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Systems pharmacology of the amyloid cascade : unfolding oligomer modulation in Alzheimer's disease

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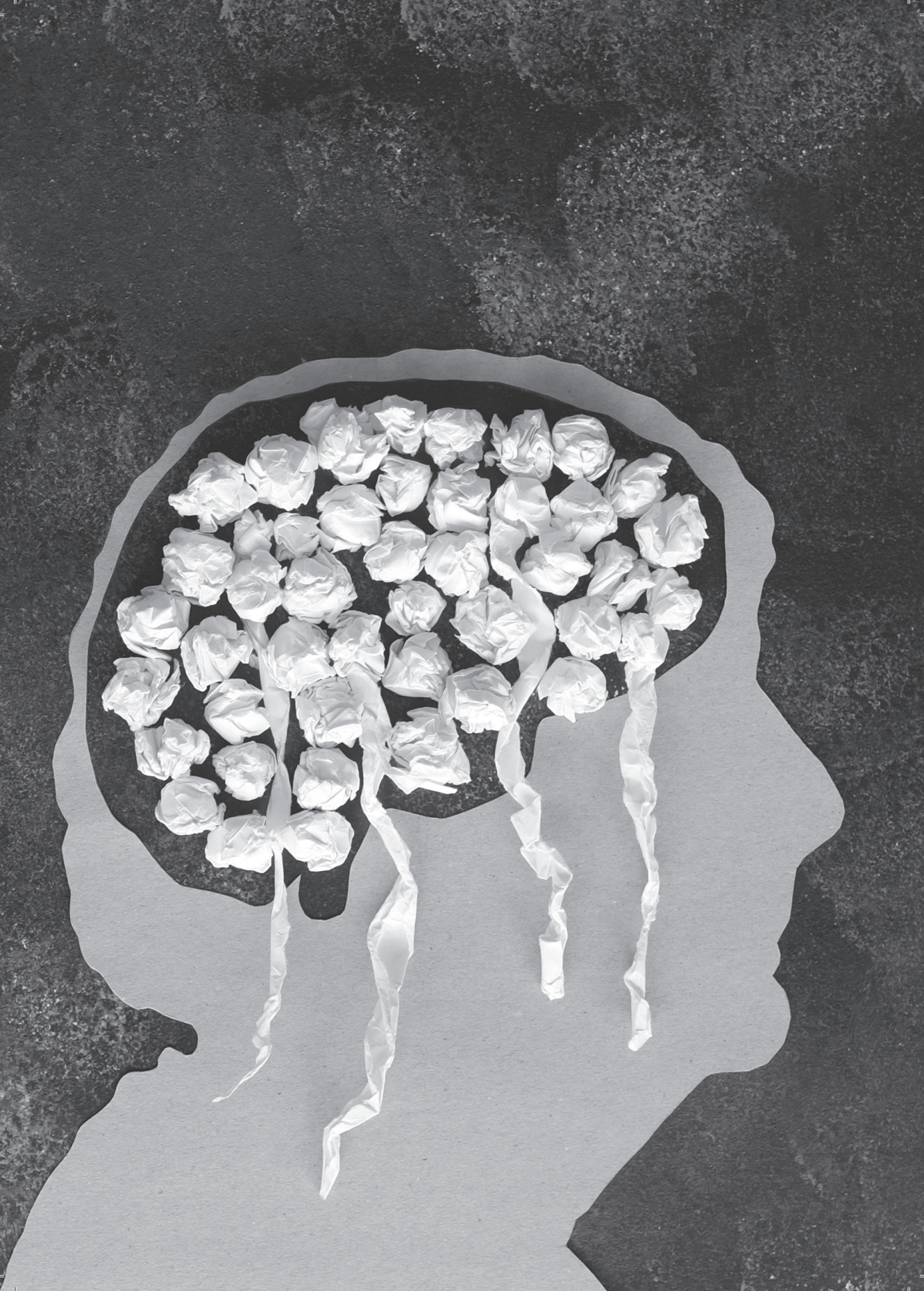


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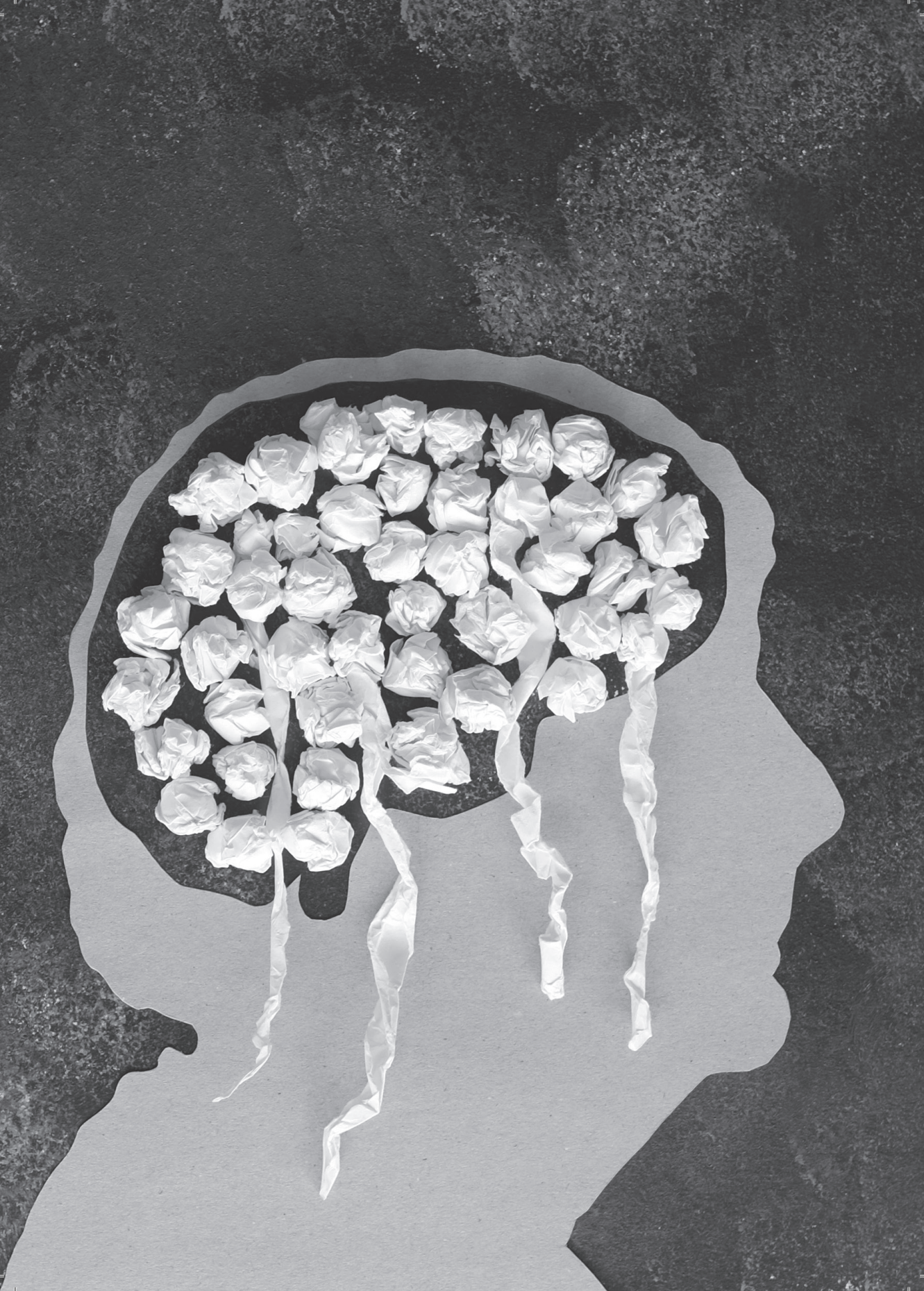
Title: Systems pharmacology of the amyloid cascade : unfolding oligomer modulation in Alzheimer's disease

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Section III

Application of a systems pharmacology model
to characterize oligomer modulation following
secretase inhibition



Chapter 6

Systems pharmacology analysis of the A β oligomer
response following γ -secretase inhibition:
Evidence for second-order kinetics of A β 42
oligomerization

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Abstract

Toxic soluble A β oligomers (A β _O) are considered to be the primary drivers of the neurodegeneration in Alzheimer's Disease (AD). Here, for the first time, the effect of BACE1 inhibition on the time course of the changes in A β _O is determined. Administration of the BACE1 inhibitor MBI-5 (30 or 125 mg/kg) resulted in a reduction of A β _O concentrations. The amyloid precursor protein (APP) metabolite (sAPP β , sAPP α , A β 40, A β 42, A β 38) and A β _O responses in CSF from cisterna-magna-ported rhesus monkeys was analysed on the basis of a recently established systems pharmacology model of the APP pathway.

The changes in A β _O were linked to the dynamics of the precursor A β 42: There was no contribution from the precursors A β 40 and A β 38 to the A β _O pool. A β 42 oligomerization was characterized to be a second-order process. Decreases in monomeric A β 42 responses following from BACE1 inhibition were partially compensated by dissociation of A β _O. The model gave an accurate description of the 6 biomarkers. The systems pharmacology analysis provided insights into A β _O reduction after treatment with a BACE1 inhibitor, and supports the hypothesis that A β 42 is the A β species prone to oligomerization. Simulations visualized that 30% reduction of the A β 42 monomeric level reduced A β _O by more than half.

Introduction

According to the amyloid hypothesis, proteolytic processing of amyloid precursor protein (APP) to form the amyloid- β ($A\beta$) peptides plays a central role in the pathophysiology of Alzheimer's Disease (AD)^{1,2}. $A\beta$ levels are increased early in the disease process, while patients remain clinically asymptomatic, forming toxic soluble $A\beta$ oligomers ($A\beta_O$) and plaques. $A\beta_O$ are considered to be a primary driver of the neurodegeneration in AD brain³.

$A\beta$ is the final product of proteolytic cleavage of the transmembrane APP in the amyloidogenic pathway and is assumed to be a precursor of $A\beta_O$. In the APP processing and clearance pathways, APP is cleaved sequentially by β -secretase 1 (BACE1) and γ -secretase⁴. BACE1 cleavage of APP releases the N-terminal secreted fragment soluble β -amyloid precursor protein (sAPP β) and C99, a C-terminal fragment which remains membrane bound. C99 is subsequently cleaved by γ -secretase, creating $A\beta$ peptides of different amino acid chain lengths, of which the most common have 38, 40 or 42 amino acids ($A\beta_{38}$, $A\beta_{40}$, or $A\beta_{42}$, respectively)⁵. A third secretase, α -secretase cleaves APP within the $A\beta$ sequence generating non-amyloidogenic soluble sAPP α and precluding $A\beta$ generation⁶.

$A\beta$ appears to aggregate into at least three different states: $A\beta_O$, which are soluble disordered clusters, protofibrils, which are prefibrillar insoluble high molecular weight $A\beta_O$ (50-1500 kDa) comprising spherical, annular, and curvilinear assemblies, and fibrils, which are long, many-chain highly structured β -sheet-like aggregates^{7,8,9}. $A\beta$ is believed to co-exist with $A\beta_O$, protofibrils and fibrils at equilibrium¹⁰. The pathway by which normal monomeric forms of $A\beta$ become fibrils is still uncertain¹¹.

One of the main therapeutic strategies for AD is to reduce $A\beta$ in the central nervous system and thereby, theoretically, preventing all downstream pathological processes. Potential therapies include inhibition of the secretases responsible for its production (BACE1 or γ -secretase inhibitors). The effect of inhibiting $A\beta$ production on $A\beta_O$ s is not fully understood.

Several studies on the pharmacokinetics (PK) and the pharmacodynamics (PD) of BACE1 and γ -secretase inhibitors have been reported^{12,13,14,15,16,17}. Such models focus primarily on the drug effect on $A\beta_{40}$ and/or $A\beta_{42}$ dynamics. A quantitative characterization of the drug effects on $A\beta_O$ is still lacking.

The drug effects on the individual attributes of the APP pathway are difficult to predict, because it involves a complicated biological network. In order to develop a model that

fully characterized the drug effects on A β monomeric and oligomeric levels, its important to consider the interactions between APP metabolites.

Systems pharmacology provides a mathematical framework for integrating understanding of biochemical/pathological pathways with basic principles of PK and PD. Recently, a systems pharmacology model of the APP processing pathway was developed to characterize APP metabolite (sAPP β , sAPP α , A β 40, A β 42) responses to BACE1 inhibition¹⁸. Throughout the article the name ' β -APP model' is used to refer this model. Using information from monomeric A β species, an A β 42 oligomer pool was identified in the β -APP model. It is of interest to know if the A β _O response to BACE1 inhibition was correctly derived from A β monomeric responses. This would verify monomeric A β as good predictor of A β _O response to A β production inhibition. To this end, A β _O measurements need to be compared to model predicted A β _O levels.

In the current crossover study in cisterna-magna-ported rhesus monkeys the effects of a BACE1 inhibitor (MBi-5; 30, 125 mg/kg) on the CSF concentrations of six biomarkers (sAPP β , A β 40, A β 42, A β 38, A β _O, sAPP α) were determined. A β _O concentrations in CSF were quantified using a novel two-site ELISA assay¹⁹. The time course of the changes in the concentration of all biomarkers were simultaneously analysed with the β -APP model. This analysis yielded predictions of the effect of MBi-5 on the A β _O concentrations (the oligomer pool). Next, these model predictions were compared to measured A β _O concentrations. Finally, the existing model was extended to include also the effect on A β _O concentrations. Specifically, the objectives of this investigation were fourfold: (i) to compare model predicted A β _O from the recently reported β -APP model with observations of A β _O; (ii) to characterize A β _O dynamics following BACE1 inhibition; (iii) to confirm that A β _O dissociates to restore the equilibrium between A β monomers and A β _O, following secretase inhibition; (iv) to investigate the relationships of A β 40, A β 42 and A β 38 monomers with the A β _O pool.

Materials and Methods

Animals

All animal studies were reviewed and approved by the MSD Institutional Animal Care and Use Committee. The NIH Guide to the care and use of Laboratory Animals and the Animal Welfare act were followed in the conduct of the animal studies (Institute of Laboratory Animal Resources, National Research Council, 1996). The CMP rhesus monkey model was reported by Gilberto et al.²⁰. The rhesus monkeys are chronically

implanted with catheters in the cisterna magna, facilitating repeated sampling of CSF and plasma in conscious rhesus monkeys. These rhesus monkeys were individually housed and captive-bred in a closed colony.

In this study, six male animals, weighing between 8.6 kg and 11.8 kg (mean, 9.7 kg), age at 9 years to 13 years (mean, 11 years) at time of the study were included.

Drug administration and sampling

Information on the effect of BACE1 on sAPP α , sAPP β , A β 40, A β 42, A β 38 and A β O was obtained following a single oral administration of MBI-5 at 30, 125 mg/kg (5 mL/kg) or vehicle (0.4% methylcellulose) in a four-way full crossover study.

Plasma and CSF drug concentrations were collected at 0 (predose) and 3, 5, 7, 9, 13, 14.5, 16, 19, 22, 25, 28, 31, 49, 55, 58, 73 and 96 h postdose, resulting in 18 plasma and CSF PK samples for each monkey per treatment group. 2 mL 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 LC-MS/MS. The concentrations of sAPP α , sAPP β , A β 40, A β 42, A β 38 and A β O were determined from CSF samples, collected at the same time points as PK samples, by established and validated ELISA-based assays (Meso Scale Diagnostics), giving 18 measurements of each biomarker for each monkey per treatment. The two-site ELISA assay used for A β O measurement was previously described by Savage et al.¹⁹.

PK-PD analysis

The PK and PD data were analysed with a non-linear mixed effects modelling approach utilizing the software package NONMEM (version 7.2.0²¹). In this approach, structural (fixed) effects and both intra- and interindividual variability are taken into account. Typical values of structural model parameters (population parameters, which define the average value for a parameter in a population) (θ), the variance and covariance of the interindividual variability (ω^2) and the variance of the residual error (σ^2) are estimated.

The models were compiled using Compaq Visual Fortran (version 6.6, Compaq Computer Corporation, Houston, Texas, USA) and executed on a PC equipped with an Intel QuadCore (Intel® Core™ i7 CPU860, 2.80 GHz, 3.24 GB RAM). Data management and model assessment was done using the statistical software package S-PLUS for Windows (version 8.0 Professional, Insightful Corp., Seattle, USA).

The best models were chosen based on the analysis of their obtained minimum value of the objective function (defined as minus twice the log-likelihood), the precision of

parameter estimates, and visual inspection of goodness-of-fit plots. A more detailed description of the modelling procedure was described in van Maanen et al.¹⁸.

To evaluate the performance of the model a visual predictive check (VPC) was performed in which the median and the 90% inter-quantile range of the data simulated with the final parameter estimates were plotted together with the observations. A validated result would have close resemblance of median observed and predicted line with 90% of the observations that fall within the 90% prediction interval.

Model description

The systems model of the APP processing pathway was developed by sequential analysis of PK and PD data following administration of MBI-5. The PK model of MBI-5 was based on simultaneous analysis of plasma and CSF PK data. The PK model of MBI-5 has been reported elsewhere by van Maanen et al.¹⁸.

The PK model adequately described the plasma and CSF concentration time profiles of MBI-5, respectively, thus the model could serve as input for PD model analysis.

The interrelationships of the absolute amounts of APP metabolite responses to BACE1 inhibition were described recently using a comprehensive systems model of the APP processing pathway¹⁸, the so-called β -APP model. To describe the effect of the BACE1 inhibitor on A β 38, the model had to be extended. Also, the oligomerization of A β was changed to a second order process.

The biomarker response profiles of MBI-5 measured in CSF were adequately described by the β -O-APP model containing compartments for seven variables: APP, sAPP β , sAPP α , A β 40, A β 38, A β 42 and A β _O (Fig. 6.2). 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 α -secretase and BACE1. As both sAPP β and C99 are products of APP cleavage by BACE1, sAPP β and C99 were presumed to follow the same kinetics and therefore sAPP β could be used in the model as surrogate precursor for A β . The production of sAPP α , sAPP β and A β were assumed to be first order, i.e. dependent on the concentration of its precursor. The interaction between APP, sAPP β , sAPP α , A β 40, A β 38, A β 42 and A β _O is described by Eq. 6.1 - Eq. 6.7:

$$\frac{d}{dt}APP = Rin_{APP} - (Rin_{\beta} \times EFF + Rin_{\alpha}) \times APP \quad (6.1)$$

$$\frac{d}{dt}sAPP\alpha = Rin\alpha \times APP - Rout_a \times sAPP\alpha \quad (6.2)$$

$$\frac{d}{dt}sAPP\beta = Rin\beta \times EFF \times APP - (Kin_{40} + Kin_{42} + Kin_{38}) \times sAPP\beta \quad (6.3)$$

$$\frac{d}{dt}A\beta_{40} = Kin_{40} \times sAPP\beta - Kout \times A\beta_{40} \quad (6.4)$$

$$\frac{d}{dt}A\beta_{38} = Kin_{38} \times sAPP\beta - Kout * A\beta_{38} \quad (6.5)$$

$$\begin{aligned} \frac{d}{dt}A\beta_{42} = & Kin_{42} \times sAPP\beta - Kout_{42} \times A\beta_{42} - Kpl \times (A\beta_{42})^{ALPH} \\ & + Krev \times A\beta_O / \left(\frac{MW_{A\beta_{42}}}{1000} \times Factor_{oligo} \right) \end{aligned} \quad (6.6)$$

$$\frac{d}{dt}A\beta_O = Kpl \times (A\beta_{42})^{ALPH} \times \frac{MW_{A\beta_{42}}}{1000} \times Factor_{oligo} - Krev \times A\beta_O \quad (6.7)$$

The exchange between the $A\beta_O$ pool and the $A\beta_{42}$ compartment is described by Eq. 6.6 and Eq. 6.7, where $ALPH$ is the power of the concentration of $A\beta_{42}$, $Factor_{oligo}$ is the conversion factor on $A\beta_O$ and $MW_{A\beta_{42}}$ is the molecular weight of $A\beta_{42}$. $Krev$ and Kpl are the dissociation rate and higher-order $A\beta_{42}$ oligomerization rate constant, respectively, which are dependent on the baseline values of $A\beta_{42}$ and the $A\beta_O$ pool ($A\beta_{42}_{base}$ and $A\beta_{O_{base}}$, resp.) according to Eq. 6.8:

$$Krev = \frac{Kpl \times (A\beta_{42_{base}})^{ALPH} \times \frac{MW_{A\beta_{42}}}{1000} \times Factor_{oligo}}{A\beta_{O_{base}}} \quad (6.8)$$

The rate of change of APP with respect to time in the presence of the inhibitor is described by Eq. 6.1, 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. 6.9.

$$EFF = 1 - \frac{C_{\text{target}}^{\text{GAM}} \times Imax}{C_{\text{target}}^{\text{GAM}} + IC50^{\text{GAM}}} \quad (6.9)$$

Where C_{target} is the target site concentration of MBI-5, $IC50$ the C_{target} that results in 50% inhibition of BACE1, $Imax$ is the maximum response and GAM is the Hill coefficient. C_{target} was derived from the PK model as:

$$C_{\text{target}} = C_{\text{plasma}} \times \frac{AUC_{\text{CSF}}}{AUC_{\text{plasma}}} \quad (6.10)$$

Where AUC_{CSF} and AUC_{plasma} are the areas under the CSF and plasma concentration time curves, respectively. C_{target} is assumed to be in steady state with C_{plasma} .

It is assumed that the system is in steady state (SS) when no treatment is given ($EFF=1$). These steady state conditions were used to derive part of the system parameters. From SS and Eq. 6.1 it follows that the source of APP (Rin_{APP}) is:

$$Rin_{\text{APP}} = Rout_a \times sAPP_{\alpha_{\text{base}}} + (Kin_{40} + Kin_{42} + Kin_{38}) \times sAPP_{\beta_{\text{base}}} \quad (6.11)$$

Where APP_{base} is the baseline level of APP, which is assumed to be equal to the sum of the baseline levels of $sAPP_{\alpha}$ and $sAPP_{\beta}$. All alternate pathways are represented by the terms for α -secretase.

Using SS conditions and Eq. 6.2 the $sAPP_{\alpha}$ formation rate (Rin_{α}), equivalent to the α -secretase cleavage step, can be derived:

$$Rin_{\alpha} = Rout_a \times \frac{sAPP_{\alpha_{\text{base}}}}{APP_{\text{base}}} \quad (6.12)$$

Where $sAPP_{\alpha_{\text{base}}}$ is the baseline level of $sAPP_{\alpha}$.

The $sAPP_{\beta}$ formation rate (Rin_{β}), equivalent to the BACE1 cleavage step, follows from SS and Eq. 6.3:

$$Rin_{\beta} = (Kin_{40} + Kin_{42} + Kin_{38}) \times \frac{sAPP_{\beta_{\text{base}}}}{APP_{\text{base}}} \quad (6.13)$$

Where $sAPP\beta_{base}$ is the baseline level of $sAPP\beta$.

Using SS conditions and Eq. 6.4, 6.6 and 6.5, respectively, the formation rates of $A\beta40$ ($Kin40$), $A\beta42$ ($Kin42$) and $A\beta38$ ($Kin38$), equivalent to γ -secretase cleavage steps, can be calculated:

$$Kin_{40} = Kin_{42} \times \frac{A\beta40_{base}}{A\beta42_{base}} \quad (6.14)$$

$$Kin_{42} = Kout \times \frac{A\beta42_{base}}{sAPP\beta_{base}} \quad (6.15)$$

$$Kin_{38} = Kin_{42} \times \frac{A\beta38_{base}}{A\beta42_{base}} \quad (6.16)$$

Where $A\beta40_{base}$, $A\beta42_{base}$ and $A\beta38_{base}$ are the baseline levels of $A\beta40$, $A\beta42$ and $A\beta38$, respectively. $sAPP\beta_{base}$ is the baseline level of $sAPP\beta$, used here as surrogate for the baseline level of C99.

The model structure includes six transit compartments (Fig. 6.2), one for each biomarker measured in CSF ($sAPP\alpha$, $sAPP\beta$, $A\beta40$, $A\beta42$, $A\beta38$, $A\beta_O$), to account for transport from the target site in the brain to CSF. These transit processes are described, in general, by Eq. 6.17:

$$\frac{d}{dt} xAx_{CSF} = Ktr * (xAx - xAx_{CSF}) \quad (6.17)$$

Where Kt is the transit rate for the particular APP metabolite xAx ($KtAP$ for $sAPP\alpha$ and $sAPP\beta$ and $KtAB$ for $A\beta40$, $A\beta42$, $A\beta38$ and $A\beta_O$).

Results

Within-study comparison

The performance of the recently reported β -APP model¹⁸ was assessed using a 'within-study comparison': model parameter values were optimized using the current study data and then A β _O was predicted and compared to the observed concentrations of A β _O in the present study. The rationale for this analysis is that different methodologies were used for the quantitation of the PD biomarkers in the current study compared to the previous study¹⁸. Consequently, biomarker baseline levels and ratios changed (see Supplemental Material). When using the β -APP model and parameter values, the difference in prediction of A β _O and data may be caused by methodology differences or model misspecification. It is impossible to distinguish the two from each other. In the 'within-study comparison', the difference in prediction of A β _O and data must be related to model misspecification and the model can be optimized accordingly.

Comparison of model predicted versus observed A β _O concentration profiles

The β -APP model parameters were optimized on a subset of the current data, using only the biomarker data of sAPP β , sAPP α , A β 40 and A β 42. Compared to the results obtained in the previous study, the estimates of the A β degradation rate (K_{out}) was significantly lower and transit rate for sAPP α and sAPP β from brain to CSF (K_{trAP}) was significantly higher (K_{out} : 0.94 h⁻¹ (95% CI, 0.689-1.19) and 0.304 h⁻¹ (95% CI, 0.198-0.41) in previous and current study, respectively; K_{trAP} : 0.0985 (95% CI, 0.0931-0.104) and 0.127 (95% CI, 0.111-0.143) in previous and current study, respectively). The IC₅₀ of the BACE1 inhibitor MBI-5 did not change significantly.

Using the parameter values, optimized for the current study data, the CSF A β _O response data in the current study was predicted. For this, similar to the compartment "Observed CSF A β 42", the compartment "Observed CSF A β _O" was added to the model, which represents the transport of A β _O from brain-to-csf (Figure 6.2). The prediction of the onset of the A β _O response to BACE1 inhibition was slow relative to the observations (Figure 6.1). Likewise, the maximum response was also underpredicted.

A conversion factor was included to account for different units of A β monomers and A β _O

It is to be noted that the concentrations of A β monomers were expressed in pM and A β _O concentrations were expressed in pg/mL. Therefore, a conversion factor needed

to be included in the model, which has a relationship with the molecular weight of the oligomers and other processes involved (e.g. differences in distribution volume). The conversion factor is implemented in the differential equations describing $A\beta_{42}$ (Eq. 6.6) and $A\beta_{O}$ (Eq. 6.7). Based on visual inspection, this factor was initially set to 0.05 for the prediction discussed above and later optimized to be 0.0178.

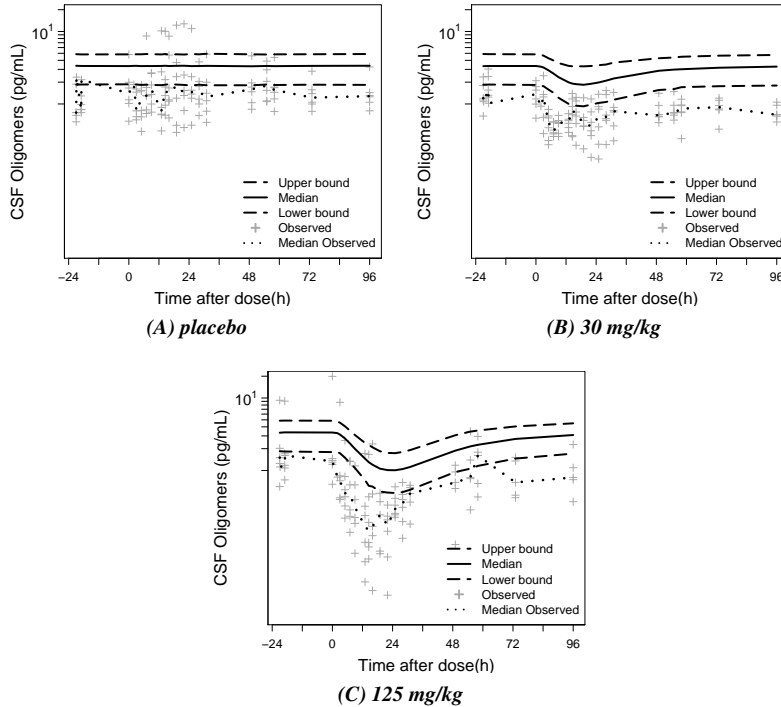


Figure 6.1: Prediction of $A\beta_{O}$ response vs. time profile of placebo (A), 30 mg/kg (B) and 125 mg/kg (C) MBI-5 in the rhesus monkeys with 90% confidence interval.

Predictions were performed with the model structure presented in van Maanen et al.¹⁸, with parameter values optimized on the current study data (*within-study comparison*). Observation sample size: $n=108$ for each APP metabolite from 6 monkeys collected over 4 days.

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

A β 42, A β 38). Further, the transit rate for brain-to-CSF transport ($KtAB$) did not differ for A β 38 compared to A β 40 and A β 42. Different formation rates were implemented for each one of the A β species. The formation rates of A β 40, A β 42 and A β 38 were calculated according to Eqs. 6.14-6.16. The highest formation rate was found for Kin_{40} (1.29 h^{-1}), followed by Kin_{38} (0.380 h^{-1}) and than Kin_{42} (0.0993 h^{-1}).

A β 42 only contributor to A β_O pool

After extension of the model for A β 38, the contribution of A β 40, A β 38 and A β 42 to the oligomer pool was investigated. These A β species were evaluated both as single contributors as combined sources of A β for the oligomer pool, by including oligomerization rates for each A β . A β 42 was identified as the only contributor to the oligomer pool.

A β oligomerization is a second-order process

The dependence of the A β_O concentration on the A β 42 concentration was investigated. The oligomerization was identified to be a higher order process, with an order of 1.81 (95% CI, 1.33-2.29), indicating that its rate is proportional to the \sim 2nd power of the concentration of monomeric species and that the oligomerization can only occur when two A β 42 peptides interact. The difference in absolute oligomer response following a 1st, 1.81 and 2nd order oligomerization process is visualized in Figure 6.3. This plot illustrates that the order of the oligomerization process affects not only the onset of the oligomer response, but also the maximum effect.

The second-order A β oligomerization means that a relatively larger change from baseline for A β_O compared to monomeric A β species is obtained following BACE1 inhibition, as is depicted in Figure 6.5B.

To better understand the relationship between A β 42 and A β_O response, the change of baseline for A β 42 was plotted against the change of baseline for A β_O (Figure 6.4). This plot exhibits a hysteresis loop between A β 42 and A β_O effects, when followed over time.

Thus, the same A β 42 concentration corresponds to two different magnitudes of A β_O effects depending on the temporal sequence in which the effect is measured (e.g. 30% reduction in A β 42 and 11% or 57% reduction in A β_O following 125 mg/kg MBI-5). The reason is because the maximum A β 42 response was achieved before the maximum of A β_O response (Figure 6.5A).

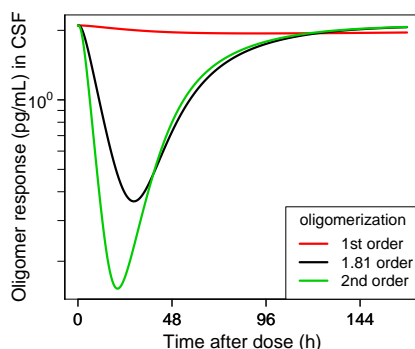


Figure 6.3: Illustration of the difference in absolute oligomer response following a 1st, 1.81 and 2nd order oligomerization process. The oligomer response was simulated after a single dose of 125 mg MBI-5, using the typical parameter estimates.

1st order: red solid line; 1.81 order: black solid line; 2nd order: green solid line.

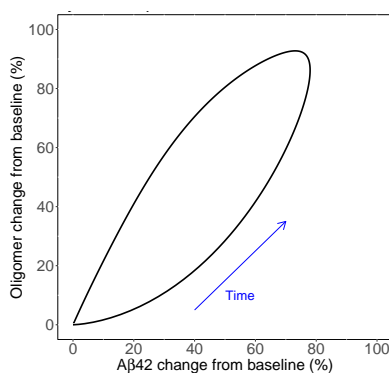


Figure 6.4: Illustration of the relationship between response of A β 42 and A β O. The A β O and A β 42 response was simulated after a single dose of 125 mg MBI-5, using the typical parameter estimates.

The β -O-APP model described APP metabolite and A β O responses to BACE1 inhibition

Figures 6.6-6.8 show the model description of each APP metabolite and A β O for each dose group. In general, an adequate description of the biomarker responses was obtained across dose groups. A slight underprediction was observed for sAPP β response at dose 125 mg/kg (Figure 6.8B) and overprediction of the A β O baseline (Figure 6.6F).

The β -O-APP model was used to simulate the biomarker interrelationships in CSF

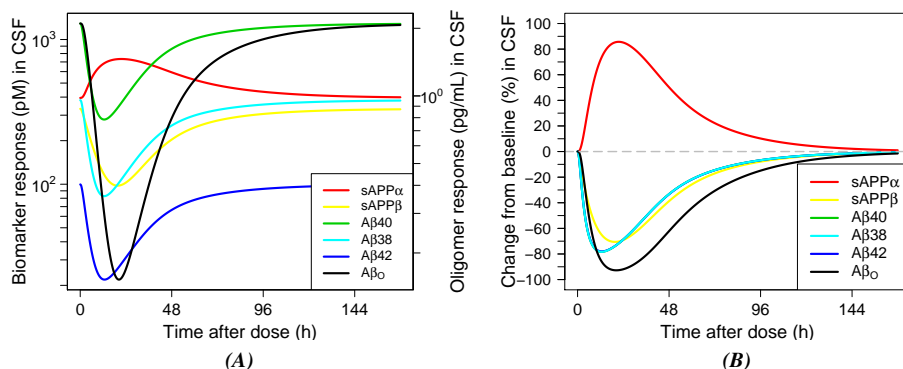


Figure 6.5: Simulation absolute biomarker responses (A) and biomarker change from baseline (%) (B). The biomarker responses were simulated after a single dose of 125 mg MBi-5, using the typical parameter estimates.

sAPP α red solid line; sAPP β yellow solid line; A β 40 green solid line; A β 38 light blue solid line; A β 42 dark blue solid line; A β _O black solid line.

after BACE1 inhibition (Figure 6.5A), illustrating that the biomarker maximum responses in CSF appear at different time sequence. Also, the response profiles of A β 40, A β 42 and A β 38 were similar, albeit at different concentration levels. When visualizing the change from baseline for these A β species, the profiles were overlapping (Figure 6.5B).

Model parameters

The population parameters and intra- and interanimal variability, optimized for the current study population, are presented in Table 6.1. The random-effects model structure was optimized by comparing the results of models with interanimal variability on different parameters. The final model included interanimal variability for the baselines of sAPP β , sAPP α and A β , modelled as lognormally distributed parameters. The same interanimal variability was included for the baselines of A β 38, A β 40 and A β 42, as these are products of the same cleavage step. For each APP metabolite (sAPP β , sAPP α , A β 40, A β 42, A β 38, A β _O) separately, a proportional error was used to describe the random residual variability.

The parameter estimate of the IC₅₀ was not significantly different from recently reported: in the recent analysis an IC₅₀ of 0.0269 μ M (95% CI, 0.0154–0.0384) was found¹⁸; in the current analysis an IC₅₀ of 0.0322 μ M (95% CI, 0.0214–0.043) was identified.

Table 6.1: Population parameter estimates including coefficient of variation (CV%)

PARAMETER	DESCRIPTION	VALUE	UNIT	CV%
<i>Structural parameters</i>				
sAPP β _{base}	baseline sAPP β	332	pM	24.9
A β 38 _{base}	baseline A β 38	381	pM	13.2
A β 40 _{base}	baseline A β 40	1290	pM	7.18
A β 42 _{base}	baseline A β 42	99.6	pM	10.3
sAPP α _{base}	baseline sAPP α	395	pM	17.5
K _{out}	degradation rate A β 40, A β 42, A β 38	0.321	h ⁻¹	14.5
R _{out_a}	degradation rate sAPP α	1.18	h ⁻¹	13.6
K _{tAP}	transit rate sAPP α and sAPP β	0.138	h ⁻¹	5.68
K _{tAB^a}	transit rate A β	10	h ⁻¹	
I _{max^a}	maximal inhibition (I _{max})	1		
IC ₅₀	median inhibition concentration	0.0322	μ M	17.1
GAM	Hill coefficient	0.749		10.3
K _{pl}	second-order oligomerization rate constant	6.59e-4	pM ⁻¹ h ⁻¹	10.3
A β _O _{base}	baseline A β _O	2.1	pg/mL	13.8
ALPH ^a	Power of the concentration of A β 42	2		
Factor _{oligo}	Conversion factor on A β _O	0.0178		45.8
<i>Interanimal variability</i>				
$\omega^2_{\text{BSAP}\beta}$ ^b	Interanimal variability sAPP β baseline	0.26		30.4
$\omega^2_{\text{BSAP}\alpha}$ ^b	Interanimal variability sAPP α baseline	0.145		30.3
ω^2_{AB} ^b	Interanimal variability A β	0.103		39.8
<i>Residual error</i>				
$\sigma^2_{\text{A}\beta 40}$ ^c	Residual variability A β 40	0.078		12.1
$\sigma^2_{\text{A}\beta 42}$ ^c	Residual variability A β 42	0.0576		19.8
$\sigma^2_{\text{sAPP}\beta}$ ^c	Residual variability sAPP β	0.0971		26
$\sigma^2_{\text{sAPP}\alpha}$ ^c	Residual variability sAPP α	0.0486		23.3
σ^2_{oligo} ^c	Residual variability A β _O	1.14		20.7
$\sigma^2_{\text{A}\beta 38}$ ^c	Residual variability A β 38	0.0711		19.5

^a Fixed.

^b Interanimal variability is assumed to follow a normal distribution with mean zero and variance ω^2 .

^c Residual variability is assumed to follow a normal distribution with mean zero and variance σ^2 .

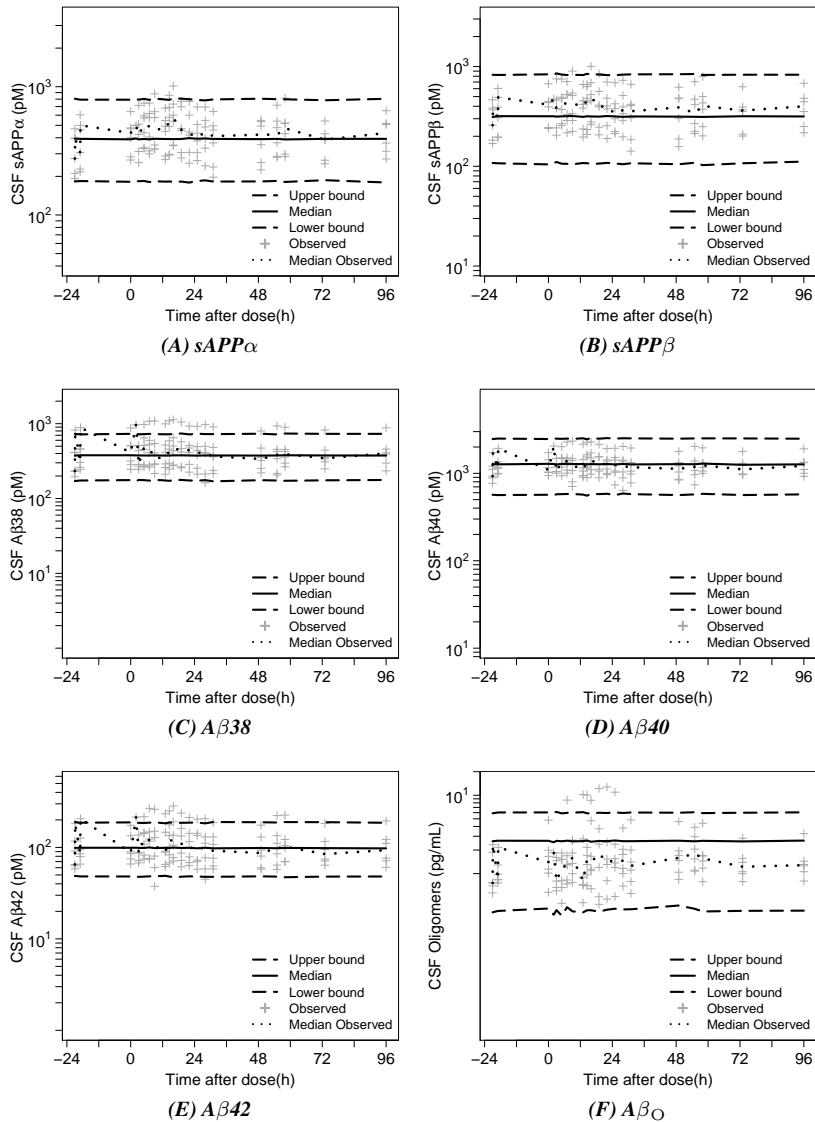


Figure 6.6: Placebo. Visual predictive check of biomarker response vs. time profile of placebo in the rhesus with 90% confidence interval. Predictions were performed with extended model ((A), (B), (C), (D), (E), (F)). Observation sample size: $n=108$ for each APP metabolite from 6 monkeys collected over 4 days.

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

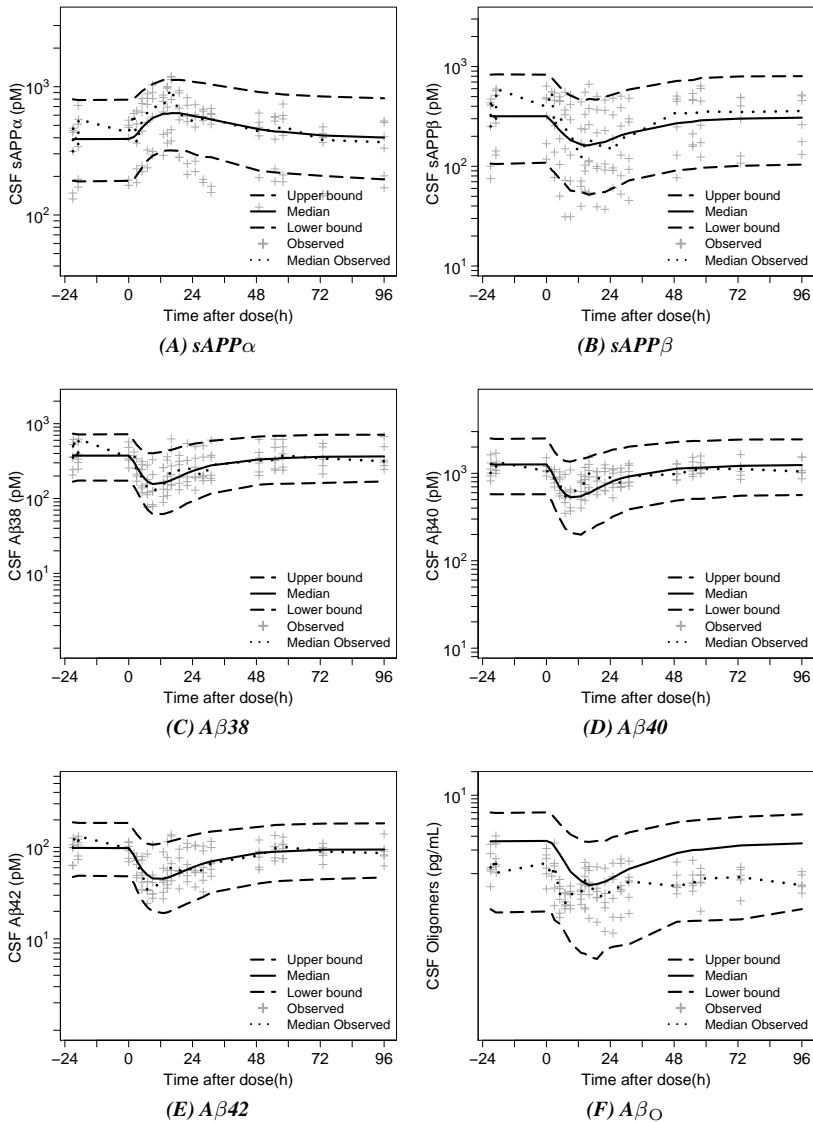


Figure 6.7: Dose 30 mg/kg. Visual predictive check of biomarker response vs. time profile of MBI-5 in the rhesus with 90% confidence interval. Predictions were performed with model with extended model ((A), (B),(C), (D), (E),(F)). Observation sample size: n=108 for each APP metabolite from 6 monkeys collected over 4 days.

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

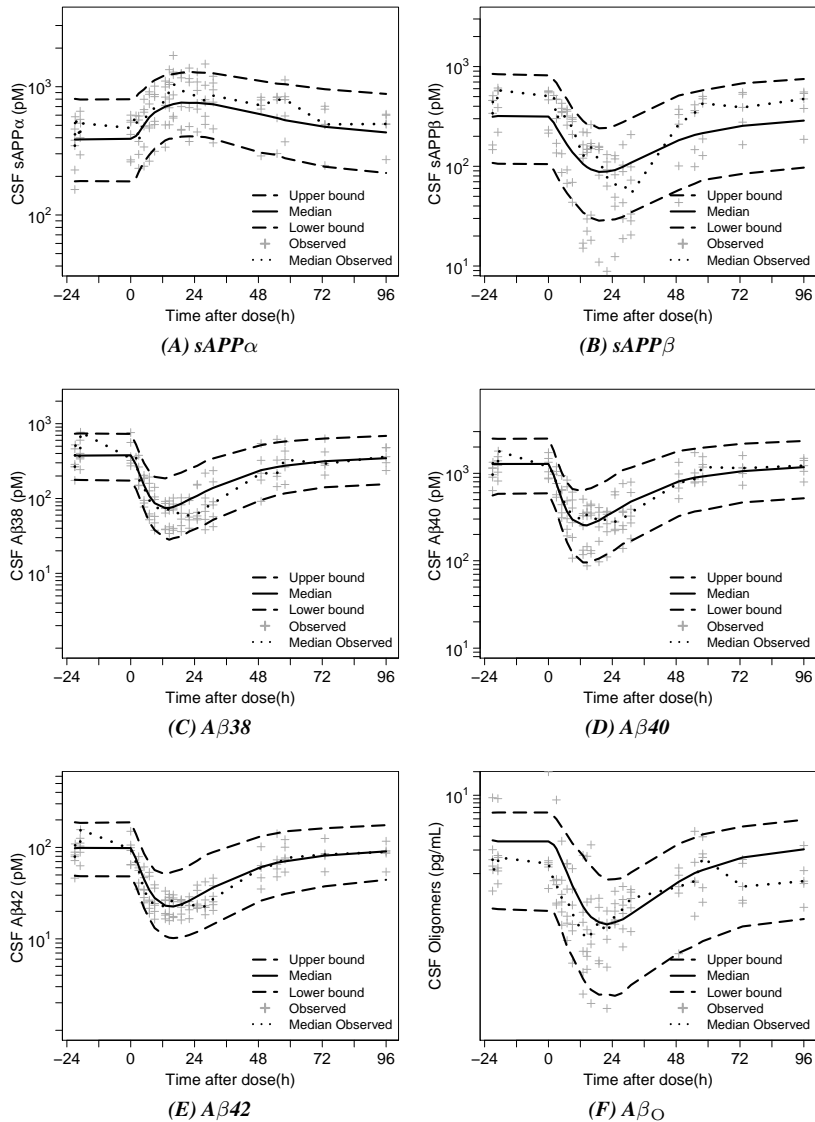


Figure 6.8: Dose 125 mg/kg. Visual predictive check of biomarker response vs. time profile of MBI-5 in the rhesus with 90% confidence interval. Predictions were performed with extended model ((A), (B),(C), (D), (E),(F)). Observation sample size: $n=108$ for each APP metabolite from 6 monkeys collected over 4 days.

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

Discussion

Soluble A β _O are believed to be responsible for the neurodegeneration or toxicity to brain tissue observed in AD. To optimize therapeutic intervention targeting A β production with the aim to reduce A β _O burden, it is important to understand and quantify the PD effects on A β _O. In that respect, it is imperative to consider the behaviour of the APP system as a whole.

The recently reported APP systems model¹⁸, the β -APP model, was extended to include A β 38 dynamics and describe A β _O response data from a novel assay. The so-called β -O-APP model successfully captured sAPP β and sAPP α concentration behaviour, A β monomeric (A β 38, A β 40, A β 42) and oligomeric concentrations and the interactions between these species.

A β oligomerization was a second order process, indicating that the concentration of A β directly affects the rate of the reaction. Specifically, doubling the concentration of A β would quadruple the rate of the oligomerization. The half-life of the oligomerization process is dependent on the initial A β concentration. The second-order kinetics of A β oligomerization means that a relatively higher change from baseline for A β _O compared to monomeric A β species is obtained following BACE1 inhibition. e.g. 30% reduction in A β 42 yields a 50% reduction in A β _O following 125 mg/kg MBI-5. By reducing A β _O levels, neuropathological alterations underlying AD may be slowed down or stopped. As such, A β production inhibition is a potential disease modifying therapy.

The β -O-APP model also contains expressions to account for the fact that decreases in monomeric A β 42 response resulting from BACE1 inhibition is partially compensated by reverse dissociation of A β _Os. A β _Os appear to dissociate in order to restore the balance between A β monomers and A β _O. This supports the belief that A β co-exist with A β _O in equilibrium and that A β _O formation is reversible to a certain extent¹⁰. As amyloid plaques and fibrils might exist in equilibrium with A β oligomeric forms, reducing A β _O levels through A β production inhibition may bring down higher ordered forms as well. Takamura et al.²² reported that antibodies raised against A β _O reduced plaques in conjunction with A β _O.

Our analysis indicated that of the measured A β species (A β 38,A β 40,A β 42) A β 42 was the only major contributor to the oligomer pool. This is in line with the findings that A β 42 is the dominant A β species in plaques and fibrils^{23,24,25}. Further, Garai and Frieden²⁶ reported greater *in vitro* oligomerization propensity of A β 42 compared to A β 40, using a fluorescent assay with tetramethylrhodamine-labelled A β . A β 42 is very self-aggregating,

while A β 40 may actually be anti-amyloidogenic^{2,27}. The additional two amino acids on the C-terminus of A β 42 makes the peptide more hydrophobic and significantly more rigid than A β 40 and susceptible to aggregation. The increased rigidity promotes entropy-driven aggregation. The high hydrophobicity of A β 42 pushes for aggregation to reduce exposure of the hydrophobic tail²⁸.

Recently, A β _O were predicted to decrease in response to BACE1 inhibition, which was at that time derived indirectly on the basis of an analysis monomeric A β response data. In the current analysis, the decrease in A β _O concentrations following BACE1 inhibition was confirmed. The within-study comparison that was used to compare model predicted versus observed A β _O indicated that the onset of the A β _O response was predicted to be slower and the predicted maximum response was lower than observed.

The addition of the parameter *Factor_{olig}* made it possible to account for differences in units of the quantification assays of monomeric A β and A β _O. This parameter has a relationship with the size of the oligomers and other process involved. A lower apparent volume of distribution of A β _O compared to A β monomers would be expected, if the measured A β _Os are high-molecular weight species. This would then be reflected in *Factor_{olig}*. The measured A β _Os were a mixture of A β _O with different number if A β monomers incorporated, of which the distribution was unknown. If, for simplicity, it is assumed that *Factor_{olig}* only has a relationship with A β _O size, it is defined as one divided by the number of subunits of A β 42 in A β _O. This would indicate that, on average, the measured A β _Os contain 56 subunits of A β . This is close to the reported size of larger amyloid oligomers of 30-50 protein molecules²⁹.

It was not possible to use the parameter estimates from the β -APP model in the current analysis, as different biomarker assays were used to determine APP metabolite concentrations. In principle, identified system parameters are attributed to the biological system and may not change from one analysis to another³⁰. However, in practice, due to experimental variation system parameters may shift. Then, it is important to understand what is measured and to realize what experimental design aspects might be different as well as those that are kept the same. With respect to the development of a system model, standardization of biomarker assays for data collection will be beneficial.

The β -APP model was extended to describe A β 38, in addition to A β 40 and A β 42 dynamics. Different formation rates were found for these A β species. Ranking the formation rates from high to low these rank: A β 40, A β 38, A β 42. This is consistent with the composition of A β species reported for human CSF, in which A β 40 is the dominant isoform, and the concentration of A β 42 was much lower than A β 40 and A β 38

concentrations³¹.

Unwanted protein aggregation, such as that of A β in AD, is generally believed to involve aggregation in a non-native state. In the case of amyloidogenic proteins, the starting reactant is the monomeric form of the protein and the product of the protein aggregation is aggregated protein fibrils. The intermediate species that are formed along the way are still uncertain. Various approaches to determine protein aggregation kinetics and understand the underlying mechanism have been reported in literature and were reviewed by Morris et al.¹¹. These were based on *in situ* and *ex situ* aggregation kinetics studies. Lomakin et al.³² investigated the fibrillation of A β 40, by following its aggregation using quasi-elastic light scattering *in vitro*. They proposed a critical protein concentration above which stepwise protein aggregation occurs: (i) monomers, (ii) micelles, (iii) nuclei, (iv) fibrils. The fiber elongation rate was proposed to be proportional to the A β 40 monomer concentration, i.e. a first order process. This cannot be directly compared to the higher order A β 42 oligomerization identified in the current analysis. As A β 42 is more prone to aggregation than A β 40 (*vide supra*), the results reported by Lomakin et al.³² might have been different for A β 42 under the same experimental conditions. Moreover, *in vitro* conditions for aggregation are less complex than *in vivo*, where processes as production, elimination, deposition and fibrillization of A β monomers are in dynamic equilibrium.

Conclusions & Perspectives

The findings reported herein indicate that the use of systems pharmacology modelling can be a very useful tool when investigating drug effects on attributes of a complicated biological network. The β -O-APP model was able to integrate information from an A β _O assay with the PK and APP metabolites concentration measurements in response to BACE1 inhibition. This yielded important information about the relationship between monomeric A β species and A β _Os: (1) Oligomerization was a higher order process. This means that a relatively larger change from baseline for A β _O compared to monomeric A β species is obtained following BACE1 inhibition; (2) A β _Os decreased in response to BACE1 inhibition; (3) Of the measured A β species A β 42 was the only major contributor to the oligomer pool.

The β -O-APP model brings us closer to optimizing the therapeutic intervention to reduce A β _O burden. In a follow-up analysis, the potential reduction of the putatively neurotoxic A β _O pool following γ -secretase inhibition will be investigated. Potential differences in effects on A β _O levels after treatment with a BACE1 *versus* a γ -secretase inhibitor will be evaluated. To this end, data following treatment with the γ -secretase inhibitor

MK-0752 from the current study will be added to further inform the model (**Chapter 7**).

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Chapter 6

Supplemental Material

Supplement to

Systems pharmacology analysis of the $A\beta$ oligomer response following β -secretase inhibition:
Evidence for second-order $A\beta_{42}$ oligomerization

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J.A. Stone, M. Danhof**

SUPPLEMENTAL MATERIAL

Study differences

The response data of A β 40, A β 42, sAPP β and sAPP α from the recent BACE1 inhibitor study¹ (hereinafter referred to as study 1) and the current BACE1 inhibitor study (hereinafter referred to as study 2) following a dose of 125 mg/kg MBI-5 is depicted in Supplemental Figure S6.1. A large between-study variability in the data was observed. This is also apparent from the plots of the ratios of A β 42:A β 40 (Supplemental Figure S6.2A), A β 40:sAPP β (Supplemental Figure S6.2B) and sAPP β :sAPP α (Supplemental Figure S6.2C) in each study. There was no overlap in the rhesus monkey individuals included in studies 1 and 2.

References

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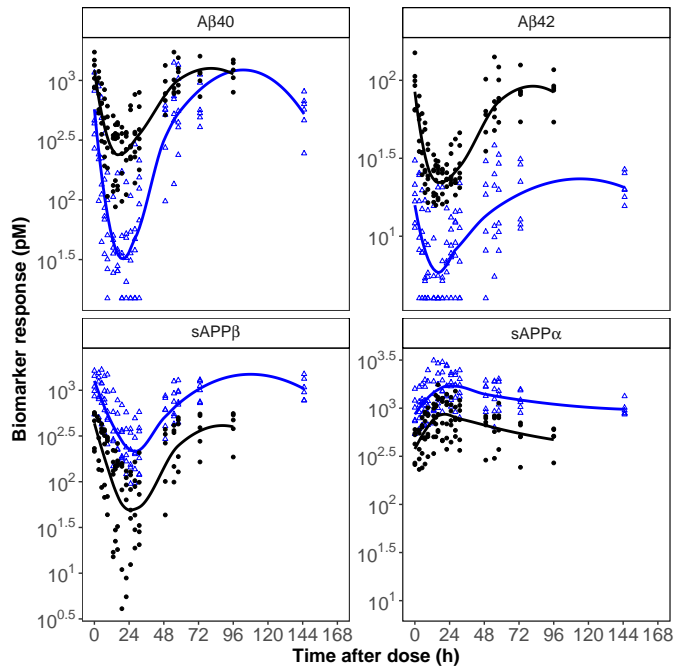


Figure S6.1: Study differences in absolute concentrations of biomarkers.
Study 1: blue line and symbols; Study2: black line and symbols; Lines are smoothers.

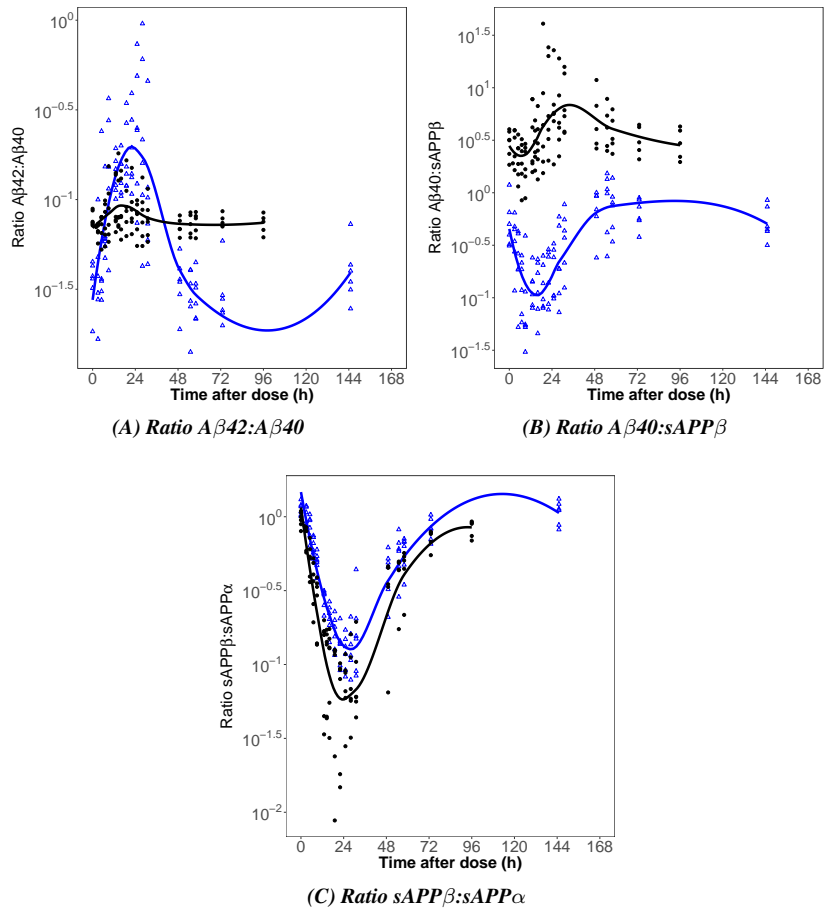


Figure S6.2: Study differences in ratios of biomarkers.

Study 1: blue line and symbols; Study2: black line and symbols; Lines are smoothers.