NEW THERAPEUTIC ALTERNATIVES FOR ALZHEIMER’S DISEASE

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ABSTRACT

Alzheimer’s disease is rapidly growing worldwide and there is no cure for it yet. Currently available drugs only provide symptomatic relief and do not intervene in disease process sufficiently enough to prevent it or cure it. Amyloid hypothesis is the most dominant hypothesis proposed for the pathogenesis of this disease. This review will put together a disease model based on known human and animal data with regards to breakdown in neuronal energy generation. The model will integrate energy deficits as the cause of neuronal dysfunction and abeta peptide production culminating in loss of cognitive functions. Also, few enzyme targets are mentioned in neuronal bioenergetics pathway for design and development of new disease modifying therapies.

KEYWORDS: Alzheimer’s disease, amyloid hypothesis, neuronal energy, new therapeutics, enzyme targets.

INTRODUCTION

Alzheimer’s disease is one of the most common forms of neurodegenerative diseases that frequently cause dementia. Ageing is a major risk factor of neurodegenerative diseases. The disease is characterized by progressive memory loss, cognitive impairment and variations in personality. Large amount of the cognitive symptom begins from a diminution of basal forebrain cholinergic neurons leading to decreased cholinergic transmission \cite{1}. Along with these symptoms, neuropsychiatric symptoms such as depression, psychosis and agitation are observed among patients. Alzheimer’s begins with a subtle decline in memory and progresses to deterioration in cognitive and adaptive functioning. Through few epidemiological investigations it is seen that midlife hypercholesterolemia, diabetes, hypertension,
inflammation, cardiovascular diseases, obesity, and viral infections may significantly contribute to development and progression of Alzheimer’s diseases.

Initially, patients display limited forgetfulness with disruption of memory impairment which is due to short term memory disruption and then leads to long term memory deficits. Besides cognitive worsening, patients display behavioral and psychological signs and symptoms of dementia (BPSD). BPSD is a broad term that includes heterogeneous group of non-cognitive symptoms and behaviors, including activity disturbances, anxieties, hallucinations, phobias and diurnal rhythm disturbances, paranoid and delusional ideation. [2]

Animal Models
Animal models are chosen so that they meet the taxonomic equivalency to humans. There are three types of animal models.
1) HOMOLOGUS- These animals have the same causes, symptoms and treatment options.
2) ISOMORPHIC- These animals share the same symptoms and treatment only.
3) PREDICTIVE- These animals are similar to a particular human disease.

Animal disease models are considered important in the development of drugs for Alzheimer’s disease. The Etiology of AD is unknown but there is still a probability in the favor of amyloid hypothesis. A broad range of animal models have been developed to mimic the human context of the disease for the purpose of developing diseases modifying agents. The goal of developing animal models is to stimulate neuropathological findings of AD. [3]

Animal models used in AD can be split into 3 categories Natural, Genetic, Intervention.

Natural
In the recent years, dogs have been considered as a useful animal model for AD due to its close proximity of canine and human brain ageing. [4] Dogs develop extensive AB and diffuse plaque deposition and the extent of AB deposition correlates with the decline of some measures of cognitive function in the absence of neurofibrillary tangles. [5] Also, the amino acid sequences of AB are fully conserved between dogs and humans. Because of its spontaneous age related AB deposition, dogs are used to evaluate intervention with the aim of reduction AB load. [6]

Among the non-human primate models, mouse lemurs seem to be potential animal model that exhibit amyloid plaque, neurofibrillary tangles and some other AD related neuropathology.
This primate lives 8-10 years and show age related changes similar to those of aging in humans. \[7\] It is seen, that the genes responsible for formation of senile plaques are highly similar between mouse lemur and humans. It is seen that, in a recent study, that cognitively impaired aged mouse lemurs have cerebral atrophy in those brain regions that are responsible for cognitive functions. \[8\] It is seen that, there is a difference in distribution of AB deposits and plaques between human and mouse lemur. But as with human AD, no diagnostic tools are available that can predict which adult mouse lemur will develop AD like symptoms. \[9\]

**Genetic**

Transgenic animal models are those that reproduce the cause of familial AD by transfecting a mutant human amyloid precursor protein. Mice have been extensively used as transgenic animal model as they are associated with AB-production, deposition and clearance and also, we can study the effects of AB on neuronal network and synapses which play an important role in cognitive decline. \[10\] The APP mouse model has successfully produced a wide range of parenchymal and vascular amyloid deposits similar to those of human AD. Although, there is morphological similarity, it is observed that there is difference in biochemical composition of deposited AB between mouse models and AD brain. \[11\] Moreover, these transgenic mouse models failed to develop NFT’s which an important histopathological hallmark of AD result from intraneuronal aggregation of hyper-phosphorylated tau protein. \[12\]

Another animal model is fruit fly Drosophila melanogaster widely used animal model of neuro-degeneration including AD. Although the fly brain has only a few fractions of cells of the human brain and different neuroanatomical organization, it is similar in the fundamental aspects of cell-biology, in terms of regulation of gene expression, neuronal connectivity, and cell-signaling. \[13\] Moreover, its short life cycle and completely sequenced genomes are added experimental advantages of this model. The fact the transparent cuticle of the larvae of fruit-fly allows the study of the disease progression in living intact animals which is rather difficult in vertebrates. \[14\]

**Interventional Models**

Here, introduction of pharmacological or chemical substances into the brain or induction of lesions in specific brain regions may replicate some of the characteristic features of AD. Many of these models involve introduction of AB peptide into the rat brain or rhesus monkey. Other chemical interventional models include scopolamine induced amnesia, introduction of inflammation with endotoxins or interference with brain metabolism. \[15\]
Major disadvantage of this model is that it includes non-specificity of the lesion, and their failure to capture disease progression.

**Validation Criteria for Animal Models**

**Face Validity:** The human model must resemble the human disease condition on a superficial level.

**Predictive Validity:** The animal model selected should be successful in discriminating between successful and unsuccessful treatments for the human disease condition.

**Construct Validity:** Here, the animal model should have good understanding about the human disease condition. [16]

**Histopathological Hallmarks**

It has been a clinical challenge to treat Alzheimer’s disease. There is evidence to suggest that single herb or herbal formulations may offer certain complementary cognitive benefits to the approved drugs. [17]

**Ayurvedic Medicine**

The Indian system of holistic medicine known as Ayurveda uses mainly plant-based drugs or formulations to treat various ailments. Rasayana tantra, one of eight major disciplines in Ayurveda is defined as a treatment to attain longevity, intelligence, freedom from age related disorders, youthful appearance, optimum strength of physique and sense organs, maintain language ability and improve memory. [20] A number of herbs and herbal preparation have been documented relevant to AD in the Ayurveda. Various plants used in traditional systems for the management of dementia and AD are Angelica archengelica, Artemisia absinthium, Bacopa monniera, Berberis aristata, Centella asiatica, Coptis chinensis, Curcuma longa, Cynodon dactylon, Cyperus rotundus and many more. Following is the list of plants used in the management of Alzheimer’s diseases. [21]
Table 1. Plants used in the management of Alzheimer’s

<table>
<thead>
<tr>
<th>PLANT/FAMILY</th>
<th>MECHANISM OF ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Angelica archangelica.</em> L. Apiaceae</td>
<td>Crude alcoholic extract have inhibited Ache activity in vitro [22].</td>
</tr>
<tr>
<td><em>Chamaecrista mimosoides</em> L. Greene</td>
<td>Methanolic extracts of C.mimosoides roots showed good antioxidant cholinesterase inhibitory activity [23].</td>
</tr>
<tr>
<td><em>Coptis chinensis</em> Franch. Ranunculaceae</td>
<td>The alkaloids obtained from this plant are AChEIn and the plant has improved a scopolamine induced learning and memory deficits in rats. [24]</td>
</tr>
<tr>
<td><em>Cynodon dactylon L.</em> Peers. Poaceae</td>
<td>It has reported antioxidant property [25, 26, 27].</td>
</tr>
<tr>
<td><em>Cyperus rotundus.</em> L. Cyperaceae</td>
<td>It has reported antioxidant property [28, 29].</td>
</tr>
<tr>
<td><em>Epimedium koreanum.</em> L. Berberidaceae</td>
<td>Methanolic extracts showed significant cholinesterase activity [30, 31].</td>
</tr>
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</table>

Herbal Formulations or Mixture of Herbal Ingredients

They may have advantages with multiple target regulation compared with single target antagonist in the view of traditional Chinese medicines, although there have been few clinical trials examining the efficacy and safety of herbal formulations in AD patients [32].

Examples of some herbal formulations are shenwu capsule (mixture of 6 herbs) is thought to reduce amyloid activity [33].

GEPT (a combination of 5 active components extracted) from Chinese herbs. They are thought to reduce the level of Aβ via inhibition of gamma secretase [34, 35].

Thus, even no serious adverse events have been reported in the use of these formulations. Single drugs or formulations may be able to complement approved drugs for AD [36].

Let’s, have a look at few of the approved drugs for AD.

Acetylcholinesterase inhibitors reversibly bind and inactivate the enzyme that degrades acetylcholine, which is involved in memory.Donepezil (Aricept) is the only acetylcholinesterase inhibitor approved for use in all stages of the disease [37].

The N-methyl-D-aspartate receptor antagonist Memantine (Namenda) is approved for treating moderate to severe disease and is thought to prevent excitatory amino acid neurotoxicity without interfering with the physiologic actions of glutamate, a neurotransmitter necessary for learning and memory [38].
Table 2. Medications for treating Alzheimer’s diseases.

<table>
<thead>
<tr>
<th>MEDICATION</th>
<th>COMMON DOSAGE</th>
<th>PHARMACOLOGICAL ACTIONS</th>
<th>ADVERSE EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANTICHOLINESTERASE INHIBITORS</strong></td>
<td></td>
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<tr>
<td>Donepezil (Aricept)</td>
<td>10mg per day</td>
<td>Centrally active, reversible and noncompetitive acetyl cholinesterase inhibitor</td>
<td>Nausea, Diarrhea, Headache, Insomnia</td>
</tr>
<tr>
<td>Galantamine (Razadyne)</td>
<td>8mg twice per day</td>
<td>Reversible, competitive acetyl cholinesterase inhibitor that also acts on nicotine acetylcholine receptor</td>
<td>Nausea, Vomiting, Cardiac arrhythmia, Dizziness</td>
</tr>
<tr>
<td>Rivastigmine (Exelon)</td>
<td>6 mg orally twice per day</td>
<td>Reversible, carbamate acetylcholinesterase inhibitor that preferentially interacts with acetylcholinesterase G1</td>
<td>Vomiting, Headache, Diarrhea</td>
</tr>
<tr>
<td><strong>NMDA RECEPTOR ANTAGONIST</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memantamine (Namenda)</td>
<td>10 mg twice per day</td>
<td>Low to moderate affinity, NMDA antagonist that binds to NMDA receptor operated cation channel</td>
<td></td>
</tr>
</tbody>
</table>

It has been seen that currently available drugs only provide symptomatic relief and do not intervene in disease process sufficiently enough to prevent or cure it [45].

**Targets for New Therapies**

Characteristic features of this disease are decline in neuronal mass and cognitive functions. The hypothesis proposed for the pathogenesis of this disease is called “AMYLOID HYPOTHESIS” which states that excessive production of amyloid peptides called abeta peptide (Aβ) is the underlying cause of neuronal dysfunction and death [46].

However, it is seen that recent drugs that were designed based on amyloid hypothesis have failed in clinical trials [47]. Early and persistent molecular events in this disease progression are energy deficiency and high oxidative stress in the neurons [48].

Brains of individual with Alzheimer’s disease exhibit neuronal degeneration and characteristic lesions referred to as amyloid plaques and neurofibrillary tangles. Currently, the only diagnosis of Alzheimer’s is the presence of these plaques [49].
The hypothesis comprises of the amyloid cascade of events

- Increased production of AB peptide
- Their aggregation leading to plaque and neurofibrillary tangle formation
- Tau phosphorylation
- Finally, cognitive decline \[^{50}\].

Figure 1. Amyloid hypothesis of Alzheimer’s disease. The classic amyloid hypothesis cascade of events is shown here. The main features are over-Production of Aβ peptides by secretase cleavage, peptide aggregation followed by neurotoxicity, tau phosphorylation, plaque and neurofibrillary tangle (NFT) formation with end result of cognitive loss and Alzheimer’s disease \[^{51,52}\].

Two molecular events which are closely related to onset, progression and pathogenesis of the disease, are high oxidative stress and reduced energy production by neurons \[^{53}\]. It is been seen that energy defects serve as a driver mechanism of Alzheimer’s initiation and progression \[^{54,55}\].

Brain’s requirement for energy needs are enormous as indicated by the fact that brain weigh only 2% of total body weight but yet consumes 20% of total energy produced \[^{56}\]. The energy of neurons are required for neurotransmission, synthesis of neurotransmitters and mylenation. It is mainly dependent on glucose utilization for energy generation \[^{57}\]. Indeed, defects in energy production by neurons are observed very early in this disease process.
Energy deficits in neurons are defined as reduction in production of ATP, the major form of energy in cells \([58]\).

It is been seen that Functional data showing reduced glucose utilization by the brain, also known as hypo metabolism, has been demonstrated in humans using positron emission tomography(PET), an imaging technique using 2-(18F) fluoro-2-deoxy-D-glucose(FDG-PET)\([59,60]\). FDG is a non degradable analog of glucose and it serves as a tracer to study energy metabolism. The concentration of FDG indicates tissue metabolic activity, in terms of regional glucose uptake. Such studies have demonstrated consistent, early and progressive reductions in glucose utilization in Alzheimer’s patients \([61]\). Also, the extent and regional location of hypo-metabolism in the brain correlate with severity of symptoms. Increasing evidence suggests that reductions in brain glucose utilization occur at the presymptomatic stages of the disease and observed before the onset of disease \([62]\).

Following are the causes of these energy deficits during the disease:

1. Depletion of nicotinamide adenine dinucleotide (NAD+) a coenzyme involved in ATP generation redox reactions. Depletion in the levels of this key coenzyme may occur due to high oxidative stress blocking its regeneration as well as consumption by enzymes such as PARP which use NAD as s substrate \([63,64]\).

2. Decrease in activities of important enzymes involved in glycolysis namely hexokinase and phosphofructokinase and consequent reduction in energy production \([65,66]\).

3. Decrease in the activities of enzymes involved in mitochondrial tricarboxylic acid cycle (TCA cycle) and oxidative phosphorylation processes, which generate most of cellular ATP.
   a) Decrease in the activity of alpha –ketoglutarate dehydrogenase complex, a rate limiting enzyme in TCA cycle \([67]\).
   b) Decrease expression and activity of cytochrome C oxidase, an important component of oxidative phosphorylation via electron transport chain \([68]\).
   c) Decrease in the activity of pyruvate dehydrogenase(PDH), a key rate-limiting enzyme in mitochondria to convert pyruvate, the end product of glycolysis, into acetyl-CoA, to initiate the TCA cycle.PDH is the first enzyme component of pyruvate dehydrogenase complex \([68]\).

The putative mechanism of decrease in PDH activity during Alzheimer’s may be its phosphorylation by glycogen synthase kinase 3b (GSK-3b)\([69,70]\). GSK-3b is a
serine/threonine protein kinase that mediates phosphorylation of serine and threonine amino acids in enzymes or regulatory proteins. Phosphorylation by GSK-3b usually inhibits the activity of target protein, as in the case of glycogen synthase, the enzyme that controls conversion of glucose to glycogen. The brain has very little stores of glycogen, making it unlikely that GSK-3b plays that role in that tissue.

It is now believed that this enzyme play a regulatory role in Alzheimer’s via two activities

- Its ability to phosphorylate microtubule-associated protein tau. Hyperphosphorylation of tau disrupts its interaction with tubulin within microtubules and leads to accumulation of neurofibrillary tangles
- Phosphorylate PDH and inactivate it \[^{71}\].

Another enzyme whose expression is modulated directly by neuronal energy deficit is BACE-1. It has been seen that energy deprivation can cause post transcriptional induction of BACE-1\[^{72,73}\]. It directly connects neuronal energy crisis to amyloid hypothesis i.e. increase in BACE-1 activity would lead to increased AB42 and initiate the amyloid cascade.

**Energy Targets and Approaches to New Therapies**

It’s clear that several pathways may contribute to neuronal energy deficiency observed in Alzheimer’s. Also, we propose that risk factors associated with Alzheimer’s disease including ageing, traumatic brain injury/stroke, genetics and metabolic diseases (e.g. type 2 diabetes) can combine to trigger detrimental molecular pathways in neurons. Specifically brain hypoxia and inflammatory cytokine production which occur in stoke or traumatic brain injury, insulin resistance and dysregulation of insulin signaling in diabetes and genetics in patients with apoE4 alleles act as switches to bring about gene expression or enzyme activity changes. The resulting chronic hypo-metabolic state by itself can trigger neuronal dysfunction and cognitive decline. This coupled with induced up-regulation of BACE-1 can further led to ab42 accumulation and fatal entry into amyloid cascade culminating in hallmark features of Alzheimer’s disease. Since the core cause of neuronal dysfunction in our model is energy driven we have termed it as bioenergetics of neurodegeneration model (BEND) \[^{74}\].
Figure 2. An “energy deprivation” based model of Alzheimer’s is proposed.

Based on above model we selected enzyme targets in our bioenergetics breakdown paradigm. The choice of targets was based on drug ability factors, i.e.

- Low possibility of toxicity when target is modulated.
- Likelihood of efficacy when target is regulated.
- Information available on crystal structure and availability of existing chemistry scaffolds to design compounds.

Following are few of the proposed energy sensitive targets for new therapeutics

<table>
<thead>
<tr>
<th>TARGETS</th>
<th>NORMAL FUNCTION</th>
<th>ROLE IN ALZHEIMER’S DISEASE</th>
<th>APPROACH TO THERAPEUTIC MODULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate dehydrogenase (PDH)</td>
<td>Rate limiting enzyme for TCA cycle to generate energy</td>
<td>Inactivation during disease genesis creates neuronal energy gaps</td>
<td>Allosteric modulation to increase activity</td>
</tr>
<tr>
<td>Glycogen synthase kinase3b(GSK 3β)</td>
<td>Phosphorylation of regulatory proteins/enzymes</td>
<td>Phosphorylation of PDH and Tau</td>
<td>Selective inhibitors</td>
</tr>
<tr>
<td>β-secretase for beta-site APP cleaving enzyme-1(BACE-1)</td>
<td>Protease which cleaves APP</td>
<td>Upregulation and overproduction of ab-42</td>
<td>Selective inhibitors</td>
</tr>
</tbody>
</table>

The selected targets are discussed below

1) PDH- The objective would be to increase PDH’s activity and enhance energy production. Pharmacologically it’s more difficult to activate an enzyme than to inhibit its activity, but in case of PDH there are avenues available to activate it through allosteric approaches \[^{[75]}\]. Also, PDH can be modulated by either pyruvate dehydrogenase kinase and GSK-3b. It would be efficient to design drugs around these kinases and GSK-3b inhibition \[^{[76]}\].
2) GSK-3b-This enzyme is an important target for several reasons. Its involvement in Alzheimer’s is at least at two separate levels-one which fits well with our energy paradigm-blocking GSK-3b activity should result in continued activation of PDH and second its role in phosphorylation of tau protein. Also, inhibition of GSK-3b has shown to decrease Aβ42 production.\cite{77,78} Thus, inhibition of this target could improve energy production as well as into the amyloid plaque formation and tau phosphorylation.

3) BACE-1-This enzyme is already being pursued vigorously for design of new therapeutics for Alzheimer’s as a way to decrease Aβ-42\cite{79}. Direct inhibition continues to be a favored approach for this target, but because of this enzyme’s involvement with other substrates, selectivity and toxicity can be a burden. In contrast, if we can modulate cellular energy and indirectly lower BACE-1 activity, it could accomplish dual objective of safety and efficacy.\cite{80}

CONCLUSION

Because of the urgent need for new safe and efficacious therapies for Alzheimer’s, large amount of investments are being made in identifying new approaches and drugs.\cite{81} Targeting amyloid hypothesis continues to be the favored approach, but other approaches must be considered since amyloid based drug candidates to have failed in the clinic.\cite{82} Based on the existing literature, we have come to a conclusion that energy crisis as the central theme and as basis of novel drug discovery approaches.\cite{83} We propose three targets: a)PDH, which is upstream of amyloid cascade. b) GSK-3b, a bridge between energy crisis and amyloid cascade and c) BACE-1, an integral part of amyloid cascade but may be modulated by energy improvements.

REFERENCES


