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ARTICLE

Intestinal P-gp activity is reduced in postmenopausal women under breast cancer therapy

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Abstract

Intestinal P-glycoprotein (P-gp) activity plays a crucial role in modulating the oral bioavailability of its substrates. Fexofenadine has commonly been used as a P-gp probe, although it is important to note the involvement of other drug transporters like, OATP1B1, OATP1B3, and OATP2B1. In vitro studies demonstrated an upregulation of P-gp protein in response to exposure to pregnancy-related hormones. The objective of this study was to investigate how intestinal P-gp activity is impacted by menopausal status. This study sampled fexofenadine plasma concentrations over 0–12 h after probe drug administration from two groups of patients with breast cancer: premenopausal ($n = 20$) and postmenopausal ($n = 20$). Fexofenadine plasma concentrations were quantified using liquid-chromatography tandem mass spectrometry. Area under the plasma concentration-time curve from zero to infinity (AUC_{inf}) was calculated through limited sampling strategies equation. Multiple linear regression was applied on AUC_{inf}, maximum plasma concentration (C_{max}), and time to C_{max}. Postmenopausal patients showed a significant increase in C_{max} (geometric mean and 95% confidence interval [CI] 143.54, 110.95–176.13 vs. 223.54 ng/mL, 161.02–286.06 and in AUC_{inf} 685.55, 534.98–878.50 vs. 933.54 ng·h/mL 735.45–1184.99) compared to premenopausal patients. The carriers of the ABCB1 3435 allele T displayed higher C_{max} values of 166.59 (95% CI: 129.44–214.39) compared to the wild type at 147.47 ng/mL (95% CI: 111.91–194.34, $p = 0.02$). In postmenopausal individuals, the decrease in P-gp activity of ~40% may lead to an increased plasma exposure of orally administered P-gp substrates.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Intestinal P-glycoprotein (P-gp) activity controls the oral bioavailability of its substrates. Fexofenadine has been used as a P-gp probe, although other drug transporters, such as OATP1B1, OATP1B3, and OATP2B1, are also involved. In vitro studies have shown that P-gp protein is increased in response to pregnancy-related hormone exposure.

WHAT QUESTION DID THIS STUDY ADDRESS?

Is menopausal status capable of influencing intestinal P-gp activity in clinical studies?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The findings of this study indicated a decrease in intestinal P-gp activity during postmenopausal status, as evidenced by a 40% increase in fexofenadine plasma exposure when compared to the premenopausal state.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

In postmenopausal individuals, the decrease in P-gp activity may lead to an increased plasma exposure of orally administered P-gp substrates.

INTRODUCTION

Pharmacokinetic changes in women based on menopausal status are not well defined. Menopause, which refers to the permanent cessation of menstruation after the loss of ovarian follicular development, may be associated with changes in phase I and phase II drug metabolism enzymes.[1,2] Although a recent study had shown a reduction in 20% of the activity of intestinal CYP3A4 in postmenopausal women compared to premenopausal women,[3] other studies considering changes in intestinal or hepatic CYP3A4 activity did not find pharmacokinetic changes of erythromycin and prednisolone in pre- and postmenopausal women.[4]

Studies conducted in both human and in vitro models have suggested that the physiological changes associated with hormonal levels, such as progesterone and estradiol, can affect the basal expression of drug transporters protein.[5–7] Specifically, in vitro studies have shown that the decrease in hormonal levels has been correlated with the decrease in P-gp and OCT activities.[6,7] Moreover, postmenopausal women are often under-represented in clinical studies, resulting in a limited understanding of the clinical implications of menopausal status on pharmacokinetic changes.[8]

Fexofenadine, a histamine H1 receptor antagonist, is widely recognized as a substrate of P-glycoprotein (P-gp) due to its favorable safety profile and minimal metabolic transformation. It has been proposed as a probe drug for assessing P-gp activity in both in vitro models and clinical studies. However, it is important to note that fexofenadine is also a substrate of other drug transporters, such as OATP1B1, OATP1B3, and OATP2B1.[9] Several in vivo investigations have demonstrated that co-administration of fexofenadine with known P-gp inhibitors leads to an increase in its area under the plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}), whereas its AUC and C_{max} decreased when administered with P-gp inducers.[10–13] Additionally, in previous studies developed in animal model knockout for the Mdr1a/1b gene,[14,15] the authors did not find any affect in the biliary excretion clearance concerning both plasma and liver fexofenadine concentrations. However, the absence of P-gp caused a sixfold increase in the fexofenadine plasma concentration after oral administration. Therefore, these results show that P-glycoprotein plays an important role in efflux transport in the small intestine but only a limited role in biliary excretion in mice. Consequently, these results provide further evidence that alterations in fexofenadine plasma concentrations can indicate changes in intestinal P-gp function.

Therefore, understanding the impact of menopausal status on drug transporter activity, specifically, P-gp, is crucial as it plays a complex role in absorption, distribution, and elimination processes. However, there is still a significant gap in our knowledge regarding this aspect. In light of this, the objective of the present study was to investigate the influence of menopausal status on P-gp in vivo activity in patients with breast cancer using fexofenadine as a probe drug.

MATERIALS AND METHODS

Patients and data collection

The study was approved by the ethics committees of the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Sao Paulo, Brazil, and of General Hospital of Ribeirão Preto Medical School, University of São Paulo, Sao Paulo, Brazil (record number: 35539714.7.0000.5403). All patients provided written consent. We recruited a total of 40 tamoxifen-treated patients with breast cancer (20 mg/day) and categorized them into two groups according to age, menopausal status, and clinical records ($n = 20$/group), as previously reported

by the research group[14]: (1) premenopausal (patients aged <50 years) and (2) postmenopausal (patients aged >60 years). In our study, menopause was defined as the cessation of menstruation for 12 months and FSH concentrations in women under age 50 years. Key exclusion criteria included a history of significant medical conditions, pregnancy or lactation, use of any concomitant medications that could influence the tamoxifen metabolism, and smoking or use of any tobacco products within 60 days of study drug administration. The patients were distributed into two groups according to menopausal status: premenopausal ($n = 20$) and postmenopausal ($n = 20$). All patients were histologically diagnosed with estrogen receptor (ER)-positive breast cancer and were under tamoxifen (20 mg/day) treatment for more than 3 months. Patients were suitable for inclusion if they were not concomitantly taking any other moderate or potent inhibitors or inducers of the drug transporter ABCB1 (P-gp). All investigated patients also received a single oral dose of fexofenadine (120 mg) as a P-gp probe drug. Serial blood samples (1 mL) were collected in heparin tubes at predose and 15, 30, 60, 90, 120, 180, 240, 360, 480, and 720 min after the administration of the probe drug. For pharmacogenetic screening, whole blood (2 mL) was collected at predose in EDTA tubes and stored at −70°C until analysis.

Fexofenadine measurements in plasma

Plasma samples of 50 µL were added with the internal standard solution (diphenhydramine, 10 ng/mL) and 150 µL of acetonitrile. Fexofenadine was resolved on an Express C18 column (Ascentis; Merck, Darmstadt, Germany) using as a mobile phase a mixture of water and acetonitrile (60:40, v:v) added with 0.1% formic acid. Fexofenadine was quantified by Quattro Micro triple-quadrupole liquid-chromatography tandem mass spectrometry (Waters, Milford, EUA) in the positive ion electrospray ionization mode, as described previously with some modifications.[15] The method had no matrix effect and showed linearity in the range of 1–2000 ng/mL of plasma. The coefficients of variation and the relative standard errors of the precision and accuracy studies were less than 15%.

Genotyping

DNA was obtained from peripheral blood leukocytes according to usual procedures with a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The following single-nucleotide polymorphisms were detected by real-time polymerase chain reaction with 5-nuclease allelic discrimination assays according to the manufacturer's instructions: *ABCB1* 1236C>T (rs1128503), 2677G>T/A (rs2032582), and 3435C>T (rs1045642).

Pharmacokinetic analyses

The observed C_{max}, and the time to reach the C_{max} (T_{max}) were obtained based on the experimental data. The prediction of the AUC from zero to infinity (AUC_{inf}) values for fexofenadine was obtained from the regression equation previously reported by the research group.[16]

$$-5.830 + 2.614^{*}C_{90min} + 4.818^{*}C_{240min}.$$

Statistical analyses

Simple and multiple linear regression were used to test associations between the log-transformed AUC_{inf} of fexofenadine and potential covariates. Each potential covariate was assessed univariately and any covariate that was found to meet the significance criteria of $p < 0.15$ were included in the multiple models. As covariates, body mass index (BMI) was analyzed as continuous variables; hormonal status (pre- and postmenopausal), self-identified "race/color" (White or non-White), *ABCB1* genotypes (1236C>T, 2677G>T/A, and 3435C>T), and diplotypes were analyzed as categorical variables. Deviations from Hardy–Weinberg equilibrium were assessed by the goodness-of-fit χ^2 test using the HardyWeinberg package (version 1.7.3). *ABCB1* diplotypes were inferred using the haplo.stats package (version 1.8.7). The statistical analyses were performed with the RStudio platform (version 2021.9.0.351), and all tests considered $p < 0.05$ as statistically significant.

RESULTS

The cohort study included premenopausal and postmenopausal groups with median ages of 47 and 65 years, respectively ($p < 0.05$). The BMI did not differ significantly between the groups, 27.74 versus 28.01 kg/m^2 for premenopausal and postmenopausal, respectively (p value = 0.32). Covariates meeting the significance criteria of $p < 0.15$ in the univariate analysis and subsequently incorporated into the multiple models for fexofenadine AUC_{inf} and C_{max} included menopausal status and the *ABCB1* 3435 allele T. The postmenopausal group showed (Table 1) a significant increase in C_{max} geometric mean values (Figure 1a) from 143.54 ng/mL (95% confidence interval [CI]: 110.95–176.13) to 223.54 ng/mL (95% CI: 161.02–286.06) compared

TABLE 1 Pharmacokinetics parameters in the study population.

Characteristic	All ($n=40$)	Premenopausal ($n=20$)	Postmenopausal ($n=20$)
AUC (ng h/mL) GM (95% CI)	800 (674–950)	686* (535–878)	933* (735–1185)
C_{max} (ng/mL) GM (95% CI)	157.22 (131–188)	127# (99.8–162)	194# (152–249)
T_{max} (h) median (CV)	2.7 (55.5)	2.5 (59)	3.0 (53.1)

Note: Significant interactions were detected by multiple linear regression analyses and were designated by * when a significant difference between pre- and postmenopausal status was detected in the AUC_{inf} ($p=0.02$), and by # when a significant difference between pre- and postmenopausal status was detected in the C_{max} ($p=0.01$).

Abbreviations: AUC, area under the plasma concentration-time curve; CI, confidence interval; C_{max}, maximum plasma concentration; CV, coefficient of variation; GM, geometric mean; T_{max}, time to C_{max}.

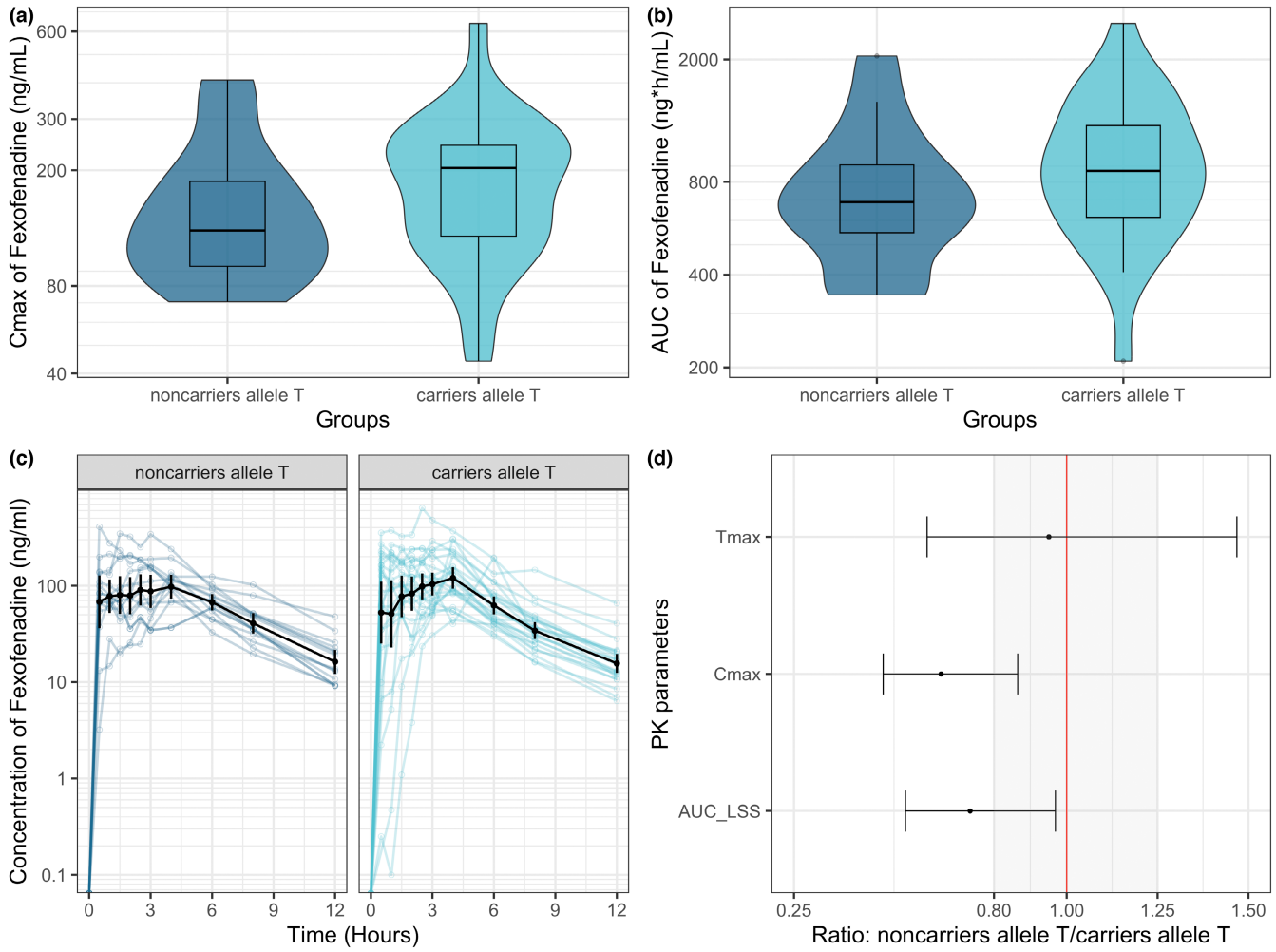

FIGURE 1 Violin plot of the AUC (panel a), C_{max} (panel b), and concentration-time profile (panel c) of fexofenadine following an oral single-dose (120 mg/day) in breast cancer patients ($n=40$) stratified by hormonal status. Pre/postmenopausal ratio of fexofenadine pharmacokinetics parameters (panel d). AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; LSS, limited sampling strategy; PK, pharmacokinetic; T_{max}, time to C_{max}.

to the premenopausal group ($p=0.01$). Moreover, there was a significant increase in AUC_{inf} geometric mean values (Figure 1b) from 685.55 ng h/mL (95% CI: 534.98–878.50) in the premenopausal group to 933.54 ng h/mL (95% CI: 735.45–1184.99) in the postmenopausal group ($p=0.02$). There are notable differences in clearance (CL/F) between the two groups. The postmenopausal group exhibited a significant increase in the geometric mean values of CL/F, which rose from 126.33 L/h (95% CI: 102.77–155.31) to 192.44 L/h (95% CI: 155.76–237.75) when compared to

the premenopausal group ($p=0.002$). The T_{max} did not differ between the groups, the premenopausal group showed a median of 2.5 h with a coefficient of variation (CV) of 58.96%, whereas the median for the postmenopausal group was 3 h with a CV of 53.11%.

The *ABCB1* genotypes 1236C>T (Chi-squared test p value = 0.77), 2677G>T/A (Chi-squared test p value = 0.54), and 3435C>T (Chi-squared test p value = 0.87) did not deviate from the Hardy–Weinberg equilibrium (Table S1). Although carriers of the *ABCB1* 3435 allele T displayed higher C_{max} (Figure 2a) geometric mean value of 166.59 (95% CI: 129.44–214.39) compared to the wild type of 147.47 ng/mL (95% CI: 111.91–194.34, $p=0.02$), displayed lower CL/F values of 139.51 (95% CI: 111.21–175.03) compared to the wild type at 169.25 L/h (95% CI: 127.67–224.38, $p=0.02$), and showed a no difference in the geometric mean AUC_{inf} (Figure 2b) value of 860.12 (95% CI: 685.61–1079.05) compared to the wild type of 708.98 ng h/mL (95% CI: 534.79–939.90, $p=0.05$), the other *ABCB1* genotypes did not show any statistically significant difference. Specifically, carriers of the *ABCB1* 2677 allele nonG and the *ABCB1* 1236 allele T did not display any significant difference with AUC_{inf} values of 864.38 (95% CI: 670.01–1115.14) and 844.78 ng h/mL (95% CI: 655.25–1089.14), respectively, compared to their respective wild type groups ($p=0.86$ and $p=0.92$). There was no significant variation in T_{max} observed among the different *ABCB1* genotypes.

The *ABCB1* diplotypes did not show any statistically significant difference in the fexofenadine C_{max} (Figure 3a) and AUC_{inf} (Figure 3b) in multiple linear regression analysis. The carriers of two copies of the reference GCG haplotype have shown AUC_{inf} of 726.05 ng h/mL (95% CI: 518.73–1016.23), the patients who carry one copy of GCG haplotype have displayed an AUC_{inf} of 836.97 ng h/mL (95% CI: 627.1–1117.07), and for those who does not carry any CGC haplotype, the AUC_{inf} was 723.66 ng·h/mL (95% CI: 457.64–1144.32). Regarding the C_{max}, in addition, there is no statistically significant difference between carriers of GCG haplotype. Patients carrying two copies of the reference GCG haplotype exhibited a C_{max} of 142.23 ng/mL (95% CI: 96.92–208.74), whereas patients harboring a single copy of the GCG haplotype demonstrated a C_{max} of 171.62 ng/mL (95% CI: 127.57–230.88). Conversely, those lacking any CGC haplotype displayed a C_{max} of 132.15 ng/mL (95% CI: 82.01–212.96). No significant variation in T_{max} was observed among the different *ABCB1* diplotypes. Additionally, when considering the CL/F values, carriers of the ABCB1 3435 allele T displayed lower CL/F values of 139.51 (95% CI: 111.21–175.03) compared to the wild type at 169.25 L/h (95% CI: 127.67–224.38, $p=0.02$).

DISCUSSION

In our previous study,[14] we examined the influence of menopausal status on the activity of CYP2D6 and CYP3A. The geometric means of midazolam oral clearances, indicative of CYP3A activity, were similar in the premenopausal (30.09 mL/min/kg, 95% CI: 21.55–42.020) and postmenopausal groups (24.10 mL/min/kg, 95% CI: 19.41–29.92). Additionally, there were no significant differences in the metabolic ratio (plasma log metoprolol/alpha-hydroxy metoprolol), representing CYP2D6 activity, between the premenopausal (0.25; 0.21–0.30) and postmenopausal (0.24; 0.19–0.29) groups. The current study aims to evaluate the impact of menopausal status on the pharmacokinetics of fexofenadine, a probe drug of P-gp activity.[9,17,18]

Fexofenadine is considered a P-gp substrate with an excellent safety profile. Furthermore, in vivo studies have shown that the fexofenadine AUC increases when

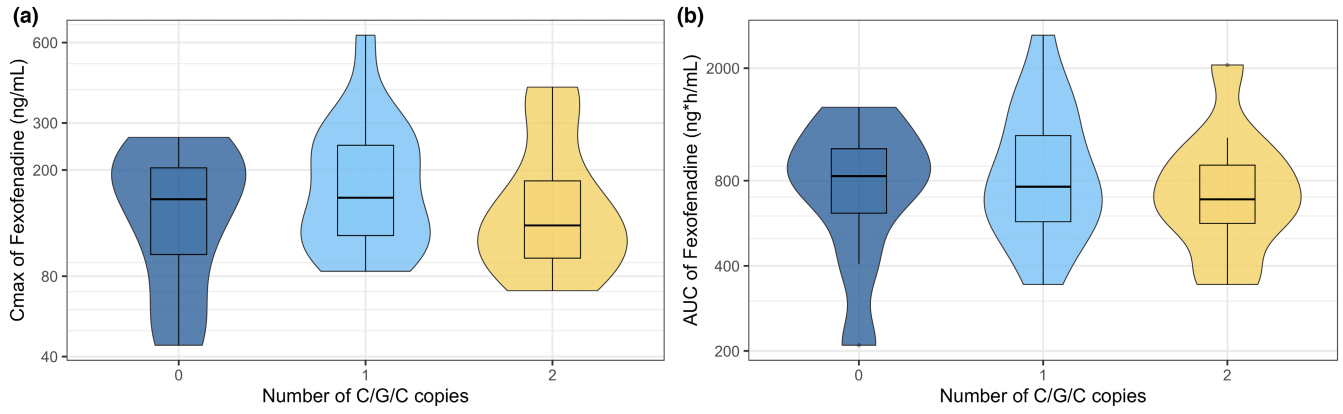

FIGURE 2 Violin plot of the AUC (panel a), C_{max} (panel b), and concentration-time profile (panel c) of fexofenadine following an oral single-dose (120 mg/day) in patients with breast cancer ($n=40$) stratified by *ABCB1* 3435C>T genotype. *ABCB1* 3435C>T genotype ratio of fexofenadine pharmacokinetics parameters (panel d). AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration.

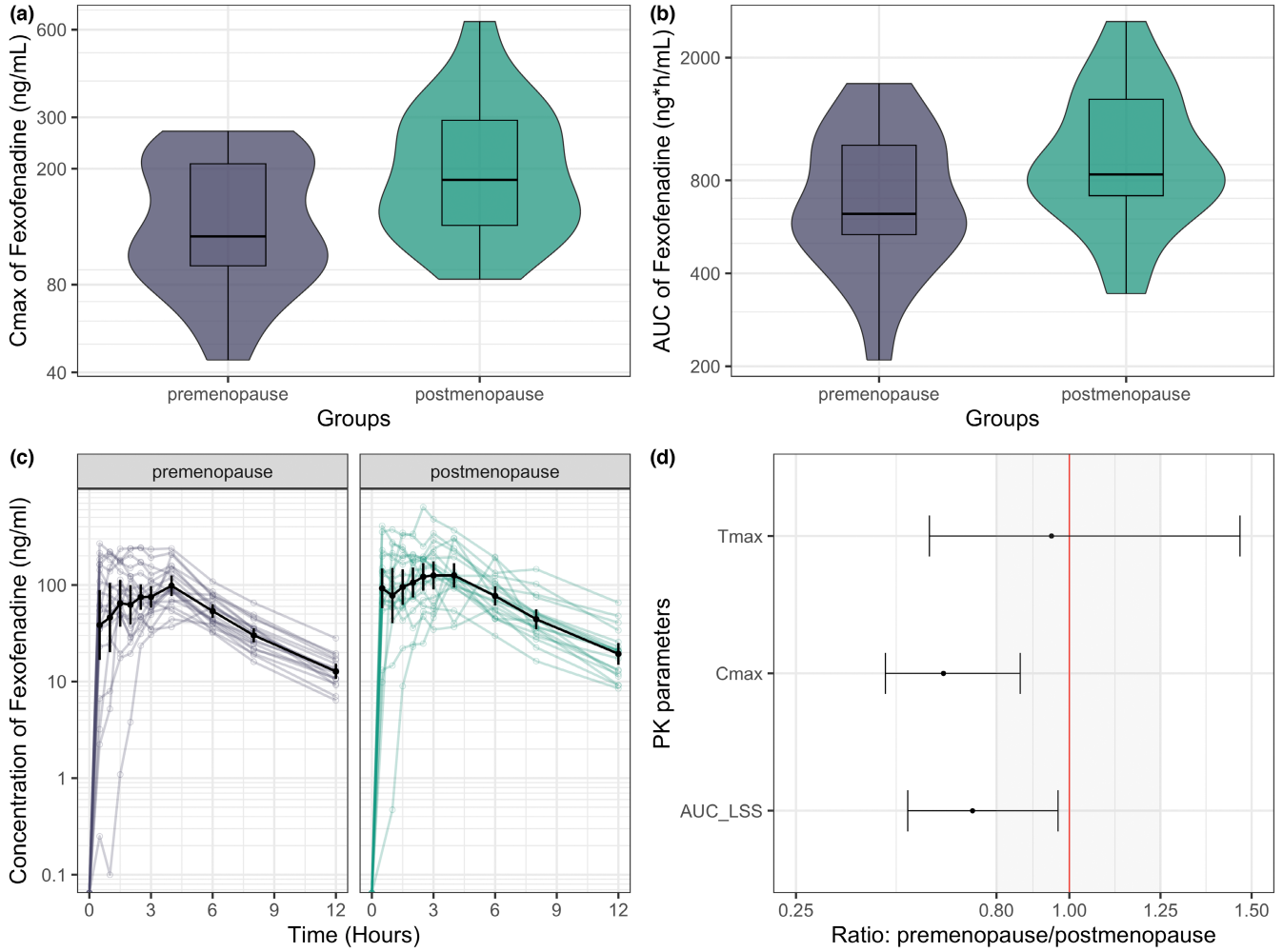

FIGURE 3 Violin plot of the AUC (panel a) and C_{max} (panel b) of fexofenadine following an oral single-dose (120 mg/day) in patients with breast cancer ($n = 40$) stratified by *ABCB1* diplotype. AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; LSS, limited sampling strategy; PK, pharmacokinetic; T_{max}, time to C_{max}.

co-administered with known P-gp inhibitors and decreases with inducers. Although fexofenadine is mainly eliminated unchanged, it cannot be considered a specific marker of P-gp because it is a substrate for the intestinal influx transporter OATP2B1, a substrate for the hepatic influx transporters OATP1B1 and OATP1B3.[19] Despite the lack of fexofenadine specificity as a P-gp probe drug, it was chosen in the present study due to its more excellent safety than others, such as digoxin, which also suffers from a lack of specificity. Dabigatran etexilate has been suggested as a potential P-gp probe with greater specificity.[19] However, further validation is needed because dabigatran etexilate is also subjected to intestinal CYP3A4 and glucuronidation metabolism, albeit to a limited extent.[20,21]

The data for fexofenadine pharmacokinetics analysis consisted of 436 samples collected from 40 tamoxifen-treated women with breast cancer who received a fexofenadine single oral dose (120 mg).

It is important to note that fexofenadine exhibits linear pharmacokinetics up to 120 mg,[22] and we used the limited sampling strategy (LSS) method to extrapolate the AUC accurately. The correlation between the predicted AUC_{inf} from the LSS equation and the observed AUC from our data demonstrates a high degree of accuracy with a correlation coefficient of 0.93 (Figure S1). During the validation step, the LSS method achieved an R2 (MD%, MAD%) of 0.96 (0.35, 7.08) and 0.94 (2.04, 8.69) for the training and validation sets, respectively, by applying the times of 90 and 240 min.[16] This demonstrates the accuracy and reliability of the LSS method for estimating AUC_{inf} in our study.

Overall, the geometric mean for fexofenadine AUC_{inf} was 800.00 ng h/mL (95% CI: 673.88–949.72 ng h/mL), and for C_{max} was 157.22 ng/mL (95% CI: 131.47–188 ng/mL). Regarding the T_{max}, the overall median was 2.7 h (CV: 55.46). These findings are in agreement with Vanhove et al.[19] who orally administered 120 mg single-dose of fexofenadine to evaluate the impact of P-gp

activity. The authors reported fexofenadine C_{max} range from 31 to 177 ng/mL in healthy volunteers. On the other hand, our results demonstrated a 1.1-fold decrease in AUC_{inf} and a 1.7-fold decrease in C_{max} compared to the findings of Dresser et al.[23] In their study, Dresser and colleagues examined the influence of grapefruit juice on the bioavailability reduction of fexofenadine in a cohort of healthy volunteers. In another study from the same group,[24] the author investigated the impact of grapefruit, orange, and apple juices on fexofenadine pharmacokinetics in healthy volunteers, the authors reported fexofenadine C_{max} at 288 ng/mL and the AUC_{inf} 1616 ng h/mL. The discrepancy between our results from the Dresser and coworkers could be explained by the tamoxifen therapy undertaken by our patients.

Tamoxifen, a selective estrogen-receptor modulator, is a standard long-term adjuvant treatment in women with hormone-positive breast cancer, often for 5–10 years. Tamoxifen has been identified as a strong P-glycoprotein inhibitor in in vitro studies.[25,26] However, it is essential to consider the concentrations used in these in vitro experiments. For example, in the study by Mao et al.,[25] a tamoxifen concentration of 10 μM was selected as the optimal concentration for experiments in ER-negative human gastric cancer cells. Similarly, Callaghan et al.[26] observed initial P-gp inhibition with 20 μM of tamoxifen in a human epidermal carcinoma cell line (KB3) and its drug-resistant derivative (KBV-1). It is important to note that a concentration of 10 μM is approximately equivalent to 5636.38 ng/mL of tamoxifen in plasma.

In contrast, our previous publication reported steady-state plasma concentrations (C_{ss}) of tamoxifen in clinical patients.[14] Postmenopausal patients exhibited increased C_{ss} compared to premenopausal patients (116.95 vs. 201.23 ng/mL). Therefore, the concentrations applied in the in vitro studies are ~50-fold higher than those observed in our clinical patient population (Ximenez et al.[14]). Bosh et al.[27] investigated tamoxifen's impact on the clearance of edoxaban, a known P-gp substrate, in patients with breast cancer. The findings from this study suggest that tamoxifen may not act as a P-gp inhibitor in a clinical context.

Given this substantial difference in concentration, it is reasonable to conclude that whereas tamoxifen may exhibit P-gp inhibitory properties in vitro at higher concentrations, the clinical relevance of such inhibition, especially at the lower, clinically relevant concentrations seen in patients, remains uncertain. Our findings suggest that the observed changes in fexofenadine pharmacokinetics are more likely due to factors other than significant P-gp inhibition by tamoxifen in our clinical population.

In our study, fexofenadine pharmacokinetics were evaluated in premenopausal patients ($n = 20$) aged from 35 to 50 years and in postmenopausal patients ($n = 20$) aged from 60 to 79 years (Table 1). The postmenopausal group when compared to the premenopausal group showed a significant increase in C_{max} (223.54 vs. 143.54 ng/mL; Figure 1a) and AUC_{inf} (933.54 vs. 685.55 ng h/mL; Figure 1b) geometric means. The linear regression model showed that postmenopausal patients have shown ~40% higher fexofenadine plasma exposure than premenopausal patients (685.55 vs. 933.54 ng h/mL, $p = 0.02$; Table 1, Figure 1b), suggesting decreased P-gp activity in postmenopausal women. Furthermore, our study has demonstrated that a daily dose of tamoxifen at 20 mg is insufficient to achieve a complete reduction in P-gp activity, as we have observed an influence of menopausal status on P-gp activity.

Based on our knowledge, this is the first study that has shown an increase in fexofenadine plasma exposure and in CL/F probably related to the increased bioavailability due to decreased intestinal P-gp activity in postmenopausal women. The impact of menopausal status on fexofenadine pharmacokinetics was not identified in previous studies[19,23,24] probably due to the lack of balance in the number of patients included in both pre- and postmenopausal groups and the consequent lack of significant statistical power. Needless to say, there are few clinical studies aimed to investigate drug transporter's activity on the drug's pharmacokinetics. The influence of menopausal status on fexofenadine plasma exposure between the investigated groups is probably due to the bioavailability differences (pre/postmenopausal ratio 90% CI, 0.66, 0.52–0.83). These results infer that the increased fexofenadine AUC in postmenopausal patients is not due to a decreased CYP activity because fexofenadine does not suffer intestinal or hepatic metabolism (Figure 1).

Hence, a reasonable hypothesis arises, suggesting that reduced P-gp activity in the gastrointestinal tract of postmenopausal patients could potentially elevate the bioavailability of fexofenadine. It is also worth noting that in postmenopausal patients undergoing treatment with a P-gp substrate, concurrent administration of a P-gp inhibitor should be approached with caution. For instance, this scenario may warrant consideration for dosage adjustments in medications, such as dabigatran etexilate, a well-known P-gp substrate, and prudent clinical monitoring of patients for signs and symptoms of bleeding when co-administered with cobicistat, a P-gp inhibitor.[28]

In a recent study, Fashe et al.[6] evaluated the impact of pregnancy-related hormones (estrone, estradiol, estriol, progesterone, cortisol, and placental growth hormone) on hepatic concentrations of non-CYP drug-metabolizing enzymes and transport proteins, including P-gp, in sandwich-cultured human hepatocytes from female donors, administered individually or in combination, across a range of physiologically relevant concentrations. The authors observed that OAT2 protein concentrations were

significantly decreased whereas OCT3 and P-gp were significantly increased in response to pregnancy-related hormone exposure. These findings corroborate our hypothesis that in the postmenopausal status, we have a lower intestinal P-gp activity.

Carriers of the *ABCB1* 3435 allele T exhibited higher geometric mean values for C_{max} (Figure 2a), with a value of 166.59 ng/mL, compared to the wild-type group with a value of 147.47 ng/mL ($p = 0.02$). However, no statistically significant differences were observed for carriers of the *ABCB1* 2677 allele nonG and the *ABCB1* 1236 allele T, with AUC_{inf} values of 864.38 and 844.78 ng h/mL, respectively, compared to their respective wild type groups ($p = 0.86$ and $p = 0.92$). In a study by Hoffmeyer et al.,[29] it was reported that plasma concentrations of digoxin were significantly higher in *ABCB1* 3435 TT carriers compared to CC carriers (with 7 subjects in each group). This finding suggests that the *ABCB1* TT genotype is associated with lower intestinal P-gp activity. However, these results contrast with those of Kim et al.,[30] who investigated *ABCB1* gene polymorphisms in a group of 37 healthy European-American and 23 healthy African American subjects. Kim et al. used fexofenadine as a substrate to compare the pharmacokinetic profiles among different genetic variant groups. Their study showed that the AUC of fexofenadine was significantly lower in the *ABCB1* 3435 TT group (with $n = 12$) compared to the CC group (with $n = 6$).

Some limitations of this study must be noted. First, this study was not designed to assess the effect of relatively rare genotypes in *ABCB1* (e.g., 2677GA and 2677AA) on fexofenadine pharmacokinetics, as study subjects were not selected based on genotype. Second, all included patients were under tamoxifen therapy, an in vitro P-gp inhibitor.

Based on the Biopharmaceutics Drug Disposition Classification System (BDDCS) proposed by Wu and Benet,[31] fexofenadine belongs to the class 3 which compounds required a drug transporter to facilitate absorption. Nevertheless, intestinal apical efflux transporters can significantly limit the absorption of such compounds, especially when there is sufficient penetration into the enterocytes via an uptake transporter. In other words, whereas the influx of class 3 compounds is typically limited by absorptive transporters, the counteractive effects of efflux transporters can still be significant as they are not saturated. These general principles also apply to class 4 compounds, although class 4 drugs may achieve sufficient solubility in the naturally occurring surfactants within gut contents, enabling them to act similarly to class 3 compounds. Therefore, the reduction of P-gp activity caused by menopausal status could increase the oral bioavailability of class 3 and class 4 drugs.

In conclusion, we have shown the impact that menopausal status has on the pharmacokinetics of fexofenadine for the first time. Postmenopausal patients have shown higher fexofenadine plasma exposure than premenopausal patients. These findings suggest that postmenopausal women's intestinal P-gp activity is reduced, resulting in a higher fexofenadine bioavailability. Regarding the *ABCB1* genotypes, apart from a higher fexofenadine C_{max} in carriers of the *ABCB1* 3435 allele T, fexofenadine pharmacokinetics were not influenced by common genetic polymorphisms in ABCB1. The findings described here suggest a plausible explanation for the clinical impact that P-gp activity has and sheds light upon a field lacking information regarding the relationship between menopausal status and drug transporter activity.

AUTHOR CONTRIBUTIONS

J.P.B.X. and V.L.L. wrote the manuscript. J.P.B.X., V.L.L., and J.M.d.A. designed the research. J.P.B.X., V.L.L., and A.R. performed the research. J.P.B.X., V.L.L., E.B.C., and G.S.-K. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

DATA AVAILABILITY STATEMENT

The source code is publicly available at https://github.com/XimenezJP/HaploStats_CTS.

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REFERENCES

1. Madla CM, Gavins FKH, Merchant HA, Orlu M, Murdan S, Basit AW. Let's talk about sex: differences in drug therapy in males and females. *Adv Drug Deliv Rev*. 2021;175:113804.
2. Franconi F, Campesi I. Pharmacogenomics, pharmacokinetics and pharmacodynamics: interaction with biological differences between men and women. *Br J Pharmacol*. 2014;171(3):580-594.
3. Paine MF, Ludington SS, Chen ML, Stewart PW, Huang SM, Watkins PB. Do men and women differ in proximal small intestinal CYP3A or P-glycoprotein expression? *Drug Metab Dispos*. 2005;33(3):426-433.
4. Harris RZ, Tsunoda SM, Mroczkowski P, Wong H, Benet LZ. The effects of menopause and hormone replacement therapies on prednisolone and erythromycin pharmacokinetics. *Clin Pharmacol Ther*. 1996;59(4):429-435.
5. Nazir S, Iqbal Z, Nasir F. Impact of menopause on pharmacokinetics of rosuvastatin compared with premenopausal women. *Eur J Drug Metab Pharmacokinet*. 2016;41(5):505-509.
6. Fashe MM, Fallon JK, Miner TA, Tiley JB, Smith PC, Lee CR. Impact of pregnancy related hormones on drug metabolizing enzyme and transport protein concentrations in human hepatocytes. *Front Pharmacol*. 2022;13:1004010.
7. Wang H, Zhou L, Gupta A, et al. Regulation of BCRP/ABCG2 expression by progesterone and 17beta-estradiol in human placental BeWo cells. *Am J Physiol Endocrinol Metab*. 2006;290(5):E798-E807.
8. Gustavson LE, Benet LZ. Menopause: pharmacodynamics and pharmacokinetics. *Exp Gerontol*. 1994;29(3–4):437-444.
9. Imanaga J, Kotegawa T, Imai H, et al. The effects of the SLCO2B1 c.1457C > T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenet Genomics*. 2011;21(2):84-93.
10. Hamman MA, Bruce MA, Haehner-Daniels BD, Hall SD. The effect of rifampin administration on the disposition of fexofenadine. *Clin Pharmacol Ther*. 2001;69(3):114-121.
11. Shon JH, Yoon YR, Hong WS, et al. Effect of itraconazole on the pharmacokinetics and pharmacodynamics of fexofenadine in relation to the MDR1 genetic polymorphism. *Clin Pharmacol Ther*. 2005;78(2):191-201.
12. Yasui-Furukori N, Uno T, Sugawara K, Tateishi T. Different effects of three transporting inhibitors, verapamil, cimetidine, and probenecid, on fexofenadine pharmacokinetics. *Clin Pharmacol Ther*. 2005;77(1):17-23.
13. Kharasch ED, Bedynek PS, Walker A, Whittington D, Hoffer C. Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics: II. Ritonavir effects on CYP3A and P-glycoprotein activities. *Clin Pharmacol Ther*. 2008;84(4):506-512.
14. Ximenez JPB, de Andrade JM, Marques MP, Coelho EB, Suarez-Kurtz G, Lanchote VL. Hormonal status affects plasma exposure of tamoxifen and its main metabolites in tamoxifen-treated breast cancer patients. *BMC Pharmacol Toxicol*. 2019;20(Suppl 1):81.
15. Muppavarapu R, Guttikar S, Rajappan M, Kamarajan K, Mullangi R. Sensitive LC-MS/MS-ESI method for simultaneous determination of montelukast and fexofenadine in human plasma: application to a bioequivalence study. *Biomed Chromatogr*. 2014;28(8):1048-1056.
16. Coelho EB, Cusinato DAC, Ximenez JP, Lanchote VL, Struchiner CJ, Suarez-Kurtz G. Limited sampling modeling for estimation of phenotypic metrics for CYP enzymes and the ABCB1 transporter using a cocktail approach. *Front Pharmacol*. 2020;11:22.
17. Ma JD, Tsunoda SM, Bertino JS, Trivedi M, Beale KK, Nafziger AN. Evaluation of in vivo P-glycoprotein phenotyping probes. *Clin Pharmacokinet*. 2010;49(4):223-237.
18. Bosilkovska M, Samer CF, Déglon J, et al. Geneva cocktail for cytochrome P450 and P-glycoprotein activity assessment using dried blood spots. *Clin Pharmacol Ther*. 2014;96(3):349-359.
19. Vanhove T, Bouillon T, de Loor H, Annaert P, Kuypers D. Fexofenadine, a putative in vivo P-glycoprotein probe, fails to predict clearance of the substrate tacrolimus in renal recipients. *Clin Pharmacol Ther*. 2017;102(6):989-996.
20. Stangier J, Rathgen K, Stähle H, Gansser D, Roth W. The pharmacokinetics, pharmacodynamics and tolerability of dabigatran etexilate, a new oral direct thrombin inhibitor, in healthy male subjects. *Br J Clin Pharmacol*. 2007;64(3):292-303.
21. Blech S, Ebner T, Ludwig-Schwellinger E, Stangier J, Roth W. The metabolism and disposition of the oral direct thrombin inhibitor, dabigatran, in humans. *Drug Metab Dispos*. 2008;36(2):386-399.
22. Robbins DK, Castles MA, Pack DJ, Bhargava VO, Weir SJ. Dose proportionality and comparison of single and multiple dose pharmacokinetics of fexofenadine (MDL 16455) and its enantiomers in healthy male volunteers. *Biopharm Drug Dispos*. 1998;19(7):455-463.
23. Dresser GK, Kim RB, Bailey DG. Effect of grapefruit juice volume on the reduction of fexofenadine bioavailability: possible role of organic anion transporting polypeptides. *Clin Pharmacol Ther*. 2005;77(3):170-177.
24. Dresser GK, Bailey DG, Leake BF, et al. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther*. 2002;71(1):11-20.
25. Mao Z, Zhou J, Luan J, Sheng W, Shen X, Dong X. Tamoxifen reduces P-gp-mediated multidrug resistance via inhibiting the PI3K/Akt signaling pathway in ER-negative human gastric cancer cells. *Biomed Pharmacother*. 2014;68(2):179-183.
26. Callaghan R, Higgins CF. Interaction of tamoxifen with the multidrug resistance P-glycoprotein. *Br J Cancer*. 1995;71(2):294-299.
27. Bosch F, Mulder F, Franken L, et al. Effect of the P-glycoprotein inhibitor tamoxifen on edoxaban plasma levels in women with breast cancer. *Thromb Res*. 2023;228:46-53.
28. Kumar P, Gordon LA, Brooks KM, et al. Differential influence of the antiretroviral pharmacokinetic enhancers ritonavir and cobicistat on intestinal P-glycoprotein transport and the pharmacokinetic/pharmacodynamic disposition of dabigatran. *Antimicrob Agents Chemother*. 2017;61(11):e01201-e01217.
29. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci*. 2000;97(7):3473-3478.
30. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther*. 2001;70(2):189-199.

31. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res*. 2005;22(1):11-23.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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