Introduction

The amyloid cascade hypothesis posits that amyloid-β (Aβ) protein peptide levels are increased early in Alzheimer’s disease (AD), leading to the formation of toxic soluble Aβ oligomers (Aβs) and plaques (Di Carlo et al., 2012). According to this hypothesis, a series of causal events initiated by abnormal Aβ levels leads to neuronal cell death and cognitive and functional decline over time (Hardy and Selkoe, 2002). Toxic Aβs are considered to be the drivers of the neurodegeneration (Benilova et al., 2012; Klein, 2013). Such soluble forms of multimeric Aβ peptides are intermediate of soluble Aβ monomers and insoluble Aβ fibrils and likely consist of a mixture of oligomeric species. Aβ dimers, trimers, larger Aβs, and structures such as soluble protofibrils have been isolated from the AD brain (Mc Donald et al., 2010; Esparza et al., 2013; Yang et al., 2013; Savage et al., 2014). Aβs are in a constant equilibrium with Aβ monomers and other Aβ aggregates (Benilova et al., 2012).

Formulation of Aβ requires proteolytic cleavage of the transmembrane protein β-amyloid precursor protein (APP). Sequential cleavage of APP by the enzymes β-secretase 1 (BACE1) and γ-secretase leads to the formation of Aβ (Esler and Wolfe, 2001), as schematically depicted in Fig. 1. Here, cleavage by BACE1 leads to the formation of both the N-terminal secreted fragment soluble β-amyloid precursor protein (sAPP) β and the C-terminal membrane-bound 99-amino acid fragment (C99). C99 is subsequently subject to cleavage by γ-secretase, yielding Aβ species of different chain lengths. The most common Aβ isoforms have 38, 40, or 42 amino acids (Aβ38, Aβ40, or Aβ42, respectively) (Wiltfang et al., 2002). In parallel, full-length APP is also cleaved by α-secretase, leading to the formation of sAPPα, which is nonamyloidogenic (Portelius et al., 2011).

Aβ production in the brain is a target for AD therapy, with the potential for a disease-modifying effect by reducing Aβ levels (Husain et al., 2008). Several BACE1 inhibitors are being tested in human clinical trials, but the optimum level of BACE1 inhibition required for the treatment of AD remains to be determined (Yan and Vassar, 2014). A quantitative understanding of the effects of secretase inhibitors on the APP
pathway may provide greater insights into dose-response pharmacology relationships.

Generally, measures of Aβ response in humans and primates can only be obtained in the cerebral spinal fluid (CSF) and not in the brain. However, it is believed that changes in Aβ concentrations in CSF reflect changes in brain Aβ (Lu et al., 2012). Thus, CSF Aβ serves as key biomarker for Aβ production–targeted therapies (Jack and Holtzman, 2013). The cisterna magna ported (CMP) rhesus monkey model enables longitudinal sampling in the CSF outflow from the cisterna magna in conscious rhesus monkeys. Because APP is completely homologous between humans and rhesus monkeys, the CMP rhesus monkey model is used to study the effects of secretase inhibitors (Podlisny et al., 1991; Gilberto et al., 2003; Cook et al., 2010).

Several studies on the pharmacokinetics (PK) and the pharmacodynamics (PD) of BACE1 and γ-secretase inhibitors have been reported (Lu et al., 2012, 2013; Parkinson et al., 2013; Janson et al., 2014). Liu et al. (2013) proposed a mechanistic PK-PD model of BACE1 inhibition in monkeys. They identified the β-secretase cleavage step as the rate-limiting step for Aβ formation. However, their model is a simplification of the underlying system because no distinction is made between the β-secretase and γ-secretase cleavage steps and Aβ was modeled as a direct product of APP. Potter et al. (2013) used compartmental modeling to investigate the APP processing pathway based on the results from a metabolic tracer study in humans with rare autosomal dominant AD. A model with 18 compartments accounting for the kinetics of Aβ38, Aβ40, and Aβ42 enrichments, including compartments representing APP and C99, was proposed. However, the reported model is structurally and numerically unidentifiable, considering that not all APP metabolites were measured.

To our knowledge, no systems pharmacology model has been reported that provides an integrated description of the effects of drugs on the APP metabolites. Systems pharmacology modeling is an extension of traditional mechanism-based PK-PD modeling, linking the system that is affected by the drug to its treatment-associated measured biomarkers. This involves computational analysis of the time course of the changes in biomarkers on the basis of a structural mathematical model that describes the underlying biologic processes, while making a strict distinction between drug-specific and systems-specific parameters. It has been demonstrated that such mechanism-based PK-PD models have much improved properties for extrapolation and prediction (Danhof et al., 2005, 2007). Systems pharmacology modeling will provide a quantitative understanding of the effects of drugs on the APP processing pathway to improve the prediction and magnitude of Aβ reducing effects.

The objective of this investigation was to characterize the multistep production of Aβ in the brain and its disposition into CSF in the rhesus monkey and to obtain an indirect impression of Aβ42, using information from the monomeric Aβ species. To this end, CSF Aβ dynamic data from CMP monkeys treated with the BACE1 inhibitor MBi-5 were analyzed. Inter-relationships of APP metabolites and their responses to MBi-5 were each measured by an enzyme-linked immunosorbent assay and metabolite responses were then integrated by means of a systems pharmacology modeling approach. Comprehensive, model-based information from MBi-5 PK and PD is integrated across time points, doses, and end points, yielding information on dose response and APP metabolite (sAPPβ, sAPPα, and Aβ) responses and inter-relationships. In this manner, invaluable information is obtained on the functioning of the integrated biologic system. The effect of BACE1 inhibition on Aβ42 is anticipated, which will be measured in future studies.

Materials and Methods

Animals. Animal use procedures conformed to the Guide for the Care and Use of Laboratory Animals (1996 National Research Council Institute of Laboratory Animal Resources) and were reviewed and approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories. The CMP rhesus monkey model was described by Gilberto et al. (2003). The rhesus monkeys were chronically implanted with catheters in the cisterna magna, allowing repeated sampling of CSF and plasma in conscious rhesus monkeys. Six male animals, weighing between 5.2 and 11.7 kg (mean 8.7 kg), aged 2–10 years (mean 8 years), were included in the study. These monkeys were captive bred in a closed colony and individually housed.

Drug Administration and Sampling. The study protocol and pharmacological profile of MBi-5 was described previously by Dobrowolska et al. (2014a). The study protocol is summarized here. In a single-dose, four-way, full crossover study, vehicle (0.4% methylcellulose) or MBi-5 was administered orally at 10, 30, 125 mg/kg (5 mg/kg), with at least 2 weeks washout between each period. Plasma and CSF drug concentrations were collected at 0 (predose) and 3, 5, 7, 9, 13, 16, 19, 22, 25, 28, 31, 49, 55, 58, 73, and 145 hours postdose, resulting in 17 plasma and CSF PK samples for each monkey per treatment group. Two milliliters of blood and 1 ml of CSF were collected at each time point. The concentration of MBi-5 in the plasma and CSF samples was determined using liquid chromatography–tandem mass spectrometry.

The concentrations of Aβ40, Aβ42, sAPPα, and sAPPβ were determined from CSF samples collected at –22, –20, and –1 hours (predose) and 2, 4, 6, 8, 12, 15, 18, 21, 24, 27, 30, 48, 54, 57, 72, and 144 hours postdose, giving 19 measurements of each biomarker for each monkey per treatment group. One milliliter of CSF was collected at each time point. The assays used for the concentration measurements were described previously (Sankaranarayanan et al., 2009; Wu et al., 2011).

PK-PD Analysis. The PK-PD model was developed and fitted to the data by means of nonlinear mixed-effects modeling using the NONMEM software package (version VI, level 2; GloboMax ICON Development Solutions, Ellicott City, MD) (Beal, 2008). This approach takes into account structural (fixed) effects and both intra- and interindividual variability. The following parameters were estimated: typical values of structural model parameters (population parameters, which define the mean value for a parameter in a population) (θ), the variance and covariance of the interindividual variability (ω²), and the variance of the residual error (σ²). A stepwise procedure was used to find the model that best fitted the data. A convergence criterion of three significant digits in the parameter estimates was used. The obtained minimum value of the objective function was used for the comparison of nested models. A decrease of 10.8 points in the likelihood ratio test exclusion of a parameter was considered significant. The first-order conditional estimation approach with ψ-ψ interaction was used for parameter estimation. Random effects at the individual level were included as exponential (eψ), reflecting lognormal distributions of the individual model parameters (eq. 1):

\[ \theta_i = \theta \times e^{\psi_i} \]  

(1)

in which \( \theta_i \) is the value for the \( i \)th individual; \( \theta \) is the typical value for the parameter; and \( \psi_i \) is an interindividual random effect, which is assumed to follow a normal distribution with mean zero and variance \( \omega^2 \).
The residual variability was explored with additive and proportional error models (eqs. 2 and 3, respectively) or a combination of both (eq. 4).

\[ y_{ijk} = f\left(\theta_i\right) + e_{ijk} \]  
\[ y_{ijk} = f\left(\theta_i\right) \times \left(1 + e_{ijk}\right) \]  
\[ y_{ijk} = f\left(\theta_i\right) \times \left(1 + e_{ijk}\right) + e_{ijk} \]  

where \( y_{ijk} \) is the \( k \)th observation on the \( j \)th occasion for the \( i \)th individual; \( f(\theta_i) \) is the corresponding model-predicted observation; and \( e \) represents the residual departure of the observed concentration from the predicted concentration, which is assumed to follow a normal distribution with mean zero and variance \( \sigma^2 \).

To evaluate the prediction of the central tendency and distribution of the observed data by the model, a visual predictive check was performed in which the median and the 90% interquantile range of the data simulated with the developed model were plotted approximately 90% of the observations falling within the 90% prediction interval.

The NONMEM software package was implemented on an Intel QuadCore (Intel Core i7 CPU@60, 2.80 GHz, 3.24 GB RAM; Intel, Santa Clara, CA) and Compaq Visual Fortran (version 6.6; Compaq Computer Corporation, Houston, TX) was used as compiler. Data management and model assessment was done using the S-PLUS for Windows statistical software package (version 8.0 Professional; Insightful Corp., Seattle, WA).

**Model Description.** The systems pharmacology model of MBi-5 was developed by sequential analysis of PK and PD data. The PK model of MBi-5 was based on simultaneous analysis of plasma and CSF PK data. The results of the PK data analysis are provided in the Supplemental Material. The PK profiles of MBi-5 observed in plasma and CSF were adequately described by a three-compartmental model (Supplemental Figs. 1 and 2) and the PK parameters were estimated with good precision (Supplemental Table 1); thus, the model could serve as input for PD model analysis.

The biomarker response profiles of MBi-5 measured in CSF were adequately described by a model containing compartments for five variables: APP, sAPP\( \beta \), sAPP\( \alpha \), A\( \beta \)40, and A\( \beta \)42 (Fig. 2). The production of APP was believed to be zero order (i.e., a constant production of APP). It was assumed that there is no alternative proteolytic enzyme cleaving full-length APP other than \( \alpha \)-secretase and BACE1. Because both sAPP\( \beta \) and C99 are products of APP cleavage by BACE1, sAPP\( \beta \) and C99 were presumed to follow the same kinetics; therefore, sAPP\( \beta \) could be used in the model as a surrogate precursor for A\( \beta \). The production of sAPP\( \alpha \), sAPP\( \beta \), and A\( \beta \) were assumed to be first order (i.e., dependent on the concentration of its precursor). The interaction between APP, sAPP\( \beta \), sAPP\( \alpha \), A\( \beta \)40, and A\( \beta \)42 is described by eqs. 5–9:

\[
\frac{d}{dt} \text{APP} = R_{\text{in}APP} - \left( R_{\text{out}APP} \times \text{EFF} + R_{\text{in}APP} \right) \times \text{APP} \\
\frac{d}{dt} \text{sAPP\( \beta \)} = R_{\text{in}sAPP\( \beta \)} - R_{\text{out}sAPP\( \alpha \)} \times \text{sAPP\( \alpha \)} \\
\frac{d}{dt} \text{sAPP\( \alpha \)} = R_{\text{in}sAPP\( \alpha \)} - \left( K_{\text{in}40} + K_{\text{in}42} \right) \times \text{sAPP\( \alpha \)} \\
\frac{d}{dt} \text{A\( \beta \)40} = K_{\text{in}40} \times \text{sAPP\( \beta \)} - K_{\text{out}A\( \beta \)40} \times \text{A\( \beta \)40} \\
\frac{d}{dt} \text{A\( \beta \)42} = K_{\text{in}42} \times \text{sAPP\( \beta \)} - K_{\text{out}A\( \beta \)42} \times \text{A\( \beta \)42} \\
\]

The rate of change of APP with respect to time in the presence of the inhibitor is described by eq. 5, in which the BACE1 cleavage inhibition is incorporated by the factor EFF. EFF is the degree of inhibition caused by MBi-5, expressed as shown in eq. 10.

\[
\text{EFF} = 1 - \frac{C_{\text{target}}}{C_{\text{IC50}}} \\
\]

where \( C_{\text{target}} \) is the target site concentration of MBi-5, \( C_{\text{IC50}} \) is the \( C_{\text{target}} \) that results in 50% inhibition of BACE1, \( \text{max} \) is the maximum response, and GAM is the Hill coefficient. \( C_{\text{target}} \) was derived from the PK model as shown in eq. 11:

\[
C_{\text{target}} = C_{\text{plasma}} \times \frac{\text{AUC}_{\text{CSF}}}{\text{AUC}_{\text{plasma}}} \\
\]
where AUC_CS and AUC_plasma are the areas under the CSF and plasma concentration time curves, respectively. Here, C_target is assumed to follow the same profile as C_plasma but at a level between C_CS and C_plasma.

It is assumed that the system is in steady state when no treatment is given (EFF = 1). At the treatment free state, the change of the variables with respect to time is shown in eq. 12:

\[
\begin{align*}
\frac{d}{dt} APP &= 0 \\
\frac{d}{dt} sAPP &= 0 \\
\frac{d}{dt} Aβ40 &= 0 \\
\frac{d}{dt} Aβ42 &= 0
\end{align*}
\] (12)

These steady-state conditions were used to derive part of the system parameters. From eqs. 5 and 12, and it follows that the source of APP ( RinAPP) is shown in eq. 13:

\[
R_{inapp} = (R_{in} + R_{inh}) \times APP_{base}
\] (13)

where APP_base is the baseline level of APP, which is assumed to be equal to the sum of the baseline levels of sAPPα and sAPPβ, because it was assumed that there is no alternative proteolytic enzyme cleaving full-length APP other than α-secretase and BACE1. Using eqs. 6 and 12, the sAPPα formation rate ( R_{inα}), equivalent to the α-secretase cleavage step, can be derived shown in eq. 14:

\[
R_{inα} = R_{outα} \times \frac{sAPPα_{base}}{APP_{base}}
\] (14)

where sAPPα_{base} is the baseline level of sAPPα.

The sAPPβ formation rate ( R_{inβ}), equivalent to the BACE1 cleavage step, follows from eqs. 7 and 12, as shown in eq. 15:

\[
R_{inβ} = (K_{in40} + K_{in42}) \times \frac{sAPPβ_{base}}{APP_{base}}
\] (15)

where sAPPβ_{base} is the baseline level of sAPPβ.

From eqs. 8 and 12, the Aβ40 formation rate ( K_{in40}), equivalent to a γ-secretase cleavage step can be calculated as shown in eq. 16:

\[
K_{in40} = K_{out} \times \frac{Aβ40_{base}}{sAPPβ_{base}}
\] (16)

where Aβ40_{base} is the baseline level of Aβ40. sAPPβ_{base} is the baseline level of sAPPβ, used here as surrogate for the baseline level of C99.

From eqs. 9 and 12, with substitution of K_{out} from eq. 16, the Aβ42 formation rate ( K_{in42}), equivalent to a γ-secretase cleavage step, is deduced as shown in eq. 17:

\[
K_{in42} = K_{in40} \times \frac{Aβ42_{base}}{Aβ40_{base}}
\] (17)

where Aβ42_{base} is the baseline level of Aβ42.

The model structure includes four transit compartments (Fig. 2), one for each biomarker measured in CSF (sAPPα, sAPPβ, Aβ40, and Aβ42), to account for transport from the target site in the brain to CSF. These transit processes are described, in general, by eq. 18:

\[
\frac{d}{dt} species_{CSF} = K_t \times (species – species_{CSF})
\] (18)

where K_t is the transit rate for the particular species ( K_AP for sAPPα and sAPPβ and K_AB for Aβ40 and Aβ42).

The system defined above can now be extended to incorporate an Aβ3 pool for Aβ42 oligomerization. The addition of the Aβ3 pool to the model structure requires adaptation of eq. 9, describing Aβ42 dynamics. The exchange between the Aβ3 pool and the Aβ42 compartment is described by eqs. 19 and 20:

\[
\frac{d}{dt} Aβ42 = \frac{K_{in42} \times sAPPβ – K_{out42} \times Aβ42 – K_{pl} \times Aβ42}{K_{rev} \times Aβ3} + K_{rev} \times Aβ3
\] (19)

\[
\frac{d}{dt} Aβ3 = K_{pl} \times Aβ42 - K_{rev} \times Aβ3
\] (20)

where K_{pl} and K_{rev} are the Aβ42 oligomerization and dissociation rate, respectively, which are dependent on the baseline values of Aβ42 and the Aβ3 pool (Aβ42_{base} and Aβ3_{base}, respectively) according to eq. 21:

\[
K_{rev} = \frac{K_{pl} \times Aβ42_{base}}{Aβ3_{base}}
\] (21)

### Results

**Separate Empirical Models Described Response of Each APP Metabolite.** Initially, empirical PK-PD models were developed to quantify the exposure-response relationships for each CSF APP metabolite (Aβ40, Aβ42, sAPPα, and sAPPβ) of the BACE1 inhibitor MBi-5 in monkeys. The typical model structure of each APP metabolite-inhibitor combination consisted of a transit model with one or two compartments, with the drug effect modeled relative or subtractive to baseline using an I_max/E_max function. A summary overview of the results of these models is depicted in Table 1. The empirical models provided consistency of drug effects across APP metabolites, with identified potencies [95% confidence intervals (CIs)] of 0.0254 μM (0.0246–0.0262) for Aβ40, 0.0455 μM (0.0351–0.0559) for Aβ42, 0.0490 μM (0.0192–0.0788) for sAPPβ, and 0.0265 μM (0.0135–0.0395) for sAPPα. The mean transit time through the compartments of the models was...
lower for Aβ40 and Aβ42 than for sAPPβ and sAPPα. This indicates that the response of Aβ40 and Aβ42 will appear earlier in CSF, even though sAPPβ is a sequentially earlier product of the amyloidogenic APP pathway.

A Systems Model to Describe APP Metabolite Responses. A comprehensive compartmental PK-PD model, incorporating MBI-5 PK and CSF APP metabolite (Aβ40, Aβ42, sAPPα, and sAPPβ) concentrations was developed to quantify APP metabolite responses to BACE1 inhibition in monkeys. The model is schematically presented in Fig. 2. The model describes production, elimination, and brain-to-CSF transport of each APP metabolite, as well as their inter-relationships (Fig. 3). The rate of APP metabolism was assumed to be close to the maximal capacity of the enzymes involved (Nelson and Cox, 2000). Thus, APP production was approximated to follow zero-order kinetics. sAPP was used in the model structure as a surrogate substrate for C99 in the γ-secretase cleavage step. Because both sAPPβ and C99 are products of the APP cleavage by BACE1, their formation rates should be the same; thus, use of sAPPβ as a surrogate for C99 was justified. To account for transport from the target site in the brain to CSF, the model included one transit compartment for each APP metabolite. The drug target site in the brain to CSF, the model included one for C99 was justified. To account for transport from the target site in the brain to CSF, the model included one transit compartment for each APP metabolite. The drug target site in the brain to CSF, the model included one

MBI-5 Increased sAPPα and Decreased sAPPβ and Aβ in a Dose-Dependent Manner. APP metabolite CSF concentrations showed a dose-dependent response in the presence of the BACE1 inhibitor. The dose-dependent increase of sAPPα and the corresponding decreases of sAPPβ and Aβ were described by the model with a single drug effect. A potency (IC50) of 0.0256 μM (95% CI, 0.0137–0.0375) was identified. This value is close to the in vitro inhibition constant (K) of 10 nM for MBI-5 inhibition of purified BACE1 and is also close to the IC50 for inhibition of Aβ production in intact cells of 24 ± 6 nM (Dobrowolska et al., 2014a). When estimated, the maximal inhibition (Imax) was close to 1. Therefore, Imax could be fixed to 1, indicating 100% inhibition of BACE1 at sufficient high drug concentrations. Figures 4–7 show the model description of each APP metabolite for each dose group.

Aβ4 Pool Required to Account for Differential Effect on Aβ40 and Aβ42. A differential effect of BACE1 inhibition was observed for Aβ40 and Aβ42: a higher response is observed in the data for Aβ40 than for Aβ42 (Fig. 7, E and G). This differential effect could be described by extending the model with an Aβ4 pool connected to the Aβ42 compartment, resulting in an adequate description of sAPPβ, sAPPα, Aβ40, and Aβ42 CSF concentration time profiles for each dose group (Figs. 4–7, respectively). Incorporating the Aβ4 pool in the model improved the description of Aβ40 response for the 30- and 125-mg/kg dose group (Figs. 6, E and F, and 7, E and F), as well as the description of the 125-mg/kg dose for Aβ42 response (Fig. 7, G and H). Furthermore, the description of the sAPPβ response for the 125-mg/kg dose (Fig. 7, C and D) was improved. Exchange of an Aβ40 monomer pool with an Aβ pool was evaluated but could not be identified.

Model Parameters. The population parameters and intra- and interanimal variability were optimized for the study population and are depicted in Table 2. To select the best random-effects model structure, a sequence of models with interanimal variability on different parameters was tested and the results were compared. The final model included interanimal variability for the baseline of sAPPβ and the IC50 of MBI-5. Both were included as exponential in nature, reflecting lognormal distributions of the individual model parameters. Because the baselines of the other APP metabolites were modeled as function of the baseline of sAPPβ, its interanimal variability reflects also on the other baselines. Residual, or unexplained, variability was described for each APP metabolite (sAPPβ, sAPPα, AB40, AB42) separately. Residual variability was assumed to follow a normal distribution. Proportional errors were used to describe the residual variability for each APP metabolite. The identified residual variability was higher for Aβ40 and Aβ42 than for sAPPβ and sAPPα. System-specific parameters could be
distinguished from drug-specific parameters (all correlations < 0.95).

Incorporating the $A\beta_0$ pool into the model improved the description and did not affect the parameter estimate of the $IC_{50}$ significantly: with the $A\beta_0$ pool, an $IC_{50}$ of 0.0269 $\mu$M (95% CI, 0.0154–0.0384) was identified; without the $A\beta_0$ pool, the $IC_{50}$ was 0.0256 $\mu$M (95% CI, 0.0137–0.0375). The incorporation of the $A\beta_0$ pool affected the Hill coefficient of the sigmoidal $I_{max}$ concentration response relationship. The $A\beta_0$ pool resulted in a Hill coefficient slightly deviating from unity: with the $A\beta_0$ pool, a Hill coefficient of 1.53 (95% CI, 1.14–1.92) was identified; without the $A\beta_0$ pool, the Hill coefficient was 1 (fixed). This mainly improved the description of the APP metabolite concentration response curves for the higher dose groups (Fig. 7).

**Higher Brain-to-CSF Transport of $A\beta$.** It was not possible to separate the rate of the $\gamma$-secretase cleavage step and brain-to-CSF transfer. For sAPP$\beta$ and sAPP$\alpha$, the transit rate was estimated to be 0.0895 h$^{-1}$. This value should be interpreted relative to the $A\beta$ transit from the brain to the CSF. For sAPP$\alpha$, the brain turnover (0.8 hours) could be distinguished from the half-life of the brain-to-CSF transfer (7.0 hours). $A\beta$ is transported from the brain to the CSF approximately 10$^5$-fold faster than sAPP$\alpha$. As a result, the response of $A\beta$ to drug treatment will appear earlier in the CSF than the response of sAPP$\alpha$, even though sAPP$\alpha$ is a sequentially earlier product of the APP pathway.

It was not possible to identify the brain turnover of sAPP$\beta$ as a separate parameter. In the model structure, sAPP$\beta$ was used as a surrogate substrate for C99 in the $\gamma$-secretase cleavage step, driving the response of $A\beta$. Therefore, the $\gamma$-secretase cleavage step could not be separated from sAPP$\beta$ elimination.

**APP Metabolite Inter-Relationships.** The proteolytic cleavage rates of APP through the action of BACE1 ($R_{\text{ina}}$) and $\alpha$-secretase ($R_{\text{ina}}$) were calculated from the model...
parameters according to eqs. 14 and 15 to be 0.314 h\(^{-1}\) and 0.404 h\(^{-1}\), indicating that 56% of full-length APP is cleaved by \(\alpha\)-secretase and 44% by BACE1. The formation rates of A\(\beta\)40 and A\(\beta\)42 were calculated according to eqs. 16 and 17. The higher \(K_{\text{in}40}\) (0.574 h\(^{-1}\)) than \(K_{\text{in}42}\) (0.020 h\(^{-1}\)) is in line with previously reported findings of the ratio between A\(\beta\)42 and A\(\beta\)40 of about 1:10 in the non-AD brain (Iwatsubo et al., 1994). A difference in A\(\beta\)40 and A\(\beta\)42 degradation rate (\(K_{\text{out}}\)) was also evaluated during the model development process, but this could not adequately capture the response profile of A\(\beta\)42.

Fig. 5. Dose of 10 mg/kg. Visual predictive check of the biomarker response versus time profile of MBi-5 in the rhesus monkey, with 90% CIs. (A–H) Predictions were performed with the model with (B, D, F, H) and without (A, C, E, G) the A\(\beta\)0 compartment. The observation sample size was 114 for each APP metabolite from six monkeys collected over 7 days. Plus symbols represent observed measurements. Dotted lines correspond to the median observed profile. Solid lines show the median simulated profiles. Long-dashed lines correspond to the 90% prediction intervals obtained from 1000 individual simulated profiles.
The developed model could be used to predict biomarker inter-relationships in response to BACE1 inhibition and visualize the response of APP and Aβ (Fig. 3A) in brain. APP increases after BACE1 inhibition and appears to be shunted down the α-secretase pathway, resulting in an increase of sAPPα product. The elevation in sAPPα in the data drives the modeling conclusion that there is some increase in APP in the setting of BACE1 inhibition but it is
fairly modest. The $\beta_\text{lo}$ level decreases after BACE1 inhibition, indicating that there is reduced formation of $\beta_\text{lo}$ by reduced levels of monomeric $\beta_42$ and that $\beta_\text{lo}$ dissociates to monomeric $\beta_42$. The latter influences the shape of the $\beta_42$ response curve, which is different than the shape of the $\beta_40$ response curve.

For sAPP$\beta$, sAPP$\beta$, $\beta_40$, and $\beta_42$, the time courses of brain versus CSF responses were predicted (Fig. 3, respectively), showing that the earlier appearance of $\beta_42$ response in CSF relative to sAPP$\alpha$ and sAPP$\beta$ arises from the slower brain-to-CSF transfer.
TABLE 2

Population parameter estimates including CV% for the extended model with $\text{A}_\beta$ pool

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{sAPP}_\beta$&lt;sub&gt;base&lt;/sub&gt;</td>
<td>Baseline sAPP$\beta$</td>
<td>$1.19 \times 10^{3}$ pM</td>
<td>11.6</td>
</tr>
<tr>
<td>$K_{\text{IC50}}$</td>
<td>Median inhibition concentration</td>
<td>0.0269 μM</td>
<td>21.8</td>
</tr>
<tr>
<td>$R_{\alpha}$</td>
<td>Oligomerization rate</td>
<td>1.53</td>
<td>13.1</td>
</tr>
<tr>
<td>$F_{\text{trans}}$</td>
<td>Baseline $\text{A}_\beta$</td>
<td>278 pm</td>
<td>41.0</td>
</tr>
<tr>
<td>$\text{IC}_{50}$</td>
<td>Median inhibition concentration</td>
<td>0.0269 μM</td>
<td>21.8</td>
</tr>
<tr>
<td>GAM</td>
<td>Hill coefficient</td>
<td>1.53</td>
<td>13.1</td>
</tr>
<tr>
<td>$K_{\text{AB}}$</td>
<td>Transit rate of sAPP$\alpha$ and sAPP$\beta$</td>
<td>2.04 h$^{-1}$</td>
<td>20.0</td>
</tr>
<tr>
<td>Interanimal variability&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Interanimal variability of baseline sAPP$\beta$</td>
<td>0.0568</td>
<td>30.1</td>
</tr>
<tr>
<td>Interanimal variability&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Interanimal variability of $\text{IC}_{50}$</td>
<td>0.279</td>
<td>35.5</td>
</tr>
<tr>
<td>Residual error&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Residual variability of $\text{A}_\beta$</td>
<td>0.240</td>
<td>12.7</td>
</tr>
<tr>
<td>Residual error&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Residual variability of $\text{A}_\beta$</td>
<td>0.161</td>
<td>12.4</td>
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<tr>
<td>Residual error&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Residual variability of sAPP$\beta$</td>
<td>0.0621</td>
<td>23.5</td>
</tr>
<tr>
<td>Residual error&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Residual variability of sAPP$\alpha$</td>
<td>0.0634</td>
<td>10.6</td>
</tr>
</tbody>
</table>

CV%, coefficient of variation.

<sup>a</sup>$\text{A}_\beta$<sub>base</sub> = $F_{\text{trans}}$<sub>base</sub> $\times$ sAPP$\beta$<sub>base</sub>.

<sup>b</sup>$\text{A}_\beta$<sub>base</sub> = $F_{\text{trans}}$<sub>base</sub> $\times$ sAPP$\beta$<sub>base</sub>.

<sup>c</sup>Residual variability of sAPP$\alpha$.

<sup>d</sup>Residual variability of sAPP$\beta$.

<sup>e</sup>Interanimal variability is assumed to follow a normal distribution with mean zero and variance $\sigma^2$.

<sup>f</sup>Residual variability is assumed to follow a normal distribution with mean zero and variance $\sigma^2$.

Discussion

A systems model of the APP processing pathway was developed describing the inter-relationships of $\text{A}_\beta$, $\text{A}_40$, sAPP$\alpha$, and sAPP$\beta$ upon inhibition of BACE1 with MBI-5. All four APP metabolites provided consistent information regarding drug potency. The MBI-5 concentration-dependent decrease of the APP metabolites could be described by incorporating a single drug effect in the model: inhibition of the formation rate of sAPP$\beta$, equivalent to the BACE1 cleavage step. The model supported the notion that higher $\text{IC}_{50}$s can provide 100% inhibition of BACE1 implies that there are no inherent mechanistic limitations of the APP pathway to blocking $\text{A}_\beta$ production. Therefore, reaching complete inhibition largely depends on drug properties such as having sufficient potency, bioavailability, and tolerability.

The relatively large interanimal variability identified on the baseline level of sAPP$\beta$ (also reflecting on the baseline levels of the other APP metabolites) and the $\text{IC}_{50}$ probably denotes the large intrinsic biologic differences in APP processing between subjects. Residual variability was higher for $\text{A}_40$ and $\text{A}_42$ than for sAPP$\beta$ and sAPP$\alpha$. Residual variability represents the uncertainty in the relationship between the concentrations predicted by the model and the observed concentration and includes any model misspecification error. The higher residual variability for $\text{A}_\beta$ could be related to the second cleavage step by $\gamma$-secretase, yielding $\text{A}_\beta$. In this analysis, no direct information was available regarding the $\gamma$-secretase cleavage step. This would require data from a $\gamma$-secretase inhibitor study. Such data may explain some of the residual variability identified for $\text{A}_\beta$.

The systems model identified a higher brain-to-CSF transport of $\text{A}_\beta$ compared with sAPP$\alpha$. This result is consistent with the identified lower mean transit time for $\text{A}_40$ and $\text{A}_42$ compared with sAPP$\alpha$ in the separate empirical models for each APP metabolite. The potencies identified in the empirical models were consistent with the single potency of 0.0256 μM (95% CI, 0.0137–0.0375) identified using the systems model.

In the systems model, the APP production was approximated to follow zero-order kinetics. In reality, APP production is regulated by various factors (above all, the synaptic activity) (Cheng et al., 2014). In this analysis, no quantitative data on the factors involved in APP production were available. It was assumed that the APP production was close to the maximum. Consequently, subtle changes in APP regulation would have little effect on APP metabolite formation.

Our modeling results imply that 56% of full-length APP is cleaved by $\alpha$-secretase and 44% by BACE1. To our knowledge, there are no quantitative data available from the literature on the ratio of APP moving down the $\alpha$-secretase pathway and BACE1 pathway. Dobrowolska et al. (2014b) compared sAPP$\alpha$ and sAPP$\beta$ levels in human CSF from the lumbar region from cognitively normal and AD participants. They identified an sAPP$\beta$/sAPP$\alpha$ ratio of 0.59 ± 0.4 (n = 15) in cognitively normal healthy controls. Wu et al. (2011) reported sAPP$\alpha$ and sAPP$\beta$ levels of 37.1 pmol/g and 50.8 pmol/g, respectively, in human brain cortex samples from elderly subjects without AD (n = 16) (Wu et al., 2011), resulting in a ratio of 0.73. However, both ratios do not directly reflect the ratio of APP cleaved by $\alpha$-secretase and BACE1, because the steady-state sAPP$\beta$ and sAPP$\alpha$ levels in the brain are the result of multiple processes such as production, degradation, and transfer from the brain to the CSF. Levels in the CSF are also affected by transfer through the lumbar region. In our analysis, the developed model facilitated the separation of the different processes involved.

The systems analysis points to a difference in biology of $\text{A}_40$ and $\text{A}_42$. First, a lower formation rate was identified...
for Aβ42 than for Aβ40. This is consistent with the composition of Aβ species reported for human CSF, in which Aβ40 is the dominant isoform (Murphy et al., 1999). The relative production of Aβ40 and Aβ42 is probably regulated through changes in the γ-secretase cleavage site (Murphy et al., 1999; Dolev et al., 2013). Second, the model included an Aβo pool in the brain for Aβ42 but not Aβ40. Inclusion of exchange of an Aβ42 monomer pool with an Aβo pool could account for the differential effect of MBi-5 on Aβ40 and Aβ42 observed in the data, in which the response for Aβ40 was higher than for Aβ42. Without incorporation of the Aβo pool into the model, the Aβ40 and Aβ42 response both could not be described adequately by a single drug effect. The identification of this Aβo constitutes the scientific basis for the identification of BACE1 inhibitor effects on higher-ordered amyloid species. Because of the dissociation of Aβ42 oligomers to Aβ42 monomers during BACE1 inhibition, the response for Aβ42 was lower than for Aβ40. The differential effect of the Aβo pool on Aβ42 is consistent with the biology of oligomer and plaque formation in which Aβ42 plays a more significant role than Aβ40 (Di Carlo et al., 2012). Aβ42 is the major constituent of plaque and other species such as oligomers (Jarrett et al., 1993; Iwatsubo et al., 1994).

The baseline level of the Aβo pool estimated by the model of 278 pM (1255 pg/ml) should be interpreted as the level of Aβ42 monomers that are incorporated in the “oligomer soup” in the brain (i.e., Aβ dimers, trimers, and high molecular weight species) (Benilova et al., 2012). Here, no distinction is made between oligomeric species, because the Aβo pool is modeled as a pool in equilibrium with monomeric Aβ42 without correction for the number of subunits in multimeric species comprising the Aβo pool.

The Aβo dissociation rate (K_u) of 1.308 × 10^{-6} s^{-1} identified here is relatively slow compared with the dissociation of Aβ aggregates acquired from in vitro analysis techniques. Grünig et al. (2013) detected the appearance of monomers from Aβ42 and Aβ40 protofibrils: the dissociation rate was 1.4 × 10^{-4} and 1.2 × 10^{-4} s^{-1} for Aβ42 and Aβ40, respectively. Narayan et al. (2012) reported Aβ40 fibrils releasing soluble Aβ40 species at a rate of 9.3 × 10^{-5} s^{-1}. Sánchez et al. (2011) identified Aβ monomer off rates of 0.6 × 10^{-2} and 1.0 × 10^{-2} s^{-1} for Aβ40 and Aβ42 fibrils, respectively. It is difficult to compare rates obtained in vivo with those determined using in vitro approaches, because the in vitro experimental settings can have a major effect. Moreover, the comparability of the dissociation rates to the value obtained in our analysis is limited, because no particular oligomeric species was characterized in the systems pharmacology approach.

Quantitative measurements of the response of Aβo to drugs targeting the APP pathway, such as BACE1 inhibition, are of interest. Initial results of a new sensitive Aβ oligomer assay (Savage et al., 2014) suggest an Aβo baseline level of approximately 1.5 pg/ml in rhesus CSF from the cisterna magna, which constitutes different oligomeric species (Savage and Kalinina, unpublished observations). This number cannot be compared directly with the model-derived Aβo baseline of 1255 pg/ml representing Aβ subunits in the “oligomer soup” in the brain. In addition, the Aβ-oligomer assay may not pick up all oligomeric species or Aβo may dissociate to monomers during sample preparation. Furthermore, it remains to be seen whether CSF Aβo measurements accurately reflect the brain Aβo concentrations (Rosenblum, 2014). The hydrophobicity of oligomers may make them very low or absent in aqueous fluids such as CSF (Yang et al., 2013). Recent data suggest that human CSF Aβo ranges between 0.1 and 10 pg/ml and human brain Aβo levels are 252 pg/ml in the AD brain and 87 pg/ml in the control brain (Savage et al., 2014). Other data suggest 1000-fold higher concentrations of Aβo than monomers in the soluble fraction of the human AD cerebral cortex (Yang et al., 2013). Relative concentrations in the CSF will not necessarily reflect the relative concentrations in the brain because oligomers are likely cleared to the CSF much more slowly than monomeric Aβ. Additional dose-ranging studies of BACE1 inhibition in rhesus monkeys, in which Aβo response is quantified, are ongoing. It is anticipated that including such data in the systems pharmacology model analysis will elucidate the relationship between the Aβo pool in the model and measurements of Aβo.

The identified Aβo pool should be interpreted with caution because an Aβo pool in rhesus monkeys may differ from an Aβo pool in patients with AD with plaque burden. Rhesus monkeys do not develop dementia and neurodegenerative changes that characterize AD (Heuer et al., 2012). It is almost certain that the rhesus monkeys used in this study had far less amyloid deposition than a human patient with AD. Therefore, the most crucial question is the nature of the identified Aβo pool in rhesus monkeys and its pathologic relevance to AD in humans. Aβ oligomerization is a separate aggregation event. Certain oligomers are off-pathway species that do not further aggregate to amyloid fibrils. Coexistence of several oligomeric populations that do or do not propagate into fibrils is possible. If it can be demonstrated that there is a relationship between the soluble Aβo identified in rhesus monkeys and the AD brain-derived soluble Aβo, and this could be correlated with neurotoxicity, then the relevance of Aβo in rhesus monkeys would be indisputable.

The lack of success of clinical trials targeting the APP pathway has been ascribed to the failure to reduce the level of toxic Aβo (Rosenblum, 2014). Plaques, toxic Aβo, and Aβ peptides should be targeted to significantly reduce soluble Aβ load because of the relationship between these three. It has been hypothesized that by decreasing Aβ levels, soluble Aβo amounts are also reduced, in turn inducing the release of Aβo from plaques to restore the balance between Aβo in the plaques and the extracellular environment (Rosenblum, 2014).

The ability to identify and estimate the oligomerization effect through modeling suggested that these efforts to model the monomer pathway may also provide information on the higher-ordered amyloid species. The ability to see this effect suggested that Aβ production inhibition by MBi-5 may also have the ability to draw down these forms as well as inhibit Aβ de novo production. In a study with APP transgenic mice, it has been demonstrated that BACE1 inhibition reduces amyloid plaque load (Kennedy and Hide, unpublished observations). This implies that if monomeric Aβ levels decrease as a result of blocked Aβ production, Aβo dissociate to restore the equilibrium between monomeric Aβ and Aβo. To confirm this, incorporation of Aβo data into the model using rhesus data is ongoing.

A comprehensive model of the APP pathway describing the effects the BACE1 inhibitor MBi-5 has been established, taking into account the kinetics and inter-relationships of sAPPα, sAPPβ, Aβ40, and Aβ42. The effect of BACE1 inhibition was incorporated into the model as inhibition of the formation rate of sAPPβ. Because sAPPβ and C99 are both products of the same BACE1 cleavage step, the response of sAPPβ could be used as a driver of Aβ response. However, sAPPβ and C99 could be subjected to different elimination processes because C99 remains membrane bound (Selkoe, 1999). The fact that the Hill coefficient of the concentration response relationship
slightly deviates from unity may be a reflection of this simplification of the underlying biologic system. To adequately separate the sequential cleavage steps of BACE1 and γ-secretase from other processes involved, data from a γ-secretase inhibitor study in CMP rhesus monkeys (Cook et al., 2010) will be added to further inform the model (ongoing).

Because BACE1 is the initiating enzyme in Aβ production, its inhibition has been proposed to decrease the amount of cerebral Aβ and to subsequently prevent the development of Aβ-associated pathologies (Cole and Vassar, 2007). With the developed systems pharmacology model, a deeper comprehension of the effects of β-secretase inhibition on the APP processing pathway and the anticipated effect on Aβ, was gained. Understanding these effects early in preclinical development could improve the anticipation of the magnitude of Aβ reducing effects in humans. The model forms the first step in developing a translational systems model to predict possible Aβ response of new drug candidates in humans, based on their estimated potency in rhesus monkeys.

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Authorship Contributions

Participated in research design: Michener, Savage, Kennedy, Kleinj, Stone.

Conducted experiments: Michener.

Data analysis: van Maanen, van Steeg, Stone.

Wrote or contributed to the writing of the manuscript: van Maanen, van Steeg, Savage, Kennedy, Stone, Danhof.

References


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SUPPLEMENTAL MATERIAL

Title: Systems pharmacology analysis of the amyloid cascade following β-secretase inhibition enables the identification of an Aβ42 oligomer pool

Authors: Eline M.T. van Maanen, Tamara J. van Steeg, Maria S. Michener, Mary J. Savage, Matthew E. Kennedy, Huub Jan Kleijn, Julie A. Stone, Meindert Danhof

Journal name: Journal of Pharmacology and Experimental Therapeutics

Pharmacokinetic Data Analysis

The exposure at the target site in the brain can rarely be quantified directly. In the cisterna magna ported (CMP) rhesus monkey model exposure can be measured in cerebrospinal fluid (CSF) in addition to plasma. The pharmacokinetics (PK) in plasma and CSF can be used to derive a measure of exposure at the target site. Therefore, a population PK model was developed that describes the PK of MBi-5 in plasma and CSF in CMP rhesus monkeys. The results of the PK analysis of MBi-5 were included in the subsequent PK-PD analysis.

The PK model was developed and fitted to the data by means of non-linear mixed effects modeling using the NONMEM software package version VI level 2 (see the Materials and Methods section in associated article).

The compartmental PK model of MBi-5 was based on simultaneous analysis of plasma and CSF PK data. The PK profiles of MBi-5 in plasma and CSF were adequately described by a model containing three compartments: a central, peripheral and CSF compartment (Supplemental Figure 1). The CSF compartment is linked to the central compartment, with exchange determined by rate constants $K_{32}$ and $K_{23}$. The model considered elimination from the central and CSF compartment, where the elimination from the central compartment ($K_{20}$) is described by the
Michaelis-Menten equation (Supplemental Equation S1).

\[ K_{20} = \frac{V_{\text{MAX}}}{K_M + \frac{A_2}{V_2}} \]  

(S1)

The rate of change in each compartment can be expressed as:

\[ \frac{d}{dt} A_1 = -K_a \times A_1 \]  

(S2)

\[ \frac{d}{dt} A_2 = K_a \times A_1 - K_{24} \times A_2 + K_{42} \times A_4 - K_{23} \times A_2 + K_{32} \times A_3 - \frac{V_{\text{MAX}} \times A_2}{K_M + \frac{A_2}{V_2}} \]  

(S3)

\[ \frac{d}{dt} A_3 = K_{23} \times A_2 - K_{32} \times A_3 - K_{30} \times A_3 \]  

(S4)

\[ \frac{d}{dt} A_4 = K_{24} \times A_2 - K_{42} \times A_4 \]  

(S5)

MBi-5 displayed nonlinear PK at different kinetic levels. The extent of the absorption decreased with an increase in dose \((K_a, \text{ from } 10.0 \text{ to } 0.144 \text{ h}^{-1} \text{ for } 10 \text{ and } 125 \text{ mg/kg, respectively). The distribution to the CSF compartment appeared to be saturable, reflected in a decrease in the rate constant from the central to CSF compartment for the 125 mg/kg dose \((K_{23}, \text{ from } 0.000488 \text{ to } 0.000116 \text{ h}^{-1}). Elimination was identified from the central and CSF compartment. As the
elimination of MBi-5 from the central compartment followed Michaelis-Menten kinetics (Supplemental Equation (S1)) the clearance in the central compartment changed as function of time and concentration (CL$_2 = K_{20}(t,C_p)\times V_2$). At the doses included in the current investigation, clearance in the CSF compartment (CL$_3 = K_{30} V_3$) was approximately $10^6$-fold greater than clearance from the central compartment, indicating that the CSF clearance route contributes remarkably.

Table 1 shows all PK parameter estimates. The volume of the CSF compartment could not be estimated and was fixed to a small value (0.0250 L). Interanimial variability was quantified for the volume of the central compartment ($V_2$). Residual variability (proportional error) was higher for the CSF than for the plasma concentration (0.628 and 0.188 for CSF and plasma, respectively).

The developed PK model gives an adequate description of plasma and CSF concentration time profiles, as can be seen from plots of the simulated and observed concentrations versus time profiles with 90% confidence interval (Supplemental Figure 2).

PK data from the CMP rhesus monkey show that there is substantial CSF exposure after oral dosing (10 fold lower than in plasma). The data suggest that MBi-5 concentrations in brain, expected to be in between plasma and CSF levels, are sufficient to adequately inhibit $\beta$-secretase activity in brain. The plasma and CSF concentrations versus time profiles predicted from the model had a good fit to the values observed in the rhesus monkeys. Thus, the model could serve as input for PD model analysis.
Supplemental Table 1: Population parameter estimates including coefficient of variation (CV%) for the PK model of MBi-5

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>DESCRIPTION</th>
<th>VALUE</th>
<th>UNIT</th>
<th>CV%</th>
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<tr>
<td><strong>Structural parameters</strong></td>
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<td>maximum velocity</td>
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<tr>
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<td>absorption rate dose10</td>
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<td>$h^{-1}$</td>
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<tr>
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<td>$K_{23}$ for dose125 as fraction</td>
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<td>$V_3^b$</td>
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<td><strong>Interanimal variability</strong></td>
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<td>$\sigma^2_{\text{CSF}}$</td>
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<td>-</td>
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</table>

$^a$ $V_4 = V_2 \times FV_4$.

$^b$ Fixed.

$^c$ $K_a$ dose125 = $K_a$ dose30 $\times FK_a$.

$^d$ $K_{23}$ dose125 = $K_{23}$ $\times FK_{23}$. 
Supplemental Figure 1: Schematic of the population PK model for MBi-5, that comprised of a dose, central, peripheral and CSF compartment. Rate constants for the individual compartments are $K_a$ (absorption), $K_{24}$ (rate constant from central to peripheral), $K_{42}$ (rate constant from peripheral to central), $K_{23}$ (rate constant from central to CSF), $K_{32}$ (rate constant from CSF to central). $A_1, A_2, A_3, A_4, V_2, V_3$ and $V_4$ are amounts (A) and volume of distribution (V) of MBi-5 in dose, central, CSF and peripheral compartments, respectively. $K_{30}$ is the elimination rate in CSF compartment. $V_{max}$ is the maximum velocity; $K_m$ is the Michaelis-Menten constant.
Supplemental Figure 2: Visual predictive check of plasma (left panels) and CSF (right panels) concentration time profile of MBi-5 in the rhesus with 90% confidence interval. The rhesus were administrated with 10 mg/kg (A) (B), 30 mg/kg (C) (D) and 125 mg/kg (E) (F) MBi-5. Observation sample size: n=102 for plasma and CSF per dose from 6 monkeys collected over 7 days.

Plus-symbols represent observed measurements. Dotted line corresponds to the median observed profile. Solid lines show the median simulated profiles. The longs-dashed lines correspond to the 90% prediction intervals obtained from 1000 individual simulated profiles.