Prevention of Alzheimer's disease by control of beta-amyloid production in the periphery

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Abstract

Using a novel approach for identification of disease modifier genes, it was demonstrated that the level of expression of the *Presenilin2* gene by the liver regulates the accumulation of pathogenic concentrations of Alzheimer's disease (AD)-initiating beta-amyloid within the brain. The anti-leukemia therapeutic imatinib (trade name Gleevec), which does not cross the blood-brain barrier, reduced liver production of beta-amyloid and lowered its accumulation in the brain below pathogenic levels. These observations suggest that Alzheimer's disease is preventable. The imatinib-related compound, imatinib para-diaminomethylbenzene trihydrochloride, is more than three-fold more potent in inhibiting beta-amyloid production than imatinib and exhibits only 1/16th of the activity of imatinib in the inhibition of Abl kinase (the imatinib target in leukemia), resulting in a selectivity ratio of nearly 60 for the AD indication. These studies suggest that prophylactic reduction of beta-amyloid at the site of its production in the livers of aging humans has the potential to lower the incidence AD and point to the identity of a drug that can accomplish that goal.

Keywords: Alzheimer's disease; beta-amyloid; imatinib

1. Background

Alzheimer's disease (AD) is characterized by the age-dependent deposition of beta-amyloid within vulnerable regions of the brain, particularly the frontal cortex and hippocampus (Terry 2006). Beta-amyloid aggregates have a pathogenic effect, leading to progressive neuronal loss that causes deterioration of the ability of those brain regions to orchestrate both higher order and basic neural processes. As the deterioration worsens, the affected individual faces dementia and a worsening quality of life, and eventually the condition is fatal. Age is the greatest known risk factor for AD with an incidence of 30–50% in people 85 years or older. For a given individual, the time at which AD manifests is the consequence of an additional series of risk factors, some of which might be due to environmental causes, but many of which are attributable to that individual's genetic endowment.

1.1 AD Genetics

Some of the genes whose protein products affect AD risk have been identified. For example, certain variations (mutations) in the gene that encodes the Amyloid Precursor Protein (APP; Tanzi 1989), a cell membrane protein produced in all body tissues, predispose individuals to early-onset AD. APP is a substrate for proteolysis by the endogenous proteases beta and gamma secretase, liberating beta-amyloid proteolytic fragments ranging from 37 to 43 amino acid residues. The 42-residue species is thought to be the most pathogenic (Wolfe 2006), and forms aggregates, which, in addition to contributing to the plaques that deposit in the AD-affected brain, are thought to initiate processes that lead to cognitive deficits (Barten & Albright 2008). AD-predisposing variations in APP cluster in the vicinity of the cleavage sites, affecting the rate at which pathogenic beta-amyloid fragments are generated, their stability, and their ability to form aggregates (Selcoe 2001). Individuals inheriting such APP variations usually show signs of AD

in their 50s, whereas sporadic AD is not common until individuals reach their 70s (Waring & Rosenberg 2008). Rare variations in two other genes, *Presenilin 1* and *Presenilin 2*, also confer high risk to early-onset AD. These two genes encode independent proteins of similar structures that function as part of the gamma secretase protein complex (Wolfe 2006). As a consequence of these genetic observations and considerable experimentation, the so-called "amyloid cascade" model that has emerged holds that biochemical events that increase the production and accumulation of beta-amyloid, particularly Aβ-42, accelerate the onset and progression of AD.

1.2 The Blood-Brain Barrier and the Implications of Regulation of beta-amyloid in Peripheral Tissues

The tacit assumption of the researchers in this field has been that local over-production of pathogenic beta-amyloid within the brain is the problem and that the likely solution would involve development of beta and gamma secretase inhibitors designed to penetrate the Blood-Brain Barrier (BBB) so as to reduce local generation of the beta-amyloid. The BBB, which protects the mostly non-regenerating cells of the brain by isolating it, is normally impermeable to small polar molecules. Efforts to develop BBB-penetrating AD therapeutics have thus far been disappointing. Recent research (Sutcliffe et al. 2011) has now shown that cerebral levels of beta-amyloid are regulated by peripheral tissues. One implication of this discovery is that therapeutic penetration of the BBB is unnecessary. This represents a fundamental change in concepts concerning AD pathogenesis.

2. Alzheimer's Modifier Gene

Although mice do not normally develop Alzheimer's-like disease, they can be "engineered" to get Alzheimer's disease by the introduction of an APP transgene that contains the so-called Swedish mutations: two mutations that in humans predispose individuals to early onset Alzheimer's (Kulnane & Lamb 2001). In some genetically pure strains of

mice (such as B6) the APP transgene causes high levels of brain beta-amyloid leading to cognitive deficits as measured in maze tests (Hock et al. 2009) and deposition of beta-amyloid in plaques (Lehman et al. 2003). Other pure strains of mice (such as D2) accumulate less beta-amyloid in the brain, do not develop cognitive deficits, nor do they get plaques. The genetic differences in the mouse genome responsible for the difference in susceptibility to Alzheimer's disease have been mapped to three broad chromosomal regions by Bruce Lamb and colleagues (Ryman et al. 2008), but the identities of the responsible genes were not known.

Forty pure strains of mice that had been generated by breeding B6 mice with D2 mice were studied (Sutcliffe at al. 2011). These 40 recombinant inbred (RI) strains had each been made "pure" by brother-sister matings over more than 20 generations. Each of the 40 RI strains inherited some (approximately half) of its genes from B6 and the remaining approximately half from D2, but each RI strain had a different mix of genes. A database that contained the activities of every one of the mouse's approximately 25,000 genes (how much mRNA product each gene made) in each of 10 tissues (including liver and several areas of the brain) in all 40 pure mouse stains was constructed. The database was queried as to whether any genes had activities that were inherited in the 40 strains in exactly the same manner in which susceptibility to Alzheimer's disease was inherited. A gene was discovered in each of the three chromosomal intervals: chromosome 1, *Psen2*; chromosome 2, *Zfhx 1b*; chromosome 7, *Cib1* (Sutcliffe et al. 2011).

It was expected that their heritable differences in activities would be observed within the brain, but that was not what was found. Instead, unexpectedly, it was discovered that the activity level of each of the three genes (how much mRNA product it made) was higher in the liver of all strains of mice that were susceptible to Alzheimer's disease than in any of those that were resistant to the disease.

For example, trait correlation regression analysis was performed between the genotype of the *Psen2* interval on chromosome 1 and the amount of Psen2 mRNA that accumulates in each of the 10 tissues in the RI mice, calculating the Pearson's product-moment correlation values (Sutcliffe et al. 2011). The values are shown in Table 1. None of the tissue samples derived from brain shows high heritability of *Psen2* expression, as was evident by the overlapping distributions of mRNA expression levels between lines carrying the alternative genotypes at the *Psen2* locus. Thus, *Psen2* expression in the brain is not a modifier of brain beta-amyloid accumulation. However, in the liver, the amount of Psen2 mRNA was highly correlated ($p = 4.99$ e-41) with the genotype at the *Psen2* locus. Furthermore, mice inheriting the B6 genotype express 2-to-8-fold more Psen2 mRNA in the liver than do D2-genotype mice.

Table 1: Heritability of Psen2 mRNA accumulation in various tissues*

	r	**r^2**
brain	$\lvert r \rvert < 0.05$	<0.0025
cerebellum	r = 0.6344	0.4025
eye	$\lvert r \rvert < 0.35$	<0.1225
hippocampus	$\lvert r \rvert < 0.36$	<0.1296
kidney	r = -0.4733	0.2240
liver	r = -0.9402	0.8840
nucleus accumbens	r = 0.7260	0.5271
neocortex	r = 0.5500	0.3025
prefrontal cortex	$\lvert r \rvert < 0.51$	<0.2601
striatum	r = 0.5329	0.2840

***** Pearson's product-moment correlation value (r), negative correlation values indicate expression of Psen2 mRNA in B6 genotypes is higher than in D2 genotypes. $\lvert r \rvert < 0.8$ represent incomplete heritabilities unlikely to account for mapped QTLs because of overlapping distributions.

Although each of the three genes (*Psen2, Zfhx 1b* and *Cib1)* was also expressed in the brain, there was no correlation between the activity level of the genes in the brain and the inheritance of Alzheimer's disease resistance. That

suggested that the genes were acting in the liver to modify the susceptibility of the mice to Alzheimer's disease. Strengthening this argument is that the chromosome 7 gene, *Cib1,* encodes calmyrin, a protein discovered because of its preferential interaction with Presenilin 2 (the *Psen2* product) in a two-hybrid screen (Stabler et al. 1999).

3. Pathogenic Amyloid Comes From Peripheral Tissues

The *Psen2* gene encodes the 448 amino acid trans-membrane protein Presenilin2. In humans, some *Presenilin2* mutations confer risk for early-onset Alzheimer's disease. The product of the *Presenilin2* gene is the probable catalytic component of the gamma secretase complex, responsible for cleavage of beta-amyloid from APP. It was hypothesized that higher activity of *Presenilin2* in the liver increases the clipping rate leading to more beta-amyloid released by the liver into the blood, where it can form small aggregates and migrate to the brain and eventually initiate pathology. Were this hypothesis correct, then one ought to be able to reduce the amount of beta-amyloid in the brain by reducing how much is made by the liver. The leukemia drug imatinib (Gleevec) was known to inhibit the APP clipping rate by interfering with the interaction between gamma-secretase and its activator, GSAP (He et al. 2010). Imatinib does not enter the brain; therefore, it had not been tested to determine whether it could reduce accumulation of beta-amyloid in the brain.

The test was conducted by injecting mice with imatinib for 1 week. Even though the drug cannot get to the brain, it reduced the amount of brain beta-amyloid by about 50% (Sutcliffe et al. 2011). That implied that a considerable portion of the brain amyloid was produced outside of the brain but rapidly entered the brain. Liver is a major source of beta-amyloid origin, but there might be other non-brain sources in addition to liver that have not been tested. The take-away message is that the amount of cerebral amyloid, hence the risk for Alzheimer's disease, is regulated by peripheral tissues. This concept has been

confirmed and the results replicated in studies (Weintraub et al. 2013) which show that hippocampal beta-amyloid accumulation accompanied by cognitive deficits initiated by endotoxin-induced peripheral inflammation is eliminated by imatinib administration, and (Cancino et al. 2008) that imatinib administration to amyloid plaque-bearing APP transgenic mice led to plaque reduction and cognitive improvement. Furthermore, it has been shown (Eisele et al. 2014) that intraperitoneal injection of beta-amyloid aggregates leads to brain amyloidosis, demonstrating directly that peripheral beta-amyloid can enter the brain to initiate disease.

4. More Selective Amyloid-lowering Compounds

Any treatment that lowered the concentration of blood beta-amyloid to an acceptable level would be a candidate for chronic use for individuals wishing to lower their risk for this frightening disease. Compounds closely related in structure to imatinib, including its natural metabolites in the human body and byproducts of its organic synthesis, were studied to assess and compare their activities in inhibiting beta-amyloid production and tyrosine kinase inhibitor function. Ideal Alzheimer's medications would have higher potency in reducing beta-amyloid but lower potency in inhibiting tyrosine kinase activity, and thus their long-term maintenance use would be less cause for concern. Two compounds were identified that are more potent than imatinib in reducing beta-amyloid production by cultured cells transfected to produce human APP and which are more selective than imatinib in sparing tyrosine kinase activity in an in vitro assay for Abl kinase, the imatinib target enzyme in leukemia. The better of the two, imatinib para-diaminomethylbenzene trihydrochloride, is more than three-fold more potent in inhibiting beta-amyloid production than imatinib and exhibits only $1/16^{th}$ of the activity of imatinib in the inhibiting Abl kinase, thus represents a 60-fold improvement over imatinib in its selectivity for the Alzheimer's indication

(Sutcliffe & Hilbush 2016), positioning it as the potential drug for disease prevention over decades of use.

5. Further Considerations

These studies identify a peripheral source of beta-amyloid as a prime target for disease prevention in the therapeutic 'window of opportunity' preceding onset of AD. The unexpected discovery that lowering the production of beta-amyloid peripherally leads to a reduction in brain amyloid implies that elevated beta-amyloid in the circulation is potentially a hallmark (or clinical biomarker) of the preclinical stage preceding AD onset. Longitudinal studies have shown unequivocally that plasma beta-amyloid levels are consistently higher in individuals destined to develop Alzheimer's as compared to healthy controls both in autosomal dominant carriers (APP, PSEN1 and PSEN2) (Bateman et al. 2012; Fagan et al. 2014) and in Down syndrome patients (Zigman & Lott 2007; Head et al. 2011). The elevation of plasma beta-amyloid (specifically Aβ-42) in mutation carriers was shown to be stable for decades preceding age of onset (Fagan et al. 2014), suggesting a prolonged period of progressive accumulation that can be halted by drugs lowering its production in the periphery.

There is a general scientific agreement that non-aggregated beta-amyloid has beneficial effects within the brain and probably on many types of cells in the body. After all, its structure and activity has been selected through evolution. It is known that in humans, some variations in the APP gene that encodes beta-amyloid confer risk for early-onset Alzheimer's, and individuals with Down syndrome who carry an extra copy of this gene develop Alzheimer's very early; so beta-amyloid can have negative effects as well. Aggregated beta-amyloid is toxic to neurons and initiates the cellular pathologies we recognize as Alzheimer's disease. The studies reviewed here suggest that, at least in mice, the beta-amyloid produced locally within the brain is benign as far as Alzheimer's.

Beta-amyloid produced by the liver aggregates in the blood and some of it enters the brain. When added to the brain-made beta-amyloid, the toxic effect over prolonged decades initiates the disease process. As larger aggregates form, they are no longer soluble and deposit as plaques. The plaques themselves are probably benign, but demonstrate the sites within the brain where toxic aggregates have initiated pathology.

6. Conclusions and Future Directions

The mouse studies, both genetic and with imatinib, suggest that lowering blood (liver-derived) beta-amyloid by as little as 20% can reduce the brain beta-amyloid burden to a level below that which is pathogenic. It is probably a bad idea to get rid of all beta-amyloid, as for example it would be a bad idea to completely rid the blood of cholesterol: you need some, but too much takes a toll over time. Imatinib and drugs with similar actions taken as pills ought to be found to prevent or greatly delay the pathogenic effects of beta-amyloid in the brain – a future world with lower Alzheimer's incidence. Patent applications were filed on the dual discoveries that pathogenic beta-amyloid has a primary source in peripheral tissues outside of the brain (particularly the liver) and that the FDA-approved anti-leukemia drug imatinib is effective in reducing amyloid accumulation in the brain, even though it does not cross the blood-brain barrier (BBB), and additional applications on the discoveries of compounds that are more potent and selective for the Alzheimer's indication. Both patents have been approved and issued in Europe (Sutcliffe et al. 2015; Sutcliffe & Hilbush 2016) and are pending in other jurisdictions. The patent portfolio has been assigned to ModGene Pharma, LLC, which is seeking investors and pharmaceutical partners to run clinical trials that would allow development of AD-preventing medications.

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