Integrating tracer kinetic data with absolute protein concentration measurements in a systems pharmacology model. Application to the APP pathway in CMP rhesus monkeys.

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Introduction

Build-up of amyloid-β (Aβ) peptide and its associated plaques in brain is hypothesized to lead to development of AD. Toxic Aβ oligomers ($Aβ_O$), in constant equilibrium with Aβ monomers, are considered to be the drivers of neurodegeneration in AD. Aβ peptides are generated by sequential cleavage from amyloid precursor protein (APP) by β-site APP-cleaving enzyme (BACE) and γ-secretase in the amyloidogenic pathway. Aβ peptide concentration in CSF is a therapeutic target for AD, with the potential for disease modifying effect by reducing Aβ levels.

Tracer kinetic studies can be used to gain understanding of the dynamics of the APP pathway. To take full advantage of the tracer kinetic data, combined analysis with absolute APP metabolite concentrations is required. Integrating biomarkers from different analytical tools can result in technical challenges. For this, a systems pharmacology modeling approach can be used, linking drug concentrations, plasma tracer enrichment and biomarker responses (absolute and fraction labeled proteins).

Objectives

To characterize the APP metabolite responses to BACE inhibition, by means of a systems pharmacology approach accounting for tracer dynamics throughout the APP pathway, and therefore aid the interpretation of the tracer kinetic data.

Methods

***In vivo* labeling protocol:**

The $^{13}C_6$-leucine infusion protocol was previously described by [1][2]. In short, a tracer is infused intravenously after drug administration and the proportion of synthesized labeled APP metabolites is monitored for some hours using stable isotope labeling kinetics (SILK).

PK-PD analysis:

A. A systems pharmacology model was used to describe the time course of the changes in APP metabolites *(ELISA)* on the basis of the underlying biological processes following BACE inhibition.

B. A two-pool PK model was developed to describe the plasma tracer enrichment.

C. The systems pharmacology model was extended to describe the time course of the changes of fraction labeled proteins *(SILK)* following BACE inhibition.

Study design

Compound:

β-site APP-cleaving enzyme inhibitor (BACEi)

Tracer:

$^{13}C_6$-leucine infusion 1h after BACEi administration

- Bolus 4 mg/kg iv 10 min + 12h infusion 4 mg/kg/hr

Study design

- Cisterna-magna-ported rhesus monkeys (n=6) [3]
- Dose-ranging SD, 4 period cross-over
  - Vehicle and active treatment (3 different doses)
- Measured:
  - PK: plasma and CSF concentrations
  - Plasma enrichment labeled leucine
  - PD in CSF:
    - sAPPβ, Aβ40, Aβ42, sAPPα *(ELISA)*
    - fraction labeled sAPPβ, fraction labeled total Aβ, fraction labeled sAPPα *(SILK)*

All animal studies were reviewed and approved by the Merck IACUC. The Guide and Animal Welfare regulations were followed in the conduct of the animal studies. Veterinary care was given to any animals requiring medical attention.

Model

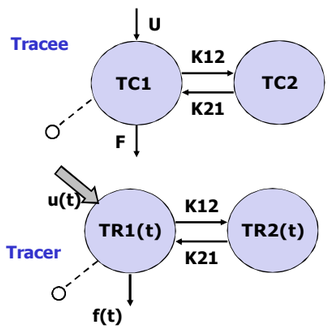
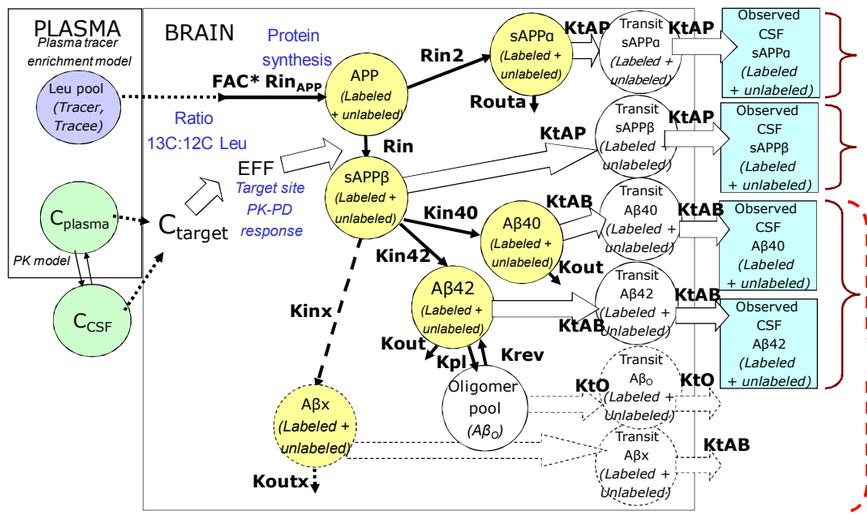


Figure 1. Schematic PK model to quantify plasma $^{13}C_6$-L tracer enrichment. Dashed line: sampling. *Arrow:* tracer infusion. *U:* production of tracee in pool 1. *u(t):* tracer infusion into pool 1. *F:* disposal of tracee from pool 1. *f(t):* disposal of tracer from pool 1. *K12:* Transit rate from pool 1 to pool 2. *K21:* Transit rate from pool 2 to pool 1.

Figure 2. Schematic systems pharmacology model to describe tracer dynamics throughout the APP pathway. Dashed arrows and compartments: additions to model structure compared to model based on ELISA data only.



$$sAPP\alpha_{total} = sAPP\alpha_{lab} + sAPP\alpha_{unlab}$$

$$Fraction\ labeled\ sAPP\alpha = \frac{sAPP\alpha_{lab}}{sAPP\alpha_{unlab}}$$

$$sAPP\beta_{total} = sAPP\beta_{lab} + sAPP\beta_{unlab}$$

$$Fraction\ labeled\ sAPP\beta = \frac{sAPP\beta_{lab}}{sAPP\beta_{unlab}}$$

$$A\beta40_{total} = A\beta40_{lab} + A\beta40_{unlab}$$

$$A\beta42_{total} = A\beta42_{lab} + A\beta42_{unlab}$$

$$Fraction\ labeled\ A\beta_{total} = \frac{A\beta40_{lab} + A\beta42_{lab}}{A\beta40_{unlab} + A\beta42_{unlab}}$$

$$Fraction\ labeled\ A\beta_{total} = \frac{A\beta40_{lab} + A\beta42_{lab} + A\beta x_{lab} + A\beta_{O\,lab}}{A\beta40_{unlab} + A\beta42_{unlab} + A\beta x_{unlab} + A\beta_{O\,unlab}}$$

Abbreviations:

APP: amyloid-β precursor protein; *Aβ:* amyloid-β-peptide; *Ctarget:* drug concentration at the target site; *Kin40:* Aβ40 formation rate; *Kin42:* Aβ42 formation rate; *Kinx:* Aβx formation rate; *Kout:* Aβ40 and Aβ42 degradation rate; *Koutx:* Aβx degradation rate; *Kpl:* oligomerization rate; *Krev:* oligomer dissociation rate; *KtAP:* transit rate sAPPα from brain to CSF; *KtAPb:* transit rate sAPPβ brain to CSF; *KtAB:* transit rate Aβ brain to CSF; *KtO:* transit rate AβO brain to CSF; *RinAPP:* production flux of new APP; *Rin:* sAPPβ formation rate; *Rin2:* sAPPα formation rate; *Routa:* sAPPα degradation rate

Results

A. A comprehensive model described the response of APP metabolite responses (ELISA) to BACE inhibition, with one common drug effect. The effect of BACEi was build-in the model as inhibition of loss of the APP precursor pool, shared by sAPPα and sAPPβ. An adequate description of all 4 biomarkers was obtained *(figure 3).*

B. A two-pool PK model related tracer infusion [mg/kg/h] to the measured enrichment, quantified as tracer ($^{13}C_6$-L) to tracee (endogenous $^{12}C_6$-L) ratio (TTR [%]).

C. The model from step A was extended to account for tracer dynamics throughout the APP pathway, utilizing BACEi PK and plasma tracer enrichment as 2 independent inputs. First, model structure and parameters were fixed to those identified in step A and used to predict fraction labeled proteins (SILK). The data indicated that an "Aβx" model component needed to be incorporated, representing Aβ isoforms other than Aβ40 and Aβ42, to account for differences in Aβ measurements in ELISA and SILK. In addition, $Aβ_O$ were included in fraction labeled Aβ. An adequate description of absolute (ELISA) and fraction labeled (SILK) protein responses (figure 4) was obtained . The developed model was used to simulate the dynamics of Aβ biomarker responses, showing the lowering effect of Aβx and $Aβ_O$ on the fraction labeled Aβ curve (figure 5a). Also, the response of sAPPα and fraction labeled sAPPα was further evaluated through simulation (figure 5b), indicating that sAPPα responds in a dose-dependent manner, whereas a dose-dependent response is absent for fraction labeled sAPPα.

Step A: Description protein concentrations (ELISA)

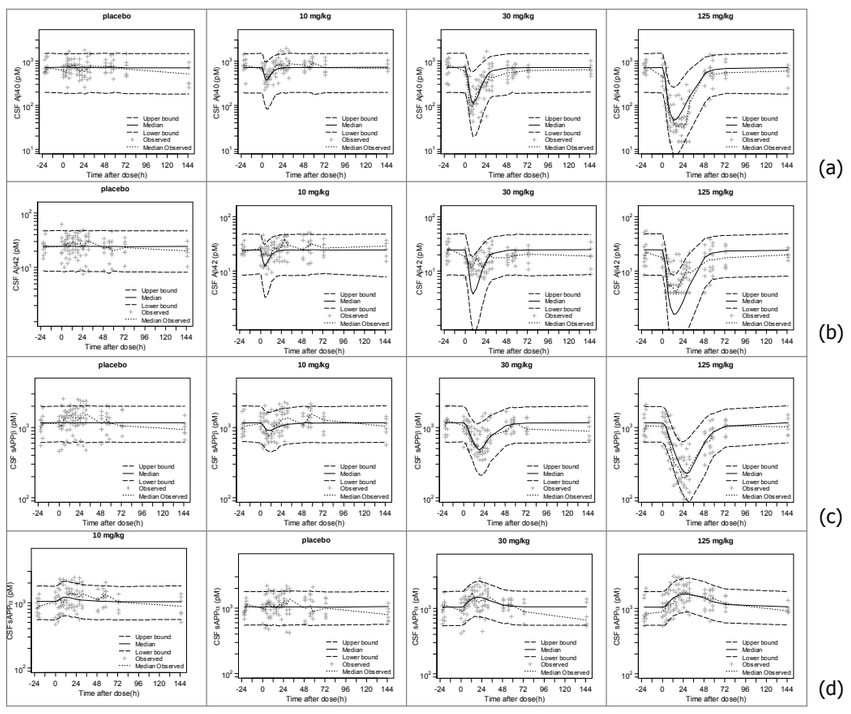


Figure 3. Description of Aβ40 (a), Aβ42 (b), sAPPβ (c) and sAPPα (d) response to BACE inhibitor by the comprehensive BACE model from step A.

Step C: Description fraction labeled proteins (SILK)

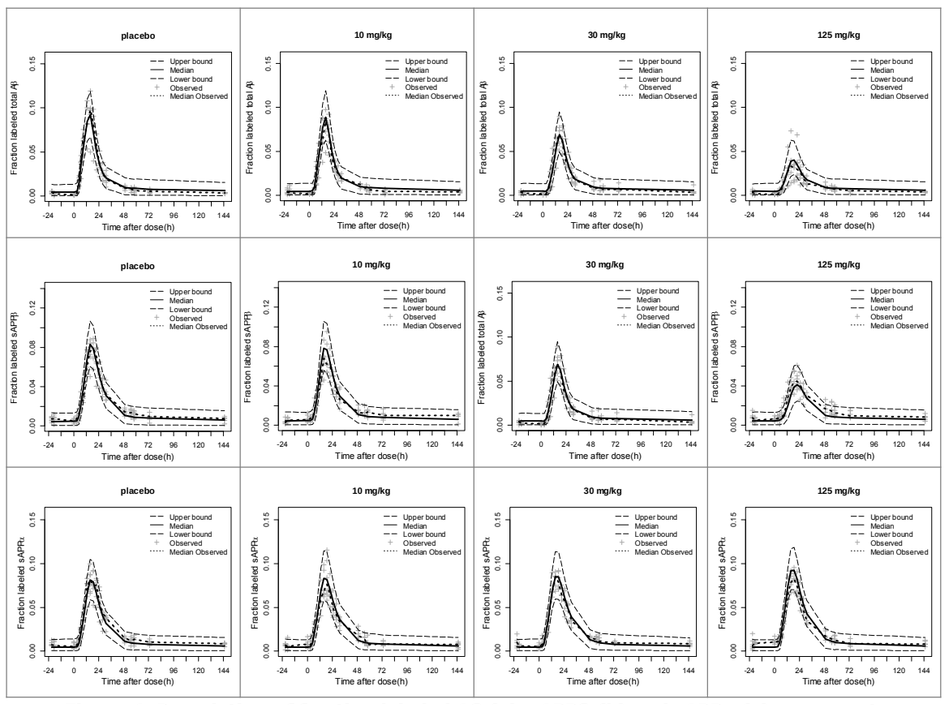


Figure 4. Description of fraction labeled Aβ (a), sAPPβ (b) and sAPPα (c) response to BACE inhibitor by the comprehensive BACE model from step C.

Step C: Simulation

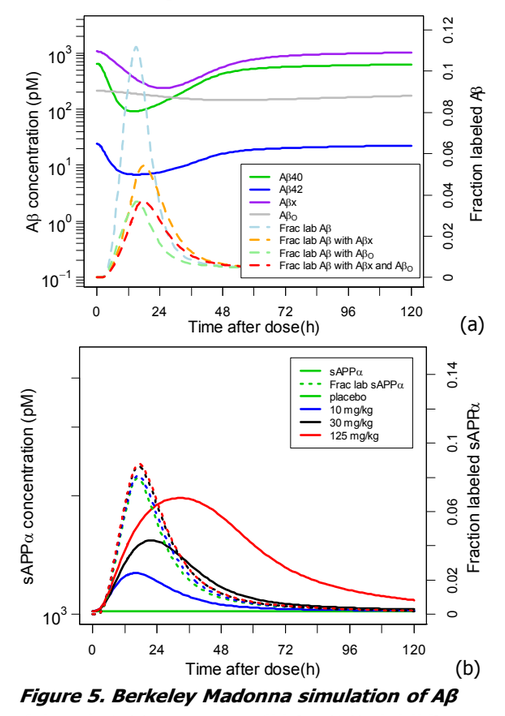


Figure 5. Berkeley Madonna simulation of Aβ (a) and sAPPα response (b) to BACE inhibitor with model as depicted in figure 2.

Conclusion & Perspectives

- A comprehensive model-based analysis was able to integrate information from tracer kinetic data *(SILK; plasma tracer enrichment and fraction labeled proteins)* with the PK and absolute protein concentration measurments *(ELISA)* of the BACEi across time points, doses and endpoints.
- Inclusion of Aβx and $Aβ_O$ in calculation of fraction labeled Aβ accounted for differences in APP metabolite responses as measured in ELISA and SILK and improved description.
  - Aβx are Aβ isoforms, other than Aβ1-40 and Aβ1-42. The antibodies used to isolate Aβ in SILK protocol were not end-specific for the N-terminus.
  - $Aβ_O$ are soluble oligomeric species of Aβ42, such as dimers, trimers and multimeric Aβ peptides, which could have been measured in SILK.
- The systems model enabled a more informed interpretation of tracer kinetic data and the APP pathway. Analysis of tracer kinetic data only may lead to misinterpretation, e.g. absence of dose response sAPPα.
- It is anticipated that adding data following γ-secretase inhibition will provide more information on the APP pathway as well as the differences in APP metabolite responses as measured in ELISA and SILK.

References

[1] Bateman RJ, Munsell LY, Chen X, Holtzman DM, Yarasheski KE. Stable isotope labeling tandem mass spectrometry (SILT) to quantify protein production and clearance rates. J. Am. Soc. Mass Spectrom. 2007;18:6 997–1006. **[2]** Cook, J. J., Wildsmith, K. R., Gilberto, D. B., Holahan, M. a, Kinney, G. G., Mathers, P. D., … Bateman, R. J. (2010). Acute gamma-secretase inhibition of nonhuman primate CNS shifts amyloid precursor protein (APP) metabolism from amyloid-beta production to alternative APP fragments without amyloid-beta rebound. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(19), 6743–50. **[3]** Gilberto, D. B., Zeoli, A. H., Szczerba, P. J., Gehret, J. R., Holahan, M. A., Sitko, G. R., … Motzel, S. L. (2003). An alternative method of chronic cerebrospinal fluid collection via the cisterna magna in conscious rhesus monkeys. *Contemporary Topics in Laboratory Animal Science*, *42*(4), 53–59