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Two-compartment pharmacokinetic model of itraconazole after single oral dose administration – gender differences

Dvoprostorni farmakokinetički model itrakonazola nakon oralne primene jedne doze leka – razlike među polovima

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Abstract

Background/Aim. Itraconazole (ICZ) is a widely used antifungal drug with hypervariable pharmacokinetics (PK), which is the result of the molecule's nature itself, as well as the influence of multiple factors. One of the factors is gender, but its importance is not yet substantiated. The aim of the study was to examine the effect of gender on ICZ PK using a two-compartment model, obtained after a single oral dose of the drug, under fed conditions, in healthy participants of both genders. **Methods.** A previously conducted bioequivalence study of two pharmaceutical formulations of a 100 mg oral dose of ICZ in 38 healthy participants (22 men and 16 women) yielded 114 sets of ICZ plasma concentrations. Of these, 64 sets (40 from men and 24 from women) were analyzed in this study using Kinetica software as they fit the two-compartment model. ICZ plasma concentrations were determined by a previously validated liquid chromatographic method with mass spectrometric detection. Statistical analyses in SPSS included Mann-Whitney *U* and Fisher's exact tests for group comparisons, along with Spearman's correlation for parameter relationships. **Results.** Poorer ICZ absorption was observed in females compared to males, accompanied by differences in the drug's distribution process between the central and peripheral compartments and *vice versa.* What's more, there are also differences in ICZ elimination between genders, with it being more effective in women. This isn't solely a result of a more prominent first-pass effect, but is also connected to the terminal phase of elimination after oral administration of the drug. **Conclusion.** The application of a two-compartment model for ICZ after its single oral dose administration under fed conditions in healthy research participants provided a more detailed insight into the variable PK of this drug, as well as into the existing gender-based differences.

Key words:
administration, oral; dose-response relationship, drug; itraconazole; pharmacokinetics; sex factors.

Apstrakt

Uvod/Cilj. Itrakonazol (ITZ) je široko korišćen antimikotik koji ima veoma varijabilnu farmakokinetiku (FK), što je rezultat same prirode molekula leka, kao i uticaja više faktora. Jedan od faktora uticaja je pol, ali njegov značaj još uvek nije potvrđen. Cilj rada bio je da se ispita uticaj pola na FK ITZ-a primenom dvoprostornog modela, koji je dobijen nakon primene leka *per os*, u jednoj dozi, na pun želudac kod zdravih ispitanika oba pola. **Metode.** Prethodno sprovedena studija bioekvivalencije dve oralne farmaceutske formulacije ITZ-a od 100 mg kod 38 zdravih učesnika (22 muškarca i 16 žena) rezultirala je sa 114 setova plazma koncentracija ITZ-a. Od toga je u ovoj studiji analizirano 64 seta (40 od muškaraca i 24 od žena) korišćenjem softvera *Kinetica*, jer je njihovom primenom dobijen dvoprostorni model. Koncentracije ITZ-a u plazmi bile su određene prethodno validiranom metodom tečne hromatografije sa masenom spektrometrijskom detekcijom. Statističke analize u SPSS-u obuhvatale su Mann-Whitney *U* i Fisher-ov egzaktni test za poređenje grupa, kao i Spearman-ovu korelacionu analizu odnosa parametara. **Rezultati.** Slabija resorpcija ITZ-a otkrivena je kod ženskog pola u odnosu na muškarce, a praćena je i razlikama koje su se pojavile u procesu distribucije leka iz centralnog u periferni prostor i obrnuto. Šta više, postoje razlike i u eliminaciji ITZ-a među polovima, koja je efektivnija kod žena. Ovo nije samo rezultat izraženijeg efekta prvog prolaza, već je povezano i sa terminalnom fazom eliminacije nakon oralne primene leka. **Zaključak.** Primena dvoprostornog modela ITZ-a nakon njegove primene u jednoj dozi *per os* kod zdravih učesnika u istraživanju omogućila je detaljniji uvid u varijabilnu FK ovog leka, kao i u razlike koje postoje među polovima.

Ključne reči:
oralna primena; lekovi, odnos doza-odgovor; itrakonazol; farmakokinetika; pol, faktor.

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Introduction

Itraconazole (ICZ), an orally active triazole, acts as an antifungal agent with a broad spectrum of activity [1–4]. However, due to its unpredictable oral bioavailability, its pharmacokinetics (PK) is variable and non-linear, characterized by prolonged clearance and slow accumulation [5–7]. The variability in oral bioavailability is attributed to the ICZ molecule itself, which is a weak base with very high lipophilicity, resulting in poor absorption from the gastrointestinal tract [8]. The extent and rate of the release of the active substance into the bloodstream significantly depend on the pharmaceutical formulation of the drug, particularly in the case of ICZ capsules [9–11]. Conventional capsules require an acidic environment in the stomach for optimal solubility, which affects drug absorption and optimal bioavailability [12]. Additionally, ICZ is extensively metabolized by cytochrome P450 3A (CYP3A) 4 – CYP3A4 in the liver, producing numerous metabolites, with hydroxy-itraconazole (OH-ICZ) being the most significant active one [13–15]. Due to its variable PK, particularly oral bioavailability, ICZ is classified as a highly variable drug. This classification indicates that intra-subject variability for parameters such as maximum drug concentration (C_{max}) and the area under the concentration-time curve (AUC) exceeds 30% [11, 16]. Furthermore, bioequivalence studies have shown that the within-subject coefficient of variation for C_{max} ranged from 44.95% to 69.1% [11, 16, 17]. ICZ volume of distribution (V_d) is about 70 L, and it is excreted mostly as inactive metabolites, approximately 35% in the urine and 54% in the feces [18, 19].

Numerous factors influence the absorption, V_d, biotransformation, and/or clearance (Cl) of drugs [20–22]. This is particularly significant for hypervariable drugs like ICZ. While the impact of different pharmaceutical formulations is well established, factors such as patient ethnicity, age, body mass index, gender, and interactions with other drugs or food have not been sufficiently investigated as sources of ICZ PK variability [10–12, 23–34]. The effect of patient age on ICZ PK remains unclear, as previous studies have reported contradictory results, even in an examination ranging from infants to adolescents [28, 31]. In our previous study [33], the tested PK parameter (AUC_∞) of both ICZ and OH-ICZ was included in the multiple linear regression analysis, and age was not a significant variable affecting their PK. This was not surprising, given that the ages of healthy participants ranged from 23 to 55 years. Similar results were observed when this analysis tested the influence of body weight on the selected PK parameter of ICZ and OH-ICZ, which was not corrected according to the body weight of the subjects. Additionally, since the participants were healthy individuals enrolled in a clinical trial – with no concomitant drug use, a standardized meal prior to drug administration, and shared ethnicity – their gender should be analyzed in more detail. In accordance with this, our previous research highlighted the importance of gender as a potential factor influencing the PK of ICZ in healthy subjects [33]. This was expedient since some authors, using a population PK model obtained after administering a single dose of the drug to healthy individuals, showed that gender did not affect ICZ PK, while others showed the opposite results [11, 28, 29].

The PK of ICZ has been assessed in numerous studies following intravenous and oral administration [17, 28, 33–38]. Non-compartment analysis has frequently been used, alongside the one-compartment PK open model, although ICZ follows multicompartment kinetics [8, 37].

The aim of this study was to further investigate the influence of gender on the ICZ PK following a single oral dose administered to healthy subjects under fed conditions, using a two-compartment open PK model.

Methods

Investigational drug

The 100 mg ICZ capsules used in the previous clinical PK study were sourced from two different manufacturers, whose bioequivalence had been established in an earlier study [17].

Participants

A total of 38 healthy participants (22 men and 16 women) were selected based on predefined inclusion criteria and participated in the study after providing informed consent and receiving comprehensive information about the study. The average age and body mass index of male participants were 38 ± 6.8 years (range 26–55 years) and 24.87 ± 2.80 (range 19.93–29.94), respectively. The average age and body mass index of female participants were 38 ± 6.7 years (range 23–50 years) and 24.82 ± 2.86 (range 19.49–28.69), respectively. All procedures related to the participants have already been explained [17]. However, considering that the participants in the study were healthy subjects, the exclusion criteria are additionally stated. These were primarily clinically relevant abnormalities in the medical history, medical examination, hematology and biochemistry tests, and urinalysis. Moreover, exclusion criteria included: use of any drugs within 14 days before the start of the study, except oral contraceptives; known drug allergy to ICZ; smoking; a recent history of drug or alcohol abuse, or a positive urine screening test for psychoactive substances; participation in other clinical studies within three months prior to the study initiation; positive test results for hepatitis B surface antigen, anti-hepatitis C virus, and/or anti-human immunodeficiency virus antibodies; unwillingness to conform to the study protocol.

Investigational study design

A randomized three-sequence, three-period, two-treatment, partially replicated crossover study in which two pharmaceutical formulations of ICZ were compared was performed [17]. The clinical protocol was approved by the Ethics Committees of the Military Medical Academy (No. 103/2024) and Medical Faculty of the Military Medical Academy (No. 2/11/2024) and approved by the Medicines and Medical Devices Agency of Serbia (No. 515-04-01565-14-1 from December 24, 2014).

Sample collection and analytical method

Since 38 subjects were enrolled, with 16 blood samples *per* subject during one period, and the protocol demanded three treatment periods, there were a total of 114 sets of ICZ plasma concentrations for analysis [17]. The previously established liquid chromatography method with mass spectrometric detection was used [17, 39].

Pharmacokinetic parameters and two-compartment model

Individual plasma concentrations of ICZ were analyzed, and PK parameters for the two-compartment model were calculated using 64 sets of ICZ plasma concentrations (40 sets from male and 24 from female participants). PK parameters were calculated using Kinetica software, version 5.0 (Thermo Fisher Scientific Inc., United States). The remaining 50 sets of ICZ concentrations (26 from women and 24 from men) could not fit the two-compartment open PK model.

PK parameters used in the two-compartment analysis included: k_a – the absorption rate constant calculated according to the equation: $k_a = \ln(2)/t_{1/2ka}$, where $t_{1/2ka}$ represents an absorption half-life; $C_{maxcalc}$ – maximum (peak) plasma drug concentration; $t_{maxcalc}$ – the time where t = C_{max}; $C_{maxcalc\ corr}$ and AUC_{corr} were obtained by dividing calculated values $C_{maxcalc}$ and AUC with the dose-to-body weight ratio; V1/F – volume of the central compartment in the two-compartment model; k_e – central compartment elimination rate constant; k_{12} – constant rate of transition from the central to peripheral compartment; k_{21} – constant rate of transition from the peripheral to central compartment; V_z/F – volume of distribution during the terminal phase after extravascular administration; α and β – exponents; A – intercept of the linear equation on log transformed data; B – slope of the linear equation on log transformed data; Cl/F – apparent total body clearance of the drug from plasma after oral administration.

Statistical analysis

Statistical analysis was performed using the SPSS software version 26.0 (IBM, USA, 2019). Comparison between genders for continuous variables was conducted using the Mann-Whitney *U* test. Fisher's exact test was used to examine the interrelation of PK parameters ($k_a < k_e$ and $k_a > k_e$) in men and women. Spearman's correlation analysis was used to assess relationships between PK parameters. The value of $p < 0.05$ was considered statistically significant.

Results

After *per os* ICZ administration, a two-compartment PK model was obtained (Tables 1 and 2). Further exploration of the defined model after the application of orally administered immediate-release formulations of ICZ (capsule) included correlations between k_a median values and other ICZ PK parameters that reflect the absorption properties of the drug *in vivo*. A statistically significant moderate correlation between k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$ parameters of ICZ, respectively, was shown (Figure 1A, B). There was no correlation between k_a and t_{max} and k_a and $C_{maxcalc\ corr}$, respectively. Evaluation of the influence of gender on ICZ absorption following single-dose oral administration, using a two-compartment model, indicated that there was no significant difference in the median values of k_a between men and women. However, a significant difference between genders was observed comparing the calculated values of the ICZ parameter C_{max} corrected by the ratio of the received drug dose and body weight ($C_{maxcalc\ corr}$). Its value was significantly lower in women (Table 1). Moreover, statistically significant moderate correlations between ICZ parameters k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively, were observed in the male gender. No such correlations were detected in women (Figure 1A, B). Furthermore, we examined correlations between ICZ parameters of absorption and distribution. We showed statistically significant moderate positive correlations between k_a and k_{12} and k_a and k_{21}, respectively, considering the total number of sets of ICZ plasma concentrations, as well as male sets of plasma concentrations. This correlation was not found in women (result not shown).

Statistically significant, very strong negative correlations between $C_{maxcalc\ corr}$ and V1/F parameters of ICZ were found when the calculation of all examined 64 sets of concentrations was performed, as well as both for male and female sets of ICZ plasma concentrations (Figure 2A). Moderate negative correlations between the parameters $C_{maxcalc\ corr}$ and V_z/F were

Table 1

Pharmacokinetic parameters of itraconazole absorption calculated from 64 sets of plasma concentrations after administration of a single oral dose of 100 mg of itraconazole obtained by the two-compartment open model

Parameters	Gender		*p*-value
	men (n = 40)	women (n = 24)	
k_a (h^{-1})	0.45 (0.08–1.26)	0.49 (0.12–1.09)	0.840
$t_{maxcalc}$ (h)	4.60 (2.79–7.44)	5.09 (1.44–6.93)	0.149
$C_{maxcalc}$ (ng/mL)	52.40 (13.25–200.65)	37.07 (14.22–172.92)	0.111
$C_{maxcalc\ corr}$ (ng/mL/mg/kg)	45.14 (8.75–190.61)	24.28 (8.53–117.59)	0.012
AUC ((h)*(ng/mL))	854.17 (241.74–7,847.21)	792.24 (202.01–2,105.82)	0.318
AUC_{corr} ((h)*(ng/mL)/mg/kg)	763.10 (137.79–6,277.77)	507.03 (169.69–1,684.66)	0.061

k_a – absorption rate constant; $t_{maxcalc}$ – time at which maximum concentration of a drug is achieved in plasma; $C_{maxcalc}$ – maximum plasma drug concentration; AUC – area under the concentration-time curve; $C_{maxcalc\ corr}$ and AUC_{corr} – values obtained by dividing calculated values of $C_{maxcalc}$ and AUC by dose-to-body weight ratio; n – number. Values are presented as median (minimum–maximum).

***Note*: *The value of $p < 0.05$ was considered significant according to the Mann-Whitney *U* test.**

Table 2

Pharmacokinetic parameters of itraconazole distribution and elimination calculated from 64 sets of plasma concentrations after administration of a single oral dose of 100 mg of itraconazole obtained by the two-compartment open model

Parameters	Gender		*p*-value
	men (n = 40)	women (n = 24)	
V_1/F (L/kg)	16.66 (0.24–92.89)	30.90 (0.50–106.84)	0.057
V_z/F (L/kg)	182.71 (9.68–2,543.09)	358.31 (7.32–6,013.46)	0.016
k_e (L/h)	0.13 (0.01–0.27)	0.11 (0.03–1.08)	0.305
k_{12} (L/h)	0.26 (0.01–16.23)	0.26 (0.15–6.53)	0.688
k_{21} (L/h)	0.04 (0.01–10.21)	0.04 (0.002–3.11)	0.560
A	116.63 (4.76–440.80)	75.28 (13.99–463.46)	0.197
α	0.42 (0.20–16.28)	0.44 (0.27–6.53)	0.739
B	8.34 (0.34–65.50)	4.69 (0.004–23.63)	0.029
β	0.01 (0.00–0.21)	0.008 (0.00–1.08)	0.228
Cl/F (L/h)	212.86 (23.17–752.12)	226.75 (0.20–900.04)	0.318

V_1/F – volume of the central compartment in two-compartment model; V_z/F – volume of distribution during the terminal phase after extravascular administration; k_e – central compartment elimination rate constant; k_{12} – constant rate of transition from the central to peripheal compartment; k_{21} – constant rate of transition from the peripheral to central compartment; A – intercept of the linear equation on log transformed data; B – shape of the linear equation on log transformed data; α (alpha) and β (beta) – exponents; Cl/F – apparent total body clearance of the drug from plasma after oral administration.

Values are presented as median (minimum–maximum). The value of $p < 0.05$ was considered significant according to the Mann-Whitney *U* test.

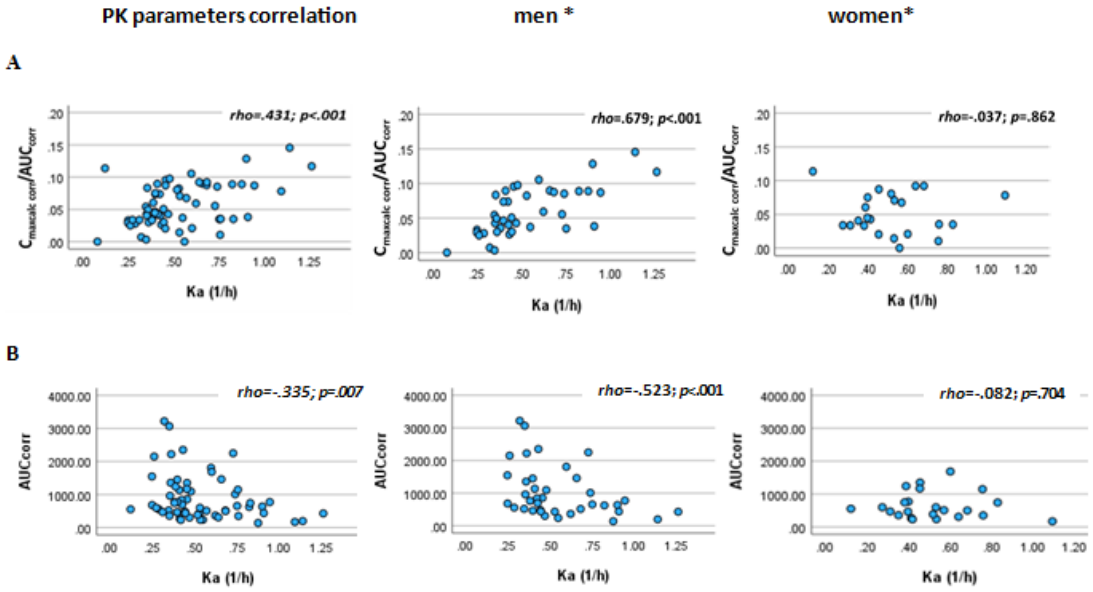

Fig. 1 – The correlations between ICZ PK parameters for the assessment of the absorption process: A) k_a and $C_{maxcalc\ corr}/AUC_{corr}$; B) k_a and AUC_{corr} of ICZ

ICZ – itraconazole; PK – pharmacokinetic; rho – Spearman's rank correlation coefficient. For other abbreviations, see Table 1.

Correlations were performed by using Spearman's correlation analysis ($p < 0.05$; $p < 0.001$ indicates significant correlation).

***Note*: *Sets of ICZ plasma concentrations obtained from men and women.**

also highly statistically significant when all examined sets of plasma concentrations were considered. This was also the case related to men, but not to women (Figure 2B). On the other hand, it was shown that correlations between parameters AUC_{corr} and V1/F were moderate and statistically significant for all 64 examined series, which was also related to the male gender, but not to the female (Figure 2C). The examined correlation of the $C_{maxcalc\ corr}/AUC_{corr}$ with the V1/F parameter showed a moderate negative correlation that was statistically significant for all examined sets of ICZ plasma concentrations, as well as for women, but not for men (Figure 2D).

In contrast, a moderate negative significant correlation between the parameters $C_{maxcalc\ corr}/AUC_{corr}$ and V_z/F was obtained, which was significant for the overall examined sets of concentrations, as well as for the male gender, but not for the females (Figure 2E). In addition, it was shown that the value of the ICZ parameter k_a was higher than the k_{12} value, and both

of these parameters had higher values than that of k_{21} ($k_a > k_{12} > k_{21}$) in 90% of men and 82% of women sets of ICZ concentrations, respectively.

Regarding parameters of the distribution, the median value of V_z/F was significantly higher for women than men, 182.71 (9.68–2,543.09) vs. 358.31 (7.32–6,013.46), respectively. Moreover, parameter B, defined as the intercept of the extrapolation of the β-phase to time zero in the two-compartment open model, was significantly lower in women than in men (Table 2).

The correlation of ICZ PK parameters of distribution in male and female genders indicated a statistically significant positive correlation between k_{12} and α parameters in both genders (Figure 3A). The correlation between the parameters k_{12} and β was not significant in men, in contrast to women, in whom a moderate negative statistically significant correlation was observed (Figure 3B). The situation was similar concerning the correlation between the ICZ parameters V1/F and k_{21}, which was positive and statistically significant in women but not in men (Figure 3C).

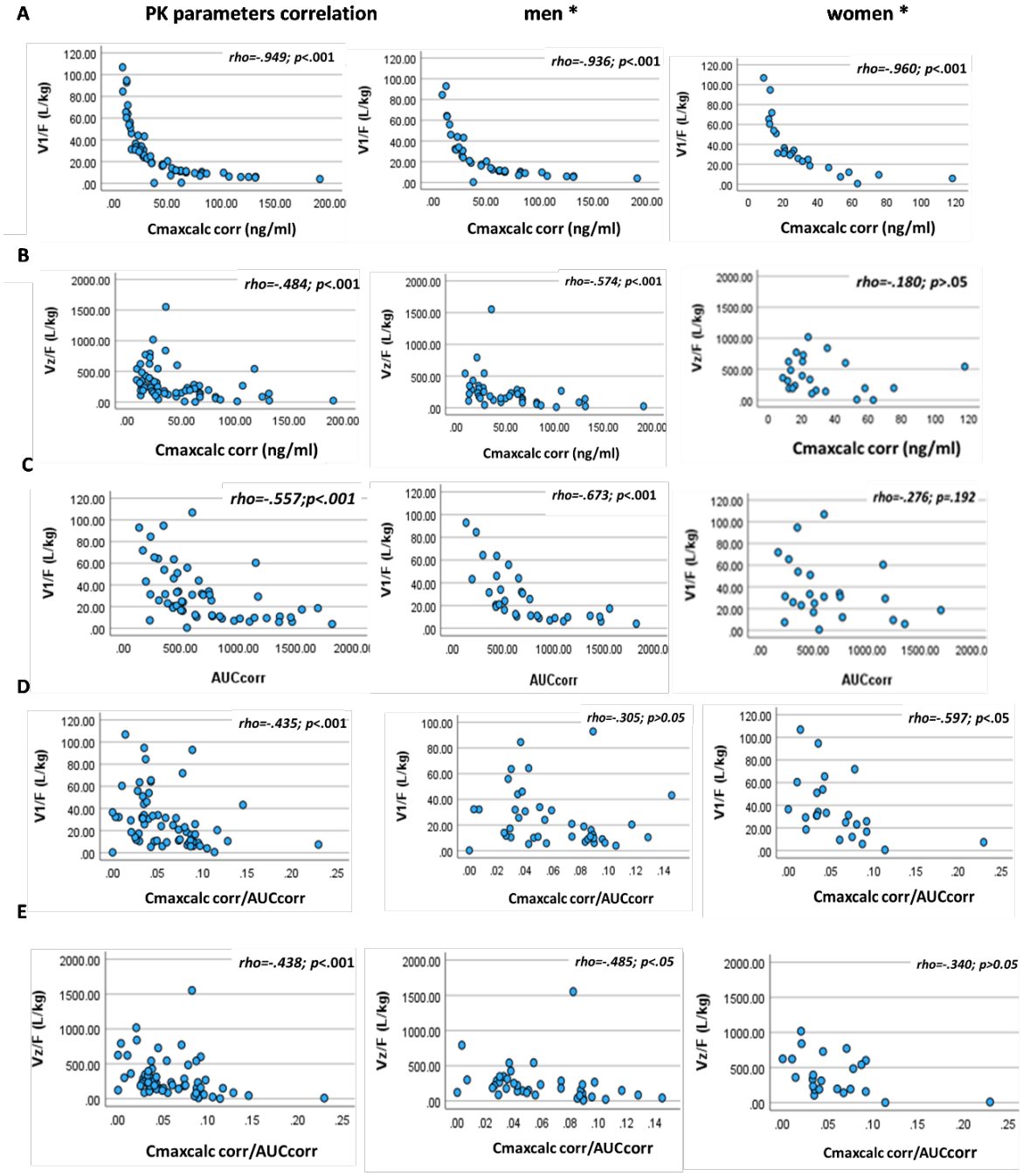

Fig. 2 – The correlations between ICZ PK parameters of absorption and distribution, respectively: A) $C_{maxcalc\ corr}$ and V_1/F; B) $C_{maxcalc\ corr}$ and V_z/F; C) AUC_{corr} and V_1/F; D) $C_{maxcalc\ corr}/AUC_{corr}$ and V_1/F; E) $C_{maxcalc\ corr}/AUC_{corr}$ and V_z/F
For abbreviations, see Figure 1 and Tables 1 and 2.
Correlations were performed by using Spearman's correlation analysis ($p < 0.05$; $p < 0.001$ indicates significant correlation).
***Note*: *Sets of ICZ plasma concentrations obtained from men and women.**

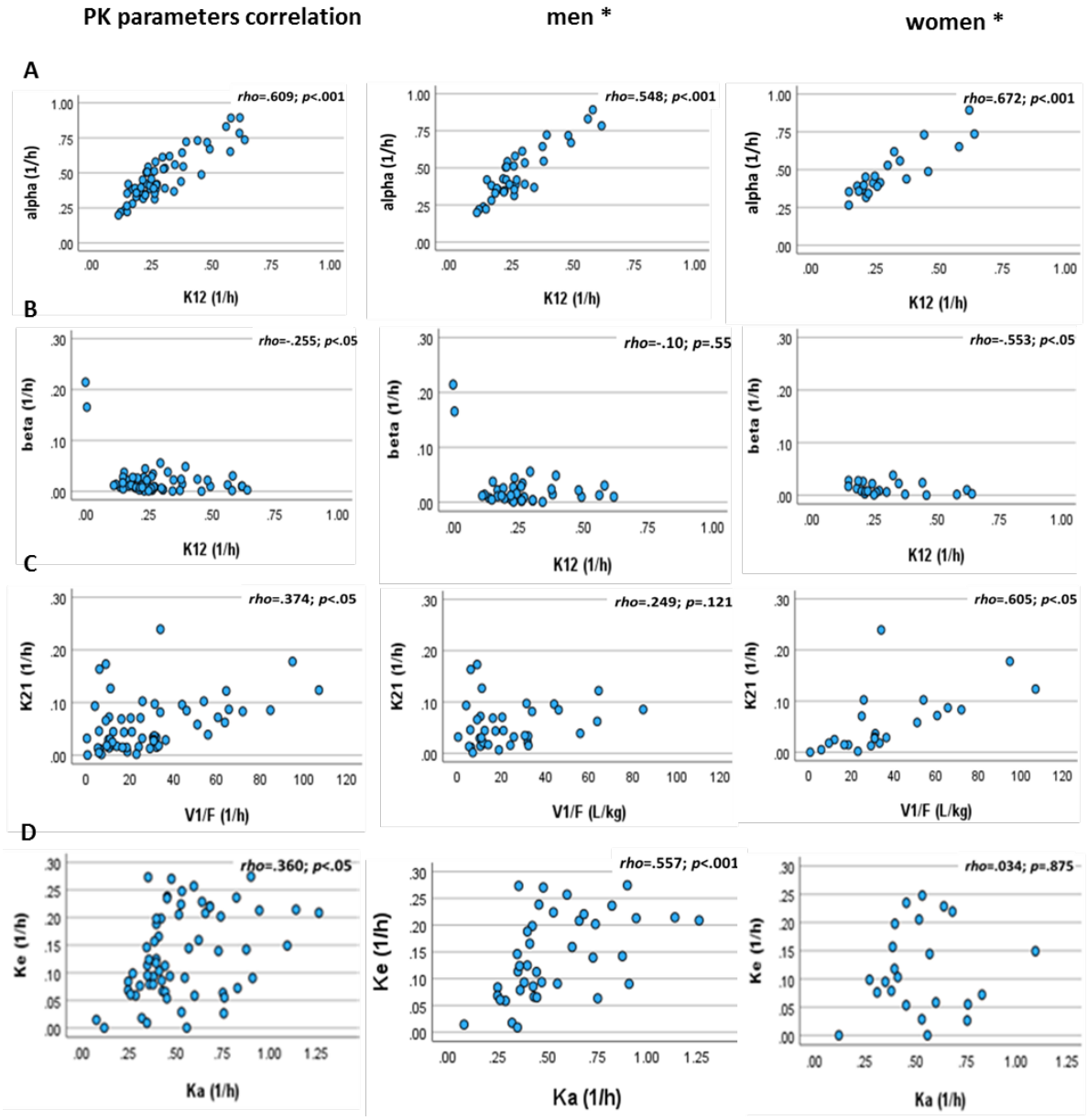

Fig. 3 – The correlations between ICZ PK parameters: k_{12} and alpha (A), k_{12} and beta (B), V1/F and k_{21} (C), k_a and k_e (D)

For abbreviations, see Table 2 and Figure 1.

Correlations were performed by using Spearman's correlation analysis ($p < 0.05$; $p < 0.001$ indicates significant correlation).

***Note*: *Sets of ICZ plasma concentrations obtained from men and women.**

On the other hand, a statistically significant positive correlation between V_1/F and V_z/F was found in men, whereas no such correlation was found in females (data not shown).

When the parameters of elimination, k_e and Cl/F, were considered, no significant differences were identified between males and females (Table 2).

The correlation between ICZ PK parameters related to absorption and elimination in male and female genders was also investigated. The correlation between ICZ parameters k_a and k_e was moderately positive and statistically significant only in the male gender (Figure 3D). On the other hand, the relation of ICZ parameters such as $k_a > k_e$ was present in 100% of male sets of drug concentrations and 87.5% of female sets ($p < 0.05$ according to Fisher's exact test). Therefore, the opposite relation, $k_a < k_e$, was not present in the male gender, while it accounted for 12.5% of female sets of ICZ concentrations ($p = 0.016$ according to Fisher's exact test).

Discussion

Our previous study included 38 healthy participants, each of whom provided 16 blood samples during a single investigation period, with the overall study designed to include three such periods [17, 33]. As a result, 114 sets of plasma concentrations of the drug itself, as well as its metabolite

OH-ICZ, were obtained and included in the PK analyses. In the present study, our results indicated that following oral administration of a 100 mg capsule in the fed state, two-compartment PK model parameters could be calculated by using 64 ICZ sets of plasma concentrations, 40 sets obtained from men and 24 from women. Additionally, in order to confirm the obtained two-compartment model, we used a novel method called the direct model [40] and presented good correlations between the following parameters: k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$.

Differences in ICZ PK parameters between genders have been observed using non-compartmental analysis and a one-compartment open model [33]. The present study employed a two-compartment model to further elucidate these differences. No significant differences were found between men and women in the median values of the parameter k_a. However, statistically significant moderate correlations were observed in male gender between ICZ parameters k_a and AUC_{corr}, as well as between k_a and $C_{maxcalc\ corr}/AUC_{corr}$. No such correlations were detected in women. Moreover, a significant difference between genders was found in the comparison of the $C_{maxcalc\ corr}$, with values being significantly lower in women. This finding is particularly noteworthy, as $C_{maxcalc\ corr}$ represents the parameter $C_{maxcalc}$ corrected according to the body weight of the subjects. This substantiates our previous findings that gender, and not the body mass index, significantly influenced the ICZ PK. Accordingly, when all 114 ICZ concentration sets were analyzed using non-compartmental analysis, women showed significantly lower median values for $C_{maxcalc\ corr}$, $AUC_{72hcorr}$, and $AUC_{\infty corr}$ compared to men. Moreover, when the results of the open one-compartment model parameters were analyzed, values of $C_{maxcalc\ corr}$ and AUC_{corr} were significantly lower in women than in men [33]. In accordance with that, a strong positive correlation was observed between parameters AUC and AUC_{corr}, as well as C_{max} and $C_{maxcalc\ corr}$ when considering one-compartment and two-compartment models, respectively [33]. All this indicates a poorer ICZ absorption in women compared to men. It is already known that certain parameters influencing the absorption process differ between genders. These differences can be attributed to variations in gastric acid secretion levels, with some studies indicating that it is lower in women [8, 22, 29]. This follows from the fact that ICZ is a very lipophilic drug, ionizing only at low pH, so the greater acid secretion in the stomach, the better solubility in water, which is evidently the case to a greater extent in men. Moreover, the speed of emptying the contents from the stomach and intestinal motility are higher in men, so this further favors greater absorption of the drug in men [22]. In accordance with this, authors Fagiolino et al. [11] analyzed the data on the ICZ bioequivalence study and concluded that women have less oral bioavailability and a more variable AUC than men. This may also explain the lack of correlation in our two-compartment model in women between ICZ parameter k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively.

When we considered ICZ distribution in a two-compartment open model, it was found that the parameter V_z/F was significantly higher in women than in men. Corresponding parameter V_d/F obtained from non-compartment analysis and one-compartment model was also significantly higher in the female gender in our previous study [33]. It can be explained by the fact that ICZ has very high lipophilicity and an extremely high volume of distribution [18, 19]. Since the amount of fat in the body does not account for lean body mass and muscles, and when the same body mass index in both genders exists, the female gender, on average, has at least 10% more body fat compared to men [41–44], these are in favor of significantly higher ICZ volume of distribution in women. In addition, our previous study showed that weight did not influence ICZ PK after single-dose oral administration, as selected ICZ PK parameters were corrected with the dose-to-body weight ratio [33]. The parameters corrected in the same way were included in the present investigation, which was performed by using a two-compartment open model. This further indicated that gender, rather than body mass, influences the ICZ PK. Furthermore, a statistically significant negative correlation was found between V_1/F and V_z/F in men, which was not the case in women. Taking into account that the parameter V_1/F shows the apparent volume of the central or plasma compartment, and V_z/F indicates the apparent volume of distribution during the terminal phase after non-intravenous drug administration in a two-compartment model, all this supports the different distribution of ICZ in males and females. Moreover, while no significant correlation between the parameters k_{12} and β was observed in men, a moderate, statistically significant negative correlation was identified in women. In contrast, the correlation between ICZ parameters V_1/F and k_{21} was significantly positive in women but not in men. These findings highlight the differences observed in the process of drug distribution between the central and peripheral compartments between genders.

Since all PK processes occur simultaneously in the body [44] results concerning values of ICZ volume of distribution are in accordance with the findings that the parameter $C_{maxcalc}$ value, corrected for body weight ($C_{maxcalc\ corr}$), is significantly lower in females. Moreover, the differences found in the correlations between gender, which refer to the relationship between absorption and distribution parameters in this study, also support different PK of ICZ in women and men (negative correlations between parameters $C_{maxcalc\ corr}$ and V_z/F; $C_{maxcalc\ corr}/AUC_{corr}$ and V_1/F, and $C_{maxcalc\ corr}/AUC_{corr}$ and V_z/F were highly statistically significant in men, but not in female gender). According to the mentioned direct model [40], the relationships among the constants $k_a > k_{12} > k_{21}$ were satisfied in 90% of men and 82% of women sets of ICZ concentrations, respectively, providing more rationale for setting the two-compartment model for this drug. Again, there were no significant correlations between parameters k_a and k_{12} and k_a and k_{21}, respectively, in the female gender, which was the case in men.

When the β exponent, also referred to as the post-distribution or terminal phase in the two-compartment model, was considered, the results indicated that the parameter B is significantly lower in women. Namely, the β hybrid constant

is related to the elimination of the parent drug from the systemic circulation through metabolism and/or excretion, including the effects of overlapping the processes of elimination and distribution, which is not yet finished [44]. ICZ is extensively metabolized by the liver *via* the CYP3A4 enzyme, as the major enzyme involved, resulting in various metabolites. However, the main metabolite, OH-ICZ, exhibits trough plasma concentrations about twice as high as those of ICZ [13–15]. In our previous investigation, not only were the values of C_{max} and AUC significantly lower for both ICZ and OH-ICZ in the female gender, but women also exhibited significantly lower medians of plasma concentrations of both the parent drug and metabolite in comparison to males 72 hrs after administration of ICZ [33]. Since it was related to the metabolite to a greater extent, it pointed out less exposure to OH-ICZ in the female gender compared with males. Therefore, gender differences related to the CYP3A4 enzyme that metabolizes ICZ predominantly in the liver could be one of the causes [45, 46]. Namely, women are thought to have approximately 1.4 times higher CYP3A4 activity than men. Moreover, Wolbold et al. [47] examined 39 human liver tissue samples and found that expression of this enzyme is twice as high in women as in men. Sakuma et al. [48] presented two possible mechanisms for the more dominant expression of the CYP3A enzyme in women. The first mechanism is that activation of the pregnane X receptor by female sex hormones plays an important role in the dominant expression of the CYP3A enzyme in women. The second one is related to the influence of growth hormone (GH) [49, 50]. In a person with GH-deficient secretion, a different expression of CYP3A enzyme exists, depending on the way of substitution therapy administration [50]. When this hormone was given continuously (imitating the way GH is secreted in the female gender), the activity of the CYP3A4 group of enzymes was increased, while when it was given in pulses (which is the way GH is secreted in the male gender), its activities were decreased. Therefore, the elimination of the ICZ is more effective in women as a result not only of the more prominent first-pass effect but also related to the terminal phase of elimination after oral administration. These differences are also substantiated by the findings that the correlation of ICZ parameters k_a and k_e was moderately positive and statistically significant only in the male gender. Furthermore, in contrast to all ICZ concentration series obtained from males in whom the parameter k_a was greater than k_e, the flip-flop model was present in 12.5% of female sets of ICZ concentrations ($p = 0.016$). This phenomenon is defined when the PK parameter k_a is less than the k_e for some drugs [44]. Related to ICZ in women, it seems that it is related to the "flip" scenario, in which limited absorption is more prominent, resulting in a slower rise in plasma concentrations after oral administration [51].

In addition to the physical and chemical properties of the drug, other factors that contribute to this are the already mentioned physiological factors, specifically related to gender, such as variations in gastrointestinal tract physiology, including gastric pH, transit time, and enzyme activity, which can affect absorption speed. As already mentioned, women have less oral bioavailability and a more variable AUC than men [11], in accordance with our findings of the lack of correlation between absorption parameters in women in two-compartment analysis.

Moreover, the application of the ICZ two-compartment PK model after its oral administration was possible by using 64 sets of ICZ plasma concentrations (40 from men and 24 from healthy female participants), out of 114 total obtained in our previous study [33]. For the remaining 50 sets of ICZ plasma concentrations, the two-compartment model analysis could not be calculated by Kinetica software version 5.0. This can be attributed to the slowed and delayed distribution phase, as it was mentioned [51]. According to the literature, when the initial distribution phase is small compared to the total AUC, the two-compartment model "falls" to the one-compartment model [9, 52–55]. This is actually in accordance with our previous results [33], since when all 114 sets of ICZ plasma concentrations were taken into analysis by a one-compartment model, all PK parameters of the drug could be calculated and used for further analysis. Moreover, this is also related to the hypervariability of ICZ during the absorption process, meaning that variability for the parameters C_{max} and AUC is larger than 30%, as was already shown in our ICZ bioequivalence study [17]. In the presented paper, it was additionally considered in the context of PK differences between genders. We strongly support the introduction of therapeutic monitoring of ICZ in everyday clinical practice, which would allow individualization of the ICZ dose by checking plasma or serum drug concentrations and adjusting its dose, especially in patients with serious fungal infections [30].

Study limitations

A limitation of the study could be the relatively small number of sets of ICZ concentrations enabling the formation of a two-compartment PK model of ICZ compared to the total number of sets of concentrations obtained after oral administration of ICZ in all study participants. However, in our previous work, positive strong correlations between one-compartment and two-compartment models for parameters AUC and AUC_{corr}, as well as $C_{maxcalc}$ and $C_{maxcalc\ corr}$, respectively, were shown [33]. Moreover, the obtained two-compartment model after extravascular administration of the drug was verified using a direct model, which was substantiated by good correlations between the parameters ka and AUC_{corr}, as well as k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively, obtained using our data.

Conclusion

Our results indicated that following oral administration of a 100 mg capsule in the fed state, two-compartment pharmacokinetic model parameters could be calculated by using 64 itraconazole sets of plasma concentrations, 40 sets obtained from men and 24 from women. Itraconazole parameter C_{max} corrected by the dose-to-body weight ratio, i.e., $C_{maxcalc\ corr}$, was significantly lower in women than in men, while statistically significant correlations between parameters k_a and AUC_{corr} and k_a and $C_{maxcalc\ corr}/AUC_{corr}$, respectively, were observed in men,

but not in women. The median value of parameter V_z/F was significantly higher in women than in men, while parameter B, the intercept of the extrapolation of the β-phase to time zero, was significantly lower in women than in men. Additionally, the correlation of itraconazole parameters k_a and k_e was positive and statistically significant only in the male gender, while the relation of itraconazole parameters such as $k_a < k_e$ was not present in the male gender, but it accounted for 12.5% of female sets of itraconazole concentrations, and the difference was statistically significant. Therefore, the two-compartment open model of itraconazole following a single oral dose under fed conditions in healthy participants provided a detailed insight into its variable pharmacokinetics and gender-based differences.

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