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ORIGINAL INVESTIGATION

Translational PK–PD modelling of molecular target modulation for the AMPA receptor positive allosteric modulator Org 26576

Roberta Bursi · Gul Erdemli · Robert Campbell · Matthew M. Hutmacher · Thomas Kerbusch · David Spanswick · Ross Jeggo · Kari R. Nations · Peter Dogterom · Jacques Schipper · Mohammed Shahid

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Abstract
Introduction The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor potentiator Org 26576 represents an interesting pharmacological tool to evaluate the utility of glutamatergic enhancement towards the treatment of psychiatric disorders. In this study, a rat–human translational pharmacokinetic–pharmacodynamic (PK–PD) model of AMPA receptor modulation was used to predict human target engagement and inform dose selection in efficacy clinical trials.
Methods Modelling and simulation was applied to rat plasma and cerebrospinal fluid (CSF) pharmacokinetic and pharmacodynamic measurements to identify a target concentration (EC_{80}) for AMPA receptor modulation. Human plasma pharmacokinetics was determined from 33 healthy volunteers and eight major depressive disorder patients. From four out of these eight patients, CSF PK was also determined. Simulations of human CSF levels were performed for several doses of Org 26576.
Results Org 26576 (0.1–10 mg/kg, i.v.) potentiated rat hippocampal AMPA receptor responses in an exposure-dependant manner. The rat plasma and CSF PK data were fitted by one-compartment model each. The rat CSF PK–

R. Bursi (✉)
Clinical PK-PD, Dev. DMPK, MSD,
PO Box 20, Oss, BH 5340, the Netherlands
e-mail: roberta.bursi@merck.com

G. Erdemli
Department of Molecular Pharmacology, Schering-Plough,
Newhouse, UK

R. Campbell
Department of Pharmacology, Schering-Plough,
Newhouse, UK

M. M. Hutmacher
Ann Arbor Pharmacometrics Group,
Ann Arbor, MI 48104, USA

T. Kerbusch
Department of M&S, Early Stage Dev., MSD,
Oss, the Netherlands

D. Spanswick · R. Jeggo
NeuroSolutions Ltd,
Coventry, UK

K. R. Nations
Clinical Research, Merck Research Laboratories,
Rahway, NJ, USA

P. Dogterom
Early Stage Development, MSD,
Oss, the Netherlands

J. Schipper
Project and Pipeline Management, MSD,
Oss, the Netherlands

M. Shahid
Department of Molecular Pharmacology, MSD,
Newhouse, UK

Present Address:
G. Erdemli
15 Churchill Terrace,
Newton, MA 02460, USA

Present Address:
R. Campbell
25 Springbank Crescent,
Hamilton, UK

PD model yielded an EC_{80} value of 593 ng/ml (90% confidence interval 406.8, 1,264.1). The human plasma and CSF PK data were simultaneously well described by a two-compartment model. Simulations showed that in humans at 100 mg QD, CSF levels of Org 26576 would exceed the EC_{80} target concentration for about 2 h and that 400 mg BID would engage AMPA receptors for 24 h.
Conclusion The modelling approach provided useful insight on the likely human dose–molecular target engagement relationship for Org 26576. Based on the current analysis, 100 and 400 mg BID would be suitable to provide 'phasic' and 'continuous' AMPA receptor engagement, respectively.

Keywords Org 26576 · AMPA receptor positive allosteric modulator · Translational pharmacometrics · PK–PD modelling and simulation · Depression · ADHD · Schizophrenia

Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian brain (see Bowie 2008; Kew and Kemp 2005; Dingledine et al. 1999; Bigge 1999). It acts on a variety of receptors, coupled with ion channels or G-proteins. Among them α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)-type ionotropic glutamate receptors are the most abundant subtype and required for fast excitatory neurotransmission. AMPA receptors characteristically display rapid desensitisation in the continuous presence of agonist. Since the first demonstration of pharmacological potentiation of AMPA receptors by Ito et al. (1990), a number of positive allosteric modulators have been shown to enhance AMPA receptor-mediated synaptic transmission in vitro and in vivo (Lynch 2006; Lynch and Gall 2006; Arai and Kessler 2007). In contrast to direct AMPA receptor agonists, these agents do not activate AMPA receptors in the absence of glutamate suggesting that they are interacting at a non-orthosteric binding site (Arai and Kessler 2007). In general, these modulators prolong the current flowing through the channel by reducing deactivation and/or desensitisation properties of AMPA receptors. In addition, AMPA receptor coupled ion channels initiate cellular depolarisation that relieves magnesium-dependant block of *N*-methyl-D-aspartate (NMDA) subtype ionotropic glutamate receptors, thus facilitating NMDA receptor-mediated glutamatergic signal transmission (Vandergriff et al. 2001; Lynch 2006; Arai and Kessler 2007). Since disturbances in glutamatergic neurotransmission have been implicated in the pathophysiology of some neuropsychiatric disorders, potentiation of AMPA receptor-mediated glutamatergic neurotransmission has been proposed to have central nervous system (CNS) therapeutic utility (O'Neill et al. 2004a, b; Lynch 2006; Arai and Kessler 2007). Animal behavioural studies have demonstrated efficacy of such modulators in enhancing learning and reducing memory impairments as well as in models of schizophrenia, attention deficit disorder and depression (Damgaard et al. 2010; Su et al. 2009; Hamlyn et al. 2009; Broberg et al. 2009; Woolley et al. 2009; Olsen et al. 2006; Porrino et al. 2005; Knapp et al. 2002; Li et al. 2001; Johnson et al. 1999; Larson et al. 1996; Hampson et al. 1998a, b). The observation that enhancement of neurotransmission through an allosteric mechanism and behavioural effects of AMPA receptor positive allosteric modulators can be achieved without an unacceptable risk of excitotoxicity has facilitated clinical evaluation (Lynch 2004; O'Neill et al. 2004a). Although limited, a body of preliminary clinical studies is emerging demonstrating positive effects on memory in elderly and in patients with psychiatric disorders (Goff et al. 2001, 2008; Wezenberg et al. 2007).

Org 26576 ([9aS]-8,9,9a, 10-tetrahydro-5H, 7H-pyrido [3,2-f] pyrrolo[2,1-c] [1,4]oxazepin-5-one) is a new AMPA receptor positive allosteric modulator (AMPA-PAM) that has been identified as a useful pharmacological tool for investigating the potential utility of glutamatergic agents in the treatment of psychiatric indications such as depression. It has been shown previously to exert its central effects through positive modulation of AMPA receptors (Erdemli et al. 2007; Jordan et al. 2005), and efficacy in animal models is suggestive of benefits in the treatment of depression (Su et al. 2009) and cognitive dysfunction (Hamlyn et al. 2009). Thus, Org 26576, like other AMPA-PAMs, has been shown to facilitate hippocampal neuronal proliferation and survival which may, at least, be partly involved in mediating the effects of this class of glutamate modulators on neuroplasticity, cognition and affective behaviour.

The absence of a PET tracer or other specific biomarkers for AMPA receptor engagement and/or efficacy has, however, posed a significant challenge with regards to appropriate dose selection for clinical proof of concept studies with Org 26576. While animal models with neurochemical or behavioural readouts have a less clear relation to modulation of AMPA receptors, AMPA evoked in vivo electrophysiology offers a relatively direct readout of target modulation (Jeggo et al. 2007; Tierney et al. 2008). This technology and translational pharmacometrics lent their support to select doses based on target modulation. To this purpose, a rat–human translational pharmacokinetic–pharmacodynamic (PK–PD) model of AMPA receptor modulation was developed. This model was used to predict target engagement in humans and to inform dose selection for efficacy trials in psychiatric disorders such as attention deficit hyperactivity disorder (ADHD) and major depressive disorder (MDD).

Method

Rat pharmacokinetics

A comprehensive set of pharmacokinetic data derived from experiments on 59 male Sprague–Dawley rats, with 356 concentration measurements, was used to develop the rat PK model. These data were from experiments designed to determine bioavailability, plasma and cerebrospinal fluid (CSF) exposure as well as temporal aspects of Org 26576 levels following animal treatment. A broad dose range (0.3–30 mg/kg), encompassing that used in the electrophysiology pharmacodynamics assay, was employed. The time points for plasma and CSF analysis ranged from 0.033 to 24 and 0.033 to 4 h, respectively. For CSF measurements, rats were implanted with guide cannula aimed at cisterna magna and allowed to recover from surgery for 3 days prior to treatment with drug. Org 26576 was administered (i.v.) in gelatin/mannitol via a jugular vein catheter and samples of CSF (25–30 μl) collected at intervals of 20, 30, 60, 120 and 240 min post-administration. Org 26576 levels were analysed and quantified (nanograms per millilitre) using liquid chromatography–mass spectrometry/mass spectrometry.

Rat in vivo electrophysiology

Two in vivo electrophysiology studies were performed in male Sprague–Dawley rats: In the first study, a vehicle and 0.1, 0.3, 1, 3 and 10 mg/kg IV Org 26576 were administered to groups of eight rats each. This first study was followed by a second confirmatory study where 0.3 and 1 mg/kg and vehicle were tested following the same study protocol.

The male Sprague–Dawley rats (270–370 g) were initially anaesthetized with an intraperitoneal injection of chloral hydrate (5% solution; 400 mg/kg). The level of anaesthesia was subsequently maintained either by intermittent administration of further anaesthetic (chloral hydrate, 100 mg/kg i.v.) or by the commencement of an infusion of anaesthetic approximately 30 min after induction (chloral hydrate; 100 mg/kg/h i.v.). The period of intermittency, or rate of infusion, was adjusted dependant on anaesthetic depth, assessed by withdrawal responses to tail-pinch and the stability of measured cardiovascular variables. Core body temperature was maintained at 37°C. The right femoral vein and artery were cannulated for the administration of drugs and the recording of arterial blood pressure, respectively. Multibarrel electrodes for iontophoresis were descended vertically to the CA1 region of the hippocampus (Bregma −4.2, lateral 2.4, 1.8–2.4 mm below pial surface; Paxinos and Watson 1982). Multibarrel electrode pipette barrels contained AMPA (5 mM in 195 mM NaCl; pH 8) and the AMPA antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX; 1 mM in 195 mM NaCl; pH 8) for current ejection and Pontamine Sky Blue (PSB; 2% dissolved in 0.5 M sodium acetate) for current balancing.

Neuronal activity was recorded using a Neurolog amplifier and a 1401 interface (Cambridge Electronic Design, UK). The amplitude and duration of current ejections were adjusted to produce an increase in firing rate that was less than 30% of maximum. AMPA-evoked activity was analysed in 5-min windows with a 10-min stable period achieved before the administration of test compounds.

In all experiments, PSB was injected via iontophoresis as a marker into the hippocampus after the termination of neuronal recording. At the end of the experiments, brains were removed and after serial cryo-sectioning the PSB marked recording sites were then visualized under a light microscope for confirmation of the location of specific recording sites. All animal experiments were carried out at NeuroSolutions Ltd. according to the Animals (Scientific Procedures) Act, 1986, and upon completion of each in vivo experiment, animals were killed by an overdose of anaesthetic (pentobarbitone sodium; 200 mg kg^{-1}) and cervical dislocation.

Human pharmacokinetics

Two clinical trials, one in healthy male volunteers and one in MDD patients, were conducted in which the safety, tolerability and pharmacokinetics of Org 26576 were investigated. For the purpose of this manuscript, attention is focussed only on the pharmacokinetics. Tolerability and safety will be thoroughly reported elsewhere (Nations et al., in preparation).

For both trials, the trial protocol, the subject information and the informed consent form were approved by the Independent Ethics Committee of the trial centre. The trials were conducted in compliance with the current revision of the Declaration of Helsinki, ICH guideline and Good Clinical Practice and current regulatory regulations.

Healthy volunteers The trial consisted of two parts: In part I, single rising doses of 5 to 250 mg were investigated, and in part II, one group received 100 mg BID for 7 days and one group received multiple increasing doses as 100 mg BID on days 1 and 2, 150 mg BID on days 3–5, 225 mg BID on days 6–8, 325 mg BID on days 9–11 and 400 mg BID on days 12 and 13 and once daily on day 14 at 12 h intervals.

PK blood sampling was performed according to a dense sampling scheme following single dose as well as multiple dose administrations. A total of 1,290 plasma concentrations contributed to the modelling and simulation analysis of this study.

MDD patients In part I of this study, four groups of MDD patients each received multiple escalating doses with different starting doses (A 100–600 BID during a 16-day treatment period; B 200–600 mg BID during a 13-day

treatment period; C 300–600 mg BID during a 10-day treatment period; D 100–300 mg BID during a 13-day treatment period). In all groups, frequent blood samples were collected on the days that doses were increased whereas in group D, continuous CSF was collected in fractions of 30 min for 2 h before the morning dose of 100 mg (day 1) and 300 mg (day 10) through 12 h after.

A total of 422 plasma concentrations and 206 CSF concentrations contributed to the modelling and simulation analysis of this study. Part II of the study focussed on safety, tolerability and pharmacokinetics during a 4-week treatment period, but data were not used in the M&S analysis.

Modelling

Rat and human PK concentrations below the quantification limit of 10 and 0.2 ng/ml, respectively, were omitted from the PK model fittings in general. All models utilized the 'log-transform both sides approach' to reduce skewness and homogenize the residual variability. Diagnostic plots were used to assess these assumptions of residual variability, as well as assess the adequacy of model fittings. Differences in the objective function value (OFV; difference denoted ΔOFV) between models were used to guide model development (Wählby et al. 2001).

Modelling was performed using the NONMEM Version VI software (ICON plc). SAS 9.1.3 (SAS Institute Inc.) and/or S-Plus 7.0 (Insightful Corp.) were used for pre-processing (e.g. constructing NONMEM data sets), post-processing such as graphics and simulation where necessary.

Results

Org 26576 enhances AMPA-mediated single hippocampal neurone activity

Iontophoretic application of AMPA onto single CA1 hippocampal neurones evoked a mean excitation of 481±35 spikes per AMPA response which was depressed by 91%–43±8 spikes per AMPA response in the presence of NBQX (40–200 nA, $n=16$, $P<0.01$ paired t test, Fig. 1). The NBQX-induced reduction in AMPA-evoked neuronal activity recovered to 420±48 spikes per AMPA response ($n=16$, 87% of control AMPA-induced responses), after NBQX iontophoresis was terminated, confirming that the evoked excitation was mediated by AMPA receptor activation (Fig. 1).

Org 26576 (0.1–10 mg/kg, i.v.) enhanced the AMPA-evoked neuronal activity in a reversible and dose-dependant manner. The maximum effect (66±12% of control values, $n=8$) on AMPA-evoked activity was observed 5–10 min after i.v. administration of 1 mg/kg Org 26576 (Fig. 2). This enhancement was statistically significant when compared to time matched vehicle control ($P<0.01$, one-way ANOVA, post hoc Dunnett's test). The effect at higher doses was similar to that observed at 1 mg/kg. The increase in neuronal activity was maintained for at least 30 min. No significant effect was observed at 0.1 mg/kg ($n=8$), and the minimum effective dose of Org 26576 was 0.3 mg/kg. At this dose, Org 26576 potentiated AMPA-evoked neuronal activity to a maximum of 42±15% of control values at 5–10 min post-administration ($P<0.05$, $n=8$, Fig. 2).

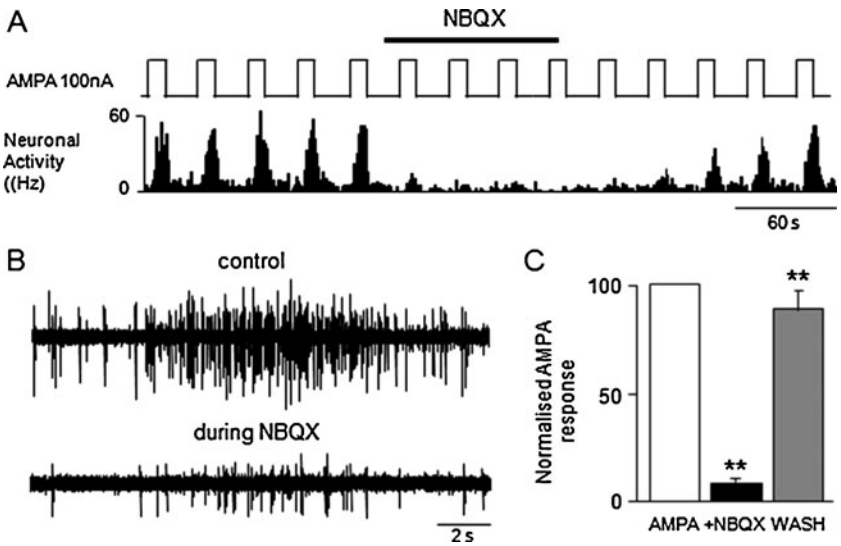

Fig. 1 AMPA receptor-mediated single neurone firing in the CA1 hippocampus in vivo. **a** A continuous rate histogram of typical AMPA-evoked neuronal activity recorded from CA1 region of hippocampus in rats under anaesthesia. AMPA (100 nA, cycle 10 s on, 20 s off) and NBQX (200 nA) were applied iontophoretically. **b** Expanded AMPA-evoked responses taken from A before (control) and during the administration of NBQX. **c** A bar chart of the group data indicating the reduction of AMPA-evoked activity in the presence of NBQX in a reversible manner ($n=16$, $^{**}P<0.01$)

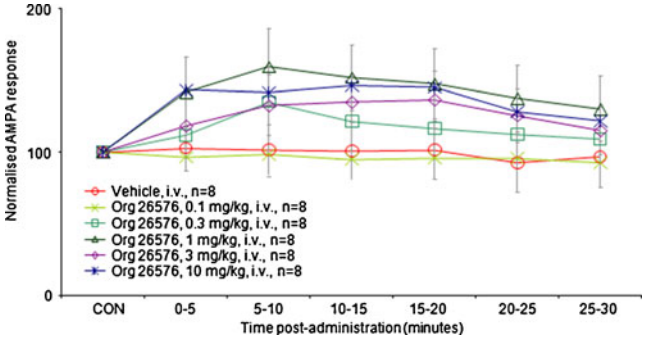

Fig. 2 Systemic administration of Org 26576 enhances AMPA-mediated single neurone firing in a dose-dependant manner. Line graph detailing the dose-dependant responses over time of AMPA-evoked neuronal activity evoked by iontophoretic applications of i.v. administrations of Org 26576, recorded from the CA1 region of the hippocampus in the anaesthetised rat. CON is the baseline value

Rat plasma and CSF PK model

The rat PK model was developed by first analysing the IV plasma data. A one-compartment model well described the disposition of Org 26576 in plasma. The CSF data were subsequently added to the dataset and fitted using a two-compartment model, but the fitting was overparameterized. Graphical inspection of the curves (see Fig. 3) revealed similar elimination rates for plasma and CSF, but no distribution phases between the two compartments were evident. A special PK model was hence derived to fit these data (Hutmacher et al. 2008). The model consisted of a one-compartment model with identical elimination rates both for plasma and CSF, but with different volumes of distribution (scale parameters) and, as consequence, with similar, but not identical, predicted areas under the curve (AUCs) for plasma and CSF. The estimated CSF volume does not bias predictions of the concentration–time profiles and thus does not affect the accuracy of estimates and predictions of the concentration–AMPA response relationship. The parameter results are provided in Table 1 and the rat PK data and model fittings are displayed in Fig. 3.

Rat PK–PD model

An E_{max} model was fit to the rat in vivo electrophysiology data. The model form implemented was

$$\ln\left(AMPA - PAM\right) = \ln\left(E_0 + \frac{E_{\max} \cdot C_{CSF}(t)^{\gamma}}{EC_{50}^{\gamma} + C_{CSF}(t)^{\gamma}}\right) + \varepsilon$$

where AMPA-PAM is the acronym for AMPA receptor positive allosteric modulator, E_0 is the placebo (non-drug) response, E_{max} represents the maximum of the drug effect, C_{CSF} is the CSF concentration of the drug, the EC_{50} reflects the drug concentration that achieves half of E_{max} and γ is the Hill coefficient. Early modelling indicated that the two electrophysiology studies, the first and the second studies, had a different response level. For the model fittings, the study-specific differences were estimated for E_0 and E_{max}, while the potency (EC_{50}) and the Hill coefficient were assumed to be the same across both studies.

The parameter estimates from the selected final model are displayed in Table 2. The second study had 26% and 114% larger baseline and E_{max} estimates, respectively. The EC_{50} estimate for the pooled data was 384 ng/ml, with an inter-subject CV of 94.4%.

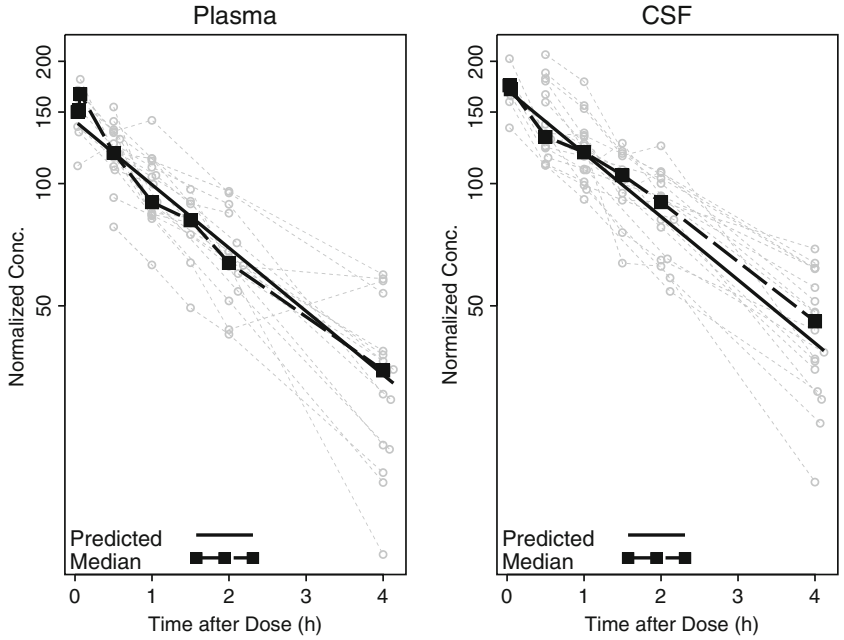

Fig. 3 Rat plasma and CSF concentration data following IV administration with model predictions

Table 1 Rat PK model parameter estimates

Parameter	Estimate (ASE)[f]	IIV (%CV)[g]
CL (L/h/kg)[a]	0.338 (0.016)	27.7
V_P (L/kg)[b]	1.04 (0.05)	16.8
$F_{Dose=5\ (\%\uparrow)}$[c]	58.2 (9.0)	–
V_{CSF} (L/kg)	0.848 (0.036)	15.6
σ_P (%CV)[d]	12.8 (2.5)	
σ_{CSF} (%CV)	11.0 (0.9)	
β (h^{-1})[e]	0.328	
$\tau_{1/2}$ (h)	2.1	

[a] Clearance

[b] Volume of distribution ($V_{P=plasma}$; $V_{CSF=CSF}$)

[c] Estimate of a different bioavailability for one group dosed 5 mg/kg

[d] Residual error variance component ($\sigma_{P=plasma}$; $\sigma_{CSF=CSF}$)

[e] Macro-constant and half-life: $\ln(2)/\beta=\tau_{½}$

[f] Estimated asymptotic standard error

[g] Inter-individual variability (IIV) of the subject-specific random effects (η) are reported as an approximate %CV (e.g. for Variance(η)=ω^2, %CV≈100·ω)

The steepness of the concentration–effect is quantified by the large Hill coefficient estimate of 3.20. Issues with estimating this steep concentration–effect curve are reflected by the large estimated standard error of the Hill coefficient (2.18)—yielding a relative standard error of 68%. This large standard error could be an indication that the doses used in the experimental designs were not optimal to capture the steep threshold-like concentration effect. Rat AMPA-PAM data and model predictions are displayed in Fig. 4.

Table 2 Final rat PK-AMPA receptor positive allosteric modulator model parameter estimates

Parameter	Estimate (ASE)[g]	IIV (%CV)[h]
E_0[a]	498 (30)	34.4
E_0 (s)[b] (% ↑)	26.0 (10.4)	
E_{max}[c]	112 (18)	–
E_{max} (s; % ↑)	114 (38.1)	
EC_{50} (ng/ml)[d]	384 (80.5)	94.4
γ[e]	3.20 (2.18)	–
σ (%CV)[f]	13.4 (0.9)	

[a] Baseline

[b] Estimated percent increase in the parameter for the second study relative to the first

[c] Maximum efficacy

[d] Potency=concentration corresponding to the half-maximal difference between E_0 and the maximal response E_0+E_{max}

[e] Hill coefficient

[f] Residual error variance component

[g] Estimated asymptotic standard error

[h] Inter-individual variability (IIV) of the subject-specific random effects (η) is reported as an approximate %CV (e.g. Variance(η)=ω^2, %CV≈100·ω)

Target modulation predictions were constructed using The EC_{80} (concentration at plateau of effect), the EC_{50} (concentration at half of maximal effect) and the EC_{20} (concentration at start of log-linear effect) predicted from the rat AMPA-PAM model. The typical predictions from the model were used to calculate the target predictions. The target predictions and 90% confidence intervals are as follows: EC_{80}=593 ng/ml (406.8, 1,264.1), EC_{50}=384 ng/ml (265.2, 554.9) and EC_{20}=248 (90.5, 430.3)ng/ml. A plot of the concentration–effect curve as a fraction of E_{max} is provided in Fig. 5.

Human plasma and CSF model

In analogy with the rat PK data, the first modelling step consisted of simultaneously fitting the plasma concentrations of healthy subjects and MDD patients. This strategy seemed also adequate in view of the fact that CSF data were sampled from only four patients. Although unable to precisely predict the peak concentration, a one-compartment disposition model adequately fitted these data. The same data did not support more complex models, such as two- and three-compartment models. To a great extent, the difficulty in describing the peak was attributed to a dose-dependant rate of absorption. A log-linear power model was used to characterize this relationship:

$$\log\left[ka\ (Dose)\right] = ka_{\text{int}} + ka_{slp}\cdot Dose^{ka_{power}}$$

Additionally, in order to reduce the influence of the data collected during the absorption phase on the estimates of the disposition parameters, different residual error parameters were fitted to concentrations <4 and ≥4 h post-dose. Further refinements were evaluated. Dose-dependant relative bioavailability (F_{REL}) was evaluated, but not found significant. Inter-individual variability (IIV) for ka was identified to be 0, with a large inter-occasion variability (IOV) of about 100%. Next, the IOV components were tested separately on CL/F and relative oral bioavailability. The IOV on CL/F showed a larger decrease in the OFV and thus was retained in the model. In general, this model well described the post-absorption profile and was considerate adequate for AUC and trough concentration estimates. Difficulties in fitting the absorption profile might reduce the utility of the model in predicting concentrations at t_{max}—noting the highly variable absorption rates within the individuals. Ultimately, a one-compartment model with dose-dependant first-order absorption was considered adequate for characterizing the plasma concentration–time profile.

The CSF concentrations obtained from four patients were subsequently added to the plasma data. Visual

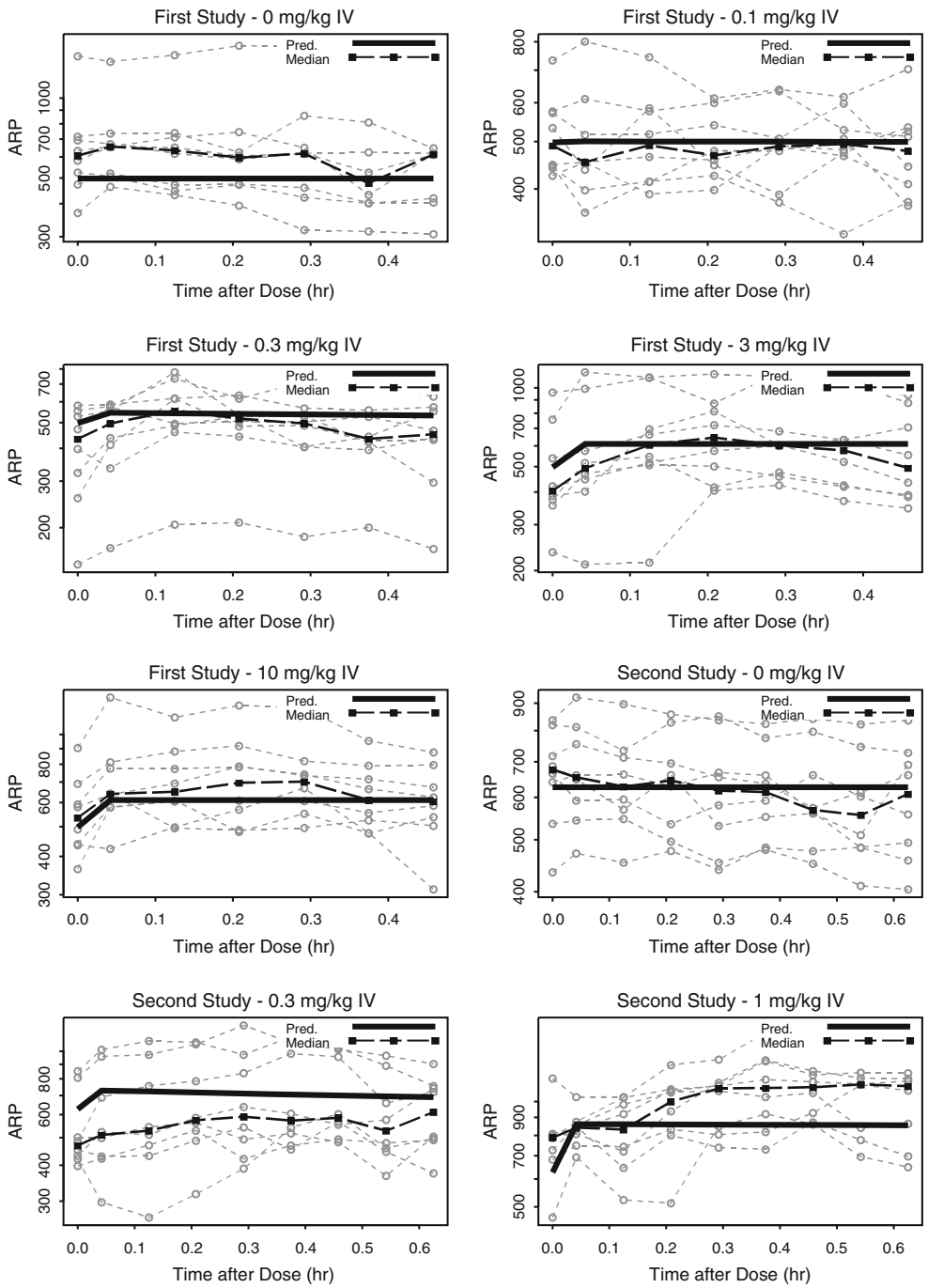

Fig. 4 Rat AMPA-PAM measurements and model predictions

inspection (see Fig. 6) indicated a time delay between peak concentrations in plasma and CSF. Three structural extensions to the previous plasma PK model were assessed with the CSF data: a two-compartment model, an equilibration model and an effect site like (keo) model. Although differences among OVFs are not anticipated to be distributed as a chi-square distribution, the two-compartment model was selected because it resulted in about a 100-point decrease in the OVF relative to the effect site model. Attempts were made to add IIV components to the CSF-related parameters. These fittings either did not converge or the variance components estimates converged to large unrealistic values—likely due to data from only four patients. A separate residual variance component was incorporated for fits to the CSF data.

The parameter estimates from the final plasma–CSF model are displayed in Table 3. In this model, most of the parameter estimates including the IIV and IOV components are similar to those obtained for the plasma-data-only model. The CSF distribution phase was estimated as α= 0.931 ($\tau_{1/2}$=0.75 h). The terminal phase parameter β= 0.270 ($\tau_{1/2}$=2.57 h) did not change appreciably from the

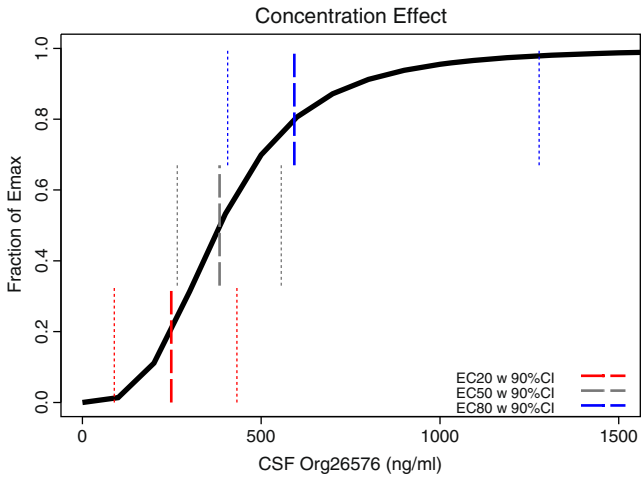

Fig. 5 Fitted concentration–effect curve for Org 26576 in rat[a]. [a]Estimated concentrations at 20%, 50% and 80% of the maximal effect E_{max}: EC_{20}=248 ng/ml, EC_{50}=384 ng/ml and EC_{80}=593 ng/ml

plasma-only fitting. The estimates of CL/F and V/F were 24.5 l/h and 65.4 l, respectively. Concentration–time profiles for this model are displayed in Fig. 6.

Simulations target modulation in humans

Duration of action as an indicator of predicted efficacy was defined by the time above EC_{80}, which was assumed to be the same between rat and human. For this purpose, the human PK model was used to simulate CSF levels for different Org 26576 doses and regimens (QD, BID) in order to identify the dose and the duration during which concentration levels will remain above EC_{80} and in order to estimate their duration above it. Examples of these simulations for several total daily doses following a QD and a BID regimen are displayed in Fig. 7.

As Fig. 7 shows, for a total daily dose of 100 mg (i.e. 50 mg BID or 100 mg QD), only following QD administration concentration levels reach the EC_{80} value of 593 ng/ml. By increasing the dose, both regimens will produce levels above it for longer durations and at doses of 400 mg BID and higher concentrations are expected to modulate the AMPA receptor target nearly for 24 h.

The relationship between time above the EC_{80} and QD and BID dosing is further illustrated in Fig. 8. Regarding the BID dosing, the 100-mg BID regimen is predicted to yield nearly 2 h above the EC_{80} upon each dosing occasion and is the first dose (at 50 mg increments) that achieves any time above the EC_{80}. The 200-mg BID regimen is predicted to yield duration of action of nearly 6 h. The 450-mg BID regimen dose is predicted to achieve 10–11 h of the dosing interval of 12 h, while doses greater than 550 mg BID are predicted to be above the EC_{80} for the entire 12-h dosing time interval, indicating no additional benefit was predicted by increasing the dose further. The QD predictions show benefit in terms of fraction of time above the dosing interval up until doses slightly greater than 200 mg. This is because of the slower rate of absorption for higher doses (needed to ensure comparable total daily dose levels). Thereafter, BID is predicted to have a greater fraction of the dosing interval above the EC_{80} for the same total daily dose.

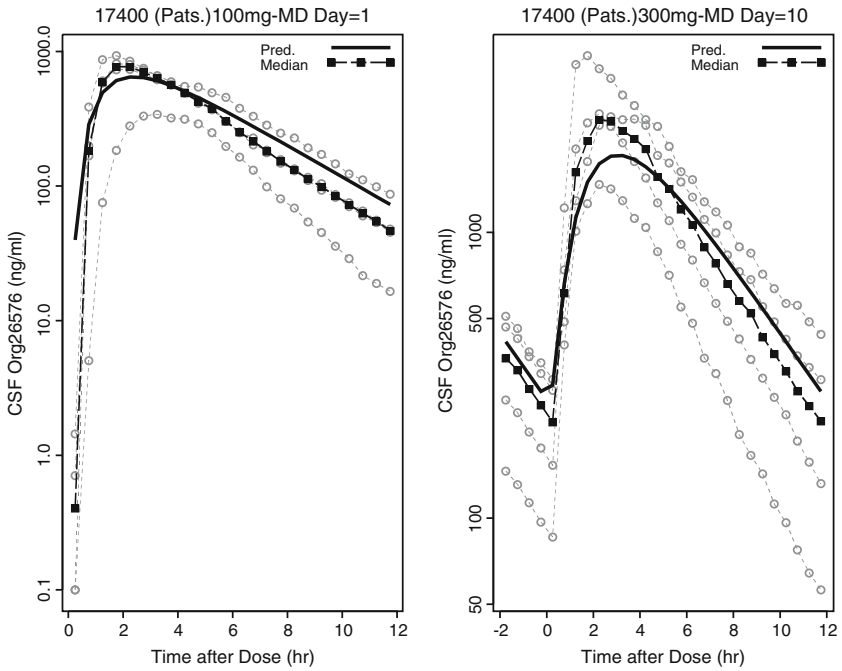

Fig. 6 Plasma–CSF model fits to the human CSF data

Table 3 Parameter estimates for the human plasma–CSF PK model

Parameter	Estimate[h] (ASE)[i]	Transformed estimate	%CV IIV (IOV)[k]
*T*lag[a]	−2.82 (0.19)	0.0596 (h)	87.7
ka_{int}[b]	3.53 (0.40)	–	0.0 (99.8)
ka_{slp}	−0.632 (0.066)	–	
ka_{power}	0.3[j]	–	
CL/*F*[c]	3.20 (0.054)	24.5 (L/h)	31.0 (11.1)
V/*F*[d]	4.18 (0.072)	65.4 (L)	23.6
Q/*F*[e] CSF	2.32 (0.17)	10.2 (L/h)	–
V/*F* CSF	2.72 (0.16)	15.2 (L)	–
σ (time<4)[f]	−1.24 (0.08)	28.9 (%CV)	
σ (time≥4)	−1.01 (0.13)	36.4 (%CV)	
σ CSF	−1.53 (0.31)	21.7 (%CV)	
α[g]	–	0.931 (1/h)	
$\tau_{1/2\alpha}$		0.75 (h)	
β	–	0.270 (1/h)	
$\tau_{1/2\beta}$		2.57 (h)	

[a] Time delay

[b] Absorption rate constant

[c] Apparent clearance

[d] Apparent volume of distribution

[e] Inter-compartmental clearance

[f] Time relative to previous dose; σ=residual error

[g] Macro-constants α and β and corresponding half-lives

[h] Estimated on the log (ln) scale

[i] Estimated asymptotic standard error

[j] Fixed

[k] Inter-occasion variability (IOV), inter-individual variability (IIV) of the subject-specific random effects (η) is reported as an approximate %CV (e.g. Variance(η)=ω^2 , %CV≈100·ω)

Discussion

The main findings from the current study indicate (a) a clear PK–PD relationship for the AMPA-PAM Org 26576 in rat, (b) dose levels of Org 26576 used in human studies yielded exposure levels consistent with those required for AMPA receptor activation and (c) translational application of PK–PD modelling can provide useful insight towards molecular target engagement and dose setting in clinical studies with Org 26576.

Model (mechanism)-based PK–PD modelling is increasingly becoming an integral part of the decision making process within the entire drug development trajectory from translational research (Mankoff et al. 2004) to late stage development and product labelling (Sheiner and Steimer 2000; Danhof et al. 2008; Mager and Jusko 2008). The translatability potential of pharmacokinetic and pharmacodynamic parameters from preclinical species to humans is generally based on the observation that pharmacokinetic parameters obey allometric principles, while drug's pharmacological intrinsic capacity, E_{max}, and sensitivity, EC_{50}, are often, but not always, similar across species. In absence of a PET ligand or specific biomarkers for target engagement and/or efficacy validated in preclinical species as well as in humans, in vitro and in vivo data may lend their support to the assumption of translatability.

This is the case of the AMPA-PAM Org 26576, for which target modulation of the AMPA receptor was shown in the rat by in vivo electrophysiology. Potentiation of AMPA receptor-evoked neuronal electrophysiological response provides a relatively direct measure of molecular target engagement and was thus preferred over other possible readouts such as neurochemical or behavioural responses which may have a more complex relationship with regards to AMPA receptor modulation. The limitation of the applied technique, however, is that it may not be validated in humans for obvious reasons. The observation of a dose–response as well as the estimate of an EC_{50} target concentration in the rat provided the opportunity to explore dosing regimens capable of modulating the AMPA target in humans. This translation, however,

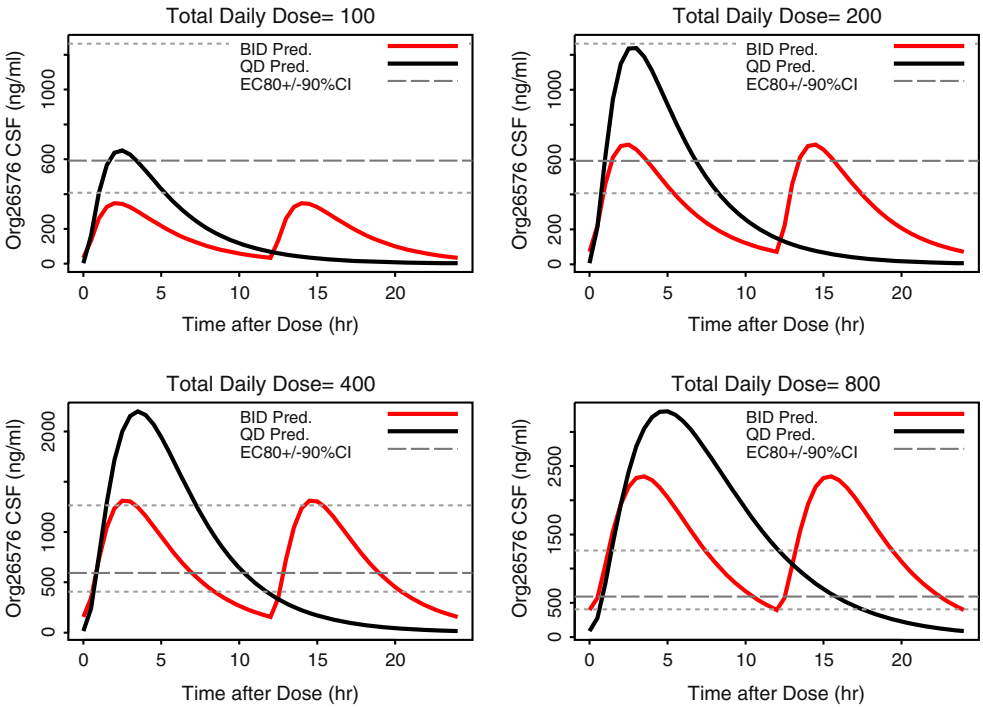

Fig. 7 Simulations for total daily doses of 100, 200, 400 and 800 mg Org 26576

can only be acceptable when the human, and the rat targets would behave similarly, i.e. the EC_{50} and the Hill coefficient would be similar. For Org 26576, the assumption of target similarity is supported by the literature (see Dingledine et al. 1999), which reports the similarity of human and rat glutamatergic receptors as well as the similarity in the receptor pharmacology of AMPA receptor potentiators at rat and human AMPA receptors (see Arai and Kessler 2007).

An additional assumption which was implicitly made in this study was the use of rat and human CSF exposures as surrogates for drug concentration at the target in the brain. As postulated in literature (de Lange and Danhof 2002), the free concentration of a drug in the brain interstitial spaces, $C_{u,brain}$, is available to interact with the majority of the CNS receptors and therefore is a true measure of receptor occupancy. CSF concentrations, C_{csf}, are expected to reflect this free concentration only in a limited number of cases where the drug will diffuse through the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier via the transcellular route (passive diffusion), and it will distribute homogeneously over the brain fluids. In these cases, however, plasma free drug concentrations, $C_{u,plasma}$, would serve to the same purpose, while being less invasive. In a recent study in rats (Watson et al. 2009), the correlations among D_2 receptor occupancy, $C_{u,brain}$, $C_{u,plasma}$ and C_{csf}, were evaluated for six marketed antipsychotic drugs. It was shown that for drugs, which quickly penetrate the brain by simple diffusion, $C_{u,plasma}$ and C_{csf} seem to predict $C_{u,brain}$ equally well, but that for efflux substrates or slowly brain penetrating compounds C_{csf} seem to be more accurate. In the clinical setting, analogous discrepant observations have been reported, all depending upon the characteristics of the drug being investigated, its intrinsic physical–chemical properties, whether it is an efflux substrate or whether it undergoes CSF local metabolism (Venkatakrishnan et al. 2007; Nikisch et al. 2010).

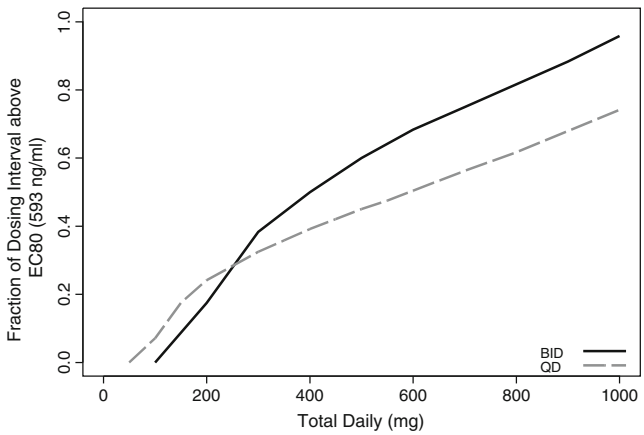

Fig. 8 Fraction of dosing interval above EC_{80}

From in silico predictions of its physical–chemical profile, Org 26576 was expected to have a good BBB penetration; from in vitro Caco-2 experiments, it did not show any efflux property (unpublished work). The consistency of the PK profiles in the different compartments and in the different species provided additional supporting evidence for the applicability of translational assumptions from rat to human. In the rat, the disposition in plasma of this AMPA receptor positive allosteric modulator is described by one-compartment model with exposure levels and terminal phases very similar to CSF. The cause for the missed estimate of a distribution

phase between the two compartments seems to lie more in the nature of the data (different experiments, different groups of rats) than in the PK characteristics of the compound. The richer sampling scheme and the bigger homogeneity of the human data enabled, on the other hand, the identification of a distribution phase between plasma and CSF, which was estimated to be less than 1 h. Also in humans, the disposition in plasma of Org 26576 was well described by one-compartment model with plasma and CSF exposures and terminal half-lives very similar to each other. The estimate of the AUC of plasma and CSF exposures yield almost identical values. All together, these observations have strongly increased the confidence level of translatability, but ultimately clearly only by means of intracerebral microdialys and/or imaging techniques can the actual concentration of the drug at the target site clearly be assessed.

Fitting of the rat PK and AMPA-PAM data yield a steep concentration effect curve with a large Hill coefficient and an imprecise EC_{50} estimate. This is reflected in the EC_{80} estimate, which is only about 2.4 times the EC_{20} estimate and by their corresponding confidence intervals which to some extent overlap with each other. Thus, the concentration–effect might appear as if generated by a threshold. The identification and quantification of this threshold, i.e. the EC_{80} concentration, was the primary objective of this study.

Once target concentrations were identified in the rat, simulations for dosing scenarios in humans could be performed to inform dose selection during the design phase of a proof of concept study. By 100-mg QD dosing, Org 26576 levels are expected to be at and above plateau for only about 2 h. In a BID regimen, the duration time above EC_{80} is therefore predicted to be about 4 h. Modulation of the target for the entire duration of the dosing interval is obtained starting at about 400 mg BID, i.e. total daily doses of 800 mg. The novel approach of modulating the AMPA receptor for therapeutic efficacy raised the question whether a ‘phasic’ (100 mg BID) or a ‘continuous’ (400 mg BID) modulation of the target would be necessary to observe efficacy in patients with psychiatric disorders. Both doses were therefore recommended for inclusion in the design of proof of concept studies in ADHD (Adler et al., in preparation) and MDD (not yet started).

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