Cerebrospinal Fluid Amyloid-β (Aβ) as an Effect Biomarker for Brain Aβ Lowering Verified by Quantitative Preclinical Analyses


Received January 31, 2012; accepted April 27, 2012

ABSTRACT
Reducing the generation of amyloid-β (Aβ) in the brain via inhibition of β-secretase or inhibition/modulation of γ-secretase has been pursued as a potential disease-modifying treatment for Alzheimer’s disease. For the discovery and development of β-secretase inhibitors (BACEi), γ-secretase inhibitors (GSI), and γ-secretase modulators (GSM), Aβ in cerebrospinal fluid (CSF) has been presumed to be an effect biomarker for Aβ lowering in the brain. However, this presumption is challenged by the lack of quantitative understanding of the relationship between brain and CSF Aβ lowering. In this study, we strived to elucidate how the intrinsic pharmacokinetic (PK)/pharmacodynamic (PD) relationship for CSF Aβ lowering is related to that for brain Aβ through quantitative modeling of preclinical data for numerous BACEi, GSI, and GSM across multiple species. Our results indicate that the intrinsic PK/PD relationship in CSF is predictive of that in brain, at least in the postulated pharmacologically relevant range, with excellent consistency across mechanisms and species. As such, the validity of CSF Aβ as an effect biomarker for brain Aβ lowering is confirmed preclinically. Meanwhile, we have been able to reproduce the dose-dependent separation between brain and CSF effect profiles using simulations. We further discuss the implications of our findings to drug discovery and development with regard to preclinical PK/PD characterization and clinical prediction of Aβ lowering in the brain.

Introduction
The amyloid cascade hypothesis postulates that Alzheimer’s disease (AD) is caused by abnormal accumulation and deposition of amyloid-β (Aβ) in the brain (Hardy and Higgins, 1992; Karran et al., 2011). Aβ is produced through sequential cleavages of the amyloid precursor protein first by β-secretase and then by γ-secretase. Inhibition of β-secretase or inhibition/modulation of γ-secretase results in lowering of Aβ40 and Aβ42 in the brain; these mechanisms are therefore recognized as potential disease-modifying treatments for AD. Programs that seek β-secretase inhibitors (BACEi), γ-secretase inhibitors (GSI), and γ-secretase modulators (GSM) have been widely pursued in academia and by the pharmaceutical industry (for reviews, see Albert, 2009; Kreft et al., 2009; Imbimbo et al., 2011; Pettersson et al., 2011).

For assessment of proximal pharmacological activities of BACEi, GSI, and GSM, the most relevant endpoint is brain Aβ. However, in clinical trials with a single dose or short-term repeated dosing treatment, which aim to establish proof...
of mechanism and exposure-Aβ response relationship, it is not feasible to measure Aβ lowering in brain. For drug development purposes, it is necessary to identify an effect biomarker in an accessible compartment that can serve as a surrogate of Aβ modulation in the brain. To this end, CSF Aβ has been chosen (Siemers et al., 2007; Ereshefsky et al., 2008; Stone, 2009; Martenyi et al., 2010; Meredith et al., 2011) with the presumption that Aβ changes in CSF reflect the pharmacological activities in brain. To inform decision making based on CSF Aβ data from clinical trials, preclinical evaluation of the effect of a BACEi, GSI, or GSM on CSF Aβ has been routinely conducted (Barten et al., 2005; Sankaranarayanan et al., 2009; Hawkins et al., 2011; Lu et al., 2011). However, the validity and utility of CSF Aβ as an effect biomarker for brain Aβ lowering have yet to be assessed rigorously. Even though qualitatively a change in CSF Aβ may indicate a change in brain Aβ (Bateman et al., 2006, 2009; Cook et al., 2010), from the quantitative perspective, current understanding is too limited to allow prediction of the effect size of brain Aβ lowering on the basis of CSF Aβ data.

The preclinical observations from in-house experiments and external publications have demonstrated complexities in the relationship between brain and CSF Aβ lowering. First, brain and CSF Aβ time courses show different temporal dynamics after a drug treatment. Compared with brain Aβ, CSF Aβ levels tend to drop quicker and return to baseline quicker (Lu et al., 2011). Second, the maximum effect size in CSF tends to exceed that in brain (Hawkins et al., 2011; Lu et al., 2011). Moreover, the disparity in the effect size increases with dose (Wang et al., 2010). These complexities have been consistently observed for compounds of distinct mechanisms (BACEi, GSI, or GSM) across multiple species (mouse, rat, and guinea pig). Consequently, it is critical to understand these CSF versus brain differences and to assess the validity of CSF Aβ as an effect biomarker for brain Aβ lowering.

In a previous study, we analyzed our Aβ time courses for four GSI in 129/SVE mice using semimechanistically based pharmacokinetic (PK)/pharmacodynamic (PD) modeling (Lu et al., 2011). The modeling outcomes suggested that the Aβ turnover kinetics is the main cause of the dissimilar temporal profiles and maximum effect sizes in the brain and CSF in the mouse and that the intrinsic PK/PD relationships for the two compartments are similar. In this report, we extended our modeling analyses to BACEi and GSM. Collectively, our results indicate that, in the pharmacologically relevant range, the brain and CSF share similar intrinsic PK/PD relationships for Aβ lowering across distinct mechanisms and species. These results support the validity of CSF Aβ as an effect biomarker for brain Aβ lowering.

### Materials and Methods

A semimechanistically based PK/PD model was used to analyze brain and CSF Aβ time course data in the mouse, rat, and guinea pig treated with BACEi, GSI, or GSM. The modeling yielded intrinsic PK/PD [concentration-Aβ generation rate, defined in Lu et al. (2011)] relationships free of confounding caused by Aβ turnover kinetics. Similar intrinsic PK/PD relationships in the brain and CSF indicate similar proximal responses to a treatment in the two compartments despite the apparent differences in Aβ temporal profiles and maximum effect sizes. The key assumption for the modeling analyses was that both brain and CSF Aβ responses were driven by the brain exposure.

#### PK/PD Data.

Studies on the three GSI, LY450139 (semagacestat), BMS-708163, and Elan-X, in 129/SVE mice have been reported previously (Lu et al., 2011). Studies on other compounds are detailed as follows.

#### Compounds. Four additional compounds (structures and in vitro potency measures in Fig. 1) investigated in this study are (4S)-4-(2,4-difluoro-5-pyrimidin-5-ylphenyl)-4-methyl-5,6-dihydro-4H-1,3-thiazin-2-amine (LY2811376) (Audia et al., 2009; May et al., 2011), N-[3-(4aS,5aS)-2-amino-4a,5-dihydro-4H-furo[3,4-d][1,3]thiazin-7a(7H)-yl]-4-fluoropyridine]-5-fluoropyridine-2-carboxamide (BACE-A) (Audia et al., 2011), (S,E)-1-(4-fluorophenyl)ethyl)-3-(3-methoxy-4-(4-methyl-1H-imidazol-1-yl)benzyl)-4-fluorophenyl)ethyl)-3-(3-methoxy-4-(4-methyl-1H-imidazol-1-yl)piperidin-2-one (E2012), and (S,E)-2-(2-(6-methoxy-5-(4-methyl-1H-imidazol-1-yl)piperidin-2-yl)vinyl]-8-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine (GSM-A) (for review, see Pettersson et al., 2011). LY2811376 and BACE-A were formulated in dimethyl sulfoxide-Cremophor-saline (5:5:90) for intravenous administration in drug development studies. The IC_{50} values for LY2811376 and BACE-A are 1050 nM (95% confidence interval 888–1240 nM, n = 19) and 65 nM (95% confidence interval 42–101 nM, n = 4), respectively, in our enzyme activity assay. E2012 and GSM-A IC_{50} values are 142 nM (95% confidence interval 135–157 nM, n = 384) and 7.2 (95% confidence interval 5.3–11.2 nM, n = 12), respectively, in our whole-cell assay.

![Fig. 1. Structures of LY2811376, BACE-A, E2012, and GSM-A investigated in this study. The in vitro IC_{50} values for LY2811376 and BACE-A are 1050 nM (95% confidence interval 888-1240 nM, n = 19) and 65 nM (95% confidence interval 42–101 nM, n = 4), respectively, in our enzyme activity assay. E2012 and GSM-A IC_{50} values are 142 nM (95% confidence interval 135–157 nM, n = 384) and 7.2 (95% confidence interval 5.3–11.2 nM, n = 12), respectively, in our whole-cell assay.](image-url)
administration to mice. E2012 and GSM-A were suspended in 20% sulfobutyl ether-β-cyclodextrin acidified with 1.5 M equivalents of 1 N HCl for dosing rats or guinea pigs.

**Animals.** Young male 129/SVE mice (18–25 g) were purchased from Taconic Farms (Hudson, NY). Male CD/Sprague-Dawley rats (250–300 g) and male Hartley guinea pigs (210–250 g) were purchased from Charles River (St. Constant, QC, Canada). All animals were acclimated for 1 week before dosing.

**Animal Treatment.** All animal studies followed the institutional animal care and use committee-approved protocol that complies with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). The study designs are listed in Table 1. Drug concentrations were measured in brain samples and Aβ in brain and CSF samples.

**Aβ Measurement.** Aβ was measured using an enzyme-linked immunosorbent assay. The measurement of Aβ in the 129/SVE mouse samples followed the protocol reported previously (Lu et al., 2011). The protocol for quantifying brain and CSF Aβx-42 (Aβ form ending at the amino acid position of 42) in the rat and guinea pig is detailed here. Hemibrains, without cerebellums, from rats or guinea pigs were homogenized in a beadbeater in 1.9 (w/v) 0.4% diethylamine-50 mM NaCl and then were incubated with shaking overnight at 4°C. The homogenate was spun at 135,000g at 4°C for 1 h, and then supernatant was collected. The brain supernatant (950 μl for rat and 900 μl for guinea pig) in duplicate was run over a Waters Oasis HLB LP 60-μg 96-well plate for concentration of Aβ. Each sample was eluted with methanol and vacufuged to dryness, and then each pellet was reconstituted with blocking buffer (95 μl for rat and 128.5 μl for guinea pig) and duplicates were combined. Rat CSF samples were undiluted, and guinea pig CSF samples were diluted by 5-fold.

Ninety-six-well plates (Greiner Bio-One, Monroe, NC) were coated with 50 μl/well of capture antibody 10G3 (6 μg/ml, Rinsa; Pfizer, New York, NY) in coating buffer (0.1 M NaHCO₃, pH 8.2). The coated plates were incubated overnight at 4°C. The plates were then washed four times with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) and 130 μl of blocking buffer (PBS-T and 1% bovine serum albumin) was added to each well. After a 1-h incubation at room temperature, the plates were washed four times with PBS-T, and 50 μl of diluted samples or standards was added to the plate in

### TABLE 1

PK/PD studies and data involved in this report

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism</th>
<th>Species</th>
<th>Study Type</th>
<th>Dose, Route</th>
<th>Time Point</th>
<th>No. of Animals/Group</th>
<th>Measurement</th>
<th>Study Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LY2811376</td>
<td>BACEi</td>
<td>Mouse (129/SVE)</td>
<td>TC</td>
<td>100, s.c.</td>
<td>1, 3, 5, 7, 18</td>
<td>8</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
</tr>
<tr>
<td>BACEi</td>
<td>Mouse (129/SVE)</td>
<td>DRC</td>
<td>1, 3, 10, 30, 100, s.c.</td>
<td>3</td>
<td>8</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>BACE-A</td>
<td>BACEi</td>
<td>Mouse (129/SVE)</td>
<td>TC</td>
<td>30, 100, s.c.</td>
<td>1, 3, 5, 7, 14, 20, 30</td>
<td>5</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
</tr>
<tr>
<td>LY450139</td>
<td>GSI</td>
<td>Mouse (129/SVE)</td>
<td>TC</td>
<td>3</td>
<td>8</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>GSI</td>
<td>Mouse (129/SVE)</td>
<td>DRC</td>
<td>5, 10, 15, 30, p.o.</td>
<td>5</td>
<td>10, 18</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>GSI</td>
<td>Mouse (129/SVE)</td>
<td>TC</td>
<td>10, p.o.</td>
<td>0.5, 1, 3, 6, 10, 18</td>
<td>5</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>BMS-708163</td>
<td>GSI</td>
<td>Mouse (129/SVE)</td>
<td>TC</td>
<td>30, 150, p.o.</td>
<td>0.5, 1, 2, 4, 6, 8, 18</td>
<td>8</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
</tr>
<tr>
<td>Elan-X</td>
<td>GSI</td>
<td>Mouse (129/SVE)</td>
<td>Single time point</td>
<td>10, p.o.</td>
<td>4</td>
<td>5</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
</tr>
<tr>
<td>GSI</td>
<td>Mouse (129/SVE)</td>
<td>Reduced TC</td>
<td>5, p.o.</td>
<td>2, 4</td>
<td>5</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>GSI</td>
<td>Mouse (129/SVE)</td>
<td>TC</td>
<td>30, p.o.</td>
<td>1, 2, 4, 7, 18</td>
<td>5</td>
<td>C₆, CSF Aβx-40, brain Aβx-40 and Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>E2012</td>
<td>GSM</td>
<td>Guinea pig (Hartley)</td>
<td>Reduced TC</td>
<td>32, 100, p.o.</td>
<td>4, 9</td>
<td>5</td>
<td>C₆, CSF and brain Aβx-42</td>
<td>N.A.</td>
</tr>
<tr>
<td>GSM</td>
<td>Guinea pig (Hartley)</td>
<td>TC</td>
<td>1, 3, 10, 30, p.o.</td>
<td>1, 3, 6, 12, 24, 48, 72</td>
<td>5</td>
<td>C₆, CSF and brain Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>GSM-A</td>
<td>Guinea pig (Hartley)</td>
<td>Reduced TC</td>
<td>100, p.o.</td>
<td>4, 9</td>
<td>5</td>
<td>C₆, CSF and brain Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>GSM</td>
<td>Guinea pig (Hartley)</td>
<td>TC</td>
<td>30, p.o.</td>
<td>1, 3, 6, 9, 24, 48</td>
<td>5</td>
<td>C₆, CSF and brain Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>GSM-A</td>
<td>Rat (Sprague-Dawley)</td>
<td>TC</td>
<td>15, p.o.</td>
<td>2, 4, 7, 24</td>
<td>3</td>
<td>C₆, CSF and brain Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>GSM</td>
<td>Rat (Sprague-Dawley)</td>
<td>TC</td>
<td>100, p.o.</td>
<td>2, 4, 7, 24, 48</td>
<td>3</td>
<td>C₆, CSF and brain Aβx-42</td>
<td>N.A.</td>
<td></td>
</tr>
</tbody>
</table>

DRC, dose-response curve study; TC, time-course study; C₆, drug concentration in brain; N.A., not applicable.
duplicate. For preparation of standard curves, rodent and human sequence Aβ1-42 (California Peptide, Napa, CA) for the rat and guinea pig Aβx-42 assay, respectively, was serially diluted in blocking buffer from 256 pg/well down to 0.25 pg/well, and 50 µl was applied to each well. Once samples or standards were applied, plates were incubated overnight at 4°C.

The plates were then washed four times with PBS-T and incubated with 50 µl of G48-biotin detect antibody (0.2 µg/ml in blocking buffer; Covance Research Products, Princeton, NJ) for 2 h at room temperature. After four washes with PBS-T, 50 µl of PerkinElmer europium-streptavidin (diluted 1:2000 in blocking buffer to a concentration of 50 ng/ml with 20 µM EDTA supplement) was added to the plates followed by a 1-h incubation at room temperature. After four washes with PBS-T, PerkinElmer Enhancement Solution at room temperature was added to the plates (50 µl/well) for a 15-min incubation at room temperature. Then europium counts were read on a Victor/Envision plate reader. Aβ levels in the samples were interpolated from the corresponding standard curves.

**Compound Concentration Measurement.** All samples were frozen at −20°C until analysis. Sample preparation and liquid chromatography mass spectrometry (LC-MS/MS) methodology was developed internally. Standard curves were prepared in plasma via serial dilution in the concentration range of 0.5 to 1000 ng/ml for LY2811376, 0.49 to 2000 ng/ml for E2012, and 0.49 to 1000 ng/ml for GSM-A, and 0.5 to 2500 ng/g for BACE-A. Frozen brain tissue was weighed and an isopropanol-water (60:40) volume equivalent to 4 µl of blank plasma was added to 50 µl of acetonitrile-containing internal standards, vor- texed for 1 min, and then centrifuged at 3000 rpm for 10 min. The supernatant (120 µl for E2012 and BACE-A and 150 µl for LY2811376 and GSM-A) was transferred to a 96-well plate. The LY2811376 samples were diluted with 100 µl of water. All samples of the other compounds were evaporated under nitrogen and recon- stituted with 120 µl of 50:50 water-acetonitrile for E2012, 100 µl of 80:20 water-acetonitrile for BACE-A, and 100 µl of 75:25 water-acetonitrile for GSM-A.

LC-MS/MS analysis was performed using a high-performance liquid chromatography system consisting of binary Shimadzu LC-20AB pumps (Shimadzu Scientific Instruments, Columbia, MD) for LY2811376, E2012, and GSM-A and binary Eksigent Express HT pumps (Eksigent Technologies, Dublin, CA) for BACE-A with a CTC PAL dual-arm autosampler (Leap Technologies, Carrboro, NC) interfaced to an API 4000 LC-MS/MS quadruple tandem mass spectrometer (AB Sciex Inc., Concord, ON, Canada). LY2811376 and a structurally similar internal standard were separated on a Supelco Ascentis Express C18 column (3 × 30 mm, 2.5-µm; Sigma-Aldrich, St. Louis, MO) by gradient elution using a flow rate of 0.8 ml/min. A 5-µl sample was injected into the column. The mobile phase consisted of solvent A (10 mM ammonium formate in 0.1% formic acid) and solvent B (acetonitrile). The gradient was as follows: solvent B was held at 5% for 0.30 min, linearly ramped from 5 to 80% in 2.0 min, held at 80% for 0.30 min, and then ramped to 5% over 0.15 min. The mass spectrom- eter was operated using positive electrospray ionization in multiple reaction monitoring mode. The ion pairs monitored were 431.5/298.2 and 516.5/238.1 for the internal standard. GSM-A and a structurally similar compound were sepa- rated on a Kinetex C18 column (30 × 3.0 mm, 2.6 µm; Phenomenex) by gradient elution using a flow rate of 1.2 ml/min. A 20-µl sample was injected onto the column. The mobile phase consisted of solvent A (10 mM ammonium formate in 0.1% formic acid) and solvent B (acetonitrile). The gradient was as follows: solvent B was held at 5% for 0.42 min, linearly ramped from 5 to 90% in 1.55 min, held at 90% for 0.26 min, and then ramped to 5% over 0.15 min. The mass spectrom- eter was operated using positive electrospray ionization in multiple reaction monitoring mode. The ion pairs monitored were 391.5/269.3 and 268/116 for the internal standard. All raw data were processed using Analyst software (version 1.4.2; AB Sciex Inc.) for LY2811376, E2012, and GSM-A and Analyst software (version 1.5.2) for BACE-A.

**Data Preparation and Statistical Analysis.** Individual Aβ levels are expressed as percentages of the time-matched means of vehicle controls. These normalized time courses were then analyzed statistically. A two-way analysis of variance was used to assess whether the Aβ lowering in CSF was different from that in brain in the compound-treated groups. After a significant main effect was identified, a Bonferroni post-test was applied to evaluate the signif- icance of effect at each time point. The statistical analyses were conducted using GraphPad Prism (version 5.01; GraphPad Software, Inc., La Jolla, CA). All data are presented as mean percentage of vehicle control ± S.E.M.

**PK/PD Modeling.** We have been using a semimechanistically based PK/PD model to analyze Aβ time course data as reported recently (Lu et al., 2011). This model assumes that steady-state Aβ (specifically, Aβ40 and Aβ42 in this study) in a given compartment is maintained via the balancing of a zero-order generation (with a rate of $K_{	ext{in}}$) and a first-order clearance (with a fractional turnover rate of $k_{	ext{out}}$). This biologically reasonable simplification of the Aβ homeosta- sis can be expressed mathematically as in eq. 1:

$$\frac{dA\beta}{dt} = K_{\text{in}} - k_{\text{out}} \times A\beta$$  \hspace{1cm} (1)

Because BACEI, GSI, and GSM inhibit Aβ generation, $K_{\text{in}}$ is modi- fied by an inhibitory sigmoidal effect, as a function of exposure (C), after a treatment. An Aβ time course then can be calculated using eq. 2,

$$\frac{dA\beta}{dt} = K_{\text{in}} \times \left(1 - \frac{I_{\text{max}} \times C}{IC_{50} + C}\right) - k_{\text{out}} \times A\beta$$  \hspace{1cm} (2)

As the driving force for the Aβ response, exposure time courses were described using compartmental PK modeling or the nonparametric, log-linear interpolation approach as appropriate. With the hypothe- sis that the lowering in CSF Aβ is caused fundamentally by the inhibition of brain Aβ generation, the brain exposure was used to drive the effect in both brain and CSF compartments. By fitting calculated Aβ time courses to the normalized Aβ data, the PK/PD model estimates those unknown parameters, $k_{\text{out}}$, $I_{\text{max}}$, $IC_{50}$ (concentration at which 50% of $I_{\text{max}}$ is achieved), and $\gamma$ (Hill coefficient). In the case in which limitations in ...
the data did not allow simultaneous estimation of all four parameters, we reduced the number of unknown parameters by fixing $\gamma$ and/or $I_{\text{max}}$ to biologically plausible values. The set of parameters ($I_{\text{max}}$, IC$_{50}$, $\gamma$, and $k_{\text{out}}$) that was biologically reasonable and yielded the lowest value of the objective function (i.e., statistically best fit of Aβ time courses) was chosen to be the final set.

With the estimates of $I_{\text{max}}$, IC$_{50}$, and $\gamma$, the relationship between exposure and Aβ generation rate (expressed as a percentage of control) can be established as in eq. 3:

$$\text{Aβ generation rate (% control)} = \frac{K_{\text{in}} \times (1 - \frac{I_{\text{max}} \times C^\gamma}{\text{IC}_{50}^\gamma + C^\gamma})}{K_{\text{in}}} = 1 - \frac{I_{\text{max}} \times C^\gamma}{\text{IC}_{50}^\gamma + C^\gamma} \quad (3)$$

This exposure-Aβ generation relationship was defined as the intrinsic PK/PD relationship for BACEi, GSI, and GSM. Unlike the observed exposure-Aβ relationship, this intrinsic PK/PD relationship is independent of PK behaviors and Aβ turnover. At steady state, this intrinsic relationship is equivalent to the exposure-Aβ lowering relationship; that is, an $x$% of inhibition of Aβ generation leads to $x$% of Aβ lowering on average at steady state. All modeling was implemented using the PK/PD computer program NONMEM V (Globomax, Hanover, MD).

Comparison of Intrinsic PK/PD Relationships. Comparisons of intrinsic PK/PD relationships between brain and CSF were made by plotting and visually inspecting curves of calculated Aβ generation rates versus free brain concentrations. If the curves for CSF and brain are similar, it indicates that the response in CSF is predictive of that in brain. As such, the validity of CSF Aβ as an effect biomarker for brain Aβ lowering can be established.

PK/PD Simulations. The impacts of $k_{\text{out}}$ and intrinsic PK/PD relationship on an Aβ temporal profile can be illustrated via simulations. In the earlier report (Lu et al., 2012), we simulated time course profiles for brain Aβx-42, Aβx-40, and CSF Aβx-40 in a nontransgenic mouse. With the incorporation of $k_{\text{out}}$ variation, the simulations reproduced the temporal differences among the three endpoints. Here, we simulated how $k_{\text{out}}$ and the intrinsic PK/PD relationship together lead to dose-dependent brain versus CSF differences in Aβ lowering.

**Results**

Aβ PK/PD Data. The PK/PD data for the three GSI, LY450139, BMS-708163, and Elan-X, were reported in Lu et al. (2011). The Aβ data for the BACEi, LY2811376 and BACE-A, in 129/SVE mice are shown in Fig. 2 and Supplemental Fig. 1, respectively. For the two GSM, GSM-A and E2012, the Aβ data are presented in Fig. 3 and Supplemental Fig. 2, respectively. Aβ data in all individual animals are listed in Supplemental Tables 1 to 5.

The dose-response data in 129/SVE mice (Fig. 2A) and guinea pigs (Fig. 3C) illustrate dose-dependent separation between the brain and CSF curves. The time course data in Figs. 2B and 3, A, B, and D [as well as in Fig. 4, D–G, in Lu et al. (2011)] demonstrate distinct temporal profiles for brain and CSF Aβ. In general, Aβ levels in CSF declined and returned to baseline quicker than those in brain, and the maximum effect sizes in CSF were greater than those in brain.

The brain exposure levels for LY2811376, BACE-A, E2012, and GSM-A in all studies are presented in Supplemental Figs. 3 to 6. In all studies, the brain exposures achieved sufficiently cover the in vitro IC$_{50}$ levels (see the legend to Fig. 1 for the IC$_{50}$ values).

**PK/PD Model Performance.** The PK/PD model described all Aβ data adequately. For demonstration of satisfactory model performance, a representative set of diagnostic
plots for modeling brain Aβx-42 in the guinea pig treated with GSM-A is presented in Fig. 4. A similar performance was achieved for description of other data sets (results not shown).

**Intrinsic PK/PD Relationships in Brain and CSF.** Figure 5 summarizes the comparisons between brain and CSF intrinsic PK/PD relationships for LY2811376 (Fig. 5A) and BACE-A (Fig. 5B) in the 129/SVE mouse, GSM-A in the guinea pig (Fig. 5C) and rat (Fig. 5D), and E2012 (Fig. 5E) in the guinea pig. The brain versus CSF comparisons for LY450139, BMS-708163, and Elan-X were presented in Lu et al. (2011). Despite some differences in $I_{\text{max}}$ and steepness of the curves, the brain and CSF curves are generally similar to each other in the upper halves.

**Brain versus CSF Aβ $k_{\text{out}}$.** The modeling-derived PD parameters, i.e., $I_{\text{max}}$, IC$_{50}$, $\gamma$, and $k_{\text{out}}$ for the GSI in the 129/SVE mouse were presented in Lu et al. (2011). The parameters for LY2811376, BACE-A, E2012, and GSM-A are summarized in Supplemental Table 6. The estimated $k_{\text{out}}$ values are worthy of particular attention. In all species, irrespective of mechanisms, the $k_{\text{out}}$ for brain Aβ seems to be lower than that for CSF Aβ. Shown in Fig. 6 are the values derived from guinea pigs and rats treated with GSM-A or E2012. The $k_{\text{out}}$ values of brain Aβ42 are 2-fold lower than that of CSF Aβ42. Likewise, in the 129/SVE mouse, the $k_{\text{out}}$ values for brain Aβ40 and Aβ42 were found to be lower than that for CSF Aβ40 (Lu et al., 2011).

A meaningful comparison of $k_{\text{out}}$ across species is prevented by the small replicates in the rat ($n = 1$) and guinea pig ($n = 2$) in this study. With a more extensive data set, we have conducted a comprehensive analysis on how the $k_{\text{out}}$ for CSF Aβ relates to the known rapid turnover of CSF and how this $k_{\text{out}}$ allometrically scales across several species (Lu et al., 2012).

**PK/PD Simulations.** Simulations were conducted to test the impacts of $k_{\text{out}}$ and the intrinsic PK/PD relationship ($I_{\text{max}}$ varying between brain and CSF) on brain and CSF Aβ profiles in a rat after drug treatment. With the PK and PD parameters specified in Fig. 7, the simulations (Fig. 7) reproduced the observed pattern of dose-dependent separation between the brain and CSF effect. At the lowest dose, 1 mg/kg, at which the brain and CSF share the same intrinsic PK/PD relationship, the Aβ time courses for the brain and CSF are similar, with the CSF curve slightly left-shifted, reflecting the impact of higher $k_{\text{out}}$ in CSF. With an elevation in dose, the brain and CSF curves are increasingly separated; at 100 mg/kg, the maximum Aβ42 lowering in brain is only 67%, in contrast to 96% in the CSF. The separation, as suggested by the simulations, is a joint result of the differences in the $k_{\text{out}}$ and the intrinsic PK/PD relationship between the two endpoints.

**Discussion**

Changes in CSF Aβ have been recognized as being reflective of the changes in brain Aβ with a therapeutic treatment (Bateman et al., 2006, 2009; Cook et al., 2010). In clinical trials, CSF Aβ has been used as an effect biomarker for indirect assessment of the pharmacological activities in the brain for Aβ-lowering agents, such as BACEi, GSI, or GSM (Siemers et al., 2007; Ereshefsky et al., 2008; Bateman et al., 2009; Stone, 2009; Martenyi et al., 2010; May et al., 2011; Meredith et al., 2011). From the drug development perspective, this presumption of the utility of CSF Aβ is faced with three challenges: 1) Aβ lowering in CSF tends to be greater than that in brain in our preclinical observations as well as those reported by others (Hawkins et al., 2011) and the reasons for this disparity are not yet understood, 2) there is a lack of rigorous quantitative evaluation of the relationship between brain and CSF Aβ lowering after therapeutic treatment, and 3) existing knowledge does not allow confident prediction of Aβ lowering in the brain on the basis of the effect in CSF. In this study, with the aid of PK/PD modeling and simulations, we strived to understand the quantitative relationship between brain and CSF Aβ lowering and to assess the validity of CSF Aβ as an effect biomarker for brain Aβ lowering.

**CSF Aβ Is a Reasonable Effect Biomarker for Brain Aβ Lowering in the Pharmacologically Relevant Range.** Preclinical and clinical evidence suggests that a modest inhibition in Aβ generation might be sufficient to alter AD progression. First, the plasma Aβ42 levels in patients with sporadic and presenilin 2 mutant (N141I) AD are increased up to 40% of those of controls (Scheuner et al., 1996), implying that a moderate elevation in Aβ generation may lead to AD. Second, the clearance from CSF of newly synthesized Aβ40 and Aβ42 is decreased by 25 and 30%, respectively, in AD versus control subjects (Mawuenyega et al., 2010). Third, an ~30% reduction in γ-secretase activity is sufficient to attenuate Aβ burden in the brain of a genetically modified mouse (Li et al., 2007). Finally, in the heterozygous PDAPP/BACE1(−−) mouse, a 12% reduction in the total

---

**Fig. 4.** Diagnostic plots demonstrate satisfactory performance for modeling brain Aβx-42 in the guinea pig treated with GSM-A. A to C, good agreement between the observed and model predicted Aβx-42 levels in the 30 mg/kg (A), 100 mg/kg (B), and dose-response groups (C, groups nudged on the x-axis for visual resolution). D to F, indicate that the model structure and the error model were reasonable.

---

**CSF Aβ as an Effect Biomarker for Brain Aβ Lowering**

371

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 15, 2017
brain Aβ level at 3 months of age led to a dramatic inhibition in Aβ deposition and neuritic dystrophy over time (McConlogue et al., 2007). These findings suggest that long-term, partial inhibition of Aβ generation may alter AD progression. For drug discovery purposes, we postulate that the pharmacologically relevant target range is up to 50% lowering in brain Aβ with long-term treatment.

We reasoned that CSF Aβ will be a reasonable effect biomarker for brain Aβ lowering if the intrinsic PK/PD relationship for CSF Aβ consistently reflects that of brain Aβ. This is indeed the case from our analyses. In the pharmacologically relevant range, the intrinsic PK/PD relationships for CSF and brain Aβ are similar, consistently demonstrated by compounds of distinct mechanisms across different species [Fig. 5 in this report and Fig. 7 in Lu et al. (2011)]. The similarity indicates that the response of CSF Aβ to a treatment is predictive of the response of brain Aβ. Therefore, CSF Aβ is a reasonable effect biomarker for assessing brain Aβ lowering.

We caution that an x% lowering of Aβ observed in CSF at a given time point does not indicate the same percentage lowering in the brain at that point, as a result of the disparity in Aβ turnover kinetics in the CSF and brain. To predict the

![Fig. 5. Brain and CSF intrinsic PK/PD relationships for LY2811376 (A) and BACE-A (B) in the 129/SVE mouse, GSM-A in the guinea pig (C) and rat (D), and E2012 (E) in the guinea pig. Despite some differences in I_{max} and steepness of the curves, the brain and CSF curves are generally similar to each other in the upper halves. Cbu, drug-free brain concentration.](image-url)
effect in brain on the basis of the effect in CSF, a PK/PD model has to be used to take into consideration the \( \alpha \) turn-over kinetics.

**Disparity between Brain and CSF Effect Is Caused by Compartment-Dependent \( k_{out} \) and \( I_{max} \)**

The brain versus CSF disconnect in \( \alpha \) lowering is a joint effect of \( k_{out} \) and \( I_{max} \) being different between the two compartments. Our modeling of numerous data sets has revealed that the \( k_{out} \) value for CSF \( \alpha \) is generally greater than that for brain \( \alpha \) and that the \( I_{max} \) in CSF is near 1 versus considerably less than 1 in brain. The simulations (Fig. 7), taking into account the compartment-specific \( k_{out} \) and \( I_{max} \), reproduced the dose-dependent separation of CSF and brain \( \alpha \) profiles.

The estimated \( I_{max} \) from modeling has limitations because brain \( \alpha \) lowering frequently does not demonstrate effect saturation, a condition for reliable \( I_{max} \) estimation. However, the brain \( I_{max} \) being less than 1 may be rationalized biologically. 1) The brain \( \alpha \)-40 in the BACE1(−/−) mouse is reduced by 80% compared with that in the background strain (Atwal et al., 2011), indicating contributions of other enzymatic activities to the \( \beta \) cleavage. Other researchers have demonstrated BACE1-like activities by cathepsin B and D (Hook et al., 2008). These findings suggest a less-than-complete inhibition of the \( \beta \) cleavage by current BACEi. 2) There may exist in the brain a static pool of \( \beta \), e.g., \( \beta \) aggregates in the brain parenchyma, not acutely affected by BACEi, GSI, or GSM. Despite the limitations in \( I_{max} \) estimation, our conclusion on the validity of CSF \( \alpha \) as an effect biomarker for brain \( \alpha \) lowering is not crippled because it is based on the well-defined upper halves of the intrinsic PK/PD relationships (i.e., postulated pharmacologically relevant range).

**Questions for Further Investigation.** Two questions closely related to the validity of CSF \( \alpha \) as an effect biomarker for brain \( \alpha \) lowering warrant further investigation. First, what are the sources of CSF \( \alpha \), and what exposure drives CSF \( \alpha \) lowering? Although it is generally believed that CSF \( \alpha \) comes from the brain, recent research argues, in a qualitative manner, that choroid plexus is a potential contributor to CSF \( \alpha \) (Crossgrove et al., 2007). For CSF \( \alpha \) to be a valid biomarker, it is essential to clarify the sources of CSF \( \alpha \) and the relative contribution of each source. Once this clarification is achieved, the driving exposure can be rationalized. In this study, we assumed the brain exposure to be the driving force. This assumption seems to be reasonable given the consistent similarity in the brain and CSF intrinsic PK/PD relationship for numerous compounds of various mechanisms across different species.

The second question requiring further investigation is to understand whether the intrinsic PK/PD relationship for CSF is still predictive of that for brain after repeated dosing. Our analyses here were based on single-dose data. Because repeated dosing is more relevant for clinical development, it is necessary to obtain preclinical understanding of the brain-CSF relationship in a repeated-dosing.

**Implications for Drug Discovery and Development.** Two implications of our results for drug discovery and development are elaborated on here. First, preclinical data must be informative for PK/PD understanding. To guide clinical trials, preclinical PK/PD characterization for brain and CSF \( \alpha \) lowering and the relationship between the two endpoints has to be adequately conducted. As we have advocated, it is crucial to use PK/PD modeling to properly define PK/PD relationships for \( \alpha \)-lowering agents (Lu et al., 2011). To that end, preclinical pharmacology studies should be carefully designed to yield informative time course data covering wide PD ranges to enable modeling (Fig. 8, left panel). Second, brain \( \alpha \) lowering in clinical trial subjects may be predicted quantitatively (Fig. 8, right panel). From CSF exposures and effects observed in trial subjects, an intrinsic PK/PD relationship for CSF \( \alpha \) lowering in those individuals may be determined. With a preclinical understanding of the brain-CSF relationship and an assumption that this relationship applies to humans, the intrinsic PK/PD relationship for brain in those individuals may then be inferred. Consequently, an average \( \alpha \) lowering at steady state is readily predicted from this intrinsic relationship. Furthermore, in conjunction with an estimated human brain \( \alpha \) \( k_{out} \) and brain exposure time course, an \( \alpha \) time course profile in the brain can be predicted.

It is necessary to point out that this study was conducted to enable establishment of proof of mechanism and understanding of the exposure mechanism-biomarker (\( \alpha \) response) relationship in early stages of clinical trials. These trials gen-

---

**Fig. 6.** The \( k_{out} \) values for brain and CSF \( \alpha \) derived from guinea pigs and rats treated with GSM-A or E2012. The \( k_{out} \) of brain \( \alpha \) is 2-fold lower than that of CSF \( \alpha \). The symbols represent the point estimates from three data sets, and the error bars represent S.E.s of estimates. A similar trend of difference was observed for brain and CSF \( \alpha \) in the 129/SVE mouse (Lu et al., 2011).

**Fig. 7.** Simulated brain and CSF \( \alpha \) profiles in a rat treated orally with a compound possessing PK/PD properties as follows: absorption rate constant \( k_{a} = 0.2/h \), total plasma clearance \( CL = 0.4 l/h/kg \), volume of distribution \( V = 4 l/kg \), brain/plasma concentration ratio \( C_{b}/C_{p} = 1 \), baseline \( \alpha \) level \( E_{0} = 100 \), brain \( \alpha \) \( k_{out} = 0.33/h \), CSF \( \alpha \) \( k_{out} = 0.68/h \), total brain IC50 for brain \( \alpha \) = 305 ng/g, total brain IC50 for CSF \( \alpha \) = 547 ng/g, Hill coefficient \( \gamma = 1 \), maximum inhibition \( I_{max} \) for brain \( \alpha \) = 0.7, and \( I_{max} \) for CSF \( \alpha \) = 1. The simulations reproduced the observed pattern of dose-dependent separation between the brain and CSF effect. At 1 mg/kg, the brain and CSF profiles are similar. With an elevation in dose, the brain and CSF curves are increasingly separated; at 100 mg/kg, the maximum \( \alpha \) lowering in brain is only 67%, in contrast to 96% in the CSF. The separation, as suggested by the simulations, is a joint result of the differences in the \( k_{out} \) and \( I_{max} \) between the two endpoints.
erally involve healthy subjects or patients with very early AD subjected to single-dose or short-term repeated (e.g., 2-week) treatment. Under these conditions, the PK/PD relationship in the brain and CSF might be predicted from preclinical understanding in healthy, nontransgenic animals. Once a proof of mechanism is achieved and a compound advances to the next stage of development, the specific exposure–biomarker relationship becomes less important than the PK/PD relationship for functional improvement in patients. At this stage, because of the longer-term (at least several months) treatment, potential plaque mobilization by treatment, and physiological and anatomical deterioration with disease progression, the PK/PD relationship for Aβ lowering in the brain and CSF in patients may differ significantly from preclinical understanding. Therefore, we caution that the learning from this study may not be generalized to disease state or long-term treatment.

In summary, through comprehensive quantitative analyses of preclinical PK/PD data for numerous BACEi, GSI, and GSM across multiple species, we found that CSF Aβ can serve as an effect biomarker for brain Aβ lowering, at least in the pharmacologically relevant range. The implications of this result on drug discovery and development are 2-fold. Preclinically, adequate PK/PD characterization should be conducted to understand the intrinsic relationship for Aβ lowering in the brain and CSF via proper experimentation combined with quantitative modeling. Clinically, Aβ-lowering effect sizes and time course profiles in the brain may be predicted through rational integration of preclinical understanding, scaled physiological parameters, and clinical CSF Aβ-lowering time course data. These predictions will guide informed decision making in drug development.

Acknowledgments

We thank the β-secretase inhibitor, γ-secretase inhibitor, and γ-secretase modulator teams at Pfizer Worldwide Research and Development for their valuable efforts.

Authorship Contributions


Conducted experiments: Hujos-Korcso, Wood, Nolan, Robshaw, Becker, Barricklow, Miller, and Osgood.

Contributed new reagents or analytic tools: O’Neill, Brodney, John- son, and Pettersson.

Performed data analysis: Lu, Zhang, and Tseng.

Wrote or contributed to the writing of the manuscript: Lu, Riddell, Bales, Wood, and Robshaw.

References


Audia JE, Mergott DJ, Sheehan SM and Watson BM (2009), inventors. Eli Lilly and Company, Audia JE, Mergott DJ, Sheehan SM, and Watson BM, assignees. Amyloid-β-lowering, at least in the pharmacologically relevant range. The implications of this result on drug discovery and development are 2-fold. Preclinically, adequate PK/PD characterization should be conducted to understand the intrinsic relationship for Aβ lowering in the brain and CSF via proper experimentation combined with quantitative modeling. Clinically, Aβ-lowering effect sizes and time course profiles in the brain may be predicted through rational integration of preclinical understanding, scaled physiological parameters, and clinical CSF Aβ-lowering time course data. These predictions will guide informed decision making in drug development.

Acknowledgments

We thank the β-secretase inhibitor, γ-secretase inhibitor, and γ-secretase modulator teams at Pfizer Worldwide Research and Development for their valuable efforts.

Authorship Contributions


Conducted experiments: Hujos-Korcso, Wood, Nolan, Robshaw, Becker, Barricklow, Miller, and Osgood.

Contributed new reagents or analytic tools: O’Neill, Brodney, John- son, and Pettersson.

Performed data analysis: Lu, Zhang, and Tseng.

Wrote or contributed to the writing of the manuscript: Lu, Riddell, Bales, Wood, and Robshaw.

References


Audia JE, Mergott DJ, Sheehan SM and Watson BM (2009), inventors. Eli Lilly and Company, Audia JE, Mergott DJ, Sheehan SM, and Watson BM, assignees. Amyloid-β-lowering, at least in the pharmacologically relevant range. The implications of this result on drug discovery and development are 2-fold. Preclinically, adequate PK/PD characterization should be conducted to understand the intrinsic relationship for Aβ lowering in the brain and CSF via proper experimentation combined with quantitative modeling. Clinically, Aβ-lowering effect sizes and time course profiles in the brain may be predicted through rational integration of preclinical understanding, scaled physiological parameters, and clinical CSF Aβ-lowering time course data. These predictions will guide informed decision making in drug development.

Acknowledgments

We thank the β-secretase inhibitor, γ-secretase inhibitor, and γ-secretase modulator teams at Pfizer Worldwide Research and Development for their valuable efforts.

Authorship Contributions


Conducted experiments: Hujos-Korcso, Wood, Nolan, Robshaw, Becker, Barricklow, Miller, and Osgood.

Contributed new reagents or analytic tools: O’Neill, Brodney, John- son, and Pettersson.

Performed data analysis: Lu, Zhang, and Tseng.

Wrote or contributed to the writing of the manuscript: Lu, Riddell, Bales, Wood, and Robshaw.

References


Audia JE, Mergott DJ, Sheehan SM and Watson BM (2009), inventors. Eli Lilly and Company, Audia JE, Mergott DJ, Sheehan SM, and Watson BM, assignees. Amyloid-β-lowering, at least in the pharmacologically relevant range. The implications of this result on drug discovery and development are 2-fold. Preclinically, adequate PK/PD characterization should be conducted to understand the intrinsic relationship for Aβ lowering in the brain and CSF via proper experimentation combined with quantitative modeling. Clinically, Aβ-lowering effect sizes and time course profiles in the brain may be predicted through rational integration of preclinical understanding, scaled physiological parameters, and clinical CSF Aβ-lowering time course data. These predictions will guide informed decision making in drug development.


Lu Y, Zhang L, Nolan CE, Becker SL, Artchison K, Robshaw AE, Pastilnik LR, Osgood SM, Miller EH, Stepan AF, et al. (2011) Quantitative pharmacokinetic/pharmacodynamic analyses suggest that the 129/SVE mouse is a suitable preclinical model for identifying small-molecule 
\( \gamma \)-secretase inhibitors. J Pharmacol Exp Ther 330:922–934.


Address correspondence to: Dr. Lu, MS#220-4546, Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Worldwide Research and Development, Eastern Point Road, Groton, CT 06340. E-mail address: yasong.Lu@pfizer.com