Study of the drug–drug interaction between simvastatin and cisapride in man

Abstract Objective: The objective of our study was to evaluate in humans the drug–drug interaction occurring during the concomitant administration of cisapride and simvastatin, two well-known substrates of CYP3A4.

Methods: Eleven healthy men aged between 20 years and 35 years gave their written informed consent to participate in the study. Each participant received repeated doses of cisapride and/or simvastatin. At first, subjects received cisapride alone, 10 mg every 8 h, for 3 days. Then, the drug was given at the same regimen during concomitant administration of simvastatin, 20 mg every 12 h for 4 days, starting on the night of day 3. Finally, cisapride was stopped and subjects received simvastatin (20 mg every 12 h) for four additional days.

Results: Simvastatin administration caused a 14 ± 20% increase in the AUC of cisapride. In contrast, plasma concentrations of simvastatin were unaltered by the co-administration of cisapride, whereas plasma concentrations of simvastatin acid, its active metabolite, were decreased by 33 ± 24%.

Conclusion: The concomitant administration of the prokinetic agent cisapride and the 3-hydroxy-3-methylglueryl CoA reductase inhibitor simvastatin resulted in altered pharmacokinetics of both drugs. Increased plasma concentrations of cisapride suggest that some patients may be at risk of toxicity while receiving both drugs, whereas the decrease in simvastatin acid plasma concentrations suggests that cholesterol lowering effects of simvastatin treatment may be blunted.

Keywords Simvastatin · Cisapride · CYP3A4

Introduction

CYP3A enzymes constitute the major cytochrome P450 (CYP) isoforms in the liver of most individuals [1, 2]. Relatively high levels of CYP3A are also present in the small intestine, and large interindividual variability observed in CYP3A activity reflects a combination of both environmental and genetic factors [1, 2]. The substrate selectivity of the CYP3A enzymes is very broad; accordingly, an extremely large number of structurally different chemicals are metabolised by these enzymes [1]. Estimates based primarily on in vitro studies suggest that the metabolism of perhaps 40–50% of drugs currently used in humans involves to some extent CYP3A-mediated oxidations. Given the numerous compounds metabolised by CYP3A, it is conceivable that competitive inhibition could be observed in patients undergoing concomitant treatment with several drugs. Thus, co-administration of CYP3A substrates would modify either efficacy or toxicity of each agent given alone.

Cisapride is a third-generation prokinetic agent that restores, normalises and facilitates motility in the gastrointestinal tract [3, 4]. The drug has a relatively low absolute bioavailability (40–50%) due to significant first-pass metabolism [3]. Cisapride is largely metabolised by N-dealkylation into norcisapride which represents 64–74% of overall recovered metabolites. Bohets et al. demonstrated that cisapride is mainly metabolised by CYP3A4 and, based on Km determination, it is unlikely that cisapride would inhibit competitively the metabolism of co-administered drugs [5]. Indeed, metabolism of cisapride is inhibited by macrolide antibacterials and
imidazole antifungals, leading to an increase in cisapride plasma concentrations during combined treatment [6, 7].

Simvastatin is effective in the treatment of hypercholesterolaemia. It is a lactone prodrug with several of its metabolites, most notably simvastatin acid, being active and capable of competitively inhibiting 3-hydroxy-3-methyl coenzyme A (HMG CoA) reductase, the enzyme responsible for the rate-limiting step of endogenous cholesterol synthesis [8, 9, 10]. CYP3As are the major enzyme subfamily responsible for the metabolism of simvastatin, but not of simvastatin acid [11, 12, 13]. Indeed, transformation of simvastatin into simvastatin acid is mediated by hepatic esterases in humans [12, 13, 14]. Increased plasma concentrations of simvastatin secondary to CYP3A inhibition has led to severe side effects including rhabdomyolysis and liver toxicity [15]. Moreover, Prueksaritanont et al. demonstrated that simvastatin was found to have a competitive inhibitory effect on hepatic CYP3A activity with a Ki value of ~10 μM, therefore a metabolic interaction with other CYP3A substrates is possible [12].

Therefore, the objective of our study was to evaluate the potential drug–drug interaction during the concomitant administration of cisapride and simvastatin in healthy volunteers.

Materials and methods

The study was approved by the Ethics Committee for Human Subjects of Laval Hospital. Eleven healthy men aged between 20 years and 35 years gave their written informed consent to participate in the study. Each volunteer had a physical examination, an electrocardiogram and routine laboratory tests judged to be normal. All volunteers were non-smokers.

Study design

Forty-eight hours before the beginning and throughout the study, volunteers were advised to stop taking caffeine-containing foods and beverages, chocolate, alcohol, grapefruit juice, and all over-the-counter and prescription drugs. Each participant received repeated doses of cisapride and/or simvastatin according to the following study design. At first, subjects received cisapride (Propulsid, Jansen-Ortho Inc., North York, Canada) alone, 10 mg every 8 h, for 3 days. Then, the drug was given during concomitant administration of simvastatin (Zocor, Merek Frost Canada Inc., Kirkland, Canada), 20 mg every 12 h for 4 days, starting on the night of day 3. Finally, cisapride was stopped and subjects received simvastatin (20 mg every 12 h) for four additional days. On the morning of study day 3, day 7 and day 11, after an overnight fast, the volunteers were admitted to the clinical Research Unit of Laval Hospital. An i.v. catheter was inserted in a forearm vein and electrodes were placed for the recording of 12-lead electrocardiogram (ECG). Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 h after drug administration on day 3 and day 7 for the analysis of cisapride. Additional samples were obtained at 9, 10, 11 and 12 h on day 7 and day 11 for the analysis of simvastatin and simvastatin acid. ECGs were obtained after each blood samples. Heparin was used to prevent catheter occlusion, the first 3 ml of each blood sample was discarded. Blood was collected in glass tubes containing ethylene diamine tetraacetic acid (EDTA; Vacutainer, Becton Dickinson, N.J.). The plasma was separated by centrifugation and frozen at −22°C until analysed. Urine was collected for a period of 8 h on day 3 and day 7 for the analysis of cisapride and its metabolite (nor cisapride). The volume of urine was recorded and aliquots were stored at −22°C. Two hours after the morning dose of the drugs, a light snack was allowed.

Electrocardiographic measurements at screening and throughout the study were obtained as vectocardiographic electrocardiograms where X (coronal), Y (lateral), and Z (sagittal) leads were recorded at a paper speed of 50 mm/s during the study. QTc intervals were measured at baseline, during day 3, day 7 and day 11 at specific times (right after blood sample draws).

Drug assays

Cisapride plasma concentrations were quantified using a slightly modified high-performance liquid chromatography (HPLC) method [16]. Metoclopramide (1 μg/mL) was used as the internal standard. The mobile phase was 48% acetonitrile and 52% potassium dihydrogen phosphate (0.05 M), pH adjusted to 5.5 with NaOH 1 M. Tritylamine (0.04 M) was added. The flow rate of the mobile phase was set at 1 ml/min and the liquid pumped through a 5-μm C18 column (25 cm×4.6 mm: Ultrasphere, Beckman Instrument, Fullerton, Calif.) with detection by ultraviolet absorbance at a wavelength of 276 nm. Plasma (1 ml) was extracted twice with 5 ml butylchloride-isopropanol 5% following addition of the internal standard (100 μl) and NaOH (2 M). A back extraction with 1 ml of 0.1 M HCl was performed and the organic phase discarded. The aqueous phase was re-extracted with butylchloride-isopropanol 5% according to the extraction method described above. The combined organic extracts were evaporated under nitrogen at 37°C. The extraction residue was dissolved in 100 μl of mobile phase. The limit of quantification of cisapride was 10 ng/ml. The intraday and interday coefficients of variation (concentrations of 20, 40 and 80 ng) were less than 10%.

Plasma levels of both simvastatin and its major metabolite simvastatin acid were determined by LC/MS/MS using lovastatin and lovastatin acid as internal standards, respectively. Plasma (1 ml) was buffered at pH 5.5 and transferred onto a pre-conditioned C-18 solid-phase extraction cartridge. The cartridge was centrifuged for 2 min at 1000 rpm and successively washed with phosphate buffer and water under centrifugation at 1000 rpm and 3000 rpm, respectively. The compounds of interest were eluted with 2 ml ethanol. The solvent was evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 100 μl of a methanol/water mixture (7:3 v/v), and 15 μl was injected into the liquid chromatographic system. Liquid chromatography was performed on a HP 1090 series II system (Hewlett Packard, Palo Alto, Calif.) equipped with a YMC Basic 2.0×100 mm, 5-μm analytical column (Waters, Milford, Mass.). This was coupled to an API 3 triple-stage quadrupole mass spectrometer (MDS-Sciei, Concord, Ontario), fitted with a Turbo Ion spray source. The mass spectrometer operating conditions were: turbo ion spray temperature 400°C, interface heater 60°C, nebulizer pressure 40 psi, curtain gas flow 1.2 ml/min and auxiliary flow 5.5 ml/min. The following MRM transitions were monitored: simvastatin m/z 419–199; lovastatin m/z 419–199; simvastatin acid m/z 419–199. Finally, the compounds of interest were quantified using a set of nine calibration standards for each compound over the 0.1-ng/ml (lower limit of quantification) to 10.0-ng/ml range. Peak area ratios of both analytes to their respective internal standards were calculated and fitted with weighted (1/C) linear regression equations. The accuracy and precision of analytical measurements was assessed in each analytical batch using duplicate quality control samples fortified with known amounts of the analytes at three different concentration levels corresponding to the low, medium and high segments of the concentration range. Intraday coefficients of variation for simvastatin and simvastatin acid (concentrations of 0.1, 0.3, 4 and 8 ng/ml) were less than 13.5%. Interday coefficients of variation for simvastatin and simvastatin acid (concentrations of 0.1, 0.3, 4 and 8 ng/ml) were less than 16%.