

Metabolism of ropivacaine in humans is mediated by CYP1A2 and to a minor extent by CYP3A4: An interaction study with fluvoxamine and ketoconazole as in vivo inhibitors

Background: Potential drug-drug interactions can be identified in vitro by exploring the importance of specific cytochrome P450 (CYP) isozymes for drug metabolism. The metabolism of the local anesthetic ropivacaine to 3-hydroxyropivacaine and (*S*)-2',6'-pipecoloxylidide was shown in vitro to be dependent on CYP1A2 and 3A4, respectively. In this in vivo model study we quantitated the role of these 2 isozymes for the metabolism of ropivacaine.

Methods: In a randomized, 3-way crossover study, 12 healthy subjects received a single dose of 40 mg ropivacaine intravenously alone or combined either with 25 mg fluvoxamine as a CYP1A2 inhibitor or with 100 mg ketoconazole as a CYP3A4 inhibitor twice daily for 2 days. Venous plasma and urine samples were collected over 10 hours and 24 hours, respectively. The samples were analyzed for ropivacaine base, 3-hydroxyropivacaine, and (*S*)-2',6'-pipecoloxylidide.

Results: Coadministration with fluvoxamine decreased the mean total plasma clearance of ropivacaine from 354 to 112 mL/min (68%), whereas ketoconazole decreased plasma clearance to 302 mL/min (15%). The relative changes in unbound plasma clearance were similar to the changes in total plasma clearance. The ropivacaine half-life ($t_{1/2}$) of 1.9 hours was almost doubled during fluvoxamine administration and the plasma concentration at the end of infusion increased slightly, whereas the corresponding parameters after ketoconazole administration remained unchanged. Coadministration with ketoconazole almost abolished the (*S*)-2',6'-pipecoloxylidide concentrations in plasma, whereas fluvoxamine administration increased the (*S*)-2',6'-pipecoloxylidide levels. The fraction of dose excreted as 3-hydroxyropivacaine in urine decreased during fluvoxamine administration from 39% to 13%.

Conclusions: CYP1A2 is the most important isozyme for the metabolism of ropivacaine. Drug-drug interactions with strong inhibitors of this isozyme could be of clinical relevance during repeated administration. A potent inhibitor of CYP3A4 causes a minor decrease in clearance, which should be of no clinical relevance. (Clin Pharmacol Ther 1998;64:484-91.)

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Drug-drug interactions can result in significant changes in the pharmacodynamics and pharmacokinetics of a compound. One approach to investigating potential metabolic drug interactions is to make use of the expanding knowledge of the substrate selectivity of isozymes involved in drug metabolism, particularly amongst the cytochrome P450 superfamily. The cytochrome P450 isozymes are responsible for the major part of the oxidative metabolism of drugs. An isozyme inhibitor increases the physiologic concentration of the parent drug by decreasing its metabolic clearance. Initial in vitro screening of enzymatic interactions in the development of new drugs will offer a focused identification of potentially important drug-drug interactions. Such findings are then to be studied in vivo to define the clinical importance of the in vitro screening results.

Ropivacaine [*S*(-)-propyl-2',6'-pipercoloxylidide] is a new long-acting local anesthetic of the amide type.¹ It contains a single chiral center and is administered as the pure *S*(-) enantiomer. Ropivacaine has now been approved for surgical anesthesia and acute pain management by epidural administration, local infiltration, and peripheral nerve blocks in adults and in children from the age of 12 years. It has also been studied as a 2-week rectal therapy for ulcerative colitis.² No in vivo racemization occurs after systemic administration of ropivacaine.³ The pharmacokinetics of ropivacaine have been shown to be linear in plasma concentrations below 2 mg/L.⁴ The plasma clearance (CL) is 400 to 500 mL/min and the elimination $t_{1/2}$ is 1.8 hours.^{4,6} Ropivacaine mainly undergoes oxidative hepatic metabolism in humans, with about 1% of an intravenous dose excreted unchanged in the urine.⁶ The 2 major metabolites of ropivacaine in humans are 3-hydroxyropivacaine and (*S*)-2',6'-pipercoloxylidide, which account for about 40% and 3%, respectively, of an administered single intravenous dose. The predominant form of 3-hydroxyropivacaine is conjugated with glucuronic acid. Both 3-hydroxyropivacaine and (*S*)-2',6'-pipercoloxylidide have significantly lower local anesthetic activity, as investigated in guinea pigs, in comparison to ropivacaine (Personal communication, March 1998, Ericson A.C., Astra Pain Control AB, Södertälje, Sweden). In vitro studies with human liver microsomes have shown that CYP1A2 is responsible for the metabolism of ropivacaine to 3-hydroxyropivacaine (Michaelis-Menten constant [K_m] = 16 μ mol/L and maximal velocity [V_{max}] = 46 pmol/mg/min) and CYP3A4 is responsible for the metabolism to (*S*)-2',6'-pipercoloxylidide (K_m = 400 μ mol/L and V_{max} = 1847 pmol/mg/min).⁷

The cytochrome P450 isozymes 3A4 and 1A2 constitute about 30% and 12%, respectively, of the total

amount of P450 isozymes in the liver,⁸ and they have so far been identified to be involved in the metabolism of about 50% (3A4) and 7% (1A2) of all prescribed drugs.⁹ Fluvoxamine is a selective serotonin reuptake inhibitor that has been shown to be a potent inhibitor of CYP1A2 in vitro¹⁰ and may potentially cause pharmacokinetic interactions with drugs metabolized by CYP1A2.¹¹⁻¹³ A single oral dose of 50 mg almost abolished the CYP1A2 activity in healthy subjects.¹³ Ketoconazole, an antifungal imidazole derivative, is an inhibitor of CYP3A4 in vitro and in vivo.¹⁴⁻¹⁶ A daily oral dose of 100 to 200 mg strongly inhibited the sulf-oxidation of omeprazole, catalyzed by CYP3A4.¹⁷ It has been suggested that caffeine could be used as a probe for CYP1A2 activity by estimating an index of the *N*-3-demethylation.¹⁸⁻²⁰

We used fluvoxamine as a CYP1A2 inhibitor and ketoconazole as a CYP3A4 inhibitor to clarify their effect on the metabolism of ropivacaine. We also assessed the CYP1A2 activity by means of a urinary test (*N*-3-demethylation index for caffeine) and related this to the plasma CL of ropivacaine.

MATERIAL AND METHODS

Subjects. Twelve subjects (6 men and 6 women) were included with a mean \pm SD age of 33 \pm 8 years and body weight of 70 \pm 12 kg. Within the previous 2 years they had been phenotyped as extensive hydroxylators with respect to debrisoquin (INN, debrisoquine) and *S*-mephenytoin, markers of CYP2D6 and CYP2C19 activity, respectively.²¹ The metabolic ratio was between 0.1 and 1.0 for debrisoquin and the *S*/*R* ratio was <0.2 for mephenytoin. The subjects were healthy as defined by medical history, physical examination, electrocardiogram, and routine laboratory analyses. They were reported not to have taken any concomitant medication for 2 weeks before or during the study. None of the subjects used tobacco, was pregnant, or used oral contraceptives. Neither grapefruit juice, grapefruit, nor alcoholic beverages were to be consumed for 2 days before or during each study session. Methylxanthine-containing food or drinks were not allowed 24 hours before or during the caffeine test. The study was approved by the Human Research Ethics Committee at Huddinge Hospital. All subjects gave their written informed consent.

Study protocol. The study was a randomized and balanced 3-period crossover study, with washout periods of at least 1 week. After an overnight fast the subjects were given the following drugs in separate study periods:

- 40 mg ropivacaine as a 20-minute intravenous infusion

- 40 mg ropivacaine as a 20-minute intravenous infusion coadministered with oral fluvoxamine, total dose of 100 mg (Fevarin, Meda, Göteborg, Sweden) given as 25 mg twice daily (8 AM and 6 PM) for 2 days
- 40 mg ropivacaine as a 20-minute intravenous infusion coadministered with oral ketoconazole, total dose of 400 mg (Fungoral, Janssen, Göteborg, Sweden) given as 100 mg twice daily (8 AM and 6 PM) for 2 days

The first dose of ketoconazole or fluvoxamine was in the morning the day before the experimental day. The ropivacaine infusion started 1 hour after intake of the morning dose on the second day of administration. Blood pressure and pulse rate were recorded at 1, 2, 4, 6, and 10 hours after the start of infusion. Standardized lunch and dinner were served, with the first meal 4 hours after the start of infusion. Venous blood samples were collected through a catheter before and at 10, 20, 30, and 45 minutes and at 1, 1½, 2, 3, 4, 6, 8, 10, and 24 hours after the start of infusion. Plasma was separated after centrifugation, transferred to polypropylene tubes (Cryotube, 5 mL, A/S Nunc, Denmark), frozen within 1 hour and stored at -20°C until analysis. Urine was collected in the intervals from 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 22, and 22 to 24 hours after the start of infusion. Aliquots were taken from each urine fraction, transferred to polypropylene tubes (Cryotube, 5 mL, A/S Nunc, Denmark), frozen within 1 hour, and stored at -20°C until assay.

The caffeine test. The caffeine urinary test was performed at least 3 days before the first study period. After emptying his or her bladder, the subject received an oral caffeine dose of 150 mg (Koffein, ACO AB, Helsingborg, Sweden) and collected urine for 24 hours. Aliquots of urine were stored at -20°C until assay.

The caffeine *N*-3-demethylation index was calculated,^{20,22} including the following metabolites: AFMU + 1U + 1X + 17U + 17X/137X (AFMU, 5-acetylaminoformylamino-3-methyluracil; 1U, 1-methyluric acid; 1X, 1-methylxanthine; 17U, 1,7-dimethyluric acid; 17X, 1,7-dimethylxanthine [paraxanthine]); 137X, 1,3,7-trimethylxanthine [caffeine]). The assays of caffeine and metabolites were performed by HPLC according to methods earlier described.^{20,22} The coefficient of variation (CV) was below 11.8% for all concentration levels and metabolites. The accuracy was between 91% and 108%.

Quantification of ropivacaine and the metabolites (*S*)-2',6'-pipercoloxylidide and 3-hydroxyropivacaine in plasma and urine. The total plasma concentration of ropivacaine base (molecular weight, 274.4) was

determined by gas chromatography with a nitrogen sensitive detector.²³ The limit of quantitation of the method was set at 0.014 mg/L. The between-day CV was 11% at 0.03 mg/L and 4.3% at 1.1 mg/L. The accuracy was between 95% and 101%.

The free plasma concentration of ropivacaine base was determined by coupled column liquid chromatography with ultraviolet detection at 210 nm after ultrafiltration of the plasma samples.²⁴ The limit of quantitation was set at 0.003 mg/L, with a between-day CV of 7.4% at 0.03 mg/L.

The total plasma concentration of (*S*)-2',6'-pipercoloxylidide was assayed in the samples taken at 8, 10, and 24 hours with use of linear-gradient coupled column liquid chromatography after ultrafiltration of acidified plasma. The metabolite was detected by mass spectrometry electrospray ionization with use of the *m/z* *M*+1 (eg, 233.2 [Unpublished method B-0018-01. Astra Pain Control AB]). The limit of quantitation was set at 0.003 mg/L and the between-day CV was 9.2% at 0.006 mg/L and 5.6% at 0.04 mg/L. The accuracy was between 94% and 102%.

The urinary concentration of ropivacaine and the metabolites (*S*)-2',6'-pipercoloxylidide and 3-hydroxyropivacaine was assayed by linear-gradient liquid chromatography.⁶ Ropivacaine and metabolites were detected by ultraviolet absorption at 210 nm. The 3-hydroxyropivacaine was determined after acid hydrolysis with 6 mol/L hydrochloric acid; that is, the sum of conjugated and unconjugated metabolite was determined. For ropivacaine the limit of quantitation was set at 0.3 mg/L, with a between-day CV of 5.5% at 0.6 mg/L and 1.3% at 3.0 mg/L. The accuracy was between 98% and 105%. The limit of quantitation of 3-hydroxyropivacaine was set at 1.5 mg/L, with a between-day CV of 2.6% at 2.3 mg/L and 1% at 11.6 mg/L. The accuracy was between 99% and 104%. The limit of quantitation of (*S*)-2',6'-pipercoloxylidide was 0.6 mg/L, and the between-day CV was 4.9% at 0.9 mg/L and 1.6% at 3.5 mg/L. The accuracy was between 99% and 102%.

Pharmacokinetic analysis. The pharmacokinetic variables were estimated according to standard non-compartmental methods with use of the pharmacokinetic program WinNonlin, version 1.1 (Scientific Consulting Inc, Apex, NC). In the calculation of pharmacokinetic parameters, all values below the limit of quantitation were set to zero. The plasma concentrations at the end of infusion (C_{stop}) and the time of peak plasma concentration were derived directly from the individual plasma concentration–time curves. The total area under the plasma concentration–time curve (AUC) was estimated by the linear trapezoidal rule up to the last

Table I. Pharmacokinetic parameters of ropivacaine in 12 healthy subjects after a 20-minute intravenous infusion of ropivacaine alone or together with either fluvoxamine or ketoconazole

Group	CL (mL/min)	$t_{1/2}$ (h)	C_{stop} (mg/L)	f_u (%)	3-hydroxyropivacaine f_m	(S)-2',6'-pipecoloxylidide f_m
Ropivacaine	354 ± 110	1.9 ± 0.5	1.23 ± 0.21	5.2 ± 1.3	0.39 ± 0.05	0.01 ± 0.02
Fluvoxamine	112 ± 27*	3.6 ± 1.1*	1.46 ± 0.24*	5.2 ± 1.1	0.13 ± 0.07*	0.17 ± 0.06*
Ketoconazole	302 ± 101	1.9 ± 0.5	1.32 ± 0.19	5.6 ± 1.9	0.47 ± 0.07	0.00 ± 0.00*

Data are mean values ± SD.

CL, Total plasma clearance; $t_{1/2}$, terminal elimination half-life; C_{stop} , plasma concentrations at the end of the infusion; f_u , unbound fraction; f_m , fraction of dose metabolized.

*95% confidence interval for the ropivacaine/fluvoxamine ratio or ropivacaine/ketoconazole ratio excludes 1; that is, a statistically significant change from ropivacaine alone; $P < .05$.

plasma concentration that was above the limit of quantitation. Total AUC was calculated as $AUC = AUC + AUC_{extrapolated}$, in which $AUC_{extrapolated}$ was C_{last}/λ . C_{last} was the last quantifiable plasma concentration and λ is the terminal rate constant estimated by linear regression of the last 3 to 6 data points on the log-linear plasma concentration curve. The terminal $t_{1/2}$ was calculated by $\ln 2/\lambda$. Plasma CL was calculated by $CL = Dose/AUC$. Mean residence time (MRT) was calculated by $MRT = AUMC/AUC - t/2$, in which t is infusion time and AUMC is the area under the first moment of the plasma concentration–time curve calculated by the linear trapezoidal rule up to C_{last} and the residual area by integration ($t \times C_{last}/\lambda + C_{last}/\lambda^2$). The apparent distribution volume (V_{ss}) at steady state was calculated by $V_{ss} = CL \times MRT$. The unbound fraction (f_u) of ropivacaine was calculated by $f_u = C_u/C$, in which C_u is the unbound concentration of ropivacaine at each sampling time and C is the total concentration at the same point of time. The unbound clearance (CL_u) of ropivacaine was calculated by taking the mean of the 2 f_u values at 1 and 2 hours and then calculating CL/f_u .

The terminal $t_{1/2}$ of (S)-2',6'-pipecoloxylidide in plasma was estimated in the ropivacaine group by use of the formula $\ln 2/\lambda$, in which λ was the slope factor determined by linear least-squares regression from the time points 8, 10, and 24 hours on the log-linear plasma concentration–time curve. Only subjects with concentrations above limit of quantitation at all 3 time points were included in this analysis. The cumulative fraction of unchanged ropivacaine excreted in urine (f_u), as well as the excretion (fraction metabolized) of the major metabolites 3-hydroxyropivacaine ($f_{m,3-OH}$) and (S)-2',6'-pipecoloxylidide ($f_{m,PPX}$), were estimated. The fractions were calculated by $A_e/dose$, in which A_e was the total amount (in micromoles) excreted. For the calculation of A_e , all values below the limit of quantitation were set to zero. The terminal $t_{1/2}$ of the metabolite 3-hydroxyropivacaine was estimated from the urinary

excretion rate estimated by linear regression of the terminal log-linear part, which could not be done for (S)-2',6'-pipecoloxylidide because of low concentrations in urine, making it impossible to estimate the $t_{1/2}$.

The hepatic extraction ratio (E_H) was estimated for ropivacaine from the formula:

$$E_H = CL_p/(C_p/C_b) \times 1/\text{hepatic blood flow}$$

in which C_p/C_b was 0.69,⁵ and hepatic blood flow was assumed to be 1.35 L/min.²⁵

Statistical analysis. The pharmacokinetic parameters for the different dose groups were compared after fitting of an ANOVA model,²⁶ taking the factors subject, period, treatment, and first-order carryover effects into account. In addition, a block factor controlling for sex (male/female) and interaction between block level and treatment group was included in the model. The analysis was made on logarithms of the data. The antilogarithm of obtained estimates then gave the results on a ratio scale. For each pharmacokinetic parameter, confidence intervals of treatment differences were calculated after adjusting for the number of comparisons using the Tukey procedure.²⁷ Consequently, the 3 pairwise confidence intervals (for the 3 group differences) all cover the true mean differences with a simultaneous confidence level of 95%. This is in contrast to the unadjusted confidence intervals for which each single interval covers the true mean difference with a confidence of 95%. A 95% confidence interval for the ratio excluding 1 was considered to be a statistically significant change with a significance level of 5%. Results are shown as mean ± SD and ratios with 95% confidence intervals (95% CI), unless otherwise stated.

RESULTS

Plasma. The concomitant administration of fluvoxamine increased the total plasma concentration–time profile of ropivacaine in a profound way, whereas ketoconazole

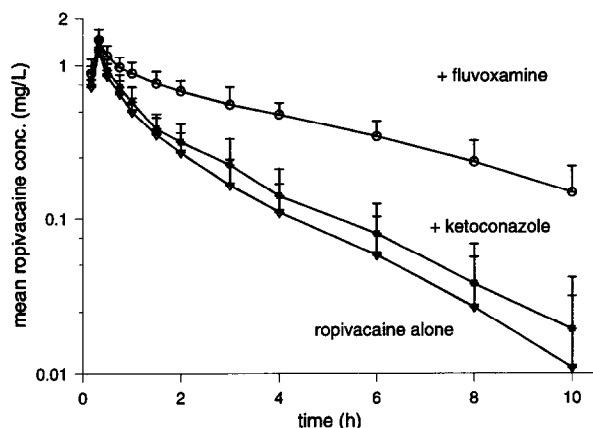


Figure 1. Mean \pm SD of total ropi vacaine concentration in plasma after 40 mg ropi vacaine given as a 20-minute intravenous infusion alone or with coadministration of either fluvoxamine, 25 mg twice daily for 2 days, or ketoconazole, 100 mg twice daily for 2 days, to 12 healthy subjects in a crossover study. Values below the limit of quantitation were set to zero.

zole had minor effects (Figure 1). When fluvoxamine was administered the total plasma CL of ropi vacaine decreased by 68% (95% CI, 62% to 72%) from 354 to 112 mL/min (Table I and Figure 2). Coadministration with ketoconazole slightly reduced CL by 15% (95% CI, 1% to 27%) to 302 mL/min. The decrease in CL after fluvoxamine administration, expressed as the percentage of the uninhibited ropi vacaine CL, showed a small variability between individuals, with the majority having a 60% to 70% reduction in CL irrespective of the level of their uninhibited CL (Figure 2). The reduction in CL in the ketoconazole group (percentage of uninhibited CL) showed a larger variability between individuals, with 1 subject having a slightly increased CL.

Fluvoxamine increased C_{stop} of ropi vacaine from 1.23 to 1.46 mg/L (ratio, 0.84; 95% CI, 0.74 to 0.95). In the ketoconazole group C_{stop} was similar to the ropi vacaine group, 1.32 mg/L (ratio, 0.93; 95% CI, 0.82 to 1.06). The highest individual plasma concentration of 1.89 mg/L was observed as C_{stop} in the fluvoxamine group.

The unbound fraction of ropi vacaine was not influenced by concomitant administration of ketoconazole or fluvoxamine, with values of 5.2% for ropi vacaine, 5.2% for fluvoxamine, and 5.6% for ketoconazole (Table I). Accordingly, the changes in CL_u were similar to the changes in CL: from 6.95 L/min in the ropi vacaine group to 2.18 L/min in the fluvoxamine group (ratio, 3.04; 95% CI, 2.24 to 4.13) and 5.68 L/min in the ketoconazole group (ratio, 1.11; 95% CI, 0.81 to 1.50).

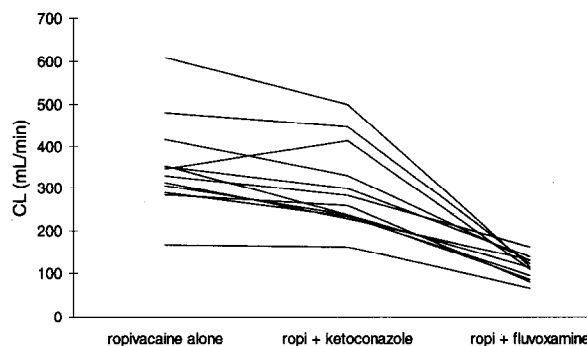


Figure 2. Individual clearance (CL) values after 40 mg ropi vacaine (ropi) given as a 20-minute intravenous infusion alone or with coadministration of either fluvoxamine, 25 mg twice daily for 2 days, or ketoconazole, 100 mg twice daily for 2 days, to 12 healthy subjects in a crossover study.

The $t_{1/2}$ increased after fluvoxamine administration from 1.9 hours to 3.6 hours (ratio, 0.51; 95% CI, 0.44 to 0.60), whereas no change was observed in the ketoconazole group, with a $t_{1/2}$ of 1.9 hours (ratio, 0.95; 95% CI, 0.82 to 1.11; Table I). V_{ss} showed a variability between the groups: 40 ± 5 L in the ropi vacaine group, 33 ± 7 L in the fluvoxamine group (ratio, 1.25; 95% CI, 1.10 to 1.43), and 38 ± 4 L in the ketoconazole group (ratio, 1.07; 95% CI, 0.75 to 0.98).

The plasma concentration of (*S*)-2',6'-pipecoloxylidide in the ropi vacaine group was 16.6 mg/L (11.0, 21.7; median, 1st and 3rd quartiles) at 8 hours after the start of infusion (Figure 3). The terminal $t_{1/2}$ of (*S*)-2',6'-pipecoloxylidide in plasma in the ropi vacaine group was 8.8 ± 2.8 hours ($n = 7$). Administration of ketoconazole strongly reduced the (*S*)-2',6'-pipecoloxylidide concentration at 8 hours to 4.1 mg/L (0.0, 6.7; median, 1st and 3rd quartiles), with only 50% of the subjects in this group having concentrations above the limit of quantitation. When fluvoxamine was administered the (*S*)-2',6'-pipecoloxylidide concentrations increased to 77.1 mg/L (62.2, 108.9; median, 1st and 3rd quartiles).

The mean \pm SD E_{H} for ropi vacaine was estimated to be 0.38 ± 0.12 , with a range from 0.18 to 0.65. No carryover effects or difference between the sexes in regard to CL, C_{stop} , or $t_{1/2}$ could be detected in the statistical analysis.

Urine. In all dose groups the excretion of unchanged ropi vacaine was no more than 1% of the given dose. The total urinary excretion of metabolites within 24 hours was lowest when fluvoxamine was coadministered (Table I). The excretion of (*S*)-2',6'-pipecoloxylidide was low, except in the fluvoxamine group where

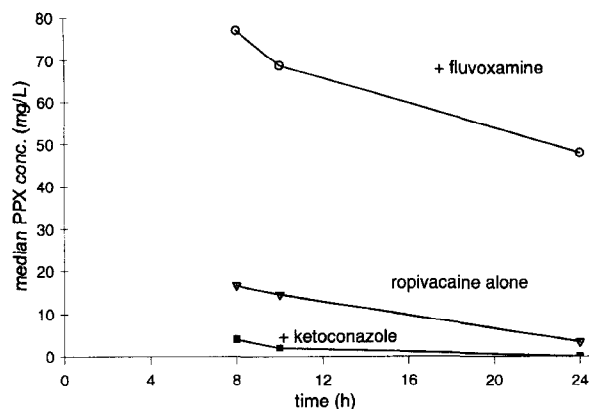


Figure 3. Median values of total (*S*)-2',6'-pipercoloxyllidide (PPX) concentration in plasma after 40 mg ropivacaine given as a 20-minute intravenous infusion alone or with coadministration of either fluvoxamine, 25 mg twice daily for 2 days, or ketoconazole, 100 mg twice daily for 2 days, to 12 healthy subjects in a crossover study. Values below the limit of quantitation were set to zero.

excretion had increased. There was a significant difference between the groups in the excretion of 3-hydroxyropivacaine, with the lowest excretion after fluvoxamine administration, although it was not completely abolished. The mean \pm SD urinary terminal $t_{1/2}$ for 3-hydroxyropivacaine was 4.4 ± 1.3 hours.

The caffeine *N*-3-demethylation index (CYP1A2 activity) ranged from 16 to 150. When the activity was related to the ropivacaine CL, no relationship between activity and CL could be found (Figure 4). In addition, no relationship was found when CL was substituted for the change in CL caused by administration of fluvoxamine. There was no relationship between the fraction of dose excreted in urine as 3-hydroxyropivacaine and the CYP1A2 activity.

Adverse events. The administration of study drugs was well tolerated by all subjects, without any clinically important adverse reactions. The majority of events that could have been related to drug administration were recorded between the caffeine dose and the first study period: headache ($n = 1$), insomnia ($n = 1$), and nausea ($n = 2$). No clinically relevant changes were observed in pulse rate or blood pressure.

DISCUSSION

This model study to assess potentially important interactions between ropivacaine and inhibitors of CYP1A2 (fluvoxamine) and CYP3A4 (ketoconazole) show that these 2 isozymes are involved in the oxidative hepatic metabolism of ropivacaine in humans

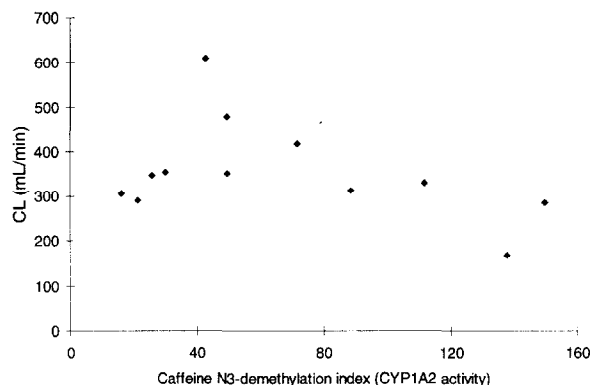


Figure 4. Relationship between plasma clearance of ropivacaine and the caffeine *N*-3-demethylation index of CYP1A2 activity. Data are from 12 healthy subjects.

because the CL of ropivacaine is reduced, as well as the formation of metabolites (f_m). Moreover, the size of the changes in CL and f_m shows that CYP1A2 inhibition has a major impact compared with CYP3A4 inhibition. Therefore these results confirm the previous *in vitro* results that the metabolism of ropivacaine is mediated by these 2 isozymes.

The dose, and consequently the plasma concentration, of the inhibitor is likely to be critical for achieving a selective inhibition when specifically studying selected isozymes. Fluvoxamine is a potent inhibitor *in vivo* of CYP1A2 and a less potent inhibitor of CYP2C19^{10,11,28,29}; that is, concentrations that are too high could give nonselective inhibition. In this study we found that a fluvoxamine dose of 25 mg twice daily strongly and with a low interindividual variability reduced the formation of 3-hydroxyropivacaine. Jeppesen et al¹³ previously concluded that a single oral dose of 50 mg fluvoxamine abolished the CYP1A2 activity, whereas CYP2C19 activity was only slightly affected. Nevertheless, we used another dosing scheme and we cannot exclude that CYP2C19 was affected. The argument for a minor impact or no impact of CYP2C19 involvement in our results are the previous *in vitro* results,⁷ which gave no indications of any involvement of CYP2C19 in ropivacaine metabolism. All of these findings indicate that the observed reduction in CL after fluvoxamine arises mainly from a decrease in 3-hydroxyropivacaine formation, likely to be mediated through CYP1A2 inhibition. The ketoconazole dose that we used has previously been shown to inhibit CYP3A4 activity.¹⁷ The larger variability observed for the change in CL in the ketoconazole group, in comparison to the fluvoxamine group, can probably be attributed to the small fraction of the ropivacaine dose that is metabo-

lized to (*S*)-2',6'-pipercoloxylidide. Nevertheless, the plasma concentrations of (*S*)-2',6'-pipercoloxylidide show that ketoconazole strongly reduced the formation of this metabolite. By use of selective inhibitors in a selected dose range, we have therefore demonstrated that less than 15% of the total CL is mediated through enzymatic pathways other than CYP1A2 and CYP3A4.

There was a noticeable increase in (*S*)-2',6'-pipercoloxylidide urinary excretion after fluvoxamine administration, even though the $t_{1/2}$ of (*S*)-2',6'-pipercoloxylidide in plasma seemed to increase. This indicates that the formation of (*S*)-2',6'-pipercoloxylidide through CYP3A4 strongly increased and, as an additional factor, further metabolic pathways of (*S*)-2',6'-pipercoloxylidide could also have been inhibited by CYP1A2 (eg, hydroxylation). One likely explanation for an increased formation of (*S*)-2',6'-pipercoloxylidide is the K_m values. The K_m values in vitro for the formation of 3-hydroxyropivacaine by CYP1A2 and (*S*)-2',6'-pipercoloxylidide by CYP3A4 are 16 and 400 $\mu\text{mol/L}$, respectively.⁷ A lower K_m value means a higher affinity for the enzyme. When metabolism through CYP1A2 was inhibited by fluvoxamine, a higher plasma concentration of ropivacaine was reached with a prolonged $t_{1/2}$, most likely increasing the concentrations of ropivacaine also in the hepatic enzymes. Therefore more (*S*)-2',6'-pipercoloxylidide could be formed through CYP3A4 despite the higher K_m , which also is compensated for by the higher capacity of 3A4 as reflected by the higher V_{max} .

The failure to determine the $t_{1/2}$ of (*S*)-2',6'-pipercoloxylidide in the urine may be explained by a collection period that is too short in relation to the $t_{1/2}$ of (*S*)-2',6'-pipercoloxylidide. This also causes the quantities of (*S*)-2',6'-pipercoloxylidide in urine to be underestimated, because more (*S*)-2',6'-pipercoloxylidide would be expected to be excreted after the 24-hour collection period, especially when fluvoxamine was administered because this prolonged the $t_{1/2}$ of ropivacaine. The fraction of dose excreted as 3-hydroxyropivacaine in the urine is more accurately estimated because the collection period was about 5 times the $t_{1/2}$. The urinary terminal $t_{1/2}$ of 3-hydroxyropivacaine in this study (4.4 hours) was slightly lower than the previously reported value of 5.7 hours.⁷

Our results did not detect any relationship between the CL or changes in CL of ropivacaine and the CYP1A2 activity as estimated by the *N*-3-demethylation index. Previous studies have shown correlations for clozapine CL¹⁸ and for fluvoxamine CL¹⁹ and CYP1A2 activity (*N*-3-demethylation). The ranges of estimated CYP1A2 activity for these 2 studies were 5 to 1000 and 15 to 250, respectively, both wider ranges

than that found in our study (16 to 150), most likely because our subjects were nonsmokers. Smoking induces CYP1A2 activity.³⁰ This narrow range in estimated activity could be one explanation for the failure to detect any relationship, depending on the sensitivity of this caffeine test.

The pharmacokinetic parameters of ropivacaine as found in this study are in agreement with previous results in healthy volunteers^{4,6} and with the extraction ratio⁶ that identifies ropivacaine as a drug with a low to intermediate extraction ratio. However, it should be emphasized that this extraction ratio is an approximation because it was estimated from a population-based value of liver blood flow and not from individual data. The lower V_{ss} in the fluvoxamine group could be an artifact caused by the longer terminal $t_{1/2}$ in this group, making the V_{ss} a more inexact estimate.

The reduction in ropivacaine CL during administration of fluvoxamine is likely to be of minor clinical relevance when ropivacaine is given as a single anesthetic dose. However, after repeated administration, toxic plasma concentrations could be reached during interaction with a potent CYP1A2 inhibitor because a reduced CL gives a higher steady-state concentration and a longer $t_{1/2}$ prolongs the time to reach steady state. This would be relevant for administration of ropivacaine by routes that could give a fast systemic absorption resulting in a high plasma concentration (eg, for repeated epidural or rectal dosing). The reduction in plasma CL after ketoconazole administration is likely to be of no clinical relevance.

This in vivo model study concluded that the in vitro results were good predictors of potential hepatic metabolic interactions for ropivacaine. CYP1A2 is the most important isozyme for the metabolism of ropivacaine, and drug-drug interactions with strong inhibitors of this isozyme could be of clinical relevance during repeated administration. A potent inhibitor of CYP3A4 causes a minor decrease in clearance, which should be of no clinical relevance.

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