ABSTRACT
γ-Secretase inhibitors are one promising approach to the development of a therapeutic for Alzheimer’s disease (AD). γ-Secretase inhibitors reduce brain β-amyloid peptide (Aβ), which is believed to be a major contributor in the etiology of AD. Transgenic mice overexpressing the human β-amyloid precursor protein (APP) are valuable models to examine the dynamics of Aβ changes with γ-secretase inhibitors in plaque-free and plaque-bearing animals. BMS-299897 2-[(1R)-1-[[4-chlorophenyl)sulfonyl][2,5-difluorophenyl]amino]ethyl]-5-fluorobenzene propanoic acid, a γ-secretase inhibitor, showed dose- and time-dependent reductions of Aβ in brain, cerebrospinal fluid (CSF), and plasma in young transgenic mice, with a significant correlation between brain and CSF Aβ levels. Because CSF and brain interstitial fluid are distinct compartments in composition and location, this correlation could not be assumed. In contrast, aged transgenic mice with large accumulations of Aβ in plaques showed reductions in CSF Aβ in the absence of measurable changes in plaque Aβ in the brain after up to 2 weeks of treatment. Hence, CSF Aβ levels were a valuable measure of γ-secretase activity in the central nervous system in either the presence or absence of plaques. Transgenic mice were also used to examine potential side effects due to Notch inhibition. BMS-299897 was 15-fold more effective at preventing the cleavage of APP than of Notch in vitro. No changes in the maturation of CD8+ thymocytes or of intestinal goblet cells were observed in mice treated with BMS-299897, showing that it is possible for γ-secretase inhibitors to reduce brain Aβ without causing Notch-mediated toxicity.

Dynamics of β-Amyloid Reductions in Brain, Cerebrospinal Fluid, and Plasma of β-Amyloid Precursor Protein Transgenic Mice Treated with a γ-Secretase Inhibitor


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ABBREVIATIONS: AD, Alzheimer’s disease; Aβ, β amyloid peptide; APP, amyloid precursor protein; BMS-299897, 2-[(1R)-1-[[4-chlorophenyl]-sulfonyl][2,5-difluorophenyl]amino]ethyl]-5-fluorobenzene propanoic acid; CTF, C-terminal fragment of amyloid precursor protein; PS, presenilin; CSF, cerebrospinal fluid; ISF, interstitial fluid; CHAPS, 3-[3-cholamidopropyl]-dimethyl-ammonio]-1-propane sulfonate; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; FA, formic acid; LY-411575, N2-[(2S)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1[7S]-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl]-L-alaninamide; TBS, Tris-buffered saline; TBST, Tris-buffered saline/Tween 20; HEK, human embryonic kidney; FACS, fluorescence-activated cell sorting; LC-MS-MS, liquid chromatography tandem mass spectrometry; TCR, T cell receptor.
also be cleaved by α-secretase, which creates a shorter CTF and a nonamyloidogenic peptide, p3, after γ-secretase cleavage. γ-Secretase is a large protein complex with presenilin (PS)-1 or PS-2, nicastrin, Aph-1, and Pen-2 as minimal critical components (Selkoe and Kopan, 2003). γ-Secretase inhibitors are being actively pursued in the pharmaceutical industry for the treatment of AD (Wolfe, 2001; Josien, 2002).

When using γ-secretase inhibitors in vivo, it is important to understand the dynamics of Aβ movement between and within brain, CSF, and plasma. Extracellular brain Aβ can exist in three basic forms: fibrillar deposits or plaques, oligomeric Aβ, and monomeric Aβ. Oligomeric and monomeric Aβ are soluble and exist in the ISF within the brain, although only a small fraction of this Aβ is freely diffusible (Cirrito et al., 2003). The ISF predominantly flows into the lymphatic system after moving through periarterial sheaths and into the cervical lymph nodes (Weller, 1998). Only an estimated 10% of CSF originates from interstitial fluid (ISF). CSF is created by the choroid plexus, which can also produce, transport, and possibly degrade Aβ (Chodobski and Szmyddynger-Chodobska, 2001; Ghersi-Egea and Strazielle, 2001). Intracellular Aβ (predominantly Aβ42) also exists and accumulates within neurons, further complicating the analysis of brain Aβ (Wilson et al., 2003). Unlike most peptides, Aβ is actively transported from brain to plasma via lipoprotein related protein (Shibata et al., 2000) and from plasma to brain via the receptor for advanced glycation end products (Deane et al., 2003). There is also evidence for a dynamic equilibrium between CSF and plasma Aβ in transgenic mice, which is altered when plaques begin to form (DeMattos et al., 2002). Plasma Aβ is known to be rapidly cleared by the liver and kidneys in an ApoE-dependent mechanism (Hone et al., 2003). If Aβ is to be used as a biomarker during clinical development of γ-secretase inhibitors, the changes in Aβ in each of these compartments should be understood.

The γ-secretase complex cleaves not only APP but also Notch 1–4, ErbB4, CD44, cadherin, and others (Selkoe and Kopan, 2003). Multiple substrates create greater possibilities for toxic side effects of γ-secretase inhibitors. In fact, major alterations in the differentiation of intestinal stem cells and lymphocytes have been reported for two γ-secretase inhibitors (Searfoss et al., 2003; Wong et al., 2004), suggesting toxicity due to Notch inhibition. Whether a therapeutic window can be achieved for γ-secretase inhibitors is a major issue that needs to be addressed.

Two structurally-related γ-secretase inhibitors have been shown to reduce Aβ in brain, CSF, and plasma of transgenic mice (Dovey et al., 2001; Cirrito et al., 2003; Lanz et al., 2003, 2004; Wong et al., 2004). In this article, BMS-299897, from a novel chemical series (Smith et al., 2000), was used to address several questions related to the potential clinical development of a γ-secretase inhibitor. What are the changes in Aβ in various compartments after administration of a γ-secretase inhibitor? Does the presence of large accumulations of Aβ in plaques alter the pharmacodynamics of Aβ decreases in plasma and CSF and is the plaque Aβ reduced? In addition, is there any Notch-related toxicity after 2 weeks of dosing at efficacious doses? These questions were addressed using Tg2576 mice.

### Materials and Methods

#### Handling of Mice

The Tg2576 mice were developed by Karen Hsiao (Hsiao et al., 1996) and licensed from the Mayo Foundation for Medical Education and Research (Rochester, MN). Male Tg2576 transgenic mice were bred to normal C57BL/6J females at the Bristol-Myers Squibb facility in Wallingford, CT. Mice were housed with a 6:00 AM to 6:00 PM light/dark cycle and allowed free access to food and water. Both male and female mice were used in these studies, and although no differences in Aβ were observed between them, only one sex was used in a single study. Young Tg2576 mice were used between 3 and 6 months of age, whereas aged animals were used at 14 to 17 months. BMS-299897 was synthesized by the Medicinal Chemistry groups of SIBIA Neurosciences, Inc. (now Merck Research Laboratories, San Diego, CA) and Bristol-Myers Squibb. Animals were dosed by oral gavage in a volume of 6 ml/kg in polyethylene glycol, average molecular weight of 400, or a vehicle consisting of 10% propylene glycol, 7.5% ethanol, and 82.5% Solutol HS-15 (polyethylene glycol/hydroxystearate; BASF Corp., Mount Olive, NJ) by weight. Mice were handled strictly according to Bristol-Myers Squibb Animal Care and Use Committee guidelines.

CSF was collected under anesthesia using a glass pulled micropette after exposure of the cisterna magna, taking care not to contaminate the CSF with blood. Two to 10 μl was routinely collected. The CSF was immediately diluted 1:10 in 1% 3-(3-cholamidopropyl)-dimethyl-ammonio-1-propane sulfonate (CHAPS) in phosphate-buffered saline (PBS) with protease inhibitors (Roche Diagnostics, Mannheim, Germany) before freezing in liquid nitrogen and storage at −80°C. Blood was collected by cardiac puncture and placed into ethylene-diaminetetraacetic acid microtainer tubes for the preparation of plasma. The cerebellum and hindbrain were collected for the determination of compound concentration, and the remaining brain was separated into quarters before freezing in liquid nitrogen.

#### Aβ40 ELISA

Human Aβ40 was measured in a standard sandwich ELISA by using an Aβ40 terminus-specific monoclonal antibody to capture the Aβ40 (TSD9S3.2; Bristol-Myers Squibb) and a monoclonal antibody recognizing human Aβ1–12 (26D6; SIBIA Neurosciences, Inc.) for detection of brain and CSF Aβ. An antibody to Aβ 17–24 (4G8, Signet Laboratories, Dedham, MA) was used for detection of plasma Aβ. Young brain tissue was prepared for assay by homogenization in 1% CHAPS + protease inhibitors. Centrifugation followed at 100,000g at 4°C for 1 h, and the supernatant was diluted 10-fold more. If aged brains were used, they were homogenized first in CHAPS as described above. The pellets were then extracted with 70% formic acid (FA), and then neutralized with 1 M Tris (pH 11). The pH of each sample was neutralized with 1 M sodium hydroxide if necessary. For the FA extracts, three to six dilutions between 2 × 105 and 105 mlg/w were examined to make sure the signal for each sample was on the linear part of the standard curve. Plasma was diluted 1:15 in 0.1% CHAPS in PBS, and mouse CSF was further diluted 1:15 in 1% CHAPS + protease inhibitors before loading on the plates.

A standard sandwich ELISA was performed using TSD9S3.2 to coat the plates. Washes between steps were done in PBS + 0.1% Tween 20. The plates were blocked with 0.1% bovine serum albumin in PBS for brain and CSF, and 1% bovine serum albumin in PBS for plasma. Samples were loaded in triplicate, and an Aβ1–40 standard curve and positive control were run on each plate. Plates were incubated overnight at 4°C (brain and CSF) or for 2 h at room temperature (plasma). Brain and CSF plates were washed before addition of horse radish peroxidase-conjugated 26D6 for 1 h, whereas the plasma samples were incubated with 4G8-biotin at 1 h before the addition of horseradish peroxidase-conjugated neurtulinid (Pierce Chemical, Rockford, IL) for 45 min. 3,3′,5,5′-Tetramethylbenzidine peroxidase substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used to develop the signal, which was stopped with 1 M phosphoric acid. Plates were read at 450 nm.
Brain α/β4 ELISA. The protocol follows that for α/β4, with the following changes. Capture of α/β4 occurred with an α/β4 terminus-specific antibody, 1163 (polyclonal, affinity purified on protein A, and then on an α/β35-42 column, Bristol-Myers Squibb). Brain CHAPS extracts were diluted to 1% CHAPS + 2.5% nonfat dry milk in PBS with protease inhibitors. The α/β40- and α/β42-specific ELISAs were shown not to detect each other by immunodepletion and spiking experiments (data not shown).

Immunoprecipitation/Western Blot Assays. For the APP and CTF assays, brain extracts as prepared for ELISA were immunoprecipitated overnight with a polyclonal antibody specific for the last 12 amino acids of APP (85461; Bristol-Myers Squibb) at 1:63 dilution and 40 μl of protein A-Sepharose-4 Fast Flow slurry (Amersham Biosciences Inc., Piscataway, NJ). The protein A beads were pre-blocked with 1% bovine serum albumin for 1 h before use. The complexes were washed twice with wash dialysis buffer (50 mM Tris, 0.15 M sodium chloride, 0.05% Tween 20 at pH 8.0), followed by one wash with Tris-buffered saline (TBS) and a final wash with 50 mM Tris, pH 6.8. The complexes were resuspended in 2× tricine sodium dodecyl sulfate sample buffer, denatured by heating at 95°C for 5 min, and electrophoresed on 16% tricine polyacrylamide gels (Novex, Rockford, IL), and then incubated with the 9E10, monoclonal antibody conjugated to fluorescein isothiocyanate and an anti-CD8 T cell antibody conjugated to Cy-Chrome (BD Biosciences PharMingen). After a 1-h incubation at 4°C, the samples were washed and resuspended in 200 μl of 2% paraformaldehyde in PBS. The samples were analyzed on an FACS Vantage SE (BD Biosciences, San Jose, CA). Live cell gating was accomplished with forward and side scatter analysis, after analyzing approximately 100,000 cells per sample.

Analysis of BMS-299897 Concentrations. The transgenic mouse plasma and brain samples were analyzed by a liquid chromatography-tandem mass spectrometry (LC-MS-MS) method. The LC-MS-MS system consisted of two Shimadzu LC-10AD pumps, a PerkinElmer series 200 autosampler, a Keystone BDS C18 2 × 20-mm (3-μm) column at 60°C, and a Micromass Quattro LC mass spectrometer. The mobile phase consisted of (A) 10 mM ammonium acetate buffer/methanol (75/25, pH 5.5) and (B) acetonitrile delivered at 0.3 ml/min using a gradient program. The initial elution condition was 15% B, which was increased to 85% B in 0.5 min and maintained for 0.5 min. It was then returned to 15% B in 0.1 min and maintained for 1.2 min. The MS-MS analysis was performed using the electrospray interface under negative ion mode with the source and desolvation temperatures at 150 and 300°C, respectively. The capillary voltage was 3.5 kV, cone voltage 35 V, and collision energy 20 eV. The mass-to-charge ratio of 510 (parent ion) and 302 (daughter ion) were used for multiple reaction mode monitoring of BMS-299897. The lower limit of detection and upper limit of detection for BMS-299897 were 3.9 and 8000 ng/ml, respectively. Plasma samples were deproteinized and extracted with two portions of acetonitrile. Brain samples (0.1 g) were homogenized in 0.4 ml of acetonitrile.

Statistical Analyses. Routine statistical analyses were done using analysis of variance and Dunnett’s post hoc test with Instat version 3.0 (GraphPad Software Inc., San Diego, CA). Results are reported as the mean ± the standard error of the mean.

Results

Treatment of Young Transgenic Mice with a γ-Secretase Inhibitor. BMS-299897 (Fig. 1) (Smith et al., 2000) is a γ-secretase inhibitor (S. Wagner, C. Polson, B. Munoz, K. Srinivasan, D. Franco, J. Hendrick, B. Robertson, A. Vinitsky, D. Izzarelli, R. Wang, J. Roome, S. B. Roberts, D. Srinivasan, D. Franco, J. Hendrick, B. Robertson, A. Vinitsky, D. Izzarelli, R. Wang, J. Roome, S. B. Roberts, D. Smith, D. Barten, M. Kounnas, and K. M. Felsenstein, manuscript in preparation). BMS-299897 preferentially inhibits cleavage of the APP CTF cleavage over that of Notch-1 in vitro (Fig. 2). In HEK293 cells, BMS-299897 had a 15-fold lower IC50 value for APP cleavage than Notch-1 cleavage.
This compound has also been described as a γ-secretase inhibitor in a detergent-solubilized in vitro assay (Tian et al., 2002). In Tg2576 mice, BMS-299897 was orally bioavailable, showing dose-dependent inhibition of brain and plasma Aβ40 (Fig. 3). The ED₅₀ values for brain (18 mg/kg) and plasma (15 mg/kg) were similar. Total brain and plasma levels of the compound increased linearly with dose, but the levels of compound in the plasma were much higher than in the brain. This was consistent with high plasma protein binding of this compound (99.6% by equilibrium dialysis of ¹⁴C-labeled compound).

The relative rates of Aβ changes in brain, plasma and CSF were examined after dosing with BMS-299897. Brain Aβ40, brain Aβ42, and CSF Aβ40 declined and recovered at similar rates (Fig. 4A). Plasma Aβ40 decreased faster than the other Aβ pools, as was investigated in more detail in a separate experiment (Fig. 4B). A delay in BMS-299897 penetration into the brain was unlikely because compound concentrations in brain and plasma tracked in a constant ratio, suggesting a rapid equilibrium (<15 min) across the blood-brain barrier. The more rapid decline of Aβ40 in the plasma of these mice was most likely due to a difference in the elimination half-life of Aβ40 in brain and plasma. Kinetic models (KineticaTM version 1.0) were used to calculate a half-life of 15 min in plasma and 38 min in the brains of these transgenic mice.

Immunoprecipitation followed by Western blots using antibodies to the C-terminal end of Aβ showed no changes in APP levels, but increases in the α- and β-cleaved CTF of APP were observed after dosing with BMS-299897 (Fig. 5A). These five CTF bands represent unphosphorylated and phosphorylated versions of each fragment, along with a CTF beginning at the 11 residue, an alternative product of γ-secretase cleavage (Buxbaum et al., 1998; Cai et al., 2001).

![Fig. 2. BMS-299897 inhibits cleavage of APP CTF more effectively than cleavage of Notch-1. Transfected HEK293 cells were treated with BMS-299897 for 5 h before the analysis of secreted Aβ or the Notch intracellular domain cleavage product.](image)

![Fig. 3. Dose-response relationship of Aβ reduction and drug concentration in young Tg2576 mice. Brain and plasma samples were collected 3 h after a single oral dose. Each group contained 8 to 20 mice and were compared with vehicle dosed animals.](image)

![Fig. 4. Reduction of Aβ levels in young Tg2576 mice after a single oral dose of 100 mg/kg BMS-299897 in Tg2576 mice. Male mice (n = 8–9) were used in A, and female mice (n = 6–8) were used in B. In A, control brain Aβ40 levels were 262 ± 7 ng/g, brain Aβ42 levels 13.9 ± 0.6 ng/g, CSF Aβ40 levels 199 ± 6 ng/ml, and plasma Aβ40 levels 14.8 ± 1.3 ng/ml. In B, control brain and plasma Aβ40 levels were 277 ± 7 ng/g and 18.5 ± 0.9 ng/ml, respectively.](image)

![Fig. 5. Immunoprecipitation/Western blot analysis of APP, CTF, and Aβ in the brains of BMS-299897-treated mice. Each lane shows a representative mouse from a group of seven mice for each treatment. Mice were dosed orally with 100 mg/kg BMS-299897. The Western blots were probed with an antibody to the C-terminal end of APP, G369 (A) or to residues 1 to 12 of Aβ 26D6 (B).](image)
All CTF were affected, as expected for a γ-secretase inhibitor. The in vivo turnover rate of CTF was high, as suggested previously (Savage et al., 1998). The CTF increased and decreased with a similar time course to the changes in Aβ (Fig. 5B). In addition, all three major isoforms of Aβ in Tg2576 mice (Aβ40, Aβ42, and Aβ38) were shown using immunoprecipitation and Western blot analysis to decline after dosing with BMS-299897, confirming the results observed by ELISA.

Brain and CSF Aβ levels of individual mice treated with BMS-299897 were compared to determine the predictive ability of CSF Aβ as a biomarker for what was occurring in the brain parenchyma (Fig. 6). The correlation between CSF and brain Aβ40 was 0.909 (r² = 0.825, p < 0.001). For young mice without Aβ accumulated into plaques, brain and CSF Aβ correlated well.

**Treatment of Young and Aged, Plaque-Bearing Transgenic Mice with a γ-Secretase Inhibitor.** It was of interest to determine whether the effects of BMS-299897 on Aβ would be consistent as the Tg2576 mice began to age and deposit plaques, creating a new, dense pool of Aβ in the brain. BMS-299897 was administered to Tg2576 mice from 4 to 14 months of age (Fig. 7). Plasma Aβ was reduced at all ages. Brain Aβ40 was sequentially extracted with CHAPS detergent, and then with FA to solubilize the deposited Aβ. In 4- to 6-month-old animals, this second FA extraction resulted in little to no signal. At 8, 10, and 14 months, the FA-extractable brain Aβ was 910, 5680, and 86,700 ng/g, respectively, and this pool of Aβ was not reduced by a single dose of BMS-299897. Although CHAPS-extractable brain Aβ did not increase in 8- and 10-month-old animals, it was reduced by BMS-299897, although only significantly so at 8 months. The CHAPS-extractable Aβ increased significantly in the 14-month-old mice having extensive plaque formation, and BMS-299897 was no longer able to reduce this pool. There were no significant differences in the concentration of the compound in animals between 4 and 14 months of age (see figure legend).

Tg2576 mice were then dosed over a 2-week period with BMS-299897 to determine whether a longer dosing regimen would enable reductions of brain Aβ in aged mice. Aged mice and a set of young mice dosed for 2 weeks were compared with a matched group given only a single dose. Aβ40 was measured in brain, plasma, and CSF. The results from this experiment are shown in Fig. 8. In young mice, plasma, CSF, and brain Aβ were all reduced after 1 dose or after 14 days of BMS-299897 treatment. In aged mice, CHAPS-extractable Aβ was elevated by 2.8-fold compared with young animals, but it was still only a small percentage (0.5%) of the FA-extractable pool. BMS-299897 did not influence either the CHAPS- or the FA-extractable brain Aβ pools in transgenic mice containing plaques within this 2-week time frame. Even so, the compound did demonstrate efficacy in both the peripheral (plasma) and central nervous system (CSF) compartments.

The efficacy of the compound after 14 days was not as great as that observed after an acute dose in both young and old animals. Concentrations of BMS-299897 were lower after repeated dosing (Table 1) in these mice, which also had significantly elevated liver weights (data not shown). These results suggest autoinduction of drug-metabolizing enzymes has occurred, a phenomenon whereby a compound triggers the up-regulation of its own metabolizing enzymes upon continual exposure. The resulting increase in compound clearance causes decreased systemic exposure. The aged animals (17-month males in this study) also had approximately 4-fold lower exposure than young animals at the same dose (Table 1) although there was no difference in exposure in the 14-month females used in the previous study. The reduced exposure is consistent with the diminished effectiveness of the same dose in the aged animals. This reduced exposure in aged males (average weight 39 g) was observed repeatedly, but it was not observed in aged females (average weight 26 g) or young males (average weight 31 g). It is unclear why such exposure differences were observed. There were no compound formulation differences between studies, not is it likely that the presence of plaques could account for the changes in BMS-299897 levels in aged males. It is speculated that weight, or other sex and age specific alterations in absorption, metabolism, or elimination of this compound mice could also contribute to this observation.

**Examination of Potential Notch Related Effects of a γ-Secretase Inhibitor in Young Transgenic Mice.** The potential effects of Notch inhibition were also investigated, focusing on T cell and intestinal cell differentiation. Unless a therapeutic window can be generated in vivo, γ-secretase inhibitors will not be useful for the treatment of AD. For T cell differentiation, the number of CD8+ T cells developing in the thymus was examined for possible reductions. Thymocyte development goes through a proscribed series of steps. CD4+/CD8− double negative thymocytes with no T cell Receptor (TCR) expression choose between the αβ and γδ TCR, and then become CD4+/CD8+ double positive thymocytes with low αβ TCR expression, and finally differentiate to CD4 or CD8 single positive cells with high αβ TCR expression (Allman et al., 2002; Germain, 2002). Thymocytes were isolated from young Tg2576 mice that were treated with BMS-299897 for 14 days. Although the inhibition of Aβ was diminished at the end of the 14-day dosing regimen, it was still significant. Brain Aβ was reduced 19% (p < 0.05) at 15 mg/kg and 56% (p < 0.01) at 100 mg/kg in this study. The total number of thymocytes isolated was the same for vehicle and for BMS-299897-treated mice at both 15 and 100 mg/kg (5.4 ± 0.4 × 10^7, 6.3 ± 1.0 × 10^7, and 5.5 ± 0.7 × 10^7 cells, respectively). The number of CD8+, αβ T cell receptor (TCR) high-expressing thymocytes was used as an indicator of mature CD8 thymocytes. Using FACS, there were no observable differences in the total number of CD8+ thymocytes (64.9 ± 3.7, 71.9 ± 1.6, and 65.5 ± 2.0% of total cells), total αβ TCR high thymocytes (21.9 ± 1.6, 18.5 ± 1.3, and 25.2 ± 1.6% of

**Fig. 6.** Brain and CSF Aβ in young Tg2576 mice. Data were collected from a number of experiments in which Tg2576 mice were treated with BMS-299897 or vehicle, n = 120.
total cells), or in the differentiated CD8+, αβ TCR high thymocytes (Fig. 9). Complete blood counts and basic serum chemistries done on a separate group of mice treated for 2 weeks with BMS-299897 also showed no differences between vehicle and treated animals. In addition, no gastrointestinal lesions or differentiation abnormalities were observed histologically (data not shown). There were no obvious Notch-based toxic effects as has previously been reported for other γ-secretase inhibitors (Searfoss et al., 2003; Wong et al., 2004).

**Discussion**

Tg2576 mice were used to study changes in Aβ levels in different biological compartments with a γ-secretase inhibitor. The mice have high levels of human Aβ in brain, plasma, and CSF, and low levels in peripheral tissues where there is some limited transgene expression (Kawarabayashi et al., 2001). We demonstrated that BMS-299897 reduced Aβ in brain, CSF and plasma of young mice with a similar time course, except for a slightly faster rate of decline in plasma Aβ within the first hour. The value of brain and plasma Aβ half-life we estimated (38 and 15 min) agrees well with previous estimates of brain Aβ turnover of 1 to 2.5 h (Savage et al., 1998) and of plasma Aβ turnover of 15 min (Savage et al., 1998; Hone et al., 2003). These results differ from those observed by Lanz et al. (2004) using LY-411575, with a significantly faster decline in Aβ levels. It is unknown how much intracellular Aβ contributes to the total pool of Aβ in the brain, but its turnover is likely to be slower than secreted Aβ. If significant, differences in the extraction of intracellular Aβ could explain their longer apparent half-life, and the increased efficacy observed with repeated dosing. The previous estimate of brain Aβ half-life was determined using a diethylamine brain extract, adding yet another extraction method to the existing data (Savage et al., 1998). Differences in the pharmacokinetics and distribution of BMS-299897 and LY-411575 within the brain itself could also account for differences in the two studies. Total brain levels of compound in both studies were measured, but differences in compound distribution between different anatomical areas of the brain (i.e., myelin-rich or -poor areas), or even within different cellular membranes (possibly through differences in lipid composition) could also contribute to different results. It is possible that it takes longer for LY-411575 to equilibrate into the compartment where brain active γ-secretase resides. These will be important questions to pursue in further studies, which should involve side by side comparisons of the two compounds.

Our studies also showed the expected increases in APP CTF with a time course mirroring the decreases in brain Aβ. Increases in CTF with γ-secretase inhibitors have been reported using N-[N-(3,5-difluorophenylacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (Dovey et al., 2001; Lanz et al., 2003). We also observed equivalent findings using BMS-299897 in wild-type APP-yeast artificial chromosome trans-
levels of Aβ oligomers. FA-extractable brain Aβ from aged mice likely represented plaque Aβ, but it may have included intracellular Aβ as well. LY-411575 also did not reduce brain Aβ in aged Tg2576 mice with a single dose (Lanz et al., 2003). After 14 days of once daily dosing, BMS-299897 was also unable to reduce plaque Aβ in aged Tg2576 mice, but it is possible that this dosing regimen was not sufficient to influence plaque Aβ. It is possible that continuous exposure to compound over 24 h or for longer than 14 days would be required to see reductions in plaque Aβ. Possible autointerduction and lower exposure of BMS-299897 in aged animals worked against increasing exposure with this compound. Attempts to give continuous exposure using BMS-299897 in chow were also limited by significant apparent autoinduction (data not shown). Reductions in plaque formation in APP transgenic mice have been reported after various treatments, all of which required 1 to 6 months of treatment (Schenk et al., 1999; Lim et al., 2000; Cherny et al., 2001; Haugaboek et al., 2001; Refolo et al., 2001; Carro et al., 2002; Jantzen et al., 2002; Permanne et al., 2002; Deane et al., 2003; Matsuoka et al., 2003; Yan et al., 2003). Two weeks of treatment with a nitric oxide-releasing non-steroidal anti-inflammatory drug was not sufficient to reduce plaque load, although 5 months of treatment was effective (Jantzen et al., 2002). In light of these reports, it is not surprising that we did not observe changes in FA extractable Aβ in these mice treated for 2 weeks, and BMS-299897 is probably not the optimal compound with which to perform these studies in transgenic mice due to its pharmacokinetic profile.

In spite of this tremendous pool of unchanging plaque Aβ in the brains of aged Tg2576 mice, CSF and plasma Aβ in aged Tg2576 mice were reduced by BMS-299897 by 1 or 14 days of treatment. These data are in agreement with those generated by others in transgenic mice (Lanz et al., 2003). Differences in ISF and CSF Aβ have also been reported using microdialysis (Cirrito et al., 2003). In addition, disparities in brain and CSF Aβ are also observed in AD patients, where soluble brain Aβ is 50 times higher than CSF Aβ (Kuo et al., 1996). The dynamics of Aβ in the brain and CSF compartments were independent in the presence of plaques, even though there was a strong correlation between brain and CSF Aβ in young mice. CSF Aβ was a valid indicator of CNS activity in any age group and could potentially be a valuable biomarker in AD patients.

Notch is part of a major signaling pathway during development. Notch was discovered in experiments with Drosophila, where homozygous loss causes major developmental de-
forms, and loss of one copy causes a notched wing phenotype. Ligand binding induces two proteolytic cleavages in the Notch receptor. The second protease activity occurs within the membrane, and requires PS. This second cleavage releases the Notch intracellular domain, which causes transcriptions of many genes. Eliminating functional PS in Drosophila, Caenorhabditis elegans, or mice has severe effects during development, similar to Notch-1 loss of function mutants. γ-Secretase inhibitors have also been shown to induce notched wings in Drosophila (Michelli et al., 2003) and to alter embryonic development in zebrafish (Geling et al., 2002).

In an adult mammal, Notch signaling plays a significant role in the differentiation of cells in the intestinal epithelium (Brittan and Wright, 2002) and during the multistep process of lymphopoiesis. Inhibition of Notch processing could prevent the development of γ-secretase inhibitors for the treatment of AD, so this is a critical point to be examined in efficacy studies with these compounds. Notch is involved in lymphopoiesis at multiple steps, including the T cell/B cell lineage decision, the γδ versus αβ TCR decision, and the CD4 versus CD8 T cell commitments (Allman et al., 2002; Germain, 2002). The data supporting a role for Notch in CD8 T cell development include experiments with transgenic mice overexpressing a truncated active form of Notch in the thymus showing an increased number of CD8 single positive thymocytes. γ-Secretase inhibitors at concentrations that eliminate activity interfere with thymocyte development in fetal thymic organ cultures at the T/B cell and αβ/γδ TCR interfaces, along with the CD4/CD8 T cell division point (Allman et al., 2002). Dose-response studies suggest that the CD4/CD8 T cell division point might be most sensitive to a γ-secretase inhibitor. Toxic, Notch-related effects of some γ-secretase inhibitors have already been described in rodents. Recently, LY-411575 was shown to profoundly affect the differentiation of T cells in the thymus, and B cells in the spleen of transgenic mice treated for 5 to 15 days (Wong et al., 2004). For intestinal stem cell differentiation, LY-411575 affects the differentiation of T cells in the thymus, and B cells in the spleen of transgenic mice treated for 5 to 15 days (Wong et al., 2004). For intestinal stem cell differentiation, LY-411575 was shown to profoundly affect the differentiation of T cells in the thymus, and B cells in the spleen of transgenic mice treated for 5 to 15 days (Wong et al., 2004). For intestinal stem cell differentiation, LY-411575 was shown to profoundly affect the differentiation of T cells in the thymus, and B cells in the spleen of transgenic mice treated for 5 to 15 days (Wong et al., 2004). For intestinal stem cell differentiation, LY-411575 was shown to profoundly affect the differentiation of T cells in the thymus, and B cells in the spleen of transgenic mice treated for 5 to 15 days (Wong et al., 2004). 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