compounds having several major clinic limitations. The sponsors (drug companies) without a clear scientific reasoning prematurely interrupted several clinical studies other muscarinic agonists.

All these limitations prevented a proper evaluation of the clinical concept. Furthermore, very few of the already developed agonists could fulfill rigorous acceptance criteria in order to be considered as a promising treatment strategy in AD patients.
1. In general, there is a need for centrally active M1 agonists. The published functionally selective agonists are partial agonists, at least some of the standard assays. Compounds like milameline and sabcomeline, lack selectivity for M1 mAChR and thus cannot be termed as M1 agonists. Some of the tested agonists like alvameline is very weak agonist for M1 mAChR. In fact, these can be considered more as M1 agonist. Notably, M1 antagonists can be detrimental to cognitive functions.

2. Some of the clinical studies employed drugs that had extremely low oral bioavailability and extensive metabolism. Eg:- Xanomeline, M4> M1 agonist has a bioavailability in humans of 0<1%, leading to adverse effects mediated most probably by a plethora of receptors.

3. Notably, Ache-inhibitors that are effective in AD have an incomparable better bioavailability: Donepezil (100%), metrifonate (90%), galanthamine (100%), rivastigmine (36%) and tacrine (17%). Most of the drugs that failed in AD had a narrow safety margin with several side effects that limit the number of patients who can tolerate higher and perhaps more effecting doses. These include agonists such as milameline, xanomeline, subcomeline and alvameline. A compound with a minimal safety margin of >100 would be preferred. However sabcomeline, for example, shows only 3-10 fold separation of cognitive vs side effects. This extremely limits the dosing protocol in AD patients milameline produced a plethora of side effects including such toxic effects as corneal opacities and urinary tract sepsis in preclinical studies and gastrointestinal flatulence and Parkinson symptoms in clinical trials.

4. All the muscarinic drugs were administered in fixed non-individualized doses, that for the non-selective agonists produced intolerable side effects.

Ach released presynaptically required when causes basic stimulation. Released Ach activates postsynaptically M1 and M4 mAChR, while M2 mAChR autoreceptors (presynaptic) control via negative feedback, the concentration of this neurotransmitters in the synapse. The entire cholinergic signal in a neuronal synapse is extremely well controlled both pre and postsynaptically. Tonic stimulation is induced by postsynaptic activation of an mAChR either by constant high
concentration of Ach or an exogenously administered muscarinic agonist. Whether cholinergic treatment in AD should produce its beneficial effect on cognition via a phasic or tonic stimulation is less than clear as outlined below.

1. The assumption is that Ach inhibitors should be more effective in intact synapses in AD and less in those synapses where there is already a presynaptic cholinergic hypofunction. Ache inhibitor increase the efficiency of cholinergic transmission by preventing the hydrolysis of released Ach, thus making more ach available at the cholinergic synapse. This may be conducive to an over excitation of interacted synapses, perhaps in a tonic rather than phasic manner. Like muscarinic agonists, ache inhibitors are assumed to take advantage of the relative, preservation of postsynaptic mAchR in AD. In this context the longer the inhibition of Ache, the better is the effect of the drug (eg. Donazepil or metrifonate,physostigmine) on cognition in AD patients. In case of prolonged inhibition of ache by an ache inhibitors the stimulation produced by elevated levels of ach in the cholinergic synapse cannot be considered as phasic. The synaptic concentration of elevated ach may well exceed the normal level needed to activate postsynaptic machR (M1- M3). In such a scenario, there is prolonged activation of these receptors. In fact, these receptors may be tonically activated (the synapse may even be flooded by ach). Thus it is not conceivable to presume that beneficial effects of ache-inhibitors in AD patients is due to a phasic activation.

2. Microdialysis studies in rat brains with the FDA approved ache-inhibitors show three fold or greater increases in ach in the extracellular fluid of hippocampus in rats. The extracellular as AChs concentration measured by intra cerebral ach concentration microdialysis technique reflects the ach concentration in the synaptic cleft. Thus the synaptic level of ach must be high if ach level is increased to that extent and it is hard to imagine this producing a phasic response. In fact Ache inhibitors may work tonically just like a postsynaptic agonist.

3. The apparent phasic aspect of ache inhibitors relates to the presynaptic inhibition of further release of ach. This, in fact, may be considered draw back for using ache inhibitors in AD. Notably, excessive autoreceptor stimulation (eg M2 mAchR) may eventually reduce the ability of presynaptic neurons to transmit
properly. Thus the activation of presynaptic autoreceptors may play a role in reducing the efficacy of ache-inhibitors. Moreover in AD, ache needs to be inhibited to such a degree that the released ach can function postsynaptically without affecting the pre-synaptic events necessary for renewed transmitter synthesis and release. This excludes a clinical response that is phasic and in fact emphases the tonic aspect.

4. Beneficial effects on cognition in AD patients can be detected with the standard AD assessment scale-cognitive (ADAS-Cog) test after a few weeks of chronic treatment with either an ache-inhibitors or a muscarinic agonist. Neither a phasic nor tonic stimulation can provide an intelligent explanation for such a delayed effect. Xanomeline improved cognitive deficits in AD patients are comparable to the effects of Ache-inhibitors. However, behavioral effects of ache-inhibitors in AD patients may differ when compared with the produced beneficial effects of xanomeline on this clinical parameter. The pharmacokinetic profile in the case of a muscarinic agonist may not reflect accurately pharmacodynamic effects relating to cognitive and psychotic behaviors in AD patients. Notably the maximal effects of xanomeline on cognition in AD patient occurred after 12 weeks whereas the behavioral improvement was observed much earlier. In such a scenario, there is no practical relevance to tonic or phasic activation of the receptors.

5. How can a phasic stimulation be induced? One feasible approach is to administer choline for phosphatidyl choline (lecithin). The physiologic precursor of Ach biosynthesis. This approach can increase, in principle, ACh levels in brain of animals and in plasma and CSF of humans but this strategy failed in AD, as it did not improve cognition in AD. Although various arguments can be proposed to explain this lack of clinical efficacy, etc, it is evident that a phasic stimulation alone may not be sufficient for a cholinergic replacement treatment to be effective in AD.

6. Studies in several animal models, particularly those in modeling the cholinergic deficiency in AD using either ache or muscarinic agonists, do not indicate a major role of phasic vs tonic modulation in ameliorating cognitive improvements. Notably when selective partial M1 agonists were compared with FDA approved
AChE inhibitor tacrine, donazepil and rivastigmine. These agonists were at least as effective as the Ache-inhibitors in restoration of cognitive impairments.

7. AChE released (from genetically modified cells that produce Ach) into denervated neocortical target region is sufficient for improving cognitive function in rats. The released ACh presumably acts in a tonic manner postsynaptic receptors, as postulated for dopamine released form grafted non-neural cells.

8. Preclinical studies indicate that memory and learning are slow processes. Activation of mAchR can induce expression of immediate early genes and transcription factors that give rise to the delayed expression of functional and morphological changes that underlie neurotrophic activity, learning and memory and other processes. As a result of such mechanism, cognitive effects induced by AF 102 B, an M1 agonist or by BTN B 99, an M2 antagonist (phasic) have a long duration of action which lasts long after the compound is no longer detectable in the body fluids or brain regions. Since M1 muscarinic agonists might also have heat neurotrophic like effects a compound with a half-life can induce eventually long-term effects reminiscent of endogenous neurotrophins. In such a scenario, the relevance of tonic Vs phasic activation of the mAChR is questionable.

9. An argument that was raised against the use of muscarinic agonists in general was, that the study of compounds via a tonic stimulation might produce tolerance following a long term treatment. However no strong evidence supports such a claim since most of the drugs tested in clinical trails are partial agonists that unlike full agonists are not supported to induce tolerance.

1.9 Different Structural Classes of Muscarinic Agonist

A deficiency of functional cholinergic neurons, particularly those extending from the lateral basalis, has been observed in patients with progressive dementia of the Alzheimer type. Cholinomimetic therapy has been directed at compensating inadequate cholinergic activity in these neurons. There has been great emphasis on the search for and study of nonquaternary ammonium based muscarinic agonist (having great lipophilic character) that will penetrate the blood brain barrier (BBB) and interact with appropriate acetylcholine receptors in the brain.
Thus conventional tertiary amine drugs such as pilocarpine and arecoline, which
demonstrate only modest cholinergic activity and are closed as partial agonists, have been
the subjects of intense structure activity studies.

1.9.1 Acetylcholine Analogs

Acetylcholine is a poor therapeutic agent. Its rapid rate of hydrolysis in the
gastrointestinal tract precludes oral administration and a similarly rapid hydrolysis by the
esterases in the blood and by acetyl cholinesterase in the nervous tissue limits its
usefulness by injection. Acetylcholine has virtually no clinical uses. The following
structural modification for acetylcholine has been done to improve muscarinic activity.

1. Alteration of quaternary ammonium head

2. Replacement of the acetyl group by other acyl moieties

3. Alteration of the ethylene bridge connecting the quaternary ammonium and the
ester groups

4. Substitution of another group for, or elimination of the ester moiety

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{O} \\
\end{array}
\]

7

Variation in amino group

\[
\begin{array}{c}
\circ \\
\text{R} \\
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{R} \\
\end{array}
\]

\[
\begin{array}{c}
\text{R} = \text{P} (\text{CH}_3)_3 \\
\text{or} \text{ As} (\text{CH}_3)_3 \\
\text{or} \text{ S} (\text{CH}_3)_3 \\
\text{or} \text{ C} (\text{CH}_3)_3 \\
\text{or} \text{ N} (\text{CH}_3)_2 \text{NH}_2
\end{array}
\]
variation in acyl group

\[
\begin{align*}
\text{O} & \quad \text{X} \quad \text{N} \\
\text{\text{\textbullet} X = S \text{ or Se or NH} } \quad 9 \\
\text{Variation in ethylene bridge} \\
\text{CH}_3\text{-CO-O-} & \text{(CH}_2\text{)}_n \text{-N-(CH}_3\text{)}_3 \\
& \quad n = 3 \text{ or } 4 \\
\text{O} \quad \text{R} \quad \text{N} \\
\text{\text{\textbullet} R = C}_2\text{H}_5 \text{ or } \text{CH}_2=\text{CH} } \\
\text{R} & \quad \text{R} \\
& \quad \text{R1 = H or CH}_3 \\
10 & \quad 11
\end{align*}
\]

Substitution of the ester group by other groups

\[
\begin{align*}
\text{R} \quad \text{O} \quad \text{N} \\
\text{\text{\textbullet} R = C}_2\text{H}_5 \text{ or } \text{CH}_2=\text{CH} } \\
\text{R} \quad \text{R} \\
& \quad \text{R = H or CH}_3 \\
12
\end{align*}
\]

1.9.2 Muscarine and Analogs

Muscarine a cyclic analog of Ach muscrone is its ketonic form

\[
\begin{align*}
\text{13} & \quad \text{14}
\end{align*}
\]
Muscarine and its analogues generally do interact with different subtypes of muscarinic receptors but lack specificity to M1 receptor.

1.9.3 Pilocarpine and its Analogs

Pilocarpine (16), the cluef alkaloid from the leaflets of shrubs of the genus pilocarpus, has a dominant Muscarinic action, but it causes anomalous cardiovascular responses, and the sweat glands are particularly sensitive to the drug.

Pilocarpine analogs (17)

Pilocarpine analogs are generally specific to the Muscarinic receptor of eye then the brain.

1.9.4 Arecoline and its Analogs

Arecoline (18), an alkaloid from areca catechu, is a cyclic “reverse ester” bioisosters of Ach.
Arecoline analogs (19)

\[ X = -\text{CO-O-R}^1 \]
\[ (R^1 = \text{CH}_3 \text{ or } \text{C}_2\text{H}_5 \text{ or } \text{C}_3\text{H}_7) \]

Various Heterocyclic rings (1, 2, 5-oxadiazole or thiadiazole or thiazole or pyrimidine or oxazole). Various analogs of arecoline have been synthesized to improve the selectivity toxicity. This will be explained in detail in connection with subsequent topics.

1.10 Different Class of Arecoline Molecules in Research and their Muscarinic Activity

Arecoline stimulates muscarinic receptor because of its structural similarity with that of acetylcholine as shown below 20 and 21.

Arecoline bioisosters have general formulae as shown in 22 and the basic nucleus is tetrahydropyridine.
Both affinity and efficacy are significantly enhanced by tetrahydropyridine series to M1 receptor, providing semirigid template, which has good affinity for the muscarinic receptor. If the molecule is flexible as that of acetylcholine, it interacts with different class of muscarinic receptors and lacks the specificity to interact with a specific muscarinic receptor. If the molecule is rigid, it is unable to stimulate different class of muscarinic receptors. Tetrahydropyridines are semirigid class, which can bind specifically to M1 receptor and also provide some kind of flexibility to stimulate M1 receptor. N-methyl group on tetrahydropyridines makes the molecule selective towards M1 than M2 receptor.

1.10.1 General Structure Activity Relationship Studies of Arecoline Bioisosters

Extensive database have been developed and continued to be developed for better pharmacodynamic and pharmacokinetic parameters. Database developed are as follows:

arecoline (20) ester group is prone to acid hydrolysis in stomach. It lacks specificity to M1 receptor and also it is carcinogenic according to studies reported.

1. Quaternization of nitrogen of the arecoline produces equipotent M1 receptor agonist as compared to arecoline itself. The secondary amine of nor arecoline (absence of CH3 group on ester of arecoline) is weaker muscarinic agonist (Bieger et al. 1970; Sauerberg et al. 1986).

2. In case of ester substituent on ester (-COOR), the affinity and biological activity increases in this order Where the triple bond of propargyl ester contributes to the receptor binding (Lambrecht and Mutschler 1981). R = CH3 < C2H5 < nC3H7 < -CH3 - CH = CH2 < CH2 = C CH

3. Reduction or removal of the ring double bond (between three and four position) reduces the muscarinic agonist activity by 250 to 1000 times (In 1, if the nitrogen
of the arecoline is substituted by sulphur (bioisoster, in 1 where N= S), activity is retained, but not active as nitrogen in arecoline (Moser et al. 1983).

4. Introduction of another nitrogen in the ring of arecoline, to produce basic structures that is pyrimidine analogue, which gives less potent derivatives than arecoline itself (Messer et al. 1992).

5. N - CH3 group of arecoline produces selectivity of the basic structure to M1 receptor (Moltzen et al. 1994). Substitution at 3rd position of the ring increases the biological activities but at 4th substitution antagonizes M1 receptor activity and other substitution does not have significant effect. The ligand is designed as tertiary nitrogen, which facilitates bioavailability (passage through blood brain barrier), and after the passage the ligand is expected to be convert positive nitrogen (Nitrenium ion) invivo oxidation in presence of mono amino oxidase, as supported by structurally related drugs or ligands (Eg. MPTP or Arecoline) and hence the molecules will be highly reactive. Due to the above structural and functional relations to M1 receptor, this basic structure and its analogues are selected for study. 3-Acetoxy quinuclidines are potent muscarinic and also thianium, piperidine derivatives of quinuclidine, also provides potent muscarinic activity (24).

\[ \text{23} \]

\[ \text{24} \]

Where \( R=\text{CH}_3 \) for arecoline and \( X \) may be 24a and 24b

\[ \text{24a} \]

\[ \text{24b} \]

Where \( R_1=\text{H, alkyl, aryl etc}; \ Y=Z=\text{O, N, S} \)
An alternative and better strategy to design arecoline derivatives is by substituting the ester (because of non-specificity to the receptor, hydrolysis in the body and carcinogenicity in nature) by five or six membered heterocyclic ring (Lambrecht and Mutschler 1981) to produce better muscarinic agonist:-

a) In Five Membered Heterocycles, (24a)

1. Electronegative atom at 1st position increases the biological activity. Order of biological activity with respect to heteroatom, is as follows (Sauerberg et al. 1991). $N > S > O$.

2. Presence or absence of electronegative atom at 5th position of the arecoline ring does not change the biological activity.

3. Electronegative atom as a part of the ring at 4th position increases the biological activity, in the order. $N > S > O$. d. SR, OR, group attached to 3rd position increases the biological activity. An increase in the carbon number of R in SR or OR up to 6 number, increases the biological activity. [Hydrophobic nature increases binding to receptor, and avoids being washed out from receptor]

b) In Six Membered Heterocycles, (24b)

1. Electronegative atom at 1st position as part of ring of sublead increases the biological activity, in the order, $N > S > O$ (Ward et al. 1992).

2. Electronegative atom at 4th position as a part of ring of sublead increases the biological activity, in the order, $N > S > 0$.

3. SR, OR attached at 3rd position increases the biological activity. As the increase in the carbon number of R in SR or OR at 2nd position of sublead up to 6 numbers, increases the agonistic activity.
1.10.2 SAR of Arecoline Bioisosters in which Arecoline Nucleus Linked to Different Substituents (R) through Various Functional Groups

**Ester linkage (25)**

![Ester linkage](image)

Ester linkage is prone to hydrolysis and some of its derivatives are carcinogenic.

1. When R substituent is H, straight or branched alkyl from one to six carbon atoms or cycloalkyl from four to eight carbon atoms, muscarinic activity increases up to two carbon atoms, beyond which the activity decreases (Butler et al. 1988).

2. When R substituent is straight or branched alkenyl from one to six carbon atoms, as carbon number increases, correspondingly activity also decreases.

3. When R is phenyl alkyl wherein, the alkyl portion is straight or branched from one to six carbons and the phenyl ring may be unsubstituted or substituted with halogen, hydroxy, alkyl from one to six carbon atoms, or alkoxy from one to four carbon atoms, muscarinic activity decreases as the carbon length increases, as the substituted group on phenyl ring become electronegative, agonistic activity also increases.

**Amide Linkage (26)**

![Amide linkage](image)
1. When R1 and R2 are independently hydrogen or alkyl from one to four carbon atoms, muscarinic activity decreases as carbon length increases in R1 and R2 independently (Butler et al. 1988).

2. When group R1 is hydrogen and R2 is cycloalkyl from three to eight carbon atoms, muscarinic activity decreases as the carbon length increases in cycloalkyl ring.

3. If Group R1 is H and group R2 is benzyloxy, it increases the activity (Kelly et al. 2001).

4. Group R1 is H and group R2 is phenyl alkyl where in, the alkyl portion is straight or branched from one to six carbon atoms, phenyl ring may be unsubstituted or substituted with halogen, hydroxy, alkyl from one to six carbon atoms or alkylloxy from one to four carbon atoms, as carbon length decreases in alkyl portion and electronegativity of substituent on phenyl ring increases, proportionately muscarinic activity increases.

Ketone Linkage (27)

1. When R is pyrrolidinyl, piperidinyl, 4-diphenyl methylene piperazinyl, azepinyl, morpholinyl, thiomorpholinyl, isoxazolyl, piperazinyl, pyrrolidinyl and isoxzolyl rings show good Muscarinic action than six numbered heterocyclic rings.

2. When R is 4-alkyl piperazinyl ring where the alkyl group may be straight or branched alkyl from one to six carbon atoms, as the carbon length of alkyl chain increases, muscarinic activity decreases (Bergmeir et al. 1995).
Oxime Ether Linkage (28)

1. When R is straight or branched alkyl chain having one to four carbon atoms, muscarinic activity decreases as carbon length increases (Bergmeir et al. 1995).

2. When R1 substituent is straight or branched alkyl from one to six carbon atoms optionally substituted with hydroxyl or alkoxyl from one to four carbon atoms, as carbon length of alkyl chain increases and electronegativity of group attached to alkyl chain increases, proportionally muscarinic activity increases.

3. When R1 is cycloalkyl of from three to eight carbon atoms where hydrocarbon chain of from one to four carbon atoms, muscarinic activity decreases as the carbon number increases in cycloalkyl ring.

1.11 General Invitro Methods to Screen Arecoline Bioisosters as M1 Receptor Agonist in AD Research

Arecoline bioisosters can be assayed as M1 agonist in AD by using either cell lines or primary cell culture involving direct experiments like muscarinic receptor binding assay (Radioligand binding assay), secondary messenger estimation (calcium, phospholipase and proteinkinase) or indirect experiments involving as result of stimulation of M1 receptor, for instance estimation of β-amyloid (40 or 42 fragment) by ELISA, β-secretase activity of α-secretase activity. Some invitro methods are explained as below:
1.11.1 M1 Receptor Binding Assay

Radioligand binding studies of muscarinic receptor 1 have been primarily carried out with aid of specific tritiated compounds \[^{3}H\] quinuclidinyl benzilate or \[^{3}H\] pirenzepine or \[^{3}H\] N-methylscopolamine. The experiment involves competition by arecoline bioisoster and radioligand to M1 receptor from either brain tissue preparation (rat or guinea pig) or cell lines (CHO K1, SK-N-MC and SK-N-SH).

1.11.2 Electrophysiological Experiment

It measures functional interaction of arecoline bioisosters with presynaptic M1 muscarinic receptors in the hippocampal slice of the rat. Muscarinic cholinergic agonists effectively suppress the electrically evoked field excitably postsynaptic potential (fEPSP) of the rat hippocampal slice. This effect is due to a decrease in release of excitatory amino acids from the Schaffer collateral / commissural fibers, probably mediated by a presynaptic M1 muscarinic receptor. The experiment is mainly used to evaluate the muscarinic agonistic properties of a test compound.73

1.11.3 Phosphoinositide (Pi) Hydrolysis Studies

PI turnover can be estimated in cell lines (CHO-K1 or SK-N-MC, SK-N-SH) or rat brain preparation was assayed for agonist stimulated PI hydrolysis according to method Berridge (1983). Cells were labeled for 16-18 h with 1 u Ci/ml \[^{3}H\] inositol and serum containing growth media; and were washed 3 times with serum-free Dulbeco’s modified Eagle (DME) medium prior to assay. DME medium including Li\(^+\) (10 mM) and HEPES (20 mM) was added 10 min price to ligand addition.

Reactions were stopped at the indicated times by scrapping the cells into 0.5 mL water, and immediately extracting the inositol phosphates. Data were corrected for the corresponding \[^{3}H\] phosphoinositides radioactivity (determined by counting radioactivity in the chloroform phase), and calculated as percent \[^{3}H\] inositol phosphates/ \[^{3}H\] inositol phosphates plus \[^{3}H\] plus \[^{3}H\] phosphoinositides74 [Guswitz and Sokolovsky, 1987].
1.11.4 Cyclic Amp Accumulation in Intact Cells

Cells were washed three times with serum free DME medium and incubated for 20 min in serum free DME medium supplemented with HEPES (20 mm) and isobutylmethyl xanthine (IBMX; 0.1 mm) prior to the addition of arecoline bioisosters. Reactions were terminated at the indicated times by scraping the cells in 0.5 ml of boiling sodium acetate buffer (50 mM, pH 4), transferring to glass tubes and boiling for additional 3 min. Cyclic AMP levels were determined by a competitive binding assay using 20,000 dpm [3H] cyclic AMP and 50 μl bovine muscle cytosol. Cyclic AMP levels were calculated from the standard curve and expressed as pmol CAMP/well.

1.11.5 Adenyl cyclase activity in isolated membranes

Using fresh rat brain membrane preparation and [X 32P [ATP], the experiment measures rate at which (20 min) cyclic AMP is formed by adenyl cyclase interlinked to M1 receptor.

1.11.6 Intracellular Ca^{2+} Estimation

In response to stimulation of M1 receptor by M1 agonists, change in intracellular calcium either in cell lines or rat brain tissue cells is measured by calcium reaching agent Fura-2A. The resulting fluorescence is measured using dual wavelength spectrofluorometer at 340/380 nm and 510 nm wavelengths.

APP secretion studies: processing of amyloid precursor protein (APP) is coupled to several neurotransmitter receptors, including M1 muscarinic (M1 Ach R), and is associated with decreased amyloid deposition. Muscarinic agonist, stimulated App secretion and membrane APP were measured in control and in NGF differentiated PC12 cells stably transferred with M1 Ach R. This secretion was markedly enhanced by either carbachol or the M1 selective agonist (arecoline bioisosters) and by NGF. App was measured by 10% PGE-SDS gels and densitometry scanning of stained band (4 chloro-1-naphthol staining).
1.11.7 Quantification of β-Amyloids (A β1-40, and A β1-42)

Either by using cultured Cells (PC 12 or SK-N-SH or SK-N-SH) or human cerebrospinal fluid (CSF) from AD patients, cells were previously incubated with M1 agonists, in response to the M1 receptor stimulation; its mediated β-amyloid (Aβ1-40 and Aβ1-42) is quantified by using Sandwich Elisa.77

1.12 Different Animal Models (Invivo) Used to Screen Arecoline Bioisosters (M1 Agonists) in AD Research

Animal models for AD are rapidly advance with further characterization of lesions in aged primates, canines, rats, mice and other large animal models with development of transgenes, having significant CNS expression of the identified genetic risk factors, APP 717 and APP 670/670 mutations, presenilin mutations and APO E 4. Different methods to develop animal models for AD research.

1.12.1 Transgenic Mice or Rat

APP or presenilin mutated mouse or rat models are used in AD research.

1.12.2 β-Amyloid Administration

β-amyloid is injected into the brain of rat or mice, to bring about AD, but this method is not satisfactory does not bring about actual condition of AD.

1.12.3 Physical Injury to Brain

Animals (Rat) brain is partially damaged to bring about memory loss. This kind of models also does not bring about actual condition of AD.

1.12.4 Injection of Aluminium chloride

Aluminium chloride is injected in the brain of rat or mice, it kills brain cells to some extent, and its research is still in advancement.
1.12.5 Quasiquilic acid Administration

Quasiquilic acid destroys specific receptors in brain (mice or rat), which can be further used as animal models for AD.

1.12.6 Scopolamine Administration

Scopolamine administration by IP to rat or mice, blocks muscarinic receptor (M1 antagonist) and brings about temporary memory loss.

1.13 Different *In vivo* Behavioral Tasks Used to Test Arecoline Bioisosters for Learning And Memory.

1.13.1 Passive Avoidance

Using passive avoidance task paradigm, the acquisition and retention of the learned task is quantified in animals (rat or mice). For this experiment rodent memory evaluation (ESCKU) is designed to measure these parameters in mice or rat. Each animal is individually trained and placed in shock zone; the time is measured in sec to reach shock free zone (SFZ). Arecoline bioisosters ability to reverse the scopolamine induced memory loss is evaluated in this experiment.

1.13.2 Water Maze

It measures spatial memory, the essential feature of this technique is that rats are placed into a large circular pool of water and can swim onto a hidden platform. The platform is hidden by its placement just below the water surface and by opaque water. Thus the platform offers no local cues to guide escape behavior. The rat can escape from swimming by climbing onto the platform and overtime the rat apparently learns the spatial location of the platform from any starting position at the circumference of the pool. Arecoline bioisosters are tested to reverse scopolamine induced spatial memory loss in terms of the time in sec to reach hidden platform.

1.13.3 Radial Arm Maze

A radical arm maze is used to evaluate the working memory in animals (Rodents). At the beginning of the trial, two food pellets are placed in each receptacle. A rat is
placed on the center hub with all guillotine doors lowered. Then, all the doors are simultaneously opened to allow the rat to choose arms freely. When the rat enters one of the arms, the doors to the remaining seven arms are closed. The open door is closed after the animal returns to the center hub. Then all the doors are raised again simultaneously. The trial is considered complete when the rat visits all eight arms or spends 10 min in the maze.

1.13.4 Plus Maze

Elevated plus maze is used to measure learning and memory in rats or mice in terms of transfer latency (TL). The time in sec the animal reach from open arm to any of the closed arms. Arecoline bioisosters are screened to reverse scopolamine induced memory loss and learning impairment in terms of TL (Pellow 1985).

1.13.5 Y Maze

Y maze is used for the evolution of spatial working and long term memory in rodents using food or sweetened water as an incentive to reach the goal. The animal is allowed to explore in three arm Y maze in sequential manner. The error committed by animal is noted and correct sequence entry is calculated in terms of percentage (percentage alteration behavior), arecoline bioisosters tested for this ability show severe scopolamine induced memory loss in terms percentage alteration behavior.

1.13.6 Stone T Maze

It is one of the oldest and more complex modular mazes (14 T mazes) used to assess the mixed spatial working, reference, cue and taxon learning skills in rats. A delicious sweetened food was placed in the goal box at the end of the maze. The animal is observed through video camera, in which animal moves in sequence to reach goal was measured. Entry into an arm, which the rat has not previously visited, is recorded as a correct response and reentry is counted as an error. The number of correct responses before committing the first error (the number of initial correct responses) is calculated as the index of radial maze performance. A trial, in which an animal made no error, or only one error at the eighth choice, can be defined as a “successful” trial. The percentage of successful trial is measured for arecoline bioisosters against scopolamine induced memory loss.
References:


23. NIA Congressional Justification for Fiscal Year 2002 Budget Request


30. For the $100 billion annual cost, this study cites figures based on 1991 data, which were updated in the journal’s press release to 1994 figures. Cited in 2001 –


34. Rice, D. P., Health Affairs, Summer 1993, 12, 164–176.


41. Tol, J., Roks, G., Rev Neurol. 1999, 155, 10-16.

42. Aerosa, S. A., Mcshare, R., Sheriff, F., Cochrane database Syst Rev. CD003154, PMID 15495043.


44. Class, L., Woods, R., Cochrane Database syst Rev.CD003260 PMID 14583963.


