Itraconazole Bioequivalence Revisited: Influence of Gender on Highly Variable Drugs

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Abstract: Highly variable drugs have been defined as drugs with a residual variability of more than 30% in terms of the ANOVA coefficient of variation. Different approaches have been proposed during the last years to deal with this problem but the topic remains controversial. Itraconazole, a highly variable drug, has low bioavailability with a high CYP3A4 presystemic biotransformation. Also, it has a very poor aqueous solubility which is very dependent on the pH of the dissolution medium.

The pregnane X receptor (PXR) has been shown to mediate the genomic effects of progesterone and estradiol in the expression of the cytochrome P-450 gene family, which plays an important role in the metabolism of hormones and xenobiotics. During the menstrual cycle both hormone concentrations vary, providing a rationale for the more variable CYP3A4 activity in women.

The analysis of the data of an itraconazole bioequivalence study involving 24 healthy volunteers (12 men and 12 women) carried out by other investigators enables us to conclude that women have less oral bioavailability and more variable AUC than men. Low bioavailability seems to be related with the higher stomach pH observed in women and variability with the aforementioned menstrual cycle incidence on both pH and CYP3A4 expression. The lower variability observed in men made it possible to discriminate differences in AUC's variability displayed by each brand.

Keywords: Highly variable drugs, bioequivalence, itraconazole, gender.

INTRODUCTION

Highly variable drugs have been defined as drugs with a residual variability of more than 30% in terms of the ANOVA coefficient of variation (CV) [1]. Residual variability, sometimes referred as within-subject variability, depends on intra-individual variability, intra-drug-product variability and any other unidentified source of variation associated with the experimental procedures of these studies. The high CV obtained with this kind of drugs makes difficult to include the 90% confidence interval (CI 90%), of the test/reference geometric mean ratio, in the [0.80-1.25] bioequivalence range (BER).

Different approaches have been proposed during the last years to deal with the problem, some of them consisted in the use of a wider BER (for maximum plasma drug concentration (Cmax)), or in carrying out a experimental design with repetition (2-products 4-way crossover design), but the topic still remains controversial.

Itraconazole, a broad-spectrum triazole antifungal agent, is a CYP3A4 substrate [2] and highly variable drug with low bioavailability (55%).

The pregnane X receptor (PXR), a member of nuclear receptors, has been shown to mediate the genomic effects of progesterone and estradiol in the expression of the cytochrome P-450 gene family, which plays an important role in the metabolism of hormones and xenobiotics [3-5]. These sexual hormones are present in a higher level in women than in men and also participate in the control of the menstrual cycle. During the female cycle both hormone concentrations vary, with maximum levels at days 14 and 22, for estradiol and progesterone respectively. During the past several years a lot of information about intra-women variability and inter-gender differences has been published [6-15].

For woman variability the conclusions reached by the different authors are sometimes contradictory, but in some cases some variability related with the menstrual cycle has been found [13,16,17]. Hormone levels fluctuation and its consequence in the metabolic activity, makes premenopausal women less constant than men.

In the case of inter-gender pharmacokinetic differences the evidence is also controversial, but the tendency is to attribute an apparent high female CYP3A4 activity [18]. The activity of several other CYP (CYP2C19, CYP2D6, and CYP2E1) isozymes and the conjugation (glucuronidation) activity may be higher in males [6-7,11-12]. As a consequence, different oral bioavailability caused by sex differences in the activity of major intestinal and hepatic metabolic enzymes may be found.

It is important to remark that pharmacokinetic parameters, often used to assess differences between genders, not always reflect the enzyme activity or its expression, since they are also dependent on physiological variables like body weight, organ size, percentage of body fat, glomerular filtration rate, gastric motility, gastric secretion and many others. Then, deductions obtained from these parameters must be carefully analyzed taking into account all the variables that could affect them.

Gender differences have been reported for various drugs: ranitidine [13], verapamil [19], beta-blockers [11], selective

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serotonin reuptake inhibitors [11], erythromycin [11], dexamethasone [8], prednisolone [20], ifosfamide [21], alosetron [22], etc.. But more than pharmacokinetic differences among individuals, and between male and female volunteers, what really affects the conclusion on average bioequivalence in highly variable drugs is the intraindividual variability of subjects and the subject-by-formulation interactions.

Our Department reviewed the original data of a bioequivalence study between two itraconazole drug-products marketed in South America, in order to find an explanation of the high variability obtained. Next section only retrieve the experimental setting that becomes relevant for the analysis of data. For more details see the original article [23].

MATERIALS AND METHODS

Clinical Protocol

Twenty-four healthy volunteers, 12 men and 12 women, aged between 18 and 28 years (mean: 22.2), with body weight between 44 and 89 kg (mean: 69.3) received a postprandial single oral dose (200 mg) of each formulation (2 capsules) separated by a 14-day washout period in a randomized study with a two-way crossover design. The sequences were balanced for each gender group, six men and six women received reference-test and the others received test-reference. Mean age difference between genders was not significant, but female body weight was significantly lower than the male one ($p<0.01$; means: 56.3 *vs.* 77.2 kg).

Pharmacokinetic Processing

From each plasma drug concentration-time curve the following parameters were determined: 1) the experimental peak concentration (Cmax); 2) the time to reach Cmax (Tmax); 3) the area under the curve from 0 to 48 hours (AUC_{0-48}); and 4) the elimination rate constant (k_{el}), which was calculated by linear regression of data points belonging to the monoexponential terminal portion of the curve. Actually, blood samples withdrawn from 12 hours post-dosing

Table 1. Results Obtained with the ANOVA Test from the Data without Ln-Transformation. The Bioequivalence Conclusion with the Data Ln Transformed is not Affected

	Parameter	Men	Women	M+W
AUC0-48 (ng*h/mL)	AUCTest	1678	1173	1425
	AUCRef	1766	1233	1499
	AUCaverage	1722	1203	1462
	T/R	0.95	0.95	0.95
	CV (%)	27	62	43
	CI 90%	0.76-1.14	0.52-1.39	0.74-1.16
	CI 90% width	0.38	0.87	0.42
Cmax (ng/mL)	CmaxTest	135.8	108.0	121.9
	CmaxRef	154.0	109.0	131.5
	Cmaxaverage	144.9	108.5	126.7
	T/R	0.88	0.99	0.93
	CV (%)	45	49	48
	CI 90%	0.57-1.20	0.64-1.34	0.69-1.16
	CI 90% width	0.63	0.70	0.47
Tmax (h)	TmaxTest	4.8	4.3	4.5
	TmaxRef	3.9	4.8	4.3
	Tmaxaverage	4.3	4.5	4.4
Kel (h^{-1})	KelTest	0.0383	0.0347	0.0304
	KelRef	0.0390	0.0353	0.0316
	Kelaverage	0.0387	0.0350	0.0310
	T/R	0.98	0.99	0.96
	CV (%)	16	42	32
	CI 90%	0.84-1.09	0.64-1.28	0.81-1.12
	CI 90% width	0.25	0.64	0.31

T/R, test-reference ratio; AUC, area under the plasma concentration time curve from 0 to 48 hours; Cmax, maximum plasma concentration; Kel, elimination constant; Tmax, time for Cmax; CV, ANOVA's coefficient of variation; CI 90%, 90% confidence interval; CI 90% width, obtained by subtracting the CI 90% boundaries.

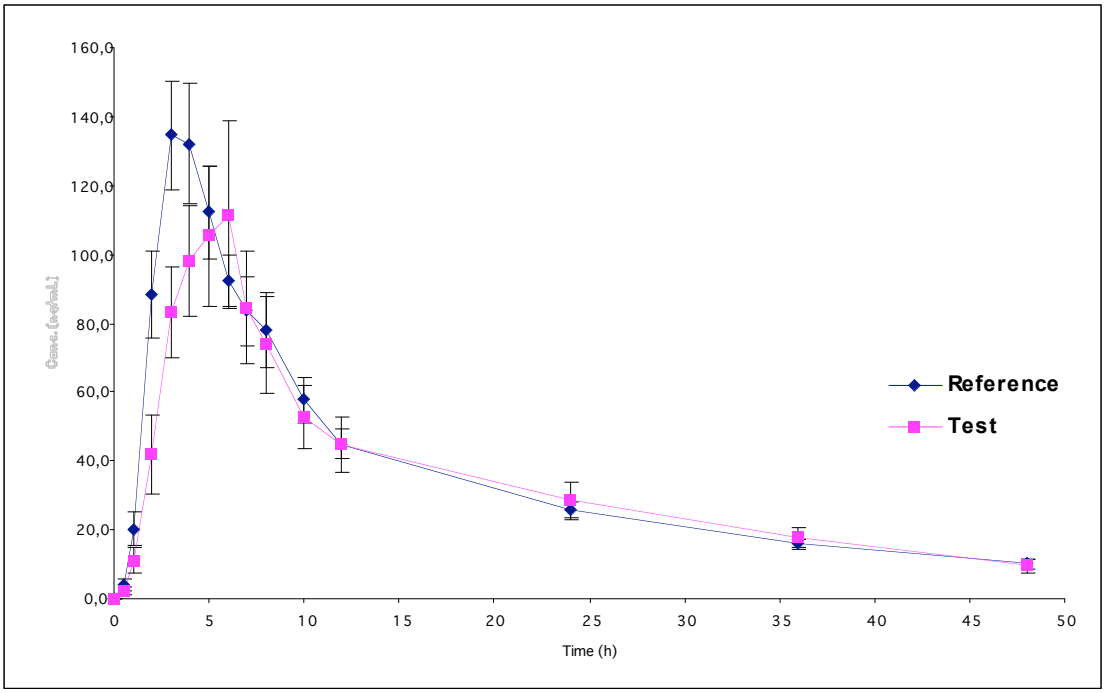

Fig. (1). Mean plasma concentration (± standard error) versus time curves for Reference and Test products, obtained in twelve healthy men.

were used to calculate k_{el} because a monoexponential profile was observed in all subjects.

Equation 1 stands for the area under the plasma drug concentration curve from zero to infinite (AUC), but since AUC_{0-48} did not represent less than 80% of the total area, it will be considered by this equation from now on. Then, AUC_{0-48} depends on the bioavailable dose (FD) and the clearance (CL) of subjects as AUC does.

$$AUC = (F.D)/CL \quad (1)$$

Since a significant gender difference in the body weights was detected, AUCs were multiplied by the respective weight of subjects ($AUC_{0-48}^{corrected}$) in order to obtain more information about gender comparison non related with their differences in size. Hence, this corrected area becomes more related with the systemic and presystemic loss of drug mass: elimination rate and bioavailability respectively.

Statistical Processing

Each pharmacokinetic parameter was analyzed by means of the ANOVA (analysis of variance) test, considering subjects, sequences, periods and treatments as sources of variation. Parameters were not log-transformed in order to calculate the coefficient of variation of the study (CV: residual standard deviation divided by reference mean), taking into account either all 24 individuals, or the 12 male subjects, or the 12 female subjects. Bayesian approach was used to estimate the 90% confidence intervals (CI) of test/reference arithmetic mean ratios (T/R), for each parameter.

Also, means, standard deviations (SD) and relative standard deviations (RSD) of each series of parameters (total, male and female) were calculated. Non-paired Student t-test or Mann-Whitney U-test were applied when necessary, depending on whether parameters displayed for each gender were normal distributed or not.

RESULTS AND DISCUSSION

The results obtained, considering 24 subjects, show that the test product can not be assessed as bioequivalent to the reference product because the 90% CI did not fall within the BER (Table **1**). The CVs obtained for AUC_{0-48} (43%) and for Cmax (48%) confirm that itraconazole, in these dosage forms, is a highly variable drug.

Table **1** also shows test-reference comparison by means of ANOVA-test, considering men and women as separated groups. Figs. (**1** and **2**) display mean curves (± standard error) obtained in both groups of volunteers. As it can be seen, women have, on average, lower AUC_{0-48} and Cmax than men, even though k_{el} displayed similar values. On the other hand, an important reduction in CV was observed for men when AUC and k_{el} were taking into consideration (27 and 16 % in men *vs.* 62 and 42% in women). This impacts on the 90% CI width for AUC_{0-48}, and then bioequivalence could have been assessed with this parameter if 24 male volunteers were recruited and the same residual variance and means were retrieved.

It was not the case for Cmax whose CVs maintain their higher value regardless of the gender of the subjects (45 and 49 % for men and women respectively). This is commonly observed with Cmax due to the higher variability that a parameter dependent on a single point data has.

Interesting results were obtained when AUC and k_{el} data were processed taking into account the brand of products. For both brands, men show higher AUC_{0-48} and $AUC_{0-48}^{corrected}$ mean values ($p<0.01$ and $p<0.001$) than women. Besides, while the test product showed gender-independent

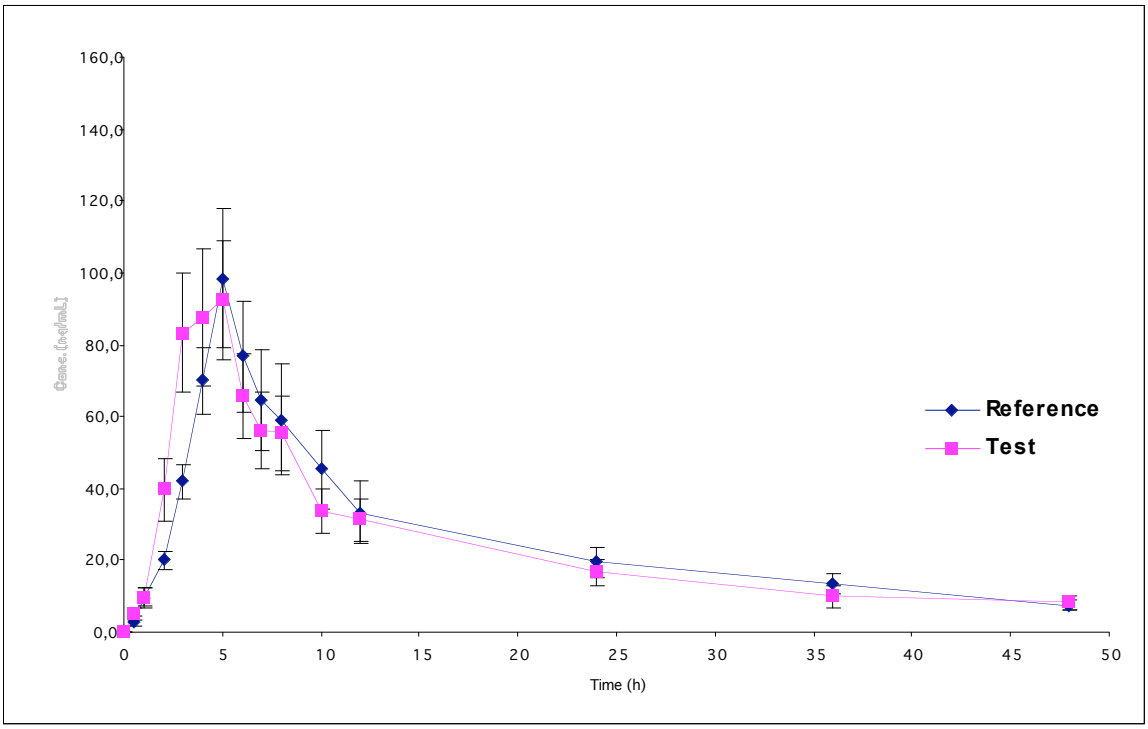

Fig. (2). Mean plasma concentration (± standard error) versus time curves for Reference and Test products, obtained in twelve healthy women.

RSD, the reference product displayed significant lower RSD in men than in women (Tables **2** and **3**).

Variability in k_{el} showed the same behavior as AUCs but in this case mean values were not different between men and women considering either reference or test product. Table **4** shows k_{el} values only for ten volunteers because calculation was very imprecise in some cases where few plasma levels were above the limit of quantification.

According to the results obtained from the reference product, AUCs differences between genders do not seem to be related with the rate of drug elimination. However the higher variability in drug elimination displayed by women might have significant impact on the higher variability in AUC. Conversely, data belonging to the test product do not enable us to detect any difference between intra-gender variabilities, then other unidentified source of variability owing to the test product could be more prevalent.

The previous paragraph could be rearranged saying that male subjects become more sensitive to the pharmaceutical form or brand variability than women due to their intrinsic low variability. Then, male volunteers might be the appropriate subjects in order to perform bioequivalence studies with this drug, not only because of their ability to conclude on average bioequivalence but also to detect differences between product variabilities.

The higher female variability could be explained by hormones fluctuations during the menstrual cycle, particularly progesterone that is an up-regulator of the CYP3A4 pathway. But their lower AUC values could not be explained by a higher clearance. This is supported by the fact that metabolic ratios (β-hydroxycortisol / cortisol), assayed in morning spot urine samples, were not different between genders [23], similarly as it was already mentioned about the elimination rate constant, k_{el}.

Itraconazole is a substrate of P-glycoprotein (P-gp) [24-27], a membrane efflux transporter which is more expressed in men than in women [11]. Enzyme and efflux carrier work concertedly in order to metabolize a drug. Even though women could have a higher expression of CYP3A4 compared to men [13, 18], the reciprocal expression in both the transporter (P-gp) and the enzyme (CYP3A4) could compensate the consequence on itraconazole metabolism and then systemic and presystemic clearance might result similar in both genders. This is in agreement with previous reports saying that clearance of P-gp substrates appear to be similar in men and women [12,15]. Besides, elimination of itraconazole was demonstrated to be straight dependent on its own inhibition, either at CYP3A4 or at P-gp level, after dose intake [23]. So, other physiologic functions, up-regulated or down-regulated by hormones, should be involved in order to understand itraconazole pharmacokinetic differences between genders, but oscillations caused by the menstrual cycle determine female higher variability.

If a similar clearance per kilogram is assumed for men and women, then female lower AUC must be caused by its lower bioavailability. Other steps apart from presystemic biotransformation should be involved. At this moment it should be taken into consideration the pKa of itraconazole and its very poor hydrosolubility. This drug only ionizes at a low pH, such as the gastric fluid [28]. For this reason, pharmaceutical companies recommend product intake a few minutes before or after a meal, in order to keep the drug in the stomach for a longer period of time.

Table 2. Analysis of AUC_{0-48} ($ng.h.mL^{-1}$) Separated by Gender and Brand

	Men		Women	
	Test	**Ref**	**Test**	**Ref**
	1207	1210	176.6	411.7
	1895	1259	957.0	461.9
	1463	1264	1102	757.7
	1377	1460	1651	807.7
	1834	1573	789.5	896.5
	1167	1692	923.3	935.3
	657.6	1704	398.1	1026
	1564	1756	2995	1353
	240.0	1950	1208	1361
	3190	2046	2172	1455
	1988	2282	832.1	2072
	3549	2991	871.0	3255
Mean =	**1678**	**1766**	**1173**	**1233**
SD =	**938.8**	**507.9**	**775.2**	**785.7**
RSD (%) =	**56**	**29**	**66**	**64**
Meansex =	**1722**		**1203**	
SDsex =	**739.5**		**763.9**	
RSDsex (%) =	**43**		**64**	

SD, standard deviation; RSD, relative standard deviation; AUC, area under the plasma concentration-time curve from 0 to 48 hours.

Table 3. Analysis of AUC_{0-48} Corrected by Weight ($ng.h.kg.mL^{-1}$), Separated by Gender and Brand

	Men		Women	
	Test	**Ref**	**Test**	**Ref**
	88132.9	88337.3	15717.4	36641.3
	144005	95653.6	57420.0	27714.0
	109755	94815.0	92551.2	63646.8
	115702	122665	83355.3	40788.9
	159541	136834	48949.0	55583.0
	94543.2	137036	50781.5	51441.5
	52608.0	136280	24682.2	63618.2
	106352	119435	161735	73045.8
	19920.0	161850	72504.0	81648.0
	274340	175930	130338	87318.0
	139139	159740	36612.4	91163.6
	227136	191411	47905.0	179003
Mean =	**127598**	**134999**	**68546.0**	**70967.7**
SD =	**69833.0**	**32938.6**	**42956.5**	**39486.2**
RSD (%) =	**55**	**24**	**63**	**56**
Meansex =	**131298**		**69756.8**	
SDsex =	**53530.3**		**40369.9**	
RSDsex (%) =	**41**		**58**	

SD, standard deviation; RSD, relative standard deviation; AUC, area under the plasma concentration-time curve from 0 to 48 hours.

Table 4. Analysis of k_{el} (h^{-1}) Separated by Gender and Brand

	Men		Women	
	Test	**Ref**	**Test**	**Ref**
	0,0218	0,0274	0,0300	0,0708
	0,0250	0,0317	0,0419	0,0238
	0,0418	0,0428	0,0223	0,0232
	0,0482	0,0379	0,0437	0,0557
	0,0511	0,0542	0,0323	0,0305
	0,0425	0,0347	0,0302	0,0595
	0,0290	0,0398	0,0494	0,0292
	0,0432	0,0381	0,0495	0,0395
	0,0484	0,0411	0,0177	0,0600
	0,0314	0,0479	0,0303	0,0240
Mean =	**0.0382**	**0.0396**	**0.0347**	**0.0416**
SD =	**0.0105**	**0.0077**	**0.0109**	**0.0181**
RSD (%) =	**28**	**19**	**32**	**44**
Meansex =	**0.0389**		**0.0382**	
SDsex =	**0.0090**		**0.0150**	
RSDsex (%) =	**23**		**39**	

SD, standard deviation; RSD, relative standard deviation; Kel, terminal elimination rate constant.

It is known that women have lower acid secretion in the stomach [11][29], and then the absorption of itraconazole might be compromised not only because of the incomplete dissolution but also due to an unrestricted presystemic metabolism at the intestine. Progesterone was identified as the main responsible for gastrointestinal motility changes that happen during pregnancy and menstrual cycle [30], as well as on biliary flow dynamics and on exocrine pancreatic function [31-32]. Conversely, men could dissolve the drug to a higher extent and perhaps this higher concentration could saturate the transporter and/or the enzymes in the enterocyte, and consequently a faster and higher absorption could be attained. So, changes in the hormonal levels during sexual cycle and the very dramatic consequence on itraconazole dissolution, due to gastrointestinal secretion and motility variation, could explain both the lower and the more variable bioavailability of drug in women.

Table **1** shows for the reference product faster peak concentration in male than in female subjects (Tmax: 3.9 *vs* 4.8 hours, $p<0.01$). Test product showed a delayed peak compared with the reference in men, which might anticipate some difficulty in dissolving the drug, and probably this fact could be the real cause for the AUC higher variability observed in the formulation test.

Even though twelve male subjects did not allow us to assess both formulation as average bioequivalent (see 90% CI in Table **1**), probably a study with twenty four individuals could have achieved this conclusion. This hypothetic study could also have obtained enough data in favor of bioinequivalence due to dissimilar variances between test and reference drug products.

CONCLUSIONS

The research carried out with the experimental data coming from a previous itraconazole bioequivalence study, enables us to conclude that women would have less oral bioavailability, mainly due to a higher gastric pH and thereafter a lower dissolution efficiency. Consequences in the presystemic elimination at the enterocyte and / or at the hepatocyte should not be discarded.

Also it has been found that women are more variable, increasing dramatically the ANOVA coefficient of variation and therefore bioequivalence assessment would be compromised in studies carried out with 24 subjects.

According to the present work, bioequivalence studies performed with male volunteers could reach a more precise conclusion, assessing both average bioequivalence and variance similarity between test and reference formulations containing itraconazole. Maybe other drugs that share the same pharmacokinetic profile as itraconazole could be better handled using men than women as subjects.

Last paragraph highlights the main goal to be achieved in bioequivalence trials. From our point of view, bioequivalence studies evaluate the *in vivo* different biopharmaceutical performances of two drug products, as a result of different manufacturing processes, formulation components, drug delivery systems, etc., in order to have a final quality control. To assess more accurately the differences in performance is necessary to reduce as much as possible the study variability. On this line of thinking the inclusion of volunteers belonging to both genders does not enhance the excellence of the trial if the final result leads to increased coefficient of variation. Here, it should not be argued that conclusions issued from

bioequivalence need to be closer to the clinical situation, because a bioequivalence study will never be at the real stage except when patients are enrolled.

Summing up, when it is clearly demonstrated that one group of individuals, representative of the population, is sensitive enough to distinguish the biopharmaceutical performance of two drug product, and if the difference observed could be included within the BER with reasonable number of subjects, then these volunteers should be enrolled into the trial. In the case of itraconazole, male subjects become the target population for bioequivalence studies.

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