Alzheimer's and Type 2 Diabetes Treatment *via* Common Enzyme Targeting

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Abstract: Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are two devastating diseases that are currently incurable. Epidemiological, clinical and pathological evidence has confirmed the co-existence of these two disorders. Moreover, there has been promising progress made in the identification of the pathological linkage between T2DM and AD in the last decade. Hence, developing common treatment strategies for these diseases is important. Currently, enzyme targeting is a potential strategy to cure many diseases. In this communication, we tried to summarize the single enzyme-targeted therapeutic approach for the treatment of AD and T2DM. This field of research continues to be active and progressive in identifying many promising enzymes that are involved in both diseases. Based on this review article, we also believe that enzyme inhibition is a promising and reliable strategy for the treatment of many incurable diseases. In the future, we expect that the scientific community will be able to develop common enzyme inhibitors for the treatment of both AD and T2DM.

Keywords: Alzheimer's disease, diabetes, enzymes, drug therapy, endocrine disorders.

INTRODUCTION

Alzheimer's disease (AD) is a complex, progressive, irreversible and neurodegenerative disorder [1, 2]. A recent report has suggested that AD affects 36 million people worldwide, which accounts for 60–80% of dementia cases [3]. AD is characterized by the aberration of multiple interactive systems, pathways and molecular mechanisms, which ultimately leads to memory loss and cognitive function [2]. The exact mechanisms of pathological defects in AD are still unknown. However, the formation and excessive deposition of β-amyloid (Aβ) oligomers and neurofibrillary tangles (NFTs) have been proposed to be a possible cause of this disorder [4]. AD is an asymptomatically progressed disorder, which still lacks effective treatment strategies and remains an incurable disease. To date, the treatment of AD is symptomatic, which moderately slows the cognitive decline. The current treatments include acetylcholine esterase inhibitors and the N-methyl-D-aspartate type glutamate receptor antagonist, memantine, to counteract excitotoxicity [5, 6]. Recently, some clinical trials have also used vaccination strategies, metal chelation, anti-inflammatory drugs, anti-oxidants and kinase inhibitors [5, 7].

Type 2 diabetes mellitus (T2DM) is a condition in which a high blood glucose level results from increased hepatic glucose production, impaired insulin production by pancreatic β -cells, altered insulin release in response to hyperglycemic stimuli or insulin resistance, which leads to medical complications that include effects on the central nervous system (CNS) [4]. The mainstay of non-pharmacological T2DM treatment is diet and physical activities. The dependence on insulin in T2DM patients is not important, but approximately one-third of T2DM patients need insulin to reduce high blood glucose levels [7, 8].

AD and T2DM share several molecular processes that suggest a possible common pathophysiology. The possible linkage has been highlighted by epidemiological, clinical and pathological evidence [4, 9, 10]. Disturbances in several biochemical and physiological pathways, which affect cell growth, cell differentiation, cellular repair mechanisms, energy metabolism and glucose utilization, have been suggested as causative factors of these disorders [11]. Both of these disorders are more prevalent with ageing and continue as a leading cause of morbidity and mortality in elderly people [4, 7]. Substantial evidence has also suggested that T2DM could lead to memory impairment and AD [4, 12]. Pathological features of T2DM, such as hyperglycemia or disturbance of insulin function, have been reported to affect synaptic plasticity, learning and memory, which could also lead to AD [13, 14]. Moreover, the pancreas is highly innervated and shares molecular similarities with the brain at the transcriptome and proteome levels [13]. An elevation of butyryl cholinesterase (BuChE) both in the pancreas and brain has also been reported, which highlights the linkage between T2DM and AD [15]. BuChE is the more studied cholinesterase (ChE) for its association with T2DM. Several

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studies have reported the relation of BuChE with both T2DM and AD in different ethnic groups [13, 16-18]. The involvement of BuChE in the pathogenesis of T2DM has been suggested either by amyloid fibril formation or by modifying other risk factors of insulin resistance [13]. Amyloid fibrils in pancreatic islets can produce excessive superoxide radicals, which lead to lipid peroxidation and nitric oxide inactivation that contributes to the apoptosis of β cells [13].

Because of the increased prevalence of these devastating diseases all over the world and especially in developed countries, finding effective therapies for more than one disease at once is critical. To the best of our knowledge, there is not any literature on the treatment strategies against AD and T2DM together, especially *via* enzyme targeting. The present communication will focus on various enzyme-targeted therapies, which can affect both T2DM and AD.

MECHANISTIC LINKAGES BETWEEN AD AND T2DM

There has been promising progress made in identifying linkage between T2DM and AD in the last decade. Apart from the role of T2DM in the induction of AD, T2DM and AD have some common clinical and biochemical pathophysiology suggesting the linkage between these disorders [9, 15, 19].

The formation and accumulation of insoluble protein aggregates with a fibrillar conformation (Aβ- and NFT-like structures) in AD are also found in T2DM as amylin in pancreatic islets [7]. These insoluble protein aggregates cause pancreatic β-cell loss in T2DM and neuronal cell loss in AD. Involvement of hormones, such as insulin, estrogen and gonadotropin, in AD pathophysiology has been an important area of research in recent years. Impaired insulin signaling has been proposed to be implicated in AD as insulin expression is inversely proportional to the Braak stage of AD progression when tau tangles begin to form [7, 20, 21]. These facts suggest that AD could also be considered as an endocrine disorder.

Oxidative stress plays a major role in diabetes and in AD and it promotes the formation of advanced glycation end products (AGEs) and lipid peroxidation products (ALEs) by the oxidative modification of proteins, lipids and nucleic acids [22]. Several studies have indicated the presence of AGEs and ALEs in AD and T2DM [22-25].

Inflammation-based mechanisms have also been proposed as a common pathological feature that coexists between AD and T2DM [15, 26]. Recent evidence has proposed aberrant cholesterol metabolism in the initiation and progression of AD through cholesterol-sensing liver X receptors, which are linked to the peroxisome proliferator-activated receptor-γ. Aberrant cholesterol metabolism has also been indicated as a major drug target of T2DM treatment [27]. Moreover, several risk factors implicated in T2DM, such as obesity, physical inactivity and genetic predisposition, have also been associated with AD [28-30].

ENZYMES: A POTENTIAL TREATMENT TARGET

Enzymes are promising targets to cure chronic diseases as both biochemical deficiency and/or overload could be corrected by activation or inhibition by acting on its specific sites to control the overall pathophysiological conditions of the body. More information about enzymes, such as the 3D structure, as well as detailed knowledge of active sites, enzyme kinetics and ligand interactions could accelerate the discovery of novel drug targets for chronic diseases. Several investigations have led to the discovery of various drugs against many incurable diseases. However, intensive studies are still underway for the search of new drugs that can act effectively for the treatment of various diseases. In the present era, enzyme inhibition is the most important treatment strategy used to treat AD [31-33]. Some studies have also reported the above mentioned strategy for the cure of T2DM [34, 35]. We believe that the enzyme-targeted therapeutic approach is a promising approach for the treatment of AD and T2DM, which are two devastating diseases that have thus far defied proper treatment.

CHOLINESTERASES

Cholinesterases are a group of enzymes that regulate cholinergic nerve and neuromuscular transmission and they terminate the action of acetylcholine (ACh) by splitting it. Acetylcholinesterase (AChE) and BuChE are the two important cholinesterases involved in this termination of cholinergic neurotransmission.

AChE is a serine hydrolase involved in the termination of chemical transmission at cholinergic synapses and secretory organs by catalyzing the hydrolysis of the neurotransmitter acetylcholine [36]. BuChE is also a serine hydrolase involved in the hydrolysis of choline esters, including acetylcholine, which is widely distributed in the nervous system and in plasma [15, 37].

The first cholinesterase inhibitor (ChEI) approved for AD treatment was tacrine (Cognex) in 1993 but it proved to be relatively short-acting and unselective between the two ChE enzymes and was associated with a high incidence of reversible hepatotoxic drugs [38]. In contrast, the second generation ChEIs, namely donepezil hydrochloride (Aricept), rivastigmine (Exelon) and galantamine (Reminyl), approved by USFDA in 1996, 2000 and 2002, respectively, are better tolerated and have been found useful in treating mild to moderate AD patients [38, 39]. However, such improvements are, unfortunately, quite modest. Recent clinical trials have triggered considerable controversy concerning the relevance of the benefit of these classes of drugs, which prompted two avenues of research as follows: one avenue to develop a new class of ChEIs with activity beyond the modest symptomatic one associated with initial agents; and one avenue to optimize current agents based on a greater understanding of enzyme/inhibitor interactions.

Cholinergic loss is the single most replicated neurotransmitter deficiency in AD and has led to the use of acetylcholinesterase inhibitors (AChE-Is) as the mainstay treatment [40, 41]. However, AChE-Is induces dose-limiting adverse effects [42]. Some potent and quickly absorbed AChE-Is, such as phenserine, tolserine and esolerine, have been reported in literature for the treatment of AD and these AChE-Is are much better than classic inhibitors. However, more studies are required to identify the potential benefits and risks of AChE-Is associated in preclinical and clinical

models [43]. Several naturally derived AChEIs have also been reported in scientific literature as follows: Huperzine A and Huperzine B (Lycopodium alkaloid isolated from the Chinese medicinal herb *Huperzia serrata*) [44]; *Nelumbo nucifera* (an aquatic plant); *Himatanthus lancifolius* (a shrub that contains several indole alkaloids) extract [45]; Galangin [46] and Cardanol derivatives [47]. However, preclinical and clinical safety as well as toxicity of these compounds have not yet been identified. Some synthetic analogues are also under preparation as potential AChE-Is, such as phenyl-5,6-dimethoxy-1-oxo-2,3-dihydro-1H-2-indenylmethanone analogues, N-alkyl-7-methoxytacrine hydrochlorides and ladostigil [43].

New agents that can affect disease progression are greatly needed. Inhibition of brain BuChE represents a new drug target for AD treatment. One study has indicated that selective butyrylcholinesterase inhibitors (BuChE-Is) elevate ACh in the brain, which augments long-term potentiation and improves cognitive performance in rodents without the classic adverse actions of AChE-Is [48]. Thereby, BuChE-Is represent a new strategy to ameliorate AD [37, 42, 48, 49], particularly because AChE activity is depleted in the brain of AD patients similar with ACh levels whereas BuChE activity is elevated [50]. Hence, the BuChE to AChE ratio changes dramatically in cortical regions affected by AD in the range of 0.2 to 11.0 [37, 49]. This altered ratio in the brains of AD patients likely modifies the normal role of BuChE in the nervous system of hydrolyzing excess ACh to depleting already reduced levels of the neurotransmitter. In one study, Omu *et al*. (2010) reported the involvement of BuChE to manage oxidative stress in gestational diabetes [51]. Because BuChE may partially compensate the action of AChE, a consistent ratio of BuChE to AChE and selective BuChE inhibition may potentially be effective in the alleviation of the cholinergic deficit and improvement of AD [32, 48]. In view of our experience and some unpublished data, we believe that BuChEIs could serve as valuable targets for future drug discovery for AD and T2DM.

GLYCOGEN SYNTHASE KINASE 3 (GSK3)

GSK3 is a versatile enzyme that regulates body metabolism by the phosphorylation of glycogen synthase (GS) and other substrates. GSK3 is identified as an important contributor of a variety of pathways viz. initiation of protein synthesis, cell proliferation, cell differentiation, apoptosis and a vital component of embryonic development through the Wnt signaling cascade [52]. In one study, Gao *et al*. (2011) reported induction of GSK3 in AD and T2DM suggesting a common pathological link between these two diseases [53]. In view of their result, these authors suggested that this enzyme could act as a novel treatment target for these diseases. A promising strategy for the treatment of neurodegenerative disorders has already been suggested by the same group *via* GSK3 inhibition, which prevents Aβ aggregation and tau protein hyper-phosphorylation.

Several potent and selective small molecule inhibitors of GSK3 (CT 99021 and CT 20026) have been identified and used *in vitro* to modulate glycogen metabolism, gene transcription, glycogen synthase activity and insulin-stimulated glucose transport [52]. Lithium cation was the first GSK3 inhibitor, which was used for the treatment of bipolar disorders [54]. Various chemically diverse families of GSK3 inhibitors have been reported during the last decade, such as ATP competitive (compounds compete with ATP in binding to GSK3) and non-ATP competitive types [55]. Martinez *et al*. (2011) suggested that non-ATP competitive types are the attractive class of GSK3 inhibitors due to lack of endogenous ATP competition, which results in better potency and lower toxicity [55]. The examples of different non-ATP competitive GSK3 inhibitors are thiadiazolidindiones, natural manzamine and sesquiter-pene palinurin [56, 57]. Several potent ATP competitive GSK3 inhibitors derived from different compounds, inclu-ding maleimides, staurosporines, indoles, paullones, pyrazol-amides, pyrimidines, furopyrimidines, oxadiazoles and thia-zoles, have been reported and reviewed recently by Kramer *et al*. [58].

Some irreversible inhibitors of GSK3, such as halomethylketones and GSK3 inhibitor VII, have also been reported as a good alternative to avoid resistance in future drug development [55]. A recently reported anti-diabetic drug, glucagon-like peptide 1, has also shown neuroprotective potential, which could be possibly linked to GSK3 modification [53]. Moreover, the important challenge for a GSK3 inhibitor in AD treatment is that it needs to cross the blood-brain barrier to exert its action in the regulation of exacerbated GSK3 brain levels [55].

Although two of the GSK3 inhibitors have been entered in clinical trials during the last five years, they failed in the first phase of development. However, it was clear that the failure was caused by the intrinsic toxicity of these compounds related to their chemical structure, the potent inhibition on the enzyme and the ATP competitiveness in their binding to GSK3. Meanwhile, NP-12 (tideglusib), the unique ATP non-competitive GSK3 inhibitor that entered clinical trials in 2006, recently passed the 2nd stage trial and may become an effective and accessible drug [55]. In view of great interest arising with GSK3, it has been proposed as a potential biomarker not only for the treatment of progression but also to stratify the patients for better clinical trial designs, which will help in proper diagnosis and on time AD neuropathology [55].

β-SITE AMYLOID PRECURSOR PROTEIN-CLEAVING ENZYME 1 (BACE1)

BACE1 is an important protease of the β -secretase family, which regulates numerous physiological functions in all living organisms. β -secretases play a crucial role in amyloid precursor protein (APP) processing, which increases the Aβ load in the brain [59]. The essential role of BACE1 on amyloid deposition within the pancreatic islets has also been reported in scientific literature and this deposition is a pathophysiological hallmark of T2DM. Involvement of BACE1 in the pathogenesis of these two chronic diseases makes it an important target for the treatment and understanding of the pathological mechanisms of these disorders.

Neuronal BACE1 levels and activity increase with age following pathological events, such as oxidative stress, hypoxia and brain injury. BACE1 levels are associated with raised Aβ [60]. Henceforth, it is plausible that the insulin resistance and impaired glucose metabolism associated with

AD may be connected to the elevated activity of this enzyme [61].

BACE1 inhibitors are recognized as a novel target of AD treatment because they affect β- and γ-secretases that cleave the APP and generate Aβ [62]. However, development of BACE1 inhibitors has been unsatisfactory, especially due to difficulties in achieving blood-brain barrier penetration by these compounds [62-64]. Mattsson *et al*. (2012) reported an *in vivo* characterization of the effect of two BACE1 inhibitors, namely cyclic sulfoxide hydroxyethylamine (NB-B4) and oxazine derivative (NB-C8), in beagle dogs [63] and they reported a CSF-Aβ pattern, which could promote development of novel therapies for the cure of AD.

To date, several BACE1 inhibitors have been reported, and some are already in clinical trials. Orally available BACE1 inhibitors, such as LY2811376, RG7129, MK-8931 and HPP854, are already in phase 1 clinical trials [65]. The BACE1 inhibitor, minocycline, has also been reported for its potential effects on pre-plaque neuro-inflammation and inhibition of BACE1 in a transgenic AD model [66].

In one study, Meakin *et al*. [61] reported that a decline in BACE1 reduces body weight, protects against diet-induced obesity and enhances glucose disposal in mice. BACE1 has already been validated as a therapeutic target for AD. Moreover, BACE1 is a useful therapeutic agent for the treatment of obesity associated with T2DM [67]. In light of the above mentioned study, BACE1 activity amelioration is an important novel approach for the treatment of diabetes and AD.

INSULIN DEGRADING ENZYME (IDE)

IDE is a zinc metalloprotease and is primarily involved in intracellular insulin degradation and its sensitivity [68]. IDE has been reported to be highly expressed not only in the liver but also in the brain [69] and it shows greater affinity towards substrates, such as insulin and Aβ, which suggests their involvement towards common etiology of T2DM and AD pathology [70, 71]. Moreover, genetic association of IDE with T2DM and AD, especially late onset AD, has also been reported in scientific literature [71, 72]. Association of this enzyme with peptides, such as insulin and Aβ, makes IDE as a promising target of drug development and new therapy for T2DM and AD. Pharmacological inhibitors of IDE have been used as early as 1955 to potentiate the hypoglycemic action of insulin [35]. Many metabolic components, such as ATP, free fatty acids, fatty acid coenzyme A thioesters, hydrogen peroxide, ubiquitin and NO, have been reported to affect IDE-mediated degradation [71, 73]. Recently, a novel IDE inhibitor (ADT-21) has been patented for the treatment of diabetes by Frenkel *et al*. [74].

The involvement of IDE in the pathophysiology of AD and T2DM has been highlighted in the literature, but its role is contradictory. Several studies have confirmed the involvement of insulin in a wide range of processes, including memory and cognition [75-77]. In one study, Leissring *et al*. [35] developed potent IDE inhibitors for the treatment of diabetes and they also suggested that IDE inhibition is a potential candidate for the management of AD. More recently, Tundo *et al*. [78] reported that IDE could function like a heat shock protein with implications in cell growth regulation, which extends its role in humans. Thus, we believe that IDE inhibitors might have multiple uses and could be a promising area of future research.

C-JUN NH_2-TERMINAL KINASE (JNK)

JNK is an important enzyme involved in the stress response pathway that is responsible for phosphorylation of the c-Jun transcription factor N-terminus. JNKs are multifunctional kinases involved in many physiological processes [79]. Several studies have indicated the association of JNK with Aβ, which is an important pathological feature of AD [80-82]. Alteration in JNK-associated activities has also been reported to be a causative of T2DM [4, 83]. The existence of JNK-interacting protein 1, a key regulator of the JNK pathway, has been reported both in the brain and islet cells, which further highlights another possible linkage between the pathogenesis of AD and T2DM [84]. Because of the involvement of JNK in the pathophysiology of both T2DM and AD, it could also be targeted for drug development for both of these diseases.

CONCLUSION

In light of the present article, we believe enzyme inhibition is a promising and reliable strategy, which could be further explored. Furthermore, additional enzymes may be identified to have a role in the pathophysiology of both AD and T2DM. In the future, we expect that the scientific community will be able to develop common enzyme inhibitors for the treatment of both AD and T2DM.

LIST OF ABBREVIATIONS

Ach	=	Acetylcholine
AChE	=	Acetylcholinesterase
AChE-Is	=	Acetylcholinesterase inhibitors
AGEs	=	Advanced glycation end products
AD	=	Alzheimer's disease
BuChE	=	Butyryl cholinesterase
BuChE-Is	=	Butyrylcholinesterase inhibitors
CNS	=	Central nervous system
ChEI	=	Cholinesterase inhibitors
ChE	=	Cholinesterase
JNK	=	C-Jun NH2-terminal kinase
GSK-3	=	Glycogen synthase kinase-3
IDE	=	Insulin degrading enzyme
ALEs	=	Lipid peroxidation products
NFT	=	Neuro fibrillary tangles
T2DM	=	Type 2 diabetes mellitus
BACE1	=	β-site amyloid precursor protein-cleaving enzyme 1

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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