Application of Population Pharmacokinetic Analysis for Quantification of *in vivo* Binding Properties in the Rat Brain by Positron Emission Tomography

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Introduction

The $GABA_A$-receptor plays a significant role in epileptogenesis. For the characterisation of the binding properties of the $GABA_A$ receptor *in vivo*, Positron Emission Tomography (PET) with ^{11}C-labelled flumazenil (FMZ) is an attractive technique. In the presented approach a single saturating dose of [^{11}C]FMZ is administered and the time courses of the concentration of FMZ in blood (using HPLC-UV) and brain (using PET) are determined.

The concentration-time curves of FMZ in blood showed dose dependent PK. It is hypothesised that this can be explained by a considerable amount of FMZ bound to the $GABA_A$ receptor in the brain. Therefore, the objective was to develop a model, which simultaneously describe the concentrations in blood and brain, and identify binding properties of the $GABA_A$ receptor *in vivo*.

Experimental Design

- Different dosages of ^{11}C-FMZ were tested: 2000 μg (n=2), 1000 μg (n=1), 500 μg (n=7), 100 μg (n=3), 50 μg (n=3), 25 μg (n=2), and 1 μg (n=6).
- The bloodsamples of the experiments with a dose of 1 μg could not be analysed because the concentrations were below the detection limit of the HPLC-UV system (25 ng/ml).

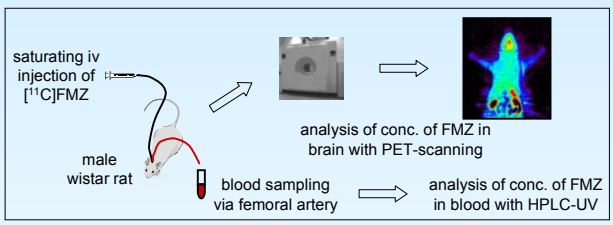

Figure 1: Experimental design.

PK-model

A user defined model (figure 2) was implemented in NONMEM's ADVAN9 subroutine. The parameters were optimised using the FO-method.

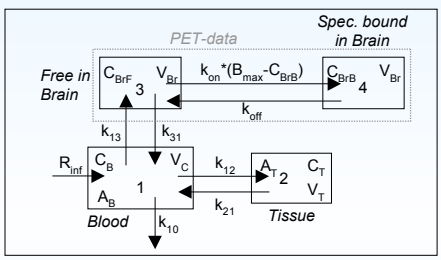

Figure 2: User defined structural PK-model.

Blood
$$\frac{dAB}{dt} = Rinf - k12 \cdot AB + k21 \cdot AT - k10 \cdot AB - k13 \cdot AB + k31 \cdot CBrF \cdot VBr$$

Tissue
$$\frac{dAT}{dt} = k12 \cdot AB - k21 \cdot AT$$

Free in Brain
$$\frac{dCBrF}{dt} = k13 \cdot \frac{AB}{VBr} - k31 \cdot CBrF - kon \cdot (Bmax - CBrB) \cdot CBrF + koff \cdot CBrB$$

Spec. bound in Brain
$$\frac{dCBrB}{dt} = kon \cdot (Bmax - CBrB) \cdot CBrF - koff \cdot CBrB$$

K_D
$$KD = \frac{koff}{kon}$$

The residual error was assumed to be proportional to the concentration in blood and brain. Furthermore, to account for the greater uncertainty in blood concentrations that are close to the detection limit an extra residual error was added. This residual variance was fixed to the square of half of the detection limit.

Results & Discussion

- The PK-model with saturable binding in the brain is indeed able to describe the observed dose dependent PK of FMZ in blood with sharper peaks at higher dose levels (figure 3).
- Model predictions at higher concentrations in the brain are slightly biased (figure 3 and figure 4), which might be explained by FMZ present in blood in the brain. This could not be taken into account with the current dataset.
- Including interindividual variability in CL and V_B resulted in better diagnostics, however, the estimation of different parameters, including B_{max} and K_D, was less accurate (%CV up to 400%).
- All parameters, except K_D are accurately estimated (table 1, model A). While V_{Br} and Q_{Br} have similar values, in the subsequent model B V_{Br} was set to Q_{Br}, which resulted in precise estimates of all parameters (table 1).
- The parameter estimates are similar to literature values: CL=19.1 *vs* 33 ml/min, $V_{d,ss}$=243 *vs* 299 ml respectively[1]. $K_{D,in\ vitro}$[2] and $K_{D,in\ vivo}$ as presently estimated did not significantly differ (7.1 ng/ml and 4.5 ng/ml respectively).

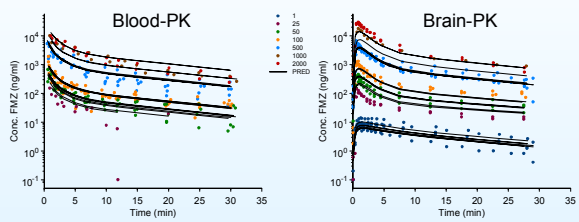

Figure 3: Concentration-time profiles for FMZ in blood and brain after different iv doses (1-2000 μg) to male Wistar rats. The dots represent the observed FMZ concentrations and the lines represent the individual predictions of model B.

Table 1: Population estimates of parameters with coefficient of variation (%) between brackets.

parameter	V_C (ml)	CL (ml/min)	V_T (ml)	Q (ml/min)	V_{Br} (ml)	Q_{Br} (ml/min)	B_{max} (ng/ml)	K_D (ng/ml)
model A	38.9 (23.3)	18.9 (4.0)	179 (15.3)	20.3 (15.7)	25.4 (16.9)	26.3 (21.7)	14.1 (39.1)	4.6 (50.8)
model B	41.9 (17.9)	19.1 (3.3)	178 (14.9)	21.4 (8.7)	23.4 (21.8)		13.8 (22.0)	4.5 (18.4)

model A: all parameters estimated
model B: $V_{Br} = Q_{Br}$

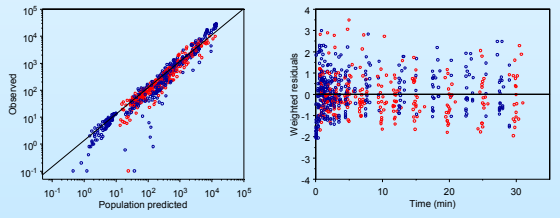

Figure 4: Diagnostic plots for FMZ concentrations in blood (red) and brain (blue).

Conclusions

- With the presented full saturation approach, using PET and population analysis, PK of FMZ in blood and brain, including binding to the cerebral $GABA_A$ receptor are determined *in vivo*.
- This method allows simultaneous, independent, and precise estimation of B_{max} and K_D in contrast to reported approaches.
- In future, studies in animal models of epilepsy will be performed using the presented approach, to investigate whether changes in $GABA_A$ receptor properties contribute to the mechanisms of epileptogenesis and pharmacoresistance.

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References:

[1] Mandema *et al.* (1991) *J. Pharmacol. Exp. Ther.* 257:472-478
[2] Mandema *et al.* (1991) *Psychopharmacology* 103:384-387