Influence of CYP2D6-dependent metabolism on the steady-state pharmacokinetics and pharmacodynamics of metoprolol and nicardipine, alone and in combination

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- 1 The metabolism of metoprolol depends in part on the genetically determined activity of the CYP2D6 isoenzyme. *In vitro* studies have shown that nicardipine is a potent inhibitor of CYP2D6 activity. Since the combination of metoprolol and nicardipine is likely to be used for the treatment of hypertension, we examined the interaction between these two drugs at steady-state.
- 2 Fourteen healthy volunteers, seven extensive and seven poor metabolisers of dextromethorphan were studied in a double-blind, randomised cross-over four-period protocol. Subjects received nicardipine 50 mg every 12 h, metoprolol 100 mg every 12 h, a combination of both drugs and placebo during 5.5 days. Steady-state pharmacokinetics of nicardipine and metoprolol were analyzed. β -adrenoceptor blockade was assessed as the reduction of exercise-induced tachycardia.
- 3 During treatment with metoprolol, alone or in combination with nicardipine, its steady-state plasma concentrations were higher in subjects of the poor metaboliser phenotype than in extensive metabolisers. β -adrenoceptor blockade was also more pronounced in poor metabolisers than in extensive metabolisers of dextromethorphan during treatment with metoprolol alone or in combination with nicardipine ($24.0 \pm 2.4\%$ vs $17.1 \pm 3.5\%$ and $24.1 \pm 2.5\%$ vs $15.4 \pm 2.7\%$ reduction in exercise tachycardia, respectively, $P < 0.01$ in each case).
- 4 Nicardipine produced a small increase in plasma metoprolol concentration in extensive metabolisers from 35.9 ± 16.6 to 45.8 ± 15.4 ng ml⁻¹ ($P < 0.02$), but had no significant effect in poor metabolisers. However, nicardipine did not alter the R/S metoprolol ratio in plasma 3 h after dosing, the plasma concentration of S-(–)-metoprolol 3 h after dosing or the β -adrenoceptor blockade produced by metoprolol in subjects of both phenotypes. The partial metabolic clearance of metoprolol to α -hydroxy-metoprolol was not altered significantly in extensive metabolisers.
- 5 Plasma nicardipine concentration and β -adrenoceptor blocking effects did not differ between the phenotypes and were not influenced by metoprolol.
- 6 We conclude that β -adrenoceptor blockade during repeated dosing with metoprolol is more pronounced in poor than in extensive metaboliser subjects, that nicardipine decreases a CYP2D6-independent route of metoprolol elimination but does not increase β -adrenoceptor blockade during repeated dosing with metoprolol.

Keywords metoprolol nicardipine pharmacogenetics CYP2D6
drug interaction enantiomers

Introduction

Hepatic metabolism of the β_1 -selective adrenoceptor antagonist metoprolol depends in part on the genetically-determined activity of the cytochrome P-450 2D6 (CYP2D6) isoenzyme. The distribution of CYP2D6 activity in the general population is bimodal. Subjects with the poor metaboliser (PM) phenotype, who lack CYP2D6 activity, account for 7% of the European population while subjects with the extensive metaboliser (EM) phenotype, in whom CYP2D6 activity is present and variable, represent the rest [1]. Oxidation phenotype is a major cause of interindividual variability in metoprolol pharmacokinetics and pharmacodynamics after oral administration of single doses of metoprolol [2]. Metoprolol is a racemic compound and its disposition kinetics are stereoselective. The hepatic clearance of R-(+)-metoprolol is impaired to a greater extent in PM than in EM subjects. Thus, the S/R metoprolol ratio is less in PM than in EM subjects [3–5]. Most of the β -adrenoceptor blocking activity resides with the S-(–)-enantiomer. Following administration of a single oral dose of metoprolol, plasma concentrations and half-life of the parent compound are 6 and 3 times greater in PM than in EM subjects, respectively, and β -adrenoceptor blockade is more pronounced in PM than in EM subjects [2]. Metoprolol α -hydroxylation accounts for 10% of the dose of metoprolol recovered in urine and correlates with CYP2D6 activity. Metoprolol *O*-demethylation, a quantitatively major metabolic route, is also decreased in PM subjects, but the population distribution is not bimodal, suggesting that it is catalysed by several enzymes including CYP2D6 [6]. While the influence of CYP2D6 activity on metoprolol pharmacokinetics and β -adrenoceptor blocking effects has been established after single doses of metoprolol, it has not been demonstrated after repeated dosing. Since the ability of CYP2D6 to metabolise its substrates may decrease when substrate concentration increases [7, 8], the differences between the β -adrenoceptor blocking effects of metoprolol in EM and PM subjects found after a single dose may not persist during repeated dosing. However, preliminary results in two PM subjects suggested that this is not the case [9].

The clinical implications of polymorphic CYP2D6 activity depend principally on the therapeutic index of active bioavailable compounds and on the activity of the parent drug relative to its metabolite(s) formed by the genetically-determined route of metabolism [8, 10]. Moreover, several drugs may compete with CYP2D6 substrates and alter the phenotypic expression of EM subjects [11, 12] with variable clinical consequences [13–16]. Nicardipine is a dihydropyridine calcium channel antagonist which is extensively metabolised in the liver. *In vitro* studies in human liver microsomes have shown that nicardipine is a potent inhibitor ($K_i = 876$ nM) of the CYP2D6-dependent oxidative metabolism of bufuralol [11]. However, no study has determined the effects of nicardipine on CYP2D6 activity *in vivo*. Combination of a β -adrenoceptor antagonist and a calcium channel antagonist is recommended for the treatment of hypertension when monotherapy does not provide sufficient control of blood pressure [17]. Thus, metoprolol and nicardipine may be combined in such

patients. The pharmacokinetic and pharmacodynamic consequences of such a combination may depend on CYP2D6 phenotype.

We performed the present study to determine whether nicardipine inhibits the polymorphic metabolism of metoprolol *in vivo* and selectively increases its β -adrenoceptor blocking effects in EM subjects. An additional objective was to confirm whether the differences in β -adrenoceptor blocking effects reported between EM and PM subjects after administration of single dose metoprolol persist during repeated dosing.

Methods

Subjects

Fourteen healthy volunteers, 12 males and two females, participated in the study. Seven were EM and seven were PM metabolisers of dextromethorphan. Their mean ($\pm$ s.d.) age was 22.6 ± 4.0 years (range 19 to 35 years) and their mean body weight was 65.1 ± 8.7 kg (range 51 to 79 kg). EM and PM subjects were matched for sex and body weight (± 5 kg). All subjects were normal on physical examination, and had normal electrocardiogram and laboratory test results. All gave their written informed consent to participate and the study protocol was approved by the local Committee for the Protection of Human Subjects in Biomedical Research.

Study design

This was a 4-period, randomised, placebo-controlled, double-blind, crossover study. One period involved the administration of 100 mg of the standard dosage form of racemic metoprolol (Lopressor 100[®], Ciba-Geigy Laboratories, Rueil-Malmaison, France) together with a placebo of racemic nicardipine every 12 h for 5.5 days (11 doses). During another period 50 mg of a slow-release form of racemic nicardipine (Loxen LP 50[®], Sandoz Laboratories, Rueil-Malmaison, France) was administered together with a placebo of racemic metoprolol every 12 h for 5.5 days (11 doses). In another period the combination of racemic nicardipine and racemic metoprolol was given in the dosing regimen mentioned above. A further period involved the administration of a placebo of racemic nicardipine and a placebo of racemic metoprolol every 12 h for 5.5 days. Subjects were asked to take the drugs at 09.00 h and 21.00 h, at least 1.5 h after meals, and to refrain from smoking and drinking caffeine-containing beverages during the study. Study periods were separated by a drug-free interval of 7 to 14 days.

Before entering the study, each subject performed two maximal exercise tests. The first test was used as a training test and the second was used to determine the individual maximal stress level (W_{max}). Each period followed the same design. On study day 1, baseline resting and exercise heart rate and blood pressure were measured before administration of the first dose of the study drug. On study day 6, following an overnight fast,

subjects were admitted to the Clinical Pharmacology Unit at Saint-Antoine University Hospital. An indwelling catheter was inserted in a forearm vein to allow blood samples to be drawn. Resting and exercise heart rate and blood pressure were again measured, subjects emptied their bladder and a blood sample was taken immediately before administration of the 11th and last dose of study drug. Additional blood samples were taken 0.5, 1, 2, 3, 4, 5, 6, 8, and 12 h after administration of this last dose. Urine was collected over the 12 h interdose interval. Plasma and urine samples were stored at -28°C until analysis. Exercise tests were performed on a bicycle ergometer, immediately after blood sampling and at 1, 2, 3, 4, 6, 8, and 12 h after the last study drug dose was administered.

Determination of CYP2D6 phenotype

Oxidation phenotype was determined by calculating the dextromethorphan urinary metabolic ratio: amount of dextromethorphan/amount of dextrorphan excreted over 8 h after administration of a single oral dose of 40 mg dextromethorphan [18, 19]. Dextromethorphan and dextrorphan concentrations in urine were measured as described previously [20, 21]. CYP2D6 activity was estimated as the log dextromethorphan metabolic ratio [21]. Oxidation phenotype was determined at least 15 days before starting the study.

Measurement of nicardipine concentration in plasma, metoprolol and α -hydroxy-metoprolol concentration in plasma and urine

Plasma nicardipine concentrations were measured by h.p.l.c. as described previously [22]. Metoprolol and α -hydroxy-metoprolol concentrations were measured in plasma and urine by h.p.l.c. with spectrofluorometer detection as described previously [23]. The limit of detection (signal to noise ratio <3) for both compounds was 5 ng ml^{-1} in plasma and 20 ng ml^{-1} in urine. The coefficients of variation for inter-day analysis of metoprolol and α -hydroxy-metoprolol were 5.9 and 10.3%, respectively, at a concentration of 25 ng ml^{-1} and 1% for both compounds at a concentration of 200 ng ml^{-1} . The accuracy of the assays ranged from 94 to 100% for metoprolol and from 97 to 100% for α -hydroxy-metoprolol.

(S)-(-)-metoprolol and (R)-(+)-metoprolol concentrations were measured at the time of peak plasma drug concentration (3 h post dose) and in 12 h urine by h.p.l.c. [24] using an AGP chiral column ($100 \times 4.0\text{ mm}$, Chromtec AB, Norsborg, Sweden). The limit of detection (signal to noise ratio <4) was 10 ng ml^{-1} in plasma and 50 ng ml^{-1} in urine. The coefficients of variation for inter-day analysis of (S)-(-)-metoprolol and (R)-(+)-metoprolol concentrations in plasma and urine were less than 10% at 5 and 20 ng ml^{-1} . The accuracy of the assay ranged from 88 to 100% for both enantiomers.

Pharmacodynamic measurements

Pharmacodynamic parameters were measured after 10 min of rest in the supine position. Resting heart rate was determined by counting heart beats from the radial

artery pulse over 1 min and resting systolic and diastolic blood pressures were measured as the mean of three consecutive recordings from a Dinamap[®] device (Critikon Inc, Tampa, Florida, USA). Exercise heart rate was determined during a standardised 4 min submaximal stress test (25%, 50% and 80% of baseline W_{max} during 0.5, 0.5 and 3 min, respectively) as the mean of the five shortest consecutive electrocardiographic RR intervals recorded at the end of the test. Exercise systolic blood pressure was determined using a mercury sphygmomanometer.

Data analysis

Pharmacokinetic data All concentrations are expressed with reference to drug base. Pharmacokinetic parameters were calculated over the last 12 h dosing interval. Interdose AUC values of metoprolol and α -hydroxy-metoprolol were calculated using the linear trapezoidal rule for increasing data points and the log-trapezoidal rule for decreasing data points [25]. Mean plasma concentrations of metoprolol and α -hydroxy-metoprolol over a dosing interval were calculated by dividing the corresponding AUC by 12. The oral clearance of metoprolol was calculated from Dose/AUC. The renal clearance of metoprolol was calculated as the amount of metoprolol recovered in 12 h urine divided by the AUC. The partial metabolic clearance of metoprolol to its α -hydroxy metabolite was calculated from the recovery of α -hydroxy-metoprolol in urine over 12 h divided by metoprolol AUC [26]. Metabolic ratios and partial metabolic clearance were calculated using molar units.

Pharmacodynamic data The β -adrenoceptor blocking effect of metoprolol was measured as the reduction in exercise tachycardia expressed as the percent change from the pre-drug value. The mean β -adrenoceptor blocking effect over a dosing interval was calculated by dividing the area under the percent reduction in exercise tachycardia vs time curve by 12.

Statistical analysis Within each study period, pharmacokinetic and pharmacodynamic data in EM subjects were first compared with corresponding data obtained in PM subjects using Wilcoxon's test. Within a given phenotypic group, Wilcoxon's signed ranks test was used to compare data obtained during treatment with metoprolol alone with that obtained after combination with nicardipine. Results are presented as means and standard deviations. Differences were considered statistically significant if the probability of erroneously rejecting the null hypothesis of no difference was less than 5%.

Spearman's rank correlation test was used to examine relationships between log dextromethorphan metabolic ratio and various metoprolol pharmacokinetic parameters. The relation between the percent decrease in exercise tachycardia and the plasma S-(-)-metoprolol concentration 3 h after dosing during treatment with metoprolol alone was compared with the corresponding relation for the combination of metoprolol with nicardipine. For this purpose, combined data from EM and PM subjects were fitted to an E_{max} model: % reduction in

exercise tachycardia 3 h after dosing = $(E_{\max} \cdot C)/(EC_{50} + C)$ where $E_{\max}$ is the maximum β -adrenoceptor blocking effect, EC_{50} is the plasma S(-)-metoprolol concentration associated with half the maximum effect and C is the plasma S(-)-metoprolol concentration 3 h after dosing. Comparison of these two fitted curves was made using an F -test [27].

Results

The pharmacokinetics of metoprolol at steady-state in EM and PM subjects

The mean plasma metoprolol concentrations over a dosing interval at steady-state during administration of metoprolol alone were 36 ± 17 ng ml⁻¹ and 185 ± 41 ng ml⁻¹ in EM and PM subjects, respectively ($P < 0.001$) (Table 1, Figure 1). EM subjects differed from PM subjects in that they had lower plasma S(-)- and R-(+)-metoprolol concentrations 3 h after dosing, higher oral metoprolol clearances, lower urinary recoveries of metoprolol and its α -hydroxylated metabolite, and higher metoprolol S/R ratios in plasma and in urine (Table 1). In PM subjects, α -hydroxy-metoprolol was undetectable in plasma and urine. However, the renal clearance of metoprolol was similar in EM and PM subjects.

The mean plasma concentration of metoprolol over a dosing interval correlated with the log dextromethorphan metabolic ratio ($\rho = 0.798$; $P < 0.001$). The oral clearance of metoprolol as well as the S/R ratios in plasma and in urine were inversely correlated with the log dextromethorphan metabolic ratio ($\rho = -0.771$, $\rho = -0.749$, $\rho = -0.802$, respectively; $P < 0.001$ in all cases).

Figure 1 Mean plasma concentrations of racemic metoprolol over a 12 h dosing interval. The mean $\pm$ s.d. and individual results are presented for EM (●) and PM (○) subjects during treatment with metoprolol, alone (Meto) and combined with nicardipine (Meto + Nicar). * $P < 0.05$.

Pharmacodynamics of metoprolol in EM and PM subjects

After 5.5 days of treatment with metoprolol, resting heart rate was 58.4 ± 6.4 beats min⁻¹ in EM subjects and 55.5 ± 5.3 beats min⁻¹ in PM subjects ($P =$ not significant). Resting systolic and diastolic blood pressures also did not differ between the two phenotypic groups. However, as shown in Figure 2, the mean percent reduction in exercise tachycardia over a dosing interval during treatment with metoprolol alone was

Table 1 Pharmacokinetics of metoprolol during treatment with metoprolol alone and in combination with nicardipine

	Metoprolol alone		Metoprolol + nicardipine	
	EM (n = 7)	PM (n = 7)	EM (n = 7)	PM (n = 7)
Plasma				
Mean metoprolol concentration (ng ml ⁻¹)	36 $\pm$ 17	185 $\pm$ 41*	46 $\pm$ 15§	181 $\pm$ 33*
Mean α -hydroxy-metoprolol concentration (ng ml ⁻¹)	75 $\pm$ 14	< LD	82 $\pm$ 20	< LD
S(-)-metoprolol concentration at 3 h (ng ml ⁻¹)	23 $\pm$ 11	74 $\pm$ 20*	21 $\pm$ 17	94 $\pm$ 32*
R-(+)-metoprolol concentration at 3 h (ng ml ⁻¹)	14 $\pm$ 8	61 $\pm$ 18*	13 $\pm$ 4	86 $\pm$ 28*
S/R metoprolol ratio at 3 h	1.76 $\pm$ 0.29	1.23 $\pm$ 0.16*	1.60 $\pm$ 0.42	1.10 $\pm$ 0.04*
Clearances				
Metoprolol oral clearance (l h ⁻¹)	172 $\pm$ 63	33 $\pm$ 11*	133 $\pm$ 42§	31 $\pm$ 6*
Metoprolol renal clearance (l h ⁻¹)	8 $\pm$ 2	11 $\pm$ 3	9 $\pm$ 2	11 $\pm$ 3
Metoprolol partial metabolic clearance (l h ⁻¹)	20 $\pm$ 8	—	17 $\pm$ 8	—
α -hydroxy-metoprolol renal clearance (l h ⁻¹)	13 $\pm$ 1	—	13 $\pm$ 3	—
Urine				
Fraction of dose excreted as metoprolol (%)	6 $\pm$ 4	36 $\pm$ 7*	7 $\pm$ 3	35 $\pm$ 6*
Fraction of dose excreted as α -hydroxy-metoprolol (%)	8 $\pm$ 2	—	9 $\pm$ 2	—
Total urinary recovery as metoprolol + α -hydroxy-metoprolol (%)	14 $\pm$ 5	36 $\pm$ 7*	16 $\pm$ 5	35 $\pm$ 6*
S/R metoprolol ratio	1.2 $\pm$ 0.3	0.6 $\pm$ 0.1*	1.3 $\pm$ 0.2	0.7 $\pm$ 0.1*

Results are presented as mean $\pm$ s.d.; * $P < 0.05$ vs EM subjects; § $P < 0.02$ vs metoprolol alone in EM subjects; LD, limit of detection.

Figure 2 Mean β -adrenoceptor blockade over a 12 h dosing interval. The mean $\pm$ s.d. and individual results are presented for EM (●) and PM (○) subjects during treatment with metoprolol, alone (Meto) and combined with nicardipine (Meto $\pm$ Nicar). * $P < 0.05$.

greater in PM than in EM subjects ($24.0 \pm 2.4\%$ vs $17.1 \pm 3.5\%$, respectively; $P < 0.05$). Similarly, the percentage reduction in exercise systolic blood pressure was $12.0 \pm 7.4\%$ in PM subjects and $4.2 \pm 4.5\%$ in EM subjects ($P < 0.05$). There was a correlation between the percentage decrease in exercise heart rate during metoprolol treatment and the log dextromethorphan metabolic ratio ($\rho = +0.749$; $P < 0.001$). Similar results were found for the percentage reduction in exercise systolic blood pressure ($\rho = +0.552$; $P < 0.02$).

Pharmacokinetics and pharmacodynamics of nicardipine

During administration of nicardipine alone, its mean plasma concentrations over a dosing interval were 14.2 ± 4.4 ng ml $^{-1}$ in EM subjects and 15.1 ± 8.0 ng ml $^{-1}$ in PM subjects ($P =$ not significant). During the combined administration of nicardipine and metoprolol, the mean plasma concentration of nicardipine over a dosing interval also did not differ in EM (17.7 ± 11.2 ng ml $^{-1}$) and PM (12.4 ± 7.0 ng ml $^{-1}$) subjects, and was not influenced significantly by metoprolol co-administration. Compared with placebo, nicardipine had no significant influence on resting or exercise heart rate or on exercise systolic blood pressure in this normotensive population (Table 2). The effects of nicardipine on heart rate and

blood pressure also did not differ between EM and PM subjects (Table 2).

Influence of nicardipine on metoprolol pharmacokinetics and pharmacodynamics

Nicardipine did not modify the differences in metoprolol pharmacokinetics found between EM and PM subjects during treatment with metoprolol alone (Table 1). However, during combination with nicardipine, the oral clearance of metoprolol was decreased selectively in EM subjects, resulting in a small but significant increase in the mean plasma metoprolol concentration compared with metoprolol alone (Table 1, Figure 1). These changes were not observed in PM subjects. The mean increase in plasma metoprolol concentration was $35 \pm 32\%$ in EM and $0 \pm 16\%$ in PM subjects ($P < 0.05$). All of the other pharmacokinetic parameters measured during treatment with metoprolol alone, including the partial metabolic clearance to its α -hydroxylated metabolite and the S/R ratios in plasma and urine, were unaltered by nicardipine co-administration.

The mean percentage reduction in exercise tachycardia over a dosing interval during treatment with the combination of metoprolol and nicardipine remained greater in PM than in EM subjects ($24.1 \pm 2.5\%$ vs $15.3 \pm 2.7\%$, respectively; $P < 0.05$). Similarly, the percentage reduction in exercise systolic blood pressure was $11.5 \pm 6.8\%$ in PM subjects and $6.7 \pm 2.2\%$ in EM subjects ($P < 0.05$). However, these pharmacodynamic effects of metoprolol were not significantly different during treatment with metoprolol alone and during treatment in combination with nicardipine.

Relationship between plasma S-(–)-metoprolol concentration and the β -adrenoceptor blocking effect of metoprolol

Figure 3 shows the relationship between plasma S-(–)-metoprolol concentration and the percentage reduction in exercise tachycardia during metoprolol alone and combined with nicardipine. During both study periods, the response of PM subjects appeared to be close to the maximum effect of metoprolol while the response of EM subjects appeared to be in the steep portion of the effect vs concentration curve. During treatment with metoprolol alone, the curve fitted to an $E_{\max}$ model gave an $E_{\max}$ value of $27 \pm 3\%$ (maximum theoretical decrease in exercise tachycardia) and an EC_{50} value of 8.8 ± 5.6 ng ml $^{-1}$ (means and 95% confidence intervals). During treatment with the combination of metoprolol and nicardipine, these values were $25 \pm 4\%$ and $6.5 \pm$

Table 2 Effects of nicardipine and placebo in EM and PM subjects

	Nicardipine		Placebo	
	EM (n = 7)	PM (n = 7)	EM (n = 7)	PM (n = 7)
Resting heart rate (beats min $^{-1}$)	68.6 ± 7.6	68.2 ± 4.9	65.0 ± 5.2	67.3 ± 2.9
Average percent reduction in exercise tachycardia (%)	-1.8 ± 3.3	-0.3 ± 1.8	-1.0 ± 1.5	-1.9 ± 3.2
Average systolic blood pressure at the end of exercise	182 ± 13	202 ± 33	180 ± 16	193 ± 27

Results are presented as mean $\pm$ s.d.
No difference was statistically significant.

Figure 3 Relationship between β -adrenoceptor blockade 3 h after metoprolol dosage and plasma S-(-)-metoprolol concentration. Data were fitted to an $E_{\max}$ model treatment with metoprolol alone (open and closed circles, solid line) and during the combination of metoprolol with nicardipine (open and closed triangles, dashed line). The curves were not significantly different. Open symbols, poor metaboliser; closed symbols, extensive metaboliser.

6.8 ng ml⁻¹, respectively. The effect vs plasma S-(-)-metoprolol concentration curve associated with treatment with the combination of metoprolol and nicardipine was not significantly different from that established during treatment with metoprolol alone.

Discussion

Our results show that differences in the pharmacokinetics and pharmacodynamics of metoprolol reported between subjects with the EM and PM phenotypes for CYP2D6-dependent oxidation after single dose metoprolol administration [1–6, 28] persist during repeated dosing. Also, nicardipine selectively inhibits metoprolol elimination in EM subjects but does not appear to alter the β -adrenoceptor blocking effects of metoprolol.

Metoprolol pharmacokinetics and pharmacodynamics in EM and PM subjects

Our results following repeated administration of metoprolol to subjects phenotyped for CYP2D6 activity show that, compared with subjects with the EM phenotype, PM subjects have increased plasma concentrations of metoprolol, decreased oral clearances, lower urinary recoveries of metoprolol and higher S/R metoprolol ratios in plasma and in urine. However, the renal clearance of metoprolol is similar in EM and PM subjects. These results are similar to those reported following single oral doses of metoprolol [1–6, 28]. In PM subjects, α -hydroxy-metoprolol was undetectable in plasma and urine. This result contrasts with previous data following single oral administration of metoprolol. Consistent with the increase in plasma metoprolol concentration in PM subjects, β -adrenoceptor blockade is more pronounced in PM than in EM subjects. Although this difference was documented after single dose metoprolol administration [2, 28], only limited information was available on its relevance to repeated dosing [9]. In EM

subjects, the ability of CYP2D6 to metabolise a substrate may decrease with increasing concentration of this substrate [7, 8]. While this has been shown for propafenone [7], our results indicate that it is not the case with metoprolol. Thus, phenotyping may be useful to predict the extent of β -adrenoceptor blockade with metoprolol in patients receiving this drug for therapeutic purposes. Our results indicate that the doses of metoprolol required to reach a given level of β -adrenoceptor blockade are higher in EM than in PM subjects.

Interaction of nicardipine with metoprolol

Pharmacokinetic interaction Silke *et al.* [29] gave single doses of metoprolol and nicardipine to 20 patients with stable coronary artery disease and found no pharmacokinetic or haemodynamic interaction. However, they did not study the influence of CYP2D6 activity on the extent of the interaction. We found a small but significant increase in plasma metoprolol concentration in EM subjects but not in PM subjects. We were expecting to detect such an interaction because *in vitro* studies have shown that nicardipine is a potent inhibitor of CYP2D6 activity [11], and several studies have shown that such inhibition is often associated with genetically-determined drug interactions [13–16]. Thus, an interaction which selectively affects EM subjects would indicate a CYP2D6-dependent mechanism [13–16]. However, several findings indicate that CYP2D6 was not involved in nicardipine-induced inhibition of metoprolol elimination in our EM subjects. Indeed, nicardipine had no influence on the plasma concentration of α -hydroxy-metoprolol, on the partial metabolic clearance of metoprolol to α -hydroxy-metoprolol or on the S/R metoprolol ratios in plasma and urine, all parameters which are known to depend on CYP2D6 activity. It has also been shown that nifedipine does not alter the clearance of sparteine or its metabolic ratio, other indices of CYP2D6 activity [30]. However, an interaction has been reported between nicardipine and propranolol [31]. It was suggested that this interaction could be explained by a decrease in the hepatic clearance of propranolol by mechanisms such as decreased microsomal drug oxidation activity or functional shunting in the liver [31]. Such mechanisms also could explain our results.

Metoprolol is regarded as a medium to high clearance drug in EM subjects and as a drug with a low systemic clearance in PM subjects [3]. Therefore, metoprolol elimination depends predominantly on hepatic blood flow in EM subjects but on hepatic enzyme activity in PM subjects. Thus, a non-CYP2D6 mediated inhibition of metoprolol metabolism by nicardipine should be associated with a selective decrease of metoprolol clearance in PM subjects, a result which we did not find. A possible explanation for this could be that nicardipine inhibits a CYP2D6-independent route of metoprolol metabolism, presumably a component of metoprolol *O*-demethylation which depends on CYP3A4 activity (M.S. Lennard, personal communication). The selective interaction observed in EM subjects might then reflect the fact that the concentrations of nicardipine were sufficient to cause limited competitive inhibition of CYP3A4 in EM subjects but were too low in PM subjects because

of the much higher metoprolol concentrations in the latter phenotype.

Also, nicardipine could increase splanchnic blood flow, thus causing a functional shunt in the liver and allowing metoprolol to escape from first pass metabolism in EM subjects (flow-dependent clearance) but not in PM subjects (enzyme activity-dependent clearance).

Since nicardipine did not alter the renal clearance of metoprolol, it is likely that the interaction that we observed was caused by an effect of nicardipine on the metabolism of metoprolol in EM subjects.

Pharmacodynamic interaction The small increase in plasma metoprolol concentration observed in EM subjects was not sufficient to be associated with an increase in mean β -adrenoceptor blockade over a dosing interval. This is consistent with the fact that the plasma concentration of S-(–)-metoprolol, the enantiomer responsible for most of the β -adrenoceptor blocking activity, 3 h after administration of the last metoprolol dose was not increased during combined treatment with metoprolol and nicardipine. Also, the plasma S-(–)-metoprolol concentration vs β -adrenoceptor blocking effect curve was similar whether or not nicardipine was combined with metoprolol. This was also the case in a study of the interaction between nicardipine and propranolol [31].

Conclusion

Our results stress the difficulties of extrapolating *in vitro* results to *in vivo* situations. Thus, despite clear evidence of potent inhibition of CYP2D6 activity by nicardipine *in vitro* [11], a selective inhibition could not be documented *in vivo*. Nicardipine did impair metoprolol elimination in EM subjects but this was independent of CYP2D6-mediated metabolism, and was too small to influence the extent of β -adrenoceptor blockade with metoprolol. Finally, this study indicates that, even at steady-state, metoprolol-induced β -adrenoceptor blockade is more pronounced in PM than in EM subjects.

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