ABSTRACT:
The present clinical trial was designed to evaluate the possible pharmacokinetic and electrocardiographic interactions of the gastroenteric prokinetic drug cinitapride with ketoconazole. The safety and tolerability of the study treatments were also evaluated. After a placebo-controlled, double-blind, crossover design, 16 healthy male (n = 8) and female (n = 8) volunteers were randomized into four treatment groups of four subjects (two males and two females): cinitapride (CTP; 1 mg t.i.d.) + ketoconazole (KET; 200 mg b.i.d.), CTP + placebo (PL), PL + KET, and PL + PL. Treatments were given for 7 days with a washout period of 14 days between crossover treatments. Cinitapride is rapidly absorbed after oral administration and is metabolized by the cytochrome P450 CYP3A4 and CYP2C8 isozymes. At steady state, coadministration with ketoconazole, a potent CYP3A4 inhibitor, increased mean Cmax,ss and AUC, by 1.63- and 1.98-fold, respectively. Measurement of mean QTc interval or baseline-corrected QTc intervals on day 7 showed small increases that were due to the effects of ketoconazole alone. Comparing CTP + KET versus PL + KET, the differences between mean increases in the QTc parameters were always less than 2 ms. Finally, no outlier increase of the QTc interval versus baseline >60 ms was identified after any treatment. The study showed that cinitapride, either given alone or after coadministration with ketoconazole 200 mg b.i.d., had no effect on cardiac repolarization as measured by changes in the heart rate-corrected QT interval on the surface electrocardiogram.

Cinitapride is a substituted benzamide gastroenteric prokinetic agent acting via complex, but synergistic effects on serotonergic 5-HT2 (inhibition) and 5-HT4 (stimulation) receptor and dopaminergic D2 (inhibition) receptors in the neuronal synapses of the myenteric plexus (Roberts, 1982; Fernandez and Massingham, 1985; Massingham et al., 1985). The pharmacology, pharmacokinetics, toxicology, and clinical profile of cinitapride have been reviewed (Fernandez and Roberts, 1991).

Cinitapride has been marketed in Spain under the trade names Cidina and Blaston since November 1990, and is available in Mexico under the name Pemix. The current indications include gastroesophageal reflux and functional disorders in gastrointestinal motility (defined as dyspepsia (35%), and gastroesophageal reflex and hiatus hernia (28%). The incidence of side effects with the dosage regimen of 1 mg t.i.d. during almost 15 years of clinical experience has been very low and, in comparative studies, not much different from that seen with placebo.

The original pharmacokinetic studies in human after oral and intramuscular administration were performed using doses substantially higher than the therapeutic dose due to the absence, at the time, of a sufficiently sensitive analytical method for the detection of plasma concentrations after very low doses of cinitapride. The absorption of cinitapride was rapid with peak levels being achieved 2 h after oral dosing (12 mg) and 1 h after subcutaneous administration (4 mg). The elimination profile was similar by either route of administration, with a half-life of some 3 to 5 h during the first 8 h and a residual half-life greater than 15 h thereafter. The plasma levels during the slow phase were negligible and the overall pharmacokinetic profile was indicative of a 3 times daily dosing being the most appropriate. In the present study, the pharmacokinetics after single and multiple doses of cinitapride at 1 mg t.i.d. is described. Plasma concentrations of cinitapride were determined by liquid chromatography-tandem mass spectrometry.
try (MS/MS) with a limit of quantification of 0.1 ng/ml that allowed an appropriate characterization of the pharmacokinetic profile of cinitapride at the therapeutic dose in human for the first time.

Additional in vitro studies with human subcellular fractions and recombinant cytochrome P450 enzymes established that cinitapride was heavily metabolized to up to 15 different metabolites and that there were four major routes of biotransformation including an oxidative N-dealkylation pathway occurring on the nitrogen atom of the piperidine ring, three monohydroxylation reactions occurring on the piperidine and substituted cyclohexyl rings, a dihydroxylated metabolite issued from the oxidation of the double bond of the cyclohexenyl ring, and a less polar compound that was attributed to an N-oxide formed on the nitrogen atom of the piperidine ring. These reactions were shown to be mainly mediated by CYP3A4 and CYP2C8 (Alberti et al., 2000). Cytochrome P4502C8 seems to be an enzyme important to cinitapride metabolism and has been a relatively neglected member of the CYP2C family, considering the number of data in the literature until 2003 (Totah and Rettie, 2005).

In view of the fact that another gastroprokinetic drug, cisapride, was recently withdrawn from many markets because of its association with the induction of the rare, but sometimes fatal, polymorphic ventricular tachyarrhythmia known as torsade de pointes (Tonini et al., 1999), it was considered prudent to investigate the electrocardiographic effects of cinitapride under conditions known to favor arrhythmogenesis with cisapride. Such conditions are those where the metabolism of cisapride is inhibited, especially by coadministration with drugs blocking the cytochrome P450 CYP3A4 isoform, such as macrolide antibiotics and antifungal azoles (Michalets and Williams, 2000; Jones et al., 2001). Under these circumstances, the metabolism of cisapride is prevented and the drug accumulates in plasma leading to concentrations sufficient to block the rapid component of the delayed rectifier potassium current passing through the cardiac hERG channel (Rampe et al., 1997). This results in a prolongation of the repolarization phase of the cardiac action potential (measured as a prolongation of the heart rate-corrected QTc interval of the surface electrocardiogram), which in turn is thought to facilitate reentry phenomenon and early after depolarization (Puisieux et al., 1996) favoring the induction of torsade de pointes arrhythmias.

In fact, according to a recent review of the relationships between preclinical electrophysiology, clinical QT interval prolongation, and torsade de pointes for some 52 diverse drugs (Redfern et al., 2003), therapeutic doses of cisapride already produce free (unbound) plasma concentrations close to those that block the hERG potassium currents and prolong the action potential and QTc interval in some nonclinical experimental models, and even in the absence of metabolic inhibition. By contrast, the concentration of cinitapride required to cause a 50% block of the hERG channel is several thousand times higher than the effective free plasma concentrations attained during clinical use (Crumb, 2000).

Also, unlike cisapride (Bohets et al., 2000; Desta et al., 2000), cinitapride is metabolized not only by the CYP3A4 isofrom of cytochrome P450, but also by CYP2C8 (Alberti et al., 2000), as well as by other phase II enzymes (De Graeve, 2001) that could eventually decrease the risk of drug-drug interactions, even with potent inhibitors of CYP3A4 such as ketoconazole. Nevertheless, in the absence of specific data to confirm this expectation, the present study was designed to compare the multiple dose pharmacokinetics and electrocardiographic effects (heart rate-corrected QTc interval) of cinitapride (1 mg t.i.d.) alone and coadministered with ketoconazole (200 mg b.i.d.). The antifungal ketoconazole used in this study is a very potent in vitro and in vivo inhibitor of CYP3A4 and a weak inhibitor of CYP2C8 (Park et al., 2004).

The primary objective of this investigation was the assessment of the pharmacokinetics after single and multiple doses of cinitapride at the therapeutic dose (1 mg t.i.d) and the pharmacokinetic interaction of cinitapride coadministered with ketoconazole. Another important objective was to evaluate the safety and tolerability of the study treatments, particularly the effects on the QT interval.

### Materials and Methods

**Ethics.** Written notification of approval was obtained from the Independent Ethics Committee and given to the investigator before starting the study, which was performed according to the regulations of the German Medicines Act, the directives of the Declaration of Helsinki for biomedical research in humans, revised version of Edinburgh (Scotland, October 2000), and the announcements for the Principles for Correct Implementation of Clinical Trials of the International Conference on Harmonisation-Good Clinical Practice Guidelines.

Before enrollment, all subjects were comprehensively informed about the trial (procedures, pharmacological effects, adverse events, consequences, risks, and hazards) and about their right to withdraw at any time without specifying reasons. All subjects gave their written informed consent before participation.

**Study Design.** The study was a phase I, single center, randomized, placebo-controlled, double-blind, double-dummy, multiple-dose, crossover clinical trial with four treatments, periods, and sequences. William’s design (Jones and Kenward, 1989) was used for the assignment of treatment sequences.

**Subjects.** Sixteen healthy male (n = 8) and female (n = 8) subjects, complying with the inclusion criteria (age 18–50 years; body mass index between 17 and 29.9 kg/m²) completed the study. At screening, subjects did not present any clinically significant abnormalities in physical examinations, vital signs, body temperature, ECGs (QTc Bazett <440 ms), and laboratory tests. Anthropometric data on subjects at screening are detailed in Table 1. The proportions of smokers, regular alcohol drinkers, and stimulant users were 59%, 65% and 34%, respectively.

**Treatment Plan.** Eligible subjects (16) were randomized into four groups of two males and two females to receive multiple doses of either cinitapride (CTP) + ketoconazole (KET), CTP + placebo (PL), PL + KET, or PL + PL for 7 days with a washout period of 14 days between treatments. All medications and placebo were produced by Laboratorios Almirall, S.A. The doses of cinitapride (1 mg t.i.d.) and ketoconazole (200 mg b.i.d.) were chosen based on the fact that 1 mg three times daily is the recommended therapeutic dose for cinitapride, and although 400 mg q.d. or 200 mg b.i.d. are the recommended maximum daily doses for ketoconazole, the higher single dose posology is not used clinically and has been shown to have, itself, a significant effect on the QT interval (Chaikin et al., 2005) that would confound the object of the study. In any event, the dose of 200 mg even once daily has been used for interaction studies with many other drugs including the classic study with terfenadine in which the maximum plasma concentrations of unchanged drug increased from undetectable (<5 ng/ml) to 25 to 55 ng/ml in four subjects and in one subject

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI (kg/m²)</th>
<th>QT (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 17)</td>
<td>34.7 ± 9.9</td>
<td>70.7 ± 17.7</td>
<td>1.72 ± 0.10</td>
<td>23.5 ± 3.9</td>
<td>390.5 ± 25.1</td>
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<tr>
<td>Males (n = 9)</td>
<td>32.2 ± 11.5</td>
<td>80.3 ± 16.7</td>
<td>1.80 ± 0.07</td>
<td>24.6 ± 3.9</td>
<td>396.5 ± 27.7</td>
</tr>
<tr>
<td>Females (n = 8)</td>
<td>37.5 ± 7.6</td>
<td>59.8 ± 11.8</td>
<td>1.64 ± 0.04</td>
<td>22.3 ± 3.8</td>
<td>385.2 ± 22.9</td>
</tr>
</tbody>
</table>

### Table 1

**Anthropometric parameters by individuals and baseline QT (mean ± S.D.)**
from 7 ng/ml to 81 ng/ml (Honig et al., 1993), indicating a high level of enzyme inhibition. The posology of 200 mg q.d. is still being used, as are those of 200 mg b.i.d. and 400 mg q.d., and both of these latter regimens may be expected to reach similar steady-state levels of enzyme inhibition. On day 1, a single oral dose of cinitapride was given to the volunteers to get information on the pharmacokinetics of a single oral dose never observed before, since the liquid chromatography methods used in the past to measure cinitapride could not detect the cinitapride in plasma compared with the liquid chromatography and tandem mass spectrometry method used in this study. Cinitapride was given as an oral solution, whereas ketoconazole and its placebo were administered as tablets. Five milliliters of cinitapride oral solution (0.2 mg/ml) followed by 50 ml of water were administered daily for 7 days at 8:00 AM (before breakfast), 4:00 PM, and 12:00 AM. On day 1, only the morning dose of cinitapride was administered. Ketoconazole tablets (or matching placebo tablets) were administered for 7 days at 10:15 AM (immediately after breakfast) and at 10:15 PM (immediately after supper); tablets were taken with 100 ml of water. Meals were served at 10:00 AM (breakfast), 2:00 PM (lunch), 6:00 PM (snack), and 10:00 PM (supper). The same schedule of meals was kept from day −2 to day 8, and in each study period, the daily meals were identical. Meals administered on study days 1 and 7 were identical.

The duration of 7 days for coadministration of cinitapride and ketoconazole was considered to be appropriate to attain sufficient enzyme inhibition, as well as steady-state pharmacokinetics for both drugs, and the 14-day washout was considered to be sufficient to ensure complete elimination of drugs and metabolites. No concomitant therapy was allowed during the study, except for treatment of adverse events.

Pharmacokinetics. Plasma samples. On days 1 and 7 of each study period, blood samples of 5 ml each were drawn by venous puncture or indwelling venous catheter and transferred into Li-heparinized Monovettes (Sarstedt AG & Co., Nümbrecht, Germany) at the following times: predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 23.5 h postdose. On days 2 to 6, a blood sample was taken before the first morning cinitapride dose. Blood samples were centrifuged at 3000g for 4°C for 10 min within 15 min of collection, and the plasma was separated and frozen at −20°C until analysis.

Drug assay. Plasma concentrations of cinitapride were determined by TNO BIBRA International Ltd. (Carshalton, Surrey, UK) using liquid chromatography-MS/MS. Plasma (0.5 ml) was aliquoted into 1.5-ml Eppendorf tubes and 25 μl (0.2 μg/ml) of the internal standard (clebopride; CAS registry 55905-53-8) were added to each tube, mixed, and centrifuged. The content of each tube was then transferred to a vial and injected onto a BioTrap 500 MS column (ChromTech Ltd., Congleton, Cheshire, UK) using 4% (v/v) 2-propanol in water containing 0.0125% ammonia at a flow rate of 3.2 ml/min as a loading solvent for 1.5 min. Cinnastrate and the internal standard were analyzed by back-flushing onto the analytical column (Hypersil BDS C18; 100/4.6 mm; 3 μm; Thermo Fisher Scientific, Runcorn, Cheshire, UK) using a mobile phase of acetate/trifluoracetic acid (buffer)/water (0.25% ammonia (55:45:5 v/v) at 1 ml/min. Under these conditions, the internal standard eluted at 3.0 to 3.2 min and cinitapride at 4.0 to 4.5 min. The BioTrap column was reequilibrated for 3.5 min before the next injection. Cinnastrate and internal standard were detected with a Micromass Quattro LC Quadrupole Mass Spectrometer (Waters, Milford, MA) equipped with electrospray ionization. The MS/MS transitions monitored using multiple reaction monitoring were 403.1 > 209.0 (cinnastrate) and 374.0 > 183.9 (internal standard). Quality control samples of 0.1, 2, and 80 ng/ml in triplicate were interspersed throughout each batch and provided the basis for accepting or rejecting the different analytical runs. The lower limit of quantification for this assay, determined in a previous method validation study, was 0.1 ng/ml using 0.5 ml of plasma. The mean values (±SD) for the quality controls (CV) for the quality controls this assay, determined in a previous method validation study, was 0.1 ng/ml (CV = 9.0%) (n = 37).

Determination of pharmacokinetic parameters. Individual plasma concentration-time data were used to calculate the pharmacokinetic parameters using a model-independent approach. The maximum concentration (Cmax) and time to Cmax (tmax) were taken from the observed values. The terminal elimination constant (λz) was estimated by linear least-squares regression analysis of the log-linear plot of plasma concentration-time data. The elimination half-life was obtained as t1/2 = ln 2/λz versus time for the terminal calculated using the linear trapezoidal method, and the elimination half-life (t1/2) was calculated from the elimination rate constant. AUC from 0 to 8 h postdosing (AUC0–8) was calculated using the linear trapezoidal rule. AUC to infinity (AUC) was only determined for day 1 using the elimination rate constant. On study day 7 the following parameters were also calculated: trough plasma concentration (Cmin,ss), average plasma concentration (Cavss = AUC/t), maximum plasma concentration (Cmax,ss), time to maximum steady-state concentration (tmax,ss), area under the concentration-time curve (AUC0–8), peak plasma concentration (Cmax), and accumulation ratio (Cmax0–8/Cmax). All pharmacokinetic parameters have been tabulated together with their descriptive statistics: mean ± standard deviation (S.D.), number of samples, and maximum and minimum values. The dosing interval t in this particular study was 8 h and, therefore, AUC0–8 is equal to AUC∞.

Pharmacodynamics. Serial 12-lead ECGs for pharmacodynamic evaluation were recorded from all subjects on days −1 and 7 during each study period at predose (−0.5 h), and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 23.5 h postadministration. The recordings of day −1 were to be used as baseline.

ECGs were read manually and blinded. In particular, QT and RR intervals were measured manually by a cardiologist in a blinded fashion as the mean of 3 to 5 beats on lead II of all electrocardiograms. Before reading, ECGs were digitized using Sigmascan technology (SPSS Inc., Chicago, IL). QT correction by heart rate was then performed according to the four different QT correction models used (see Statistical Analysis).

Safety. The safety was evaluated by adverse events, physical examination, vital signs, clinical laboratory tests, 12-lead ECG, and continuous cardiac telemetry.

The 12-lead ECGs were recorded at screening and on day 8 of each study period before the subjects left the research unit. Continuous cardiac telemetry was performed on all subjects in all study periods, which started predose on day 1 until day 8, i.e., approximately 24 h after the last dosing.

For safety assessments, ECG readings were performed automatically (as recorded in the ECG printout). The following parameters were recorded by automatic device: heart rate (beats per minute), RR, PR, QRS, QT, and QTcB (QTcB = QT/RR, Bazett formula) in milliseconds. Only clinically significant abnormal findings were to be recorded as an adverse event.

Statistical Analysis. Sample size justification. A total of 16 subjects (4 subjects per treatment sequence, 2 males and 2 females) was considered a sufficient number to provide at least 90% power to detect a 20% difference between the log-transformed AUC means of the two treatment groups (CTP+PL and CTP+KET), assuming a 30% CV of AUC values, and a correlation coefficient between treatment periods of 0.7, with a 0.05 two-sided significance level.

Pharmacokinetics analyses. The comparison of AUC0–8, AUC, and Cmax between treatment groups (CTP+PL versus CTP+KET) was based on a standard analysis of variance (ANOVA) model for crossover designs (Jones and Kenward, 1989; Snedecor and Cochran, 1989). If the treatment-by-sex interaction was statistically significant, treatment comparisons were to be performed separately for males and females. Otherwise, treatment comparisons were to be performed on the treatment factor (CTP+KET versus CTP+PL) only, Tmax, tmax, and λz were analyzed by means of nonparametric tests.

Pharmacodynamic analyses. The relationship between QT and RR was analyzed, for all subjects and by sex, by means of a linear regression model. For each treatment, Pearson and Spearman rank correlation coefficients between QT and RR were also estimated by sex and overall.

Manual QT intervals were corrected by means of the following four different models (Bazett, 1920; Fridericia, 1920; Malik and Camm, 2001; Batchvarov et al., 2002; Malik et al., 2002; ICH Guidance for Industry E14, 2005).

Model A: Linear QTcL = QT + α × (1 − RR)
Model B: Individualized parabolic log/log QTcL = QT/RR
Model C: Fixed parabolic log/log model (α = 1/2) (Bazett) QT = β × RR−1/2
Model D: Fixed parabolic log/log model (α = 1/3) (Fridericia) QT = β × RR−1/3

For each subject and QT correction model, the relationship between QT and RR intervals was described by means of the corresponding regression models:

1. Model A: Linear model QT = β + α × RR
2. Model B: Individualized parabolic log/log model QT = β × RR−1/2

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3. Model C: Fixed parabolic log/log model ($\alpha = 1/2$) (Bazett) $QT = \beta \times \frac{RR}{\sqrt{R}}$

4. Model D: Fixed parabolic log/log model ($\alpha = 1/3$) (Fridericia) $QT = \beta \times \frac{RR}{\sqrt[3]{R}}$

In each case, the QT and RR interval values for each subject were derived from the ECGs corresponding to the drug-free periods. The $\alpha$ parameter of heart rate correction in models A and B was calculated for each subject under conditions where the correlation coefficient between QTc values and RR values equaled 0 (i.e., when the QTc interval was completely independent of heart rate). The $\alpha$ parameter of heart rate correction in models A and B and $\beta$ coefficient for each subject were calculated by means of the SAS nonlinear procedure (PROC NLIN; SAS Institute, Cary, NC).

The appropriateness of individual $\alpha$ values was studied for each subject by means of the Pearson and Spearman rank correlation coefficient ($\rho$) analysis between the values of RR and QT corrected according to the models described above. If $\rho$ was not significantly different from zero ($p > 0.05$), then the corresponding $\alpha$ value was considered appropriate for that particular subject.

For each QT correction model, QTc mean values and mean changes from baseline in QTc were compared between treatments by means of the Wilcoxon signed rank test overall and by sex.

Differences between treatments in QTc mean, mean changes from baseline QTc mean, and mean normalized areas were also analyzed by means of an ANOVA model for factorial designs. Treatment and interaction effects in this factorial model were estimated by least square means with S.E.M. and 95% confidence interval (95% CI). In all statistical tests, the probability of type I error was set at 0.05 two-tailed.

Results

Of the 17 subjects initially enrolled in the study, 16 completed all four treatment periods. One subject withdrew from the study at his own request. All subjects fulfilled the inclusion criteria and none of them met an exclusion criterion. Subjects were between 18 and 49 years old, their body weight was 44.6 to 101.8 kg, their height 1.56 to 1.94 m, and body mass index 18.1 to 29.5 kg/m$^2$ (Table 1).

Safety. No changes were observed in physical examination parameters during the trial compared with screening. Forty-two ($n = 42$) treatment-emergent adverse events (AEs) corresponding to 44 AE episodes occurred in 15 (88.2%) of the 17 study participants. There were no severe AEs or withdrawals due to these events. The percentage of subjects with any AE was 35.3% for CPT+PL, 50% for CTP+KET, 50% for KET+PL, and 43.8% for PL.

No differences were observed between male and female volunteers with regard to the number of subjects presenting any AE (8 of 17 and 7 of 17, respectively) or total number of AEs (21 AEs with 22 episodes in both sexes). All AEs were of mild or moderate intensity. Except for one AE (headache of moderate intensity) medically treated with a single 500-mg dose of paracetamol, all AEs had resolved spontaneously by the end of the study.

Laboratory parameters including hematology, biochemistry, and urinalysis did not indicate any clinically significant abnormalities, and in particular, no treatment-related changes from pre- to post-treatment were seen at approximately 24 h after the last dosing (days 1 and 8). Predose to postdose changes were small after all treatments and generally did not differ from those observed after placebo treatment.

Vital sign assessments were compatible with findings in healthy, normotensive subjects throughout the study. Although slight increases and decreases in systolic blood pressure, diastolic blood pressure, and pulse rate were noted, there was no evidence of a relationship to treatment. The fluctuation in systolic and diastolic blood pressure was within the expected diurnal variation and also occurred with placebo treatment. No treatment-related effects were observed from qualitative and quantitative safety ECG evaluations (screening versus day 8 at 24 h after the last dose). Continuous cardiac telemetry did not reveal clinically significant findings in the judgment of the investigator.

Pharmacokinetics. Plasma concentration-time profiles of cinitalapride on days 1 and 7 of treatment with and without ketoconazole are presented in Fig. 1. On day 1 of treatment, concentrations of cinitalapride were highly variable with mean (±S.D.) $C_{max}$ values of 0.33 ± 0.17 ng/ml (CTP+PL) and 0.41 ± 0.25 ng/ml (CTP+KET) and mean (±S.D.) AUC$_{0-8}$ of 1.05 ± 1.24 ng·h/ml (CTP+PL) and 1.43 ± 1.94 ng·h/ml (CTP+KET). No differences related to the coadministration of ketoconazole were detected in the cinitalapride absorption rate, with the mean (±S.D.) $t_{max}$ values being 1.4 ± 0.6 h (CTP+PL) and 1.5 ± 0.6 h (CTP+KET). No reliable estimates for the elimination kinetics could be obtained in any volunteer because of the low plasma concentrations during this phase. Steady-state pharmacokinetics was achieved by day 3 with cinitalapride alone and cinitalapride with ketoconazole (Fig. 2). On day 7 of treatment, concentrations of cinitalapride were detected in all subjects, being less variable and higher than those on day 1. The mean (±S.D.) $C_{max,day}$ was 0.20 ± 0.12 ng/ml (CTP+PL) and 0.40 ± 0.22 ng/ml (CTP+KET), and mean (±S.D.) AUC$_{day}$ was 3.05 ± 1.77 ng·h/ml (CTP+PL) and 5.33 ± 2.91 ng·h/ml (CTP+KET). The accumulation factors were 2.9 (CTP+PL) and
3.7 (CTP+KET) (Table 2). Other parameters such as $t_{\text{max}}$ remained unchanged at steady state compared with day 1 of treatment. Ketoconazole moderately increased cinatapride AUC$_{r,ss}$ and $C_{\text{max,ss}}$ by 1.98- and 1.63-fold, respectively (see Table 3).

The increase in CTP concentrations after coadministration with KET was statistically significant for $C_{\text{max,ss}} (p < 0.001)$ and AUC$_{ss,ss}$ ($p < 0.001$) (Table 3). CTP concentrations were slightly higher in women, although relative increases due to KET inhibition were similar in males and females (data not shown).

Pharmacodynamics. The pharmacodynamic evaluation in this study comprised a thorough evaluation of the QTc after multiple-dose treatment with cinatapride alone or in combination with ketoconazole.

No relevant findings were observed in the standard ECG parameters. Minor rhythm and/or conductance disturbances were observed as isolated episodes. The morphology of the ECGs was considered as being normal and there were no signs of clinically significant abnormalities in any of the ECG traces.

A statistically significant correlation was found between QT and RR in all treatment groups, and that pattern was not modified by treatment with cinatapride alone or in combination with ketoconazole.

When comparing the QTc means, or the mean changes from baseline QTc mean) is the recommended variable to be considered for calculating effects on the QT (ICH Guidance for Industry E14, 2005).

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No outlier >60 ms was identified after any treatment (data not shown) and no subject had a QTc >500 ms (see ICH Guidance for Industry E14, 2005). Thus, in this trial, cinatapride has not shown any potential for inducing QTc changes at therapeutic doses of 1 mg t.i.d.

Heart rate showed similar mean values for all four treatment regimens. No correlation was found between QTc and RR in each QT correction model with correlation coefficients ($r^2$) ranging from 0.02 to 0.04 using Pearson’s analysis and 0.03 to 0.07 using Spearman’s analysis. No trends toward sex differences were observed. Finally, no correlation was found between QTc or delta QTc and cinatapride concentrations, as these were within approximately 0.8 to 1.7 ms for all QTc parameters used for the comparison of the treatment CTP+PL versus PL+PL (Table 4). Moreover, the mean increases in these QTc parameters were always below 2 ms, if the treatments CTP versus PL were within approximately 0.8 to 1.7 ms for all QTc parameters used for the comparison of the treatment CTP+PL versus PL+PL (Table 4). Moreover, the mean increases in these QTc parameters were always below 2 ms, if the treatments CTP+KET versus PL+KET were compared.

These differences are clinically irrelevant and mainly due to QTc variability and small sample size. Further analysis by means of factorial ANOVA models showed that these increases were due to the effect of ketoconazole alone (statistically significant in models A, B, and D, overall and in women). No significant effect was observed either for cinatapride alone or for the interaction cinatapride-ketoconazole in any of the factorial models fitted. Thus, the factorial model analysis results suggest that cinatapride does not induce QTc prolongation either when given alone or during coadministration with ketoconazole 200 mg b.i.d.

No outlier >60 ms was identified after any treatment (data not shown) and no subject had a QTc >500 ms (see ICH Guidance for Industry E14, 2005). Thus, in this trial, cinatapride has not shown any potential for inducing QTc changes at therapeutic doses of 1 mg t.i.d.
plasma levels (matched data) in the four QT correction models (data not shown).

Discussion

Drug interactions with inhibitors of the cytochrome P450 system have been shown to be of particular clinical importance for gastrokinetic agents such as cisapride (Desta et al., 2000) and H1-receptor antagonists such as astemizole and terfenadine (Dresser et al., 2000). Ketoconazole increases the plasma concentrations of drugs metabolized by CYP3A4, and this enzyme is known to be involved in the metabolism of cin萘apride. By contrast to cisapride metabolism, CYP2C8 has been shown to also be an important enzyme in cin萘apride metabolism and, although also inhibited by ketoconazole, in this case, the inhibition is weak compared with the inhibition of CYP3A4. Thus, the present study was designed to assess the influence of ketoconazole 200 mg b.i.d on the pharmacokinetics and cardiac safety of therapeutic doses of cin萘apride 1 mg t.i.d. The presence of ketoconazole resulted in only a small increase in cin萘apride plasma concentrations as reflected by a 1.63-fold increase in steady-state Cmax and a 1.98-fold increase in AUC0, without prolongation of the t1/2. This finding supports the conclusion that CYP2C8 retains some capacity for removing cin萘apride from blood despite the effect of ketoconazole blocking CYP3A4-mediated metabolism. A study carried out in the urine obtained in the present study demonstrated that oxidative metabolism occurs and that conjugation was also an important pathway contributing to the rapid clearance of the molecule (De Graeve, 2001).

As a consequence, the safety profile of cin萘apride also appears to be unaffected by concomitant administration of ketoconazole as suggested by the similar incidence and intensity of AEs in both treatment groups. Forty-two (n = 42) treatment-emergent AEs corresponding to 44 AE episodes occurred in 15 (88.2%) of 17 subjects of the safety evaluation. There were no serious adverse events or withdrawals due to AEs. No treatment-related effects were observed for mean QTc

### Table 4

**Comparisons of QTc means and mean changes from baseline QTc mean between treatments (mean ± S.E.M.)**

Models are: A, linear, QTcI; B, parabolic log/log, QTcI; C, fixed parabolic log/log (α = 0.5), QTcI (Bazetti); D, fixed parabolic log/log (α = 0.33), QTcF (Fridericia).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
<th>Model D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP + KET vs. CTP + PL</td>
<td>5.11 ± 1.85</td>
<td>5.17 ± 1.87</td>
<td>3.56 ± 2.25</td>
<td>4.78 ± 1.79</td>
</tr>
<tr>
<td>CTP + KET vs. PL + PL</td>
<td>0.85 ± 2.78</td>
<td>0.89 ± 2.84</td>
<td>0.90 ± 3.03</td>
<td>1.19 ± 2.81</td>
</tr>
<tr>
<td>CTP + KET vs. PL + PL</td>
<td>4.66 ± 2.49</td>
<td>4.80 ± 2.53</td>
<td>2.75 ± 2.63</td>
<td>4.38 ± 2.45</td>
</tr>
<tr>
<td>CTP + PL vs. PL + KET</td>
<td>-4.27 ± 2.78</td>
<td>-4.28 ± 2.80</td>
<td>-2.66 ± 2.87</td>
<td>-3.59 ± 2.75</td>
</tr>
<tr>
<td>CTP + PL vs. PL + KET</td>
<td>-0.45 ± 2.56</td>
<td>-0.38 ± 2.62</td>
<td>-0.81 ± 2.72</td>
<td>-0.40 ± 2.65</td>
</tr>
<tr>
<td>PL + KET vs. PL + PL</td>
<td>3.81 ± 1.72</td>
<td>3.90 ± 1.79</td>
<td>1.85 ± 1.44</td>
<td>3.19 ± 1.73</td>
</tr>
</tbody>
</table>

### Table 5

**Mean changes from baseline QTc mean (ms)**

Models are: A, linear, QTcI; B, individualized parabolic log/log, QTcI; C, fixed parabolic log/log (α = 0.5), QTcI; D, fixed parabolic log/log (α = 0.33), QTcF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Model A (Mean ± S.E.M.)</th>
<th>Model B (Mean ± S.E.M.)</th>
<th>Model C (Mean ± S.E.M.)</th>
<th>Model D (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP + PL</td>
<td>All</td>
<td>-8.2 ± 2.8</td>
<td>-8.2 ± 2.9</td>
<td>-3.4 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>-6.0 ± 5.3</td>
<td>-5.9 ± 5.3</td>
<td>-0.4 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>-10.3 ± 2.3</td>
<td>-10.4 ± 2.4</td>
<td>-6.4 ± 2.4</td>
</tr>
<tr>
<td>CTP + KET</td>
<td>All</td>
<td>-2.1 ± 2.8</td>
<td>-2.2 ± 2.8</td>
<td>0.7 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>-1.2 ± 4.9</td>
<td>-1.4 ± 4.8</td>
<td>1.9 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>-3.0 ± 3.0</td>
<td>-3.0 ± 3.2</td>
<td>-0.5 ± 4.3</td>
</tr>
<tr>
<td>PL + KET</td>
<td>All</td>
<td>-2.5 ± 2.2</td>
<td>-2.5 ± 2.3</td>
<td>-1.1 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>-2.5 ± 2.3</td>
<td>-2.5 ± 2.4</td>
<td>-2.2 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>-2.6 ± 4.0</td>
<td>-2.6 ± 4.1</td>
<td>-0.0 ± 4.5</td>
</tr>
<tr>
<td>PL + PL</td>
<td>All</td>
<td>-9.6 ± 2.5</td>
<td>-9.7 ± 2.5</td>
<td>-3.8 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>-12.3 ± 4.2</td>
<td>-12.4 ± 4.2</td>
<td>-3.7 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>-6.9 ± 2.6</td>
<td>-6.9 ± 2.7</td>
<td>-3.8 ± 2.9</td>
</tr>
</tbody>
</table>
changes on day 7 compared with screening. Continuous cardiac telemetry did not reveal clinically significant findings in the judgment of the investigator.

Detailed evaluation of the possible potential of cinitapride to induce a QTc prolongation involved the use of four different QT correction models applied to the manually evaluated ECGs before and on day 7 of the four study treatments. There is an ongoing discussion about the most appropriate method(s) for correcting QT interval for changes in heart rate. In this article, we have employed the two most used population methods of QT correction [Bazett and Fridericia, models C and D (Bazett, 1920; Fridericia, 1920)] and two models of subject-specific QT correction [linear and individualized log/log, models A and B (Malik and Camm, 2001; Malik et al., 2002)].

Mean QTc changes from mean baseline values showed a decrease for all treatments with all four correction models (except for QTcB under CTP+KET, where a minimal increase was observed, probably due to overcorrection). Heart rate showed similar mean values for all four treatment regimens.

QTc mean changes, baseline-corrected QTc means, or normalized area under the QTc curve on day 7 indicated a small QTc increase of approximately 5 to 6 ms during both PL area under the QTc curve on day 7 indicated a small QTc increase of four treatment regimens.

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QTc mean changes, baseline-corrected QTc means, or normalized area under the QTc curve on day 7 indicated a small QTc increase of approximately 5 to 6 ms during both PL+KET and CTP+KET treatment on average. Mean changes during cinitapride treatment ranged from −0.8 to 1.7 ms compared with placebo treatment and were obviously not significant.

The results of the factorial analysis performed on QTc data (see Table 4) are consistent, since they show that, whatever QT correction method is applied, the QTc prolongation effect is entirely due to KET. The contribution of the combined treatment (CTP+KET) in the increase of QTc changes from baseline compared with placebo alone is clearly not different, from both a clinical and a statistical point of view, from the contribution of KET alone (PL+KET).

The small increase produced by ketoconazole was not significant in the factorial analysis either, which also confirmed that cinitapride did not induce QTc prolongation, either when given alone or during coadministration with ketoconazole 200 mg b.i.d. Accordingly, no correlation was found between QTc or delta QTc and plasma concentrations of cinitapride in any of the four QT correction models. In addition, no pharmacokinetic or pharmacodynamic interaction was noted regarding clinical adverse events or laboratory findings, further demonstrating the safety of concomitant administration of cinitapride and ketoconazole.

In conclusion, concomitant administration of ketoconazole 200 mg b.i.d. has very little influence on the pharmacokinetic profile of cinitapride and does not modify its safety profile. Cinitapride did not induce changes in the QTc at the therapeutic dose of 1 mg t.i.d., either when given with placebo or when given with ketoconazole 200 mg b.i.d. This is in contrast with previous findings with cisapride, a gastrokinetic drug that has been associated with pronounced QTc prolongation and fatal arrhythmias.

### References


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