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ORIGINAL ARTICLE

Pharmacokinetics of cyclophosphamide enantiomers in patients with breast cancer

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Abstract
Purpose Adjuvant chemotherapy with cyclophosphamide (CYC) is used for the treatment of breast cancer. CYC is used as a racemic mixture, although preclinical data have demonstrated differences in the efficacy and toxicity of its enantiomers, with (S)-(−)-CYC exhibiting a higher therapeutic index. The present study investigated the enantioselectivity and influence of CYP2B6, CYP2C9, CYP2C19, and CYP3A on the kinetic disposition of CYC in patients with breast cancer.
Methods Fifteen patients previously submitted to removal of the tumor and treated with racemic CYC (900 or 1,000 mg/m^2) and epirubicin were included in the study. The in vivo activity of CYP3A was evaluated using midazolam as a marker drug. Serial blood samples were collected up to 24 h after administration of the first cycle of CYC.
Results The kinetic disposition of CYC was enantioselective in patients with breast cancer, with plasma accumulation of the (S)-(−)-CYC enantiomer (AUC 195.0 vs. 174.8 μg h/mL) due to the preferential clearance of the (R)-(+)CYC enantiomer (5.1 vs. 5.7 L/h). Clearance of either CYC enantiomer did not differ between the CYP2B6, CYP2C9, and CYP2C19 genotypes or as a function of the in vivo activity of CYP3A evaluated by midazolam clearance.
Conclusions The pharmacokinetics of CYC is enantioselective in patients with breast cancer concomitantly treated with epirubicin and ondansetron. Genotyping or phenotyping did not contribute to adjustment of the CYC dose regimen in patients included in this study.

Keywords Cyclophosphamide · Enantiomers · Breast cancer · Pharmacokinetics · CYP · Midazolam

Introduction

Breast cancer is a major public health issue worldwide [1]. It is the most frequent type of cancer worldwide among women, and it is responsible for nearly 500,000 deaths each year [2]. Combination chemotherapy using cyclophosphamide-containing regimens is integral to standard adjuvant therapy of node-positive breast cancer, resulting in significant improvements in disease-free survival and overall survivals [3].

Although CYC is a chiral drug which is administered as a mixture of its two enantiomers, (R)-(+)-CYC and (S)-(−)-CYC, there are no clinical studies regarding the effect of stereoselectivity on CYC pharmacodynamics. All available data were observed in preclinical studies, and most of them have showed that the (S)-(−)-CYC not only exerted higher antitumor effects (Lewis lung carcinoma, 16/C mammary adenocarcinoma and B16 melanoma) than (R)-(+)-CYC but also revealed higher therapeutic indices as well [4–6]. However, (R)-(+)-CYC was more effective

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than (S)-(−)-CYC against L 120 and P 388 lymphoid leukemias in vivo transplantable tumor models [7].

The liver is the main site of CYC bioactivation and/or detoxification. Oxidation at the C-4 position of CYC generates 4-hydroxycyclophosphamide (4-OH-CYC), which is in equilibrium with its ring-open tautomer aldophosphamide. This compound is then transported to the target tissue and spontaneously decomposes into phosphoramide mustard (ultimate metabolite responsible for the alkylating effect) and acrolein (metabolite responsible for urotoxicity). CYP isoenzymes including CY2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A, and CYP3A5 are involved in the bioactivation of CYC. Detoxification of CYC metabolites occurs through aldehyde dehydrogenases, or conjugation with glutathione by glutathione-S-transferases. CYC can also be directly detoxicated by N-dechloroethylation mediated by CP3A4/CYP3A5 leading to the formation of 2-dechloroethylcyclophosphamide (no cytotoxic effects) and chloroacetaldehyde (neurotoxic) [8, 9].

Genetic factors are thought to play a role in the interindividual variation in both response and toxicities associated with CYC-based therapies. A better understanding of the pharmacogenetic factors influencing the variation in CYC response offers an opportunity to individualize the treatment [9].

Genetic impact of CYP2B6, CYP2C9, and CYP2C19 gene variants on CYC metabolism has been investigated in order to better understand the mechanisms underlying the variation among individuals with respect to the formation of 4-OH-CYC.

In human liver microsomes, the CYP2B6*6 (G516T and A785G) carriers show higher CPA-4-hydroxylation [10]. The CYP2B6 G516T polymorphism, responsible for the enhanced catalytic activity by autoactivation, is one of the most common mutations in the CYP2B6 gene, with a frequency of 19.9% in Japanese [11], 28.6% in Germans [12] and 16.4% in human liver microsomes of Sweden [10]. Homozygotes CYP2B6*6 patients with malignant lymphoma or breast cancer show higher CYC clearance than heterozygotes and homozygotes of CY2B6*1 [13]. However, no differences were found in the CYC 4-hydroxylation between carriers of the CYP2B6 G516T variant allele ($n = 8$) and carriers of the CYP2B6 wild-type ($n = 21$) in patients with hematological malignancies [14].

CYP2C19 may partly contribute to the bioactivation of CPA in human liver microsomes, while the role of CYP2C9 appears minor. Human liver microsomes data revealed no differences in the intrinsic clearance of 4-OH-CYC formation between CYP2C9*1*1 ($n = 13$) and those subjects with one or two variant CYP2C9 alleles ($n = 19$) or between CYP2C19*1*1 ($n = 27$) and CYP2C19*1*2 ($n = 5$) [15].

However, the investigation of 124 Caucasian patients indicated that variant alleles in the CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, GSTA1, GSTP1, ALDH1A1, and ALDH3A1 genes do not explain the interindividual variability in CYC and 4-OH-CYC pharmacokinetics and probably are not the cause of toxicity variability [16]. Factors including the number of investigated patients, CYC dose or comedication might be responsible for the discrepancy among the different studies.

Despite the large number of studies on the clinical pharmacokinetics of CYC, few reports have investigated the enantioselectivity in the kinetic disposition of CYC, with no difference in pharmacokinetic parameters being observed between the (S)-(−)- CYC and (R)-(+)- CYC enantiomers [17–20].

Particular attention has been paid to the influence of chirality on 4-hydroxylation and on the detoxication products of CYC. Preliminary data indicate that different enzymes are responsible for the N-dechloroethylation of both CYC enantiomers considering that phenytoin pretreatment increases the formation of active metabolites from both CYC enantiomers and increases only the (S)-(−)-N—dechloroethylcyclophosphamide formation [17]. The pharmacokinetics of CYC in cancer patients ($n = 12$) indicate an enantioselective difference in the metabolism of CYC with the formation clearance of (R)-(+)-N-dechloroethylcyclophosphamide being almost twice as high as that of (S)-(−)-N-dechloroethylcyclophosphamide (0.25 vs. 0.14 L/h) [18]. The kinetic disposition of intravenous CYC is not enantioselective in cancer patients [19, 20], although these studies involved a small number of patients with various types of cancer. However, CYC kinetic disposition is enantioselective in patients with lupus nephritis resulting in higher exposures of the (S)-(−)-CYC (AUC 152.1 vs. 129.25 μg h/mL) when compared to (R)-(+)-CYC [21]. Thus, the results regarding the enantioselective pharmacokinetics of CYC in humans are still inconclusive.

The objective of the present study was to investigate the influence of enantioselectivity on the pharmacokinetics of CYC in a homogenous group of patients with breast cancer treated with CYC in combination with epirubicin and genotyped for CYP2B6, CPY2C9, and CYP2C19 and whose CYP3A activity was evaluated in vivo.

Patients and methods

Clinical protocol

The study was approved by the Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo. Patients with invasive breast cancer and an indication of CYC in combination with

Table 1 Individual data of the breast cancer patients investigated ($n = 15$)

Patient	Age (years)	BMI (kg/m^2)	Rac-CYC dose (mg)	Midazolam Cl (L/h kg)
1	55	25.0	900	0.35
2	25	24.4	1,000	0.06
3	41	15.3	1,000	1.68
4	54	24.1	900	0.32
5	33	22.2	1,000	1.08
6	35	22.6	1,000	0.18
7	51	24.8	900	1.01
8	56	24.2	1,000	0.86
9	53	33.9	1,000	0.46
10	40	20.1	1,000	0.25
11	49	32.4	1,000	0.33
12	43	28.1	1,000	1.18
13	36	23.6	900	0.63
14	47	22.3	900	0.47
15	57	27.7	1,000	0.31
Median	47	24.2	–	0.46
Mean (95% CI)	45 (39.6–50.4)	24.7 (22.2–27.2)		0.61 (0.36–0.86)

epirubicin were included in the study after they had signed a free informed consent form. The patients studied ranged in age from 25 to 57 years (Table 1) and presented hepatic and renal function within normal limits. The patients underwent clinical examination and histological and cytological tests. All patients included in the study received a single intravenous dose of 8 mg ondansetron immediately before the beginning of chemotherapy.

The patients were treated with 4 to 6 doses of injectable racemic CYC (Genuxal®, Asta Médica, São Paulo, SP, Brazil) at individual doses of 900 or 1,000 mg infused over a period of 1 h and administered at intervals of 21 days. The patients were hospitalized during the first chemotherapy cycle and, on that occasion, received an intravenous dose of 1 mg midazolam (Dormonid®, Roche, Rio de Janeiro, RJ, Brazil) used as a marker drug of in vivo CYP3A activity. Blood samples were collected into heparinized syringes (5,000 IU Liquemine®, Roche, São Paulo, SP, Brazil) immediately before administration of the drug and 15, 30, 45, and 60 min and 1.25, 1.5, 2, 3, 5, 8, 12, 16, and 24 h after the beginning of infusion. The samples were centrifuged at 2,000*g* and frozen at −70°C until the time of chromatographic analysis.

Enantioselective analysis of CYC in plasma

The CYC enantiomers were analyzed by LC–MS/MS according to the method previously developed by our research group [22]. Briefly, chromatographic separation was carried out on a Chiralcel® ODR column (Chiral Technologies Inc., Exton, PA, USA) and Lichrospher 100 RP-18 pre-column (Merck, Darmstadt, Germany). The mobile phase consisted of water:acetonitrile (75:25, v/v) supplemented with 0.2% formic acid. For sample preparation, 0.2 mL plasma was supplemented with 25 µL antipyrine (internal standard, 0.1 mg/mL in methanol) and 5 mL of extraction solvent (ethyl acetate:chloroform, 75:25, v/v). After extraction for 30 min and centrifugation at 2,000*g* for 10 min, the organic phase was transferred to conical tubes and evaporated to dryness under vacuum. The residues were dissolved in 200 µL of the mobile phase and 100 µL n-hexane, and 40 µL was submitted to chromatographic analysis. The protonated ions and their respective ion products were monitored at the following transitions: m/z 241 > 160 for the CYC enantiomers and 189 > 104 for the internal standard. The method was linear within a concentration range of 2.5–25,000 ng/mL plasma for each enantiomer. The coefficient of variation for precision and accuracy was less than 15%.

Analysis of midazolam in plasma

Midazolam was analyzed in plasma by LC–MS/MS as described in a previous study from our group [23]. Plasma aliquots (1 mL) supplemented with the internal standard (clobazam) were extracted in basic medium (0.1 M NaOH) with 4 mL toluene-isoamyl alcohol (100:1, v/v) for 30 min in a mechanical horizontal shaker. After centrifugation at 2,000*g* for 5 min, the organic phases were separated and evaporated to dryness. The residues were dissolved in 50 µL of the mobile phase (acetonitrile and 10 mmol/L ammonium acetate, 50:50) and analyzed by LC–MS/MS. Midazolam and the internal standard were separated on an RP-18 column using the mobile phase described above.

Protonated $[M + H]^+$ species and their respective ion products were monitored at transitions of m/z 301 > 259 for the internal standard and 326 > 291 for midazolam. The analytical curve was constructed over a concentration range of 0.1–100 ng/mL plasma.

Pharmacokinetic and statistical analyses

The kinetic disposition of midazolam and of the CYC enantiomers was analyzed with the WinNonlin software, version 4.0 (Pharsight Corporation, Mountain View, CA, USA) using a monocompartmental model and first-order kinetics. The area under the plasma concentration versus time curve ($AUC^{0-\infty}$) was determined by the trapezoidal linear method from time zero to 24 h for CYC and from time zero to 8 h for midazolam, with extrapolation to infinite. Pharmacokinetics parameters were evaluated according to the standard equations of the software.

The results are expressed as median, mean, and 95% confidence interval determined with the Graphpad Instat® software, version 3.0 (Graphpad Software Inc., San Diego, CA, USA). The Wilcoxon test was used to evaluate (R)-(+)/(S)-(−) enantiomer ratios, different from unity, with the level of significance set at $P \leq 0.05$. Differences in the pharmacokinetic parameters of each enantiomer among genotypes were evaluated by the Mann–Whitney or Kruskal–Wallis test at a level of significance of $P < 0.05$.

The orthogonal regression test was used to evaluate the relationship between Cl of midazolam and Cl of each CYC enantiomer according to the equations described by Schellens et al. [24] with the level of significance set at $P \leq 0.05$. Orthogonal regression was performed with the GMC program (Geraldo Maia Campos, Biological Research, version 6.6, Ribeirão Preto, Brazil).

Extraction of genomic DNA

Genomic DNA was isolated from peripheral leukocytes (5 mL whole blood) using the salting out technique. CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, and CYP2C19*17 were identified by a polymerase chain reaction (PCR) amplification with use of the allele-specific primers. PCR products were digested with *Ava*2 (CYP2C9*2), *Ava*3 (CYP2C9*3), *Sma*I (CYP2C19*2), *Bam*HI (CYP2C19*3), and MN1 (CYP2C9*17) (New England Biolabs, Inc., Beverly, MA, USA), and analysed on 2% agarose gels stained with ethidium bromide. The allelic variants CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, and CYP2C19*17 were identified based on the electrophoresis band patterns [25–28].

For analysis of the frequency of the CYP2B6 G516T polymorphism, genomic DNA segments were amplified by PCR amplification with use of the allele-specific primer described by Jacob et al. [29], digested with *Bsr*1 (New England Biolabs, Inc.), and submitted to 10% acrylamide gel electrophoresis [09].

Results

Figure 1 shows the plasma (R)-(+)-CYC and (S)-(−)-CYC concentration versus time curves for 15 patients with breast cancer treated with 900 or 1,000 mg/m^2 racemic CYC infused over a period of 1 h. The pharmacokinetic parameters calculated for the (S)-(−)-CYC and (R)-(+)-CYC enantiomers are reported in Table 2 as median, mean, and 95% confidence interval. The patients presented enantioselectivity in the pharmacokinetics of CYC with plasma accumulation ($AUC^{0-\infty}$ 195.0 vs. 174.8 μg h/mL), lower clearance (5.1 vs. 5.7 L/h), and higher distribution volume (46.0 vs. 45.6 L) of the (S)-(−)-CYC enantiomer.

Table 3 shows the frequency of the CYP2B6, CYP2C9, and CYP2C19 gene polymorphisms in 13 patients. The genotype of two patients could not be evaluated because of the lack of good quality DNA. The results showed 61.54% patients with the CYP2C9*1/*1 genotype, 38.6% patients with the genotype CYP2C19*1/*1, and 53.85% patients with the genotype CYP2B6 G/G.

Table 4 shows the clearance of the (S)-(−)-CYC and (R)-(+)-CYC enantiomers in patients genotyped as CYP2B6 G/G or G/T. A significant difference ($P \leq 0.05$, Wilcoxon test) in clearance between (S)-(−) and (R)-(+) enantiomers was observed for the G/G polymorphism ($n = 7$; 5.8 vs. 6.3 L/h). However, no significant difference in enantiomer clearance was observed between the G/G and G/T polymorphisms ($P \leq 0.05$, Mann–Whitney test).

Table 4 also shows the clearance of the (S)-(−)-CYC and (R)-(+)-CYC enantiomers in patients genotyped for the CYP2C9 and CYP2C19 polymorphisms. A significant

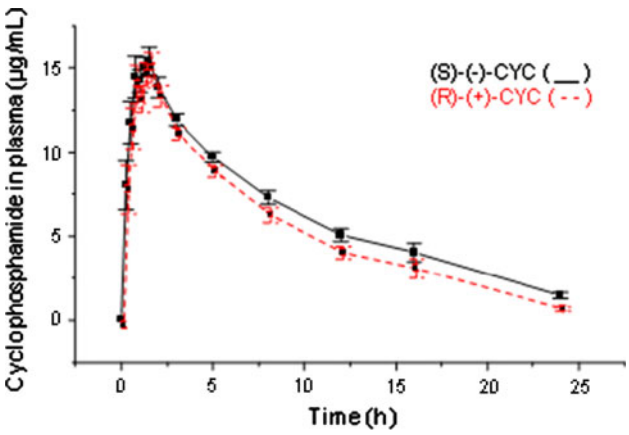

Fig. 1 Plasma concentration versus time curves obtained for the (R)-(+)-CYC and (S)-(−)- CYC enantiomers in patients with breast cancer ($n = 15$). Data are reported as mean and SEM

Table 2 Kinetic disposition of the (S)-(−)-CYC and (R)-(+)-CYC enantiomers after intravenous administration of a single dose of racemic CYC to patients with breast cancer ($n = 15$)

Parameter	(S)-(−)-CYC	(R)-(+)-CYC	*P*
$t_{½}$ (h)	5.8	5.1*	0.0001
	5.9 (5.1–6.7)	5.2 (4.4–5.9)	
Kel (h^{-1})	0.12	0.14*	0.0001
	0.12 (0.11–0.14)	0.14 (0.12–0.17)	
C_{max} (μg/mL)	19.7	19.7	0.3028
	21.5 (17.3–25.8)	21.6 (17.4–25.9)	
$AUC^{0-\infty}$ (h μg/mL)	195.0	174.8*	0.0001
	188.8 (158.8–218.9)	168.1 (139–197.3)	
Cl (L/h)	5.1	5.7*	0.0001
	5.6 (4.6–6.6)	6.4 (5.1–7.8)	
Vd (L)	46.0	45.6*	0.0084
	46.1 (39.0–53.2)	45.3 (38.6–52)	

Values are expresses as median, mean, and 95% confidence interval

AUC values were normalized to the dose of 1,000 mg

* $P \leq 0.05$, Wilcoxon test

Table 3 Frequency of CYP2B6, CYP2C9, and CYP2C19 gene polymorphisms in the breast cancer patients investigated ($n = 13$)

Patient	Genotype		
	CYP2B6	CYP2C9	CYP2C19
1	G/T	*1/*2	*1/*1
2	G/G	*1/*1	*1/*2
3	G/G	*1/*3	*1/*1
4	T/T	*1/*1	*1/*1
5	G/G	*1/*2	*1/*17
6	G/G	*1/*1	*1/*2
7	G/T	*1/*1	*17/*17
8	G/G	*1/*1	*1/*3
11	G/T	*1/*2	*1/*1
12	G/T	*1/*1	*17/*17
13	G/G	*2/*3	*1/*1
14	G/G	*1/*1	*1/*17
15	G/T	*1/*1	*1/*17
$n = 13$	G/G: 7 (53.85%)	*1/*1 = 8 (61.54%)	*1/*1 = 5 (38.46%)
	G/T: 5 (38.46%)	*1/*2 = 3 (23.07%)	*1/*2 = 2 (15.38%)
	T/T: 1 (7.69%)	*1/*3 = 1 (7.69%)	*1/*3 = 1 (7.69%)
		*2/3 = 1 (7.69%)	*1/*17 = 3 (23.08%)
			*17/*17 = 2 (15.38%)

(G) Wild-type allele; (T) mutant allele

Table 4 Clearances of the CYC enantiomers as a function of the different CYP genotypes in the breast cancer patients investigated ($n = 13$)

Polymorphism	Total clearance (L/h)	
	(S)-(−)-CYC	(R)-(+)-CYC
CYP2B6		
G/G ($n = 7$)	5.8	6.3
	5.8 (4.1–7.6)	6.7 (4.3–9.1)
G/T ($n = 5$)	4.9	5.3
	5.6 (2.5–8.7)	6.4 (2.4–10.4)
CYP2C9		
*1/*1 ($n = 8$)	5.0	5.5
	5.6 (3.9–7.4)	6.4 (4.2–8.6)
*1/*2 and *1/*3 ($n = 4$)	4.5	5.2
	5.3 (1.4–9.2)	6.3 (0.8–11.8)
CYP2C19		
*1/*1 ($n = 5$)	4.0	4.8
	4.5 (3.0–6.1)	5.3 (3.3–7.3)
*1/*2 and *1/*3 ($n = 3$)	5.1	5.7
	5.5 (1.1–9.9)	6.0 (1.0–11.1)
*1/*17 and *17/*17 ($n = 5$)	5.8	6.3
	6.7 (3.6–9.8)	7.9 (3.6–12.2)

Values are expressed as median, mean, and 95% confidence interval

Mann–Whitney or Kruskal–Wallis test showed *P* values ≥ 0.05 among genotypes

difference ($P \leq 0.05$; Wilcoxon test) in enantiomer clearance was observed for the CYP2C9*1/*1 polymorphism (5.0 vs. 5.5 L/h, respectively for (S)-(−)-CYC and (R)-(+)-CYC). However, there was no difference in enantiomer clearance when the CYP2C9*1/*1 were compared with CYP2C9*1/*2 plus CYP2C9*1/*3 polymorphisms. With respect to genotype CYP2C19, no significant difference between enantiomers was observed for the CYP2C19*1/*1 polymorphism. No difference in enantiomer clearance was observed when polymorphisms CYP2C19*1/*1 were compared with CYP2C19*1/*2 plus CYP2C19*1/*3 or when polymorphisms CYP2C19*1/*1 were compared with CYP2C19*1/*17 plus CYP2C19*17/*17.

Figures 2 and 3 show the lack of significant correlations between the clearance of the CYC enantiomers and in vivo activity of CYP3A evaluated by clearance of midazolam administered intravenously immediately before CYC infusion. No correlation was observed between the clearance of midazolam and clearance of either CYC enantiomer.

Discussion

Enantioselectivity in the pharmacokinetics of CYC was evaluated in a group of 15 patients with breast cancer. The patients investigated first underwent surgery for removal of the tumor and then received adjuvant chemotherapy with

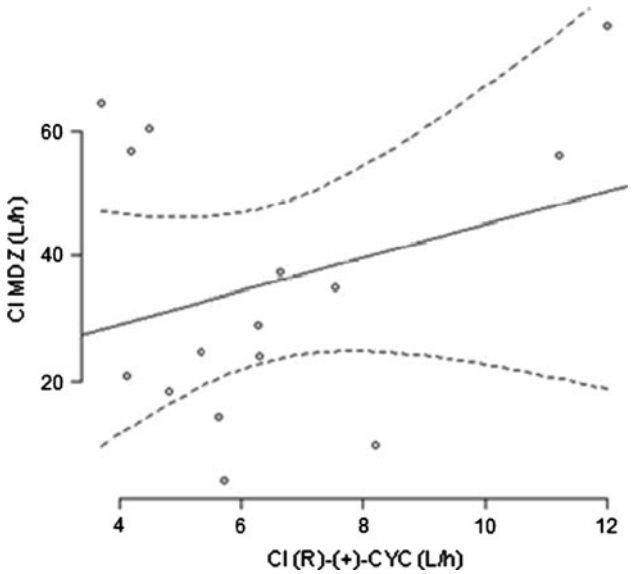

Fig. 2 Correlation between the clearance of the (R)-(+)-CYC and in vivo activity of CYP3A evaluated by midazolam clearance (Cl MDZ) administered intravenously. Confidence bands for the adjusted line: $y = 18.67 + 2.63x$; $r = 0.29$, $P = 0.29$

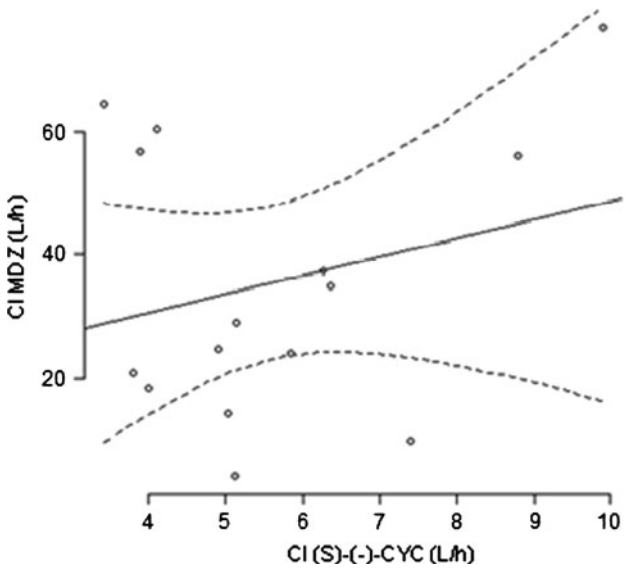

Fig. 3 Correlation between the clearance of the (S)-(−)-CYC and in vivo activity of CYP3A evaluated by midazolam clearance (Cl MDZ) administered intravenously. Confidence bands for the adjusted line: $y = 18.87 + 2.97x$; $r = 0.25$, $P = 0.36$

CYC. Ten patients received a single CYC dose of 1,000 mg, and the other 5 received a single dose of 900 mg, both administered intravenously. The analysis of an elevated number of patients ($n = 15$) and of a homogenous group in terms of gender (women), type of disease (breast cancer), associated drugs (epirubicin and ondansetron), and dose administered (900 and 1,000 mg) permits a higher consistency in the evaluation of the enantioselectivity of CYC in humans. Ondansetron, a drug widely employed to prevent chemotherapy-induced emesis, increases the clearance of CYC by 17% [30]. With respect to epirubicin, we found no studies investigating its pharmacokinetic interaction with CYC.

CYC pharmacokinetics has been reported as no enantioselective. Williams et al. [18] investigated 12 patients with different types of tumors, 9 of them receiving a single CYC dose of 2.1 g/m^2 infusion over 1 h, whereas the other 3 patients received a dose of 60 mg/kg infusion over 2 h daily for 2 days. Jarman et al. [31] investigated the metabolism of CYC enantiomers in 4 patients (1 g intravenous) who had squamous cell carcinoma of the lung, including one woman and three men. Holm et al. [20] evaluated CYC pharmacokinetics in 9 patients (3 women, 6 men, and 1 unknown) treated for various neoplastic diseases after intravenous administration of doses ranging from 614 to 1,270 mg.

However, the pharmacokinetics of CYC was enantioselective in the investigated patients (Table 2). The results showed significant differences in the volume of distribution between the (S)-(−)-CYC and (R)-(+)-CYC enantiomers (46.0 vs. 45.6 L). Jarman et al. [31] found that the binding of CYC to plasma proteins is not enantioselective. The percentage of binding is almost identical when the enantiomers are incubated individually, with a value of 35% for (R)-(+)-CYC and of 36% for (S)-(−)-CYC. These authors reported a similar volume of distribution for the (S)-(−)-CYC and (R)-(+)-CYC enantiomers (49.6 ± 8.5 and 50.6 ± 11.4 L, respectively). Volumes of distribution of 0.45 ± 0.08 L/kg and 0.43 ± 0.07 L/kg were reported by Corlett and Chrystyn [18] for the (S)-(−)-CYC and (R)-(+)-CYC enantiomers, respectively.

In the present study, significant differences in total clearance were observed between the (S)-(−)-CYC and (R)-(+)-CYC enantiomers (5.1 vs. 5.7 L/h) (Table 2). This difference in CYC enantiomer clearance has not been reported in other studies. In this respect, Williams et al. [18] and Corlett and Chrystyn [19] reported total clearance values of 6.9 L/h and 0.049 ± 0.021 L/h kg for (R)-(+)- CYC and of 7.2 L/h and 0.048 ± 0.02 L/h.kg for (S)-(−)- CYC, respectively. The observation of differences in total clearance between CYC enantiomers increases the discussion about the contribution of each isomer to the final pharmacological effect after administration of the racemic mixture. Paprocka et al. [6] observed a higher antitumor effect and higher therapeutic indices for the S-enantiomer in three models of solid tumors (Lewis lung carcinoma, 16/C mammary adenocarcinoma, and B16 melanoma) when compared to the R-enantiomer. Furthermore, the same group showed later that (R)-(+)-CYC is more effective than (S)-(−)-CYC in leukemia cells [7]. In the present investigation, the plasma concentrations of the (S)-(−)-CYC enantiomers were significantly higher than

those of the (R)-(+)-CYC antipode ($AUC^{0-\infty}$ 195.0 vs. 174.8 μg h/mL; Table 2).

In the present study, the enantiomers differed in terms of their elimination half-life (Table 2), a finding not observed in studies reported in the literature. Williams et al. [18] reported elimination half-lives of 5.8 h for (R)-(+)-CYC and 5.7 h for (S)-(−)-CYC in cancer patients. Similar values of 6.82 ± 2.27 and 7.13 ± 1.84 h have been reported by Corlett and Chrystyn [19] for the (R)-(+)-CYC and (S)-(−)-CYC enantiomers, respectively, in six patients with cancer. Jarman et al. [31] reported elimination half-lives of 7.3 ± 1.8 h for (R)-(+)-CYC and of 7.9 ± 1.7 h for (S)-(−)-CYC in 4 patients with lung carcinoma. Although not statistically significant, data reported in the literature show a tendency toward a more prolonged elimination half-life for the (S)-(−)-CYC enantiomer, in agreement with the present results (5.9 vs. 5.0 h for (S)-(−)-CYC and (R)-(+)-CYC, respectively).

Jarman et al. [31] reported equal urinary excretion of the two enantiomers or a slightly favored excretion of the (S)-(−)-CYC enantiomer in patients with lung carcinoma. Formation clearance of the dechloroethylcyclophosphamide metabolite was found to differ between enantiomers, with higher values for (R)-(+)-CYC (0.25 vs. 0.14 L/h) in 12 patients with different types of cancer. Although (R)-(+)-CYC is preferentially dechloroethylated, only a small percentage of the dose is converted to this metabolite (3.6 vs. 1.9%) [18]. The authors did not observe differences in the formation clearance of the active metabolite 4-OH-CYC between enantiomers (5.1 vs. 5.6 L/h for (R)-(+)-CYC and (S)-(−)-CYC), respectively). The plasma accumulation of the (S)-(−)-CYC enantiomer observed in the present study ($AUC^{0-\infty}$ 195 vs. 174.8 μg.h/mL) might be explained, at least in part, by the preferential dechloroethylation of the (R)-(+)-CYC enantiomer.

The results in Table 4 show that clearance of either CYC enantiomer did not differ between patients genotyped as G/G ($n = 7$) or G/T ($n = 5$) for CYP2B6; *1/*1 ($n = 8$) or *1/*2 plus *1/*3 ($n = 4$) for CYP2C9, and *1/*1 ($n = 5$) and *1/*2 plus *1/*3 ($n = 3$) or *1/*17 plus *17/*17 ($n = 5$) for CYP2C19. According to our data and other clinical previous studies with a larger number of patients [14, 16] CYP2B6, CYP2C19, and CYP2C9 genotypes do not interfere in CYC pharmacokinetics as enantiomeric mixture or individual enantiomers.

CYP3A in vivo activity was also evaluated in the present study in order to clarify one of many possible mechanisms that could explain the observed inter-patient variability in the CYC pharmacokinetics considering the role of CYP3A4 in its activation and deactivation pathways [8]. Midazolam clearance is the most widely accepted and tested CYP3A probe. Studies with human liver samples have shown that midazolam clearance following intravenous administration correlates with hepatic CYP3A concentrations [32]. We observed a 25-fold variability in midazolam clearance in the investigated patients (0.06–1.68 L/kg h, Table 1), which was also observed by other studies [33]. Similar clearance values were observed for intravenous midazolam in 37 women by Chen et al. [34] (0.50 ± 0.08 L/h.kg; mean ± SD) and in 41 women by Kharash et al. [35] (8.9 ± 2.4 mL/min kg). No correlation was observed between the clearance of midazolam and the clearance of either CYC enantiomer in the patients investigated (Figs. 2 and 3).

In conclusion, the pharmacokinetics of CYC is enantioselective in patients with breast cancer concomitantly treated with epirubicin and ondansetron.

The clearance of either CYC enantiomer did not differ between the CYP2B6, CYP2C9, and CYP2C19 genotypes or as a function of in vivo CYP3A activity, suggesting that genotyping or phenotyping did not contribute to adjustment of the CYC dose regimen in patients included in this study.

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