Alzheimer's & Dementia 10 (2014) S411–S419

Lessons from a BACE1 inhibitor trial: Off-site but not off base

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Abstract Alzheimer's disease (AD) is characterized by formation of neuritic plaque primarily composed of a small filamentous protein called amyloid-β peptide (Aβ). The rate-limiting step in the production of Aβ is the processing of Aβ precursor protein (APP) by β-site APP-cleaving enzyme (BACE1). Hence, BACE1 activity plausibly plays a rate-limiting role in the generation of potentially toxic Aβ within brain and the development of AD, thereby making it an interesting drug target. A phase II trial of the promising LY2886721 inhibitor of BACE1 was suspended in June 2013 by Eli Lilly and Co., due to possible liver toxicity. This outcome was apparently a surprise to the study's team, particularly since BACE1 knockout mice and mice treated with the drug did not show such liver toxicity. Lilly proposed that the problem was not due to LY2886721 anti-BACE1 activity. We offer an alternative hypothesis, whereby anti-BACE1 activity may induce apparent hepatotoxicity through inhibiting BACE1's processing of β-galactoside α-2,6-sialyltransferase I (STGal6 I). In knockout mice, paralogues, such as BACE2 or cathepsin D, could partially compensate. Furthermore, the short duration of animal studies and short lifespan of study animals could mask effects that would require several decades to accumulate in humans. Inhibition of hepatic BACE1 activity in middle-aged humans would produce effects not detectable in mice. We present a testable model to explain the off-target effects of LY2886721 and highlight more broadly that so-called off-target drug effects might actually represent *off-site* effects that are not necessarily *off-target*. Consideration of this concept in forthcoming drug design, screening, and testing programs may prevent such failures in the future.

Keywords: Aging; Animal model; Brain disorder; CNS; Dementia; Demyelination; Drug trial; Liver damage; Human studies; Melatonin; Neuronal death; Sialylation; Secretase; Side effects; ROS

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly population of the United States [1]. AD is clinically characterized by a loss of cognition and memory, and this form of progressive dementia ultimately leads to changes in the patient's personality, incapacitation, and, eventually, loss of life. Major hallmarks of AD are deposits of amyloid plaques comprising mostly fibrillar amyloid-β (Aβ) peptide and neurofibrillary tangles comprising hyperphosphorylated tau [1]. Molecular mechanisms and environmental factors, both epigenetic and genetic, that underlie these pathologic findings are still unclear [2,3]. Current drug therapies exist for AD but mostly aim to treat the symptoms of this devastating disease. There is an urgent need for developing therapeutics to slow or potentially prevent the development of AD. In response, new therapeutic strategies and experimental drugs for AD are emerging [4–6]. Many clinical drug trials have been undertaken, but initial results have not been encouraging. Some of the issues with the clinical trial failures have been discussed recently [7–9]. Therefore, there is a need to better understand the biochemical and pathologic mechanisms of AD, which in turn may shed light on reasons underlying these recent failures and guide drug design with improved clinical outcomes.

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1552-5260/$ - see front matter
http://dx.doi.org/10.1016/j.jalz.2013.11.004

In this work we propose a plausible explanation for the recent failure of an Eli Lilly drug trial of β-site APP-cleaving enzyme and offer a testable model to explain the off-target effects of the drug, with a focus on learning lessons that would help prevent such failures in the future.

1.1. BACE1 as a relevant target for AD?

Neuropathologically, AD is characterized by the presence of amyloid-β (Aβ) peptide plaques in the hippocampus and cerebral cortex of the brain, which provides a primary diagnostic criterion of AD [1]. AD is believed to result from the dysregulation of the production and/or turnover of Aβ [10]. Hence, the β-site APP-cleaving enzyme 1 (BACE1), the rate-limiting enzyme in the pathway that produces Aβ peptide from the Aβ precursor protein (APP) [11], is considered a promising target for prevention or modification of AD [12].

BACE1 mRNA is transcribed from a 30.6-kb region of chromosome 11q23.2–11q23.3, consisting of 9 exons and 8 introns [13]. BACE1 genomic structure and functional characterization reveals that both the promoter region and 5'- and 3'-untranslated regions (UTR) are subjected to regulation [14–16]. Indeed, transcriptional regulation of BACE1 by p25/cdk5 leads to enhanced amyloidogenic processing [17]. Thus, changes in the activity of the promoter region could play an important role in regulating the level of BACE1 and accompanying activity in neurons [14]. By analogy, drug-based inhibition of the enzyme may have a similar effect as regulating promoter activity (i.e., changing the overall BACE1 activity level) and prove effective in treating AD. Production of Aβ from APP also involves the γ-secretase complex. However, inhibition of γ-secretase runs the risk of interfering in the broadly functional notch signaling pathway [18]. BACE1 knockout mice have not been reported to exhibit any dramatic ill effects over the course of their lifespan [19], although less attention has been paid to reports of timidity and reduced exploratory behavior that accompanies BACE1 knockout [20]. Thus, assuming the validity of the amyloid hypothesis, drug-induced inhibition of BACE1 activity would appear to be an ideal anti-AD strategy.

1.2. Failure of a BACE1 inhibitor clinical trial

Unfortunately, a recent phase II trial of the LY2886721 BACE1 inhibitor from Eli Lilly and Co. may have, at least temporarily, called this anti-AD strategy into question, due to signs of liver toxicity in test subjects [21]. Lilly has indicated that they believe this to be consequent to a secondary effect, unrelated to the drug's mechanism of action. At first, this appears to be a reasonable conclusion. After all, BACE1 knockout mice are viable and grow to adulthood without obvious liver injury [19]. Of potentially greater interest, BACE1 knockout mice have a variety of what would be presumed to be indicators of superior health, including lower fat, greater insulin sensitivity, and higher levels of brown adipose tissue [22]. However, in light of the LY2886721 trial outcome, deeper examination of BACE1 activity on substrates other than APP may indicate mechanisms that require additional attention.

2. BACE1 catalyzes more than Aβ cleavage

2.1. Implications of BACE1 off-site inhibition: Aberrant spindle formation, demyelination, and impaired motor coordination

In addition to APP processing, BACE1 plays an important role in other pathways. For example, peripheral nerves in newborn BACE1 knockout mice are thinly myelinated [23,24]. In a recent study, researchers reported that mice require BACE1 to form and sustain muscle spindles [25]. The study further demonstrated that normal spindle function was impaired not only during development but also into adulthood in BACE1 knockout animals. They showed that BACE1 deficiency in adult mice impairs motor coordination. Movement defects arise from abnormal morphology and low numbers of muscle spindles. Interestingly, similar deficiencies developed in wild-type mice treated with a structurally different BACE1 inhibitor, LY2811376, a Lilly agent that was found to lower Aβ within the cerebrospinal fluid (CSF) of healthy volunteers. Lilly terminated development of this compound consequent to its toxic properties in rats [26].

Spindle formation and maturation require BACE1 processing of neuregulin 1 (Nrg1), a transmembrane protein that regulates myelination. BACE1 and the disintegrin and metalloproteinase, ADAM10, have recently been described to cleave Nrg1 to generate a fragment that signals in a paracrine manner and rescues myelination in BACE1 knockout zebrafish [27,28].

2.2. Implications of off-site BACE1 inhibition: Liver damage susceptibility

In addition to its muscle tissue and neurologic activity, BACE1 is the major enzyme that cleaves β-galactoside α-2,6-sialyltransferase I (ST6Gal I) within the liver [29,30]. This generates a sialyl α-2,6-galactose residue, and the enzyme is secreted from the cell after proteolytic cleavage [31]. ST6Gal I cleavage is necessary for its secretion, and it is the secreted ST6Gal I that is active in glycoprotein sialylation in response to radiation stress. This protein modification is an essential step in resistance to radiation-induced cellular damage, since elimination of glycoprotein sialylation results in greater radiation-induced cytotoxicity [32]. BACE1 knockout mice have one third as much plasma ST6Gal I as control mice [30].