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Original Communication

Influence of food and sex on the pharmacokinetics and pharmacodynamics of furosemide

Laura Magallanes[1], Pietro Fagiolino[1,*], Marta Vázquez[1], Nikoletta Fotaki[2], Manuel Ibarra[1], Marianela Lorier[1], Virginia Bértola[3] and Anna Barindelli[3]

[1]Department of Pharmaceutical Sciences, Faculty of Chemistry, Bioavailability and Bioequivalence Center for Medicine Evaluation, Universidad de la República, Uruguay.
[2]Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom.
[3]Department of Clinical Laboratory, Faculty of Medicine, Universidad de la República, Uruguay.

ABSTRACT

The goal of this study was to investigate if food coadministration might change the pharmacokinetic and the pharmacodynamic responses of a 40-mg oral dose of furosemide in a similar way in male and female subjects. Twelve healthy Caucasian (8 women and 4 men) subjects that participated in a previous bioequivalence study under fasting condition, now received the same two oral formulations [Lasix® (Reference, R) and Furosemide EFA® (Test, T)] with food. Urinary excretion of unchanged drug (PK), and of chloride, sodium and potassium (PD) was monitored throughout time. PK and PD parameters were calculated from the respective excretion rate versus time curve. Since one of the volunteers (woman) abandoned the trial, ten subjects were retained for bioequivalence evaluation in order to maintain the TR/RT sequences of administration balanced. The furosemide excretion rates were significantly reduced in both formulations, but to a higher extent in men, whereas the chloride excretion rate was reduced similarly in both women and men after food coadministration. Sex-related differences in the relative bioavailability between the two formulations obtained after fasting state were overridden when furosemide formulations were coadministered with food. Food intake yielded similar gastric emptying in both sexes. Technological differences between the formulations could not be evidenced under fed condition. Physiological changes are evident not only in the luminal space of the gastrointestinal tract after the ingestion of food, but also in the cardiovascular system, showing intensive blood flow fraction redistribution among the organs. As the subjects were maintained in a resting state during the administration of the formulations both under fast and fed conditions, the increased blood flow fraction destined to the splanchnic region might have come from the renal region, and thereafter, the furosemide renal excretion and its saluretic effect diminished because of food ingestion. These hemodynamic changes could have had a higher impact on males since their renal blood flow fraction is normally higher in comparison with female subjects under fasting conditions.

KEYWORDS: furosemide, oral absorption, food interaction, sex-by-formulation interaction, PKPD correlations

INTRODUCTION

Furosemide is a loop diuretic that is commonly used in the treatment of edematous states associated with cardiac, renal and hepatic failures, and in the treatment of uncontrolled hypertension with abnormal renal function. Its oral bioavailability is low (67%) even if a solution of 40 mg is given

*Corresponding author: pfagioli@fq.edu.uy

to healthy male subjects [1]. In a previous study carried out with women and men [2], absolute bioavailability under fasting state was reduced to 51% revealing a sex-related influence on the oral absorption of furosemide. Sustained-release formulations rendered even lower drug absorption [3, 4]. Switching from intravenous bolus to oral slow-release dosage forms, more than three quarters of the administered dose were not absorbed. Therefore, drug dissolution, drug arrival at the absorption site and its intestinal permeation play significant roles in furosemide absorption as previously reported [5-7].

MATERIALS AND METHODS

Equipments

Furosemide urine concentrations were measured by high performance liquid chromatography coupled with ultraviolet detector (HPLC-UV) using the Dionex® Ultimate 3000 system equipped with a Phenomenex® Luna C18 reverse phase column (5 µm, 150 x 4.6 mm) kept at 40 °C.

Electrolytes (chloride, sodium, and potassium) in urine were measured with Cobas c311 analyzer (Cobas® 4000 analyzer series, Roche/Hitachi).

Chemicals

Assayed formulations were two brands marketed in Uruguay as immediate-release tablets containing 40 mg of furosemide: Furosemide EFA (Antia Moll, batch 028, expiration date Jan/2019, Test formulation), and Lasix® (Sanofi Aventis, batch 1D034M, expiration date May/2017, Reference formulation). Furosemide powder used as standard was obtained from Antia Moll Laboratory.

The organic solvents used for extraction of furosemide from the urine of volunteers (n-hexane [from Merck (Darmstadt, Germany)] and ethyl acetate [from Dorwil (Argentine)]) and for preparing standard solutions (methanol [from Pharmaco & Aaper (USA)]) were of analytical grade. Buffer prepared with potassium dihydrogen phosphate and phosphoric acid from Labsynth (Sao Paulo, Brazil), together with acetonitrile HPLC-grade (from Merck [Darmstadt, Germany]) was used as mobile phase for drug quantification in urine. The internal standard, phenytoin, was obtained from *Fármaco Uruguayo* Laboratory (Uruguayan pharmaceutical company).

Subjects and study design

Twelve Caucasian healthy volunteers (eight women and four men) between 21 and 37 years old with mean body weight (SD) of 64 (5) and 77 (21) kg for women and men, respectively, were enrolled. The study was composed of two phases. In phase 1, subjects were given two formulations containing 40 mg of furosemide under fasting condition. Results obtained from this phase were already published [7]. In phase 2, one month after the previous one, the same two formulations were administered to the subjects under fed conditions. Results obtained from this second phase constitute the subject of this communication and they are compared with those obtained at the first phase. The study with food coadministration was carried out with one tablet (40 mg) of Test formulation (T), or Reference formulation (R), administered with 200 mL of water immediately after breakfast intake, in a randomized, two-period, two-sequence (TR and RT), balanced crossover design. A one-week washout period was kept between both administrations. Study Protocols (phases 1 and 2) and Informed Consent forms 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 consents were obtained from all subjects before their entry in the phase 1 study. The complete study (phase 1 plus phase 2) was performed in the Bioavailability and Bioequivalence Centre for Medicine Evaluation, situated at the "Dr. Juan J. Crottogini" Hospital (Montevideo, Uruguay).

For the phase 2 study, volunteers came to the centre the first day of each week after an eight-hour overnight fasting period. Urine samples were collected before receiving breakfast and dosing and at 20, 40, 60, 80, 100, 120, 140, 160, 180, 210, 240, 300, 360, 420, 480, 600, and 720 min after dosing. The volume of urine was registered and two aliquots, one for electrolytes determination (chloride, sodium, potassium) and the other for furosemide quantification, were stored until analysis, refrigerated (2-8 °C) and frozen (-20 °C) respectively. Breakfast consisted of toasts with cheese and jam accompanied with 250 mL of yogurt. Standardized meals (lunch, tea, and dinner) were provided at 5, 8, and 12 h after dose administration. From 40 to 180 min post-dose,

and following each urine sampling, 70 mL of rehydration fluid, prepared after water dilution of Rehidroms®, was given to the subjects. After the collection of each urine sample at 210 and 240 min, 50 mL of rehydration fluid was given. From 4 h post-dose, volunteers were rehydrated with 100 mL of fluid after each urine sample collection. The fluid contained 15 mmol/L sodium, 4 mmol/L potassium, 13 mmol/L chloride, 15 mmol/L glucose, and 2 mmol/L citrate.

Chemical analysis of urine

Quantification of furosemide in urine was carried out by HPLC, based on a previously published method [8] with minor modifications. In brief, the assay was a liquid-liquid extraction of furosemide (with n-hexane/ethyl acetate [50/50]) from the urinary matrix (250 µL) by the addition of 250 µL of phosphoric acid (2.5 M) and 50 µL of a methanolic solution of internal standard (phenytoin, 40 µg/mL), followed by ten seconds of gentle agitation by vortex, separation of the organic phase, and evaporation until dryness under stream of nitrogen. Reconstitution of the residue was performed with mobile phase (buffer phosphate 0.02 M pH 3.5/acetonitrile [65/35]). A volume of 20 µL was injected onto the reversed-phase column and eluates were monitored at 230 nm. Retention times of analytes were 5.5 min (furosemide) and 7.7 min (phenytoin). Linearity was assessed between 0.5 to 20 µg/mL of furosemide in urine. The lower limit of quantification was 0.5 µg/mL, since intra-and-inter-day precision was below 20%, in terms of coefficient of variation, and accuracy. Otherwise, precision and accuracy was comprised between ±15% and within the 85-115% interval, respectively.

Chloride, sodium, and potassium electrolytes were measured in accordance with the instructions given by the manufacturers (Roche/Hitachi, package insert).

Pharmacokinetic (furosemide) and pharmacodynamic (electrolytes) analysis

The total volume of urine was multiplied by the concentrations of furosemide and ions in urinary samples in order to obtain the amount of analyte (ΔA) in each interval of time (ΔT). By summing all ΔA, the total amount of each analyte excreted in the urine was obtained for the 12-hour study period ($E_{0\text{-}12}$). Urinary excretion rates for each analyte (ER) were calculated by dividing ΔA by ΔT and the result was assigned to the middle of the ΔT interval. Urinary excretion rates of electrolytes were recorded as the pharmacodynamic responses (PDs) of the formulation given, and urinary excretion rate of furosemide as the corresponding pharmacokinetic response (PK). Non-compartmental PK-PD analysis for each analyte was performed over each ER *versus* time curve (Microsoft Office Excel 2007 software). The maximum ER (ER_{MAX}) and the time-to-peak (T_{MAX}) were recorded from the experimental data of each volunteer and analyte. The area under the ER-time curve from zero to infinite (AUC_{INF}) for furosemide was calculated using the trapezoidal rule until the last experimental time ($AUC_{11\text{-}hour}$), and extrapolated to infinite adding the term ER_{11}/k_{EL}, where k_{EL} is the first order elimination rate constant calculated from the slope of the terminal log-linear ER-time regression of data. AUC_{INF} corresponds to the total amount of furosemide excreted in urine (E_{INF}). Furosemide half-life ($T_{1/2}$) was calculated as $Ln(2)/k_{EL}$.

Statistical analysis

ER *versus* time curves for both electrolytes and furosemide are graphed as mean values ± standard error (SE). However, electrolyte versus furosemide ERs are presented graphically as mean values ± standard error of the electrolyte only, since it is set to assess if the same pharmacokinetic response could be associated with different pharmacodynamic responses. If there was a significant difference ($p < 0.05$) between two excretion rates of the electrolyte, which were associated with a similar excretion rate of furosemide, then a significant hysteresis for the PK-PD relationship was assessed.

Pharmacokinetic parameters for furosemide (ER_{MAX}, $E_{0\text{-}12}$, and E_{INF}), in logarithmic scale, were processed by analysis of variance (ANOVA, Microsoft Office Excel 2007 software), considering subjects, sequences, periods and treatments as variation sources. Coefficient of variation (CV) of the ANOVA was calculated according to the equation (1):

$$CV = 100\sqrt{e^{S^2} - 1} \qquad \text{(Equation 1)}$$

where S^2 is the residual variance of the ANOVA performed on the log-transformed parameters. A T-Wilcoxon test was used to evaluate the Test-Reference difference for T_{MAX}, since a non-normal

distribution was assumed. Average bioequivalence between T and R formulations, considering all the subjects, was confirmed if the 90% confidence intervals (90% CI) for the T/R ratio of the geometric means for each parameter were within the range of 0.80-1.25, and T_{MAX} did not differ significantly.

RESULTS

At the end of the whole study (phases 1 and 2) only one subject (female, 24 years, 68 kg) did not participate in the trial under fed condition. The remaining eleven subjects completed the assay with food coadministration. Table 1 summarizes the main pharmacokinetic parameters obtained for the subjects assayed under fed condition. Parameters for all electrolytes are summarized in table 2.

Data in table 1 and in our previous report [7] show that E_{INF} (±SE) for formulation R decreased from 9.49 (±0.91) to 8.35 (±0.95) mg in women, and from 16.7 (±2.5) to 9.36 (±2.17) mg in men, when food was coadministered. While for formulation T, E_{INF} (±SE) fell from 10.4 (±0.59) to 8.29 (±1.22) mg in women, and from 16.3 (±2.2) to 10.0 (±1.86) mg in men. Considering ER_{MAX}, the comparison between fasting and fed conditions shows: 3876 (±456) and 2447 (±311) µg/h for R, and 4332 (±360) and 2579 (±273) µg/h for T in women, and 7980 (±1452) and 2954 (±446) µg/h for R, and 6960 (±1470) and 3075 (±479) µg/h for T in men.

PD responses to formulations expressed as chloride ER are shown here in table 2 and in Magallanes *et al*. ((2016) [7]), for fed and fasting conditions. The $E_{0\text{-}12}$ (±SE) for formulation R decreased from 198 (±20) to 196 (±14) mmol in women, and from 278 (±35) to 225 (±49) mmol in men, when food was coadministered. While for formulation T, $E_{0\text{-}12}$ (±SE) fell from 213 (±19) to 193 (±16) mmol in women, and from 224 (±18) to 236 (±39) mmol in men. Considering ER_{MAX}, the comparison between fasting and fed conditions shows: 116 (±6.9) and 80.5 (±6.9) mmol/h for R, and 120 (±9.2) and 77.6 (±9.2) mmol/h for T in women, and 157 (±18) and 90.3 (±13.4) mmol/h for R, and 114 (±17) and 91.7 (±19.5) mmol/h for T in men.

In order to balance the sequence of administration within the female group in the study under fed condition another woman (30 years, 65 kg) was dropped off from the statistical analysis. Hence, ten subject were retained either for T *versus* R or for fed *versus* fasting comparisons. Bioequivalence between formulations was not possible to be assessed under fed condition because of the low number of subjects (n = 10) and the high variability obtained in ER_{MAX} (CV = 32%; 90% CI of ER_{MAX} T/R ratio = [0.71-1.20]). Otherwise, the 90% CI

Table 1. Arithmetic means [± standard error] of pharmacokinetic parameters obtained in 11 healthy subjects (4 men and 7 women) after a single oral dose of 40 mg of furosemide from Test (T) or Reference (R) formulations under fed condition.

	T_{MAX} (h)[a]		ER_{MAX} (µg/h)		$E_{0\text{-}12}$ (mg)		E_{INF} (mg)		$T_{1/2}$ (h)	
	T	**R**	**T**	**R**	**T**	**R**	**T**	**R**	**T**	**R**
MALE	2.00	2.33	3075	2954	9.76	9.30	10.0	9.36	2.06	2.07
	[1.17-3.75]	[0.83-2.83]	[479]	[446]	[1.44]	[1.54]	[1.86]	[2.17]	[0.18]	[0.25]
FEMALE	2.50	2.83	2579	2447	7.54	8.32	8.29	8.35	2.28	1.70
	[0.83-3.75]	[1.17-3.25]	[273]	[311]	[0.47]	[0.85]	[1.22]	[0.95]	[0.20]	[0.29]
TOTAL	2.17	2.50	2759	2632	8.35	8.68	8.73	8.80	2.19	1.81
	[0.83-3.75]	[0.83-3.25]	[243]	[254]	[0.65]	[0.75]	[0.81]	[0.90]	[0.14]	[0.19]

[a]Median and range are given for T_{MAX}.

Table 2. Arithmetic means [± standard error] of pharmacodynamic parameters obtained in 11 healthy subjects (4 men and 7 women) after a single oral dose of 40 mg of furosemide from Test (T) or Reference (R) formulations under fed condition.

	T_{MAX} (h)[a]		ER_{MAX} (mmol/h)		E_{0-12} (mmol)	
	T	**R**	**T**	**R**	**T**	**R**
MALE						
Chloride	1.50 [0.83-2.50]	2.00 [0.83-2.50]	91.7 [19.5]	90.3 [13.4]	236 [39]	225 [49]
Sodium	1.50 [0.83-2.50]	1.83 [0.83-2.50]	79.9 [19.5]	79.3 [13.8]	205 [40]	209 [50]
Potassium	2.17 [0.83-2.83]	1.50 [0.83-2.50]	11.1 [1.1]	10.7 [1.3]	50 [3.9]	43 [2.9]
Urine	1.50 [0.83-2.50]	1.83 [0.83-2.50]	12.9 [1.9][b]	13.0 [1.8][b]	2433 [208][c]	2403 [369][c]
FEMALE						
Chloride	1.83 [0.83-3.75]	2.50 [0.83-3.25]	77.6 [9.2]	80.5 [6.9]	193 [16]	196 [14]
Sodium	1.83 [0.83-3.75]	2.50 [0.83-3.25]	71.5 [10.1]	73.9 [7.3]	184 [19]	186 [16]
Potassium	2.50 [2.17-2.83]	2.83 [2.50-3.25]	10.6 [0.9]	11.0 [1.0]	42 [3.9]	43 [4.0]
Urine	1.83 [0.83-3.75]	2.50 [0.83-3.25]	12.8 [1.4][b]	14.0 [0.8][b]	2311 [171][c]	2300 [150][c]
TOTAL						
Chloride	1.83 [0.83-3.75]	2.17 [0.83-3.25]	82.7 [8.9]	84.1 [6.3]	209 [17]	207 [19]
Sodium	1.83 [0.83-3.75]	2.17 [0.83-3.25]	74.6 [9.0]	75.8 [6.5]	192 [18]	194 [20]
Potassium	2.50 [0.83-2.83]	2.50 [0.83-3.25]	10.8 [0.7]	10.9 [0.8]	45 [3.0]	43 [2.7]
Urine	1.83 [0.83-3.75]	2.50 [0.83-3.25]	12.9 [1.1][b]	13.6 [0.8][b]	2355 [127][c]	2337 [154][c]

[a]Median and range instead of mean and standard error for T_{MAX}.
[b]Excretion rate of urine (mL/min).
[c]Volume of urine (mL).

of the E_{0-12} T/R ratio was comprised within [0.85-1.07] interval, revealing a similar amount of furosemide excreted in urine from both formulations. Mean furosemide ER curves in both sexes are shown in figure 1.

An exhaustive analysis was previously done by our group [7] on the influence that sex had on the urinary excretion rate of furosemide from these same assayed formulations under fasting state. Figure 2 shows the impact that sex has on each formulation when they were administered under fed condition.

Figures 3 and 4 show the impact that food intake had on each formulation in each subject. Similarly as under fasting state, a highly significant linear correlation between ER of urine (diuresis) and ER of chloride (data not shown) was obtained. Because of this, chloride ER was selected as the PD response of formulations. A clockwise hysteresis loop for the PK-PD relationship was evident and significant in both sexes. Figure 5 shows this PK-PD relationship without and with food coadministration.

DISCUSSION

Relative bioavailability between formulations in both sexes

Formulation R was characterized as having faster disintegration and slower dissolution with respect to formulation T [7]. These distinctive properties allowed R to start the absorption from the intestine of men prior to T because of the faster gastric emptying they have, and to absorb from the intestine of both women and men at a slower rate than T, when both formulations were given under fasting condition. Besides, the absorption

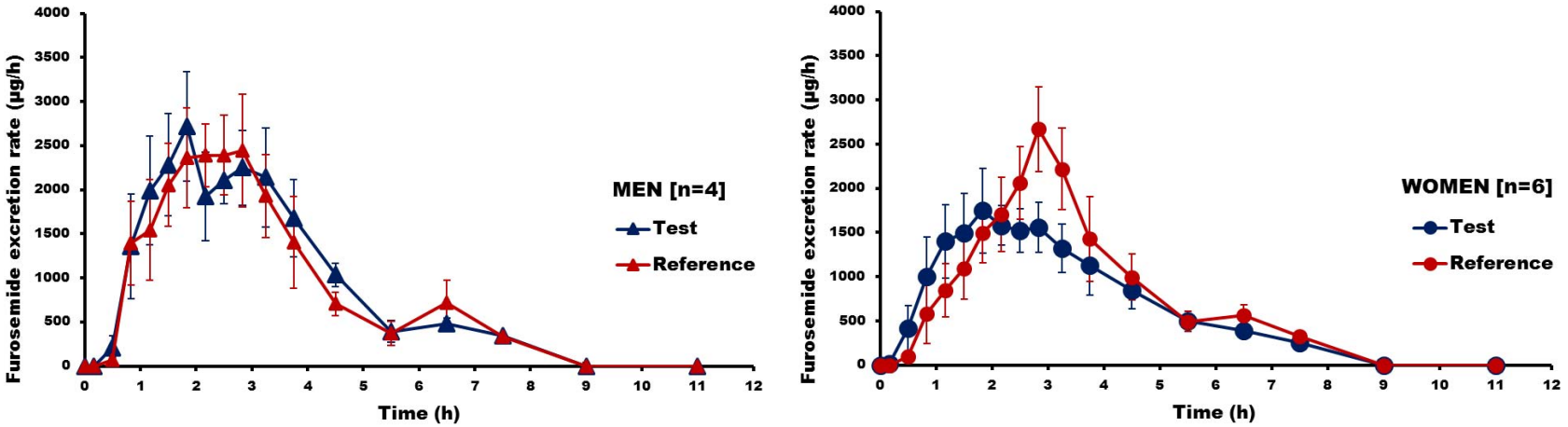

Figure 1. Mean (± S.E.) urinary excretion rate of furosemide following a 40-mg oral dose of Lasix® [Reference] and Furosemide EFA® [Test] administration to 10 healthy Caucasian subjects [4 men (left) and 6 women (right)] under fed condition.

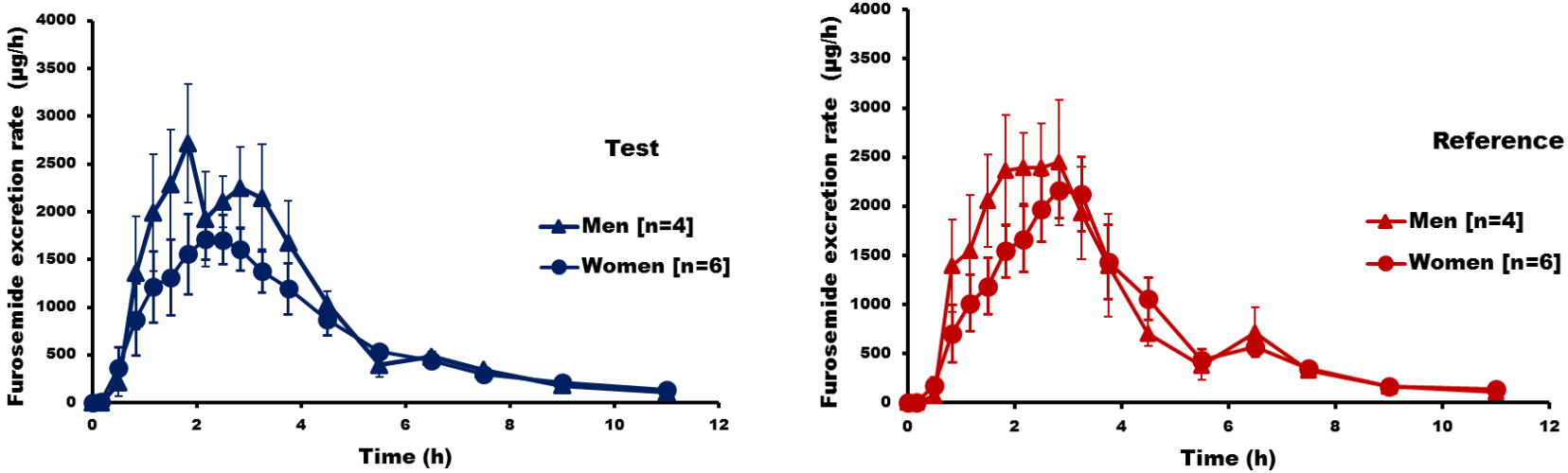

Figure 2. Mean (± S.E.) urinary excretion rate of furosemide following the oral administration of 40 mg of drug from Furosemide EFA® [Test] (left) and from Lasix® [Reference] (right) to 10 healthy Caucasian subjects under fed condition.

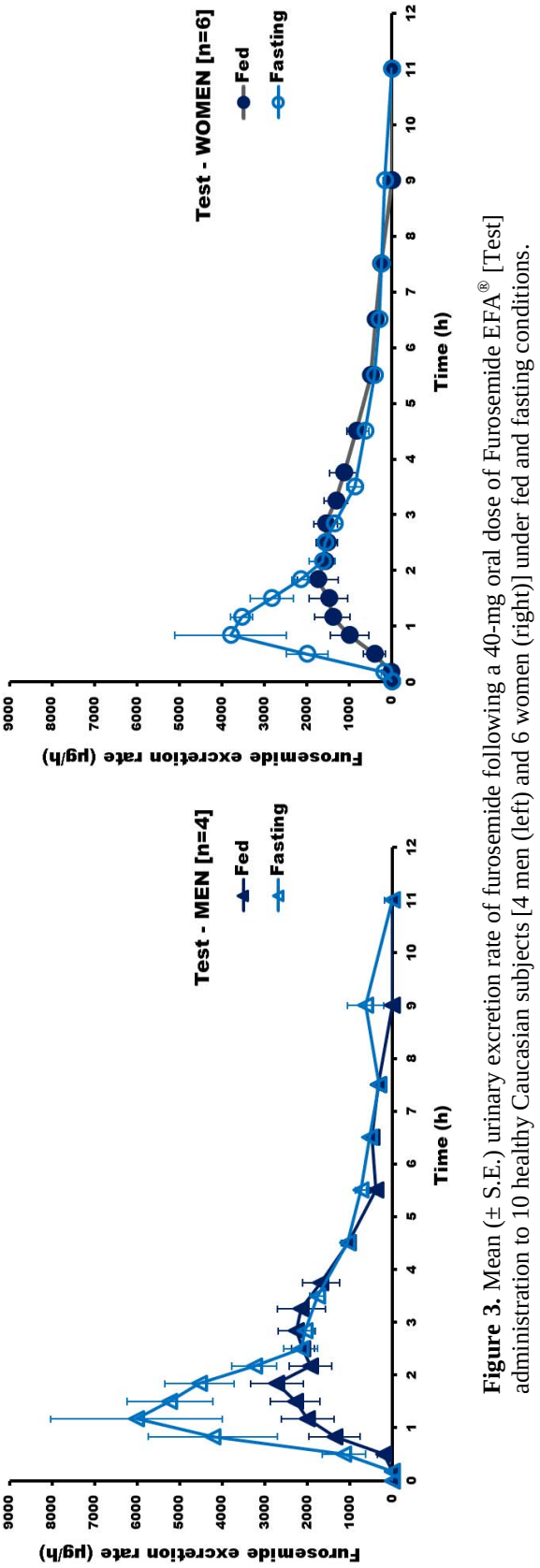

Figure 3. Mean (± S.E.) urinary excretion rate of furosemide following a 40-mg oral dose of Furosemide EFA® [Test] administration to 10 healthy Caucasian subjects [4 men (left) and 6 women (right)] under fed and fasting conditions.

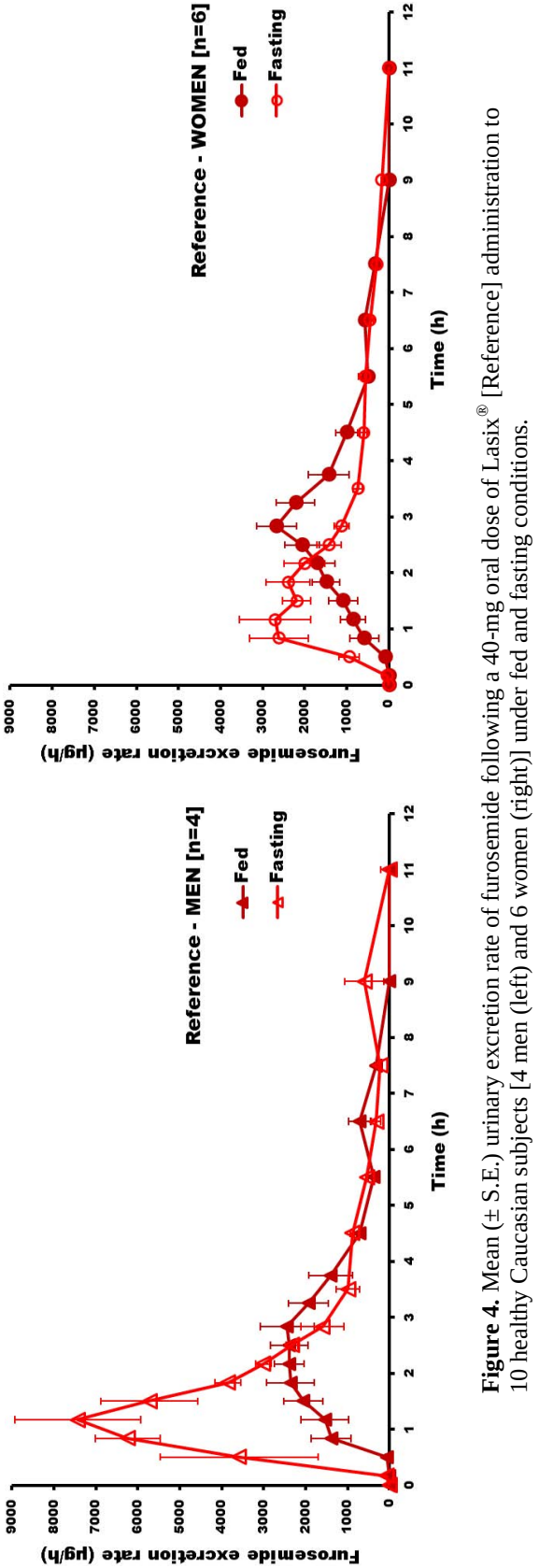

Figure 4. Mean (± S.E.) urinary excretion rate of furosemide following a 40-mg oral dose of Lasix® [Reference] administration to 10 healthy Caucasian subjects [4 men (left) and 6 women (right)] under fed and fasting conditions.

window for furosemide was concluded to be wider in men compared to women because of the lower intestinal pH men exhibit. This fact allows the drug to be absorbed to the same extent from both formulations in men but to a lesser extent from R in women [7].

T and R resulted to be bioequivalent under fasting condition [7]. Probably bioequivalence would have been maintained after food coadministration if the number of subjects had been higher (Figure 1). As it can be seen in figures 3 and 4, men extended the absorption of furosemide after food coadministration, diminishing drastically the rate and the extent, independently of the formulation they had received. Women also prolonged the absorption from both formulations, but the extent of absorption from R would fall in a less proportion than from T.

Our findings in men under fed state could be understood as a consequence of the prolonged gastric emptying food intake provoked, allowing both formulations to reach the intestine at the same time. Thus the absorption of furosemide was entirely controlled by the gastric delivery when food is coadministered. The situation seems to be similar in women, but even with a more extended gastric emptying (Figure 2). It was reported that because of the effect of progesterone, women show a slower gastric emptying caused by slower and weaker peristaltic movements [9-12], and hence, the passage of solid particles towards the duodenum requires a longer time with regard to liquid contents. This is the reason why the faster dissolving formulation, T, took a shorter time to leave the stomach. Conversely, formulation R might stay longer at the stomach since its dissolution is much slower at gastric pH. According to figures 2 and 4, it seems that the stomach of women increases the discharge of R to the duodenum throughout time, starting with the dissolved fraction (smaller content) and continuing with the solid one (higher content of drug).

Chloride ER throughout time, characterized by T_{MAX}, ER_{MAX} and $E_{0\text{-}12}$, showed the same behavior as the profile of furosemide ER. Taking into consideration bioequivalence, and considering all the subjects, these PD responses validate our conclusion reached under fasting state [7] and reaffirm our presumption that T and R would also be bioequivalent under fed state, which is in agreement with the analysis of PK responses discussed above. As a conclusion, T and R could be assessed as bioequivalent.

Relative pharmacodynamic response between both sexes

As it is shown in figure 5, similar PK-PD relationships were found under both fasting and fed conditions. Clockwise hysteresis loops were observed in both phases of the study, which is significant ($p < 0.05$) in women with both formulations under fasting and fed conditions, and in men with R in fasting state and with both formulations after food coadministration. As it was informed previously [7], under fasting condition, hysteresis could be observed in those cases where the formulation (R in men and women) or the individual physiology (women with T and R) allows the drug to be absorbed slowly. In men, drug from formulation T was so rapidly absorbed in fasting state that it was practically impossible to observe such hysteresis loop. Otherwise, after food intake, either in men or in women, with T or R, the slower gastric emptying controlled the absorption of furosemide.

Our results are in accordance with previous published findings [13, 14] and with those, where the administration of extended-release dosage forms showed a more efficient diuretic effect [4, 15]. Since the arterial plasma concentrations are the ones that command the arrival of a drug at its action site, a continuous supplying of molecules from the absorption site could maintain the effect for a longer period [16-18]. This is because the slower furosemide input could maintain its plasma concentration at a more intensive diuretic level while the absorption persisted. As shown under the 'Results' section, PD responses fell to a much lesser extent than their respective PK responses when comparing fasting and fed states, denoting that the slower absorption caused by food could maintain effective arterial concentrations and thus compensate the important decrease in the extent of absorption.

As a conclusion, in spite of the lower bioavailability of immediate-release formulation of furosemide after food intake, the reduction in its saluretic effect did not reach the same extent as in its bioavailability. This was because of an extended absorption of the drug.

The effect of food on furosemide excretion rate

ER of furosemide is related with its plasma concentration (C) by means of the renal excretion clearance (CL_{RE}) as shown in equation 2:

$$ER = CL_{RE} * C \quad \text{(Equation 2)}$$

Provided the CL_{RE} to the total clearance (CL_{total}) ratio remains constant when the formulations are switched, furosemide ER might surrogate plasma concentration and then bioavailability comparisons between T and R could be assessed from urinary data, as it was here done either under fasting [7] or fed conditions. However, for a given formulation, the assumption of CL_{RE}-to-CL_{total} constancy should not be longer accepted for inferring the fed-to-fasting bioavailability ratio from urinary data.

To understand this point we should consider the important blood flow redistribution that happens after food ingestion. Cardiac output fraction destined to the splanchnic region increases significantly during the digestion of food since great volumes of fluid are secreted to the gastrointestinal lumen, and the important metabolisms that take place in their cells require additional blood supplying. This increase in blood flow to this region leads to a decrease in blood flow to other parts of the body. To achieve efficient blood flow redistribution, well-perfused body territories such as the kidneys should be involved. Thus, if blood flow redistributions between renal and splanchnic regions were assumed, their respective regional furosemide clearances (CL_{RE} and CL_{SP}) would change in accordance with their cardiac output fraction changes [19-22]. Because of the short half-life of furosemide, its major plasma exposure practically overlaps with the interval of time spent in breakfast digestion. Then, blood flow changes may have significant impact on furosemide pharmacokinetic, diminishing its CL_{RE} and increasing its CL_{SP}. If blood flow fractions in the renal and the splanchnic regions were practically similar, as it could be assumed under resting state [12], and if during food digestion any changes in the renal blood flow were transferred entirely to the porta vein, a reciprocal change in both clearances would be expected.

Furosemide was shown to be excreted unchanged in the urine as 70% of the available dose [2, 20]. Moreover, its glucuronidation takes place mainly at the kidneys at a rate of 15% [14]. Therefore, nearly 85% of the clearance of furosemide operates at the renal region and the other 15% might take place at the splanchnic one (CL_{SP}). According to these data, food coadministration would reduce the CL_{total} significantly because of the higher impact that CL_{RE} reduction has in relation with the augmentation of CL_{SP}.

It was reported that the area under the furosemide plasma concentration–time curve (AUC) was reduced to a lesser extent than the furosemide urinary recovery moving from fasting to fed conditions [2]. Some doubts now arise about how accurate the information given by plasma data is, since according to our predictions the bioavailability loss when changing from fasting to fed might be underestimated because of the higher-than-predicted AUC with food, due to the lower CL_{total}. Urinary drug recovery is not more accurate than plasma concentration because it overestimates the actual loss of bioavailability, since food coadministration reduces CL_{RE} in a higher extent than CL_{total}. Then, the actual loss of bioavailability when furosemide is coadministered with food should be between urinary and plasma estimation. According to our data, when only urinary samples were collected, and considering the average of T and R, such loss could be estimated to be less than 17% (100*[1-[8.29+8.35]/[10.4+9.49]]) in women and less than 41% (100*[1-[10+9.36]/[16.3+16.7]]) in men.

It is known that gastric pH increases with the presence of food until supplementary gastric juice is secreted [12]. Food digestion continues in the intestine by means of both bile and pancreatic alkaline secretions, and furosemide suddenly increases its dissolution due to a strong ionization of the molecule. This ionized moiety is unable to permeate the intestinal mucosa and hence furosemide could go ahead along the gut without being absorbed [23]. This may be the cause of its decreased bioavailability in men when it is administered with food. In women, this loss of bioavailability might be lower since the intestinal pH at fasting condition was already more alkaline than in men [24], and consequently the food-related change in pH may be of less significance.

Nevertheless, one should bear in mind the effect that diuresis might have on the ER of furosemide.

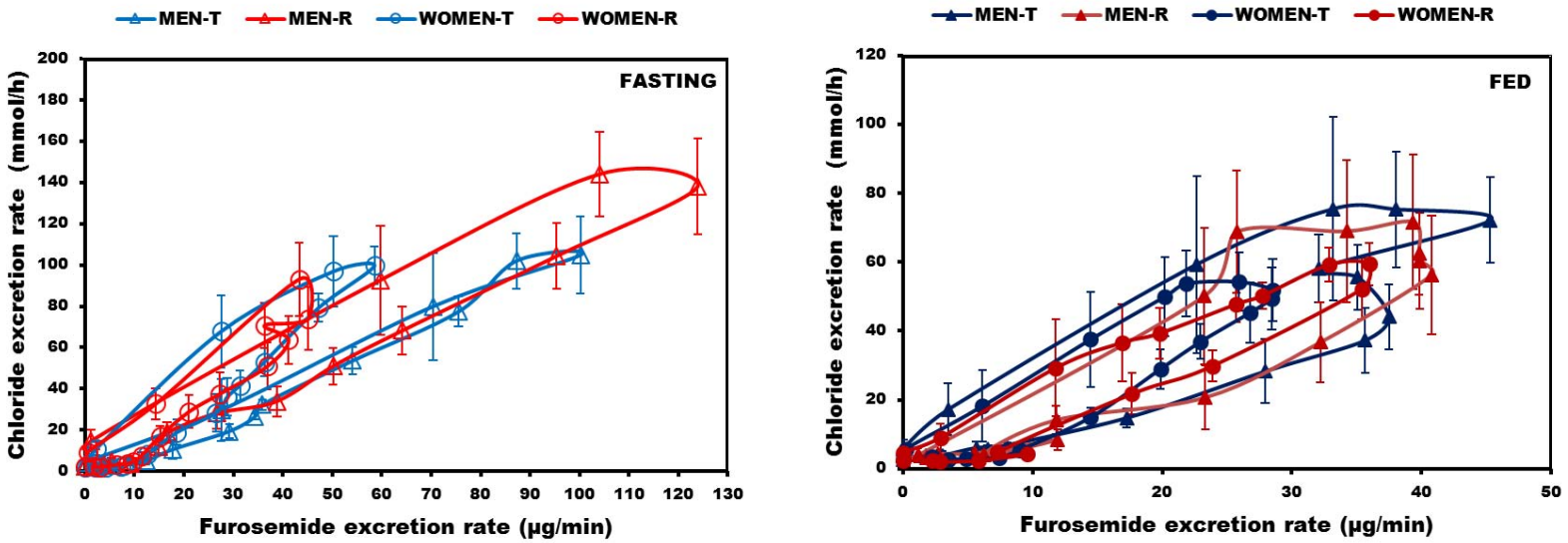

Figure 5. Mean (± S.E.) urinary excretion rate of chloride *versus* mean urinary excretion rate of furosemide after a 40-mg oral administration of Lasix® [R] and Furosemide EFA® [T] to 12 subjects under fasting condition (left) or 11 subjects under fed condition (right).

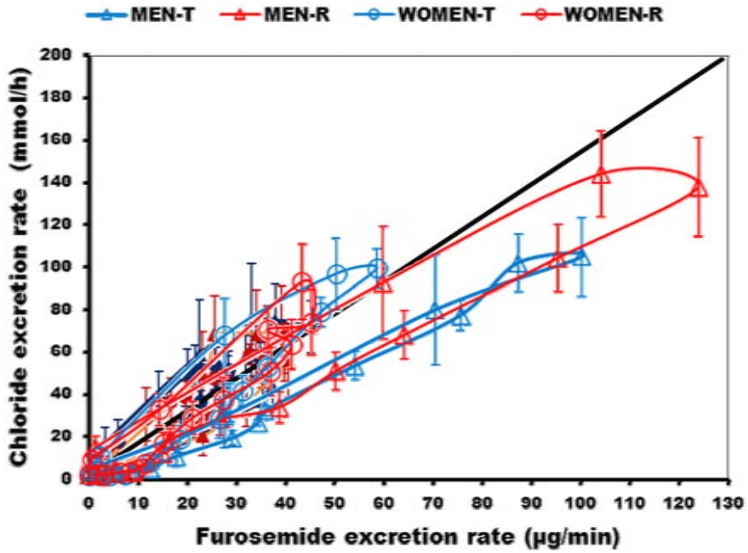

Figure 6. Superimposed graphs shown in figure 3 (upper) and the effect of straightening the furosemide urinary excretion rate just in men by a factor of 0.80 (lower).

Because of the higher cardiac output fraction destined to the kidneys in men [25], they would have a higher ER of furosemide than women under fasting condition [7]. Figure 5 (left) displays this difference between man and woman on the PKPD relationship. A higher furosemide ER was obtained in men at a given excretion rate of chloride. Under food coadministration, however, men and women seemed to display superimposed PK-PD hysteresis loops (Figure 5, right). Maybe food intake overrode such difference in renal cardiac output fraction.

Also noteworthy (Figure 6) is the superimposition of the hysteresis loops in women under both modes of administration, denoting a small redistribution of blood flows after food intake. Furthermore, there is an important change in the cardiac output distribution in men, maybe due to a significant reduction in the renal blood flow because of food digestion. This significant change in male might even overestimate the bioavailability loss when the urinary recovery of furosemide is measured.

As a conclusion, and considering the different magnitude of bioavailability loss overestimation that both sexes might have, the coadministration of food would reduce the extent of furosemide absorption from immediate-release formulations by a 15% in women, and by a two-fold higher percentage in men.

CONCLUSION

Men and women have dissimilar furosemide oral bioavailability of immediate-release formulations probably due to their differences in the intestinal pH, which in turn produces different absorption windows. The favorable intestinal environment that men had under fasting condition, with a higher absorption window, was drastically changed after food intake, leading to a severe loss in furosemide bioavailability. Conversely, the loss in bioavailability was lower in women when the formulations were given with food. Nevertheless, in both sexes, food coadministration extended the absorption of furosemide due to the prolongation of gastric emptying.

Saluretic effect was also affected by food but to a lesser extent. The clockwise hysteresis loop, which is characterized by a higher diuretic effect during drug absorption, opposed to what happens when only its elimination is operating, would have partially compensated the loss in bioavailability. This relevant fact observed in women under fasting condition, and in both sexes after food coadministration, emphasizes the importance of modulating the release of furosemide from the stomach by the use of gastroretentive formulations, and thus improving the efficiency of this drug.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interests to declare.

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