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Pharmacokinetic-pharmacodynamic modelling in the early development phase of anti-psychotics: a comparison of the effects of clozapine, S 16924 and S 18327 in the EEG model in rats

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1 The use of pharmacokinetic/pharmacodynamic (PK/PD) analysis in early compound development was investigated in the rat for two developmental anti-psychotic compounds with clozapine as a positive control.

2 Three plasma samples were collected from each of eight animals according to a pre-defined sampling matrix allowing a total of 12 time points for PK analysis. Quantitative electroencephalography (QEEG), particularly the theta and beta frequencies, was used as a measurement of pharmacological effect.

3 PK/KD modelling of the sparse PK data available relative to a rich set of PD data was achieved using a population approach in NONMEM (IV). Individual PK parameter estimates were incorporated into a PK/PD model.

4 Qualitative EEG changes in rat and human were similar for clozapine, but different for the two developmental compounds, suggesting that changes in these PD parameters may not be specifically related to the anti-psychotic activity.

5 Although no definitive data are available concerning the signal specificity of EEG frequency bands with respect to dopaminergic or serotonergic receptor activity, qualitative and quantitative differences seen in EEG parameters are likely to result from the multiple receptor occupancy for these compounds.

6 The results confirm the value of population PK/PD modelling in conjunction with sparse sampling to enable determination of concentration effect relationships in the pre-clinical development programme of CNS-active drugs.

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Abbreviations: CI, confidence interval; CL, clearance; CV, coefficient of variation; E_0, baseline effect; EC_{50}, concentration at 50% of the maximal effect; E_{max}, maximum observed effect; K_{eo}, rate of drug disappearance from the effect site; LC-UV, liquid chromatography with ultraviolet detection; LOQ, limit of quantitation; N, sigmoidicity factor (Hill coefficient); ω^2, inter-individual variability; PK/PD, pharmacokinetic/pharmacodynamic; Q, inter-compartmental clearance; QEEG, quantitative electroencephalography; r.p.h., revolutions per hour; σ^2, residual variability; TNW, total number of waves; V1, central volume; V2, peripheral volume

Introduction

It has been suggested for some time that implementation of pharmacokinetic/pharmacodynamic studies in the pre-clinical stage of drug development may serve to accelerate the overall process by enabling earlier identification of suitable doses for Phase I and II studies (Peck *et al*., 1992; Danhof *et al*., 1993; Voseh *et al*., 1996). A pre-clinical study was designed to allow characterization of the PK/PD relationships of two developmental anti-psychotic compounds, S 18327 and S 16924, administered to rats. Both compounds have multiple receptor activities on dopaminergic and serotonergic systems, as does the atypical neuroleptic clozapine, used as a positive control in this investigation. Since the success of using quantitative EEG in the determination of the central nervous system effects of a range of psychotropic drugs has been extensively reported (Herrmann & Irrgang, 1983; Dingemanse *et al*., 1988; Della Pasqua *et al*., 1998), it was selected as the measurement of pharmacological effect here. In addition, EEG parameters have been demonstrated to provide quantitative information useful for later clinical development of a number of compounds (Mandema & Danhof, 1992; Danhof *et al*., 1993). The continuous nature of EEG provides a rich set of PD data, which in this case exceeds the PK observations collected. This situation is uncommon, since PD measurements are often more limited than PK data, thereby providing little information on inter- and intraindividual variability. In this analysis, a population PK approach using mixed effects modelling was used to describe the sparse concentration data, enabling feedback of individual parameter estimates for subsequent PK/PD modelling. This type

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of modelling can be successfully used for the small databases generated for these compounds, with the purpose only of adequately describing individual profiles by *post-hoc* Bayesian feedback. This enables rapid model implementation with relatively few data and should allow timely decision making regarding the pre-clinical development program.

Methods

Study design and animals

The study protocol was approved by the Ethical Committee for Animal Experimentation of the University of Leiden. The EEG effect was determined in three groups of rats according to a parallel group design. Male Wistar-derived rats (200–300 g, Broekman BV, The Netherlands) were used throughout the study. The animals were housed individually in plastic cages under constant temperature (21°C) and 12-h light/dark cycle. Laboratory chow (Standard Laboratory Rat, Mouse and Hamster Diets, RMH-TM, Hope Farms, Woerden, The Netherlands) and water were available *ad libitum*, except during the experimental procedures.

Drugs

The following anti-psychotics were compared: clozapine: 8-chloro-11(4-methyl-1-piperazinyl)-5H-dibenzo[b,e] diazepine; S 18327: 1-{2-[4-(6-fluoro 1,2-benzisoxazol-3-yl) piperid-1-yl] ethyl} 3-phenyl imidazolin-2-one; S 16924: ((R)-2-{1-[2-(2,3-dihydro-benzo[1,4]dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl}-1-(4-fluoro-phenyl)-ethanone).

Pharmacokinetic/pharmacodynamic experiments

For the measurement of EEG signals, seven chronic cortical electrodes were implanted into the skull of each animal a week prior to experiments (Mandema & Danhof, 1990). One day prior to EEG recording, indwelling cannulae were implanted into the right jugular vein (for drug administration) and the femoral artery (for blood sampling) under light anaesthesia.

Rats (eight per compound) were dosed with 6.25 mg kg^{-1} (S 18327), 4.0 mg kg^{-1} (S 16924) or 17.5 mg kg^{-1} (clozapine) as a 10-min intravenous infusion of free base dissolved in dimethylacetamide (S 18327 and clozapine) or acidified distilled water, pH = 5 (S 16924). Three blood samples (500 μl) were collected from each animal according to a pre-determined random sampling matrix (Table 1), in order to assess the pharmacokinetics of each compound. Plasma was prepared by centrifugation and stored at −20°C until analysis. The output from bipolar leads was continuously recorded using a Nihon Kohden EEG system consisting of a bioelectric input box JB-682G, a bioelectric amplifier AB-621G and a bioelectric input panel PB-680G (Nihon Kohden Corp., Tokyo, Japan). EEG was recorded from 15 min prior to drug administration until around 7 h after dosing. During the recording period rats were placed in a slowly rotating drum (10 r.p.h.) to control the level of vigilance. The activity of the fronto-central EEG lead on the left hemisphere was subjected to on-line aperiodic analysis for quantification (Lifescan EEG monitor, Neurometrics Inc., San Diego, U.S.A.). The analysed EEG data set was stored as the frequency in Hz, amplitudes in $\mu V\ s^{-1}$ and time of occurrence. From these data the average amplitudes and total number of waves (TNW) in the 0.5–2.5 Hz (delta), 3.0–8.0 Hz (theta), 8.0–11.0 Hz (alpha) and 11.5–30.0 Hz (beta) frequency bands were calculated and used as a measure of drug effect. Data points (about 47 per animal) were processed by averaging at least one minute of consecutive EEG signals.

Drug analysis

S 18327, S 16924, clozapine and desmethylclozapine, one of the main metabolites of clozapine were quantified using reversed phase LC-UV methods following liquid-liquid extraction as briefly described below.

S 18327 Plasma (250 μl), internal standard (250 μl S 17828-1, 2 $\mu g\ ml^{-1}$ in water), cyclohexane (6 ml) and sodium hydroxide (5 M, 100 μl) were mixed, vortexed (2 min), shaken (15 min) and centrifuged (2600 r.p.m., 5 min). The organic layer was taken to dryness and the residue reconstituted in hydrochloric acid (0.01 M, 250 μl). An aliquot (100 μl) was injected onto a C8 column equipped with an in-line filter and precolumn. The mobile phase consisted of water: 0.1% trifluoracetic acid in acetonitrile (73 : 27 v v^{-1}) at a flow rate of 0.6 ml min^{-1} with detection by UV at 246 nm. The assay was validated over the range 5–1000 ng ml^{-1} with a limit of quantification (LOQ) of 5 ng ml^{-1}.

S 16924 Plasma (250 μl), water (1 ml), internal standard (50 μl S 14399-1, 125 ng ml^{-1}), sodium hydroxide solution (1 N, 50 μl) and cyclohexane/diethyl ether (70/30; v v^{-1}, 6 ml) were mixed, shaken (15 min) and centrifuged

Table 1 Random sampling matrix for the pharmacokinetic phase of the study

Rat No.	*Sampling time* (h)											
	0.17	*0.25*	*0.33*	*0.5*	*0.75*	*1*	*1.5*	*2*	*3*	*4*	*6*	*8*
1	#				#				#			
2	#						#				#	
3		#					#					#
4		#				#				#		
5			#		#					#		
6			#					#	#			
7				#		#						#
8				#				#			#	

# Denotes blood collection. Sampling times are relative to the start of infusion.

(3000 r.p.m. for 5 min at 15°C). The organic layer was taken to dryness and the residue reconstituted in hydrochloric acid (0.1 M, 200 μl). An aliquot (80 μl) was injected onto a C18 column. The mobile phase was acetonitrile/trifluoroacetic acid in water (0.02%; v v^{-1})/methanol (34/61/5; v v^{-1}) at a flow rate of 0.5 ml min^{-1} and detection by UV at 210 nm. The assay was validated over the range 2–500 ng ml^{-1} with an LOQ of 2 ng ml^{-1}.

Clozapine, desmethylclozapine Plasma (100 μl), water (100 μl), amoxapine (2 μg ml^{-1}, 100 μl), sodium carbonate buffer (1 ml) and diethyl ether (6 ml) were mixed, shaken for 15 min and centrifuged (3000 r.p.m., 5 min). The organic phase was evaporated to dryness and the residue reconstituted in a mixture of carbonate buffer (pH 9.7)/acetonitrile (70/30; v v^{-1}, 100 μl). An aliquot (40 μl) was injected onto a C18 column. The mobile phase consisted of an acetonitrile gradient (25–60%) in sodium dihydrogenphosphate (1 g l^{-1} in deionized water adjusted to pH 2.6). Detection of clozapine, desmethylclozapine and the internal standard amoxapine was by UV at 245 nm. The assay was validated over the range 50–1500 ng ml^{-1} for both clozapine and desmethylclozapine. The LOQ was 20 ng ml^{-1}.

For all assays, concentrations were determined by reference to a calibration curve, and quality control standards were included within the sample analysis.

Protein binding Protein binding was determined experimentally over a range of concentrations for ^{14}C-radiolabelled S 16924 and S 18327, and for ^{3}H-labelled clozapine by ultrafiltration of plasma followed by liquid scintillation counting of the ultrafiltrate, retentate and initial plasma samples.

Data analysis

The pharmacokinetics was determined for each compound using a population pharmacokinetic approach in NONMEM IV (Beal & Sheiner, 1992). Up to 24 plasma concentrations were collected per compound according to a pre-defined sampling matrix. No modelling was attempted for desmethylclozapine, due to the erratic nature of the profile. For each compound, the model definition targeted at a final model with the fewest structural and variability parameters needed to adequately describe the plasma concentration *versus* time profiles. No covariates were incorporated into the model. Where possible, inter-subject variability in structural parameters was included. Residual variability, which comprises measurement and model error was also evaluated for each compound. Although these models were only intended for descriptive purposes, goodness of fit and model performance were assessed by simulation of 10 further sample matrices using the final population model parameter estimates. Following refitting, the outcome was compared to the 95% confidence intervals of the final population model parameter estimates. Individual Bayesian feedback parameters were obtained for incorporation into a second model describing the relationship between plasma concentrations and EEG effects. The increase in amplitudes in both theta and beta frequency bands was selected as a measure of the pharmacological effect. Due to the delay in effect observed relative to plasma concentrations, an effect compartment was required to assess the concentration-effect relationship. The sigmoid-E_{max} model was used to describe the relationship between effect-site concentration and increase in the beta frequency band. Interestingly, however, the sum of two sigmoid E_{max} models was required to characterize the concentration-effect relationship in the theta frequency band (equation 1) .

$$E = E_0 + \sum_{i=1}^{2} \frac{E_{max,\,i} \cdot C^N_i}{EC_{50,\,i} + C^N_i} \quad (1)$$

The selection of the additive model was based on the mechanisms underlying the pharmacological activity of both compounds, i.e. multireceptor interactions. E_0 is defined as the baseline EEG value, E_{max} the maximal effect, EC_{50} the plasma concentration at half maximal change in effect and N a constant describing the sigmoidicity of the concentration-effect relationship. The rich pharmacodynamic data enabled estimation of inter-subject variability for some parameters as well as residual variability.

Results

Pharmacokinetics

A two-compartmental model following IV infusion adequately described the pharmacokinetics of total plasma concentrations for each compound with estimation of clearance (CL), central volume (V1), inter-compartmental clearance (Q) and peripheral volume (V2). For clozapine, no variability could be estimated on structural parameters due to the limited amount of data and considerably higher assay noise. When appropriate, variability on CL and Q was best characterized by a multiplicative model. Likewise, a multiplicative model was used to characterize the residual variability of all three compounds. Structural model parameters were generally well estimated with coefficients of variation (CVs) less than 25% (6.9–23%) except for V2 (clozapine) and V1 (S 18327) (Table 2). Population CL and V1 were estimated as 0.76 l h^{-1} and 1.0 l (clozapine), 0.96 l h^{-1} and 0.35 l (S 16924), and 0.28 l h^{-1} and 0.14 l (S 18327) respectively. Inter-individual variability on parameters ranged between 27 and 64% with residual variability of 24% (clozapine), 16% (S 16924) and 19% (S 18327). Observed and model predicted plasma concentrations for each compound are presented in Figure 1. Apparently, clozapine concentrations observed immediately at the end of the infusion could not be adequately described by the pharmacokinetic model. Despite the poor fit for maximal clozapine concentrations, prediction over the remaining time course was appropriate. Since clozapine has a high intrinsic affinity, the observed differences are unlikely to affect pharmacodynamic parameter estimation. Examples of Bayesian feedbacks for typical animals are given in Figure 2. Assessment of goodness of fit of the final models performed using simulated datasets indicated that most parameter estimates derived from simulated data were within the range of the 95% confidence intervals (CI) for final population model parameters. Despite the erratic profile for desmethylclozapine, plasma concentrations were in the range of those seen for the parent compound and declined in parallel with clozapine. Estimates

Table 2 Population pharmacokinetic parameters for clozapine, S 16924 and S 18327

Parameter		*Estimate*	*SE*	*CV* (%)	*Variability* (%)	*95% CI* *Lower*	*Upper*
			Clozapine				
CL	l h^{-1}	0.76	0.067	8.9	–	0.63	0.89
V1	l	1.0	0.12	12	–	0.80	1.3
Q	l h^{-1}	0.29	0.066	23	–	0.16	0.42
V2	l	13	5.3	42	–	2.3	23
ω^2		0.056	0.024	43	24	9.4	32
			S 16924				
CL	l h^{-1}	0.96	0.098	10	–	0.77	1.1
V1	l	0.35	0.029	8.2	–	0.29	0.41
Q	l h^{-1}	0.48	0.064	13	–	0.35	0.60
V2	l	1.9	0.20	11	–	1.5	2.3
σ^2 CL		0.072	0.040	55	27	0*	39
σ^2 Q		0.16	0.091	57	40	0*	58
ω^2		0.025	0.017	70	16	0*	24
			S 18327				
CL	l h^{-1}	0.28	0.045	16	–	0.19	0.36
V1	l	0.14	0.054	39	–	0.033	0.24
Q	l h^{-1}	0.47	0.081	17	–	0.31	0.63
V2	l	0.31	0.022	6.9	–	0.27	0.36
σ^2 CL		0.42	0.25	60	64	0*	95
ω^2		0.036	0.013	36	19	10	25

*CI truncated to zero.

of the protein binding (expressed as per cent bound), were 87% (S 16924), 97% (S 18327) and 88% (clozapine) and were approximately linear over the range of concentrations measured in this study.

Pharmacodynamics

Overall percentage changes observed in amplitudes for the EEG frequency bands for each compound are tabulated (Table 3). Clozapine and S 16924 showed similar qualitative increases in theta and beta, but differed for delta and alpha. No modelling was attempted for S 18327 since only a slight decrease in the alpha frequency band and an increase in total number of waves for the beta frequency band were seen. For the two other compounds, amplitudes in the theta and beta frequency bands were selected as measure of the pharmacological effect. Quantitative differences in changes in the beta and theta frequency bands were observed for clozapine and S 16924 (Tables 4 and 5) despite similar baseline estimates of 77 to 80 μV s^{-1}. Estimates of the half-life for the rate of drug disappearance from the effect site (K_{eo}), were short but significant in all cases. They differed for compounds but were similar for both beta (β) and theta (θ) frequency bands (clozapine: 2.4 (β) and 2.9 (θ) min; S 16924: 9.9 (β) and 15.5 (θ) min). For the beta frequency band, E_{max} was 45 μV s^{-1} (clozapine) and 80 μV s^{-1} (S 16924). The unbound EC_{50} for S 16924 (125 ng ml^{-1}) was higher than that for clozapine (62 ng ml^{-1}). N was similar for both compounds (clozapine: 1.6, S 16924: 2.1). Multiplicative inter-individual variability was estimated for all structural model parameters (clozapine), and on E_0, E_{max} and N (S 16924). Variability ranged from 11 to 135% with additive residual variability of 8.3 μV s^{-1} (clozapine) and 7.1 μV s^{-1} (S 16924). For the theta frequency band, a double peak observed in the profiles for most animals following clozapine administration was modelled using the sum of two E_{max} models linked to a single effect compartment. Parameters from this model were not compared with those from the single E_{max} model applied to describe the PK/PD relationship of S 16924 (Table 5). For clozapine, estimates for E_{max} and $E_{max(2)}$ (18 and 29 μV s^{-1}), unbound EC_{50} and $EC_{50(2)}$ (154 and 12 ng ml^{-1}) and N and N_2 (5.9 and 2.4) were obtained. Multiplicative inter-individual variability estimated on E_0, E_{max} and $E_{max(2)}$ was 24, 19 and 75%, respectively. The residual variability was determined to be 7.6 μV s^{-1}. Parameter estimates for S 16924 were 16 μV s^{-1} (E_{max}), 40 ng ml^{-1} (EC_{50}, unbound) and 1.5 (N). Inter-individual variability on E_0 and EC_{50} was 16 and 151% with a residual variability of 5.9 μV s^{-1}. Bayesian feedback enabled individual pharmacodynamic parameter estimates to be obtained. Figure 3 shows predicted and observed effect profiles for clozapine and S 16924 for the beta and theta frequency bands, respectively with *post-hoc* profiles from the previous PK analysis (the same animal being depicted for both frequency bands).

Table 3 Overall changes in amplitude for the four main EEG frequencies

Band	*Clozapine*	*S 16924*	*S 18327*
δ	↑ (60%)	↓ (60%)	–
θ	↑ (90%)	↑ (30%)	–
α	↑ (80%)	↓ (20%)	↓ (20%)
β	↑ (60%)	↑ (60%)	↑ (TNW)

Where percentages relate to maximum changes in amplitudes. TNW is the total number of waves.

Discussion

It has been suggested that dose finding for clinical studies based on a PK/PD guided approach is more reliable than when based on toxicity data alone (Breimer & Danhof, 1997;

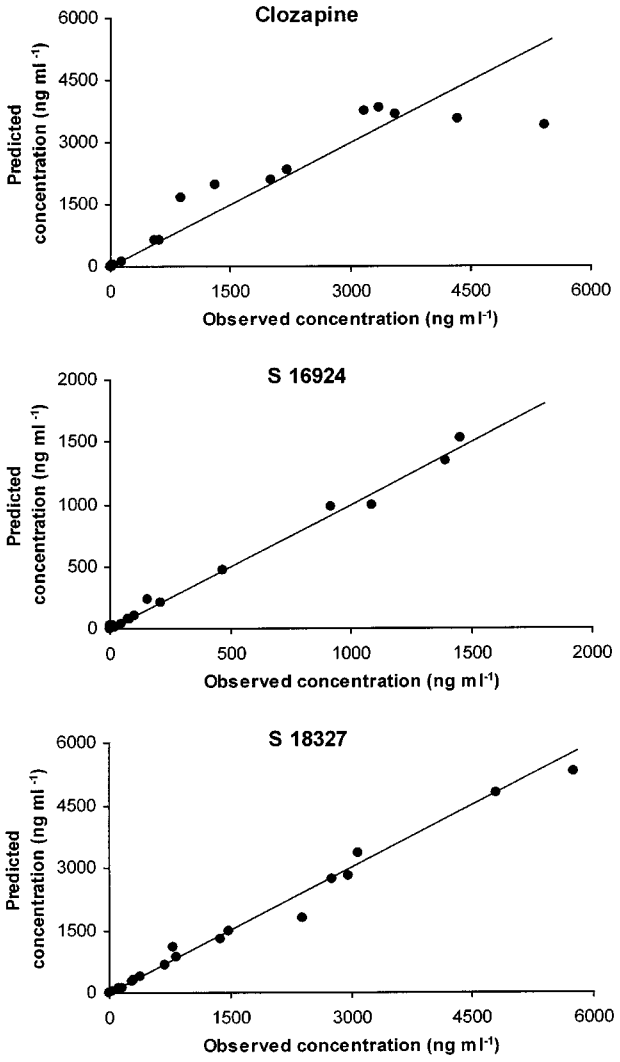

Figure 1 Individual observed *versus* predicted concentrations for final PK models with line of identity.

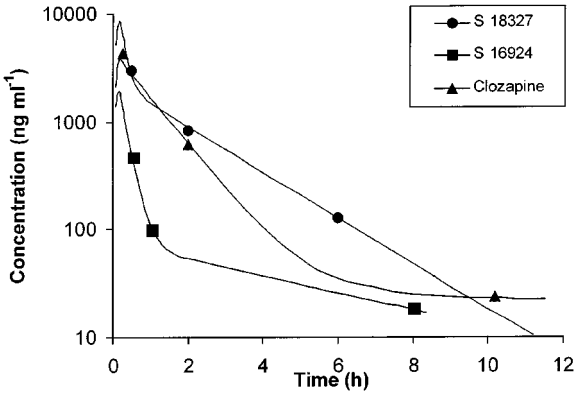

Figure 2 Bayesian feedback of a typical plasma concentration profile for each of clozapine, S 16924 and S 18327.

Reigner *et al*., 1997). The present study was undertaken primarily to evaluate the use of pre-clinical PK/PD modelling of EEG parameters in the rat for two developmental anti-psychotic compounds, S 18327 and S 16924, using clozapine as a positive control. Additional plans to assess the relevance of such findings in later clinical development were not pursued fully due to early termination of the development. Quantitative pharmaco-EEG was chosen as tool to assess the pharmacological effect for two reasons. It has already been shown to provide relevant, quantitative information for clinical development (Mandema & Danhof, 1992; Danhof *et al*., 1993), and has enabled the characterization of the central nervous system effects of a range of psychotropic drugs (Herrmann & Irrgang, 1983; Dingemanse *et al*., 1988; Greenblatt *et al*., 1994). Indeed, clinical studies with clozapine have shown that EEG changes are strongly related to positive clinical outcome in schizophrenics (Lacroix *et al*., 1995; Jin *et al*., 1995; 1998).

The success of clozapine in the treatment of schizophrenia and other psychotic disorders is probably attributable to its combined receptor activity on both dopaminergic and serotonergic systems (Metzler *et al*., 1989). It has weak antagonistic properties at D_2 receptors with higher antagonism of D_1, D_4, 5-HT_2, H_1, α_1, α_2 and cholinergic receptors (Fitton & Heel, 1990). This combination may enable anti-psychotic effects to be achieved below the threshold for unwanted extrapyramidal effects. The developmental anti-psychotics in the present study show receptor activity similar to clozapine, but S16924 is a more potent partial agonist at the $5HT_{1A}$ receptor subtype (Millan *et al*., 1998), whilst S 18327 has a lower affinity for histaminic and muscarinic receptors to prevent the autonomic adverse effects observed with clozapine (Millan *et al*., unpublished observations).

Changes in EEG signals in healthy volunteers and schizophrenic patients following administration of clozapine comprise an increase in the delta, theta, fast beta and/or low alpha frequency bands, with a concomitant decrease in the slow beta frequency band (Lacroix *et al*., 1995; Galderisi *et al*., 1996; Jin *et al*., 1998). In the present study, aperiodic analysis of the EEG signal showed increase in the amplitudes of all four frequency bands after clozapine administration. Although the beta frequency band was not split into slow and fast components here, it appears that both EEG bands are qualitatively related to the clinical outcome observed for clozapine. For S 16924, the increase in theta and beta frequency bands was accompanied by a decrease in alpha and delta frequency bands. A recent clinical study with S 16924 in healthy male volunteers showed a dose-related increase in theta and alpha1 frequency bands, accompanied by a decrease in alpha2 and beta frequency bands (unpublished data). In the aforementioned study, however, analysis of the EEG signal was not performed by aperiodic analysis. The absence of significant changes in the EEG signal following administration of S 18327, as well as the observed differences between pre-clinical and clinical outcomes for S 16924, suggest that a more suitable surrogate marker needs to be found for these types of anti-psychotics.

Our results show, on the other hand, that the EEG signal can potentially be used to differentiate drug multireceptor activity. Aperiodic analysis of the theta frequency band revealed two peaks in the effect *versus* time course profile for the clozapine, which was modelled using a second E_{max} term in the PK/PD model. Such an additive E_{max} model has been

Table 4 Pharmacodynamic parameters for beta EEG frequency

Parameter		Estimate	SE	CV (%)	Variability (%)	95% CI Lower	95% CI Upper
			Clozapine				
K_{eo}	h^{-1}	18	6.4	37	–	5.0	30
E_0	$\mu V\ s^{-1}$	79	6.6	8.4	–	66	91
E_{max}	$\mu V\ s^{-1}$	45	6.7	15	–	32	58
EC_{50}	ng ml^{-1}	508	62	12	–	386	630
N		1.6	0.083	5.1	–	1.5	1.8
σ^2 E_0		0.051	0.035	69	22	0*	35
σ^2 E_{max}		0.20	0.096	49	44	9.7	62
σ^2 EC_{50}		1.2	0.62	51	110	3.0	156
σ^2 N		1.8	0.61	34	135	79	173
ω^2		68.7	13.7	45	8.3	6.5	9.8
			S 16924				
K_{eo}	h^{-1}	4.2	0.54	13	–	3.2	5.3
E_0	$\mu V\ s^{-1}$	77	3.3	4.3	–	70	83
E_{max}	$\mu V\ s^{-1}$	80	18	23	–	44	115
EC_{50}	ng ml^{-1}	925	98	11	–	734	1116
N		2.1	0.40	19	–	1.4	2.9
σ^2 E_0		0.013	0.0049	38	11	5.9	15
σ^2 E_{max}		0.058	0.030	52	24	0*	34
σ^2 N		0.36	0.22	78	60	0*	89
ω^2		51	7.2	38	7.1	6.0	8.0

*CI truncated to zero.

Table 5 Pharmacodynamic parameters for theta EEG frequency

Parameter		Estimate	SE	CV (%)	Variability (%)	95% CI Lower	95% CI Upper
			Clozapine				
K_{eo}	h^{-1}	14	2.4	17	–	9.7	19
E_0	$\mu V\ s^{-1}$	80	6.7	8.4	–	67	93
E_{max}	$\mu V\ s^{-1}$	18	2.5	14	–	13	23
EC_{50}	ng ml^{-1}	1260	171	14	–	925	1595
N		5.9	2.7	–	–	–	–
E_{max} (2)	$\mu V\ s^{-1}$	29	8.0	28	–	13	44
EC_{50} (2)	ng ml^{-1}	99	7.7	7.8	–	84	114
N (2)		2.4	0.36	–	–	–	–
σ^2 E_0		0.057	0.022	39	24	12	32
σ^2 E_{max}		0.036	0.030	82	19	0*	31
σ^2 E_{max} (2)		0.56	0.30	53	75	0*	107
ω^2		58	11	19	7.6	6.0	8.9
			S 16924				
K_{eo}	h^{-1}	2.7	0.48	18	–	1.7	3.6
E_0	$\mu V\ s^{-1}$	77	4.4	5.7	–	69	86
E_{max}	$\mu V\ s^{-1}$	16	2.6	17	–	10	21
EC_{50}	ng ml^{-1}	297	52	17	–	196	398
N		1.5	0.28	–	–	–	–
σ^2 E_0		0.027	0.0095	35	16	9.2	21
σ^2 EC_{50}		2.3	1.5	66	151	0*	229
ω^2		35	9.2	51	5.9	4.1	7.3

*CI truncated to zero.

used to describe the analgesic effect of clonidine taking into account its agonistic activity at different receptors at different concentrations (Paalzow, 1981). In a similar way, the profile observed for clozapine seems to reflect its multiple receptor activity. Conversely, the second peak could have been due to desmethylclozapine, a metabolite of clozapine, which is active at similar receptor sites (Jann *et al*., 1993). However, concentrations attained in rat brains were observed to be only around 1% of those of the parent at doses up to 60 mg kg^{-1} clozapine (Baldessarini *et al*., 1993), such that any activity is unlikely to be due to this metabolite. In addition, the second peak was not clearly observed in profiles for the beta frequency band, suggesting an unlikely correlation with metabolite concentrations.

Conclusion

Although no definitive data are available concerning the specificity of theta and beta frequency bands with respect to dopaminergic or serotonergic receptor activity, qualitative

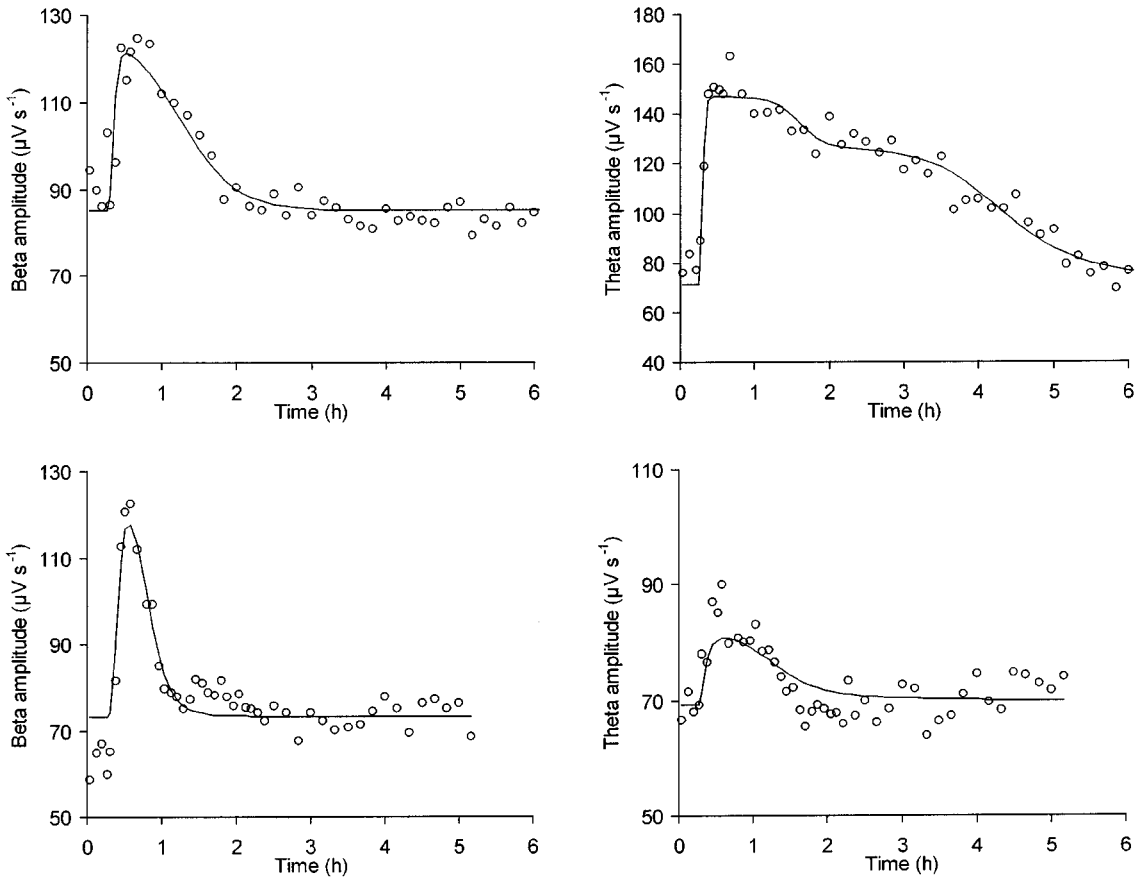

Figure 3 Individual changes in beta (left) and theta (right) EEG frequencies for clozapine (top) and S 16924 (bottom).

and quantitative differences seen in EEG parameters are likely to result from the multiple receptor occupancy for these compounds. Qualitative EEG changes in rat and human were either absent or different for the two developmental compounds, indicating that a more suitable surrogate marker needs to be found to describe the anti-psychotic properties of these types of compounds. However, our results do illustrate the value of population PK/PD modelling in conjunction with sparse sampling to enable determination of concentration-effect relationships in pre-clinical studies.

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