CHITIN AND DERIVATIVES AS ANTI-ALZHEIMER’S AGENTS

Aarti Sharma, Preeti Kothiyal
Pharmacology, Shri Guru Ram Rai Institute of Technology and Sciences/ Uttarakhand Technical University, India.

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ABSTRACT

Alzheimer’s is the most common cause of dementia in adult life and is associated with the selective damage of brain regions and neural circuits critical for memory and cognition. The neurons in the neocortex, hippocampus, amygdala, and the basal forebrain cholinergic system are the most affected brain regions. The major factors leading to Alzheimer’s are 1) amyloid plaque disposition in brain. 2) Disruption in the cholinergic activity. 3) Oxidative stress in brain 4) inflammation accompanying the disease. The continuing expansion of life expectancy, leading to a fast growing number of patients with Alzheimer’s disease, has led to an enormous increase in research focused on the discovery of drugs for primary, secondary or tertiary prevention of the disease. Despite all scientific efforts, at the moment, there are no effective pharmacotherapeutic options for prevention and treatment of Alzheimer’s disease. The subject of our study in this review is Chitin that is a naturally abundant polysaccharide. In fact, chitin is a constituent of the outer structure of insects, fungi and crustaceans. Chitin is also significant because of its relationship to some components of foods of animal, and fungal origin, and its potential medical and pharmaceutical uses. Current understanding of the role of chitin like polysaccharides in the pathogenesis of amyloid deposition of Alzheimer disease is developing fast. Polysaccharides may play a broader role in light of 1) the role of amyloid in Alzheimer disease pathogenesis. 2) anticholinesterase activity. 3) oxidative stress in Alzheimer’s disease and 4) inflammation accompanying the disease. Considering the side effects of synthetic neuroprotective agents, the search for natural neuroprotective agents has received great attention. Hence, the objective of this review is to discuss neuroprotective properties of chitin and its derivatives.


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INTRODUCTION

Dementia is increasingly being recognized as one of the most fearsome medical problems in elderly people with its prevalence rising from 1% at the age of 60 to at least 35% at the age of 90. Within the spectrum of dementias, Alzheimer’s disease is the most prevalent subtype, accounting for about 60% of all dementias. Alzheimer’s disease is a primary degenerative disease of the brain, characterized by progressive memory impairment. A patient with Alzheimer’s disease will generally present a gradual onset and a progressive and sequential decline in cognitive, behavioral and motor functions [1]. This decline will eventually interfere with the individual’s daily functioning and quality of life. Cognitive symptoms include loss of short-term memory, language impairment, and disorientation to time, place and people. In the early stages, patients may exhibit symptoms of depression. At the later stages, behavioral and psychiatric symptoms such as agitation, aggressivity, delusions, and hallucinations may develop. Finally, in the more advanced stages, motor functions decline and patients may become incontinent and, at times, bedridden [2].

Research advances have enabled detailed understanding of the molecular pathogenesis [3] of the hallmarks of the disease—ie, plaques, composed of amyloid β [4] (Aβ), and tangles, composed of hyperphosphorylated tau [5]. Neuro-inflammation is thought to be mainly associated with the activity of glial cells in immune surveillance and host defense [6]. Toxicity of free radicals contributes to proteins and DNA injury, inflammation, tissue damage and subsequent cellular apoptosis. The amyloid cascade hypothesis and cholinergic hypothesis explain the pathophysiology of Alzheimer’s disease to a great extent.

**Amyloid cascade hypothesis**

At the microscopic level, the characteristic lesions in Alzheimer’s disease are senile or neuritic plaques and neurofibrillary tangles in the medial temporal lobe structures and cortical areas of the brain, together with a degeneration of the neurons and synapses [7]. Several pathogenic mechanisms that underlie these changes have been studied, including Aβ (Amyloid Beta) aggregation and deposition with plaque development, tau hyperphosphorylation with tangle formation and neurovascular dysfunction [8].

According to this hypothesis, the central event in the disease pathogenesis is an imbalance between Aβ production and clearance, with increased Aβ production in familial disease and decreased Aβ clearance in sporadic disease [9]. Aβ oligomers could directly inhibit hippocampal long-term potentiation and impair synaptic function, in addition to the inflammatory and oxidative stress caused by aggregated and deposited Aβ. These processes impair neuronal and synaptic function with resulting neurotransmitter deficits and cognitive symptoms. Tau pathology with tangle formation is regarded as a downstream event, but could contribute to neuronal dysfunction and cognitive symptoms [10].

**Cholinergic hypothesis**

In neuropathological studies, Alzheimer’s disease is associated with a decline in cholinergic function in the basal forebrain and cortex. As deficiencies in cholinergic neurotransmission are considered to perform an essential function in the diminution of the learning and memory of Alzheimer’s disease patients [11, 12], it has also been reported that the activation of the cholinergic function via the inhibition of cholinesterase may prove a clinically effective method for the treatment of Alzheimer’s disease. Inhibition of
cholinesterase enzyme in such patients provides a enough neuroprotection in the brain [13]. In the mammalian brain, the majority of the AChE exists in the membrane-bound G4 form, but its levels decline as the neurons degenerate. It performs functions in the regulation of several physiological reactions via the hydrolysis of the neurotransmitter acetylcholine in the cholinergic synapses. Cholinomimetic/Parasympathomimetic drugs[14] produce actions similar to that of Acetyl Choline, either by directly interacting with cholinergic receptors (cholinergic agonists) or by increasing availability of Acetyl Choline at these sites (anticholinesterases). In the mammalian brain, the majority of the Acetyl Choline Esterase exists in the membrane-bound G4 form, but its levels decline as the neurons degenerate. Anti-cholinesterase drugs hence show efficacy in Alzheimer’s by normalizing the levels of acetylcholine in brain [15, 16].

The continuing expansion of life expectancy, leading to a fast growing number of patients with dementia, particularly Alzheimer’s disease, has led to an enormous increase in research focused on the discovery of drugs for primary, secondary or tertiary prevention of the disease [17]. Despite all scientific efforts, at the moment, there are no effective pharmacotherapeutic options for prevention and treatment of Alzheimer’s disease.

To date, established treatments are only symptomatic in nature, trying to counterbalance the neurotransmitter disturbance of the disease. However the disease modifying treatments are also gaining importance [18]. Mechanisms and strategies used in order to protect against neuronal injury, apoptosis, dysfunction, and degeneration in the central nervous system are recognized as neuroprotection. Neuroprotection could be achieved through several classes of natural and synthetic neuroprotective agents [19]. However, considering the side effects of synthetic neuroprotective agents, the search for natural neuroprotective agents has received great attention. Recently, an increasing number of studies have identified neuroprotective properties of chitin and its derivatives [13].

The goal of neuroprotection is to limit neuronal dysfunction or death after CNS injury in an attempt to maintain the highest possible integrity of cellular interactions in the brain, thus minimizing disturbance to neural function [5]. Many categories of natural and synthetic compounds have been reported to possess a neuroprotective activity [20]. However, these synthetic neuroprotective agents are believed to have certain side effects such as dry mouth, tiredness, drowsiness, sleepiness, anxiety or nervousness, difficulty to balance, etc. [21]. Hence, nowadays researchers have a great interest to study natural bioactive compounds that can act as neuroprotective agents.

The marine environment [22] is known as a rich source of bioactive chemical structures with promising biological activities such as neuroprotection [6]. Based on several studies, it is reported that Chitin and its derivatives have remarkable potential as a neuroprotective anti-Alzheimer’s agents owing to their anti amyloid, anti-cholinesterase, antioxidant and anti-inflammatory attributes[23]. Chitin is one of the most abundant biopolymers in nature and the most widespread amino-polysaccharide [24,25]. Chitin is used as a raw material for the industrial production of chitin-derived products such as chitosans, derivatives of chitin/chitosan, oligosaccharides and glucosamine (GlcN) [26, 27]. The main industrial sources of raw material for the production of chitin are cuticles from various crustaceans, mainly crab and shrimp[28-31]. Over the last three decades, there have been a growing number of publications on chitin, chitosan and their derivatives in the pharmaceutical industry [32,33]. Chitosan is a linear polysaccharide that consists of β-(1→4)-2-acetamido-d-glucose and β-(1→4)-2-amino-d-glucose units derived from partial deacetylation of chitin [34,35]. However, this polysaccharide has poor solubility, making it difficult to be used in food and biomedical applications [36]. Considering this property limitation, some researchers are interested in converting chitosan into oligosaccharides [37]. Chitooligosaccharides (COS), oligosaccharides form of chitosan, are readily soluble in water due to their shorter chain lengths and free amino groups in D-glucosamine units [38]. Similar to chitosan, COS have positive charges resulting from removal of acetyl units from D-glucosamine residues. These properties enable COS to interact with negatively charged polymers, macromolecules and polyanions in an aqueous environment [39, 40]. Recent reviews have been published regarding the pharmaceutical and biomedical applications of chitin, chitosan and their derivatives. This review, however, focuses specifically on neuroprotective and anti-Alzheimer’s properties of chitin, chitosan and their derivatives.

1. Chitin and derivatives as Beta secretase inhibitors

The pathological hallmark of Alzheimer’s disease is the deposition of senile plaques (SPs) and neurofibrillary tangles (NFTs) [41]. SPs are composed of the β-amyloid (Aβ) peptides, which are cleaved from amyloid precursor proteins (APPs) by proteolytic enzymes such as β- and γ-secretase[42-44]. In APP proteolysis, it seems that the key enzyme is β-secretase, which is also known as β-amyloid cleavage enzyme (BACE-1), since it initiates the formation of Aβ [45,46]. Hence, BACE-1 represents a candidate biomarker, as well as a drug target for Alzheimer’s disease.

Drugs to block this enzyme (BACE inhibitors) in theory, would prevent the buildup of beta-amyloid and may help slow or stop Alzheimers disease. BACE-1 inhibition activity of chitosan derivatives have been reported by Je et al [34]. They prepared chitosan with two degrees of deacetylation (90% and 50%) and grafted amino functionality into chitosan to improve the solubility and bioactivity. The results showed that AE-chitosan (90%) prepared from 90% deacetylated chitosan exhibited the highest BACE1 inhibitory activity, the BACE1 inhibition pattern of AE-chitosan was found to be non-competitive.

Bakrudeen Ali Ahmed et al [47] declared, chemical substitution of COS is a water soluble human safe BACE-1 inhibitor, after studying different chemically substituted chitooligosaccharides. They synthesized novel COS derivatives with different chemical substitution groups and evaluated their BACE 1 inhibitory activities. The results showed that DEAE (diethyl amino ethyl)-COSs prepared from chitooligosaccharides (MW 3-8 kDa) exhibited the highest BACE 1 inhibitory activity. In addition, the BACE 1 inhibition pattern of DEAE-COSs was found to be non-competitive, and the inhibition constant (K_i) was 100 μg/mL. This result suggests that the amino group plays an important role in BACE 1 inhibitory activity. The findings are illustrated graphically as follows in Figure 2.
Concentration-dependent inhibition of β-secretase by chemically modified compounds
(AE-COSs, DEAE-COSs, DMAE-COSs)

Figure 2

AE-COSs = aminoethyl chitooligosaccharides, DEAE-COSs = diethyl aminoethyl chitooligosaccharides, DMAE-COSs = dimethyl aminoethyl chitooligosaccharides.

Chitosan oligosaccharides protect rat primary hippocampal neurons from oligomeric β-amyloid 1-42-induced neurotoxicity. This was the finding of the study conducted by Shigong Zhu et al. [48]. The results provided enough evidence that the neuroprotective effect of COSs were mediated, at least in part, via scavenging ROS, decreasing oxidation of lipids, and depressing the activation of apoptosis.

Based on the hypothesis that the increase of an important product of oxidation may induce Aβ formation, Khodagholi et al. [49] studied the effect of chitosan in NT_2 neuron cells induced by H_2O_2 and FeSO_4. NT_2 neuronal cells are a widely accepted experimental model to study the regulation of Amyloid precursor protein metabolism and the pathogenesis of Alzheimer’s disease [50]. In their study, they found that Aβ formation by NT_2 neurons pretreated with chitosan was significantly lower than that of control cells exposed only to H_2O_2. The Aβ levels rose from 30.96 pg/mL in H_2O_2-treated cells to 22.2 and 18.35 pg/mL in the presence of 0.1 and 0.5% w/v chitosan, respectively. This study indicates that Aβ level can be controlled by treatment with this chitosan, suggesting a protective effect of chitosan in Alzheimer’s disease.

2. Acetylcholinesterase Inhibitory Activity of Chitin and derivatives

In neuropathological studies, Alzheimer’s disease is associated with a decline in cholinergic function in the basal forebrain and cortex [51]. As deficiencies in cholinergic neurotransmission are considered to perform an essential function in the diminution of the learning and memory of Alzheimer’s disease patients [52], it has also been reported that the activation of the cholinergic function via the inhibition of cholinesterase may prove a clinically effective method for the treatment of Alzheimer’s disease [53]. Cholinesterases (ChEs) including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are key enzymes that perform pivotal functions in cholinergic transmission via the hydrolysis of the neurotransmitter acetylcholine [54]. AChE, which consists of multiple subunits, is a membrane bound enzyme and present in the brain, muscles and cholinergic neurons. In the mammalian brain, the majority of the AChE exists in the membrane-bound G4 form, but its levels decline as the neurons degenerate. It performs functions in the regulation of several physiological reactions via the hydrolysis of the neurotransmitter acetylcholine in the cholinergic synapses. In the normal brain, AChE predominates but BChE activity rises, whereas AChE activity remains unchanged or diminished in the brains of Alzheimer’s disease patients. Therefore, a drug that inhibits both AChE and BChE may be preferable to selective AChE or BChE inhibitors. Because of the adverse side-effects of existing ChEs inhibitors [55,56], the development of non-toxic ChEs inhibitors as alternatives to existing drugs is of substantial interest in the treatment of Alzheimer’s disease. The inhibitory activities of synthesized COS derivatives against ChEs were evaluated by N.Y. Yoon et al. [57], (Table 1). Among the COS derivatives, DEAE COS evidenced the most potent AChE inhibitory activity with IC50 values of 9.2 ± 0.33 lg/ml. AE- and DMAE-COS evidenced marginal inhibitory activity toward AChE with IC50 values of 24.1 ± 0.39 and 56.5 ± 0.26 lg/ml, respectively.

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC50 (µg/ml) *</th>
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<tbody>
<tr>
<td>AE-COS</td>
<td>56.5 ± 0.26</td>
</tr>
<tr>
<td>DMAE-COS</td>
<td>24.1 ± 0.39</td>
</tr>
<tr>
<td>DEAE-COS</td>
<td>9.2 ± 0.33</td>
</tr>
<tr>
<td>Eserine</td>
<td>0.0089 ± 0.00005</td>
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* Each value represents the mean ± SD of three determinations. b=Eserine was used as a positive control.
Furthermore, Yoon et al [47] synthesized COS derivatives with different substitution groups. In their study, the synthesis of COS derivatives was accomplished by the displacement of the hydroxyl group at the C-6 of the pyranose ring and replaced with aminoethyl (AE), dimethylaminoethyl (DMAE) and diethylaminoethyl (DEAE) groups. The chemical structures were determined as AE-COS, DMAE-COS and DEAE-COS, in sequence.

![Chemical Structures of COS Derivatives](image)

Eserine, a parasympathomimetic and a reversible cholinesterase inhibitor, was used as the positive control in their study. Among three COS derivatives, DEAE-COS has the strongest AChEIs activity with IC50 values of 9.2 ± 0.33 μg/mL. DMAE- and DEAE-COS were identified as competitive AChEIs according to the Lineweaver–Burk plot. These findings suggest that the chemical modification will enhance the utilization of COS as AChEIs, and their inhibitory activity depends on the hydrophobic nature of the group that is introduced to them.

3. Anti-inflammatory and anti-oxidant activity of Chitin and derivatives

Neuroinflammation is thought to be mainly associated with the activity of glial cells in immune surveillance and host defense. Under pathological conditions, inflammation of the brain [58, 59] is closely involved in pathogenesis of several neurodegenerative diseases [60], such as Parkinsons disease, Alzheimers disease, human immunodeficiency virus-associated dementia and multiple sclerosis. Microglias are the principal immune effector cells in the central nervous system. Upon phagocytosis of invading bacteria, microglia are activated [61] and produce pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-), interleukin-1 (IL-1), IL-6, and prostaglandin E2 (PGE2), as well as nitric oxide (NO) and reactive oxygen species (ROS), which are thought to contribute to neuronal injuries and progression of the neurodegenerative diseases. Therefore, the modulation of microglial activation is an effective therapeutic approach against neurodegenerative diseases [62].

Chito-oligomers (COS) have attracted increasing attention due to absence of toxicity, and superior biocompatibility. Aminoethyl-COS (AE-COS) have important biological properties in medicinal and pharmaceutical applications such as angiotensin converting enzyme inhibitory, acetylcholinesterase inhibitory and anti-proliferative effects. However, the antioxidant and anti-inflammatory activities of AE-COS remains to be evaluated. Se-Kwon Kim et al [63] examined the antioxidant and anti-inflammatory activities of AE-COS on murine microglial cells (BV-2 cells) under lipopolysaccharide stimulation. It was found that AE-COS reduced the level of nitric oxide (NO) and prostaglandin E2 production by diminishing the expression of inducible NO synthase and cyclooxygenase-2 without significant cytotoxicity. Moreover, the inhibitory activities of AE-COS on generation of tumor necrosis factor-alpha and interleukin-1beta were performed. Collectively, these results indicate that AE-COS possess potential antioxidant and anti-inflammatory activities in brain microglia, and could be a useful therapeutic agent for the treatment of neuroinflammatory diseases.

Novel water-soluble chitin derivative was prepared by Jae-Young Je and Se-Kwon Kim [64] by chemical modification to evaluate antioxidant activities by free radical scavenging potential using electron spin resonance spin trapping technique. Aminoethyl-chitin (AEC) exhibited free radical scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, superoxide, and peroxyl radicals. AEC quenched DPPH and peroxyl radical over 55% and 59% at 4 mg/mL, and also suppressed superoxide radical over 58% at 2 mg/mL.

AEC was more active against hydroxyl radical, and scavenging ratio was 92.2% at 0.12 mg/mL. These results suggested that free amino group in the -CH₂CH₂NH₂ plays an important role in the free radical scavenging activity. In addition, cytotoxic effect of AEC was assessed using human lung fibroblast (MRC-5) cell line, and AEC showed less toxic against MRC-5. As illustrated above, the study conducted by Khodagholi et al [49], focused on the anti-neuroinflammatory effect of chitosan and its derivatives on NT₂ neuronal cells. Chitosan exerts anti-neuroinflammatory action by upregulation of heat shock protein 70 (Hsp-70) and inhibits the activation of NF-κB. The anti-inflammatory mechanism of Hsp-70 is mediated by the binding of Hsp-70 to NF-κB and its subsequent inhibition [65].

CONCLUSION

Chitin has been the focus of a dramatic increase in academic study during the past twenty years,” said John Vournakia, a biologist at Dartmouth College in New Hampshire. “But academic research is very different from getting products off the ground.” In fact, a number of universities and the U.S. government - as part of its $45 million Sea Grant - research program — have worked on chitin and developed more than 200 patents for its use. But so far, the chitin revolution is yet to take place for reasons that
range from the usual birth pains of an emerging biotech industry struggling to get attention in a world seemingly crammed full of emerging industries to the normal regulatory problems even the richest of pharmaceutical companies face in getting their products into drug stores [66]. In fact, the interest in chitin, chitosan and their derivatives for the treatment of neurological disorders appear to be an emerging field. Some of the representative’s examples are presented in this review with a focus on anti-alzheimer’s properties of chitin, chitosan and their derivatives. According to presented data, it seems that chitin and its derivatives are promising neuroprotective agents, as they showed neuroprotective properties such as: suppression of β-amyloid formation, AChEIs, anti-neuroinflammatory activity and anti-oxidant activity. Uptil now, most neuroprotective activities of chitin chitosan and its derivatives have been observed in vitro. Therefore, further studies are needed in order to investigate their activity in mouse model systems and/or human subjects. Consequently, chitin and derivatives can emerge as a promising anti-alzheimer’s drug in near future.

Authors’ Statements

Competing Interests
The authors declare no conflict of interest.

REFERENCES


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