

DRUG INTERACTIONS

Metoprolol-pridopidine drug–drug interaction and food effect assessments of pridopidine, a new drug for treatment of Huntington's disease

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AIMS

Pridopidine is an oral drug in clinical development for treatment of patients with Huntington's disease. This study examined the interactions of pridopidine with *in vitro* cytochrome P450 activity and characterized the effects of pridopidine on CYP2D6 activity in healthy volunteers using metoprolol as a probe substrate. The effect of food on pridopidine exposure was assessed.

METHODS

The ability of pridopidine to inhibit and/or induce *in vitro* activity of drug metabolizing enzymes was examined in human liver microsomes and fresh hepatocytes. CYP2D6 inhibition potency and reversibility was assessed using dextromethorphan. For the clinical assessment, 22 healthy subjects were given metoprolol 100 mg alone and concomitantly with steady-state pridopidine 45 mg twice daily. Food effect on a single 90 mg dose of pridopidine was evaluated in a crossover manner. Safety assessments and pharmacokinetic sampling occurred throughout the study.

RESULTS

Pridopidine was found to be a metabolism dependent inhibitor of CYP2D6, the main enzyme catalysing its own metabolism. Flavin-containing monooxygenase heat inactivation of liver microsomes did not affect pridopidine metabolism-dependent inhibition of CYP2D6 and its inhibition of CYP2D6 was not reversible with addition of FeCN₃. Exposure to metoprolol was markedly increased when coadministered with pridopidine; the ratio of the geometric means (90% confidence interval) for maximum observed plasma concentration, and area under the plasma concentration–time curve from time 0 to the time of the last quantifiable concentration and extrapolated to infinity were 3.5 (2.9, 4.22), 6.64 (5.27, 8.38) and 6.55 (5.18, 8.28), respectively. Systemic exposure to pridopidine was unaffected by food conditions.

CONCLUSIONS

As pridopidine is a metabolism-dependent inhibitor of CYP2D6, systemic levels of drugs metabolized by CYP2D6 may increase with chronic coadministration of pridopidine. Pridopidine can be administered without regard to food.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Pridopidine is a novel drug in development for treatment of Huntington's disease. It is believed to have partial affinity for the dopamine D2 receptor and its effects on motor symptoms may be related to its binding to the sigma-1 receptor.
- A single dose of pridopidine undergoes hepatic metabolism by CYP2D6 with an elimination half-life of 6 and 15 h for extensive and poor CYP2D6 metabolizers, respectively. Pridopidine exposure is thus higher in poor CYP2D6 metabolizers after a single administration.
- Following repeated administration, the pridopidine elimination half-life is 10–14 h regardless of metabolizers genotype and the pridopidine exposure is similar for extensive and poor metabolizers under steady-state conditions.

WHAT THIS STUDY ADDS

- Pridopidine is a metabolism-dependent inhibitor of its own metabolizing enzyme, CYP2D6.
- Exposure to metoprolol, a probe substrate for CYP2D6 inhibition, was markedly increased when coadministered with pridopidine.
- Food does not impact the exposure to pridopidine.

Tables of Links

TARGETS	
G protein-coupled receptors [2]	Enzymes [4]
dopamine D2 receptors	CYP1A2
Other protein targets [3]	cytochrome P450 2D6 (CYP2D6)
sigma-1 receptors (S1R)	CYP2B6
	CYP2C8
	CYP2C9
	CYP2C19
	CYP2D6

LIGANDS
Dextromethorphan
Metoprolol
NADPH
Paracetamol/acetaminophen
Paroxetine
Risperidone
Sertraline

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2–4].

Introduction

Pridopidine (4-[3-(methylsulfonyl)phenyl]-1-propylpiperidine; formerly known as ACR16, is an investigational drug under development by Teva Pharmaceutical Industries for treatment of Huntington's disease (HD; e.g., PRIDE-HD, NCT02006472). HD is a rare neurodegenerative disorder of the central nervous system (CNS) with an autosomal dominant model of inheritance and a prevalence of 1/100 000 to 5/100 000 [5, 6]. In HD, progressive neurodegenerative processes in the CNS, particularly in the striatum, lead to motor impairment, cognitive decline and abnormal psychiatric symptoms [6, 7].

Pridopidine belongs to a new class of compounds known as dopidines and appears to normalize regulation of psychomotor behaviours in preclinical models [8]. Although the entire scope of biological activity for pridopidine is not established, it is known to bind to dopamine D2 receptors [9] and shows highly selective and robust affinity for sigma-1 receptor (S1R) [10]. Recent findings suggest that the effects of pridopidine on motor behaviour abnormalities may be related to its binding to the S1R [11]. The S1R is an endoplasmic reticulum chaperone protein involved in cellular differentiation and neuroplasticity [12]. In Phase 2 trials conducted in patients with HD (i.e. HART, MermaiHD),

the observed effects in the primary endpoints of the studies, the modified motor score of the Unified HD Rating Scale, in the pridopidine-treated cohorts vs. the placebo group did not reach statistical significance although the directionality of the changes suggested a benefit of treatment with pridopidine. However, pridopidine improved the secondary endpoint, the total motor score [13–15]. In both studies, pridopidine was considered safe and well tolerated.

Pridopidine is absorbed relatively rapidly after oral administration with time (t_{max}) to reach maximum observed plasma concentration (C_{max}) of roughly 2 h [16]. Pridopidine is *N*-dealkylated by the polymorphic enzyme, cytochrome P450 2D6 (CYP2D6) to an inactive metabolite TV-45065 (formerly known as ACR30 [17]). Pridopidine's elimination half-life ($t_{1/2}$) after a single dose is approximately 6 and 15 h for extensive or poor metabolizers, respectively, and is approximately 10–14 h after repeated administration in both populations [16]. Accordingly, pridopidine exposure in poor metabolizers (11 192 ng h ml⁻¹) is almost three times higher than in extensive metabolizers (3782 ng h ml⁻¹) after a single dose. However, at steady state, poor and extensive metabolizers had comparable exposures (12 080 ng ml⁻¹ and 9338 ng h ml⁻¹, respectively) due to a reduction in pridopidine elimination in extensive metabolizers over time. A similar pattern is seen for TV-45065 with regard to half-life.

That is, the elimination half-life is different between extensive and poor metabolizers after a single dose administration (approximately 8 and 32 h, respectively) but is similar after repeated administration (17 and 19 h, respectively).

The primary objective of this report is to describe the *in vitro* studies that identified potential interactions of pridopidine with cytochrome P450 activity and the subsequent clinical study that characterized the impact of pridopidine on CYP2D6 activity *in vivo*. Following the European Medicines Agency guidelines, metoprolol was used as the CYP2D6 probe of choice for this study because it is a substrate of this CYP isoenzyme and is acknowledged for use as a pharmacological marker to evaluate the inhibitory potential of the drug in question [18]. Dosing of pridopidine to steady state represented the more clinically relevant scenario, which was equally important given autoinhibition of CYP2D6 by pridopidine. Additional objectives of the clinical study included the assessment of the effect of food on the pharmacokinetics (PK) of pridopidine as well as the safety and tolerability of pridopidine.

Methods

In vitro inhibition/induction of cytochromes P450

Evaluation of pridopidine as a CYP inhibitor. The ability of pridopidine and TV-45065 to inhibit and/or induce the *in vitro* activity of major drug metabolizing enzymes was assessed according to standard practices [18–20].

Inhibition experiments assessed changes in enzymatic activity in human liver microsomes (HLM) by quantitation of the relevant metabolic transformation of probe substrates specific for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 following incubation in the presence of pridopidine or TV-45065. Briefly, pridopidine and TV-45065 were incubated with probe substrates and pooled HLM at concentrations ranging from 0.5 to 500 $\mu\text{mol l}^{-1}$ pridopidine and 0.05 to 50 $\mu\text{mol l}^{-1}$ TV-45065. These concentrations represent the levels administered clinically and up to >100-fold higher. Time- or metabolism-dependent CYP inhibition was assessed by preincubating pridopidine (or TV-45065) with microsomes for 30 min in the absence or presence of NADPH, respectively.

For the determination of CYP2D6 inhibitor potency (K_i), activity was assessed using dextromethorphan concentrations ranging from 2.25 to 75 $\mu\text{mol l}^{-1}$ and pridopidine concentrations ranging from 9.25 to 370 $\mu\text{mol l}^{-1}$. Additionally, the reversibility of CYP2D6 inhibition by pridopidine was assessed by incubating pridopidine and microsomes for 30 min with NADPH followed by 10 min in the presence or absence of 2 mmol l^{-1} ferricyanide.

Freshly isolated cultured human hepatocytes were treated with pridopidine (at 0.1–100 $\mu\text{mol l}^{-1}$) for 3 consecutive days followed by isolation of microsomes [21]. Isolated microsomes were preincubated with pridopidine and NADPH as described above, and then re-isolated using ultracentrifugation of the incubation mixtures and washed

prior to 5-min incubation with dextromethorphan. Metabolism-dependent inhibition (MDI) of CYP2D6 by pridopidine was further evaluated by incubating pridopidine with human liver microsomes hourly (1–4 h) prior to a dextromethorphan O-demethylation assay.

To assess whether flavin-containing monooxygenase (FMO) enzymes may be contributing to the observed MDI of CYP2D6 by pridopidine, FMO was inactivated by exposing human liver microsomes to 50°C for 2 min prior to 2 h preincubation and dextromethorphan O-demethylation as described above. Additional details can be found in Supplementary Tables S1 and S2.

Evaluation of pridopidine and TV-45065 as a CYP inducer.

Induction experiments assessed changes in enzymatic activity and mRNA levels for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 in cultured human hepatocytes following treatment with pridopidine or TV-45065. Three preparations of freshly isolated human hepatocytes, each from a single donor [22–24] were treated daily for 3 days with vehicle control, one of four concentrations of test article (0.1, 1, 10, or 100 $\mu\text{mol l}^{-1}$ pridopidine or 0.05, 0.5, 5 or 50 $\mu\text{mol l}^{-1}$ TV-45065), or one of three known human CYP enzyme inducers: omeprazole (100 $\mu\text{mol l}^{-1}$), phenobarbital (750 $\mu\text{mol l}^{-1}$) or rifampicin (10 $\mu\text{mol l}^{-1}$).

Approximately 24 h following the final treatment, microsomes were isolated [22], protein levels were quantified using BCA (bicinchoninic acid) methodology [25], and CYP activity was assessed as described above, except that protein concentrations ranged from 0.020 to 0.1 mg ml^{-1} and reactions were allowed to proceed for 10 or 30 min. RNA was isolated and purified, and its integrity and levels were determined. Quantitative RT-PCR was performed in triplicate. Relative quantity of target cDNA was compared to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) cDNA using the $\Delta\Delta\text{Ct}$ method. Further details can be found in the Supplementary Appendix.

Data analysis. Where possible, IC_{50} calculations were performed using nonlinear regression (per the Levenberg–Marquardt algorithm) and K_i values were determined by processing data using a LIMS (including Galileo version 3.3, Thermo Fisher Scientific Inc., and reporting tool, Crystal Reports 2008, SAP). The entire data set (i.e., reaction rates at all concentrations of pridopidine and TV-45065, at all marker substrate concentrations) was fit with Michaelis–Menten equations for competitive, noncompetitive, uncompetitive and mixed (competitive-noncompetitive) inhibition by nonlinear regression analysis. The goodness of fit to each inhibition model was indicated by a lower Akaike information criterion value, which provided an initial basis for selection of the type of inhibition.

Induction was evaluated through folds increase of relevant CYP isoform activity in separated HLM, and through increase in relevant CYP isoform mRNA levels following incubation of the hepatocytes with pridopidine or TV-45065 compared to vehicle control and with comparison to a relevant prototypical inducer positive control, where applicable.

Clinical evaluation

Study design. The phase-1 clinical study was conducted at the Early Phase Clinical Unit of Parexel, International GmbH Berlin, Germany, and was approved by the responsible Independent Ethics Committee (Landesamt für Gesundheit und Soziales, Ethik-Kommission Berlin, Germany) and relevant Competent Authority (BfArM, Bundesinstitut für Arzneimittel und Medizinprodukte, Germany). All subjects signed informed consent, and the study was conducted in compliance with International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki.

A total of 22 healthy subjects (11 male and 11 female; age 18–65 years) participated in the study. The subjects were genotyped to exclude poor metabolizers for CYP2D6. The following alleles were analysed: *2, *3, *4, *5, *6, *7, *8, *9, *10, *35, *41 and *MxN; and poor metabolizer genotype was assigned if the subject had two nonfunctional CYP2D6 alleles.

Other standard inclusion/exclusion criteria for clinical pharmacology studies in healthy volunteers were applied, such as limitations on smoking (<10 cigarettes/day), the presence or history of any clinically significant diseases known to interfere with absorption, distribution, metabolism or excretion of drugs as judged by the investigator, and prohibition of concomitant medications other than acetaminophen and those used to treat an adverse event.

The randomized open-label study consisted of a single-sequence crossover drug–drug interaction (DDI) evaluation and a randomized crossover food effect (FE) assessment in three periods. As described below and in Figure 1, the DDI evaluation single-sequence part was carried out in Period 1 (metoprolol alone) and Period 3 (metoprolol with steady-state pridopidine) while the FE assessment was conducted in Period 2 and 3 when pridopidine was administered as a single

dose. Given the time-dependent inhibition of CYP2D6, and pridopidine being a substrate of CYP2D6, it was deemed more clinically relevant for the assessment to occur when pridopidine was at steady-state.

Use of prescribed medication or over-the-counter (OTC) medication within 2 weeks prior to dosing, or use of any drug or substance that was known to induce or inhibit metoprolol for 30 days or 5 half-lives (whichever was longer) prior to admission (Period 1, Day –1), except for paracetamol was excluded. Inhibitors and inducers of CYP2D6, antidepressant and medication known for causing significant QT-prolongation such as antiarrhythmic drugs class Ia and II were not allowed within 30 days prior to admission to the clinic.

Xanthine-containing products, alcohol or alcohol-containing products were restricted from 2 days prior to admission until end of treatment phase. Grapefruit or grapefruit-juice-containing products (including Seville oranges, bitter oranges and pomelos) were excluded from 7 days prior to admission (Period 1, Day –1) until end of treatment phase.

Screening took place within 28 days prior to dosing of metoprolol in Period 1. Subjects were housed in the clinic for the duration of the study (Periods 1–3) apart from days 4–7 of Period 3. During Period 1, 100 mg metoprolol [26] was given as a single oral dose on Day 1 after an overnight fast, and PK samples for metoprolol concentration were collected from predose up to 24 h postdose. After 3 days of washout, subjects were randomly assigned to receive a single 90-mg oral dose of pridopidine (two capsules of 45 mg) under fed or fasted conditions, with PK samples being collected from predose up to 48 h postdose. The composition of the test meal, which was given 30 min prior to dosing, was per FDA Guidance on Food-Effect Bioavailability and Fed Bioequivalence Studies [27] (high-fat, high-calorie).

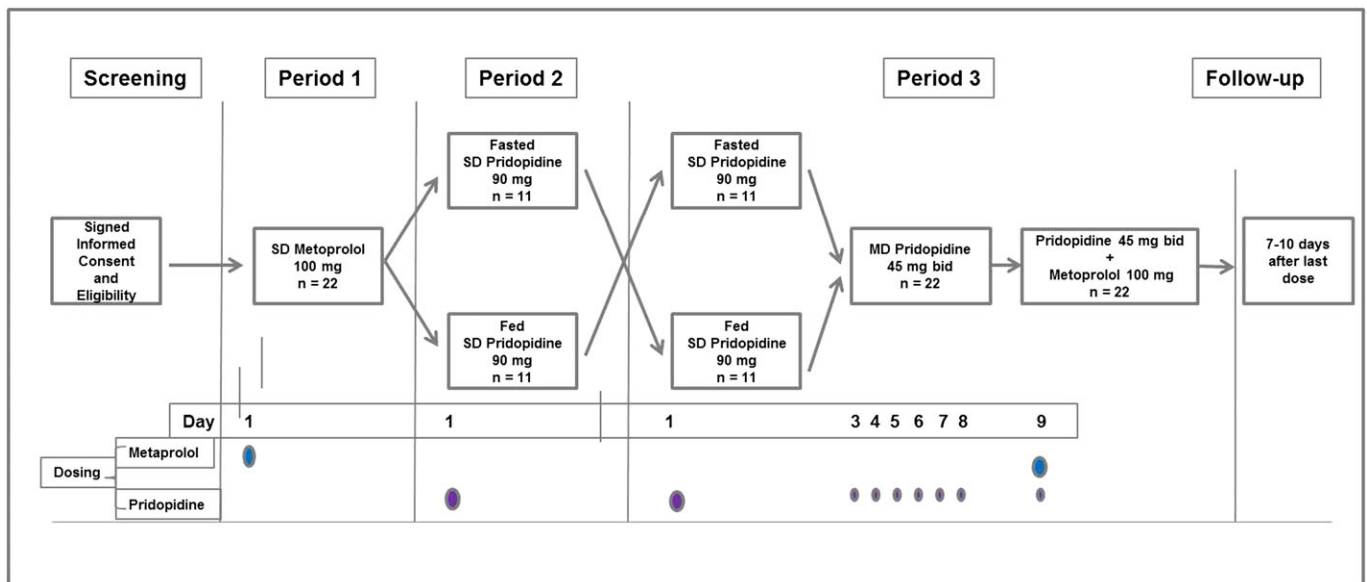


Figure 1
Study design

After an additional 4 days of washout and on Day 1 of Period 3, a second single 90-mg oral dose of pridopidine was administered under fed or fasted conditions (crossover with FE Period 2). Starting on day 3 of Period 3, multiple doses of 45 mg pridopidine were administered twice daily (BID) for 7 days. A 6-h period separated the morning and afternoon doses of pridopidine, similar to the dosing instruction given to patients with HD. Trough PK samples were collected on Days 6–8 to confirm attainment of steady state. On the last day of dosing (Day 9 Period 3), 100 mg metoprolol was administered with the afternoon dose of pridopidine, 6 h after the morning pridopidine dose. PK sampling for pridopidine and metoprolol were collected predose and at multiple times through 66 h postdose. A follow-up visit was conducted 5–10 days after the last dose of study drug.

Safety of study subjects was assessed by physical examinations, laboratory parameters (clinical chemistry, haematology including coagulation and urinalysis), vital signs and electrocardiograms (ECGs), concomitant medications, and adverse events monitoring. ECGs were obtained on Day 1 of each treatment period and on Day 9 of Period 3 before the morning dose, and at 1, 2 and 12 h postdose. On all other days except baseline (Day –1 of each period) ECGs were obtained before the morning dose.

Bioanalytical methods. Pridopidine, TV-45065 and metoprolol concentrations in plasma were determined using validated LC–MS/MS methods. Pridopidine, TV-45065, and their respective internal standards were extracted from human EDTA plasma by solid phase extraction using Evolute CX cartridges and 1.25% ammonia in methanol–water (95:5, v/v) elution. Metoprolol and its d_7 internal standard were extracted from EDTA plasma by liquid–liquid extraction into tert-butyl-methyl-ether at alkaline pH. Following extraction samples were injected into a liquid chromatograph equipped with a tandem mass spectrometry detector. Separations were performed on a reversed-phase column (XBridge C₁₈, 50 × 2.1 mm ID, 5 μm, Waters) with a mobile phase of 60% water with 0.05% ammonia and 40% acetonitrile. Calibration range was 1.41–1400 ng ml⁻¹ for pridopidine, 0.240–120 ng ml⁻¹ for TV-45065 and 0.300–300 ng ml⁻¹ for metoprolol. The assay passed linearity for pridopidine ($r = 0.9996$), TV-45065 ($r = 0.9991$) and metoprolol ($r = 0.9991$) over each of the calibration ranges tested. Mean accuracy values for pridopidine, TV-45065 and metoprolol were ±5%, ±8.6% ±3.6%, respectively. Selectivity was confirmed and no interference of any of the analytes of any of the other analytes was observed.

PK analysis. PK parameters were calculated by standard noncompartmental methods using Phoenix WinNonlin version 5.2 (Pharsight, St Louis, MO, USA). Concentrations below the LLOQ were set to zero when preceding the first measurable concentration and set to LLOQ/2 when between measurable samples and excluded following the last quantifiable sample. The terminal elimination rate constant (λ_z) was estimated by linear regression of logarithmically-transformed concentration–time data. Terminal elimination $t_{1/2}$ was calculated as $\ln(2)/\lambda_z$. The maximum observed plasma concentration (C_{max}) and time to reach C_{max} (t_{max})

were obtained directly from the concentration–time data. Area under the plasma concentration–time curve from time 0 to the time t of the last quantifiable concentration (AUC_{0-last}) was calculated by means of the mixed log-linear trapezoidal rule. The AUC from time zero to infinity ($AUC_{0-\infty}$) after a single dose was calculated as the sum of AUC_{0-last} and AUC_{extrap} where AUC_{extrap} is C_{last}/λ_z . The area under the plasma concentration–time curve from time zero to the end of the dosing interval, τ , for the morning and evening dose (AUC_{AM} , AUC_{PM}) was calculated by means of the mixed log-linear trapezoidal rule. As the afternoon dose of pridopidine was given 6 h after the morning dose of pridopidine, the AUC intervals from the timing of the morning dose were 6, 18 or 24 h. The steady-state equilibrium ratio was calculated as $AUC_{0-24PM,ss}/AUC_{0-\infty,ss}$. The accumulation ratio (R_{acc}) was calculated as $AUC_{0-24PM,ss} / AUC_{0-24,ss}$.

Statistical analysis. The drug interaction between pridopidine and metoprolol was evaluated according to guidelines [28–30]. The point estimate and 90% confidence interval (CI) for the ratio of geometric means of AUC_{0-last} , $AUC_{0-\infty}$ and C_{max} of metoprolol (with or without pridopidine) were compared to the bioequivalence range of 0.80–1.25. The primary endpoints were calculated with a linear model on the log-transformed parameters including sequence, treatment and period as fixed effects and subject (sequence) as random effect. As a secondary analysis, t_{max} and $t_{1/2}$ of metoprolol were analysed for differences by calculating the Hodges–Lehmann estimator for the median difference together with the corresponding exact 90% CI for small sample sizes.

To evaluate the FE, AUC_{0-last} , $AUC_{0-\infty}$ and C_{max} of pridopidine were log-transformed and analysed for differences between treatments (fed vs. fasted) using an analysis of variance model including period (Periods 2 and 3), treatment and sequence as fixed effects and subjects within sequence as random effect. The primary endpoints ($AUC_{0-\infty}$ and C_{max}) and secondary endpoint (t_{max}) were analysed as described above for the DDI analysis. Additionally, $AUC_{0-\infty}$ of Day 1 in fasting condition pooled vs. AUC_{0-24} of Day 9 in fasting condition (Period 3), were compared to examine PK linearity at steady-state equilibrium using 90% confidence intervals constructed for the ratio of the geometric means.

Results

In vitro results

Evaluation of pridopidine and TV-45065 as CYP inhibitors.

Pridopidine did not show any direct or time- (or metabolism-) dependent inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP3A4/5 when investigated in HLM at concentrations of 0–500 μmol l⁻¹ (Supplemental Table S1). Weak direct inhibition was observed towards CYP2D6. Further investigation revealed pridopidine as a competitive inhibitor of CYP2D6 with a K_i of 33 μmol l⁻¹ (Figure 2).

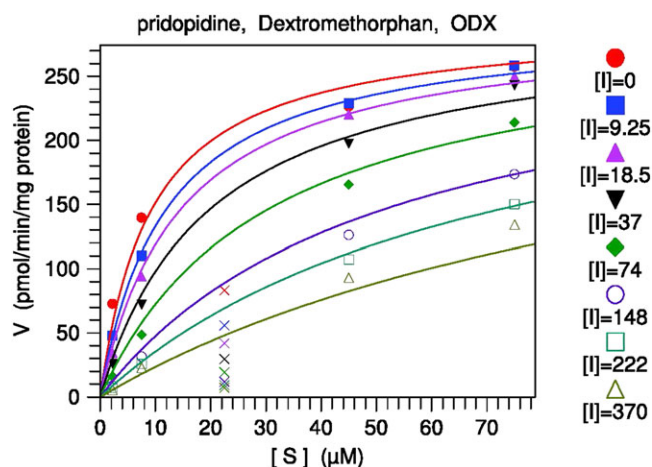


Figure 2

Inhibition of CYP2D6 by pridopidine: K_i determination

Very weak metabolism-dependent inhibition (NADPH-dependent) was observed after a 30-min preincubation (less than a 1.3-fold decrease in IC_{50} values). The time-/metabolism-dependent inhibition of CYP2D6 was further investigated in human liver microsomes with preincubation times of 2, 3 and 4 h. After 4 h of preincubation with NADPH, an approximate 14-fold shift was observed in the IC_{50} value, indicating metabolism-dependent inhibition. In agreement with the inhibition experiments described above, treatment of hepatocyte cultures with up to $100 \mu\text{mol l}^{-1}$ pridopidine resulted in a statistically significant, concentration-dependent decrease in CYP2D6 activity of up to 64.2% when compared to the vehicle control (0.1% DMSO). The metabolism-dependent inhibition of CYP2D6 was not reversed with microsomal re-isolation or by the addition of potassium ferricyanide, suggesting that pridopidine is an irreversible metabolism-dependent inhibitor of CYP2D6. In addition, heat treatment of the human liver microsomes to inactivate FMO did not affect pridopidine CYP2D6 MDI extent, suggesting that FMO was not involved in the metabolic conversion resulting in the observed metabolism-dependent inhibition (Table 1).

Table 1

In vitro evaluation of pridopidine drug–drug interaction liability – metabolism-dependent inhibition of CYP2D6 dextromethorphan O-demethylation in human liver microsomes

Preincubation time (h)	Direct inhibition (no preincubation) IC_{50} ($\mu\text{mol l}^{-1}$)	Time-dependent inhibition (preincubation without NADPH) IC_{50} ($\mu\text{mol l}^{-1}$)	Metabolism-dependent inhibition (preincubation with NADPH) IC_{50} ($\mu\text{mol l}^{-1}$)
2	39	36	5.5
2 ^a	35	33	6.1
3	39	44	3.9
4	39	42	2.8

^aMicrosomes were heat treated to inactivate flavin-containing monooxygenase

The main metabolite of pridopidine, TV-45065, did not show any direct inhibition of CYP1A2, CYP2C8/9, CYP2C19, CYP2D6 or CYP3A4. However, time-/metabolism-dependent inhibition of CYP2D6 was also observed for TV-45065, although the IC_{50} value was above $50 \mu\text{mol l}^{-1}$, suggesting that TV-45065 is unlikely to be directly responsible for the observed MDI. Irreversible inhibition parameters, K_{inact} and K_i , could not be determined, due to the *in vitro* instability of CYP2D6 in the presence of its probe substrate (data not shown).

Evaluation of pridopidine and TV-45065 as CYP inducers.

Treatment with up to $100 \mu\text{mol l}^{-1}$ pridopidine caused little (<2-fold change) or no increase in CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 mRNA levels (data not shown) in any of the three tested hepatocyte cultures. In addition, treatment of cultured human hepatocytes with up to $100 \mu\text{mol l}^{-1}$ pridopidine had little (less than 2-fold change) or no effect on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP3A4/5 activity (data not shown).

Overall, treatment of cultured human hepatocytes with up to $50 \mu\text{mol l}^{-1}$ TV-45065 for three consecutive days caused little or no effect on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP3A4/5 activity, or mRNA expression (on average, ≤ 2.0 -fold increase and $\leq 20\%$ as effective as the respective positive control CYP inducers).

In vivo results

Subject disposition and demographics. A total of 11 females and 11 males of Caucasian ethnicity were enrolled in the study. Average demographic characteristics of subjects were as follows: mean (SD) age 51.3 (6.7) years, height 172 (10.8) cm, weight 74.3 (11.8) kg and body mass index 25.0 (2.72) kg/m^2 . Of the enrolled subjects, safety assessments were completed for all subjects, and PK was assessed for 21 subjects, as one subject withdrew on Day 15 from the study after breaking her arm, which was not considered study drug-related.

Safety. Study treatments were well tolerated in general, apart from minor deviations from normal laboratory ranges, there

were no findings in haematology, clinical chemistry, coagulation or urinalysis that constituted an adverse effect (AE). An increase in pulse rate by up to 12 beats min⁻¹ was observed in subjects treated with pridopidine 45 mg BID. No clinically significant changes in remaining vital signs, 12-lead ECG parameters or physical examination were observed. There was a total of 40 recorded AEs in 15 subjects (68.2%) in this study, six AEs during the food-effect portion and 34 AEs during the DDI portion of the study (Supplement Table S3). All AEs were of mild (*n* = 35) or moderate (*n* = 5) intensity. The most frequent AE in the DDI single-sequence part was *feeling hot* (*n* = 8; 36.4%) during the coadministration of metoprolol and pridopidine, followed by nausea (18.2%) during the period of BID dosing of pridopidine. It is noteworthy that amnesic aphasia was

reported by two subjects (9.1%) following BID administration of pridopidine. These events occurred on the 3rd and 6th day of Period 3, respectively. In the FE crossover part, AEs occurred only as single incidences; therefore, a most frequent AE could not be specified.

Impact of pridopidine on metoprolol pharmacokinetics. The plasma concentration–time plots of metoprolol alone and under coadministration of pridopidine at steady state showed that the exposure to metoprolol was larger when it was coadministered with pridopidine (Figure 3). The exposure to metoprolol was markedly increased when coadministered with pridopidine as shown by the 3.5-fold increase in mean *C*_{max}, 6.5-fold increase in mean *AUC*_{0–last} and 5.1-fold increase in mean *AUC*_{0–∞}. As shown in Table 2, the geometric mean ratio and 90% CIs entirely exceeded the upper no-effect boundary of 1.25 for *C*_{max} and *AUC*. The *t*_{max} (approximately 2 h) values were comparable between the two periods; however, the range of *t*_{max} was longer during the coadministration with pridopidine (1–4 h) compared to metoprolol alone (0.75–2 h). This difference is not considered to be of clinical relevance. The median *t*_{1/2} was prolonged from 3.7 to 5.6 h when metoprolol was coadministered with pridopidine, which is consistent with the inhibition of CYP2D6 by pridopidine.

Pridopidine pharmacokinetics. The plasma concentration-*vs.*-time plot of pridopidine is shown in Figure 4. The PK parameters resulting from the morning and afternoon pridopidine dosing at steady state are shown in Table 3. As expected, the mean *C*_{max,ss}, *C*_{avg,ss} and *AUC* were lower after the morning dose than following the afternoon dose. The median *t*_{max,ss} was approximately 1.5–2 h after each dose.

Exploratory examination of morning trough plasma levels during the multiple dosing part of period 3 indicates that steady state of pridopidine was reached after 7 days of BID dosing, which was prior to coadministration of metoprolol.

Impact of food on the pharmacokinetics of pridopidine. The plasma concentration–time plots of pridopidine showed approximately similar curves under both conditions with

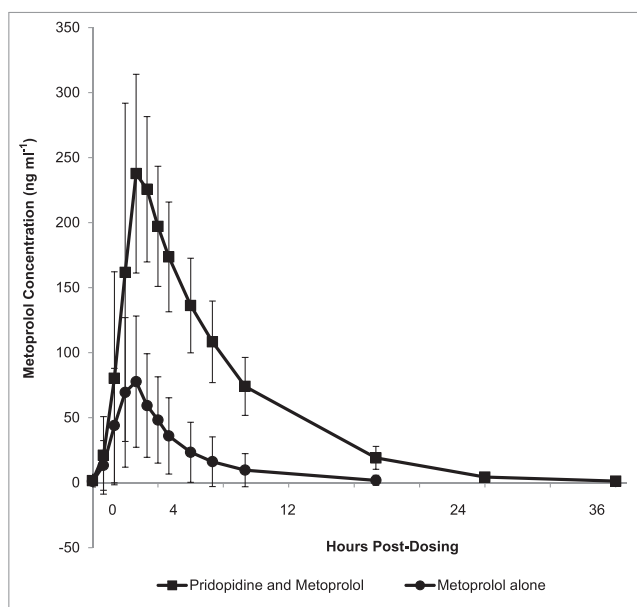


Figure 3 Mean (SD) plasma concentrations of metoprolol with and without pridopidine

Table 2

Pharmacokinetic parameters of metoprolol administered with and without pridopidine at steady state

	Mean (SD) ^a		Least square mean ^b		
	metoprolol alone	Metoprolol + pridopidine	geometric mean ratio	90% CI lower	90% CI upper
<i>C</i>_{max} (ng ml⁻¹)	84.0 (53.0)	257 (80.0)	3.50	2.90	4.22
<i>AUC</i>_{0–last} (ng·h ml⁻¹)	447 (400)	2330.52 (662)	6.64	5.27	8.38
<i>AUC</i>_{0–∞} (ng h ml⁻¹)	460 (429)	2340 (665)	6.55	5.18	8.28
<i>t</i>_{max} (h)	1.98 (0.75–2.03)	2.00 (1.00–4.00)	NC	NC	NC
<i>t</i>_{1/2} (h)	3.69 (0.83)	5.60 (0.87)	NC	NC	NC

^aMedian (range) reported for metoprolol *t*_{max}.

^bCalculated using a linear model of the log-transformed parameters including sequence, treatment and period as fixed effects and subject (sequence) as a random effect. The 90% CIs for the mean differences in log scale were then re-transformed to obtain 90% CIs for the geometric mean ratios. CI, confidence interval; NC, not calculated; SD, standard deviation

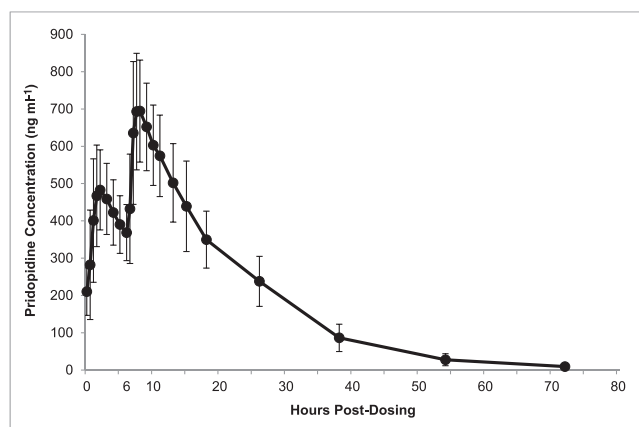


Figure 4

Mean (standard deviation) pridopidine concentrations after 7 days of dosing at 45 mg twice daily. [†]Pridopidine doses 45 mg were administered twice daily 6 h apart (AM and PM doses) on days 3–10. PK sampling for pridopidine and TV-45065 began on day 7 immediately after the AM dose and continued for 72 h

the exception that the peak of the mean concentrations in the fed state was delayed and slightly reduced compared to the fasted state (Figure 5 and Table 4). The 90% CIs of pridopidine C_{max} and AUC were entirely within the bioequivalence boundaries.

The absorption appeared to be delayed in the fed state as the median t_{max} showed a difference of 1.5 h in comparison to the fasted state. TV-45065 C_{max} and AUC were similar between fed and fasted states with 90% CIs also entirely within the bioequivalence boundaries (data not shown).

Discussion

Of the CYP enzymes tested *in vitro*, pridopidine showed inhibitory potential only for CYP2D6. The direct,

competitive inhibitory potential (K_i) of pridopidine ($33 \mu\text{mol l}^{-1}$ or 9300 ng ml^{-1}) was roughly 30-fold higher than mean peak concentrations of pridopidine in plasma following a single 45-mg dose (303 ng ml^{-1}) and roughly 15-fold higher than mean peak concentrations of pridopidine in plasma at steady state following 14 days of 45 mg BID dosing (620 ng ml^{-1}) as reported in a previous study of pridopidine [16]. Since the I/K_i ratio is smaller than the conservative threshold of 0.1, a significant interaction would not be expected solely due to reversible, competitive inhibition of CYP2D6 [28]. However, the irreversible metabolism-dependent inhibition of CYP2D6 seen in both microsomes and hepatocytes provides evidence that pridopidine could cause CYP2D6-related interactions through mechanisms other than competitive inhibition.

To test the interaction potential in a clinically relevant dosing regimen, pridopidine was dosed to steady state, and the FDA-recommended CYP2D6 probe substrate, metoprolol, was coadministered. Pridopidine caused statistically significant and clinically relevant increases in metoprolol AUC (up to 5-fold) and peak concentrations (up to 3-fold), and prolonged the elimination phase, indicating that pridopidine is a strong inhibitor of CYP2D6 activity *in vivo*. As steady-state pridopidine concentrations are reached, pridopidine inhibits CYP2D6, which in turn reduces its own hepatic clearance pathway leaving renal excretion as the primary route of elimination [31]. The pridopidine $\text{AUC}_{0-24,ss}$ calculated in this study is consistent with those reported by Lindskov [16] for CYP2D6 extensive metabolizers and CYP2D6 poor metabolizers, indicating that the auto-inhibition of CYP2D6 acts as a source of phenoconversion. This finding is supported by the 2-fold ratio of AUC_{0-24PM} of the 45-mg pridopidine dose at steady state to the $\text{AUC}_{0-\infty}$ of the 90-mg single dose of pridopidine which would have been expected to be 0.5, if pridopidine displayed linear kinetics. In essence, multiple doses of pridopidine decrease the activity of CYP2D6 in extensive metabolizers into the range of CYP2D6 poor metabolizers.

Table 3

Pharmacokinetic parameters of steady state pridopidine

	Mean (SD) ^a		
	AM (0–6 h)	PM ^c (6–18 h)	AM + PM (0–24 h)
$C_{max,ss}$ (ng ml ⁻¹)	525.40 (121.78)	727.30 (152.38)	
AUC_{ss} ^b (ng h ml ⁻¹)	2443.75 (544.95)	8052.03 (1659.34)	10495.78 (2184.34)
$C_{av,ss}$	407.29 (90.82)	447.33 (92.19)	
t_{max} (h)	1.93 (0.50–3.02)	1.50 (1.00–5.00)	
R_{acc} ^c		2.39 (0.51)	
R_{ss} ^d		2.10 (1.90–2.32)	

^aMedian (range) reported for t_{max} .

^b AUC_{ss} calculated as AUC_{0-6} following the morning dose, AUC_{0-18} following the afternoon dose and AUC_{0-24} following both AM + PM doses.

^cCalculated as $\text{AUC}_{0-24 PM,ss} / \text{AUC}_{0-24}$ after a single 90 mg pridopidine dose in fasted state.

^dCalculated as $\text{AUC}_{0-24 PM,ss} / \text{AUC}_{0-\infty}$ after a single 90 mg pridopidine dose in fasted state, reported as GMR (90% CI).

^eMetoprolol administered with PM pridopidine dose.

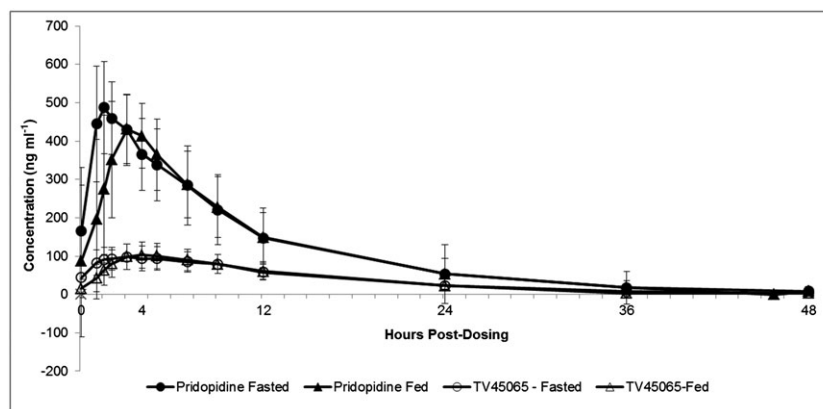


Figure 5

Mean plasma concentrations of pridopidine and TV-45065 with and without food

Table 4

Pridopidine exposure following administration of a single 90-mg pridopidine dose in the fasted and fed states

	Mean (SD) ^a		Least square mean ^b		
	Fasted	Fed	Geometric mean ratio	90% CI lower	90% CI upper
C_{max} (ng ml⁻¹)	526.50 (104)	473.80 (117)	0.89	0.8	0.95
AUC_{0-last} (ng h ml⁻¹)	5152.51 (2093)	4929.35 (1787)	0.96	0.89	1.04
AUC_{0-∞} (ng h ml⁻¹)	5268.35 (2287)	5036.81 (1904)	0.96	0.89	1.04
t_{max} (h)	1.50 (0.98–3.0)	3.00 (0.5–5.0)	NC	NC	NC
t_{1/2} (h)	7.6 (1.9)	7.7 (1.7)	NC	NC	NC

^aMedian (range) reported for pridopidine t_{max} and t_{1/2}.

^bCalculated using a linear model of the log-transformed parameters including sequence, treatment and period as fixed effects and subject (sequence) as a random effect. The 90% CIs for the mean differences in log scale were then re-transformed to obtain 90% CIs for the geometric mean ratios. CI, confidence interval; NC, not calculated

The results of this study are important for treatment of HD patients as coadministration of pridopidine once approved with other CYP2D6 substrates may result in clinically significant DDIs. Specifically, systemic levels of coadministered CYP2D6 substrates may increase with chronic dosing of pridopidine and approaches those observed in CYP2D6 poor metabolizers. Myriad CNS-active drugs, including multiple drugs used concomitantly in patients with HD (e.g. risperidone, sertraline, paroxetine), are also metabolized by CYP2D6 [32, 33]. Adequate clinical monitoring should therefore be exercised when pridopidine is coadministered with other CYP2D6 substrates.

The FE portion of this study demonstrated that the plasma concentration profiles of pridopidine meet bioequivalence criteria between the fed and fasted states, indicating that there is no significant FE and that pridopidine can be administered with or without meals. While the C_{max} and AUC values were similar, the t_{max} was slightly delayed as expected due to the slower gastric emptying rate under fed conditions [34].

In summary, the results from this study provide valuable data on factors that may impact dosing of medications metabolized by CYP2D6 when concomitantly administered with pridopidine.

Competing Interests

L.R.-G., L.S., H.H., G.P. and O.S. are employees of Teva Pharmaceutical Industries, who are sponsoring the clinical development of pridopidine for the treatment of Huntington's disease. P.M. was employed by NeuroSearch, the company that sponsored pridopidine development before Teva and is currently a full-time employee of UCB Pharma Belgium.

The *in vitro* studies were sponsored by Neurosearch A/S and conducted by Xenotech LLC (Lenexa KS USA). The *in vivo* phase 1 study was sponsored by Neurosearch A/S and conducted by Parexel International GmbH Berlin Germany. The authors thank

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Contributors

Neurosearch A/S (author P.M.) sponsored the trial, participated in the interpretation of trial data and reviewed the manuscript. Data evaluation and manuscript writing was performed by L.R.-G., L.S., H.H., G.P., and O.S.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

<http://onlinelibrary.wiley.com/doi/10.1111/bcp.13317/supinfo>

Table S1 Summary of assay conditions to determine *in vitro* CYP enzyme activity in human liver microsomes

Table S2 *In vitro* evaluation of pridopidine drug–drug interaction liability initial screen in human liver microsomes

Table S3 Adverse events