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Review Article

Multiple target combinatorial approach of Acetyl-cholinesterase inhibitors, NMDA receptor antagonist and a phytoconstituent for the possible treatment of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a severe condition in ageing societies. Alzheimer's disease (AD) is a type of dementia in which cognitive functions decline. More than 50 million people are affected by this disease across the world. Many kinds of research on Alzheimer's Disease are advancing rapidly, but very limited numbers of drugs are effective and have found a clinical application for AD patients.

The currently widely used medicines such as Donepezil and Galantamine transiently improve the symptoms of patients with mild to moderate AD. In the long history of the development of traditional Chinese medicine, many herbs and their phytoconstituents have been discovered and employed to treat dementia diseases. In recent decades, several agents were isolated from these herbs and their efficacies against AD were tested. Some flavonoids, alkaloids, phenylpropanoids, triterpenoid saponins, and polysaccharides were demonstrated to have potential efficacies against AD *via* targeting multiple pathological changes of AD. Because regeneration of neurons is a complicated process due to the involvement of multiple pathways, a combination of drugs that can work through multiple pathways could prove to be effective in treating AD. Based on prior studies and different mechanisms involved in the treatment, a new hypothesis has been proposed that a combination of galantamine, memantine and liquiritin is anticipated to produce better activity as compared to the current therapies available in the market for the treatment of Alzheimer's disease. In this article, we reviewed Acetylcholinesterase inhibitors, NMDA receptor antagonists and a phytoconstituents combination for the possible treatment of Alzheimer's disease.

Keywords: Alzheimer's disease, Neuroinflammation, Acetylcholinesterase inhibitors, NMDA receptor antagonist, phytoconstituents, memantine, galantamine, Liquiritin, the combinatorial approach, and Antioxidant.

INTRODUCTION:

AD is a progressive neurodegenerative brain disorder and characterized by memory loss, confusion and deterioration of intellectual and social functions. AD has become one of the major global health challenges of the century since it is considered the most common cause of cognitive impairment in the elderly population.

In India, more than 4 million people are estimated to be suffering from Alzheimer's and other forms of dementia, giving the country the third highest caseload in the world, after China and the US. India's dementia and Alzheimer's burden is forecast to reach almost 7.5 million by the end of 2030. AD is the 6th most common cause of death in the world and affects nearly 2% of the population, primarily occurring after the age of 60. With the continuing increase in the percentage of elderly, the incidence of AD is expected to triple in the next 50 years according to authoritative forecasts. The scenario appears increasingly severe, and the huge impact of AD on health care costs, including direct and indirect medical and social services, is currently

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estimated to increase. Indeed, in AD amyloid plaque deposition is thought to play a pivotal role in the inflammatory response within the brain including the overproduction of inflammatory mediators that have been implicated in the pathogenesis of AD.

Monotherapy also has substantial limitations. Many diseases are products of multiple pathophysiological pathways. Single drug blocks a single step in a complex pathogenic network often cannot block all crucial disease-propagating mechanisms. Combination therapy permits the deployment of agents that block multiple targets, thus increasing the likelihood of arresting or delaying the pathogenesis of a disease. In addition, administering a single agent at high doses may lead to adverse effects and for stimulation of compensatory mechanisms that attenuate effectiveness, in contrast to administering combinations of agents at potentially lower doses.

Currently approved pharmacotherapy for AD includes three cholinesterase inhibitors and one N-methyl D-aspartate (NMDA) receptor antagonist.

The NMDA receptor is a glutamate receptor subfamily broadly involved in synaptic plasticity and memory. Dysregulation of its physiological function triggers the excitotoxic effects of glutamate leading to neuronal damage and learning deficits associated with AD.

Memantine (MEM), an antagonist of NMDA receptor, is widely used in the treatment of moderate-to-severe stages of AD since it selectively blocks glutamate excitotoxicity, which in turn maintains neuronal function and alleviates the symptoms of the disease. It has been well established that inflammation contributes to neuronal damage and subsequent cognitive impairments in AD patients.

Accordingly, the use of drugs with anti-inflammatory effects is associated with a delay in the progression of AD; thus, MEM is receiving much attention as pharmacotherapy of AD since it is an NMDA receptor blocker and showed anti-inflammatory effects in several studies. Acetylcholinesterase (AChE) is responsible for the cessation of impulse transmission by rapid hydrolysis of acetylcholine (ACh) in almost all cholinergic pathways throughout the nervous system. Thus, it is a therapeutic target for improving the quality of life in AD patients. AChE-Is prevent enzymatic hydrolysis of ACh, thus increasing its level and duration of action.

Considering that oxidative stress and apoptosis are contributing factors in the pathogenesis of AD. The anti-oxidative and anti-apoptotic properties of liquiritin may also be involved in the therapeutic effect. Liquiritin is the active ingredient from the leguminous plant *Glycyrrhiza uralensis* Fisch (GuF), which has an anti-inflammatory and antioxidant effect and can protect tissues and cells from damage by oxidative and inflammatory factors.

Review of work done on Combination Therapy for Alzheimer's disease

Patel et al., 2011 reviewed combination therapy for Alzheimer's disease: This review covers key studies of the efficacy, safety and tolerability of combination therapy in AD, this review shows that combination therapy for AD seems to be safe, well-tolerated and may represent the current gold standard for treatment of moderate to severe AD and possibly mild to moderate AD as well (1).

Hartmann and Mobius., 2003 reported a surveillance study conducted among German physicians who, during routine clinical practice, treated patients with dementia with memantine in combination with an AChE inhibitor. The findings suggest that memantine in combination with AChE inhibitors have a good safety profile and are well tolerated (2).

Review of work done on the selected drugs for Alzheimer's disease

Miguel and Paul et al., 2001, The study hypothesized that memantine would prevent Aβ (1-40) induced cognitive impairment, neurodegeneration and apoptosis in hippocampal neurons of rats and improve performance in active avoidance testing (3).

Wojciech and Chris, 2003, concluded from their research that in contrast to cholinesterase inhibitors, memantine is likely to show neuroprotective effects at therapeutic concentrations used in the treatment of AD and to slow down disease progression. Clinically relevant

doses of memantine produce improvements in synaptic plasticity and learning under conditions of tonic NMDA receptor activation suggested occurring in AD (4).

Yang et al., 2008, investigated the protective effects of liquiritin on primary cultured rat hippocampal neurons. Liquiritin is also capable of enhancing the effects of nerve growth factors in extending neuroaxons. The neuroprotective and neurotrophic effects make liquiritin a promising agent against AD (5).

Current therapeutic approaches for Alzheimer's disease patients

There was no innovative medication endorsement for AD later in 2003, (6) and the present treatment choices provide only symptomatic relief (7), (8). Acetylcholinesterase inhibitors such as galantamine, donepezil, and rivastigmine are utilized to refine memory and consideration in AD patients assisting in expanding the intensities of acetylcholine by forestalling its break at the synapsis. While galantamine and rivastigmine are utilized for slight-to-modest AD, donepezil is utilized for all phases of AD. Tacrine (TAC) is the major prescription given for AD treatment, as an AChE inhibitor. As it was receding from the market due to its hepatic toxicity at prescribed dosages, it has shown an extensive margin as the most utilized AChE inhibitor in the progress of multitarget anti-AD drugs. Another alternative for modest-to-severe AD which is appropriate is recognized as the N-methyl-D-aspartate (NMDA) receptor antagonist, with slight side effects, for

example, dizziness, gastrointestinal irritation, and headache [7]. During various potential therapeutics, including numerous disease-modifying agents, these came into the clinical trials, and many of them are in phase III. Importantly, Aducanumab, ANAVEX2-73, ALZT-OP1a/b, CAD106, Crenezumab, and E2609 cover a fair proportion of these developing drugs, that is, 61% of the total drugs under phase-III trial. A majority of these therapeutic candidates aim at amyloid-related pathologies. Be that as it may, different targets, for example, neurotransmitter based, tau-based, antioxidant-based, and anti-inflammation-based targets, are additionally under clinical trial. Two of these preliminaries are focusing on inflammatory pathways and recommend neuroinflammation as a significant causative element for AD-related pathophysiology (6).

The proposed hypothesis

In this review, the potential treatment and different targets of AD have been discussed. Five medicines for the treatment of AD have been approved by the FDA. These medicines work by reducing the symptoms of AD by two given mechanisms i.e., AChE inhibition and NMDA glutamate antagonism. Tacrine, Donepezil, Galantamine, Rivastigmine are AChE inhibitors, whereas memantine is an NMDA glutamate antagonist (9). For the treatment of disease, the combination therapy of NMDA glutamate inhibitor and AChE inhibitor is very supportive in treating AD. As per literature sources, Phytoconstituents too are useful in treating AD via one or more of the aforenamed mechanisms. Some of these phytoconstituents include withanolides, curcumin, liquiritin, bacosides, and fisetin etc. while manifold factors are related to AD, the combination of drugs that can work through manifold pathways could be more efficient in treating AD.

Liquiritin is among such phytoconstituents that is obtained from *Glycyrrhiza uralensis* Fisch. It holds very high anti-inflammatory, antioxidant and antiproliferative effects. Owing to these effects, liquiritin has a significant prospective to treat AD. Being an herbal origin drug, liquiritin is recognized to be free of side effects and related toxicity. Therefore, looking at the beneficial effects of liquiritin, combination therapy has been suggested that includes concomitant administration of memantine, galantamine and liquiritin for effective treatment of AD with reduced side effects.

The rationale of the proposed hypothesis

Cholinergic hypothesis

Several studies have reported that in the post-mortem reports of AD patients' brains the progressive diminishing effect of ACh has been found. Individual's memory can be affected by the degeneration of ACh in the cerebral cortex and hippocampus region of the brain. The two ACh receptors i.e., muscarinic and nicotinic receptors are bounded in the postsynaptic neurons. In both

synapse and presynaptic neurons metabolising enzymes, AChE is found. This AChE enzyme metabolises ACh to the acetyl and choline, therefore reducing ACh levels in the postsynaptic receptor. Degeneration and Metabolism of ACh build AD's symptoms. Among the five approved drugs of AD by the FDA tacrine is no longer used clinically. AChE inhibitor drugs inhibit AChE enzymes, which in turn, cease the metabolism of Ach so that more ACh neurotransmitter reaches to post-synaptic neurons. Among AChE inhibitors, galantamine showed better effect as compared to donepezil and rivastigmine. Galantamine by inhibiting the AChE enzyme activates the α7 subtype of nicotinic receptor. Numeral clinical studies have shown that galantamine and memantine bring about better effects against AD (10).

Glutaminergic hypothesis

In the physiology of CNS, neurotransmissions of excitatory glutamate neurotransmitters play numerous explanatory roles and are also accountable for the emergence of definite memories. A vital role is played by NMDA receptors in controlling memory function and synaptic plasticity. NMDA glutamate receptor is an ionotropic glutamate receptor. Glutamate phosphorylates the NMDA type of glutamate receptor by binding. Further, calcium/calmodulin-dependent protein kinase II (CaMKII) is phosphorylated by this process. Excitotoxicity can be caused by the overstimulation of the NMDA type of glutamate receptor which can increase the influx of $Ca2^{+}$in the cells. Degeneration of neuronal cells and apoptosis is caused by the higher level of intracellular $Ca2^{+}$. Memantine (Namenda®) is an NMDA glutamate antagonist. Eli Lilly first patented memantine as an anti-diabetic drug in 1968. Belatedly, Merz Pvt. Ltd. reported that memantine helped improve cognitive functions (11). Memantine produces antagonistic effects by binding with NMDA glutamate receptors and results in reducing intracellular $Ca2^{+}$ions and excitotoxicity. Eventually, by the reduction in degeneration of the neuronal cells in the CNS, it enhances memory and cognitive functions. Memantine is also used along with an AChE inhibitor. Donepezil and memantine have been approved for the treatment of AD by the FDA.

Liquiritin

Glycyrrhiza uralensis Fisch (GuF) is one of the prehistoric herbal nutraceuticals used frequently in traditional oriental medicinal herbs of Leguminosae. Presently, GuF is known to contain more than 20 triterpenoids and 300 flavonoids (13). The compounds that have been isolated from GuF have been reported to exhibit various activities, such as antitumor, anti-viral, anti

inflammatory, immunoregulatory, recovery and neuroprotective activities (11)(12).

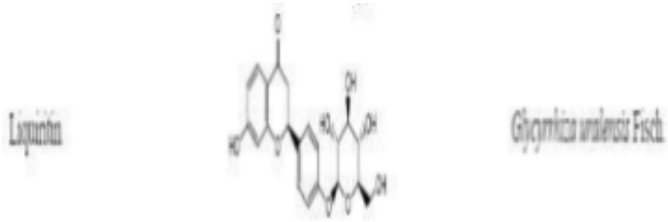

Fig. 1: Liquiritin

Chinese herbs used to improve learning memory and prevent senile dementia are natural, multi effective and usually less toxic than prescribed medications. Several studies have been done on plant extracts for brain function improvement and senile dementia treatment (13). Several reports have indicated that liquiritin or isoliquiritin may act as an antioxidant in various cell types to protect against various forms of stress (13) (14). It has also been revealed that liquiritin potentiates neurite outgrowth induced by nerve growth factors in PC12 cells (15). These results

suggest that liquiritin or isoliquiritin affects particular types of cells such as neurons in the

central nervous system.

Yang et al. (11) have established a rat hippocampal neuronal damage model and demonstrated that it had neuroprotection and neurotropism effects on primary cultured hippocampal cells. Chen et al. (15) indicated that Liquiritin might be a good candidate for treating various neurodegenerative diseases including Alzheimer's disease or Parkinson's disease.

Liquiritin is an extract from the root of *Glycyrrhiza uralensis Fisch*. (5). Yang et al. investigated the protective effects of liquiritin on primary cultured rat hippocampal neurons (16). They found that pre-treatment with liquiritin for 6 h decreased the elevated levels of intracellular $Ca2^{+}$ concentration and neuron apoptosis caused by Aβ25-35. Liquirtin is also capable of enhancing the effects of nerve growth factors in extending neuro axons (16). It is worth noting that liquiritin could also specifically inhibit the activity of acetylcholinesterase and promote the differentiation of neuronal stem cells into cholinergic neurons (16). The neuroprotective and neurotrophic effects make liquiritin a promising agent against AD.

Recently, several researchers have reported that some traditional herbal medicines that contain GuF could improve disorders of the central nervous system *in vivo*, such as by antidepressant-like effects (17), (18). Moreover, it has been demonstrated that GuF extract can ameliorate various disorders of the central nervous system induced by experimental procedures *in vivo* (19-20).

FDA has approved a combination therapy of donepezil and memantine for the treatment of AD. The mechanism of this combination therapy includes AChE inhibition and NMDA glutamate receptor antagonism. This combination of drugs has been reported to enhance the cognitive function of AD's patients and reduce excitotoxicity in the brain (21). In addition to this combination, we are proposing a hypothesis to combine galantamine, memantine and liquiritin for the treatment of AD. This combination is anticipated to be able to offer better neuroprotective and cognitive enhancement activity based on the multiple pathways involved to treat AD by these drugs.

Conclusion

In the long history of the development of traditional Chinese medicine, many herbs have been discovered and employed to treat dementia diseases. In recent decades, with the development of chromatographic and spectroscopic techniques, many agents have been isolated from these herbs and their efficacies against AD have been tested both in vitro and in vivo. The endeavours, on one hand, illustrated the principle of evidence-based medicine to clinically use these medicines to treat AD, and, on the other hand, discovered many monomer compositions as promising drugs or lead compounds for drug design in the treatment of AD.

The presently used medications for the treatment of AD are chiefly symptom-management drugs. Although they do ameliorate symptoms such as memory disorders and play an indispensable role in the treatment of AD, these drugs are incapable of reversing the progression of AD. In the context of the pathogenic complexities of AD, it is probably unlikely that single-target drugs will achieve satisfactory curative effects. Some agents in the class of flavonoids and phenylpropanoids show multiple biological properties that intend to remove the main reason for AD inception and may represent the prospects of the new drug development.

Numerous pathological pathways have been associated with AD. Hence, combination therapy of drugs and phytoconstituent with different mechanisms of action could be more effective than monotherapy to combat the disease. In this review, the combinational role of galantamine, memantine and liquiritin has been highlighted against AD due to their multiple roles as anti-proliferative, anti-apoptosis, antioxidant, anti-inflammatory, anti-AChE and antagonistic effect on NMDA glutamate receptors.

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