BACE1 inhibition reduces endogenous Abeta and alters APP processing in wild-type mice

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Abstract
Accumulation of amyloid beta peptide (Abeta) in brain is a hallmark of Alzheimer’s disease (AD). Inhibition of beta-site amyloid precursor protein (APP)-cleaving enzyme-1 (BACE1), the enzyme that initiates Abeta production, and other Abeta-lowering strategies are commonly tested in transgenic mice overexpressing mutant APP. However, sporadic AD cases, which represent the majority of AD patients, are free from the mutation and do not necessarily have overproduction of APP. In addition, the commonly used Swedish mutant APP alters APP cleavage. Therefore, testing Abeta-lowering strategies in transgenic mice may not be optimal. In this study, we investigated the impact of BACE1 inhibition in non-transgenic mice with physiologically relevant APP expression. Existing Abeta ELISAs are either relatively insensitive to mouse Abeta or not specific to full-length Abeta. A newly developed ELISA detected a significant reduction of full-length soluble Abeta 1–40 in mice with the BACE1 homozygous gene deletion or BACE1 inhibitor treatment, while the level of x-40 Abeta was moderately reduced due to detection of non-full-length Abeta and compensatory activation of alpha-secretase. These results confirmed the feasibility of Abeta reduction through BACE1 inhibition under physiological conditions. Studies using our new ELISA in non-transgenic mice provide more accurate evaluation of Abeta-reducing strategies than was previously feasible.

Keywords: Alzheimer's disease, amyloid beta, beta-site amyloid precursor protein-cleaving enzyme 1, enzyme-linked immunosorbance assay, secretase, soluble amyloid precursor protein alpha.

BACE1 knockout mice have been crossed with transgenic mice that overexpress human mutant APP. As expected, mice obtained by crossing BACE1−/− mice and APP transgenic mice showed virtually no Abeta production (Luo et al. 2003). Conversely, double-transgenic mice obtained by crossing transgenic mice that overexpress BACE1 and APP showed enhanced Abeta generation and exacerbated Abeta pathology (Willem et al. 2004). These results suggest that BACE1 is a strong candidate for anti-AD pharmacological intervention.

Abeta-lowering strategies, such as pharmacological BACE1 inhibition, are commonly investigated in APP transgenic mice. However, APP expression is not necessarily increased in sporadic AD, which represents the majority of AD patients. Consequently, testing Abeta-lowering approaches in transgenic mice may not be optimal. Particularly in studies of secretase inhibitors, the amount of substrate (APP) and the efficiency of APP cleavage are important factors that may determine Abeta production. Commonly-used APP transgenic mice carry the Swedish mutation APP, which is cleaved by BACE1 much more efficiently (> 50-fold) than wild-type (Tomasselli et al. 2003). These factors may compromise the outcome of therapeutic studies using APP transgenic mice. Thus, testing BACE1 inhibitors in wild-type mice with physiologically relevant APP expression may provide more meaningful results. However, commonly available ELISAs have a preference for human Abeta peptide, and do not have sufficient sensitivity to measure endogenous mouse Abeta. In addition, there are multiple cleavage sites in the Abeta domain, and various Abeta fragments are generated; thus, measurement of full-length Abeta is essential for accurate evaluation of Abeta-lowering strategies. However, currently available ELISAs use N-terminus region (but not end-specific) antibodies, and the results may reflect changes in various Abeta fragments. Our recently developed ELISA using the N-terminus end-specific antibody, 82E1, measures only full-length Abeta, but cross-reactivity to mouse Abeta is limited (Horikoshi et al. 2004).

In this study, we developed a full-length Abeta ELISA detecting endogenous Abeta in non-transgenic (wild-type) mouse brain. We used this tool to investigate how BACE1 activity affects Abeta levels and APP processing, using BACE1 knockout mice and BACE1 inhibitor treatment, in mice with physiologically relevant levels of non-mutant wild-type APP expression.

Materials and methods

Development and characterization of mouse/human cross-reactive N-terminus end-specific anti-Abeta antibody, clone 14F1
We used a partial Abeta peptide consisting of amino acid residues of Abeta 1–16 (DAEFRHDSGYEVHHQK) as an immunogen. This peptide was conjugated to the carrier protein, bovine thyroglobulin, through an additional cysteine residue at the C-terminus. Mice (BALB/c, Charles River, Yokohama, Japan) were immunized weekly with this antigen (50 µg/mouse).

After four to six immunizations, the spleen was isolated and fused with X63Ag8 myeloma cells. Epitopes and cross-reactivity to mouse and human Abeta of selected clones were determined by a microplate assay using various human and mouse Abeta fragments, including fragments starting with the second and third amino acid residue of Abeta. A 96-well plate (Maxisorp, Nunc, Rochester, NY, USA) was coated with Abeta fragments, and non-specific binding was blocked. The plates were incubated with selected antibodies for 30 min at 37°C, then with a horseradish peroxidase (HRP)-coupled anti-mouse IgG antibody (500 ng/mL, Immuno-Biological Laboratories (IBL), Takasaki, Japan), and visualized by O-phenylenediamine dihydrochloride (OPD) substrate (Sigma, St Louis, MO, USA).

For western blotting analysis, we used synthetic Abeta peptides and brain homogenate from APP-overexpressing transgenic mice, Tg2576 (Hsiao et al. 1996) (Taconic, Hudson, NY, USA). All samples were run on a 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gel and transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with 3% non-fat dried milk, the membrane was probed with an anti-Abeta antibody, clones 14F1, 82E1 and 6E10 (1.5, 1.0 and 1.0 µg IgG/mL, respectively), followed by HRP-coupled anti-mouse IgG.

For histochemical analysis, APP transgenic mice, Tg2576 (Hsiao et al. 1996) were perfused with 10 ml phosphate-buffered saline, pH 7.4 (PBS), followed by fixedate consisting of 4% paraformaldehyde. After dehydration with 20% sucrose in 0.1M phosphate buffer, brains were cut into 4 µm thick sections. Serial sections were incubated with the anti-Abeta antibody, clone 14F1 (2 µg IgG/mL), along with other N-terminus end (specific to the beta cleavage site) and N-terminus region-specific antibodies, clones 82E1 (Horikoshi et al. 2004) and 6E10 (1 and 2 µg IgG/mL), respectively, in 1% bovine serum albumin (BSA) and 0.05% sodium azide in PBS, pH 7.4, overnight at 4°C. Then, sections were incubated with biotinylated anti-mouse IgG for 30 min and visualized using a kit ( Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA).

Development of Abeta ELISA detecting endogenous mouse Abeta in non-transgenic mice
A 96-well plate (Maxisorp) was coated with an anti-Abeta 40 antibody, clone 1A10 (Horikoshi et al. 2004), overnight at 4°C. After blocking overnight at 4°C, standards (synthetic mouse Abeta peptide 1–40) and samples were loaded and incubated overnight at 4°C. The plate was incubated with HRP-coupled detection antibody, 14F1, and visualized using a 3,3′,5,5′-tetra methyl benzidine (TMB) substrate. To determine the specificity to full-length Abeta 1–40, we incubated Abeta fragments, 2–40 and 3–40, and developed the plate as described above. We also used human Abeta 1–40 and mouse Abeta 1–40 to determine the mouse/human cross-reactivity.

In vitro studies using primary cultured neurons from mice
Mouse primary neurons were prepared from the cerebral cortex and hippocampal formation of mouse embryos (ICR, Charles River) at embryonic days 17–18, and cultured in NeuroBasal medium supplemented with B-27 (Invitrogen, Carlsbad, CA, USA). Cells were studied on the sixth day after preparation. A gamma secretase
The brain was isolated from BACE1 knockout mice (Cai et al. 2001) and a BACE1 inhibitor, N-[N-(3,5-difluorophenacyl)-l-alanly]-S-phenylglycine t-butyl ester (DAPT; Calbiochem, San Diego, CA, USA) (Dovey et al. 2001) and a BACE1 inhibitor, N-[1S,2R]-1-benzyl-3-(cyclopropylamino)-2-hydroxypropyl)-5-(methyl(methylsulfonyl)amino-N'-(1R)-1-phenylethyl)isophthalamide (Inhibitor IV; Calbi-ochem) (Stachel et al. 2004), were dissolved in dimethylsulfoxide (DMSO) and added to culture medium at a 1 : 100 dilution. For the control, we added DMSO to yield 1% concentration in the culture medium. We treated cells for 24 h and determined Abeta levels in the culture medium by ELISA. After collecting the culture medium, cells were further incubated for 2 h with a tetrazolium salt, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium monosodium salt, and the cell toxicity was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) (Mosmann 1983)-based cell assay kit (WST-8 kit; Kishida Chemical, Osaka, Japan).

In vivo studies using BACE1 knockout mice
The brain was isolated from BACE1 knockout mice (Cai et al. 2001) carrying wild-type, heterozygous and homozygous (BACE+/+, BACE/+– and BACE–/–, respectively) genotypes (three female mice at 11 weeks-of-age in each genotype). The brain was homogenized in a 10-fold volume of 50 mM Tris-HCl buffer, pH 7.6, containing 11 weeks-of-age in each genotype). The brain was homogenized in 1% Nonident P-40, 150 mM NaCl and 1 mM EDTA, and used as positive controls for immunoblotting.

For positive controls, full-length APP, soluble amyloid precursor protein alpha (sAPPalpha) and sAPPbeta were prepared. In brief, the full-length DNA of human APP was amplified from human brain cDNA (Clontech, Mountain View, CA, USA) using a forward primer, 5′-CGGTCGACTCGCGATGC-3′ and a reverse primer, 5′-CGGCCGCCGGTCTAGTTCGATCATGCCTC-3′. The amplified products were digested with Sall and Notl, ligated into pGEX-6P-1 vector (Amersham, Piscataway, NJ, USA) and transformed into Escherichia coli (E. coli) JM109. After the sequence of cloned APP (APP695) was confirmed, sAPPalpha and sAPPbeta cDNAs were amplified using APP695 cDNA as a template. We used forward primer, 5′-CGGTCGACTCGGATGATGAACTT-3′ and a reverse primer, 5′-CGGCCGCCGGTCTAGTTCGATCATGCCTC-3′. The amplified products were digested with Sall and Notl, ligated into pGEX-6P-1 vector (Amersham, Piscataway, NJ, USA) and transformed into Escherichia coli (E. coli) JM109. After the sequence of cloned APP695 (APP695) was confirmed, sAPPalpha, sAPPbeta cDNAs were amplified using APP695 cDNA as a template. We used forward primer, 5′-CGGTCGACTCGGATGATGAACTT-3′ and a reverse primer, 5′-CGGCCGCCGGTCTAGTTCGATCATGCCTC-3′. The amplified products were digested with Sall and Notl, ligated into pGEX-6P-1 vector and used to transform E. coli JM109 cells. We confirmed the sequence of cloned sAPPalpha and sAPPbeta. APP695, sAPPalpha and sAPPbeta cDNAs in pGEX-6P-1 vector were transfected into pcDNA3.1(+) (Invitrogen) and transfected into COS-1 cells using FuGENE6 (Roche Diagnostics, Basel, Switzerland). After 2 days, cells were harvested in 10 mm Tris, pH 8.0, consisting of 1% Nonident P-40, 150 mM NaCl and 1 mM EDTA, and used as positive controls for immunoblotting.

For sAPPbeta and sAPPalpha detection, crude homogenates were mixed with diethylamine to yield 0.4% concentration, then centrifuged at 100 000 g for 45 min. The resultant supernatant fluids were mixed with SDS sample buffer and run on a gel. Proteins were transferred to a PVDF membrane and the membrane was incubated with non-fat skim milk to minimize non-specific binding. The membranes were probed with a primary antibody overnight at 4°C. We used the commercially available antibodies mouse monoclonal anti-sAPPalpha antibody (clone 2B3, 5 µg IgG/mL; IBL, Gunma, Japan) and a rabbit polyclonal anti-wild-type sAPPbeta antibody (2 µg IgG/mL, IBL). The membranes were then incubated with an HRP-coupled secondary antibody for 2 h at room temperature (20°C), and protein bands were visualized using a chemiluminescence kit (Pierce, Rockford, IL, USA). Protein bands were densitometrically analyzed (Quantity One, Bio-Rad, Hercules, CA, USA), and statistical significance was determined by t-test.

In vivo studies using BACE1 inhibitor
Non-transgenic wild-type male mice (ICR, Charles River) at 8 weeks-of-age (body weight 34.8 ± 1.5 g) were used (n = 10 in each vehicle- and drug-treated group). A BACE1 inhibitor, Inhibitor IV (Stachel et al. 2004) (10 µg Inhibitor IV in 2 µL 40% DMSO/mouse) was stereotaxically administered into the cerebral ventricle (Bregma −0.7 mm, 1.8 mm lateral, 2.5 mm depth). For control, we injected 40% DMSO in saline (vehicle). After 4 h, the brain was isolated and the samples were prepared as described above.

Results
Antibody characterization and development of a full-length Abeta ELISA sensitive enough to detect endogenous mouse Abeta in wild-type mouse brain
We screened numerous clones and selected 14F1, which is highly sensitive to mouse Abeta. The plate assay using various Abeta fragments revealed that the epitope of clone 14F1 locates amino acid residues 1–4 of Abeta. Clone 14F1 detects both human and mouse Abeta synthetic peptides, but has a preference for mouse Abeta (Fig. 1ai, lane H vs. M). As amino acid residues 1–4 of Abeta are identical in humans and mice, conformational differences between human and mouse Abeta presumably determine the mouse preference of 14F1. Our N-terminus end-specific antibody, clone 82E1 (epitope: 1–5), and a commercially available N-terminus region antibody, clone 6E10 (epitope: 3–8), show a preference for human Abeta because the fifth amino acid residue, which is specific to humans, is within their epitopes (Figs 1ai and ii, lane H vs. M). These antibodies detected Abeta in APP Tg2576 transgenic mouse brain homogenate (Fig. 1a, lane Tg). Because clone 14F1 has a preference for mouse Abeta, the 14F1-detected Abeta band in transgenic mouse brain homogenate was weaker than the 82E1- and 6E10-detected bands. An ELISA study using human and mouse Abeta 1–40 determined that 14F1/1A10 ELISA has approximately 50% cross-reactivity to human Abeta. Clone 6E10 detected uncleaved APP in addition to Abeta (Fig. 1aii), while clones 82E1 and 14F1 did not (Figs 1ai and iii).
14F1 stained Abeta plaques in APP Tg2576 mouse brain sections in a manner similar to other Abeta antibodies, clones 82E1 and 6E10 (Fig. 1b). We also found that clone 14F1 can be used for immunoprecipitation (data not shown).

We used two Abeta ELISAs, 14F1/1A10 and 12B2/1A10, in this study (Fig. 1c and Table 1). An ELISA composed of 12B2/1A10 detected human and mouse Abeta equally, and detected endogenous Abeta in non-transgenic mouse brain homogenate. However, the epitope of 12B2 (epitope: within 17–28) is located between the alpha and gamma secretase cleavage sites. Thus, the 12B2/1A10 ELISA measures the alpha and gamma secretase-cleaved fragment, P3, in addition to Abeta (Horikoshi et al. 2004).

The epitope of our newly developed clone, 14F1, is located between amino acid residues 1–4 of Abeta, and the ELISA combining 14F1 with the Abeta 40-specific 1A10 antibody measures full-length Abeta 1–40. To confirm the specificity to full-length Abeta 1–40, we examined cross-reactivity to Abeta 2–40 and 3–40, using Abeta fragments, and determined this to be 0.78% and 0.08%, respectively. Our new 14F1/1A10 ELISA achieved sensitivity to single digit fmol/mL (equivalent to sub pg/mL), and detected endogenous full-length Abeta 1–40 in non-transgenic mouse brain homogenate (Fig. 3) and cell culture medium from primary cultured neurons (Fig. 2).

Full-length Abeta ELISA provides accurate assessment of secretase inhibitor-mediated Abeta reduction in primary cultured neurons

Primary cultured neurons prepared from mouse embryos were treated with a beta and gamma secretase inhibitor, Inhibitor IV (Stachel et al. 2004) and DAPT (Dovey et al. 2001), respectively, and Abeta levels in the culture medium were determined by 14F1/1A10 and 12B2/1A10 ELISAs. In the primary cultured neurons treated with the gamma secretase inhibitor, both full-length 1–40 (14F1/1A10) and P3 cross-reactive x-40 (12B2/1A10) Abeta ELISAs provided similar results (Fig. 2a). In the case of the BACE1
inhibitor (Fig. 2b), Abeta levels determined by P3 cross-reactive Abeta x-40 ELISA (12B2/1A10) were always higher than full-length Abeta determined by 14F1/1A10 ELISA. At the highest tested concentration, 1 μM, full-length Abeta ELISA indicated virtually complete inhibition of Abeta production, while P3 cross-reactive Abeta x-40 ELISA detected only 67% Abeta reduction (Fig. 2b, the difference is indicated by bilateral arrow). At all testing concentrations, MTT determined cell toxicity to be < 10% of vehicle-treated cells.

Genetic and pharmacological BACE1 inhibition lowered brain Abeta levels in wild-type (non-transgenic) mice We determined brain Abeta levels using 1–40 and x-40 Abeta ELISAs in BACE1 knockout mice and mice treated with a BACE1 inhibitor, Inhibitor IV. Full-length 1–40 Abeta ELISA (14F1/1A10) determined that levels in BACE1–/– mice were at the background level (OD = 0.067–0.073, compared with the background level, OD = 0.055–0.071 (OD, optical density). Full-length Abeta 1–40 level in BACE1+/– mice was virtually the same as in BACE1+/+ controls (Fig. 3ai). While full-length Abeta 1–40 is diminished in BACE1–/– mice, P3 cross-reactive Abeta x-40 ELISA (12B2/1A10) detected a much less significant Abeta reduction (only 33% reduction compared with BACE1+/+ control, p < 0.01, Fig. 3aii).

In mice receiving the BACE1 inhibitor, Inhibitor IV, there was significant reduction in Abeta 1–40 level (30% reduction compared with vehicle-administered mice, p < 0.01, Fig. 3bi). However, the Abeta x-40 level did not change significantly (13% reduction, no significance, Fig. 3bii).

With systemic administration, Inhibitor IV did not reduce brain Abeta, although it reduced plasma Abeta (36% reduction compared with vehicle-treated mice, 100 mg/kg.
subcutaneous injection). Inhibitor IV is a substrate of P-glycoprotein brain efflux transporter (Stachel et al. 2006); therefore, we suspect that the lack of efficacy following systemic administration might be due to insufficient drug concentration in the brain.

Up-regulation of alpha secretase cleavage in response to genetic and pharmacological BACE1 inhibition

We used commercially available sAPPalpha- and sAPPbeta-specific antibodies to determine how BACE1 inhibition alters APP processing. Western blotting confirmed the specificities of these antibodies, and that sAPPalpha- and sAPPbeta-specific antibodies did not cross-react with each other or uncleaved APP (Fig. 4a).

In BACE1−/− mice, the levels of sAPPbeta were diminished (protein bands were undetectable) (Fig. 4bi) and sAPPalpha was significantly elevated (250% of control, p < 0.001; Fig. 4bii). Intracerebroventricular injection with a BACE1 inhibitor, Inhibitor IV, significantly reduced sAPPbeta level (59% reduction, p < 0.01; Fig. 4ci) and significantly elevated sAPPalpha level (60% elevation, p < 0.05; Fig. 4cii).

Discussion

Determination of Abeta change under physiological condition is more relevant to sporadic AD

Beta and gamma secretases are promising therapeutic targets in AD, and transgenic mice that overexpress mutant human APP have been commonly used for evaluation of potential treatments. However, early onset AD cases with increased generation of Abeta caused by genetic mutation represent a small percentage of the overall population suffering from AD (Rocca et al. 1991). There is no clear evidence that APP expression is up-regulated in sporadic AD cases. Therefore, testing therapeutic agents under physiologically relevant conditions, i.e. in mice with physiological APP expression, is important. We previously developed an N-terminus end-specific antibody, clone 82E1, which detects full-length Abeta in combination with C-terminus antibodies. However, 82E1 includes the fifth amino acid residue unique to humans within the epitope, and it was not sensitive enough to detect endogenous Abeta in non-transgenic (wild-type) mouse
brain. In this study, we developed an N-terminus end-specific antibody, clone 14F1, with an epitope within amino acid residues 1–4 of the Abeta peptide. Although amino acid residues 1–4 of Abeta are identical in humans and mice, clone 14F1 showed preference for mouse Abeta. We suspect that conformational differences between human and mouse Abeta determined this species preference of clone 14F1. The ELISA, which is composed of 14F1 and the Abeta 40-specific clone, 1A10 (Horikoshi et al. 2004), detects full-length endogenous Abeta 1–40 in non-transgenic mouse brain homogenate. This is the first full-length Abeta ELISA to detect Abeta change in wild-type mice.

Multiple secretases are involved in Abeta generation. Beta-secretase cleaves APP and generates sAPPalpha and the C99 fragment, then gamma-secretase cleaves C99 and generates C83 and generates P3. In addition to these primary cleavage sites, additional cleavage sites are known. For example, cells overexpressing BACE1 generate C89 (the secondary beta-cleavage product starting with the Glu11 amino acid of the Abeta domain) in addition to C99 (the primary beta-cleavage product starting with Asp1 of the Abeta domain) (Haass et al. 1992; Gouras et al. 1998; Vassar et al. 1999). There are some mouse cross-reactive antibodies, and they typically have epitopes within amino acid residues 14–28, a region that is identical in human and mouse Abeta. A commonly used mouse cross-active Abeta ELISA includes clone BNT77 (Suzuki et al. 1994), which has an epitope within amino acid residues 11–16 of Abeta (Fukumoto et al. 1999). The BNT77 ELISA is not cross-reactive to P3, but it still detects the Abeta 11–40 fragment [generated by cleavage at the beta’ (Glu11)-site], which is undesirable. Determination of full-length Abeta is essential for the accurate evaluation of Abeta changes, and our newly developed 14F1/1A10 ELISA provides the most accurate and useful results currently available.

**BACE1 as a therapeutic target**

BACE1-lowering approaches, including secretase inhibition, are being extensively pursued as potential disease-modifying therapies for AD (Aisen 2005). BACE1 initiates Abeta generation, and BACE1 enzyme activity and/or BACE1 protein level were elevated in sporadic AD brains (Fukumoto et al. 2002; Holsinger et al. 2002; Yang et al. 2003). Thus, BACE1 is among the most aggressively pursued pharmacological targets in AD.

In our study, we found that endogenous full-length mouse Abeta is diminished in BACE1−/− mice, confirming that BACE1 is primarily responsible for Abeta generation. While complete BACE1 gene deletion yielded expected results, the effect of partial gene deletion seemed complicated. Heterozygous BACE1 knockout mice were crossed with Swedish APP transgenic mice, and Abeta levels were investigated. At a pre-pathological stage, Abeta was slightly reduced (approximately 20%) (Luo et al. 2003). Abeta plaque load was significantly reduced (37%) at the moderate pathological stage, but the efficacy was lost at the severe pathological stage (Laird et al. 2005). Transient BACE1 gene silencing through intrahippocampal injection with small interfering RNA (siRNA) reduced Abeta load in APP transgenic mice (Laird et al. 2005; Singer et al. 2005). While partial gene deletion reduced Abeta level in mice overexpressing Swedish APP, we found no change in endogenous mouse Abeta in non-transgenic mice with physiological APP expression. Under conditions of excess substrate (APP), BACE1 is more sensitive to inhibitory actions, and the impact on Abeta levels may be exaggerated. In the case of gamma secretase inhibition, the amount of the substrate affected the Abeta reduction in vivo. A gamma secretase inhibitor, 2-[(1R)-1-[[4-chlorophenyl]sulfonyl]2,5-difluorophenyl]amino]ethyl]-5-fluorobenzepropanoic acid (BMS-299897), showed greater efficacy in APP transgenic mice with excess APP, compared with APP-YAC mice (Lamb et al. 1993) and guinea pigs with more physiologically relevant APP expression (Anderson et al. 2005; Barten et al. 2005). This suggests that testing secretase modulating approaches in APP overexpressing transgenic mice may yield artificially amplified results, resulting in overly optimistic efficacy projections. It should also be noted that Swedish mutation APP is a highly efficient BACE1 substrate (> 50-fold compared with wild-type) (Tomasselli et al. 2003), and this may confound the results. Plaque-forming transgenic mice are essential for testing disease modifying effects, but the effect of the APP mutation on outcome should be carefully considered.

A potential pharmacological agent and complete gene deletion significantly reduced endogenous brain Abeta levels in non-transgenic mice. This suggests that both approaches, enzyme inhibition and protein expression inhibition, may have therapeutic value. As heterozygous gene deletion did not reduce endogenous Abeta levels, substantial reduction (beyond 50%) of BACE1 protein level is presumably necessary. A potential enzyme inhibitor (IC$_{50}$ = 6 nM in primary cultured neurons) showed significant but moderate efficacy (30% reduction). It is unknown how much Abeta reduction would be required for clinical efficacy in AD; simultaneous inhibition of additional targets, such as gamma secretase, may be required. Along with the benefit of Abeta reduction, BACE1 inhibition elevated sAPPalpha levels in vivo, as suggested by a cell culture study (Skovronsky et al. 2000). sAPPalpha is not only non-amyloidogenic but also, neurotrophic and neuroprotective (Mattson et al. 1993, 1997; Schubert and Behl 1993). BACE1 inhibition reduces harmful Abeta, and the resulting compensatory increase in sAPPalpha has a favorable effect; this reconfirms BACE1 as a promising therapeutic target.
In conclusion, we developed a full-length Abeta ELISA that facilitates the evaluation of endogenous Abeta in non-transgenic mice. We previously developed a full-length human Abeta ELISA using the N-terminus end-specific antibody, clone 82E1 (Horikoshi et al. 2004). However, clone 82E1 has strong preference for human Abeta, and the ELISA using 82E1 does not detect endogenous Abeta in wild-type mouse brain. Our new antibody, 14F1, is also specific to the N-terminus end, and the ELISA composed of 14F1 with an anti-Abeta 40 antibody detects endogenous full-length mouse Abeta. This new ELISA facilitates in vivo investigations of Abeta-lowering strategies using wild-type (non-transgenic) mice with physiologically relevant APP expression. With this tool, we confirmed the feasibility of Abeta reduction through modulation of BACE1 using BACE1 knockout mice and a pharmacological agent. Testing in non-transgenic mice may be essential, because excess APP in transgenic mice may exaggerate the therapeutic impact of BACE1 inhibition and other Abeta-lowering strategies. In addition, testing candidate interventions in wild-type mice is rapid and cost-effective, and hence, this approach is valuable as first-line in vivo testing of secretase inhibitors and other Abeta-lowering strategies. However, non-transgenic mice do not recapitulate Abeta plaques; testing lead drug candidates in plaque-forming mice is essential to explore disease-modifying effects.

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References


