World Journal of

Pharmacology

WORLD JOURNAL OF PHARMACOLOGY

A peer-reviewed, online, open-access journal of pharmacology

Editorial Board

2011-2015

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ABOUT COVER

Editorial Board Number of *World Journal of Pharmacology*, Zhi-Gen Jiang, MD, Professor, Oregon Hearing Research Center, Department of Otolaryngology, Head and Neck Surgery, NRC-04 Oregon Health and Science University, Portland, OR 97239-3098, United States

AIM AND SCOPE

World Journal of Pharmacology (*World J Pharmacol*, *WJP*, online ISSN 2220-3192, DOI: 10.5497) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

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INDEXING/ABSTRACTING

World Journal of Pharmacology is now indexed in Digital Object Identifier.

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REVIEW

Therapeutic targets and delivery challenges for Alzheimer's disease

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Abstract

Dementia, including Alzheimer's disease, the 21st Century epidemic, is one of the most significant social and health crises which has currently afflicted nearly 44 million patients worldwide and about new 7.7 million cases are reported every year. This portrays the unmet need towards better understanding of Alzheimer's disease pathomechanisms and related research towards more effective treatment strategies. The review thus comprehensively addresses Alzheimer's disease pathophysiology with an insight of underlying multicascade pathway and elaborates possible therapeutic targets- particularly anti-amyloid approaches, anti-tau approaches, acetylcholinesterase inhibitors, glutamatergic system modifiers, immunotherapy, anti-inflammatory targets, antioxidants, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors and insulin. In spite of extensive research leading to identification of newer targets and potent drugs, complete cure of Alzheimer's disease appears to be an unreached holy grail. This can be attributed to their ineffective delivery across blood brain barrier and ultimately to the brain. With this understanding, researchers are now focusing on development of drug delivery systems to be delivered *via* suitable route that can circumvent blood brain barrier effectively with enhanced patient compliance. In this context, we have summarized current drug delivery strategies by oral, transdermal, intravenous, intranasal and other miscellaneous routes and have accentuated the future standpoint towards promising therapy ultimately leading to Alzheimer's disease cure.

Key words: Neurofibrillary tangles; Alzheimer's disease; Dementia; Amyloid β; Tau; Neurodegeneration; Blood brain barrier; Transdermal; Nasal

Core tip: Dementia, including Alzheimer's disease, the 21st Century epidemic, is one of the most significant social and health crises which has currently afflicted nearly 44 million patients worldwide and is on rampant rise. This portrays the unmet need towards better understanding

of Alzheimer's disease pathomechanisms and related research towards more effective treatment strategies. The review thus focuses on thorough understanding of Alzheimer's disease pathophysiology, pharmacotherapy in terms of explored therapeutic targets and drug delivery systems towards better delivery of anti-Alzheirmer actives and a possible way ahead.

INTRODUCTION

Dementia, including Alzheimer's disease (AD), the 21st Century epidemic, is one of the most significant social and health crises impacting families, social service and healthcare delivery systems.

The incidence of dementia and AD escalates almost exponentially with age[1]. The prevalence of dementia nearly doubles every five years after the age of 60 in which AD accounts for between 50%-70% among all dementia cases[2]. The age-standardized occurrence for those aged 60 or older is 5%-7%; among persons aged 60-64 years is 7%-18%, but among those aged over 90 years is 29%-64%[3-6]. It is generally believed that men and women are equally at risk of AD. However, there are more women patients than men possibly due to higher longevity of women as compared to men. Further, it is devastating to note that nearly one in four people with AD hide or conceal their symptoms, citing social stigma or dread of being ostracized[7] and four out of ten sufferers report being excluded from the familiar and comforting routines of everyday life[8].

Worldwide, approximately 44 million patients are reported to be afflicted with AD or other dementias and about 7.7 million new cases are reported every year[9]. The numbers are estimated to reach 76 million by 2030 and more than 135 million by 2050[4,10,11], with 90% increase in Europe, 226% in Asia, 248% in America and 345% in Africa[12]. In fact, most countries are woefully unprepared for the dementia epidemic and have not structured their health care programs to cope with the foreseen increase in numbers. Despite the urgent need for action, only 13 of the 193 World Health Organization members have instigated national dementia plans, precisely all of them in the developed world[13].

On the other hand, as per the current statistics, the number of cases of AD in Asia and Africa is lower than that reported in developed countries. There are several possible reasons like undiagnosed AD, the lack of awareness, poor access to technologically advanced health care, *etc*., or there may be lower incidence of risk factors[14]. Research in India and Africa proposes that the AD risk was possibly greater for urban as compared to rural areas. The reason for this difference is not clear whether it is increased life expectancy, lifestyle or diet?

AD though has a genetic predisposition in terms of mutations in specific genes (discussed in subsequent section), the expected hike in AD afflicted population can be attributed to increased exposure to AD risk factors that include ageing, oxidative stress (age and lifestyle induced), cardiovascular disorders, brain injuries, occupational hazards, *etc*.[12,15,16].

Further, the annual cost of AD related drug sales is reported to be increasing proportionally at growth rate of 33% from $500 million (year 1999) to approximately $6 billion (year 2008) and the estimated AD market is expected to cover a market size of $9.5 billion to $15 billion by year 2015-2017 (Figure 1)[16].

These huge statistical numbers clearly portray the unfulfilled need in AD therapeutic research and better management strategies. The major hurdle in this context is not only the identification of potential targets and discovery of potent therapeutic agents but also their effective delivery across brain.

With due consideration to these burning issues, the review focuses on thorough understanding of AD pathophysiology, pharmacotherapy in terms of explored therapeutic targets and current state of art in drug delivery systems towards better delivery of AD actives and a possible way ahead.

AD: PATHOLOGY AND SYMPTOMS

AD is a progressive brain disorder wherein the patients show clinical symptoms after a significant manifestation of disease which can take as long as 20 years[15,17]. The symptomatic appearance of AD results from progressive neurodegeneration resulting from alteration in normal anatomy and physiology of central nervous system (CNS). This primarily includes abnormal appearance of extracellular senile plaques and intracellular neurofibrillary tangles (NFTs) in CNS that interfere with classical neuronal activity triggering the neuronal death.

The senile plaques comprise toxic Amyloid β [$A\beta_{(1-42)}$] protein fragments resulting from atypical amyloidogenic cleavage of amyloid precursor protein (APP). These Aβ fragments undergo sequential aggregation process to form insoluble senile plaques that get deposited in extracellular neuronal matrix. These plaques then interfere with synaptic signal transfer and induce stress signals that activate microglia, lysosomes and synaptic mitochondria ultimately causing neuronal death[15,18-21].

The intracellular NFTs are predominantly made up of hyperphosphorylated tau protein inter-tangles that impede neuronal nutrient supply leading to neuronal death. Additionally, other pathological variations like inflammation, activated microglias, elevated levels of proinflammatory cytokines, *etc*., accelerate the neuronal death.

From the site specific AD manifestation *per se,* the early neurodegeneration is observed in the cholinergic region of basal forebrain that results in cholinergic neuronal death. This results in acetylcholine (ACh)

Figure 1 Schematic of Alzheimer's disease afflicted patient population and associated therapy market data. AD: Alzheimer's disease.

imbalance leading to early symptoms and memory loss *via* interference in both nicotinic and muscarinic receptor activities[18,19]. This early clinical stage of AD is commonly identified with mild to moderate forgetfulness in routine activities, apathy, depression, *etc*. These symptoms are broadly classified under a general class of dementia. An important point to note here is that forebrain region is associated with memory formation and thus early manifestation of AD leads to loss of recent memory followed by the old memory as the disease advances[15,19,20].

As the disease progresses senile plaques and NFTs deposition gets extrapolated to other regions of brain that predominantly include parietal and temporal lobes, hippocampus and entorhinal cortex[19,22-24]. This worsens the neuropsychiatric symptoms resulting in delirium, disorientation, lack of judgment, withdrawal from social appearance, difficulty in performing routine activities like eating, talking, walking, writing, *etc*.[15,19].

As the disease progresses, the brain shows high degree of shrinkage and debris deposition due to excessive neuronal death in all regions of brain. This impairment makes the patients dependent on help even for performing routine daily activities and this is identified as the final stage of the disease. At this stage, the excessively deprived brain function deprives the control on all the other body functions. This makes the patient highly vulnerable to secondary diseases like cardiac/pulmonary complications and out borne infections like pneumonia, *etc*., which forms the predominant reason for patient's death[15].

AD: THERAPEUTIC TARGETS

From the ongoing multidirectional research on AD etio-

logy, it is well evident that there is no unanimous opinion suggesting a single mechanistic pathway. Hence the pathophysiological and symptomatic advents associated with AD are believed to be resulting from a multicascade pathway leading to neurodegeneration. To understand this gradual and irreversible cognitive decline, various hypotheses have been proposed that include, formation of Aβ and extracellular fibrillation thereof, development of intracellular hyperphosphorylated tau and associated NFTs, oxidative stress, *etc*., ultimately resulting in neuronal death (Figure 2).

An extensive research on these variable pathways has resulted in identification of multiple therapeutic targets which are summarized below.

Amyloid cascade and therapeutic targets
This hypothesis was proposed by Hardy and Higgins in early 1990's and till date it is the most-researched and conceptual framework for AD which has markedly influenced drug development over a period of last 25 years[21]. The hypothesis is based on formation and accumulation of toxic Aβ$_{(1-42)}$ fragments resulting from abnormal amyloidogenic cleavage of trans membrane APP resulting from mutation in APP and presenilin gene (*PS-1*, *PS-2*) that regulate the entire pathway (familial origin)[25,26]. The so formed insoluble Aβ fragments further associate to form senile plaques, diffuse plaques, and cerebrovascular deposits which are the hallmarks of AD and being toxic they result in synaptic loss, neuronal death (predominantly cholinergic neurons) leading to progressive cognitive impairment[18,22-24].

Conventionally, 3 enzymes that play a crucial role in natural proteolytic cleavage of APP are α, β, and γ secretase. The first step herein comprises cleavage

Figure 2 Schematic representation indicating interlinks among important Alzheimer's disease pathogenetic hypotheses that majorly include amyloid cascade hypothesis, taupathy, mitochondrial dysfunction hypothesis, cholesterol induced mechanism, insulin signal downregulation, calpain-cathepsin hypothesis. APP: Amyloid precursor protein; PS: Presinilin; APoE: Apolipoprotein E; PI3K: Phosphoinositide-3-kinase; AKT: Protein kinase B; ROS: Reactive oxygen species.

of extracellular fragment by α-secretase (non-amyloidogenic and predominant pathway under normal condition) or β-secretase (amyloidogenic pathway predominant under AD) leading to 83 or 99 amino acid peptide residues respectively that remain attached as a trans membrane fragment. Further, these fragments are invariably cleaved by γ-secretase which leads to formation of toxic Aβ(1-42) fragments in case of amyloidogenic pathway and initiates the extracellular plaque formation[22,24] (Figure 3).

Thus, targeting Aβ cascade presents the most important strategy towards management of AD. Several of such approaches include inflection of Aβ formation, augmentation of Aβ degradation, inhibition of Aβ assembly, and immunization (passive and active) to raise antibodies that target and remove Aβ and are discussed in subsequent sections.

α-secretase stimulators: This approach came in scientific limelight with an *in vivo* study that demonstrated the potential of enzyme ADAM 10 (a member of disintegrin and metalloproteinase family) that functions as α-secretase to prevent plaque formation and additionally it offered neuronal protection in hippocampal region[27]. This is attributed to the fact that α-secretase cleaves APP in a non-amyloidogenic pathway (Figure 3) and thus, up regulation of this enzyme is postulated to arrest Aβ formation. In this context, naturally occurring retinoids are reported to possess α-secretase stimulator activity and one such molecule acitretin is at phase 2 trial

Figure 3 Schematic representation of amyloid cascade hypothesis. Three enzymes α, β, and γ secretase play a crucial role in the proteolytic cleavage of APP. The first step, extracellular fragment of APP is cleaved by α-secretase (non-amyloidogenic and predominant pathway under normal condition) or β-secretase (amyloidogenic pathway predominant under AD) leading to 83 or 99 amino acid peptide residues respectively that remain attached as a trans membrane fragment. These fragments are ultimately cleaved by γ-secretase which leads to formation of toxic Aβ(1-42) fragments in case of amyloidogenic pathway and initiates the extracellular Aβ plaque formation. APP: Amyloid precursor protein; AD: Alzheimer's disease.

stage[28]. This finding suggests the use of natural retinoid rich food which includes spinach, carrots, soy products, *etc*., as a possible nutritional supplement for AD patients. Apart from natural sources, synthetic agonists of α-secretase are under thorough investigation and one such molecule, EHT-0202 has shown very promising results both *in vitro* and *in vivo* and is currently under 3-mo phase 2 clinical evaluation in 35 AD subjects[29-31].

β-secretase modulators: The β-secretase enzyme initiates the amyloidogenic pathway and thus it is a prime requisite to develop inhibitors of the same. The enzyme is very large structurally and poses difficulties in producing an inhibitor especially with an ability to cross the blood brain barrier (BBB). Thus, small molecules are being designed to inhibit the enzyme at the active site. CTS-21166, a β-secretase inhibitor is successfully reported to reduce plasma Aβ levels in phase 1 study conducted in 48 healthy volunteers at 6 different doses

up to 225 mg and phase 2 study is planned[32]. In another study, central Aβ levels were lowered by the orally administrable non peptide molecule LY2811376 (molecule by Eli Lilly Inc.) in preclinical studies but further progress was halted as it affected animal retinal epithelium[33,34]. Other β-secretase inhibitor KMI-429 is being developed and human trial data is awaited[35]. Thus, this strategy is in its infancy and has to undergo a battery of safety and efficacy studies prior to becoming a market reality.

γ-secretase modulators: γ-secretase, the ultimate enzyme in amyloid cascade pathway, presents the next probable target to arrest amyloid cascade. With this in vision, MK-0752 (Merck), a γ-secretase inhibitor was developed which is in phase 2 trial as phase 1 trial was successful and indicated significant reduction in cerebrospinal fluid (CSF) Aβ levels in healthy volunteers[29,36].

Structurally, γ-secretase is a trans-membrane complex of four proteins: presenilin, presenilin enhancer 2, nicastrin, and anterior pharynx-defective 1[29,37] that play role in proteolysis of type-1 transmembrane proteins. Thus, it is worthy to note here that, apart from APP, γ-secretase has other substrates like Notch, E-cadherin, ErbB4, CD44, tyrosinase, alcadein which play a crucial role in embryogenesis and development[37]. Thus, non-selective inhibition of this protein may lead to side/adverse effects. As an instance, semagacestat (non-selective γ-secretase inhibitor) has advanced in therapeutic trials for AD but a phase 2 trial (14 wk) in 51 subjects (15, 22 and 14 subjects received placebo, 100 mg and 140 mg drug daily respectively) have shown high risk of skin rash and hair colour change which was reversed with treatment withdrawal[38]. Thus designing of an inhibitor to this enzyme desires meticulous selection. Owing to these observations, the new molecules are being developed with an aim to modulate the enzyme which will retain the therapeutic efficacy but overrule the adverse drug reactions[39].

Inhibitors of Aβ aggregation: Another encouraging approach for the development of novel therapeutics for treating AD is to prevent Aβ fibril formation especially by the small molecules. Neurochem Inc., a Canadian company, has developed a glycosaminoglycan mimetic Alzhemed™ which has an ability to bind to Aβ peptides and thereby inhibits the formation of Aβ aggregates. The molecule has successfully completed Phase 2 clinical trial and Phase 3 trial results are recently published wherein the data is very promising[40]. Metal ions like Cu^{2+} and Zn^{2+} are reported to augment Aβ aggregation and associated toxicity[41]. In consistency with this, a Cu/Zn chelator, clioquinol is reported to reduce CNS Aβ deposition after a 9 wk treatment in rodent model. The additional benefit of this molecule is its inherent tendency to cross BBB which is anticipated to ensure the therapeutic efficacy[42].

Aβ removal approaches: Aβ plaques are degraded by some proteases such as plasmin, neprilysin, insulin degrading enzyme, endothelin converting enzyme, angiotensin converting enzyme and metalloprotein-ase[43]. The levels of these Aβ degrading enzymes are observed to decline in AD and may contribute to Aβ accumulation[44]. In consistency to these observations, experimental evidence has suggested that inhibitors of plasminogen activator decrease the plasma and brain Aβ levels in transgenic animals[45] and increasing neprilysin levels through viral vector-delivered gene expression shows beneficial effects in animal models[46]. Additionally, the peptide hormone somatostatin is also reported to enhance Aβ clearance through activation of neprilysin[47]. Therefore targeting neprilysin with somatostatin or its analogs is an encouraging option in AD. This approach is quite in its infancy and demands thorough investigation.

Immunotherapy against Aβ: Immunotherapy was first explored by Schenk *et al*[48] for treatment of AD in a preclinical experiment involving Aβ(1-42) active immunization using PDAPP transgenic mice. So far numerous studies have shown encouraging results by both active (vaccination) and passive (monoclonal antibody) immunization. In active immunization, the Aβ peptide or fragment conjugated to a carrier protein and adjuvant which holds potential to stimulate cellular and humoral immune response is administered to the host which results in generation of anti-Aβ antibody. In passive immunization, the Aβ peptide specific antibody is directly injected into the host, thus evading the step of stimulating the host immune systems. The exact mechanism by which immunotherapy executes anti-AD activity is still not clear. However, studies conducted so far have given substantial proofs based on which few of the hypotheses are proposed *viz*. microglia-mediated phagocytosis, antibody mediated Aβ monomer sequestration, antibody mediated prevention of Aβ aggregation and neutralization of Aβ toxicity and antibody mediated peripheral clearance of Aβ[49-57]. Figure 4 illustrates the diagrammatic representation of the various mechanistic pathways of immunotherapy in AD.

Bard *et al*[49] and Hartman *et al*[50] administered Aβ monoclonal antibody to PDAPP transgenic mice and further noticed significant immunoreactivity within the microglia and macrophages. The study clearly indicated that the generated antibodies were able to cross the BBB and bind to Aβ plaques, provoking the Fc receptors (FcR)-mediated microglial phagocytosis. Numerous studies conducted by active and passive immunization have suggested that the (FcR)-mediated microglial phagocytosis might play a crucial role in clearing Aβ load from brain[49,50].

In yet another study, Yamada *et al*[51] found that administration of certain anti-Aβ monoclonal antibody m266 has selectively sequestered soluble Aβ monomers in the brain and terminated its progression to oligomers and plaques, thus circumventing associated neuro-toxicity.

Another hypothesis suggests that certain anti-Aβ monoclonal antibodies have ability to by-pass the BBB and interact with Aβ oligomers and fibrils[52,53] to either disassemble or dissolve the existing plaques[54-56].

DeMattos *et al*[57] were the first to reveal the ability of antibodies to clear the Aβ levels from the systemic circulation. In this study, Aβ mid-region antibody (m266) which bears high affinity for soluble Aβ administered to PDAPP transgenic mice showed notable reduction in Aβ burden from the plasma. This mechanism was further confirmed by both active[58,59] and passive immunization[60-62]. These mechanisms indicated that anti-Aβ antibodies directly interact with plasma Aβ and enhance its clearance. This in turn imbalance the plasma to brain Aβ ratio and there by hasten the Aβ removal

Figure 4 Schematic diagram explaining different mechanistic pathways hypothesized for immunotherapy to exert Amyloid β clearance from brain and plasma. Immunotherapy exerts its activity by active and passive ways. In active immunization, the Aβ peptide or fragment is injected into the host which in turn stimulates cellular and humoral immune response to generate anti-Aβ antibody. In passive immunization, directly Aβ peptide specific antibody is injected into the host. The generated anti-Aβ antibodies provoke anti-AD activity by one of the or combination of following ways: microglia-mediated phagocytosis, antibody mediated Aβ monomer sequestration, antibody mediated prevention of Aβ aggregation and neutralization of Aβ toxicity and antibody mediated peripheral clearance of Aβ. Aβ: Amyloid β; BBB: Blood brain barrier. AD: Alzheimer's disease.

from the brain.

Despite the promising outcome in *in vitro* and preclinical studies, application of immunotherapy in clinical trial using synthetic Aβ peptide AN1792/QS-21 in AD patients with mild to moderate severity has turned out to be fatal and resulted in abrupt termination as 8 out of 300 patients developed meningoencephalitis during the study[63]. The study concluded that, there was significant plaque reduction in patients treated with AN1792/QS-2 but unfortunately it augmented T cell activation leading to meningoencephalitis. A different clinical trial with Aβ immunization, indicated noteworthy clearance of amyloid plaque in AD patients but lacked the ability to arrest neurodegenerative progression[64].

In an attempt to evade the potentially harmful T cell responses, emergence of advanced vaccines consisting sole antibody epitope(s) that lack T-cell reactive sites is a step further in the development of immunotherapy for AD. The existing data has shown promising effects while clinical reports are still awaited. In all, despite promising

outcome of immunotherapy by curtailing the Aβ load and improvement of cognitive function, threat of adverse reactions still remains to be the unresolved issue[64].

Although several drugs have been investigated to be active at their intended targets, none have yet been proven to have significant clinical benefits. In 2011 and 2012, two negative trials of secretase inhibitors, semagacestat and avagacestat, and several negative trials of monoclonal antibodies, bapineuzumab and solanezumab were reported[64-68]. Recent studies have demonstrated that reducing Aβ in the brain is possible but that decreasing production or reducing fibrils or plaques is not clearly associated with clinical improvement and could be associated with toxicity[64-68]. These issues must be critically evaluated while development of newer therapeutic molecules against the specific targets in amyloid cascade hypothesis.

Taupathy and therapeutic targets
The second major hallmark of AD is formation of NFTs

which comprise hyperphosphorylated form of tau protein. Tau is a protein that under normal physiological conditions stabilizes microtubules, allowing transport of vesicles and other products of neuronal cell bodies down the axon to the synapse. By regulating microtubule assembly, tau controls the morphology and growth of axons[69]. The protein has several phosphorylation sites, and the microtubule binding property of tau is dependent on the phosphorylation state. The phosphorylated tau binds microtubules with a lesser affinity leading to microtubule instability[70,71].

The trigger for this abnormal tau fibrillation initiates with stress signals (Aβ, mechanical damage, ROS, *etc*.) that activate primary microglial cells in CNS. This activation results in release of proinflammatory cytokines (TNFα and interleukins) that leads to neuronal alteration initiating tau hyperphosphorylation. This hyperphosphorylated form presents an aggregation tendency forming initial paired helical filaments that further precipitate to form NFTs. These intracellular NFTs elicit toxic effect on neurons leading to neuronal death. Upon neurodegeneration, these NFTs are released in extracellular matrix that in turn augments the microglial activity *via* positive feedback mechanism[72,73].

As per this hypothesis, AD pathology starts with formation of pretangles in proximal axons of the noradrenergic locus ceruleus which spreads *via* trans-synaptic transport to entorhinal cortex, hippocampus, and neocortex. This cascade of reactions is known as taupathy and is reported to worsen the AD in conjunction with senile plaques[72] (Figure 2).

Thus, it is well conceived that tau hyperphosphorylation, microtubule disruption and formation of NFTs play a crucial role in AD pathomechanisms. Therapeutically, several of these mechanisms can be targeted to arrest AD progression *viz*. inhibition of tau kinases to lower tau hperphosphorylation and associated aggregation, enhancing clearance of tau aggregates with drugs or antibodies and microtubule stabilization by enhancing phosphatase activity. Few of such approaches have progressed from preclinical to advanced clinical trials[74,75]. In this context, the main focus is now shifting on glycogen synthase kinase 3, a prime enzyme involved in tau phosphorylation. Lithium and valproate are reported to have inhibitory action on this enzyme and have shown promising results in terms of reducing taupathy and NFT formation in transgenic mice[76,77]. Further, it should be noted that tau pathology is not specific to AD, and occurs in several other disorders, including frontotemporal dementia, corticobasal degeneration, progressive supranuclear palsy, *etc*., and these approaches can be extrapolated in treatment thereof[72].

Recently, scientists have reported close relation between taupathy and synaptic mitochondrial dysfunction that leads to ROS augmentation. With this support, mitochondrial dysfunction cascade hypothesis is gaining wide attention in AD pathomechanisms[73].

Mitochondrial dysfunction and therapeutic targets

Mitochondria being the energy hose of the cells, decline in mitochondrial function allies itself with ageing and AD. The prime assumption of this hypothesis is based on genetic predisposition of AD that is presented by low genetic mitochondrial baseline function which is predominantly inherited from mother's genome and this mitochondrial baseline function is inversely proportional with AD progression[73].

In addition to genetic predisposition, AD patho-mechanisms are also reported to manifest mitochondrial dysfunction. Aβ is reported to be present in mitochondria and is observed to be interacting with complex II of respiratory chain, mitochondrial membrane and Hsp 60 (a mitochondrial chaperon matrix protein) leading to mitochondrial abnormalities. This not only alters the regular mitochondrial function but also causes abnormal increase in mitochondrial fission and reduced fusion that severely affects the mitochondrial morphology. This alteration in morphology is proposed to augment mitochondrial fragmentation and was confirmed using confocal and electron microscopic analysis in APP overex-pressed neurons[78]. This can be additionally explained by the unwanted interaction of Aβ and NFT with dyna-min-related protein 1, the protein that maintains the mammalian mitochondria. This interaction results in increased mitochondrial fragmentation, their restricted axonal transport and subsequent neurodegeneration[79].

Apart from genetic and AD associated factors, other environmental factors like heavy metal exposure, oxidative stress, insulin resistance, *etc*., are reported to down regulate the mitochondrial function *via* positive feedback pathway. Under normal scenario, mitochondrial biogenesis can take care of this external environmental burden but fails in case of patients with already declined neuronal activity like in case of AD. Thus, these environmental factors are postulated to cause additional affliction by hastening the mitochondrial dysfunction ultimately leading to progressive AD associated synaptic damage and symptomatic manifestation[73,80].

Oxidative stress not only causes mitochondrial dysfunction but also triggers Aβ deposition, tau hyperphosphorylation and oxidation of other neuronal components like lipids, proteins, nucleic acids, *etc*., causing neuronal damage[70]. The relationship between oxidative stress and AD suggests that oxidative stress is the key component of AD pathophysiology. In this context, use of antioxidants to reduce oxidative burden on cells holds a strong rationale[81].

Antioxidants for AD therapy

Antioxidant treatment is proposed to be a promising approach to slow down the disease progression by attenuating phospholipid peroxidation, protein and DNA oxidation[82]. Flavonoids and carotenoids, a group of ubiquitous antioxidants have also shown neuroprotective effect in several experiments[83,84]. Rutin, a flavonoid

compound, protected rats from stress induced damage and streptozotocin induced neuronal inflammation[85]. Lutein, a natural carotenoid with cytoprotective effect[86]; when supplemented in combination with docosahexaenoic acid, memory scores and rate of learning improved in elderly women[87]. The spice curcumin has shown several beneficial roles (antioxidant, anti-inflammatory, and amyloid disaggregating properties) in experimental studies[88,89].

Melatonin is another antioxidant compound which is anticipated to be a potent anti-AD active. This is attributed to N-methyl-D-aspartate (NMDA) receptor modulation, inhibition of Aβ generation, formation of amyloid fibrils, attenuation of tau hyperphosphorylation, mitochondrial protection, and antiapoptotic effect[90]. Vitamin E has shown marked reduction in lipid peroxidation and plaque deposition when administered in transgenic AD rodent model but has failed to produce similar convincing results in humans[91]. Also, the combination of vitamin E with donepezil did not provide additional benefit in patients with AD or mild cognitive impairment[92]. Evidence for the protection offered by antioxidants including vitamins (E, C, and carotenoids), phytochemicals and synthetic compounds in AD is inconsistent[83].

Antioxidants though have shown positive results, their translation to clinic as a solitary AD therapy is not successful due to varied epidemiological data. However, it is worthy to note that they are emerging as nutritional supplements to decrease the incidence or to delay the progression of AD.

Acetylcholinesterase inhibitors for AD therapy

As discussed in earlier section (AD: Pathology and Symptoms), cholinergic neuronal death is the classical manifestation of AD leading to ACh imbalance and associated cognitive decline[18,19]. Thus restoration of CNS ACh levels is believed to offer early symptomatic relief.

In this context, cholinergic neuronal death restricts the opportunity to augment the ACh release from neurons and thus restoration of CNS ACh levels by arresting its degradation was thought to be a promising strategy. With this in view, Acetylcholinesterase (enzyme responsible for degradation of Ach) inhibitors (AChEIs) were the first amongst the pharmacological treatments sanctioned by the United States Food and Drug Administration (FDA) for AD. Currently four AChEIs are available in market: tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Razadyne®, Reminyl®). Their therapeutic efficacy may be attributed to their ability to sustain cognitive function over a prolonged period of therapy[93,94]. Studies have also shown that these drugs can arrest neurodegeneration and thus can delay AD progression, if the therapy is initiated at earliest in patients with mild to moderate AD. Rivastigmine blocks butyrylcholinesterase, levels of which are reported to be augmented in the brain of patients

with AD[95] and this may have an advantageous effect on prolonged cholinesterase inhibition ensuing disease stabilization. On the other hand, galantamine binds to the nicotinic ACh receptor sites which opens the ionic channels and improves the receptor responsiveness to Ach[96]. Tacrine is hardly prescribed nowadays due to its high dosing frequency and associated hepatotoxicity. Other adverse effects associated with AChEIs are nausea, vomiting, diarrhoea, anorexia, *etc.*, and are observed to worsen during dose escalation[97]. Patients with bradycardia are at higher risk and should be given additional attention[98]. Gastrointestinal (GI) effects can be minimized by simultaneous administration of food and an anti-emetic. As the cholinergic neurons decline with disease progression (severe forms of AD), the AChEI treatments becomes inefficient.

The mechanism of action by which these drugs act is by arresting the breakdown of the neurotransmitter Ach *via* inhibition of the acetylcholinesterase enzyme. Acetylcholinesterase is also found to enhance Aβ plaque formation; therefore inhibition of this enzyme will not only provide symptomatic relief but also arrest AD progression[97].

Glutamatergic system modifiers for AD therapy

Glutamate is the major excitatory neurotransmitter in the CNS which is involved in a variety of functions, including synaptic neurotransmission, neuronal growth and development, synaptic plasticity, *etc*[98,99]. Glutamatergic neurotransmission is also observed to be very crucial in learning and memory[64,65] which is greatly hampered in case of AD patients. Moreover, the glutamatergic neurons are observed in the brain regions affected by AD, particularly in the neocortex, cortex and hippocampus[99-101].

Glutamate induced up regulation of the NMDA receptor augments the intracellular calcium level leading to neuronal death which is the hallmark of AD[102]. Memantine (Namenda®, Axura®, Ebixa®), a non-competitive NMDA receptor antagonist was approved by FDA in October 2003 for treatment of moderate to severe AD. It inhibits the neurodegeneration resulting from protracted glutamate release[103] but does not interfere with cognition at therapeutic doses[104]. At higher doses, clinical studies have shown the potential of drug to cause functional decline with delay in cognition but have overruled the possibility of any severe adverse effects[105,106]. Some studies also suggest that memantine may synergies the AChEIs therapeutic efficacy if given in combination. In one such study, it is proven that the combination was effective and well tolerated by majority of AD subjects without any severe side effects[107]. With such a promising data combination therapy can be envisioned as a better AD treatment regime.

Anti-inflammatory agents for AD therapy

The perception of AD being an inflammatory disease has appeared with two unique pathophysiological findings

in AD patients. Firstly, the increased size and number of microglial cells in the brain and second the overexpression of complement system *via* amyloid plaques. Results from several studies, based on follow-up design and prescription based data have shown substantial reduction in the incidence of AD with prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs)[108].

Investigation of AD brain has confirmed the incidence of inflammation at and around the sites of Aβ plaques deposits, NFTs and degenerating neurons. The aggregation and deposition of Aβ plaques tend to provoke the activation of microglia and astrocytes. This event is further accompanied by overexpression of complement system, particularly C1q, C3b, C3a, membrane attack complex (MAC), cytokines and chemokines. During the Aβ aggregation process, binding of C1q and C3b to amyloid plaques activates the complement system causing dystrophic neuritis and up regulating C3a and MAC (Figure 5). C3b further boosts the complement activation. C3a stimulates the activation of microglial cells which clears the Aβ through phagocytic mechanism. MAC causes cell lysis and results in toxic effects. The uncleared Aβ escalates the deposition and provokes the microglial cell activation and release of cytokines, chemokines and neurotoxins which cause neuronal loss and synaptic dysfunction[108].

Multiple mechanistic pathways have been cited for NSAIDs to elicit anti-AD activity. First of which is native anti-inflammatory action (Figure 5). NSAIDs mainly exert their anti-AD activity by suppressing the synthesis of inflammatory prostaglandin executed *via* Cyclooxygenase inhibition[109]. In support of this, Kotilinek *et al*[110] also recorded that the major improvement in the memory aspect of transgenic mice is associated with decreased prostaglandin E2 and is attributed to COX-2 inhibition. In another mechanism the NSAIDs are known to inhibit the nuclear transgenic factor Kβ which is up regulated in AD patients and is involved in regulation of many cellular target genes[111].

Yet in another investigation, the role of nuclear located peroxisome proliferator-activated receptor-γ (PPARγ) was unveiled where it is involved in regulation of pro-inflammatory genes associated with the pathogenesis of AD. The NSAIDs like ibuprofen and naproxen are known to stimulate PPARγ receptor and subsequently cause anti-inflammatory effect. Also, PPRAγ receptor mediated release of pro-inflammatory cytokines is associated with reduction of β-secretase expression and Aβ secretion[112].

The second proposed mechanism of anti-AD activity of NSAIDs is attributed to inhibition of amyloidogenic APP processing, Aβ formation and its aggregation. Studies conducted by Avramovich *et al*[113] have shown that NSAIDs in particular like indomethacin and ibuprofen stimulate non-amyloidogenic α-secretase pathway and cause marked release of neurotrophic and neuroprotective APP ectodomain in neuronal cells.

Further, effect of ibuprofen causing down regulation

of α1-antichymotrypsin, a protein responsible for triggering Aβ pathogenesis proves another mechanistic pathway. They are known to exhibit a direct effect by inhibiting Aβ oligomer formation and subsequent deposition by interacting with Aβ peptide[114].

Despite generation of enormous data at cellular and preclinical level, replication of similar effects at clinical level has been still a matter of debate. Ten years of comprehensive study conducted in Canada to assess the incidence of AD has shown that use of NSAIDs is associated with reduced incidence of AD[115]. Contradictory to this, several studies conducted with other anti-inflammatory drugs like prednisone, hydroxychloroquine, COX-2 selective inhibitors (celecoxib, rofecoxib) and non-selective COX inhibitors (naproxen) have failed to show any advantageous effect[108,109].

3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins) for AD therapy

The relation between cholesterol and AD was brought to notice for the first time by Sparks *et al*[116] and statistical studies have suggested that individuals on statins therapy (drugs used in cholesterol management) have very low incidence of AD.

It had been observed that elevated intracellular cholesterol induces the β-secretase activity leading to enhanced Aβ production. Inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase by statins, reverses this elevated intracellular cholesterol level (cholesterol dependent pathway) (Figure 6) and thereby blocks the Aβ formation. Another proposed mechanism by which statins act against Aβ progression is through cholesterol independent effect or pleiotropic effect that grants neuroprotection (Figure 6)[117,118]. Herein, statins enhance nitric oxide (NO) mediated anti-inflammatory activity and facilitate the systemic Aβ clearance by stimulating endothelial NO synthase[117,118]. Owing to these multi-target activities, statins are now emerging as promising pharmacological agents for AD treatment. The clinical trials conducted with statins like atorvastatin, lovastatin (for longer duration approximately 1 year) have shown beneficial effect by reducing the plasma Aβ levels but were not observed to be reproducible[117,118]. This could be attributed to study variations that include differences in cognitive test employed, experimental protocol, study duration and the stage of AD manifestation and dose. Thus, there is need for thorough investigation of this strategy towards its clinical approval.

Insulin for AD therapy

Apart from diabetes, insulin is reported to play a critical role in glucose uptake and neurotransmission across the brain[119-122]. Further, the proteins important for transmission of insulin signal were identified to be up regulated in AD sensitive brain regions *viz*. hippocampus and temporal lobe. This instigated an interest of researchers to investigate possible correlation between

Figure 5 Diagram representation elaborating various mechanistic pathways by which anti-Alzheimer's disease activity is executed by non-steroidal anti-inflammatory drugs. The aggregation and deposition of Aβ plaques provoke the activation of microglia, astrocytes and complement system (C1q, C3b, C3a, MAC, cytokines and chemokines). The Aβ plaques which evades the clearance process forms deposit and provokes microglial cell activation and release of cytokines, chemokines and neurotoxins which consequently results in neuronal loss and synaptic dysfunction. NSAIDs are reported to act by multiple ways to elicit anti-AD activity which includes suppresion of oligomer formation, PPRAγ, COX, NFKβ, α1-ACT and non-amyloid α secretase pathway. Aβ: Amyloid β; APP: Amyloid precursor protein; MAC: Membrane attack complex; PPARγ: Peroxisome proliferator-activated receptor-γ; COX: Cycloxygenase; α1-ACT: α1-antichymotrypsin; BACE1: Beta-secretase 1.

Figure 6 Diagrammatic illustration of anti-amyloid activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors *via* cholesterol independent and cholesterol dependent mechanistic pathways. Cholesterol independent pathway: Statins cause NO mediated anti-inflammatory activity and facilitate the clearance of systemic Aβ by stimulating endothelial nitric oxide synthase. The statins with the virtue of its antioxidant effect cause reduction in lipid peroxidation which is escalated due to elevated levels of NO in AD brain. Cholesterol dependent pathway: The systemic cholesterol enters the brain in form of HDL. The astrocytes originated ApoE facilitates the uptake of extracellular free cholesterol and release into the neuronal cells *via* LRP. This act reduces free cholesterol and curtails the Aβ genesis whereas un-sequestered free ApoE aggravates the Aβ formation. Statins inhibits the HMG-CoA reductase and subsequently reverse the elevated intracellular cholesterol levels and blocks the Aβ formation. Also, ACAT inhibition leads to reduction in Aβ levels by an unknown mechanism. HDL: High density lipoprotein; BBB: Blood brain barrier; Aβ: Amyloid β; ApoE: Apolipoprotein E; LRP: LDL receptor-related protein; HMG-CoA reductase: 3-hydroxy-3-methyl-glutaryl-CoA; ER: Endoplasmic reticulum; NO: Nitric oxide; eNOS: Endothelial nitric oxide synthase; nNOS: Neuronal nitric oxide synthase; iNOS: Inducible nitric oxide synthase; ACAT: Acetyl-coenzyme a acetyltransferase. AD: Alzheimer's disease.

insulin and AD[123,124]. Remarkably, research groups both at cell-culture and clinical level have shown that normal insulin signaling under an AD condition confers beneficial effects like protection against Aβ oligomer-mediated insulin receptor loss and synaptic deterioration[125], boosting Aβ trafficking at cell membrane and clearance thereof[126].

Briefly, insulin signaling pathway is initiated when tyrosine phosphorylated insulin receptor substrate interacts with insulin receptor. Upon interaction, phosphatidylinositol 4,5-bisphosphate (PIP2) is transformed to phosphatidylinositol 3,4,5-triphosphate (PIP3) at the plasma membrane *via* cascade of mechanisms that finally activate Phosphoinositide-dependent kinase-1 which triggers the insulin signal. Though the exact mechanism by which insulin enhances cognitive function is unknown, the possible mechanisms can be down regulation of insulin receptors and other signaling intermediates discussed above[124-127].

Moreover, evidences also show that Aβ acts as a competitive inhibitor of insulin at the insulin receptor. This inhibition results GSK-3 stimulation by negative feedback mechanism which subsequently increases tau phosphorylation (Figure 7). Thus, insulin therapy is expected to arrest both Aβ generation and tau hyperphosphorylation[124-127].

Two dose clinical study performed in young, cognitively normal subjects (1.5 mU/kg per minute and 15 mU/kg per minute) showed improved memory performance and attention at high serum levels of insulin[127]. Other additional studies conducted in elderly impaired individuals by Craft *et al*[128,129] demonstrated improvement in declarative memory at dose level of 1.0 mU/kg per minute infusion. Since treatment with insulin infusion is associated with hypoglycemia, direct delivery of insulin to the brain proves to be a viable approach. In this context, intranasal administration of insulin has gained wide attention wherein a 8 wk therapy (40 IU/dose, 4 × per day) showed good performance in recalling the words in young, cognitively normal subjects[130] whereas 21 d (20 IU, 2 × per day) treatment promoted story recall and attention in cognitively impaired subjects and individuals with AD[130-132]. These effects have shown insulin to be a promising anti-AD agent.

In addition to aforementioned targets some new hypotheses are emerging with advances in understanding of AD pathmechanisms. One such recent observation is presence of autophagic vacuoles in degenerating neurons. It is proposed that these vacuoles formation results from lysosomal autophagy induced by oxidative stress, Aβ, calcium ion imbalance and cleavage of heat shock protein (Hsp 70.1) that plays a crucial role in lysosomal integrity. Accumulation of these vacuoles further results in autophagy induced neurodegeneration[133]. Thus lysosomal stabilization can be seen as a near future approach to treat AD. Thus to summarize, multivariate targets have been identified as treatment avenues for AD and are currently in various stages of clinical development. However, it must be noted that clinicians have now started believing in multitarget approach as a better therapy module to address both disease progression and symptomatic relief.

AD: DRUG DELIVERY SYSTEMS

With emergence of newer therapeutic targets as discussed in earlier section, there comes a great hope for successful AD treatment modalities in near future. Though very lucrative, the prime challenge in getting these potent drugs from bench to bedside lies in their effective delivery across BBB and ultimately to the brain. BBB is identified as an obstructive interface between blood and CNS that restricts the entry of variety of molecules to the brain *via* tight junctions and also serves as CNS microvasculature. This overprotective phenomenon of BBB turns out to be the rate limiting step in effective transport of drug to the brain. Further, during AD progression, BBB undergoes certain pathophysiological changes in terms of altered expression of certain transporter receptors, altered glucose transport, impaired P-gp efflux system, leaky vasculature, release of neurotoxins and oxidative stress induced changes in BBB permeability. These are being studied extensively in recent years for better understanding of disease pathophysiology. In coming years, understanding of these will play a crucial role in designing smart delivery systems to surpass BBB[134,135].

Besides drug delivery system, the route of administration also plays a significant role in drug absorption, distribution, and passage across BBB, *etc*. With this understanding, researchers are now focusing on development of drug delivery systems and suitable route of delivery that can synergistically circumvent BBB and enhance patient compliance[134-136]. This section describes design and development of plethora of drug delivery systems for AD therapeutics with a special attention on route of delivery. Table 1 abridges the novel strategies under investigation and Table 2 summarizes currently marketed products and ongoing clinical studies.

Oral drug delivery systems
Peroral route is most preferred route of drug delivery owing to the associated patient compliance and convenience of administration. This is marked from the fact that the first approved dosage form for treatment of AD was an oral capsule of reversible AChEI drug tacrine (Cognex®).

However for AD treatment, in addition to BBB, GI stability and permeation of drug into systemic circulation become superfluous rate limiting step upon oral administration. This is well evident from the reports that Cognex® is prescribed 4 times daily which resulted in poor therapeutic compliance due to meager oral bioavailability, severe first-pass metabolism and peripheral side effects. Thus to leverage the benefits of oral delivery towards effective treatment strategy in case of AD, researchers are working towards the modified oral dosage forms that ensure better stability and permeation across GI tract.

Various drugs investigated in literature towards modified oral dosage form include AChEIs, polyphenols, metal chelators, peptides, *etc*. Polyphenols are a class of molecules which are extensively explored for AD treatment. One such polyphenol is (-)-epigallocatechin-3-gallate (EGCG) which is reported to be a potent activator of α-secretase that activates nonamyloidogenic processing APP but is a poor candidate for oral delivery. In this context, Smith *et al*[137] developed the nanolipid particles of EGCG using a co-solubilization method at drug to excipient ratio ranging from 1:1 to 1:32. The *in vitro* studies in murine neuroblastoma cells indicated significant enhancement in α-secretase activity above the ratio of 1:8 and activity was attributed to the better encapsulation and stability of drug in nanolipid matrix. Further, the oral pharmacokinetic studies performed in male Sprague Dawley rats exhibited over two fold enhancement in oral bioavailability with nanolipid EGCG formulation as compared to plain drug when administered at dose of 100 mg/kg of EGCG. These results signify importance of modified dosage form towards better oral bioavailability and can be anticipated to also enhance the brain uptake owing to the nanolipid matrix[137]. On similar lines, Dube *et al*[138] reported chitosan-tripolyphosphate nanoparticles of EGCG and performed pharmacokinetic studies in Swiss Outbred mice. The studies indicated almost 1.5 fold improvement in oral bioavailability as compared to EGCG suspension and interestingly indicated higher permeation from the jejunum region of GI tract indicating better potential of these NPs to enter systemic circulation.

Among the various AD treatment targets antiprogesterone drugs like mifepristone are also reported to arrest the cognitive impairment and thus offer symptomatic relief[139-141]. To enhance oral bioavailability, He *et al*[139] have reported polylactide-co-glycolide (PLGA) nanoparticles of this drug and have shown significantly high oral bioavailability of mifepristone as compared to plain drug. Thus lipid as well as polymeric encapsulation of actives presents a promising strategy towards enhanced stability and permeation of drug

Figure 7 Schematic representation explaining the role of insulin in Alzheimer's disease treatment. Interaction of IRS with insulin receptor initiates the event of insulin signaling. This act triggers a cascade of activities which subsequently results in expression of PIP3 on the plasma membrane. This further leads to the activation of PDK-1 which interacts with AKT to generate the transmission of the insulin signal. In AD, the crucial components for insulin signaling like insulin receptors, IRS, PI3K and AKT; are down regulated. Also, Aβ competes with insulin to bind to insulin receptors, inhibits the PI3K-AKT interaction and enhances the GSK-3 activity which subsequently increases tau phosphorylation leading to impaired signaling in AD. IRS: Insulin receptor substrate; PI3K: Phosphoinositide-3-kinase; PIP: Phosphatidylinositol phosphate; PDK1: Phosphoinositide-dependent kinase-1; AKT: Protein kinase B; GSK-3: Glycogen synthase kinase-3; AD: Alzheimer's disease.

across GI tract. A step ahead in this field is to explore dual modalities towards enhanced GI as well as BBB permeability. For this, Mittal *et al*[142,143] developed estradiol entrapped PLGA nanoparticles coated with tween 80 using single emulsion technique (particle size approximately 170 nm). The rationale here was to enhance GI uptake of estradiol by nanopolymeric encapsulation and to enhance the brain uptake by tween 80 coat which is reported to be recognized and actively taken up across BBB by apolipoprotein receptors present on the BBB. Upon oral administration in Sprague Dawley rats, the estradiol level was found to be 1.969 ± 0.197 ng/g brain tissue in case of tween 80 coated nanoparticles which was almost 2 fold as compared to 1.105 ± 0.136 ng/g tissue as in case of uncoated nanoparticles. Interestingly, the drug levels obtained in brain with tween 80 coated nanoparticles given orally were almost similar to that obtained after intramuscular injection (2.123 ± 0.370 ng/g tissue) of estradiol

indicating the superiority of targeted nanoparticles. Also, the pharmacodynamic studies performed using elevated plus maze test indicated marked reduction in anxiety behaviour with nanoparticulate formulation as compared to plain drug[142,143].

Oral therapy though poses issues w.r.t. low oral bioavailability, chronic oral treatment is reported to be effective probably for drugs that offer minimal or no peripheral side effects. This can be attributed to the time dependent accumulation and slow clearance from brain. In support of this, very recently Kazim *et al*[144] reported that the chronic oral treatment of neurotrophic factors results in reduction of neural plasticity and associated cognitive impairment. In this study, a ciliary neurotrophic factor P021 (Ac-DGGLAG-NH2) was given orally over a period of 12 mo to both moderate and severe stages of AD in transgenic mice. It was also observed that there was a significant down regulation of hyperphosphorylated tau and Aβ and thus chronic

Table 1 Novel therapeutic strategies for Alzheimer's disease management

Active	Mechanism of action	Drug delivery system	Efficacy study model	Ref.
		Oral		
Rutin	Antioxidant	Only drug	Preclinical rodent streptozotocin induced AD model	[85]
(-)-Epigallocatechin-3-gallate	Antioxidant, α-secretase activator	Nanolipid carriers	*In vitro*, preclinical rodent model	[137]
		Chitosan	Preclinical rodent model	[138]
Mifepristone	Antiprogesterone activity, AD symptomatic relief	PLGA nanoparticles	Preclinical rodent model	[139-141]
Estradiol	Estrogenic activity, AD symptomatic relief	Tween 80 coated PLGA nanoparticles	Preclinical rodent model	[142,143]
CNTF P021 (Ac-DGGLAG-NH$_2$)	Neurotrophic factor	Only drug	Preclinical rodent AD transgenic model	[144]
Clioquinol	Metal ion Cu/Zn chelator	Only drug	Preclinical rodent AD transgenic model	[145,146]
Galactose	Glucose restoration	Only drug	Preclinical streptozotocin induced rodent model	[147]
		Transdermal		
Galantamine	AChEI	Drug in adhesive type patch	Preclinical rodent model	[151]
Donepezil (base and salt form)	AChEI	Fatty acid based topical formulation	*In vitro* skin model, preclinical rodent model	[152]
Huperzine A	AChEI	Microemulsion, solid lipid nanoparticles, nanostructured lipid carriers	*In vitro* skin model, preclinical rodent model	[153]
Donepezil	AChEI	Iontophoresis, Wearable Electronic Drug Delivery System patches	Preclinical rodent model	[154]
Rasageline and selegiline	MAO-B inhibitors	Solution and carbopol based gel - iontophoresis	*In vitro* skin model	[155]
Memantine	NMDA receptor modulator	Iontophoresis with penetration enhancers	*In vitro* skin model	[156]
Aβ(1-42) antigen	AD immunovaccine	Microneedles	Preclinical rodent model	[161]
		Intravenous		
Clioquinol	Cu/Zn chelator	Only drug	Preclinical rodent AD transgenic model	[42]
Lithium	GSK-3 inhibitor of tau phosphorylation	Only drug	Preclinical rodent AD transgenic model	[76,77]
Valproate sodium	GSK-3 inhibitor of tau phosphorylation	Only drug	Preclinical rodent AD transgenic model	[76,77]
Nerve growth factor	Cholinergic neuron protection	Polysorbate 80 coated PBCA nanoparticles	Preclinical scopolamine induced rodent model	[162]
		PEG chemical conjugate	Preclinical rodent model	[163]
		Antitransferrin antibody chemical conjugate	Preclinical rodent model	[163,164]
Galantamine	AChEI	Peptide targeting ligand functionalized liposomes	*In vitro* cell line	[165]
Aβ binding peptide QSH	Aβ reduction	Targeted PEGylated polylactic acid nanocarriers	Preclinical AD induced rodent model	[166]
Thiflavin T	Specific Aβ plaque binding	PBCA Nanoparticles	Preclinical rodent model	[168,169]
Anti- Aβ antibody	AD immunotherapy	Only drug	Preclinical rodent AD transgenic model	[49,50]
BAM -10 antibody	AD immunotherapy	External targeting with trans-cranial application of magnetic and ultrasound energy	Preclinical rodent AD transgenic model	[186,187]
Cholic acid	Cholinergic management	Fluorescent labeled PEG-PLGA nanoparticles, external ultrasound	Preclinical rodent model	[188]
Octapeptide NAP derived from the neurotrophic factor	Neuroprotective activity	Only drug	Preclinical rodent model	[196,197]
Mesenchymal stem Cells	Neuronal growth	Only drug	*In vitro*, Preclinical transgenic AD rodent model	[203,204]
		Intranasal		
Insulin	Multicascade anti AD activity	Only drug	Preclinical rodent model	[130-132]
Tacrine	AChEI	Cyclodextrin coated bovine serum albumin nanospeheres	Ex *vivo* permeation model	[175]
Curcumin	Antioxidant, anti- Aβ activity	Nanoemulsion	Ex *vivo* permeation model	[177]
siRNA	AD associated gene silencing	Cell penetrating peptide TAT conjugated polycaprolactone- PEG Micelles	Early development	[184]
Gene delivery	AD associated gene silencing	Exosomes	Early development	[185]
Pituitary adenylate cyclase-activating polypeptide	Neuroprotective activity	Only drug	Preclinical rodent model	[198]

		Implants		
Rivastigmine	AChEI	BTM and SAM based organogel implants, subcutaneous administration	Preclinical rodent model	[195]

Aβ: Amyloid β; AD: Alzheimer's disease; PLGA: Polylactide-co-glycolide; CNTF: Ciliary neurotrophic factor; AChEI: Acetylcholinesterase inhibitors; MAO-B: Monoamine oxidase B; NMDA: N-methyl-D-aspartate; GSK-3: Glycogen synthase kinase 3; PEG: Polyethylene glycol; PBCA: Polybutylcyanoacrylate; BTM: N-behenoyl L-tyrosine methylester; SAM: N-stearoyl L-alanine methylester.

Table 2 Current market status and ongoing clinical investigation for Alzheimer's disease therapeutics

Active	Mechanism of action	Drug delivery route	Clinical status	Ref.
Approved drugs				
Tacrine (Cognex®)	AChEI	Oral	USFDA approved	[205]
Donepezil (Aricept®)	AChEI	Oral	USFDA approved	
Galantamine (Razadyne, Reminyl®)	AChEI	Oral	USFDA approved	
Rivastigmine (Exelon®)	AChEI	Transdermal patch	USFDA approved	
Memantine (Namenda, Axura®, Ebixa®)	NMDA receptor inhibitor, glutaminergic system modifier	Oral	USFDA approved	
Drugs under clinical investigation				
EHT-0202	α-secretase activator	Oral	Phase 2	[29]
CTS-21166	β-secretase inhibitor	Oral	Phase 2	[35]
MK-0752	γ-secretase inhibitor	Oral	Phase 2	[29,36]
Immunoglobulin	AD immune activity	Intravenous	Phase 2	[170]
Omega-3-fatty acid treatment, nutritional supplement, physical exercise and cognitive stimulation	AD symptomatic management	Oral	Phase 3	[199]
Cerebrolysin and AChEI	Neirotropic, AChEI	Oral	Phase 4	[200,201]
Alzhemed™	Activity Aβ aggregation inhibitor	Oral (dietary supplement)	Phase 3	[206]

USFDA: United States Food and Drug Administration; AChEI: Acetylcholinesterase inhibitors; NMDA: N-methyl-D-aspartate; AD: Alzheimer's disease.

therapy can be visualized to be an effective strategy for such drugs that do not offer peripheral side effects but pose problem with oral bioavailability alone. In one such study, clioquinol (metal ion Cu/Zn chelator) was given orally over a period of 9 wk to aged APP2576 transgenic mice with advanced AD. As hypothesized, the results indicated significant reduction in both cerebral and serum Aβ levels[145,146].

Nutrient imbalance is generally observed with AD and among all, glucose hypometabolism is one such hallmark condition. With an aim to restore the brain glucose levels, Salkovic-Petrisic *et al*[147] supplemented streptozotocin induced AD rats with oral galactose (200 mg/kg per day) over a period of 1 mo. The pharmacodynamic evaluation with Morris Water Maze and Passive Avoidance test indicated restoration of cognitive function in galactose treated group as compared to control. Thus, it can be well noted that chronic treatment with nutrient sugars that can be converted to glucose *via* alternative pathways can be a supportive therapy ensuring symptomatic relief.

Transdermal drug delivery systems
Considering life-long therapy, transdermal delivery of AD actives is considered to be an ideal route as it offers sustained drug delivery over prolonged period of time with reduced dosing frequency. This certainly ensures patient compliant therapy module and positively reduces patient dependence on caretaker. Additionally, it also overrules the adversities associated with oral route *viz*. peripheral side effects, first pass metabolism, fluctuations in plasma drug concentration, *etc.*[148,149]. Owing to these lucrative advantages and feasibility of dosage form development, first AChEI drug rivastigmine transdermal patch (Exelon®) was introduced in market in year 2007 for treatment to mild to moderate AD. In a multicentric study with approximately 2000 AD patient caregivers, it was observed that 94.3% of caregivers preferred transdermal form of rivastigmine over oral therapy and corroborated better efficacy and symptomatic relief in patients[150]. With this great success of Exelon®, various conventional patches have been investigated for transdermal delivery of AD therapeutics. In one such study, Park *et al*[151] formulated drug in adhesive type patch of galantamine with a series of pressure sensitive adhesives. Among all, DT-2510 was found to be the most suitable pressure sensitive adhesive and this optimised patch demonstrated sustained drug plasma levels over a period of 24 h with 80% bioavailability. Thus, the successful delivery of galantamine *via* transdermal route proves to be a promising alternative to oral therapy ensuring relief from peripheral side effects like severe vomiting, nausea, *etc*.

Drug as well drug carrier both play a crucial role in delivery of drugs across the skin. To understand this phenomenon, Choi *et al*[152] investigated the permeation of AChEI drug donepezil in its base as well as salt form and further studied the influence of fatty acids as penetration enhancers on permeation behavior. The *in vitro* permeation studies performed using mouse

and human cadaver skin indicated a good degree of correlation and confirmed a parabolic relationship between drug permeation and fatty acid chain length wherein the oleic acid and palmitoleic acid were observed to be optimum for the base and the salt form respectively. *In vivo* pharmacokinetic studies performed in rat model indicated that the base form of the drug exhibited 6 fold higher bioavailability as compared to the salt form, when formulated and applied topically with the respective fatty acid. This confirmed that the base form is more permeable and can be correlated to the lipophilic nature of drug along with the fatty acid matrix that ensured better permeation.

Considering the need of AD therapeutics to cross BBB, one must ensure higher plasma drug levels so as to felicitate BBB transport. Use of transdermal delivery presents a hurdle here, as stratum corneum, the uppermost layer of skin epidermis acts as a strong barrier towards the permeation of drug from skin into the systemic circulation. Thus higher degree and extent of drug permeation across skin demands modifications in transdermal delivery systems.

Lipid matrix based formulations are anticipated to enhance transdermal permeation and if formulated in nanocarriers are expected to cross BBB *via* passive transport. In context of this, Patel *et al*[153] developed microemulsion (ME), solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) based gels of an AChEI drug Huperzine A (particle size less than 150 nm) and compared their efficacy both *in vitro* and *in vivo. In vitro* skin permeation studies performed using rat skin resulted in highest flux observed with ME based formulation followed by NLCs and SLNs respectively. This enhanced permeation with ME based formulation can be ascribed to unique properties of ME to cause structural alteration in stratum corneum by virtue of the ME excipient matrix. Further, *in vivo* pharmacodynamic studies performed using elevated plus maze test in scopolamine induced mice amnesia model displayed significant reduction in transfer latency period indicating better cognition with nanoformulations as compared to orally administered drug suspension.

In order to further enhance permeation across the skin, various techniques *viz*. iontophoresis, sonophoresis, microneedles are being investigated. Among these techniques iontophoresis has gained the widest attention and is being explored for varied arena of AD therapeutics *viz*. AChEI, monoamine oxidase B (MAO-B) inhibitors, metal chelators, NMDA receptor antagonists, *etc*.

one such study Saluja *et al*[154] studied the ionto-phoretic delivery and effect of electric current on drug permeation *via* Wearable Electronic Drug Delivery patches using donepezil as a model drug. The done-pezil gel loaded electronic patches were applied on hairless rats and *in vivo* pharmacokinetic studies were performed. The studies revealed that at current intensity of 0.13 mA, 0.26 mA and 0.39 mA, the C_{max} level of

drug in plasma was observed to be 0.094 μg/mL, 0.237 μg/mL and 0.336 μg/mL respectively indicating that current density has a proportional effect on drug permeation. The imperative role of electric current was also confirmed by the fact that during iontophoresis linear pharmacokinetics were observed and it altered to flip flop kinetics after iontophoretic intervention.

Kalaria *et al*[155] investigated anodal iontophoresis as a technique to check permeation of two MAO-B inhibitor drugs rasagiline and selegiline, both from solution and carbopol gel form. *In vitro* studies performed using porcine and human skin revealed that rise in electric current intensity increases the permeation flux proportionally and the major mechanism of permeation was electromigration in presence of counter ions. Further, the degree of permeation from solution form was better as compared to the carbopol gel and was attributed to slower diffusion of drugs from the gel matrix. Thus, the studies suggested that simple transdermal patches can be employed for effective iontophoretic delivery wherein the patch system plays a very crucial role on rate of permeation and thus demands a meticulous selection.

With an aim to compare the potential of chemical penetration enhancers and iontophoresis, del Rio-Sancho *et al*[156] conducted 2 sets of *in vitro* skin permeation studies wherein the skin was pre-treated over a period of 12 h with various classes of chemical permeation enhancers *viz*. decenoic acid, R-(+)-limonene, oleic acid, cineol, laurocapram followed by *in vitro* permeation with memantine drug solution. In other set, iontophoresis was investigated as a drug permeation technique at the current density of 0.5 mA/cm^2. Amongst the various penetration enhancers, R-(+)-limonene exhibited maximum transdermal flux of 91.9 ± 8.2 μg/cm^{2}per hour. Iontophoresis exhibited transdermal flux of 158 ± 6 μg/cm^2 per hour which was almost 1.5 fold higher than the optimized permeation enhancer. Thus, iontophoresis can be visualized as an effective technique and additionally this opens up newer doors to investigate the co-application of both the techniques towards better permeation profiles.

As discussed in earlier section, AD immunovaccines are now emerging as a different dimension to AD therapeutics. Initial studies have proven potential of Aβ antigen injections in mouse but clinical trials were withdrawn from phase 2 due to meningoencephalitis induced by TH1 cells[63,157,158]. To overcome this adverse reaction, transcutaneous immunization is now gaining attention in recent years as it involves immune response *via* Th2 pathway[159,160]. Matsuo *et al*[161] amalgamated the concept of transcutaneous immunization with novel transdermal devices and have developed Aβ(1-42) antigen incorporated microneedle array, MicroHyala that is dissolved upon incorporation into the skin releasing the antigen. The scientists have proven the induction of immune system activation and have suggested that further modification is desired in delivery systems to achieve higher immune response and better cognitive

regain.

Intravenous drug delivery systems

Intravenous delivery of AD actives is being investigated extensively as it results in 100% bioavailability. This ensures higher systemic levels of drug that presents higher probability of drug permeation across the BBB. Further, this serves as an effective research tool to investigate the effect on drug carrier system on BBB permeability as upon intravenous administration the only major rate limiting step towards entry of drug in brain is BBB.

In recent times, with a rationale to augment the delivery of nanoparticulate carriers across BBB, use of targeting ligands is gaining wide attention. Having understood the potential of Nerve growth factor (NGF) in maintenance of cholinergic neurons, targeted delivery of NGF is under extensive exploration. Kurakhmaeva *et al*[162] developed polybutylcyanoacrylate (PBCA) nanoparticles of NGF followed by a polysorbate 80 coat that serves as a targeting ligand across BBB. Upon intravenous administration in scopolamine induced amnesic rodent model, significant improvement in cognition was observed and was corroborated with higher levels of NGF detected in murine brain as compared to plain NGF.

Several other approaches that involve drug ligand chemical conjugation are also reported in literature for delivery of NGF that include covalent ligation of NGF to polyethylene glycol (PEG) that warrants long circulation time in systemic circulation boosting the chances of BBB uptake[163], conjugation to antitransferrin antibody enabling receptor mediated active transport across BBB[164,165]. Mufamadi *et al*[165] recently reported peptide targeting ligand functionalized liposomes (size approximately 150 nm) incorporating galantamine and they have shown selective uptake of these targeted nanoliposomes across PC12 cells in contrast to the non-targeted liposomes.

Use of multiple targeting ligands is also being investigated. Zhang *et al*[166] employed two targeting ligands on PEGylated polylactic acid (PLA) nanoparticles. The targeting ligands were TGN, a 12 amino acid ligand specific for BBB transport and an Aβ binding peptide QSH. The hypothesis here was to achieve a better permeation across BBB *via* active transport of target specific nanocarriers followed by selective binding to Aβ plaques. The *in vivo* biodistribution studies followed by intravenous administration of these nanoparticles to AD induced mice indicated almost 1.5 fold higher uptake in cerebellum and hippocampus as compared to blood indicating the preferential uptake in brain.

In another approach, Bana *et al*[167] have reported development of liposomes with phosphatidic acid and a derivative of ApoE-peptide as a dual targeting strategy. The *in vivo* biodistribution studies in rodent model upon intravenous administration revealed a higher uptake as compared to monofunctionalised liposomes. This

signposts that concurrent targeting strategy can be used as a synergistic method to enhance BBB permeability.

The nanoparticulate systems are also extrapolated for diagnostic purpose. Thioflavin T is a fluorescent marker and is reported to possess specific binding affinity towards Aβ plaques. Taking advantage of this, scientists have developed polymeric PBCA nanoparticles of this dye and have shown imaging potential of this dye towards AD diagnosis. These results are very encouraging as these nanoparticles are reported to be taken across BBB from the systemic circulation and thus the current techniques of direct CNS intervention *via* intracerebral or intracortical or intrahippocampal injections for diagnostic purpose can be circumvented[168,169].

Intravenous immunoglobulins are reported to contain anti-Aβ antibodies and are under clinical studies for their therapeutic assessment. In an open study, 8 patients with mild AD were subjected to intravenous immunoglobulins for a period of 6 mo followed by a break that was continued with further treatment for 9 mo. Studies publicized significant reduction in Aβ levels in CSF and a symptomatic progress was observed with 2.5 points increase in Mini-mental state[170]. In extension to this, phase 2 double blind studies in patients with mild to moderate AD suggested the efficacy of treatment and confirmed safety. However they have reported need for longer trials with higher number of patients for more clinically significant data generation[171].

Intranasal drug delivery systems

Olfactory pathway is being reconnoitered comprehensively towards the brain delivery of therapeutic actives as it is the most accessible route for circumventing BBB that allows entry *via* peripheral olfactory neurons and lamina propria in the CNS (Figure 8)[172-174]. In this context, both passive as well as active targeting approaches are well reported in literature. Considering the nasal epithelium and permeability of olfactory pathway, nanoparticle mediated intranasal drug delivery for treatment of AD is most widely investigated for both passive as well as active delivery modules.

Natural polymeric nanocarriers *viz*. albumin, chitosan, *etc*. are amongst the highly explored nanoparticles because of high degree of mucoadhesion, negligible nasal mucosa irritation and compatibility. Luppi *et al*[175] developed cyclodextrin coated bovine serum albumin nanospheres of tacrine (size approximately 300 nm) using coacervation technique. These nanospheres presented strong mucoadhesive properties and *ex vivo* permeation studies using sheep nasal mucosa indicated complete permeation within 100 min. This suggests a better possibility of brain delivery *via* nasal route as complete dose permeated well within the nasal mucosa clearance time (approximately 4 h).

Gao *et al*[176] developed 6-Coumarin, a fluorescent dye loaded lectinised nanoparticles as a tool for targeted uptake *via* olfactory epithelium. Briefly, Ulexeuropeus agglutinin I is reported to bind specifically to l-fucose,

Figure 8 Schematic representation of nose to brain uptake by olfactory pathway circumventing blood brain barrier that allows entry *via* peripheral olfactory neurons and lamina propria in the central nervous system. Modified from[172]. CSF: Cerebrospinal fluid.

a lectin binding domain located on olfactory epithelium. Using this as a targeting ligand decorated on PEG-PLA nanoparticles, almost 1.7 fold enhancement in brain bioavailability was seen as compared to the non-targeted PEG-PLA nanoparticles. Further, the developed targeted nanocarrier exhibited higher affinity towards olfactory mucosa than respiratory mucosa and was attributed to nanoparticulate surface immobilization of carbohydrate binding pockets present in the nasal mucosa. This evidently ensured the selective passage of targeted nanoparticles *via* olfactory pathway upon nasal administration.

Researchers have also developed nanoemulsion based formulation of anti-AD drugs with a view of better permeation possibility achieved using selective surfactants. In this area, Sood *et al*[177] formulated nanoemulsion of curcumin using high hydrophilic lipophilic balance surfactant with size less than 100 nm (optimized using Box-Behnken model). Further, with a rationale to synergise permeation by better mucoadhesion, the formulation was additionally loaded with 1% chitosan solution. Interestingly, in *ex vivo* permeation studies across sheep nasal mucosa, the mucoadhesive nanoemulsion exhibited an increased flux

(445.1 ± 37.48 μg/cm^2 per hour) in contrast to plain nanoemulsion (359.9 ± 36.85 μg/cm^2 per hour). This could be attributed to opening of tight nasal epithelial junctions with enhanced permeation resulting from the combination approach. Thus, multiple permeation pathways may enhance the brain drug delivery *via* nasal route.

As discussed in earlier sections, Aβ is the major culprit causing AD induced neuronal death. Since past two decades, serious efforts are being directed to use this as a diagnostic marker. In this context, it must be understood that detection of Aβ in blood is a difficult challenge as it gets assimilated in blood only after sufficient progression of disease and is present at very low concentration as compared to the brain. This makes AD diagnosis a difficult task and if at all diagnosed it is only after a significant progression of disease. Thus, it is of prime concern to identify other efficient diagnostic techniques. For this, Kameshima *et al*[178,179] did a systematic study in Tg2576 mice wherein they not only indicated significant amount of Aβ(1-42) in nasal cavity but also proposed that it reaches nasal cavity *via* non blood pathway. They further proved that there is positive correlation between nasal and brain Aβ(1-42)

levels which was not observed in case of serum and CSF. This is worthy to note as using nasal cavity for AD diagnosis will not only avail early diagnosis of AD but will also enable to monitor disease progression.

The efficacy of nasal route to deliver therapeutic actives is well perceived with pilot clinical studies performed for insulin delivery in patients with AD and mild cognitive disorders. The CSF biomarkers were positively identified ascertaining delivery of insulin *via* nasal route. Further, the study results were promising in terms of attention and memory improvement corroborating that insulin signaling pathway plays a crucial role in cognition[180]. This favors further exploration of this route for delivery of peptides which is otherwise difficult by other routes.

With this promising milestone achieved, the research here is paving a new path towards nasal delivery of siRNA, dsDNA, miRNA, *etc*. These molecules are recognized to be playing a key role in gene silencing especially for the pathways that lead to Aβ generation and thus represent a newer therapy module. From AD *per se* miR-107, miR-206 are recognized as potential actives inhibiting β-secretase and brain-derived neurotrophic factor (BDNF) respectively, whereas miR-34 is selective in terms of reducing stored BDNF levels[181-183]. Though it is lucrative, one must understand that the biggest challenge here is their site specific delivery owing to their extremely fragile nature and lack of permeability[183]. The smart formulation approach is anticipated to come to the rescue here wherein scientists have shown improved delivery of siRNA intranasally when given *via* cell penetrating peptide TAT conjugated polycaprolactone-PEG micelles as carriers[183,184]. Alternatively, exosomes, specialized vesicles resulting from plasma membrane like structures are coming in limelight as delivery vehicles *via* nasal route after proving their better efficacy *via* intravenous route. But research in this direction is in quite infancy and desires thorough investigation[183,185].

Altering BBB permeability

Thorough insights of AD pathophysiology and treatment strategies conclude in one major understanding that BBB serves as a major milestone to be overcome towards effective management of AD.

In supplement to various active and passive targeting strategies to cross BBB, transitory increase in BBB permeability using magnetic resonance or ultrasound is proposed to be the most site specific and sophisticated targeting strategies as it allows external control over BBB permeability and can further be focused to the particular site in the CNS.

Using this technology, Jordão *et al*[186] proposed the targeted immunotherapy approach for AD treatment. In this study, anti-Aβ antibody BAM-10 was administered intravenously with magnetic resonance imaging (MRI) and focused ultrasound contrast reagents in transgenic AD mice model. This was followed by trans-cranial application of magnetic and ultrasound energy. The results indicated immediate entry of contrast agents along with the antibody across the BBB and were confirmed with significant binding of antibody to Aβ plaques in brain cortical region. The similar group further explored the potential of only ultrasound energy to achieve selective BBB permeation. In the study, they injected a single dose anti-Aβ antibody with ultrasound contrast agent in transgenic AD mice model and demonstrated a significant reduction in Aβ plaques post 4 d of treatment. They also observed that the ultrasound application resulted in activation of glial cells and astrocytes in brain region which is assumed to further reduce the Aβ plaque load[187].

This strategy can be extended to ensure synergistic penetration of nanoparticles by virtue of their size along with increased permeability of BBB. To study this phenomenon, Nance *et al*[188] prepared fluorescent labeled PEG-PLGA nanoparticles (size approximately 60 nm) of cholic acid and administered them intravenously with contrast reagents. Upon application of brain focal MR-guided ultrasound, a significant increase in fluorescent intensity was observed as compared to when administered without MR-guided ultrasound. In another study, Treat *et al*[189] have demonstrated better penetration of liposomal formulations across BBB using ultrasound treatment.

These strategies are also extrapolated for AD diagnostics[190-192]. Under current protocol, an intra cerebroventricular injection of MRI contrast agent is given for imaging based diagnosis of microscopic Aβ plaques. To avoid the CNS intervention, Santin *et al*[192] have reported a novel ultra sound-Gd-staining protocol wherein they propose to administer clinically approved MRI contrast agent Dotarem® and microbubbles Sonovue® intravenously followed by external ultrasound treatment that ensures partitioning of contrast agent inside BBB. With preliminary studies in mouse model, the group has demonstrated fast imaging within 30 min with a resolution upto 29 μm which is similar to the one achieved with intra cerebroventricular injection alone.

These studies reveal the potential of transient improvisation of BBB permeability towards better diagnostics and therapeutic efficacy. Though non-invasive, one must thoroughly study the effect of prolong use of such strategies on BBB. To understand this Xie *et al*[193] employed the ultrasound frequency of 1 MHz over temporal bone of higher animal model, *i.e.*, pigs for a period of 30 min and tracked the permeation of using MRI and a dye evans red. Studies revealed significant retention up to 90 min post exposure but was not observed at 120 min. This indicates that enhanced BBB permeability with ultrasound is a temporary and reversible mechanism but demands detailed investigation of chronic use of such techniques as AD treatment is a life-long therapy desiring regular therapeutic intervention.

Miscellaneous

With an aim to achieve sustained delivery of AD actives

over a prolonged period of time, implants are emerging as novel delivery tools in this arena[194]. Sustained release biodegradable polymeric implants of PLGA, Polylactic acid, Lecithins, organogels of safflower oil, N-stearoyl L-alanine methylester (SAM), N-behenoyl L-tyrosine methylester (BTM), *etc.*, and hydrogels are very well explored. In this class of biodegradable implants, organogel based implants (identified as a 3-dimensional gelator network entrapping an organic phase component and drug) are gaining wide attention in AD therapeutics due to their unique properties of insitu implant formation, better entrapment and control over the release of low molecular weight polar compounds.

In congruence with this hypothesis, Bastiat *et al*[195] developed BTM based organogels of rivastigmine and compared its efficacy w.r.t. SAM based organogels. These organogels were injected subcutaneously in rodent model wherein they formed an implant *in situ*. *In vivo* pharmacokinetic studies herein resulted in 2.5 fold enhancement in bioavailability with BTM based organogels as compared to SAM based organogels over a period of 35 d with minimum foreign body response thus proving the potential of these oraganogels in AD treatment modality.

Not only the drug delivery systems but AD therapeutic research is also witnessing the discovery of newer molecules originating from native brain protective factors. One such example is an octapeptide NAP derived from the neurotrophic factor (molecular weight = 825 Da). Researchers have shown the significant accumulation of this octapeptide in cortex and cerebellum of brain with reduced levels of hyperphosphorylated tau and Aβ in transgenic mouse[196,197]. In extension to this, Gozes *et al*[197] studied the effect of this oligopeptide on memory retention and reported improved short-term spatial memory behavior in cognition impaired rat model. This finding suggests the potential use of this peptide towards both pathophysiological and symptomatic relief in AD therapy.

One such other peptide under investigation is pituitary adenylate cyclase-activating polypeptide (PACAP) which is a strong α-secretase activator and possesses neuroprotective activity. Upon continuous intranasal application Rat *et al*[198] demonstrated that PACAP upregulated non amyloidogenic processing of resulting in reduction of Aβ and synergistically improved the levels of brain derived neurotrophic factor. These results are promising towards development of newer therapy modalities for AD and nasal route can be anticipated as an emerging choicest route for delivery of such molecules as it will not only ensure effective delivery to brain but will also confer stability to these fragile peptides otherwise difficult to deliver by other routes.

FUTURE PROSPECTS

With thorough insight of current state of art in AD rese-

arch and due consideration to few burning facts *viz*. clinical failure of majority of drug candidates especially those targeted towards reduction of Aβ load, lack of any new drug approval and market entry since almost a decade necessitates identification of newer AD targets and/or modified treatment strategies.

In this setting, multi-domain treatment strategies are expected to become mainstream in coming years. For instance, a Multi-domain Alzheimer Prevention Study (MAPT) is currently ongoing in France that involves combination of omega-3-fatty acid treatment, nutritional supplement, physical exercise and cognitive stimulation in patient population over the age of 70. The results have shown very positive outcome opening a newer opportunity for AD management[199]. Also a combination of neurotrophic peptide drug, Cerebrolysin (Ever Neuro Pharma Ltd.) and AChEIs have shown very promising synergistic results and a phase 4 clinical trial is on-going[200,201].

Additionally, epigenetics is recently being discussed as a key reason of AD pathomechanisms and is correlated with alterations due to methylation of DNA and/or acylation of histones. Enzymes involved in these reactions are thus drawing wide attention of researchers and identification of potential inhibitors of these driver enzymes (histone acetyl transferase, *etc.*) is underway. Additionally a concept of epigenetic diet that includes vitamins (B6, B12, folate, *etc.*) is getting streamlined as these vitamins act as essential cofactors for the enzymes that control methylation homeostasis. Thus, a supplementary therapy to the existing treatment can be seen as a next step in AD therapeutics[200].

Neurodegeneration being the major pathophysiological cause of AD, stem cells induced neuroregeneration is becoming lucrative avenue which will not only arrest disease progression but will also offer symptomatic relief. *In vitro* studies have shown that mesenchymal stem cells (MSCs) augment neuronal cell differentiation, neurite growth and more importantly are resistant to taupathy[202]. Another study has recently suggested that human placental MSCs elicit significant immunomodulatory and paracrine effects leading to marked improvement in spatial and memory functions in AD transgenic mice[203]. With such promising research leads, it is envisioned that stem cells will soon serve as a survivor to AD patients.

CONCLUSION

Literature gives a wide spectrum of possibilities towards future AD treatment but in current setting complete AD cure appears to be an unreached holy grail. As a fact, 413 AD clinical trials have been conducted from year 2002 to 2012 and only 0.4% of trials have shown positive results[196]. This portrays the unmet need towards more better and deeper understanding of AD pathomechanisms and related research towards more effective treatment strategies. In this context, it is predicted that even if one succeeds in achieving

two-year delay in both the onset and progression of AD, it will possibly reduce both AD prevalence and the last stage of disease by more than 20% and 30% respectively. This will in turn reduce the individual, social, and economic burden of the disease[197]. Thus the great challenge of coming decades will be to find financial and humanitarian resources towards better management of those afflicted with AD and to refine and redouble research efforts.

With more awareness and worldwide programs like AD Neuroimaging Initiative, Alzheimer's Drug Therapy Initiative we are optimistic that the collaborative streamlined research will soon come up with a promising therapy for AD treatment and ultimately the cure.

ACKNOWLEDGMENTS

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