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Sex- and smoke-related differences in gastrointestinal transit of cyclosporin A microemulsion capsules

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ABSTRACT

The aim of this work was to study the effect of the sex and the smoking status on the pharmacokinetics and the bioequivalence assessment of a branded and a generic cyclosporine A microemulsion formulation in soft-gelatin capsule.

Sixteen healthy volunteers (eight women and eight men) participated in a CyA bioequivalence study, with nine of the volunteers being smokers. Sandimmun Neoral® (brand formulation; Reference) and Sigmasporin Microral® (generic formulation; Test) were administered under fasting conditions. Pharmacokinetic parameters were calculated through non compartmental analysis. Bioequivalence was declared based on the 90% confidence intervals (90% CI) for the T/R ratio of the geometric means for each parameter. *In vitro* determination of the capsules opening time was performed in simulated gastric fluid without enzyme with USP Apparatus 2.

The extent of absorption was similar between both products for all subjects or each sex-group. The absorption rate was similar for both products when considering all subjects, whereas a significant difference in the T_{MAX} between the two products was observed for the male subjects only, which relates to its slower capsule opening time observed *in vitro* (12.4 versus 6.0 min). No differences were observed in women that could relate to their slower gastric emptying. Differences in drug exposure were observed between smokers and non-smokers.

Sex- and smoke-related differences in the gastrointestinal transit should be considered when the on-set time would be determinant for the treatment success of a drug.

1. Introduction

Cyclosporin A (CyA) is a non-ribosomal cyclic-neutral-hydrophobic peptide of eleven amino-acids, which is produced by the fungi *Tolypocladium inflatum Gams* and is extracted from the mycelia (Borel et al., 1995). The clinical usefulness of CyA is to prevent graft rejection following organ transplantation and also for the treatment of autoimmune diseases (Krensky et al., 2010; Sandimmune®, 2010).

CyA gastrointestinal absorption is incomplete and variable. Compared with an intravenous infusion, the absolute bioavailability of soft capsules is approximately 30% (Sandimmune®, 2010). This low and variable bioavailability is determined by many factors including: solubility, emulsification, and pre-systemic hepatic and intestinal metabolism, which is mediated by the concerted action of P-glycoprotein (P-gp) efflux transporter and the CYP3A4 isozyme, located in the liver and several extra-hepatic sites, most notably, the epithelium of the upper intestine (Christians et al., 2000; Fahr, 1993; Saeki et al., 1993; Wu et al., 1995; Kolars et al., 1991; Webber et al., 1992). Grevel et al. (1986) suggested the existence of an absorption window for CyA located in the upper portion of the intestine.

Microemulsion formulations (Sandimmun Neoral ®-Novartis, and several generic brands) have improved its therapeutic performance in comparison with the emulsion formulation (Sandimmun® – Novartis), and these formulations exhibited dose-proportionally in AUC over a wide and clinically relevant dosage range and an increase in the rate and extent of CyA absorption. The relative bioavailability of CyA after administration as a microemulsion ranges from 174% to 293% compared to Sandimmun® (Mueller et al., 1994). Another benefit of the microemulsion formulation is the decreased inter-individual variability observed

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since CyA becomes ready to be absorbed once it is released from the soft capsules (Mueller et al., 1994; Qazi et al., 2006).

There is strong evidence supporting sex differences on gastric emptying, with female subjects having longer lag-periods for solid meals, lower gastric emptying rate and higher gastric residence time (Bennink et al., 1998; Datz et al., 1987; Hermansson and Sivertsson, 1996; Hutson et al., 1989; Knight et al., 1997; Sadik et al., 2003). Differences were also reported for transit time throughout large intestine, which seems to be shorter in men (Sadik et al., 2003; Degen and Phillips, 1996). Reports are controversial regarding transit time in small intestine, as some studies reported no sex differences while others found a significantly slower transit in females (Freire et al., 2011).

Sex-specific pharmacokinetic differences related with CyA metabolism have been also reported. On the one hand, a higher CYP3A4 activity observed in females (Wolbold et al., 2003; Greenblatt and von Moltke, 2008; Scandlyn et al., 2008) would decrease oral bioavailability and increase systemic clearance. On the other hand, only one-third to one-half of the P-gp expression observed in men was reported in females (Schuetz et al., 1995). Substrates of both CYP3A4 and P-gp have showed higher bioavailability and clearance in women (Schwartz, 2007). Consequently, these facts might preclude a similar CyA exposure between sexes.

Beyond the consequence that these differences may cause on drug clinical response, they can have a major impact on bioequivalence between oral formulations containing P-gp and CYP3A4 substrates due to the sex-by-formulation interaction. Men and women can have different capabilities to discriminate two drug-products (Chen et al., 2000; Ibarra et al., 2012), a phenomenon usually neglected in conventional average bioequivalence studies. Kees et al. (2007) conducted two crossover studies in which effects of two formulations and food intake on CyA bioavailability were studied, and found that food significantly increased maximum concentration only in male subjects.

Tobacco smoking has also been related with effects on gastric physiology, although debate persist as some studies reported a delayed gastric emptying of solids for smokers (Nowak et al., 1987; Miller et al., 1989; Johnson et al., 1991) while others associated tobacco and nicotine with increased gastric motility and faster emptying rates of solid and liquid contents (Ferreira et al., 2002; Grimes and Goddard, 1978; Sanaka et al., 2005; Hanson and Lilja, 1987; Graff et al., 2001). Graff et al. (2001) and Lagrue et al. (2006) also found an increased intestinal peristalsis secondary to nicotine intake.

A pharmacokinetic analysis on data coming from a CyA bioequivalence study will be presented here below. The aim of this work was to study whether the sex and the smoking status of individuals could affect both the pharmacokinetics and the bioequivalence assessment between two CyA soft-capsulated microemulsion formulations.

2. Materials and methods

2.1. Materials

2.1.1. Equipments

GFL Destilator 2008 (Germany), Distek dissolution apparatus 2100C (NJ, United States), Oakton ph6 pH-meter (IL, United States), and Abbott AxSYM™ system for immunoassays (Abbott laboratories, IL, United States) were used.

2.1.2. Chemicals

Following reagents were used: hydrochloric acid from Dorwil (BA, Argentine) and potassium chloride from Carlo Erba (Ml, Italy). Distilled water was obtained in our laboratory. Abbott reagents for AxSYM CyA determination based on fluorescence polarization immunoassay (FPIA) were provided by Bioerix (MVD, Uruguay).

Assayed formulations were soft gelatin capsules containing 100 mg CyA microemulsion: Test (T) Sigmasporin Microral® (lot number 408935), EMS Sigma Pharma, Brazil; and Reference (R) Sandiummun Neoral® (lot number S0181) Novartis, Switzerland.

2.2. Methods

2.2.1. In vitro determination of the capsules opening time

Six units of each product were tested. The *in vitro* assay was performed using the simulated gastric fluid without enzyme (aqueous solution of HCl and KCl, pH 1.2), according to the United States Pharmacopoeia (USP, 2009). This medium is considered biorelevant by the World Health Organization (WHO, 2006). The conditions were: USP Apparatus 2 (paddle); 50 rpm stirring speed; volume 500 mL; temperature 37 ± 0.5 °C. Each dosage unit was visually inspected up to the capsule shell rupture time. This time was recorded unit by unit in separated assays.

2.2.2. Bioequivalence study

2.2.2.1. Subjects and study design. Sixteen Caucasian healthy volunteers (eight women and eight men) between 18 and 37 years old with mean body weight (SD) of 58 (7.5) and 82 (12) kg, respectively, were enrolled in a CyA bioequivalence study. Nine of the volunteers were smokers (4 men and 5 women) and seven non-smokers (4 men and 3 women). The study was carried out with two capsules (100 mg) of CyA: Test formulation (T) or Reference formulation (R), administered under fasting conditions with 100 mL of water, in a two-period and two-sequence (TR and RT), randomized, compensated-crossover design. A two-week washout period was kept between both administrations. The study protocol and informed consent form were designed according to the ethical guidelines for human clinical research and were approved by the Institutional Ethics Review Committee of the Faculty of Chemistry (Uruguay). Written informed consent was obtained from all subjects before their entry in the study. The study was performed in the Bioavailability and Bioequivalence Centre for Medicine Evaluation, situated in "Dr. Juan J. Crottogini" Hospital (Montevideo, Uruguay).

Volunteers came to the Centre the first day of each week, with an eight-hour overnight fasting period. Standardized meals (lunch, tea, dinner and breakfast) were provided at three, seven, eleven and twenty-two hours after dose administration. Twenty-four hours post-dose, the volunteers were released from the Centre, returning at thirty-four and forty-five hours post-dose for blood sampling. During the second day of the study, there were no food restrictions.

2.2.2.2. Sampling and chemical analysis. Blood samples were drawn from the antecubital vein through cannulation and placed into heparinized tubes immediately. The samples were drawn before dosing (0 h) and at 0, 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 34, and 45 h after dosing. Blood samples were stored refrigerated (2–8 °C) until analysis.

Quantification of CyA in blood was done by Fluorescence Polarization Immuno Assay (FPIA) using AXSYM (Abbot™) equipment, according to the instructions given in the package insert. The lower limit of quantification was determined to be 12.5 ng/mL, since intra-and-inter-day precision was below 20%, in terms of coefficient of variation, and accuracy was comprised between 85% and 115%.

2.3. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using the software tool Kinetica 5.0 (Thermo Fisher Scientific,