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REVIEW ARTICLE

Potential Roles of α-amylase in Alzheimer's Disease: Biomarker and Drug Target

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Abstract: Alzheimer's disease (AD), the most common form of dementia, is pathologically characterized by the deposition of amyloid-β plaques and the formation of neurofibrillary tangles. In a neurodegenerative brain, glucose metabolism is also impaired and considered as one of the key features in AD patients. The impairment causes a reduction in glucose transporters and the uptake of glucose as well as alterations in the specific activity of glycolytic enzymes. Recently, it has been reported that α-amylase, a polysaccharide-degrading enzyme, is present in the human brain. The enzyme is known to be associated with various diseases such as type 2 diabetes mellitus and hyperamylasaemia. With this information at hand, we hypothesize that α-amylase could have a vital role in the demented brains of AD patients. This review aims to shed insight into the possible link between the expression levels of α-amylase and AD. Lastly, we also cover the diverse role of amylase inhibitors and how they could serve as a therapeutic agent to manage or stop AD progression.

Keywords: Alzheimer's disease, biomarker, drug target, alpha-amylase, enzyme inhibitor, amyloid-beta, neurological disorders, drug repurposing.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is presented as a principal cause of dementia. AD affects nearly 40 million people globally and is envisioned to reach approximately 80 million by 2040. It is the 6th cardinal root of death in the United States, and related medical costs may surpass $1.1 trillion by 2050 [1, 2]. The disease was first described by Dr. Alois Alzheimer in 1906 when he examined the brain histology of a 51-year-old woman who had passed due to an unknown but deteriorating mental status. The patient has presented severe brain tissue changes and characteristics such as memory loss, linguistic issues, and abnormal behavioral changes [3].

Two kinds of AD exist, early-onset and late-onset AD. Early-onset AD typically affects people younger than 65 years old. Early-onset, known as familial, constitutes roughly 5% of AD cases with patients as young as their 40s, and symptoms may start to develop between 30 and 40 [4, 5]. In contrast, late-onset AD strikes 65 years and above and ultimately represents the larger proportion of AD cases. Nonetheless, the disease is characterized by a loss of cognitive abilities that are adequately severe to obstruct everyday tasks. The most apparent early symptom manifested by patients is short-term memory loss (amnesia or minor forgetfulness), which becomes more conspicuous as the disease progresses with mild preservation of older memories and cognitive impairment [6]. Atypical clinical manifestations include hallucinations, visual and learning difficulties, behavioral changes, and psychosocial incompetence.

The progression of AD originates from pre-clinical stages, mild cognitive impairment (MCI), and subsequently dementia due to AD. However, these stages that characterize AD progression did not consider essential disease prognostic factors, such as the coexistence of other disease conditions. The presence of additional conditions that are co-occurring with the primary condition is known as comorbid diseases. It may occur prior to or concurrently with AD and could detrimentally influence the overall patient's clinical status and AD progression. In addition, emerging evidence has shown the association of AD with other chronic diseases such as cardiovascular disease, cerebrovascular disease, and diabetes [7, 8].

Type 2 diabetes mellitus (T2DM) is a well-known risk factor for AD and is occasionally referred to as type 3 diabetes. Research has shown a two to a three-fold increase of developing dementia in diabetes patients, and subjects with T2DM displayed poor performance in cognitive domains compared to those with normal glucose metabolism [9]. Diabetic patients also demonstrated a 1.25-1.9-fold increase in cognitive impairment and dementia [10]. Moreover, shreds of evidence from epidemiological, clinical, and pre-clinical studies point towards insulin modification and altered glucose metabolism as a link between diabetes and AD with dysregulated phosphatidylinositol-3-kinase (PI3K)-Akt signaling as the common pathway affected [11]. There were also noticeable morphological changes in the brains, mainly

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in the hippocampus of diabetic subjects, with post-glucose-loaded hyperglycemia. The brain scans revealed a significantly lower hippocampal volume and atrophy than non-diabetic subjects [12]. This also aligns with the morphological brain changes of AD patients in the hippocampus, amygdala, and medial temporal lobe in the early stages of the disease [13].

Amylases are from a large group known as glycosyl hydrolase enzymes that function to catalyze the breakdown of complex carbohydrates. There are three main classes of amylase enzymes, namely (1) α-amylase is found in animals, plants, fungi, and bacteria [14], (2) β-amylase that is found in plant seeds, bacteria, and fungi [15] and (3) ɣ-amylase found in animals, yeast and fungi [16]. This review aims to uncover the pathophysiology of AD by focusing on a key player, α-amylase and the possible association with T2DM. Further, we aim to shed light on the functionality of the enzyme in AD and how it can be made as a therapeutic target point.

2. CHARACTERISTICS OF THE HUMAN α-AMYLASE

α-Amylase (EC 3.2.1.1) is one of the most established and studied amylolytic enzymes. It is a ubiquitous hydrolase synthesized by plants, animals, and microorganisms and functions by catalyzing the non-specific cleavage of internal α-(1-4)-glycosidic linkages in starch, glycogen, and other related oligosaccharides to yield progressively smaller polymers composed of glucose units [17]. The human α-amylase requires two cofactors for its activity: (a) calcium ion as the enzyme are metalloenzymes and coordinated between eight ligands from the main amino acid chain, side chains, and three water molecules and (b) chloride ion which closely binds to the active site and is considered to act as an allosteric activator [18]. In the human body, salivary α-amylase is expressed in salivary, mammary, and lacrimal glands, whereas the pancreatic isoform is expressed only in the pancreas. The pancreatic isoform constitutes approximately 5-6% of the total protein present in pancreatic secretions and possesses no inactive precursor, unlike other pancreatic enzymes. Ultimately, α-amylase can be secreted into the bloodstream, and homeostasis (rate of production versus the rate of clearance) is tightly regulated [19, 20].

3. ROLE OF α-AMYLASE IN GLUCOSE METABOLISM

The enzyme α-amylase is a membrane-bound endoenzyme that catalyzes the first step in the digestion of starch, a main carbohydrate of the human diet. The catalytic role of α-amylase gradually hydrolyses the polysaccharide, which results in maltose, maltotriose, and limit dextrins as the main products [21]. Hence, the hydrolysis by α-amylase would lead to relatively high glucose concentrations in the blood circulation, causing postprandial hyperglycemia. α-amylase plays an essential role as a marker in glucose metabolism as salivary amylase levels were significantly higher in diabetics subjects than those in healthy non-diabetics [22]. The higher enzyme activity incurs excessive conversion and increases the blood sugar level in tissues, causing hyperglycemia [23]. Thus, α-amylase expression is widely used in diagnosing and monitoring glucose levels [24]. The pathophysiological states of α-amylase in other diseases could also serve as an excellent diagnostic tool. Traditionally, the distribution α-amylase reflects its plasma concentration and the source organ whereby increased levels or activity of α-amylase in human serum has clinical applicability, as mentioned in Table **1** below.

4. POSSIBLE LINK BETWEEN α-AMYLASE AND AD

The brain demands the most energy compared to other organs in our body, and brain cells require a continual supply of glucose to function properly. The energy demand in the brain is usually met by ATP production during glucose oxidation or oxidative phosphorylation in the mitochondria. In low glucose availability, the physiological function of neurons can be disrupted, which can lead to dendritic alterations, synaptic loss, and neuronal cell death [35]. Hence, to circumvent inadequate glucose access and meet energy demands, the brain uses glycogen storage in the astrocytes *via* the astrocyte-neuron lactate shuttle (ANLS). The shuttle is regulated by neuronal activity to enhance astrocytic glycogenolysis and downstream lactate secretion for long-term potentiation, memory forming processes, and neurotransmitter production [36, 37]. On this note, the presence of polysaccharides and glycogen in the brain likely suggests the existence of α-amylase expression in the brain, too [38]. From a human postmortem hippocampal tissue, α-amylase was found in the astrocytes [39], pericytes and neurons [38]. Interestingly, neuronal α-amylase was found in structures resembling dendritic spines, which was clearly visible in non-demented healthy individuals, but the levels were lesser or lost in AD patients [38, 40].

However, large glycogen units can accumulate abnormally in AD patients when homeostasis is disrupted. Increased activity of the glycogen-degrading enzyme α-amylase was detected in the homogenates of the hippocampus in AD patients, suggesting the involvement of this enzyme in increasing astrocytic glycogenolysis events [38]. Additionally, in a neurodegenerative state, the brain has an imbalanced glucose metabolism or mitochondrial dysfunction with altered levels of α-amylase, leading to impaired glucose utilization and hypometabolism. In a study using the APP/PS1 mouse model, lactate levels in the frontal cortex persisted with aging. The increased glycolysis and accumulation of lactate may exacerbate Aβ deposition, suggesting a possible link between region-specific glycolysis and the development of AD pathology [41]. On the contrary, a reduction in the relevant metabolites involved in glycolysis was observed when AD patients were sampled for their cerebrospinal fluid (CSF) [42]. This suggests a decrease in glycolysis events in AD patients. The discrepancy could be attributed to the different stages of AD, whether the patient is at the advanced or preclinical stages.

Cellular and molecular studies have indicated shared pathology between type 2 diabetes mellitus and AD. In AD patients, the impaired glucose metabolism could be due to the compromised insulin growth factor (IGF)-1 receptor and insulin receptor signaling, which also suggests impaired insulin signaling is closely associated with AD pathology [43, 44]. Further studies have shown that reduction or deletion of

Table 1. Pathophysiological states and influence on plasma amylase levels.

Disease	Description	α-amylase Level
Periodontal conditions	Includes dental plaque and caries formation. Adhesion of the α-amylase-binding bacteria facilitates the hydrolysis of dietary starch and consecutively produces additional glucose and lactic acid [25]. The existing acid pool in the plaque and bacterial reactions increases, causing tooth demineralization [26]	Individuals with higher levels of salivary α-amylase are increasingly predisposed to dental caries [27]
Familial hyperamylasemia	There are four types of hyperamylasaemia: pancreatic, salivary, macroamylasemia, and their combinations. It is a benign condition that can occur sporadically or in familial forms [28]	An increase by approximately more than 10% of the normal upper limits of serum amylase and/or lipase found on more than three occasions, lasting more than six months [29]
Renal failure	Often reversible reduction or impairment in kidney function, as measured by glomerular filtration rate (GFR) [30]. There could be a decreased clearance level of the kidneys and increased oxidative stress and inflammatory states of the pancreas [31]	Clearance of amylase is reduced and serum amylase increased [32]
Acute pancreatitis	Inflammation of the pancreas. Related to systemic inflammatory response and can impair the function of other organs. Acute pancreatitis is one of the most common gastrointestinal causes of hospitalization in the United States [33]	Diagnosis of pancreatitis includes serum amylase or lipase activity which has increased to three times the upper limit of the normal value [34]

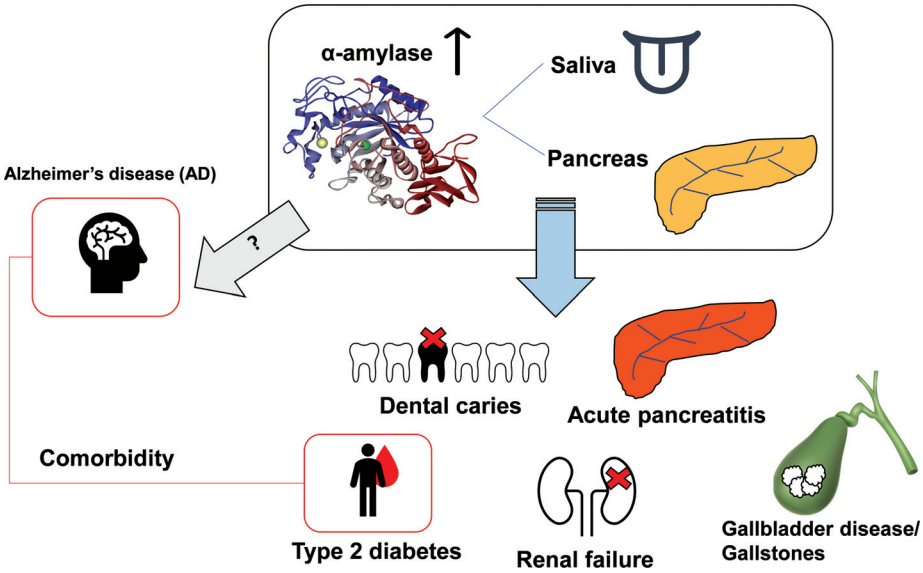

Fig. (1). Potential pathophysiological implications from altered levels of α-amylase. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

IGF-1 receptor in astrocytes could incur hippocampal-dependent memory impairments, impaired glucose and Aβ uptake in mice, suggesting that IGF-1 receptor signaling contributes to hypoglycemia and Aβ deposition in AD brains [45]. The imbalance between glycogenesis and glycogenolysis has also been linked to increased aggregation and contributes to the plaque burden [46]. Hence, it is tempting to speculate that dysregulated glycogenolysis and glycogenesis could underlie cognitive and memory impairment and could be considered as a pathway contributing to AD pathogenesis. Conversely, it was hypothesized that individuals with high neuronal α-amylase could be warranted neuroprotection against AD and possess better cognitive abilities due to a higher amount of glycogen degrading α-amylase in the synapses [40]. The discrepancy could be addressed by further research to dissect the essential role of α-amylase in glycogenolysis which is vital for neuronal activity and whether the levels of this enzyme (high or low) could produce the similar effect in other cellular structures of the brain and in different stages of AD.

Nonetheless, α-amylase levels could serve as an indicator for AD and be used as a therapeutic target to prevent AD progression. As a result, the pathological changes observed in various neurodegenerative diseases, including AD, Parkinson's disease (PD) or Huntington's disease (HD), suggest that glucose metabolism regulation by α-amylase and maintenance of mitochondrial homeostasis are critical for brain function.

5. α-AMYLASE AND AD PATHOLOGY

5.1. Amyloid-β (Aβ) Deposition

In AD pathogenesis, the amyloid precursor protein is cleaved by β-secretase, also known as the β-site APP-cleaving enzyme 1 (BACE 1) [47, 48]. Subsequently, the membrane-bound C99, containing 99 amino acids, is exposed to γ-secretase cleavage, producing variable Aβ fragments and the most common forms are the 40-amino acid ($A\beta_{1-40}$) and 42-amino acid ($A\beta_{1-42}$) variants. Both are neurotoxic, with the latter heightening the rate of misfolding and toxic aggregation, corresponding to AD pathogenesis [49]. Furthermore, these plaques can be deposited extracellularly and cause a cascade of pathological changes such as disrupting the surrounding synaptic network and the death of neurons [50].

The link between the glycogen degrading property of α-amylase and the $A\beta_{1-42}$ has been delineated using *in vitro* studies whereby the results signified an increased α-amylase immunoreactivity in human fetal primary astrocytes, with more significant effect after stimulation from fibril $A\beta_{1-42}$. This suggests that fibril $A\beta_{1-42}$ could influence the post-translational modifications of α-amylase than gene expression [51]. Additionally, there is a strong pancreatic amylase immunoreactivity in astrocytes adjacent to the $A\beta_{1-42}$ plaques [39]. Apart from its role in glycogen degradation, the colocalization of α-amylase with the $A\beta_{1-42}$ plaques could be attributed to its ability to interact with other proteins by forming complexes. Studies have revealed that α-amylase could act as a protein carrier, transporter, modulator, and protects the interaction with other proteins [52]. It would be interesting to use an *in vivo* AD model to further confirm colocalization between the two and map out any possible interaction.

5.2. Tau Pathology

Tau is a microtubule-associated protein whereby its expression can be abundantly found in neuronal cells, particularly in the axons of neurons. The tau protein plays a vital biological function in the regulation of microtubule dynamics [53]. The microtubule tau protein can also be found in oligodendrocytes. It influences neurite development, cytoskeleton stabilization, and regulation of synaptic functions found in the axonal processes of the neurons by binding to microtubules [54]. The physiological role of tau protein is modulated in the neuronal cytoplasm through the equilibrium state of phosphorylation and dephosphorylation, maintained by kinases and phosphatases, respectively [55]. The atypical hyperphosphorylation of tau protein in certain neurons present in demented patients induces deconstruction of microtubules. Tau protein molecules are then liberated and clump into paired helical filaments through hydrophobic interactions, making up the principal constituent for neurofibrillary tangles production [55, 56] in neuronal cell bodies and neurites senile plaques.

The colocalization between salivary α-amylase with the tau protein-positive tangles has been confirmed in microscopy imaging and double immunofluorescence staining, particularly in the Hirano bodies formation in AD pathology. The presence of α-amylase levels demonstrated a positive correlation in AD patients compared to non-demented controls [38]. The study not only suggests that α-amylase is present in the human brain, but its gene expression, concentration, and activity levels are also altered in a neurodegenerative brain such as AD. Injured brain structures or reduced brain function have upregulated brain tau-protein and α-amylase levels, suggesting that both tau and α-amylase in the brain of old rats could be used as cognitive impairment markers [56]. On top of its role to degrade polysaccharides, it could be deduced that α-amylase is a key player in the modifications of glucose availability and neuropathological changes observed in AD patients.

Overall, several studies have demonstrated the colocalization of α-amylase with $A\beta_{1-42}$ plaques and neurofibrillary tangles production. From the studies, it is tempting to speculate that higher α-amylase activity could be determined when a specific assay that accurately quantitates brain α-amylase activity is developed and applied. Nevertheless, the prominent role of the enzyme in the glucose metabolism of the brain has been documented in a few studies, and when the brain is in a neurodegenerative state, the metabolism is impaired and could contribute to AD pathogenesis.

6. CURRENT BIOMARKERS FOR AD

The progression spectrum of AD usually starts with pre-clinical stages of AD, followed by mild cognitive impairment (MCI), and subsequently dementia due to AD. At the pre-clinical stages, there are no evident and significant clinical symptoms yet. However, at the MCI stage, symptoms of memory loss are noticeable and measurable. Lastly, 40 to 60% of the MCI patients are likely to develop full-blown clinical symptoms and progress to AD dementia after several years of pre-clinical onset [57]. Hence, it is essential to detect and differentiate between pre-clinical and MCI stages as delayed diagnosis usually worsen prognosis and impedes effective therapy implementation. Biomarkers are crucial to develop an effective treatment plan or a personalized medicine approach. They could be essential in defining the stages of the disease, tracking the progression, and documenting the effect of treatment.

Cerebrospinal fluid (CSF) is a common fluid biomarker used for AD. It is the most logical source as the interstitial fluid in the brain has direct contact with the CSF *via* an unrestricted bidirectional flow of proteins, and the CSF is secluded from the direct impact of the peripheral system through the restricted transportation of molecules and proteins by the blood, making it highly sensitive for AD-specific biomarkers such as Aβ and tau protein with specificity between 90% to 95% [58, 59]. CSF biomarkers are related to the three main pathological changes: amyloid-β (Aβ) deposition into extracellular Aβ plaques, intracellular neurofibrillary tangles (NFT) formation, and neuronal loss. The CSF $A\beta_{42/40}$ ratio pattern can better predict anomalies of the cortical amyloid deposition than with CSF $A\beta_{42}$ alone. Subsequently, this will result in fewer false-positive cases of low CSF $A\beta_{42}$ or false-negative of high CSF $A\beta_{42}$ and the CSF $A\beta_{42/40}$ ratio could be used as an amyloid status proxy in AD clinical trials and eventually in clinical care settings [60].

Peripheral body fluids, such as the blood, have also been used as a diagnostic or prognostic AD biomarker. Abnormalities or early changes have been reported in red blood cells, white blood cells, and platelets in AD patients, prompting a

multicellular testing panel. Studies have shown that blood cells experience morphological changes, oxidative stress, and altered energy production due to Aβ deposition [61, 62]. Additionally, protein kinase C (PKC), which is crucial for red blood cell signaling, synapse formation, and memory, is also affected. In erythrocytes, PKC activity can be altered and disrupted by increased Aβ toxicity, suggesting that blood cells are affected in AD patients [63]. However, AD-specific biomarkers in blood pose difficulty in isolation due to minute concentration. It also requires a highly sensitive technical approach to quantify [64].

6.1. α-Amylase in AD: useful Diagnostic Tool

Salivary biomarkers are increasingly important, especially in toxicology, drug testing, and endocrinology, and can be used for the early diagnosis of certain oral diseases. Additionally, there is a possible association between salivary biomarkers with AD as studies have shown that AD-related species such as Aβ and tau were expressed in salivary glands through Western blot detection and enzyme-linked immunosorbent assay (ELISA) [65, 66]. Salivary flow and composition are also modulated by the autonomic nervous system. Salivary α-amylase demonstrates a positive correlation with activation of the sympathetic nervous system and local parasympathetic nerves regulating the release of α-amylase [67, 68]. This suggests a possible relationship between saliva and the nervous system as well as the possible linkage that neurodegenerative diseases can be detected in saliva. Hence, salivary biomarkers could be promising in AD diagnosis.

There are few reported studies on utilizing α-amylase as a biomarker. One research studied the saliva samples collected from 15 AD patients and ten non-demented, healthy controls without neurological disease. In the study, five salivary markers; $A\beta_{1-40}$, $A\beta_{1-42}$, insulin-like growth factor (IGF)-I, IGF-II, α-amylase, interleukin 1β (IL-1β), and tumor necrosis factor α (TNF-α) were analyzed. It was revealed that there were significant differences, particularly salivary α-amylase, between patients and controls, which suggests that α-amylase can be represented as an appropriate diagnostic biomarker for AD [69]. Moreover, combining the salivary biomarkers, matrix metalloproteinase-8 (MMP-8), amylase, and myoglobin (MYO) could robustly differentiate healthy individuals and AD patients. As a result, salivary MMP-8, amylase, and MYO are patented as a Salivary AD Index due to the significantly higher level of these biomarkers found in AD patients than healthy individuals [70]. Interestingly, it was found that α-amylase reflects M_3 muscarinic agonist activity in AD. The authors conducted a study on the safety/tolerance of two doses of xanomeline tartrate (100 mg and 115 mg) given to 12 AD patients and measured serum amylase (pancreatic and salivary) as a potential marker for M_3 activity. The salivary amylase levels were altered and presented a trend in the 115 mg group compared to pancreatic, which did not show any change, suggesting that salivary α-amylase may better serve as a marker for M_3 activity [71].

Using salivary biomarkers, especially when detecting the levels and activity of α-amylase, can be a robust alternative for AD. On top of its non-invasiveness, it does not incur side effects, is inexpensive, and can be regarded as a universal diagnostic fluid. In order to establish α-amylase as a biomarker, there needs to be a routine procedure for collection, standardized tests, and analysis. Currently, passive drooling is a highly recommended method of collection as it does not compromise salivary flow and composition, thus allowing analytes in saliva to be quantified accurately [72]. However, demented patients may not be able to cooperate physically, and the duration of sampling could be unnecessarily long, which may lead to degradation or instability of α-amylase activity.

Additionally, various methods can be used to analyze saliva samples such as ELISA, western blot, immunofluorescence, flow cytometric assays, multiplex array assays, infrared spectroscopy, chromatography, mass spectrometry, and Ellman's colorimetric method [73, 74]. Although the variety is convenient, sensitivity levels could differ with each instrument and possibly affect the statistical significance of the analyte concentration. Hence, establishing a standardized method or test with the highest accuracy and sensitivity would minimize variations in results and allow robust predictions of α-amylase activity in AD. Moreover, drugs such as anxiolytics, antidepressants, analgesics, and antihypertensives could influence the salivary flow and qualitative composition of saliva [75].

Overall, implementing salivary α-amylase as an important biomarker could foster a new research direction for AD. However, in order to establish salivary α-amylase as a golden standard to diagnose and screen neurodegenerative diseases such as AD, limitations such as the inconsistencies and lack of standardization should be addressed.

6.2. Repurposing Drugs for AD: α-amylase Inhibitors

The usually prescribed drugs for the symptomatic treatment of AD include cholinesterase (ChE) inhibitors and currently consist of donepezil, rivastigmine, and galantamine. The overall mode of action of these inhibitors is to facilitate central cholinergic neurotransmission activity by reducing the physiological breakdown of the neurotransmitter, acetylcholine (ACh) *via* inhibiting the acetylcholinesterase (AChE) activity in the synaptic cleft [76]. These inhibitors were prescribed to treat mild and moderate AD.

Other therapeutic agents approved for moderate to severe AD include the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, memantine. It selectively binds the NMDA receptor and alleviates the deleterious effects of elevated glutamate levels that cause neuronal dysfunction [77]. There is also a newly approved drug, aducanumab (marketed as Aduhelm), by the Food and Drug Administration (FDA). Aducanumab, a monoclonal antibody, selectively binds to human B-cell clones triggered by neo-epitopes present in pathological Aβ aggregates, reducing brain Aβ plaques [78, 79]. However, the approved treatments only provide symptomatic relief. Furthermore, they may not be effective to all AD patients due to varying stages of pathogenesis, while the efficacy of aducanumab is too early to tell. There were also many clinical trial failures due to an incomplete understanding of the AD pathophysiology, inappropriate dosage, inaccurate choice of treatment target, and unfitting therapy time during disease development. Hence, there is a need to discover diverse and probably multi-targeted therapeutic targets for AD, constantly.

The development of α-amylase inhibitors is of interest as it makes excellent therapeutic agents for the treatment of

Table 2. Current pharmacological approaches against α-amylase.

Marketed Drugs	Description
Acarbose	- Antidiabetic drug which acts by inhibiting α-amylase and α-glucosidase [87]. - Regulates blood glucose and postprandial glucose levels [23].
Phytotherapeutics	-
Phenolic acid	- Gallic acid can be found in food sources such as fruits, vegetables, spices, and tea [88]. - Mild inhibitor of α-amylase [89].
Flavonoids	- Luteolin and quercetin selectively inhibit α-amylase activity, covering the wide and shallow catalytic groove [90].
Plants	- Certain types of grains, rice, flowers, seed, and fruit rinds such as *Gingko biloba*, *Punica granatum*, and *Hibiscus sabdariffa* [91]. - It has been documented and summarized in the following literature [91].

disorders such as diabetes and obesity. Furthermore, based on the earlier-mentioned connection between α-amylase, diabetes, and AD, α-amylase inhibitors might be suited to be repurposed for AD treatment. The currently available anti-diabetic drug that inhibits α-amylase is acarbose. The drug demonstrates a competitive, reversible inhibition of α-amylase and lowers glycosylated haemoglobin levels by reducing plasma glucose in T2DM patients [80]. Although the idea that α-amylase inhibitors could be repurposed is welcomed, there are several studies on the pharmacokinetics-pharmacodynamics relationship of acarbose, whereby it was found to have low blood-brain-barrier (BBB) permeability [81, 82]. However, strategic structural modifications could be made to the inhibitors in order to increase lipophilicity and, hopefully, the permeability of the BBB.

Interestingly, incretin-based therapies that consist of GLP-1 agonists and the dipeptidyl peptidase-4 (DPP4)-inhibitors are applied as second-line therapies in T2DM and can cross the BBB *via* passive diffusion [83]. In medicinal chemistry, natural products such as luteolin, a flavone, improve glucose metabolism by inhibiting pancreatic α-amylase and can be detected in the brain tissues, suggesting its permeability through the BBB [84]. Additionally, phenolic compounds such as phenolic acids and flavonoids demonstrate the ability to bind covalently to α-amylase and modify its activity by forming quinones or lactones that react with nucleophilic groups on the enzyme [85]. Other possible natural products include aqueous extracts of vegetables such as the Indian spinach and brinjal extracts, whereby a study demonstrated their ability to inhibit α-amylase activity regarding obesity, diabetes, and hypertension [86]. However, *in vivo* determination of α-amylase inhibitory activities using suitable AD animal models could be suggested to confirm the potential health benefits in improving cognition and memory in animals as well as to identify potential BBB permeation activity. By administrating α-amylase inhibitors with good BBB permeability, it is postulated to reduce α-amylase activity in the brain and subsequently decreased AD-associated biomarkers such as the $A\beta_{1\text{-}42}$ and tau protein or associated comorbidity. Hence, it is inviting to hypothesize that α-amylase inhibitors are beneficial by offering a multi-target strategy to address the metabolic complexity and pathology in AD patients.

Nevertheless, it is vital to consider the suitability of the long-term use of these inhibitors in AD patients, especially those with other pre-existing conditions, as suppression of α-amylase activity can lead to hypoglycemia and gastrointestinal complications [92]. Additionally, α-amylase is required in post-synapses to provide quick and continuous glucose access. Hence, the administration of α-amylase inhibitors could suppress this action and possibly result in a deleterious impact on the neuronal synapses [38]. It is currently unknown whether α-amylase inhibitors could provide a definitive advantage at the end-point, and significant concerns regarding its safety or tolerability, pharmacokinetic and pharmacodynamic profiles in AD patients must be considered. Also, the exact mechanisms that underlie the association between diabetes mellitus and dementia are not fully delineated. The multifactorial nature of both disorders needs to be understood to buttress the link between diabetes and AD and develop novel routes for therapeutic interventions. However, conducting clinical trials can elucidate the short- and long-term benefit versus risk profile of these agents, giving a better insight into their roles as a therapeutic option for AD patients. Last but not least, it is also essential to identify the optimal sequence or timeline of α-amylase inhibitors administration. It is a critical point to consider as individual patients go through different phases of AD, which poses difficulty in managing their symptoms with the best risk-benefit ratio for both acute and chronic users.

CONCLUSION

Alzheimer's disease (AD) is debilitating and complex, and is associated with other chronic comorbidities such as diabetes. The α-amylase enzyme, one of the key enzymes in regulating the digestion of carbohydrates, was recently reported to be present in the human brain. It is also suggested that the α-amylase could play a role in the neurodegenerative AD pathology. In disease diagnostics, α-amylase could potentially serve as a quantifiable novel biomarker for AD progression in affected individuals. However, present limitations such as irregularity in the testing kits used need to be addressed in order to establish α-amylase as a robust biomarker that is specific and sensitive for diagnosis. Additionally, α-amylase could possibly be made into a desirable target for therapeutic interventions. By repurposing existing

or novel α-amylase inhibitors, it is possible to address the altered enzymatic level in AD patients and associated comorbidity such as diabetes, which has been a risk factor. Overall, understanding the pathophysiology of α-amylase in AD patients could provide more information on the underlying mechanisms and paves the way for new therapeutic avenues.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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