SUPPLEMENTAL MATERIAL

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

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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).

$$K_{20} = \frac{V_{MAX}}{K_M + \frac{A_2}{V_2}} \tag{S1}$$

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

$$\frac{d}{dt}A_1 = -K_a \times A_1 \tag{S2}$$

$$\frac{d}{dt}A_2 = K_a \times A_1 - K_{24} \times A_2 + K_{42} \times A_4 - K_{23} \times A_2 + K_{32} \times A_3 - \frac{V_{MAX} \times A_2}{K_M + \frac{A_2}{V_2}} \tag{S3}$$

$$\frac{d}{dt}A_3 = K_{23} \times A_2 - K_{32} \times A_3 - K_{30} \times A_3 \tag{S4}$$

$$\frac{d}{dt}A_4 = K_{24} \times A_2 - K_{42} \times A_4 \tag{S5}$$

MBi-5 displayed nonlinear PK at different kinetic levels. The extent of the absorption decreased with an increase in dose (K_a, from 10.0 to 0.144 h^{-1} for 10 and 125 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}, from 0.000488 to 0.000116 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 ($CL_2 = K_{20}(t,Cp) \times V_2$). 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 β-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

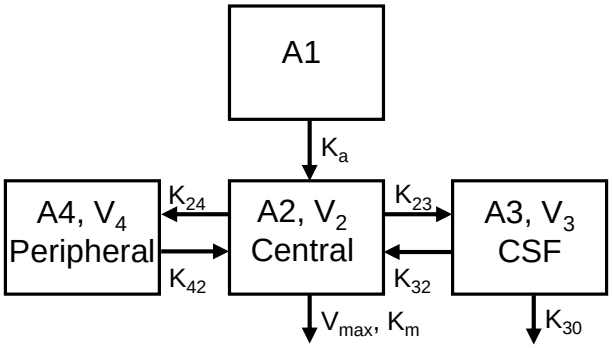
PARAMETER	DESCRIPTION	VALUE	UNIT	CV%
Structural parameters				
V_2	central volume	122	L	18.9
Q_4	intercompartmental clearance	2.01	$L.h^{-1}$	53.2
FV_4[a]	peripheral volume as fraction of central volume	0.488		42.8
K_m	Michaelis-Menten constant	6.24	μM	24.4
V_{max}	maximum velocity	1.04	$\mu M.h^{-1}$	25.7
K_a dose10[b]	absorption rate dose10	10.0	h^{-1}	-
K_a dose30	absorption rate dose30	0.250	h^{-1}	47.6
FK_a[c]	absorption rate dose125 as fraction of K_a dose30	0.579		37.0
K_{23}	rate constant from central to CSF	0.000488	h^{-1}	37.7
FK_{23} dose 125[d]	K_{23} for dose125 as fraction	0.239		23.5
K_{30}	elimination rate CSF compartment	34.5	h^{-1}	21.2
V_3[b]	volume CSF compartment	0.0250	L	-
Interanimal variability				
${\omega^2}_{\mathrm{V2}}$	Interanimal variability central volume	0.0612		27.0
Residual error				
${\sigma^2}_{\mathrm{plasma}}$	Residual variabiliy plasma	0.188		10.5
${\sigma^2}_{\mathrm{CSF}}$	Residual variabiliy CSF	0.628		26.4

[a] $V_4 = V_2 \times FV_4$.

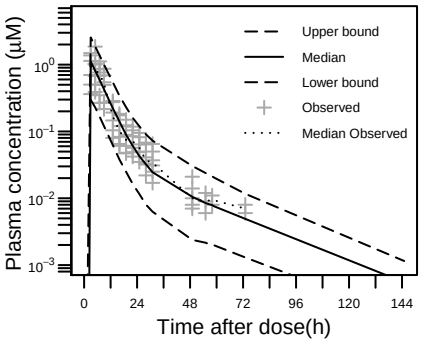
[b] Fixed.

[c] K_a dose125 = K_a dose30 $\times FK_a$.

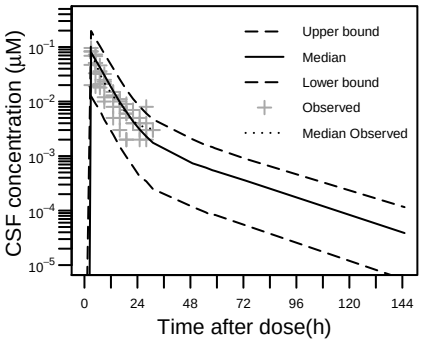
[d] K_{23} dose125 = $K_{23} \times FK_{23}$.



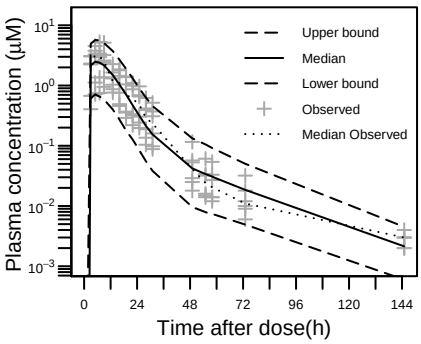
Supplemental Figure 1: Schematic of the population PK model for MBi-5, that comprised of a dose, central, peripheral and CSF compartment. Rate constants for the individual compartments are K_a (absorption), K_{24} (rate constant from central to peripheral), K_{42} (rate constant from peripheral to central), K_{23} (rate constant from central to CSF), K_{32} (rate constant from CSF to central). *A1*, *A2*, *A3*, *A4*, 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.



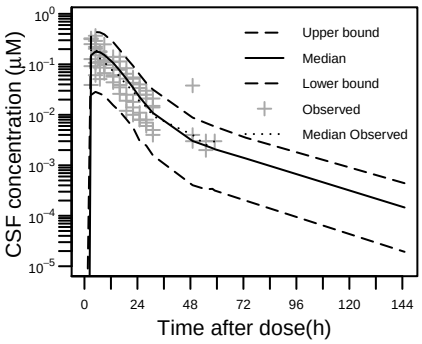
(A) 10 mg/kg MBi-5 plasma



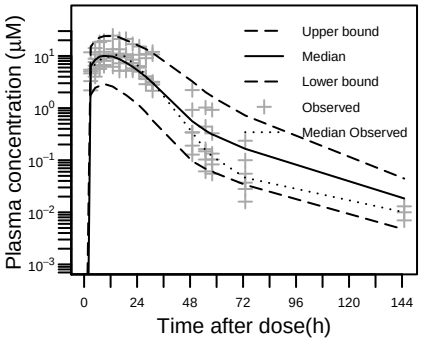
(B) 10 mg/kg MBi-5 CSF



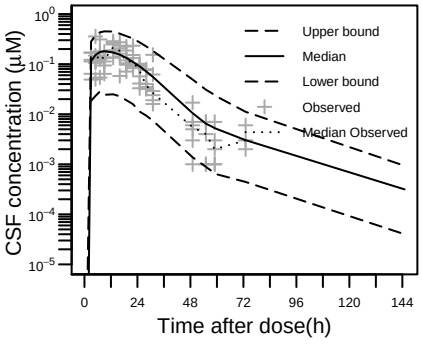
(C) 30 mg/kg MBi-5 plasma



(D) 30 mg/kg MBi-5 CSF



(E) 125 mg/kg MBi-5 plasma



(F) 125 mg/kg MBi-5 CSF

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.