Clinical Pharmacokinetics

Effect of pH and Comedication on Gastrointestinal Absorption of Posaconazole
Monitoring of Intraluminal and Plasma Drug Concentrations

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Supplemental Digital Content

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**Detailed Methodology**

**Study Materials**

Posaconazole 40 mg/mL oral suspension (Noxafil®) and esomeprazole 40 mg tablets (Nexium®) were kindly provided by the University Hospitals Leuven (Leuven, Belgium). Posaconazole (700.78 g/mol) was kindly provided by the Chemical Research Division of MSD (Whitehouse Station, NJ, USA). The internal standard, itraconazole, was obtained from Janssen Research Foundation (Beerse, Belgium). Stock solutions were prepared in DMSO (Acros Organics, Morris Plains, NJ, USA). Diethylether was purchased from Fisher Scientific (Loughborough, UK). BDH Laboratory Supplies (Poole, UK) provided NaOH. Methanol, HCl (1N) and sodium acetate were obtained from VWR International (West Chester, PA, USA). Chem-labo (Zedelgem, Belgium) supplied acetic acid. HPLC-grade water was obtained by purification with a Maxima system (Elga Ltd, High Wycombe, UK).

**Solubility Experiments**

The equilibrium solubility of posaconazole was determined in different media. An excess of Noxafil® suspension (25 µL of the 40 mg/mL suspension) was added to 0.5 mL of medium and mixed on a 25D controlled environment incubator shaker (New Brunswick Scientific, Edison, NJ, USA) at 37°C. After 24 hours, samples were centrifuged (10 minutes, 21000 g, 37°C) and the supernatant was diluted ten times with mobile phase. Concentrations of posaconazole in the diluted supernatants were measured by a reversed-phase HPLC method with fluorescence detection (see below).

**In Vivo Study: Sampling**

After administration of the dosage form, samples of human gastric and intestinal fluid (sample volume between 0.5 and 4 mL) were aspirated every 10 minutes during the first hour and subsequently every 15 minutes for up to 5 hours. Upon measuring the pH (Hamilton Slimtrode, Bonaduz, Switzerland), the gastrointestinal aspirates were transferred to microcentrifuge tubes and immediately centrifuged (room temperature, 9860 g, 5 minutes), after which the supernatant was diluted ten times with mobile phase. These diluted samples were then stored at −30°C until analysis. It should be noted that although the gastrointestinal fluids were aspirated for up to 5 hours, the Results section presents the AUC3h values. This was justified because beyond the 3-hour time point, no differences in concentration-time profiles between the four conditions were observed.

In parallel to the sampling of gastrointestinal fluids, venous blood samples were collected in heparinized tubes (BD Vacutainer Systems, Plymouth, UK) at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 24 and 48 hours after drug intake. Plasma samples were obtained by centrifugation (4°C, 1800 g, 10 minutes) and stored at −30°C until analysis.

**Analysis of Posaconazole in Gastrointestinal Fluid Samples**

After aspiration, centrifugation and dilution of the gastrointestinal fluid samples, as described above, concentrations of posaconazole in the diluted supernatants were measured by a validated reversed-phase HPLC method with fluorescence detection. After whirl mixing of the thawed diluted samples for 10 seconds and centrifugation (4°C, 20817 g, 5 minutes), a volume of 10 µL (for the gastric fluid samples) or 100 µL (for the duodenal fluid samples) of the supernatant was injected into an Hitachi Elite LaChrom HPLC system (VWR International) consisting of an L-2130 pump, an L-2200 autosampler and a Waters Novapak C-18 column under radial compression (particle size 4 µm, dimensions 8 × 100 mm). Fluorescence signals (excitation wavelength 240 nm, emission wavelength 385 nm) were detected on a Hitachi Elite LaChrom L-2480 fluorescence detector (VWR International). The column was equilibrated with a mobile phase consisting of 20 mmol/L sodium acetate buffer (pH 3.3) and methanol (18 : 82 v/v).
The flow was maintained at a rate of 1 mL/min. The retention time of posaconazole amounted to 5.3 minutes. After elution, the column was flushed with 100% methanol for 3 minutes and re-equilibrated with mobile phase for 4 minutes.

Calibration curves were generated using spiked mobile phase. The calibration curves were linear between 0.098 and 50 µmol/L for the gastric fluid samples (injection volume of 10 µL), and between 0.012 and 6.25 µmol/L for the intestinal fluid samples (injection volume of 100 µL), respectively. Samples were diluted to fit within this range. Intra- and interday precision and accuracy were assessed by analyzing standard samples (n = 5) at 0.4, 4 and 40 µmol/L for posaconazole in blank gastric fluids diluted 1/10 with mobile phase and at 0.05, 0.5 and 5 µmol/L for posaconazole in blank intestinal fluids diluted 1/10 with mobile phase. The relative error of the intraday validation amounted to −2.29%, −0.22% and +0.13% at 0.4, 4 and 40 µmol/L, respectively, for the gastric fluid samples, and to −3.42%, −3.41% and −1.62% at 0.05, 0.5 and 5 µmol/L, respectively, for the intestinal fluid samples. The relative error of the interday validation remained below −1.6% for posaconazole in gastric fluid samples, and below −3.3% for posaconazole in intestinal fluid samples. Determination of the intraday repeatability resulted in relative SDs below 1.3% at all concentrations, for both the gastric fluid and intestinal fluid samples (except for 2.7% at 0.4 µmol/L in gastric fluid). Determination of the interday repeatability resulted in relative SDs below 1.9% at all concentrations, for both the gastric fluid and intestinal fluid samples.

Analysis of Posaconazole in Plasma Samples
Before HPLC/fluorescence analysis, posaconazole was extracted from the plasma samples. After adding 100 µL internal standard solution (2.5 µmol/L itraconazole in 0.2 N HCl) to 1000 µL plasma, the sample was diluted with 500 µL NaOH (2N). After extraction with 4 mL diethylether and centrifugation (4°C, 2680 g, 5 minutes), the water layer was discarded and the organic layer was evaporated to dryness under a gentle stream of air. The residue was dissolved in 400 µL of a MeOH : H₂O solution (50 : 50 v/v). After centrifugation of the diluted solution (4°C, 20817 g, 5 minutes), the posaconazole concentration in the supernatant was measured by reversed-phase HPLC and fluorescence detection. A volume of 50 µL was injected into the HPLC system and analyzed in the same way as described in the previous section.

To determine concentrations, a calibration curve was generated with spiked blank human plasma, which was treated in the same way as the samples. Linearity was obtained between 0.008 and 4 µmol/L. Intra- and interday precision and accuracy were assessed with five replicates of standard samples at 0.1 and 1 µmol/L. The relative error of the intraday validation amounted to +1.73% and +2.64% at 0.1 and 1 µmol/L, respectively. The relative error of the interday validation amounted to +0.2% and +1.3% at 0.1 and 1 µmol/L, respectively. Determination of the intraday repeatability resulted in relative SDs below 1.2% at all concentrations. Determination of the interday repeatability resulted in relative SDs below 2.2% over the linear concentration range.

Data Presentation and Statistical Analysis
AUC values were calculated by the trapezoidal rule. All concentration-time profiles are presented as mean ± SD for five subjects. Posaconazole concentrations in these profiles are presented in µmol/L units. Conversion to ng/mL can be done by applying the following conversion factor: 1 µmol/L equals 700.8 ng/mL (molecular weight of posaconazole = 700.8 g/mol). The C_{max} and AUC parameters of the concentration-time profiles for the four different conditions were compared using repeated measures ANOVA, followed by a Dunnett’s multiple comparison test. Differences were considered statistically significant at p < 0.05. As we were specifically interested in the absorption phase of the concentration-time profiles, and a full pharmacokinetic analysis was beyond the scope of this study, the study design did not enable calculation of further pharmacokinetic parameters (limited sampling time).