Review

Insulin, insulin-degrading enzyme and amyloid-β peptide in Alzheimer’s disease: review and hypothesis

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Abstract

Clinical and epidemiological studies have found that type 2 diabetes, and hyperinsulinaemia, increased the risk of developing Alzheimer’s disease (AD) in the elderly. The link between hyperinsulinaemia and AD may be insulin-degrading enzyme (IDE). This enzyme degrades both insulin and amylin, peptides related to the pathology of type 2 diabetes, along with amyloid-β peptide (Aβ), a short peptide found in excess in the AD brain. We review the current evidence, which suggests that hyperinsulinaemia may elevate Aβ through insulin’s competition with Aβ for IDE. Genetic studies have also shown that IDE gene variations are associated with the clinical symptoms of AD as well as the risk of type 2 diabetes. The deficiency of IDE can be caused by genetic variation or by the diversion of IDE from the metabolism of Aβ to the metabolism of insulin. It is intriguing to notice that both hyperinsulinaemia and IDE gene variations are related to the risk of AD when the Apolipoprotein E4 (ApoE4) allele, the major risk factor of late-onset AD, is not present. Further studies of the role of IDE in the pathogenesis of AD, which may uncover potential treatment target, are much needed.

Keywords: Insulin; Amyloid-β-peptide; Insulin-degrading enzyme; Type 2 diabetes and Alzheimer’s disease

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1. Introduction

The United States Census Bureau projects that between 2005 and 2025 the total population will grow 20%, but the demographic of age 65 and over will increase by nearly 50%.

As the population ages, type 2 diabetes and Alzheimer’s disease (AD) are becoming surging epidemics. Both diseases are chronic and complicated, and they are the leading causes of morbidity and mortality in the elderly [88]. Several epidemiology studies have shown that type 2 diabetes increased the risk of AD in both cross-sectional and prospective populations. Further, elevated peripheral insulin, a common biomedical sign of type 2 diabetes, has
been singled out as a possible independent risk factor of AD.

To date, it has been found that the Apolipoprotein E4 (ApoE4) allele is a major risk factor of late-onset AD [85]. However, 50% of AD patients do not possess ApoE4; other risk factor(s) might contribute to the pathogenesis of the disease. In the process of elucidating other contributing factors the association between hyperinsulinaemia and AD was found to be particularly strong in populations lacking ApoE4. While the mechanism behind such a relationship is still unclear, it is noted that the two diseases share a common protease, insulin-degrading enzyme (IDE). This review will summarize the relevant data on the relationship of type 2 diabetes and hyperinsulinaemia with AD. We will also present current researches indicating a relationship among IDE and the two diseases.

2. Type 2 diabetes and Alzheimer’s disease

Cross-sectional studies in epidemiology using different large populations have shown that the percentage of type 2 diabetes among AD patients is significantly higher than among age-matched non-AD controls [49,67,83]. At the same time, type 2 diabetes patients are also shown to suffer more from cognitive impairment, lower Mini-Mental Status Exam (MMSE) score and lower rate of correct Clock Drawing Test (numbers and hands), as compared with the non-diabetic subjects [80].

In longitudinal studies following large populations prospectively, type 2 diabetes subjects as compared to the non-diabetic control group have double the risk of AD [4,34,51,68,70,96]. Moreover, the rate of onset of AD is higher among patients who have suffered from type 2 diabetes for more than 5 years compared to those with a disease duration of less than 5 years [51]. Such an observation indicates that the pathological process of association is slow, and probably accumulative. The oral diabetic medications that stimulate pancreatic β cells to release more insulin have been shown to elevate the incidence of AD among diabetes patients [56,68]. Insulin treatment caused even greater risk of AD among these subjects.

In AD, one of the neuropathological hallmarks is the presence of neuritic plaques in the brain, which contain extracellular deposits of Aβ that have formed filaments; another neuropathological character is neurofibrill tangle [33]. To date, multiple studies show that Aβ is the cause of AD [78] (reviewed by Selkoe), and yet soluble oligomers of Aβ possess more neurotoxic components [19,69,91]. The Honolulu study is a cohort study of ethnic Japanese males with neuropathological diagnosis made by autopsies. It showed that comorbid diabetes caused a higher number of neuritic plaques, neurofibrillary tangles and cerebral amyloid angiopathy in the AD brain [70]. Another study confirmed this finding, and also found that islet amyloid, the pancreas pathology found in some type 2 diabetics, was more frequent and extensive in the AD patients without type 2 diabetes than non-AD controls [41].

Despite the strong evidence that type 2 diabetes increases the risk of AD in these studies, two cross-sectional studies reported lower rates of type 2 diabetes when the AD patients at the nursing home or at the clinic were compared to the controls [65,87]. Since these studies were based on severely ill patients, which were different from the community-based studies, those who suffered from both type 2 diabetes and AD were more likely not to be included if their life span was shorter due to the combination of the diseases. We summarize these current studies related to type 2 diabetes and AD in Table 1. In fact all the prospective studies, the majority of cross-sectional studies and the neuropathological analyses support the conclusion that type 2 diabetes increases the risk of AD. A possible cause of disagreements among research findings is that hyperinsulinaemia, which is variable present in diabetic patients, may be more specifically related to AD than type 2 diabetes per se.

3. Hyperinsulinaemia and the risk of Alzheimer’s disease in the absence of ApoE4

In the pre-clinical syndrome of type 2 diabetes, hyperinsulinaemia precedes hyperglycemia by many years [93], and as a result, the insensitivity of the insulin receptor or the defect of signal transduction, probably due to chronic
over-stimulation, is considered to be the cause of the disease. After the onset of type 2 diabetes, hyperinsulinoma is present among many but not all diagnosed cases [50]. Several studies have shown that insulin concentrations in serum were higher among AD patients as compared to a control group [14,30,49,75]. One cross-sectional study reported that 37% of AD subjects suffer from impaired glucose tolerance, presumably also having elevated plasma insulin, versus 19.9% of non-AD subjects in the same population [49]. Insulin levels under fasting conditions are associated more with AD than are insulin levels 2 h post glucose loading. It has been shown that hyperinsulinemia (>89.4 pmol/l) is associated with a high risk of AD among the subjects who do not carry ApoE4 allele (7.5% versus 1.4%, P=0.0004), but has no effect on the risk when the ApoE4 gene is present [17,49].

Insulin-mediated energy metabolism was lower in the AD patients without ApoE4 than the AD patients with ApoE4 [16].

A recent longitudinal study conducted by Mayeux et al. has further indicated that hyperinsulinemia increased the risk of AD [55]. Craft et al. have also shown that the plasma concentration of insulin is positively correlated with the severity of AD [18]. Strikingly, the same group has demonstrated that peripheral infusion of insulin in the elderly people (mean age 68.7) increased the level of Aβ in CSF within 120 min, which also correlated with the decreased memory function [92]. Such phenomenon was not observed among the younger subjects (mean age 60.8) in the study. Nevertheless, unlike the studies of the peripheral insulin level and the risk of AD, the relationship between the insulin level in cerebrospinal fluid (CSF) and AD remains unclear. One study has reported that the insulin level in CSF was decreased among AD patients [18], yet another report showed no difference in the CSF insulin levels between the AD cases and controls [60].

Cognitive impairment, the clinical symptom of AD, is also shown to be associated with elevated plasma insulin in the absence of clinical diabetes syndrome. One case report showed that a patient with insulinoma presented with cognitive impairment, which was not resolved after blood glucose concentration was corrected [38]. Two cross-sectional studies have demonstrated that both lower Mini-Mental State Exam (MMSE) score and poor long-term memory were significantly correlated with higher insulin level [84,89]. The association was present with and without cardiovascular disease, and also present after excluding subjects with diabetes [84].

Unlike the positive correlation between type 2 diabetes and AD described above, which was not demonstrated in the samples of nursing home and ambulatory patients, all the epidemiological studies have consistently shown the relationship between increased peripheral insulin levels and the risk of AD. In summary, these studies suggest that elevated insulin rather than type 2 diabetes alone leads to AD pathology, especially in populations without ApoE4 allele.

4. Insulin-degrading enzyme

There are probably several mechanisms underlying the relationship between type 2 diabetes and the increased risk of AD. For example, the formation of advanced glycation end product (AGE) in diabetes has been shown to be aggregated with Aβ in plaques of AD brain. Diabetes also causes cerebrovascular changes that are associated with AD [20] (reviewed by de la Torre). However, these could not explain that elevated insulin itself without the clinical syndrome of type 2 diabetes is strongly related to AD [55,84]. We thus hypothesize that IDE plays the critical role in the mechanism associating hyperinsulinemia and type 2 diabetes with AD. We will present the evidence that AD might be caused in some cases by increases of Aβ due to the failure of clearance by IDE or the deficiency of IDE itself.

IDE is a neutral thiol metalloprotease, which requires both a free thiol and bivalent cations for its activity as a protease. It is a single polypeptide with a molecular weight of 110 kDa, and dimers or trimers of it have been purified under non-denaturing conditions [66]. Zn²⁺ is the metal bound to IDE. The active site of IDE consists of the sequence His-Glu-aa-His (HEXXH) in which the two histidines coordinate the binding of the zinc atom and the glutamate plays an essential role in catalysis [5,7]. IDE was co-isolated with the multicatalytic proteasome, suggesting that IDE might be involved in a protein complex [10]. An endogenous 14 kDa inhibitor of IDE appears to regulate its activity [66]. In addition, ubiquitin forms a complex with, and inhibits the activity of, IDE within the cells [77].

The gene encoding IDE is located on chromosome 10 q23–q25 in humans [2]. It spans approximately 120 kb, and contains 24 exons and large sequences of introns. The coding sequence is highly conserved during evolution from E. coli, to Drosophila, to human. The homologous regions of the IDE gene among eukaryots are contained in exons as well as in introns. This suggests that the protease function of IDE, which is mediated by the exons of IDE, and the regulation of IDE gene expression, which is likely buried in the surrounding sequences of exons, are conserved during evolution.

IDE is ubiquitously expressed, with its highest expression in the liver, testes, muscle and brain [46]. Its expression is regulated during cell differentiation and growth with IDE mRNA level increased in the brain and testes when development proceeds [6]. Further, IDE expression is affected by aging, with IDE activity significantly decreased in both the muscles and liver of old animals as compared to young animals [76].

The subcellular localization shows that IDE is abundant in cytosol and peroxisomes [5] (reviewed by Authier). In addition, IDE is also found in rough endoplasmic reticulum (RER), plasma membrane as well as in the extracellular compartment [73]. Although the mechanism of IDE to locate outside the cells is unclear, we identified intact IDE in human CSF, further indicating that IDE does exist in extracellular fluid under physiological condition. Biochemical studies have shown the presence of IDE in the soluble fractions from
human brains, which contain both extracellular and cytosolic compartments [57,71]. To exclude the possibility that IDE merely sticks to the plasma membrane during the subcellular separation, Vekrellis et al. biotinylated the surface of intact cells and still found the labeled IDE in the plasma membrane fraction [90]. IDE’s subcellular location seems regulated during development and differentiation. In undifferentiated neuronal PC 12 cells, IDE is found present on the cell surface as well as released into the extracellular space. When the cells differentiate in response to growth factor, IDE is no longer secreted [90].

5. Insulin-degrading enzyme degrades insulin, amylin and amyloid-β peptide

To date, all the identified genes with missense mutations that predispose an individual to AD either increase Aβ production or enhance Aβ fibrillation. The actual amount of neurotoxic Aβ in the brain is determined by (1) Aβ production through Amyloid Precursor Protein (APP) processing and (2) Aβ degradation and clearance. Several proteases are involved in Aβ degradation in vitro, but the two major enzymes in vivo are IDE and Neprilysin (NEP) [54].

Several short peptides with molecular weights of 3–10 kDa have been shown to serve as the substrates of IDE, including insulin [44], insulin-like growth factors I and II [58], amylin [9], Aβ [48,57,62,73] and others. The peptide substrates share little to no homology of primary amino acid sequence, but have similar secondary structure and amyloidogenic character [8,47]. Therefore, IDE likely plays a role in catabolic regulation, especially in preventing formation of amyloid deposits by cleaving the component peptides. Among the substrates of IDE, insulin and amylin are related to the pathogenesis of type 2 diabetes, while Aβ is involved in AD pathology.

IDE has been shown to play a major role in the degradation and clearance of insulin in vivo. A cross-linking study has shown that insulin binds IDE specifically in the intact cells [37]. Overexpression of IDE in cells in culture has been found to increase the rate of insulin degradation [45]. On the other hand, the injection of IDE specific antibodies into the cells inhibited the process of insulin degradation [79]. The GK rat is an animal model of type 2 diabetes. IDE gene mutations are the genetic cause of diabetes in these animals [26]. The mutated form of IDE expressed in these rats increases insulin level as a result of reduced insulin degradation, and causes symptoms typical of human type 2 diabetes syndrome [28].

Another pathological feature of type 2 diabetes is the presence of islet amyloid deposits composed mainly of amylin that causes pancreatic beta cells dysfunction [42] (reviewed by Kahn). In biochemical analyses, amylin is also specifically cross-linked to, and degraded by, IDE [9]. Thus the action of IDE in type 2 diabetes is complex, involving more than regulation of insulin levels alone.

Since Aβ is secreted extracellularly, and deposits outside the neuronal cells in the AD brain, we took an approach to screen any secreted protease(s) in neuronal and non-neuronal cell culture media for the ability to degrade Aβ. Among all secreted proteases from the cells, only IDE degraded Aβ. We found that under physiological conditions IDE is secreted at high levels from the microglial cells, and degrades Aβ extracellularly [73]. Purified IDE from rat liver and brain was shown to degrade Aβ effectively. IDE is present in the soluble fractions from human brains, and binds and degrades Aβ specifically [57,71]. Primary cultured neurons were also shown to clear Aβ via extracellular IDE as well as IDE on the cell surface [90]. IDE from brain homogenates degrades different forms of Aβ: Aβ40, Aβ42 and an Aβ mutant in one type of AD (Dutch Variant 1-40 Q) [61,71]. Aβ42 is the longer form of Aβ and more abundant in the AD brain.

Aβ degrading activity by IDE was shown to be lower in AD brains than in the controls [71]. Moreover, the amount of hippocampal IDE protein was also found to reduce in AD brains as compared to the controls [15]. When the IDE gene was deleted in mouse model, Aβ levels in the brain were elevated [27,58], suggesting IDE activity is critical in determining the amount of brain Aβ in vivo. More significantly, enhanced IDE activity in the IDE and APP double transgenic mice decreased their brain Aβ levels, and prevented the formation of AD pathology [52].

IDE has multiple substrates in vivo with different  , They can compete with each other to be degraded by IDE. One working hypothesis is that the imbalance of the substrates could affect the degradation process by IDE, and thus influence the pathogenesis of AD or type 2 diabetes. Indeed, adding increasing amounts of insulin, a substrate of IDE with low  ( ), specifically inhibited enzyme activity for degradation of Aβ ( ) [74] in the cell culture model for secreted IDE. Therefore, if the insulin level increases in the brain, it would inhibit IDE to degrade Aβ effectively, which could cause Aβ neurotoxicity, and then AD.

Insulin and insulin receptors are found abundantly in the brain [39], and the imbalance of insulin itself and its signal transduction in the brain might also contribute to the AD pathogenesis. Both insulin and its receptors are involved in synaptic transmission, and appear to play a role in learning and memory [94,97]. Insulin and insulin receptors were shown to decrease in a normal brain with aging, but increase in AD brains [29]. Several basic science studies have explored and shown the relationship between the increased insulin and AD pathology in the aspects other than Aβ degradation alone. For example, insulin increases the secretion of Aβ into extracellular space [31], stimulates tau phosphorylation to form neurofibrillary tangles, and impairs insulin signal transduction [32,40] (reviewed by Gasparini and Hoyer). Insulin also affected APP processing in vivo, a critical molecular step in generating Aβ, to secrete sAPP [13,16,81]. In addition, Aβ reduces insulin binding to insulin receptors [95].
6. Genetic variation of IDE has been associated with AD as well as type 2 diabetes

Recent genetic studies have shown that chromosome 10 contains potentially important novel gene(s) for late-onset AD as well as type 2 diabetes [21,22,64,86]. Since the IDE gene is located on chromosome 10 [11], and IDE demonstrates an ability to degrade insulin, amylin and Aβ, it is reasonable to hypothesize that IDE as a candidate gene for both type 2 diabetes and AD.

Several association studies using a single nucleotide polymorphism (SNP) approach have investigated the relationship between IDE and AD. Some of them have found that the variations at the intron and surrounding sequences of the IDE gene were related to late-onset AD in the absence of the ApoE4 allele. Edland et al. found that in the absence of ApoE4, certain haplotypes (the pattern of DNA variations) of the IDE gene predicted risk of late-onset AD in a case-control study \( (P = 0.008) \). In the presence of ApoE4, a risk of developing AD existed regardless of IDE genotype [23,24]. In another case-control study, which combined clinical symptoms, CSF analysis and neuropathological examination using four different populations, genetic variations in the IDE gene increased both the risk for developing AD and the severity of the disease [72]. In contrast, two other genotypes at the same locus, within a 276 kb linkage disequilibrium block identified by linkage studies on chromosome 10q that segregates with AD, were not found to be associated with AD in this study. Specifically two haplotypes of the IDE gene: H2 (odds ratio = 0.7, \( P \) value = 0.01) and H5 (odds ratio = 0.5, \( P \) value < 0.0001) were associated with a decreased risk of late-onset AD. Another IDE haplotype, H4, increases the risk of AD with an odds ratio of 2.9 and \( P \) value of <0.0001. The same genetic variations did not predict any risk of the disease in early-onset AD in their study. Interestingly, the IDE gene variations were more related to the specific clinical measurements of AD such as MMSE scores, tau levels in CSF, etc. than to the general diagnosis of AD alone. In addition, Ertekin-Taner et al. recently reported that IDE gene variations are also associated with plasma Aβ levels and the disease itself [25]. These results suggest that variations of the IDE gene likely modify the phenotype and the severity of AD. However, in the other two studies paid no regard to ApoE4 status, the IDE polymorphisms were not shown to be associated with the risk of AD [1,12].

Further, several studies have independently mapped an increased risk of AD and its quantitative traits to chromosome 10 using genome wide scans. Bertram et al. have performed the linkage analyses of seven genetic markers on chromosome 10q, and six of them mapped near the IDE gene [11]. The highest LOD score was observed in ApoE4-negative sub-sample at a marker (D10S1710) mapped 9 cM away from IDE. Furthermore, an allele-specific association between the risk of AD and a marker located within 195 kb of the IDE gene (D10S583) has been detected, and the finding was later confirmed by an independent case-control study [3]. In addition, the evidence for linkage to age at onset was identified \( \sim 9 \) cM from the IDE gene in AD families [53]. Myers et al. have reported a possible interaction between ApoE4 allele and chromosome 10 loci, and a peak region for linkage on this chromosome was as far as \( \sim 77 \) cM from the IDE gene [63,64]. Therefore, it still remains unclear whether the peak reported between D10S583 (115 cM) and D10S1671 (127 cM) [11] and the more proximal peak at D10S1225 (81 cM) [63] represent linkage to one or two underlying loci. It is also possible that the IDE gene or a gene close to IDE, impacts AD in the absence of ApoE4 allele, while the other one at the locus of chromosome 10 distant from IDE gene relates to the risk of AD only in the presence of ApoE4.

Like AD, type 2 diabetes is a disease with multiple etiologies, and several genetic loci have been shown to be related to the disease, including a locus (D10S587) on chromosome 10 [22]. IDE haplotypes at the 3′ region were shown to be associated with the diagnosis of type 2 diabetes in male subjects from the Framingham Heart Study population [43]. However, it has been found that fasting plasma glucose and Hba1c were associated with IDE polymorphisms at \( P \) value of 0.001–0.025 regardless of gender [43]. When only male subjects were analyzed, the associations between the clinical symptoms of type 2 diabetes and the IDE gene variation became even more prominent reaching the \( P \) value of 0.0001–0.0019. Interestingly, IDE polymorphisms were found not to be associated with type 2 diabetes in another case-control study [35]. Again, unlike the Framingham Heart study design described above [43], this study was conducted with both male and female subjects, and it used the diagnosis of type 2 diabetes as an outcome instead of specific clinical characteristics of the disease. In addition, the variation of the IDE gene also contributes to the different levels of plasma insulin [36].

Animal studies have revealed that dysfunctional IDE protease causes the diabetic and neuropathology in GK rats [26,28]. The studies described above did not consider the possible relationship between the variation of coding sequences of the IDE gene and the two diseases: type 2 diabetes and AD in humans. It suggests that the regulation of the IDE expression likely plays some role in the pathogenesis and the severity of AD and type 2 diabetes in humans. Nevertheless, these studies do not exclude the possibility that the gene(s) in close vicinity to IDE instead of the IDE gene itself increases the risk of AD.

7. Summary and future research

IDE degrades both insulin and amylin, which are related to type 2 diabetes; it also degrades Aβ, a peptide involved in AD pathology. The enzyme activity, including substrate affinities, presents a logical mechanism for the fact that hyperinsulinaemia and type 2 diabetes increase the risk of AD in elderly. We hypothesize that the deficiency of IDE, which leads to increased Aβ and thus AD pathology in the brain,
Gene therapy of AD: ApoE4 allele. The risk of AD in the subgroup of patients who do not carry the IDE gene variations is associated more strongly with Aβ degradation then the IDE work when degrading. This results in a relative deficiency of IDE. The authors thank Peg AtKisson, Igna Peter, Timothy Liu, she worked in his laboratory, and for his continuous support. W.Q.Q. thanks Dr. Dennis J. Selkoe for his encouragement and by grants from GREFF award (GCRC of New England Medical Center) and NEMC recruitment fund for W.Q.Q.

can be a result of either diversion of IDE from the metabolism of Aβ to the metabolism of insulin (Fig. 1A) or IDE genetic variation (Fig. 1B). Furthermore, hyperinsulinaemia as well as the IDE gene variations are associated more strongly with the risk of AD in the subgroup of patients who do not carry ApoE4 allele.

IDE offers new avenues to the prevention and treatment of AD:

1. Gene therapy. It has been demonstrated that increased gene dosage of IDE prevents and treats the AD pathology formation in an animal model of transgenetic mice [52]. As the biotechnology of gene therapy matures, this approach could become an effective way to treat AD.

2. Enzyme induction. Hersh’s group has found a novel peptide to induce IDE activity [82]. Other research teams are also actively searching for similar molecules. This approach is less invasive, and could be an ideal way to prevent AD.

3. Treating type 2 diabetes. Instead of compensating insulin resistance by the administration of exogenous insulin, researchers should focus on alternative treatments which reduce insulin resistance for the subgroup of type 2 diabetes patients with hyperinsulinaemia. Avoiding excessive insulin would allow more native IDE to be available for Aβ degradation, and thus reducing the risk of AD.

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