Non-Linear Mixed Effects PK/PD Modelling of Acute Autoinhibitory Feedback Effects of Escitalopram on Extracellular Serotonin (5-HT) Levels in Rat Brain

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Introduction

Escitalopram (ESC) is a selective serotonin (5-HT) reuptake inhibitor (SSRI) with antidepressant as well as anxiolytic activity (Sánchez et al., 2004; Thase, 2006). SSRIs selectively block neuronal 5-HT reuptake, which results in increased neurotransmitter concentrations at the synaptic and somatodendritic level. The release of 5-HT from the neurons is negatively regulated by 5-HT autoreceptor feedback-loops (Fig. 1) (Hjorth et al., 2000). Consequently, following acute drug administration, the firing activity of 5-HT neurons is reduced and the enhancement of release of new 5-HT into the synapse is dampened. Recently, a structural PK/PD feedback turnover model that mechanistically describes the acute ESC-induced effects on brain extracellular 5-HT has been developed (Bundgaard et al., 2006).

Objectives

The aim of this study was to characterise the PK/PD relationships including inter-individual variability (IIV) of ESC-induced 5-HT response after acute administration to rats. For this purpose, the mechanistic turnover feedback model was assessed using a non-linear mixed effects (NLME) modelling approach.

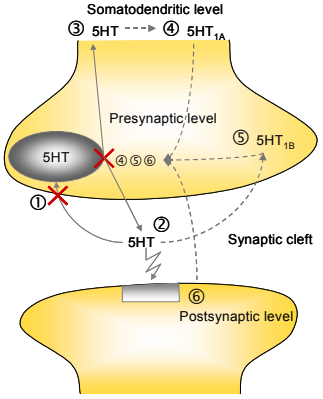


Fig. 1. Illustration of the presumed auto-regulatory mechanisms following blockage of the 5-HT transporter with SSRIs. By blocking the reuptake (1), the SSRIs increase the concentration of 5-HT at the synaptic (2) and somatodendritic level (3). The increased 5-HT levels act on somatodendritic and terminal 5-HT autoreceptors (4 and 5) as well as on postsynaptic 5-HT receptors (6), which exert negative feedback on the output of 5-HT into the synaptic cleft. In the proposed PK/PD model, the moderator M includes the sum of all negative feedback originating from (4), (5), and (6) (see Fig. 2). Solid lines denote release/uptake pathways and dashed lines control pathways.

Experimental Methods

Rats (n=17) were infused with 2.5, 5, or 10 mg/kg ESC or vehicle over 60 min. Extracellular 5-HT in hippocampus was continuosly monitored using intracerebral microdialysis. Simultaneously, serial blood was sampled at regular time intervals for ESC unbound plasma levels. Analysis of escitalopram in plasma (50 µl) and 5-HT in dialysates (20 µl) were accomplished by HPLC.

Measured total ESC plasma concentrations were converted into free, unbound concentrations by correcting for a mean protein binding of 50%, determined by ultrafiltration. Hippocampal 5-HT levels were converted to percentage of basal pre-dose levels normalised to 100% for each animal.

References

Bundgaard C., Larsen F., Jørgensen M., Gabrielsson J., 2006. *Eur. J. Pharm. Sci.* 29, 394-404.

Hjorth S., Bengtsson H.J., Kullberg A., Carlzon D., Peilot H., Auerbach S.B., 2000. *J. Psychopharmacol.* 14, 177-185.

Sánchez C., Bøgesø K.P., Ebert B., Reines E.H, Bræstrup C., 2004. *Psychopharmacology*, 174, 163-176.

Thase M.E., 2006. *Expert Opin. Pharmacother.* 7, 429-440.

Modelling

The structural PK/PD model was a turnover model with drug-induced inhibition of loss of response (k_{out}) and an inhibitory feedback moderator function resembling the acute mechanism of action of ESC (Bundgaard et al., 2006). The model is schematically illustrated in Fig. 2. The acute 5-HT response, R, was described by

$$\frac{dR}{dt} = \frac{k_{in}}{M} - k_{out} \cdot R \cdot I(C_p) \qquad \text{(eq. 1)}$$

where k_{in}, M, k_{out} and $I(C_p)$ are the turnover rate, moderator, fractional turnover rate and drug inhibitory function expressed as a sigmoid I_{max} function (incl. IC_{50} and shape ($Hill$) factor). The turnover rate k_{in} of R was parameterized with the baseline value R_0 and fractional turnover rate k_{out} by

$$k_{in} = R_0^2 \cdot k_{out} \qquad \text{(eq. 2)}$$

The turnover of the moderator, M, was governed by the first-order rate constant k_{tol}, which determined the onset and offset of inhibitory feedback ('tolerance') to the test compound according to

$$\frac{dM}{dt} = k_{tol} \cdot R - k_{tol} \cdot M \qquad \text{(eq. 3)}$$

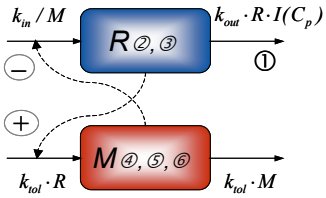


Fig. 2. Schematic illustration of the proposed negative feedback model. Response R acts linearly on the production of the moderator M. M is then 'fed back' by inversely inhibiting the production of R. Solid lines denote build-up and loss and dashed lines indicate how R and M influence each other. See also Fig. 1.

NONMEM VI (Globomax) was used for the modelling and S-Plus® for the other calculations and plots. In the first step, the PK model was fitted and the final parameter estimates were fixed in the combined PK/PD analysis. The turnover PD model was described by differential equations (ADVAN9). IIV was modelled using exponential errors. The residual variability was proportional for the PK and additive for the PD. The final model was evaluated using 95% predictive performance plots and bootstrap analysis.

Results

PK model

Parameter Estimates and Standard Errors from the Two-Compartment Model for Escitalopram Intravenous Administration Data (FOCEI)

Parameter	Final Parameter Estimate		Interindividual Variability (%CV)	
	Mean	%RSE	Final estimate	%RSE
CLintercept (L/min)	0.207	7.5	17	36
slope (L/min·mg/kg)	0.00803	19	ne	na
V1 (L)	2.12	14	45	42
Q (L/min)	0.219	8.3	15	78
V2 (L)	10.5	3.3	ne	na
RV (%CV)	18	27	na	na

RSE relative standard error; RV residual variability
ne not estimated; na not applicable
V1, V2 central and peripheral volume of distrbution
Q intercompartmental clearance
TVCL typical value of clearance
slope slope of the linear relationship between dose and TVCL (TVCL = 0.207 + 0.00803 · Dose)
FOCEI First Order Conditional Estimation with Interaction

PD model

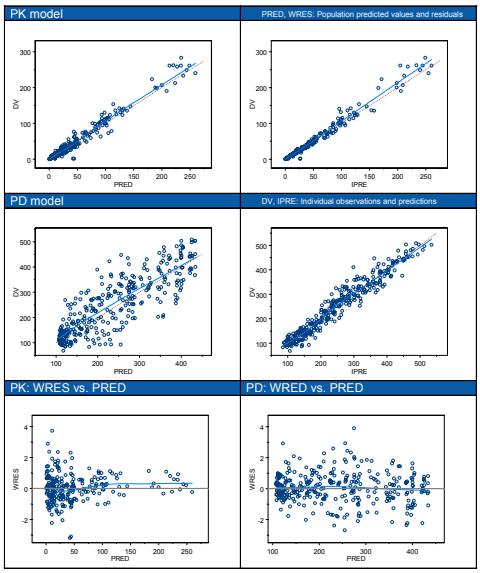
Parameter Estimates and Standard Errors from the Autoinhibitory Feedback Model of 5-HT in the Brain after ESC Intravenous Administration Data (FO)

Parameter	Final Parameter Estimate		Interindividual Variability (%CV)	
	Mean	%RSE	Final estimate	%RSE
k_{out} (min^{-1})	0.146	29	ne	na
k_{tol} (min^{-1})	0.0017	42*	ne	na
R_0 (%)	118	7.5	16	45*
Hill	1.5	8.5	ne	na
I_{max}	0.745	5.0	14	39
IC_{50} (ng/mL)	6.8	20	82	41*
RV (%CV)	30	16	na	na

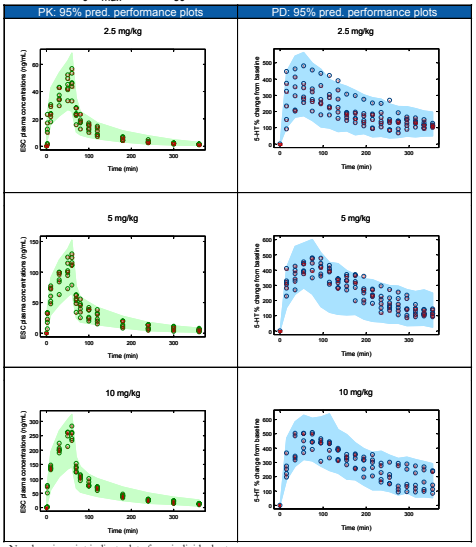
Akaike's Information Criterion (AIC) = 3558 (condition number = 13.0)
RSE relative standard error ; RV residual variability
ne not estimated; na not applicable
Without inhibitory feedback (k_{in}): AIC = 3583 (+25) (condition number = 31.2 (+18.2))
*Bootstrap variability >100%
FO: First Order estimation method

Results continued

A two-compartment PK model (ADVAN3, TRANS4) adequately described the ESC plasma levels. IIV was estimated for CL, V1 and Q (cf. Table). Dose level on CL was the only significant covariate. 5-HT levels were significantly increased following drug administration. However, at high doses, the mean response-time curves were almost identical. Therefore, a simple intrinsic turnover model was considered inappropriate. The final model included no further covariates and fitted all the response-data well (cf. goodness of fits plots below) and resulted in parameter estimates with acceptable precision. IIV was identified for R_0, I_{max} and IC_{50}. The half-life of the tolerance development was about 7 hrs ($\ln 2/k_{tol}$). The residual variability was 18% for the PK and 30 response units (%) for the PD.



The bootstrap analysis (n=200) confirmed the high precision for the PK model, while for the PD, the %RSE were higher than estimated by NONMEM, especially for k_{tol}, IIV_{R0} and IIV_{IC50} (>100%). The 95% predictive performance plot (n=200, shown below) were made from the final model with covariance estimates added for R_0, I_{max} and IC_{50}.



Numbers in point indicate data from individual rats

In conclusion, the NLME autoinhibitory feedback model was successfully implemented. Predictive performance appeared adequate and variability estimates were low (PK) to moderate (PD). Considerably higher variability was seen for IIV of the *in vivo* potency (IC_{50}) and also I_{max} and R_0 from the bootstrap analysis. The model may serve as a tool to compare the PK/PD behaviour of different SSRIs after acute administration.

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