Influence of Quinidine, Cimetidine, and Ketoconazole on the Enantioselective Pharmacokinetics and Metabolism of Metoprolol in Rats

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ABSTRACT Metoprolol is a β-blocker and its racemic mixture is used for the treatment of hypertension. In the present study we investigated the influence of CYP2D and CYP3A on the stereoselective metabolism of metoprolol in rats. Male Wistar rats ($n = 6$ per group) received racemic metoprolol (15 mg/kg) orally, with or without pretreatment with the CYP inhibitor ketoconazole (50 mg/kg), cimetidine (150 mg/kg), or quinidine (80 mg/kg). Blood samples were collected up to 48 h after metoprolol administration. The plasma concentrations of the stereoisomers of metoprolol, *O*-demethylmetoprolol (ODM), α-hydroxymetoprolol (OHM) (Chiralpak® AD column), and metoprolol acidic metabolite (AODM) (Chiralcel® OD-R column) were determined by HPLC using fluorescence detection ($\lambda_{exc} = 229$ nm; $\lambda_{em} = 298$ nm). CYP3A inhibition by ketoconazole reduced the plasma concentrations of ODM and AODM and favored the formation of OHM. CYP2D and CYP3A inhibition by cimetidine reduced the plasma concentrations of OHM and AODM and favored the formation of ODM. The inhibition of CYP2D by quinidine reduced the plasma concentrations of OHM and favored the formation of ODM. In conclusion, the results suggest that CYP3A is involved in the formation of ODM and CYP2D is involved in the formation of AODM. *Chirality 21:886–893, 2009*. © 2009 Wiley-Liss, Inc.

KEY WORDS: metoprolol; cytochrome P450 inhibitors; metabolites; pharmacokinetics; rats; enantiomers

INTRODUCTION

Metoprolol, a selective ß$_1$-adrenergic receptor antagonist, is used in clinical practice in the racemic form for the treatment of myocardial infarction, heart failure, and arterial hypertension. The (S)-metoprolol enantiomer has about 500-fold more affinity for the ß$_1$-adrenergic receptor than its (R)-antipode.[1,2]

In rats, the kinetic disposition of metoprolol is enantioselective, although inverse and less marked when compared to humans. Oral clearance of the (−)-(S) enantiomer is higher than that of the (+)-(R) enantiomer in rats treated with racemic metoprolol at a dose of 20 mg/kg (2.26 versus 1.99 ml/min/kg). The elimination half-life is similar (35 min) for the (+)-(R)- and (−)-(S)-metoprolol enantiomers, indicating that rats are an animal model with a behavior similar to that of Caucasian extensive metabolizers of metoprolol.[3]

Metabolism is the main process responsible for metoprolol elimination.[4] The metabolites of metoprolol (see Fig. 1) are generated by aliphatic hydroxylation (α-hydroxymetoprolol, OHM), deamination (H104/83), and *O*-dealkylation (*O*-demethylmetoprolol, ODM) followed by oxidation (acid metabolite, AODM).[5] The rate of α-hydroxylation exceeds *O*-demethylation by about 30% in rat liver microsomal preparations.[6] In these oxidations, CYP2D is associated with α-hydroxylation and an additional CYP enzyme is responsible for *O*-demethylation in human liver microsomes.[6,7]

The α-hydroxylation pathway shows a high degree of product stereoselectivity in rat liver microsomes, mainly yielding the 1′R-hydroxy product (1′R/1′S > 12).[6] Boralli et al.[8] observed that the kinetic disposition of unchanged metoprolol and the formation of ODM are not enantioselective in rats but the metabolism of α-OHM mainly yields the 1′R product. As CYP2D is only partly responsible for *O*-demethylation of metoprolol in rats,[6] the involvement of another CYP isoform in the metabolic pathway might be assessed by chemical inhibitor studies.[9]

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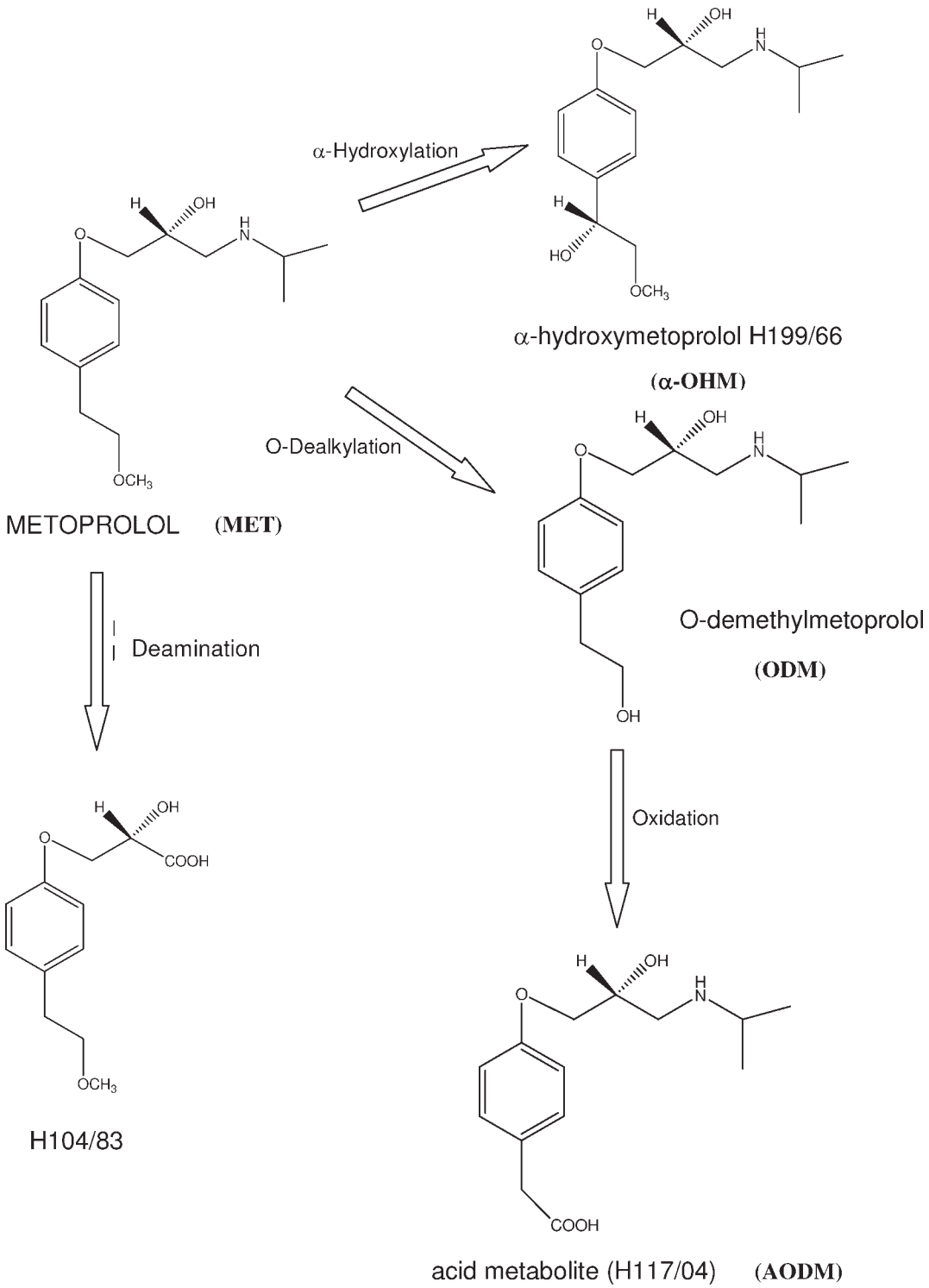

Fig. 1. Metabolism of metoprolol by oxidative pathways in rats and man (adapted from Borg et al.[5]).

Coulbourne et al.[10] demonstrated an interaction between metoprolol and desipramine in rats, a substrate and inhibitor of CYP2D, respectively. The authors observed an increase up to 20-fold in plasma metoprolol levels when the drug was administered in combination with desipramine, indicating that in rats metabolism also occurs through CYP2D6.

In vitro and in vivo studies have shown that quinidine inhibits CYP2D. Law et al.[11] reported that quinidine administered to rats at a dose of 80 mg/kg inhibited the 4-

hydroxylation of amphetamine, which primarily depends on CYP2D. The authors also demonstrated a positive correlation between the urinary excretion of 4-hydroxyamphetamine and CYP2D activity in rat liver microsomes.

Studies conducted on rats have indicated that ketoconazole inhibits CYP3A and P-gP.[13] Yuan et al.[12] demonstrated the inhibition of CYP3A after intraperitoneal administration of ketoconazole to rats at a dose of 50 mg/kg. Similar results have been reported by Zhang et al.[13] using an oral dose of 30 mg ketoconazole/kg, whereas Kageyama et al.[14] observed CYP3A inhibition in rats after intravenous administration of 10, 15, and 20 mg/kg.

In contrast, cimetidine, an H2 receptor antagonist used for the treatment of peptic ulcers and associated diseases, shows an interesting behavior. In male rats, cimetidine inhibits CYP2C11 as well as CYP2C6.[15]

Considering that the inhibition of CYPs involved in the metabolism of metoprolol (CYP2D and probably CYP3A) might be enantioselective, with consequent changes in the plasma concentrations of the eutomer, the objective of the present study was to investigate the influence of quinidine, cimetidine, and ketoconazole on the kinetic disposition and metabolism of metoprolol enantiomers in rats.

MATERIALS AND METHODS

Experimental Protocol

The study was approved by the Ethics Committee for the Use of Animals of the Ribeirão Preto Campus, University of São Paulo, in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the U.S. National Institutes of Health. Male Wistar rats (200 ± 20 g) were kept for 3 days before the experiment in a room at a controlled temperature (21–23°C) and humidity (40–60%) and on a 12-h light-dark cycle. The animals had free access to ration and water throughout the experiment.

Control animals fasted for 12 h (n = 6 per sampling time) received 0.1 ml i.p. physiological saline acidified with 5% (v/v) HCl at a final pH of 4.0. Two hours later, rats received by gavage racemic metoprolol (Sigma, St. Louis, MO) dissolved in water at a dose of 15 mg/kg.[16] Blood samples (5 ml) were collected by decapitation at zero, 2, 4, 6, 8, 10, 20, 30, 40, and 50 min and 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 10 h after metoprolol administration. Heparin (Liquemine®, 5000 IU, Roche, Taboão da Serra, SP, Brazil) was used as anticoagulant. The plasma samples obtained after centrifugation were stored at −70°C until the time for analysis.

Animals in the quinidine group were fasted for 12 h (n = 6 per sampling time) and received quinidine (Buchler, São Paulo, Brazil) i.p. at a dose of 80 mg/kg dissolved in physiological saline acidified with 5% (v/v) HCl at a final pH of 4.0.[11] Four hours after quinidine administration, the animals received by gavage racemic metoprolol dissolved in water at a dose of 15 mg/kg. Blood samples (5 ml) were collected and stored as described for the control group.

Animals in the cimetidine group were fasted for 12 h (n = 6 per sampling time) and received cimetidine (FURP, Guarulhos, Brazil) i.p. at a dose of 150 mg/kg dissolved in physiological saline acidified with 5% (v/v) HCl at a final pH of 4.0.[15] Ninety minutes after the administration of cimetidine, the animals received by gavage racemic metoprolol dissolved in water at a dose of 15 mg/kg. Blood samples (5 ml) were collected and stored as described for the control group.

Animals in the ketoconazole group were fasted for 12 h (n = 6 per sampling time) and received ketoconazole (CIMED, São Paulo, Brazil) i.p. at a dose of 50 mg/kg dissolved in physiological saline acidified with 5% (v/v) HCl at a final pH of 4.0.[12] One hour after the administration of ketoconazole, the animals received by gavage racemic metoprolol dissolved in water at a dose of 15 mg/kg. Blood samples (5 ml) were collected and stored as described for the control group.

TABLE 1. Ratios of log AUC and C_{max} inhibitor/control

	Ketoconazole	Cimetidine	Quinidine
AUC			
(+)-R-metoprolol	1.86 (1.85)	1.50 (1.52)	1.55 (1.57)
	1.76–1.97[a]	1.40–1.61[a]	1.44–1.67[a]
(−)-S-metoprolol	1.96 (1.82)	1.55 (1.54)	1.58 (1.57)
	1.83–2.08[a]	1.45–1.65[a]	1.49–1.66[a]
C_{max}			
(+)-R-metoprolol	1.09 (1.08)	1.08 (1.09)	1.05 (1.05)
	1.04–1.13	1.07–1.10	1.02–1.08
(−)-S-metoprolol	1.06 (1.05)	1.07 (1.07)	1.00 (0.99)
	1.02–1.12	1.04–1.10	0.96–1.03

Data are expressed as mean (median) and 90% confidence interval (n = 6).
[a]Inhibition for log AUC ratios outside the interval of 0.80–1.25.
Inhibition for log C_{max} ratios outside the interval of 0.70–1.43.

Analytical Methods

The isomers of metoprolol, ODM and OHM were analyzed in 1.0 ml plasma samples after liquid-liquid extraction procedure. The isomers were resolved simultaneously using a Chiralpak AD column and fluorescence detection according to the procedure described by Boralli et al.[8] The quantification limit was 1 ng/ml plasma for individual metoprolol and ODM and 5 ng/ml for the individual α-OHM isomers. AODM was analyzed in 0.4 ml plasma samples after protein precipitation followed by a cleanup procedure. AODM isomers were resolved on a Chiralcel OD-R column and fluorescence detection according to the method described by Cerqueira et al.[17] The quantification limit was 17.0 ng/ml plasma for each enantiomer. Both methods were validated, with the observation of intra- and interday variations of less than 15%.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters were calculated on the basis of the plasma concentration versus time curves for the enantiomers using the WinNonlin software, version 4.0 (Pharsight, Mountain View, CA) and first-order kinetics.

TABLE 2. Influence of drug inhibitor on AUC enantiomeric ratios for metoprolol and its metabolites

		AUC Ratio			
		Control	Ketoconazole	Cimetidine	Quinidine
MET	(S)/(R)	0.76	0.95	0.88	0.77
ODM	(S)/(R)	1.11	1.03	1.32	1.29
OHM	2(S)/2(R)	1.05	1.33	0.82	1.00
OHM	1′(R)/1′(S)	10.90	6.23	14.50	5.41*
AODM	(S)/(R)	1.11	1.03	1.05	1.06

Data are expressed as median.
*$P < 0.05$, Kruskal-Wallis test; control group vs. inhibitor group.

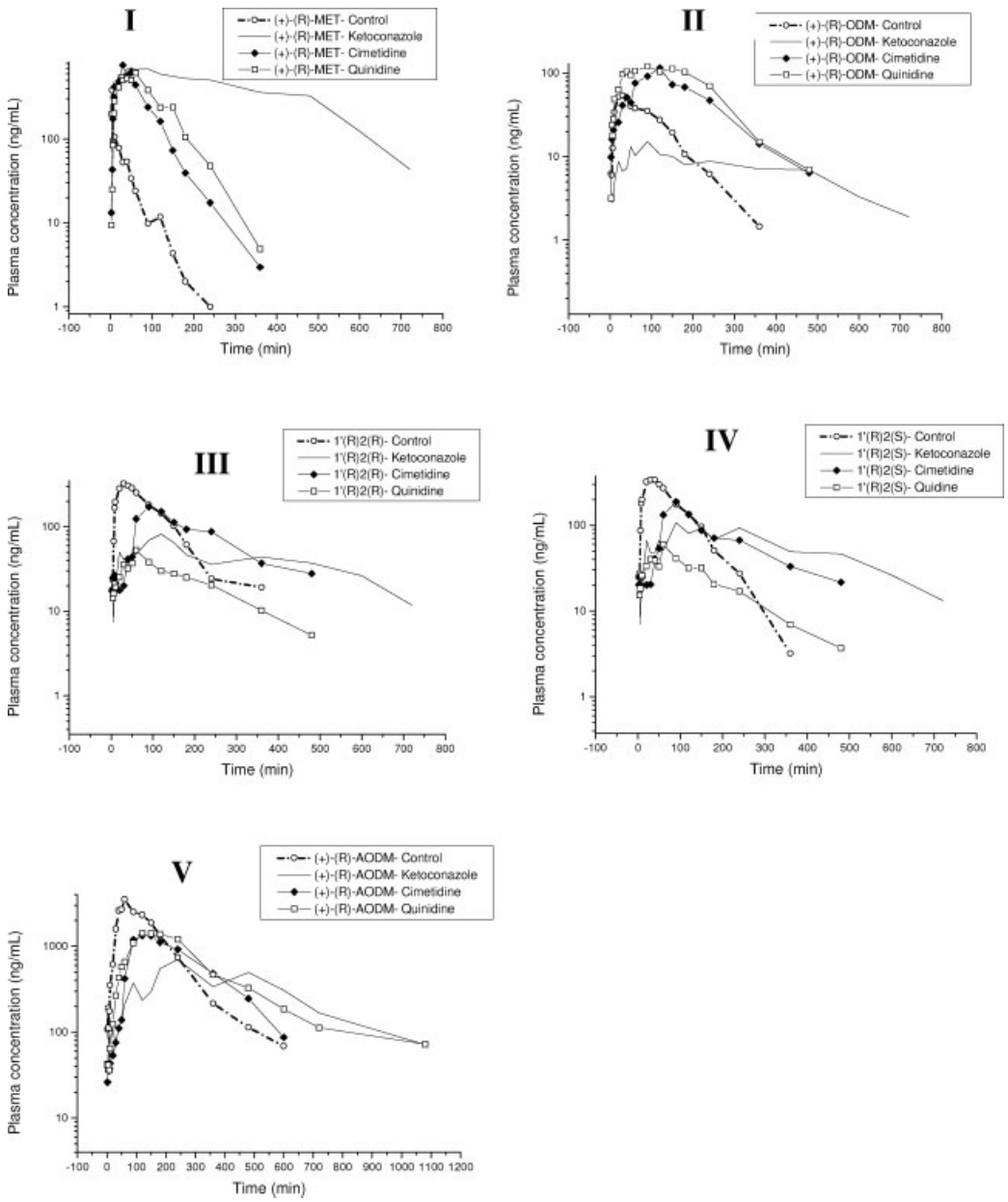

Fig. 2. Plasma concentration versus time curves of the (+)-(R)- isomers of metoprolol and its metabolites in all groups. Data are expressed as the median. I: (+)-(R)-metoprolol; II: (+)-(R)-ODM; III: 1′(R)2(R)-OHM; IV: 1′(R)2(S)-OHM; V: (+)-(R)-AODM.

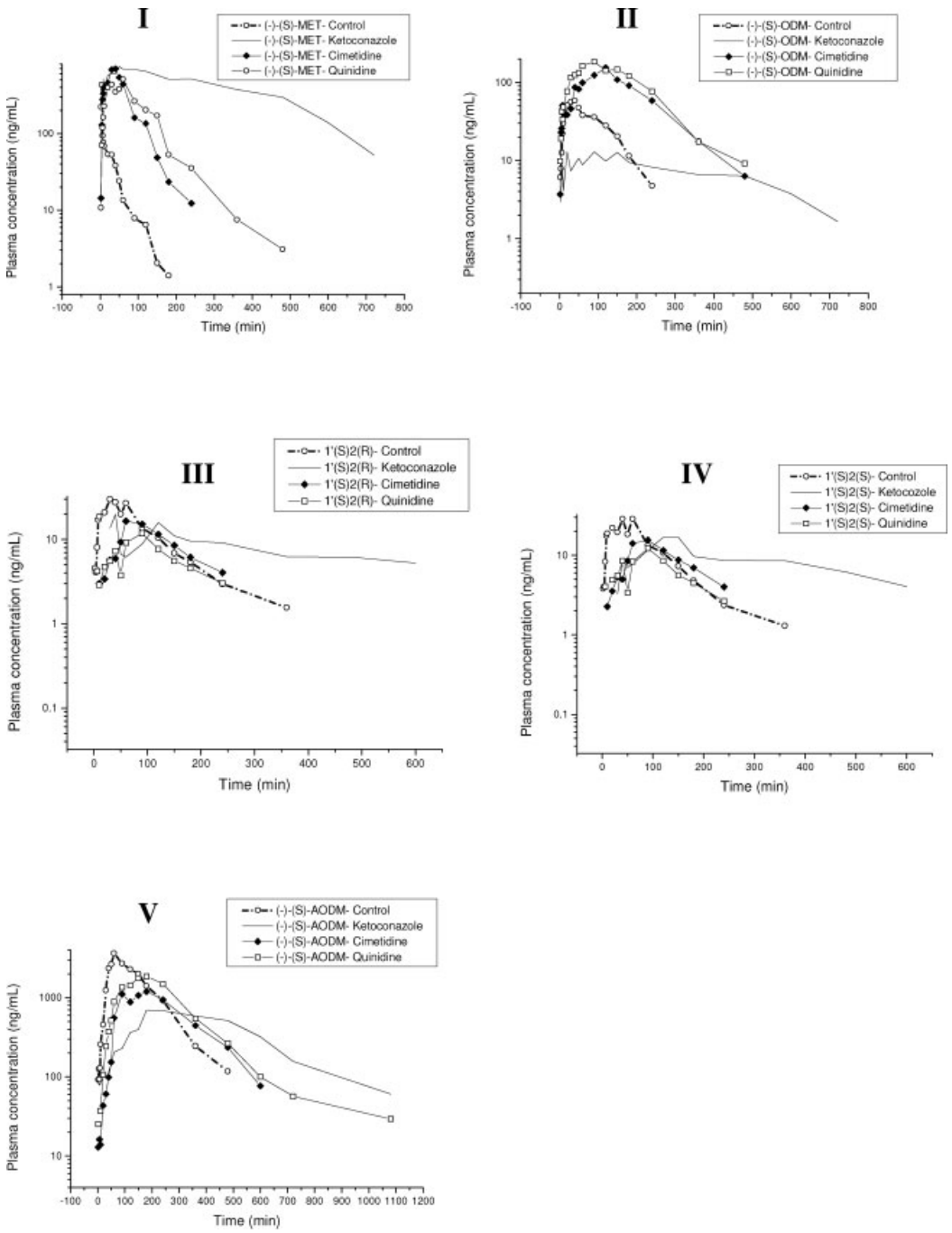

Fig. 3. Plasma concentration versus time curves of the S-(−) isomers of metoprolol and its metabolites in all groups. Data are expressed as the median. I: (−)-(S)-metoprolol; II: (−)-(S)-ODM; III: 1′(S)2(R)-OHM; IV: 1′(S)2(S)-OHM; V: (−)-(S)-AODM.

Statistical analysis was performed using the Graphpad Instat® software for the calculation of the mean, the median and the 95% confidence interval. Within group comparison of metoprolol and its metabolites was performed by the Wilcoxon test, with the level of significance set at $P \leq 0.05$. The Kruskal-Wallis test followed by Dunn's post-test was used to compare the enantiomers of metoprolol and its metabolites among groups.

According to Tucker et al.,[18] a drug-drug interaction study should be evaluated statistically based on confidence intervals (90%) of differences. As a default position for most drugs, it is reasonable according to their proposal to conclude that no interaction occurred if the 90% confidence interval of the ratio of log AUC or C_{max} after single-dose administration in the presence and absence of an inhibitor is within 0.80–1.25 for AUC and 0.70–1.43 for C_{max}.

TABLE 3. $AUC^{0-\infty}$ values obtained for the evaluation of ketoconazole, cimetidine, and quinidine as inhibitors of the metabolism of metoprolol

	$AUC^{0-\infty}$ (ng h/ml)			
	Control	Ketoconazole	Cimetidine	Quinidine
(+)-(R)-metoprolol	101.0	4480.5*	916.2*	1109.9*
(−)-(S)-metoprolol	83.4	4470.5*	767.1*	830.8*
(+)-(R)-ODM	121.2	97.3*	367.0*	508.1*
(−)-(S)-ODM	126.4	99.5*	484.9*	627.1*
1′(S),2(R)-OHM	58.3	99.0*	41.3*	33.7*
1′(S),2(S)-OHM	57.1	109.0*	41.1*	34.5*
1′(R),2(R)-OHM	638.2	678.6*	534.0*	178.1*
1′(R),2(S)-OHM	659.6	753.5*	553.6*	165.4*
(+)-(R)-AODM	8319.0	5439.0*	5516.0*	7899.9
(−)-(S)-AODM	9596.2	5845.5*	6095.5*	8462.5

Values are reported as median ($n = 6$ per sampling time).
*$P < 0.05$, Kruskal-Wallis test; control group *vs* inhibitor group.

RESULTS

Table 1 shows the 90% confidence intervals for the ratios of log AUC and C_{max} inhibitor/control of metoprolol. The 90% confidence intervals of the ratios of log C_{max} of the (+)-(R)- and (−)-(S)-metoprolol enantiomers were within the interval of 0.70–1.43 proposed by Tucker et al.[18] for studies on metabolism inhibitors. However, the 90% confidence intervals of ratios of log AUC of both metoprolol enantiomers were higher than the interval of 0.8–1.25 proposed by Tucker et al.,[18] a finding suggesting that ketoconazole, cimetidine, and quinidine inhibit the metabolism of metoprolol in a nonenantioselective manner. AUC enantiomeric ratios for metoprolol and its metabolites are not influenced by CYP inhibitors as shown on Table 2, excepted quinidine which showed significant difference when compared to control group in relation to metabolite OHM [1′(R)/1′(S) ratios 5.41 vs. 10.90].

The plasma enantiomer concentrations of metoprolol and its metabolites versus time in the control and inhibitor-treated groups are shown in Figures 2 and 3. Table 3 illustrates the AUC values obtained for the two enantiomers of metoprolol and its metabolites in the presence and absence of ketoconazole, cimetidine, and quinidine.

The AUC values obtained for both metoprolol enantiomers in the ketoconazole group were ∼50 times higher than those observed in the control group (Table 3). With respect to the metoprolol metabolites, the AUC values obtained for both enantiomers of ODM and AODM were ∼1.3–1.6 times lower in the ketoconazole group, whereas those of the 1′(S)-OHM enantiomers were ∼1.8 times higher in this group. C_{max} was increased for both enantiomers of metoprolol and reduced for all metabolites studied (Table 4).

In the group of animals treated i.p. with 150 mg/kg cimetidine, an ∼9-fold increase in AUC values was observed for both metoprolol enantiomers (Table 3). The AUC values obtained for the enantiomers of AODM and OHM were found to be reduced in this group. However, more than 3-fold increase in the AUC values obtained for the ODM enantiomers was observed in the cimetidine group when compared with control. C_{max} was increased for both enantiomers of metoprolol and ODM and was reduced for the other metabolites (Table 4).

Treatment with quinidine led to an increase in AUC values for both enantiomers of metoprolol (∼10 times) and of ODM (4.5 times) (Table 3). AUC values were reduced 1.73, 1.65, 3.58, and 3.99 times for the 1′(S)2(R), 1′(S)2(S), 1′(R)2(R), and 1′(R)2(S) isomers of OHM, respectively. C_{max} was found to be increased for both enantiomers of ODM and reduced for the other metabolites (Table 4).

DISCUSSION

The present study investigated the influence of the CYP inhibitors quinidine, cimetidine, and ketoconazole on the enantioselective pharmacokinetics of metoprolol and its metabolites in male Wistar rats. There are no studies evaluating the interaction between metoprolol (single-enantiomer compound or racemic mixture) and quinidine, cimetidine, or ketoconazole in rats or humans.

The kinetic disposition of metoprolol and its metabolites ODM, OHM or AODM was not enantioselective in rats treated with a single oral dose (Table 2). Pretreatment with ketoconazole did not lead to the enantioselective inhibition of metoprolol metabolism. However, pretreatment with cimetidine resulted in the plasma accumulation of the (+)-(R)-metoprolol enantiomer because of the preferential inhibition of the formation of the (+)-(R)-ODM metabolite. Pretreatment with quinidine also resulted in the plasma accumulation of (+)-(R)-metoprolol not only because of the preferential inhibition of the (+)-(R)-ODM metabolite but also of (+)-(R)-AODM.

Rats treated with a single i.p. dose of quinidine (80 mg/kg) presented higher AUC values (Table 2) for both metoprolol enantiomers and lower AUC values for the four stereosisomers of OHM when compared to the control group, confirming the participation of CYP2D in the α-hydroxylation of metoprolol and its predominant involvement in the formation of 1′(R)-hydroxylated metabolites. Considering

TABLE 4. C_{max} values obtained for the evaluation of ketoconazole, cimetidine, and quinidine as inhibitors of the metabolism of metoprolol

	C_{max} (ng/ml)			
	Control	Ketoconazole	Cimetidine	Quinidine
(+)-(R)-metoprolol	450.0	761.5*	775.5*	597.5*
(−)-(S)-metoprolol	513.4	726.0*	796.3*	487.5*
(+)-(R)-ODM	55.6	15.1*	115.4*	142.5*
(−)-(S)-ODM	62.0	16.0*	151.1*	194.0*
1′(S),2(R)-OHM	36.3	13.8*	18.0*	12.5*
1′(S),2(S)-OHM	32.1	17.0*	17.1*	12.6*
1′(R),2(R)-OHM	336.2	87.5*	202.8*	53.7*
1′(R),2(S)-OHM	360.6	110.0*	196.9*	54.5*
(+)-(R)-AODM	3253.7	742.5*	1419.1*	1777.0*
(−)-(S)-AODM	3282.2	872.3*	1262.3*	1970.5*

Values are reported as the median ($n = 6$ per sampling time).
*$P < 0.05$, Kruskal-Wallis test; control group vs. inhibitor group.

that quinidine is a CYP2D inhibitor in rats,[11] the data also suggest the participation of this CYP isoform in the formation of AODM since the C_{max} values for the two AODM enantiomers were significantly reduced (Table 4) and the elimination half-lives were prolonged (Figs. 2 and 3).

The AUC and C_{max} values obtained for both metoprolol enantiomers in rats treated with a single i.p. dose of ketoconazole (50 mg/kg) were significantly higher than those observed for the control group (Tables 3 and 4), whereas the AUC and C_{max} values observed for the OHM and AODM metabolites were reduced ($P < 0.05$). These results suggest that ketoconazole inhibits the metabolism of both metoprolol enantiomers.

Studies have shown that the formation of AODM depends on CYP2D and on other enzymes that differ from CYP2D in terms of substrate affinity.[6,7,9] As ketoconazole is a selective inhibitor of CYP3A in rats,[12,13] the differences in AUC values for metoprolol and its metabolites ODM and AODM between the control and ketoconazole groups (Table 3) suggest the participation of CYP3A in the *O*-demethylation of metoprolol. The inhibition of CYP3A by ketoconazole probably shifts the equilibrium of the enzymatic reactions in a direction that favors the availability of metoprolol for α-hydroxylation, which would explain the higher AUC values observed for all OHM isomers (Table 3). The suggestion of the equilibrium shift in the enzymatic reactions is based on the high dose used in the study, as well as on the fact that CYP2D is not induced in any circumstance.[19]

Cimetidine administered as a single i.p. dose (150 mg/kg) to rats resulted in an increase of the AUC and C_{max} values of both metoprolol enantiomers (Tables 3 and 4) and in a reduction of the AUC values of both AODM enantiomers (Table 3). Previous studies have shown that cimetidine inhibits CYP2C6, CYP2C11,[15] CYP2D[20], and CYP3A[21] in rats. The reduction in the AUC values of both AODM enantiomers might be a consequence of the inhibition of CYP3A and CYP2D by cimetidine. The increase in the AUC of the precursor metabolite ODM cannot be explained by the reduced formation of AODM since in the ketoconazole group the AUC of both ODM and AODM was found to be reduced. The results suggest that cimetidine inhibits CYP3A and CYP2D involved in the metabolism of ODM and AODM. With respect to the OHM isomers, the reduction in the AUC is the result of inhibition of CYP2D.

In summary, CYP3A participates in the formation of the ODM metabolite and CYP2D is involved in the formation of AODM in rats treated with a single oral dose of racemic metoprolol.

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