Therapeutic Doses of Eltrombopag do not Inhibit Hepatic BCRP in Healthy Volunteers: Intravenous Ceftriaxone as a Model

Daniel Valente Neves, Carolina Pinto Vieira, Adriana Rocha and Vera Lucia Lanchote

Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.

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ABSTRACT - PURPOSE: Ceftriaxone elimination occurs through breast cancer resistance transporter (BCRP) and multidrug resistance-associated protein 2 (MRP-2) which are expressed on the canalicular membrane of hepatocytes. Eltrombopag, a thrombopoetin receptor agonist used in the treatment of immune thrombocytopenic purpura, is reported in in vitro studies as an inhibitor of intestinal BCRP but not an inhibitor of hepatic BCRP. Thus, the present study evaluates the effect of therapeutic doses of eltrombopag on the clinical pharmacokinetics of intravenous ceftriaxone. **METHODS**: Healthy adult (n=12) were treated with oral doses of eltrombopag (0, 25 or 50 mg) 28 and 4 h prior to intravenous ceftriaxone administration (1g). Serial blood samples were collected up to 48 h after ceftriaxone administration and plasma samples were analysed by LC-MS/MS using 50 µL aliquots (total concentration) and 100 µL (unbound concentration). **RESULTS**: A method to analyze total and unbound ceftriaxone in plasma using LC-MS/MS was developed and validated with linearity from 1 to 200 µg/mL. Both methods are sensitive, precise and accurate with coefficients of variation less than 15% in the study of inter- and intra-assay precision and accuracy. Ceftriaxone pharmacokinetics in healthy adults were described using a bicompartmental model, with a mean clearance of 0.96 L/h (CI95% 0.71-1.20) and $AUC_{0-\infty}$ of 1106 µg.h/mL (CI95% 811-1400) for volunteers that received only ceftriaxone; clearance of 0.95 L/h (CI95% 0.77-1.13) and $AUC_{0-\infty}$ of 1083 µg.h/mL (CI95% 876-1290) for volunteers that received ceftriaxone plus 25 mg of eltrombopag and clearance of 0.96 L/h (CI95% 0.74-1.19) and $AUC_{0-\infty}$ of 1072 µg.h/mL (CI95% 872-1273) for volunteers that received ceftriaxone plus 50 mg of eltrombopag. **CONCLUSIONS**: The results do not support the existence of a clinical pharmacokinetic drug interaction involving hepatic BCRP in human subjects receiving intravenous ceftriaxone and oral eltrombopag.

INTRODUCTION

Breast cancer resistance protein (BCRP) is an efflux protein of importance in pharmacokinetics and in drug-drug interactions (DDI) with other inhibitory drugs or substrates of this protein (1,2). BCRP is located in various tissues of humans, such as the intestine (apical brush border membrane of the enterocyte), liver (hepatocyte membrane canaliculi), kidneys (apical brush border membrane of proximal renal tubular cells), testis, placenta and blood-brain barrier, and its activity can affect the absorption and elimination of drugs that are substrates of this drug transporter (2,3).

The clinical relevance of BCRP in drug disposition has been demonstrated in several pharmacogenetic studies investigating the cDNA polymorphism c.421C>A (Q141K, rs2231142), in which the transport function of this efflux protein is decreased (421CA or AA) and the plasma concentrations of several drugs, such as rosuvastatin and atorvastatin, are increased (4–6). Thus, inter-individual differences in BCRP function contribute to the variability in drug exposure and efficacy of drugs that are BCRP substrates (5). In addition to genetic polymorphism, concomitant administration of BCRP inhibitor drugs may also increase systemic exposure of BCRP substrates. Patients with solid tumors treated with oral topotecan with elacridar, an inhibitor of BCRP and P-glycoprotein (P-gp), showed a significant increase of the oral bioavailability of topotecan, from 40 to 97% (7).

Experimental studies have also investigated the potential role for BCRP in DDIs. Intravenous administration of methotrexate, a BCRP substrate, in *Bcrp1* knockout mice or in wild-type mice pre treated with pantoprazole, an inhibitor of BCRP -

Corresponding Author: Vera Lucia Lanchote, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Av. do Café, s/n, Campus da USP, Ribeirão Preto, SP, Brasil; lanchote@fcfrp.usp.br

and P-gp, reduced methotrexate clearance (1.5 vs 0.8 L/h/kg) (8). Concomitant administration of eltrombopag (75 md/day for 5 days) with rosuvastatin 10 mg single oral dose increased plasma rosuvastatin exposure in healthy volunteers by 55%. The study authors speculated that this effect could be due to eltrombopag inhibition of OATP1B1 (reducing the hepatic uptake) and/or BCRP (enhancing rosuvastatin absorption and/or by inhibiting rosuvastatin elimination in the bile) (9). Later, Takeuchi et al. (2014) showed that the plasma concentrations of oral rosuvastatin were much higher in *Bcrp1* knockout mice when compared to *Bcrp1* wild-type mice, but the difference between the two strains was not so marked after intravenous administration of rosuvastatin (10). The cited authors also showed that transcellular transport of rosuvastatin in the apical to basal direction across Madin-Darby canine kidney II/BCRP/PDZ domain-containing 1 (MDCKII/BCRP/PDZK1) cells was elevated in the presence of 10μM eltrombopag and similar to that across (MDCKII/mock/PDZK1) cells, demonstrating that eltrombopag is a high-affinity substrate for BCRP and a potent inhibitor of intestinal BCRP (10). Elsby et al. (2016) showed that eltrombopag inhibited BCRP-mediated rosuvastatin transport with IC_{50} value of 2.1 μM in colorectal adenocarcinoma (Caco-2) cells BCRP inhibition assay utilizing rosuvastatin as BCRP probe substrate (6). Furthermore, eltrombopag was predicted to cause a DDI through intestinal BCRP inhibition in vivo (maximum theoretical gastrointestinal concentration/absolute inhibition constant ratio of 323), but not a DDI through hepatic BCRP inhibition in vivo (maximum unbound liver inlet concentration/absolute inhibition constant of 0.11), supporting that inhibition of intestinal BCRP may be the principal cause of the clinically observed BCRP-mediated DDIs. The cited authors reported that solitary inhibition of the intestinal BCRP by fostamatinib (100 mg twice daily for 5 days) increased up to a 2-fold rosuvastatin exposure in healthy volunteers (6).

Ceftriaxone is a third generation cephalosporin used in the treatment of bacterial infections caused by susceptible gram-positive and gram-negative microorganisms (11). It is used in paediatric and adult patients in the treatment of various infections, such as urinary tract and respiratory tract infections, septicemia, meningitis and the treatment of nosocomial infections caused by multidrug resistant pathogens (12–15). Ceftriaxone is highly bound to plasma proteins (83-96%) and eliminated in urine (40-67% of dose) and bile as the unchanged drug. Ceftriaxone is one of the cephalosporins with the highest biliary elimination (40-50% of the dose) compared to other beta-lactam antibiotics (16–18). The elimination of ceftriaxone through the biliary tract occurs through BCRP and MRP-2 (Multidrug Resistance-Associated Protein-2), which are expressed in the hepatocyte canalicular membrane (19). Ceftriaxone is a hydrophilic drug (log P = -2.1) with a volume of distribution of approximately 9 L, an elimination half-life of 6.2 h and a total clearance of 1 L/h in healthy adults (16,20).

Eltrombopag is a thrombopoietin receptor agonist, indicated for oral use in the treatment of immune thrombocytopenic purpura (21). Hepatic uptake of eltrombopag, mediated at least in part by OATP1B1, is the rate-limiting process in its overall elimination. Eltrombopag can act as an inhibitor of OATP1B1 and also as a potent inhibitor of BCRP in the small intestine, with saturation being observed at around 10 μM, inferring that inhibition of BCRP by eltrombopag may occur at the clinical doses (10).

Results from pharmacokinetic modelling of in vitro studies predicted that orally administered eltrombopag will not inhibit hepatic BCRP (6); however, this prediction has not been tested clinically. The present study investigates for the first time in a clinical study, whether eltrombopag is also a hepatic BCRP inhibitor. Ceftriaxone was administered intravenously to healthy volunteers as a probe drug, since 40-50% of its elimination is biliary and dependent on hepatic BCRP. In addition, this study also reports the analysis of total and unbound ceftriaxone in small plasma aliquots (150 μL) via LC-MS/MS.

MATERIALS AND METHODS

Chemicals and reagents

Ceftriaxone (ceftriaxone hemi-heptahydrate disodium salt, purity: 98%) was purchased from Toronto Research Chemical (Ontario, Canada). Cefazolin was kindly supplied by Eli-Lilly (São Paulo, Brazil). The solvents and solutions used in the extraction procedures (acetonitrile (ACN), JT Baker, Xatoloc, Mexico; potassium phosphate monobasic (KH_2PO_4), VETEC QUIMICA, Sigma-Aldrich, Brazil and chloroform, JT Baker, Phillipsburg, NJ, USA) and the mobile phase of the chromatographic system, were obtained from Merck (Darmstadt, Germany) as chromatography grade reagents. Diethylamine (JT Baker, Phillipsburg, NJ, USA) was PA grade. Water was obtained from the Milli-Q® Plus purification system (Millipore Corp., Bedford, MA, USA).

The stock solution of ceftriaxone was prepared at a concentration of 2000 μg of the freebase/mL of

water. Working solutions were prepared by diluting the stock solution to the final concentrations of 2, 8, 16, 40, 80, 160, 240 and 400 µg/mL water.

The internal standard solution (IS) of cefazolin was prepared at a concentration of 200 µg/mL of water. From this solution, dilution was performed to obtain a solution of 100 µg of cefazolin/mL water.

Chromatographic analysis

The HPLC system consisted of a Shimadzu chromatograph (Kyoto, Japan) equipped with an LC-10 AD pump and a CTO-10 AS oven. Ceftriaxone elution was performed on the Nova Pak® C18 column (Waters) with particles of 4 µm (150 x 3.9 mm) and the mobile phase ammonium acetate ($AcNH_4$)(10 mmol/L, pH 6.57 adjusted with diethylamine, 95%): ACN(5%) with a flow rate of 1 mL/min at 30 °C.

The mass spectrometry detection system was the Quattro Micro LC triple quadrupole (Micromass, Manchester, UK) equipped with an electrospray interface (ESI). Analyses were performed in the positive ionization mode. The capillary voltage in the ESI was 3 kV. The source and desolvation temperatures were maintained at 150 and 280 °C, respectively. Nitrogen was the nebulization gas at a release speed of 400 L/h, and argon was the collision gas at an approximate pressure of 2.00 x 10-4 mbar. The cone voltage was kept at 15 V for ceftriaxone and 20 V for the IS. The collision energy was 15 eV for ceftriaxone and IS.

Optimization conditions in MS/MS were obtained by the direct infusion of standard solutions of ceftriaxone (100 µg/mL of water) and cefazolin (100 µg/mL of water) incorporated in the mobile phase, through a diffusion pump that released 10 µL/min. Analyses were conducted in the MRM mode (Multiple Reaction Monitoring). Protonated ions $[M+H]^+$ and respective ion products were monitored in transitions 554 > 396 for ceftriaxone (see Fig. 1) and 455 > 323 for IS (see Fig. 2). Data acquisition and sample quantification were performed with the MassLynx program, version 3.5 (Micromass, Manchester, UK).

Sample preparation

Blank plasma of healthy volunteers (not treated with ceftriaxone) were provided by the Hemocenter of the University Hospital, Faculty of Medicine, University of São Paulo, Brazil. For the analysis of total ceftriaxone, aliquots of 50 µL of blank plasma (local hospital) spiked with 25 µL of IS solution (cefazolin, 2.5 µg) were added to 25 µL of water and 50 µL of KH_2PO_4 buffer (1 mol/L, pH 8.0) and precipitated with 200 µL of acetonitrile. After shaking in the mixer (30 s) and centrifugation at 10 min at 3000 g, 300 µL of the supernatant was collected, and 100 µL of chloroform was added. The tubes were again shaken in the mixer (30 s), 70 µL of the supernatant was collected, and 40 µL was submitted for analysis. For the analysis of unbound ceftriaxone, aliquots of 100 µL of plasma were centrifuged in the Centrifree® device (Millipore Corporation, Billerica, MA, USA) for 80 min, and 50 µL aliquots of the ultrafiltrate were added to 200 µL of acetonitrile and 25 µL of IS. After shaking in the mixer (30 s), 200 µL of the supernatant was collected, and 60 µL was submitted for analysis.

Determination of the matrix effect

The matrix effect was evaluated with 8 plasma samples, including 4 normal, 2 lipemic and 2 hemolyzed samples. Plasma samples were processed according to item sample preparation. The plasma extracts were spiked with the IS and with ceftriaxone at concentrations equivalent to low quality control (LQC) and high quality control (HQC). For each sample, the normalized matrix factor was calculated by the internal standard normalized matrix factor (ISTD), according to the following formula:

ISTD = (Analyte response in matrix/Response of internal standard in matrix)/(Response of analyte in solution/Response of internal standard in solution).

The results were expressed as coefficients of variation (CV) of the ISTD for all samples, LQC and HQC. A matrix effect was considered absent if ISTD had a CV below 15%.

Validation

The methods were validated according to the recommendations of the U.S. Food and Drug Administration guide for the industry in the validation of bioanalytical methods (22). To determine linearity, calibration curves were constructed by analyzing aliquots of 50 µL of blank plasma for total ceftriaxone and 100 µL of blank plasma for unbound ceftriaxone spiked with 25 µL of each of the ceftriaxone solutions. Peak area ratios (analyte/IS) were plotted according to respective plasma concentrations (1, 4, 8, 20, 40, 80, 120 and 200 µg of unbound and total ceftriaxone per milliliter) with a weighting factor of 1/X. The linear regression equations and the correlation coefficients were calculated.

The method was considered linear until the highest plasma concentration showed a deviation less than or equal to 15% of the nominal

concentration and with a linear correlation coefficient equal to or greater than 0.98.

The limit of quantification was defined as the lowest plasma concentration of ceftriaxone quantified with a precision of 20% and an accuracy of 80-120%. Thus, 5 replicates were analyzed at the concentration of 1 μg ceftriaxone/mL plasma.

Quality controls were prepared in blank plasma at concentrations of 1.0 (lower limit of quantification quality control-LLQC), 2.4 (low quality control-LQC), 80 (medium quality control-MQC), 160 (high quality control-HQC) and 1600 (dilution quality control-CQD) μg of ceftriaxone/mL plasma.

Recovery was analyzed with blank plasma samples spiked with ceftriaxone (2.4 and 160 μg/mL) and IS. Recovery was calculated via the direct comparison of the peak areas of extracted samples with ceftriaxone standards peak areas and IS in solution, directly injected into LC-MS/MS.

The precision and accuracy of the methods were evaluated in intra- and inter-assay experiments. The plasma solution containing ceftriaxone was prepared using blank plasma spiked with ceftriaxone standard solutions at three different known concentrations: 2.4, 80 and 160 μg of ceftriaxone/mL. Subsequently, aliquots of these solutions were stored at -70 ° C until analysis. For the quantification of intra-run precision and accuracy, five aliquots of the concentrations mentioned above were analyzed in a single analytical assay and the results were calculated with a freshly prepared calibration curve. For the quantification of inter-assay precision and accuracy, five aliquots of the concentrations were analyzed on three consecutive days, and the results were calculated with a calibration curve newly prepared for each analytical run.

The dilution procedure was analyzed for samples originally above the upper limit of quantification. For this analysis, a 1600 μg/mL plasma solution was prepared similarly to the solutions mentioned above. A volume of 25 μL of this plasma solution was added to 225 μL of blank plasma (dilution 1 to 10). After homogenization for 30 s in *vortex*, 25 μL of this diluted sample was processed as described in the sample preparation section.

To evaluate the stability of the samples, short duration, freeze-thaw and post-processing cycles were performed. For the evaluation of short-term stability, three aliquots of plasma ceftriaxone solution were kept at room temperature for 4 h, after which time the samples were analyzed as described previously. For the evaluation of the stability of the freeze-thaw cycles, three aliquots of each solution were frozen at -70 °C for 24 h and then thawed at room temperature. Successive cycles of freezing and thawing were performed and the samples were analyzed after the third cycle. For the evaluation of post-processing stability, extracts from three aliquots of ceftriaxone solution were maintained within the automatic injection system at 5 ± 1 °C for 12 h prior to LC-MS/MS injection. The results obtained were compared with those obtained by analyzing freshly prepared samples and expressed as relative standard error (% inaccuracy).

Clinical protocol

The study was approved by the Research Ethics Committee of the Hospital das Clínicas of the Medical School of Ribeirão Preto, University of São Paulo, Brazil. The sample size was calculated with a Power and Sample Size Calculation program (23) using the AUC of ceftriaxone in healthy volunteers (AUC 1006 μg.h/ml, standard deviation 118 μg.h/mL) (24). The inclusion of 4 volunteers in each group resulted in a significance level of $p < 0.05$, a test power of 80% and a difference between AUC means of at least 30% (ceftriaxone vs. ceftriaxone + 25 mg eltrombopag or ceftriaxone + 50 mg eltrombopag).

This study enrolled twelve healthy adult volunteers. The volunteers were informed in detail about the study proposal, duration and possible risks involved. After signing the *Informed Consent Term*, the volunteers underwent clinical examination and laboratory tests to evaluate liver (ALT, AST, gamma GT, bilirubin and total proteins) and renal functions (urea, plasma creatinine and creatinine clearance (Cockroft-Gault formula)). After 8 h of fasting, the volunteers received a single intravascular dose of 1 g of ceftriaxone (at a concentration of 0.1 g/mL by intravascular infusion for 3 minutes) (Rocefin®, Roche, RJ, Brazil) with or without eltrombopag (Revolade, GlaxoSmithKline, RJ, Brazil). The studies were conducted in full compliance with the principles of the Helsinki Declaration and local laws and regulations related to clinical trials.

The clinical protocol consisted of 3 groups according to the presence (two different doses) or absence of eltrombopag. The first group (n = 4), after 12 h of fasting, received 1 g of ceftriaxone diluted in 10 mL of water in the morning (intravenous administration for 3 minutes). The second group (n = 4) was treated orally with two doses of 25 mg of eltrombopag: the first dose was administered 28 hours prior to ceftriaxone administration, and the second dose was administered four hours prior to ceftriaxone administration. The third group (n = 4) was treated orally with two doses of 50 mg of eltrombopag; the

first dose was administered 28 hours prior to ceftriaxone administration, and the second dose was administered four hours prior to ceftriaxone administration. The standard hospital diet was served 3 hours after ceftriaxone administration. Blood samples (5 mL) were collected in heparinized syringes (Liquemine® 5000 IU, Roche) 0, 0.5, 1, 2, 4, 8, 12, 18, 24, 32, 40 and 48 hours after the initiation of the ceftriaxone infusion. Plasma aliquots for chromatographic analysis were obtained by centrifugation of the blood samples (3000 g for 10 min) and then stored at -70 °C until analysis.

Pharmacokinetics and statistical analyses

The pharmacokinetic analysis of ceftriaxone was performed with Phoenix® WinNonlin®, version 6.4 (Pharsight Corp, St Louis, MO, USA). Pharmacokinetic parameters were calculated based on experimentally obtained plasma concentrations. Plasma concentration *versus* time data following the intravenous administration of ceftriaxone were analyzed based on the two-compartment model. Statistical analysis was performed, using ANOVA and Tukey post test and graphs were created with R (R Foundation for statistical computing, Vienna, Austria, 2014, version 3.1.2 R).

RESULTS

Analysis of ceftriaxone in plasma

Figures 1 and 2 show the protonated ions and their respective product ions monitored at transitions 554>396 and 455>323 for ceftriaxone and IS, respectively.

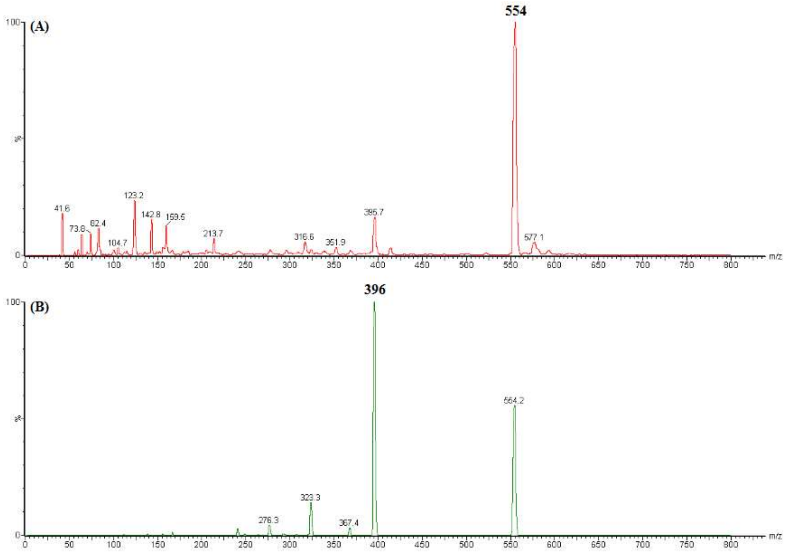

Figure 1. Mass spectra of the protonated molecular ion (A) and the product (B) ion of ceftriaxone.

Figure 2. Mass spectra of protonated molecular ion (A) and product (B) ion of cefazolin.

Figure 3 shows the chromatograms related to the analysis of total ceftriaxone and Figure 4 shows the chromatograms related to the analysis of unbound ceftriaxone in healthy volunteer plasma 0.25 h after the administration of 1 g of ceftriaxone IV. The retention times for ceftriaxone and IS are shown in Table 1 as ceftriaxone total plasma concentration and as ceftriaxone unbound plasma concentration. Table 2 shows the ceftriaxone stability data as total plasma concentration.

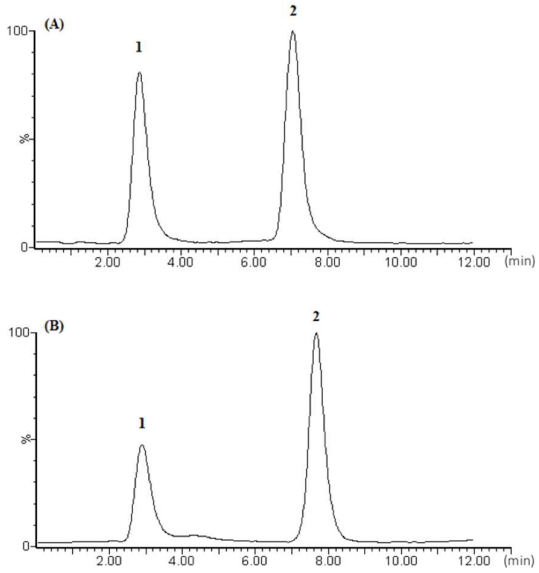

Figure 3. Chromatograms referring to the analysis of total plasma ceftriaxone. (A) Human plasma spiked with ceftriaxone (200 µg/mL) and cefazoline (50 µg/mL). (B) Plasma volunteer 0.25 h after administration of 1 g ceftriaxone IV. 1-ceftriaxone, 2-cefazoline

Table 1. Retention times of ceftriaxone and cefazolin when analysed as total and unbound plasma concentration

Drugs	Retention time (min)	
	Total	Unbound
ceftriaxone	2.85	1.10
cefazolin (IS)	7.62	1.12

Table 3 depicts the validation data regarding total and unbound plasma ceftriaxone concentrations. Precision and accuracy results are acceptable for a quantification limit of 1 µg/mL plasma, linearity from 1 to 200 µg/mL plasma. The recovery of LQC (2.4 µg/mL) and HQC (160 µg/mL) ranged from 106-117%, and IS recovery was approximately 107%.

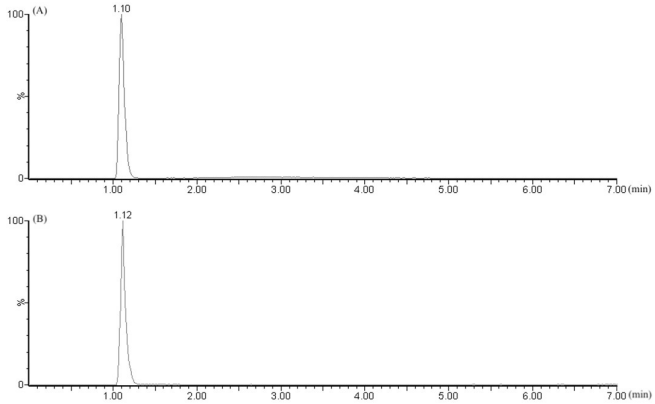

Figure 4. Chromatograms show the analysis of unbound ceftriaxone in plasma. Volunteer plasma 0.25 h after administration of 1 g ceftriaxone IV, (A) ceftriaxone and (B) cefazoline (IS; 50 µg/mL).

Table 2. Stability study of the analytical method of total plasma ceftriaxone concentration.

Condition	% Loss
4 h duration,	
2.4 µg/mL	2.38
160 µg/mL	0.27
Freezing / thawing cycles	
2.4 µg/mL	2.20
160 µg/mL	1.75
Post-processing (12h)	
2.4 µg/mL	3.27
160 µg/mL	6.42

Characteristics of volunteers

The physical characteristics of volunteers are presented in Table 4 as the mean and 95% CI. Age, weight, height, BMI and creatinine clearance values did not differ ($p \leq 0.05$) among the eltrombopag 0, 25 and 50 mg groups.

Ceftriaxone pharmacokinetics

The pharmacokinetics of total ceftriaxone are shown in Figure 5 (according to the use of eltrombopag) and in Table 5 (mean and 95% CI). The data show similar pharmacokinetic parameters among the 3 groups (eltrombopag 0 mg, eltrombopag 25 mg, eltrombopag 50 mg). Table 5 shows the percentage of unbound ceftriaxone observed in 12 healthy volunteers.

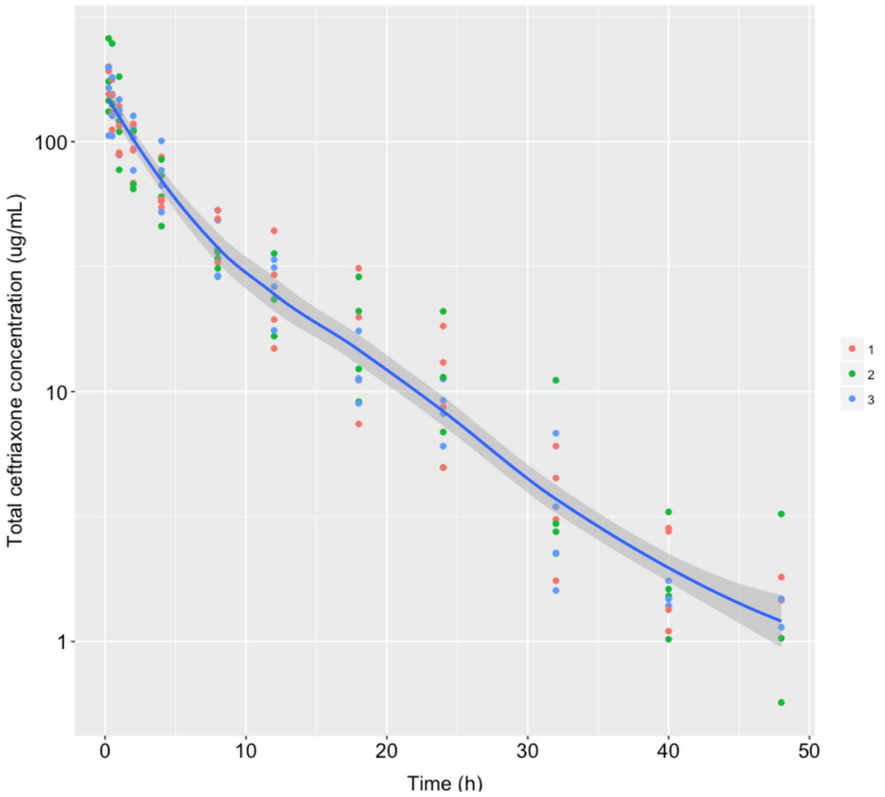

Figure 5. Plasma concentration *versus* time of ceftriaxone in healthy volunteers (n = 12) treated with single IV dose of 1g ceftriaxone. The graph represents the mean and 95 % confidence interval (CI) for the data. (1) Volunteers treated only with ceftriaxone, (2) volunteers treated with ceftriaxone and 25 mg of eltrombopag, (3) volunteers treated with ceftriaxone and 50 mg of eltrombopag

Table 3. Parameters validation of the analytical methods of total and unbound plasma ceftriaxone concentrations.

	Total ceftriaxone	Unbound ceftriaxone
Absolute recovery (%)		
2.4 µg/mL	117	---
160 µg/mL	106	---
Linearity (µg/mL)	1 – 200	1 – 200
Linear equation	y=0.005x+0.001	y=0.006x+0.001
Coefficient of determination	$r^2 = 0.99$	$r^2 = 0.99$
Limit of quantification (µg/mL)	1.0	1.0
Precision (CV %, n = 5)	16.4	14.0
Accuracy (Inaccuracy %)	2.8	0.2
Intra-assay precision (CV %)		
2.4 µg/mL (n=5)	9.0	6.9
80 µg/mL (n=5)	9.3	1.9
160 µg/mL (n=5)	10.5	3.9
Inter-assay precision (CV %)		
2.4 µg/mL (n=15)	9.6	13.6
80 µg/mL (n=15)	13.8	13.2
160 µg/mL (n=15)	14.3	5.4
Intra-assay accuracy, %		
2.4 pg/mL (n=5)	-14.1	-4.9
80 pg/mL (n=5)	-4.5	12.7
160 pg/mL (n=5)	9.3	14.6
Inter-assay accuracy, %		
2.4 µg/mL (n=15)	-4.3	-4.9
80 µg/mL (n=15)	-2.8	12.7
160 pg/mL (n=15)	-2.5	14.6

CV = coefficient of variation; % Inaccuracy= $[(C_{obs}-C_{added})/C_{added}]$x 100

Table 4. Anthropometric characteristics of the investigated healthy volunteers (n = 12). Data are expressed as mean (95% CI).

	eltrombopag 0 mg (n=4)	eltrombopag 25 mg (n=4)	eltrombopag 50 mg (n=4)
Sex m/f	2/2	2/2	2/2
Age (years)	28 (20-35)	28 (27-32)	35 (28-43)
Weight (kg)	70 (52-87)	76 (54-97)	73.1 (53.9-92.2)
Height (m)	1.69 (1.59-1.78)	1.73 (1.61-1.85)	1.69 (1.59-1.80)
BMI (kg/m^2)	24.1 (20.2-28.0)	24.8 (21.0-28.6)	25.0 (21.5-28.5)
Creatinine *clearance* ($mL/min/1.73m^2$)*	120 (93-146)	122 (106-139)	110 (86-134)
Serum albumin (g/dL)	4.28 (4.13-4.42)	4.35 (4.22-4.48)	4.35 (4.22-4.48)

*Cockcroft-Gault formula.

Table 5. Total ceftriaxone pharmacokinetics in healthy volunteers (n = 12) treated with single IV dose of 1g of the drug according to eltrombopag association. Values are expressed as mean (95% CI).

Parameter	Eltrombopag 0 mg	Eltrombopag 25 mg	Eltrombopag 50 mg
Cmax (μg/mL)	207 (124-290)	207 (129-285)	170 (122-217)
$AUC^{0-\infty}$(μg.h/mL)	1106 (811-1400)	1083 (876-1290)	1072 (872-1273)
$t_{1/2}$ (h)	8.3 (6.8-9.8)	8.3 (6.3-10.2)	8.1 (5.1-11.1)
Vd_c (L)	5.5 (2.9-8.0)	5.3 (3.4-7.1)	6.3 (4.2-8.5)
Vd_{ss} (L)	9.3 (6.6-12.0)	9.2 (6.6-11.8)	8.8 (6.0-11.6)
MRT (h)	9.9 (8.1-11.6)	9.9 (6.4-13.4)	8.98 (7.7-10.3)
Cl (L/h)	1.0 (0.7-1.2)	1.0 (0.8-1.1)	1.0 (0.7-1.2)
Unbound (%)	9.9 (6.0-13.7)	10.9 (8.7-13.2)	10.0 (7.1-13.0)

ANOVA complemented with the Tukey post-test, $p<0.05$ (Eltrombopag 0mg vs Eltrombopag 25 mg) e Eltrombopag 0 mg vs Eltrombopag 50 mg).

DISCUSSION

The primary aim of the study was to clinically evaluate previous in vitro study predictions that oral eltrombopag acts as an inhibitor of intestinal BCRP but not hepatic BCRP (6). To test this question the pharmacokinetics of intravenous ceftriaxone (a predominately BCRP and MRP2 substrate) (18, 19, 25) was followed in healthy volunteers in the absence versus presence of therapeutic oral doses (25 or 50 mg) of eltrombopag.

Sensitive, selective and reproducible LC-MS/MS methods for the quantitation of total ceftriaxone from 50 μL of plasma and unbound ceftriaxone from 100 μL of plasma were developed and validated. The methods are suitable for pharmacokinetic and therapeutic drug monitoring studies up to 48 hours after the administration of a single dose of 1 g intravenous ceftriaxone. A previous LC-MS/MS reported by Ongas et al. (2017) reported volumes of 350 μL plasma for the quantification of total and unbound ceftriaxone

(26). Lefeuvre et al. (2017) reported an LC-MS/MS method for quantification of total ceftriaxone in plasma samples using plasma volumes of 100 µL (27). Then, the small volumes of plasma used in the present study represents an important advantage, in special for pediatric studies.

One of the first studies to investigate the pharmacokinetics of ceftriaxone in adults was performed by Patel et al. (24). The authors reported the use of three single doses of ceftriaxone (0.5, 1 and 2 g) in a 30-min intravenous infusion in 12 healthy adult volunteers, with serial blood collections up to 24 hours after ceftriaxone infusion. The pharmacokinetic parameters reported by the authors at a dose of 1 g were similar to those obtained in the present study (Table 5), with the harmonic mean elimination half-life of 6 h, a mean AUC of 1006 µg.h/mL, a Vd of 9 L and a Cl of 1 L/h. Pollock et al. reported single dose and multiple dose pharmacokinetic parameters of ceftriaxone in healthy adult volunteers (28). The average pharmacokinetic parameters in the first dose were: Cl 1.057 L/h, Vd 9.66 L and $t_{1/2}$ 6.4 h. Payasi et al. evaluated the pharmacokinetics of ceftriaxone and sulbactam in 8 healthy adult volunteers. The results were also similar to those of the present study, revealing an average Cmax of 152.06 µg/ mL, a $t_{1/2}$ of 5.2 h and an AUC_{0-24} of 760.16 µg.h/mL (29).

The pharmacological activity of antibiotics depends on the unbound fraction in plasma and consequently, the concentration at the site of infection (30). The present study shows that the unbound ceftriaxone fraction in adults ranged from 6 to 13% (Table 5), which is similar to values reported in the literature (31-33). Stoeckel et al. reported that the binding of ceftriaxone to plasma proteins in adults ranged from 4 to 16.7% and was concentration-dependent (34). Popick et al. reported that ceftriaxone is highly bound to plasma proteins (95.3%) at a low plasma concentration (25 µg/mL) and decreased to 64.2% as the concentration was increased to 800 µg/mL (35). Schaad & Stoeckel quantified the unbound fraction of ceftriaxone in infants and children receiving intravenous ceftriaxone at a dose of 50 mg/kg, and the mean result was that 15.8 ± 0.03% of the ceftriaxone fraction was unbound to plasma proteins in infants, and 16.4 ± 0.03% was unbound to plasma proteins in children (36).

The inclusion of 4 volunteers in each group resulted in a significance level of $p < 0.05$, a test power of 80% and a difference between AUC means of at least 30% (ceftriaxone vs. ceftriaxone + 25 mg eltrombopag or ceftriaxone + 50 mg eltrombopag). So, this difference of 30% in AUC is significant in clinical research studies and it is considered a high difference in the pharmacokinetic studies by U.S. Food and Drug Administration (37).

Although the lack of eltrombopag plasma concentrations remain as a limitation in this study, therapeutic doses of eltrombopag (two doses of 25 or 50 mg administered 28 h and 4 h before intravenous ceftriaxone administration) did not alter the pharmacokinetics of ceftriaxone in healthy volunteers, suggesting that the eltrombopag does not inhibit hepatic BCRP. Thus, the results of our clinical study are in agreement with the in vitro results reported by Elsby et al. (2016) regarding eltrombopag as an inhibitor of only intestinal BCRP (6). Rosuvastatin exposure was higher (AUC by 55% and Cmax by 103%) in healthy volunteers following eltrombopag administration (75 mg po daily for 5 days) (9) and rosuvastatin exposure was also higher (AUC by 1.96-fold and Cmax by 1.88-fold) in healthy volunteers following fostamatinib administration (100 mg po twice daily for 5 days) due to intestinal BCRP inhibition.

CONCLUSIONS

The results do not support the existence of significant clinical pharmacokinetic drug interaction involving hepatic BCRP in human subjects receiving intravenous ceftriaxone (1g) and oral eltrombopag. possibly due to the lack of inhibition of hepatic BCRP by eltrombopag. Clinically, intravenously administered BCRP substrate drugs including ceftriaxone should not require dose adjustments in subjects taking oral eltrombopag.

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CONFLICT OF INTEREST

The authors report no conflicts of interest related to this work.

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