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Ximelagatran, an Oral Direct Thrombin Inhibitor, Has a Low Potential for Cytochrome P450-Mediated Drug-Drug Interactions

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Abstract

Background: Ximelagatran is an oral direct thrombin inhibitor currently in clinical development for the prevention and treatment of thromboembolic disorders. After oral administration, ximelagatran is rapidly absorbed and extensively bioconverted, via two intermediates (ethyl-melagatran and hydroxy-melagatran), to its active form, melagatran. *In vitro* studies have shown no evidence for involvement of cytochrome P450 (CYP) enzymes in either the bioactivation or the elimination of melagatran.

Objective: To investigate the potential of ximelagatran, the intermediates ethyl-melagatran and hydroxy-melagatran, and melagatran to inhibit the CYP system *in vitro* and *in vivo,* and the influence of three CYP substrates on the pharmacokinetics of melagatran *in vivo*.

Methods: The CYP inhibitory properties of ximelagatran, the intermediates and melagatran were tested *in vitro* by two different methods, using heterologously expressed enzymes or human liver microsomes. Diclofenac (CYP2C9), diazepam (CYP2C19) and nifedipine (CYP3A4) were chosen for coadministration with ximelagatran in healthy volunteers. Subjects received oral ximelagatran 24mg and/or diclofenac 50mg, a 10-minute intravenous infusion of diazepam 0.1 mg/kg, or nifedipine 60mg. The plasma pharmacokinetics of melagatran, diclofenac, diazepam, *N*-desmethyl-diazepam and nifedipine were determined when administered alone and in combination with ximelagatran.

Results: No inhibition, or only minor inhibition, of CYP enzymes by ximelagatran, the intermediates or melagatran was shown in the *in vitro* studies, suggesting that ximelagatran would not cause CYP-mediated drug-drug interactions *in vivo*. This result was confirmed in the clinical studies. There were no statistically significant differences in the pharmacokinetics of diclofenac, diazepam and nifedipine on coadministration with ximelagatran. Moreover, there were no statistically significant differences in the pharmacokinetics of melagatran when

ximelagatran was administered alone or in combination with diclofenac, diazepam or nifedipine.

Conclusion: As ximelagatran did not exert a significant effect on the hepatic CYP isoenzymes responsible for the metabolism of diclofenac, diazepam and nifedipine, it is reasonable to expect that it would have no effect on the metabolism of other drugs metabolised by these isoenzymes. Furthermore, the pharmacokinetics of melagatran after oral administration of ximelagatran are not expected to be altered by inhibition or induction of CYP2C9, CYP2C19 or CYP3A4. Together, the *in vitro* and *in vivo* studies indicate that metabolic drug-drug interactions involving the major human CYP enzymes should not be expected with ximelagatran.

Ximelagatran (Exanta™[1], AstraZeneca) is an oral direct thrombin inhibitor currently in clinical development for the prevention and treatment of thromboembolic disorders. Following oral administration, ximelagatran is rapidly absorbed and extensively bioconverted to its active form, melagatran, with low interindividual variability in plasma concentrations of melagatran.[1-3] Melagatran is a potent direct inhibitor of human α-thrombin,[4,5] and the antithrombotic effect of melagatran has been demonstrated in animal[4,6-10] and human[3] models of experimental thrombosis. Ximelagatran administered in fixed-dose regimens without coagulation monitoring or dosage adjustment has been shown to be effective in the prophylaxis of thromboembolism after total hip or total knee replacement surgery[11-16] and treatment of acute deep vein thrombosis.[17] The promising efficacy of ximelagatran for the prevention of stroke and systemic embolic events has also been shown in patients with nonvalvular atrial fibrillation receiving long-term treatment with ximelagatran.[18]

The formation of melagatran from ximelagatran occurs via two intermediates, ethyl-melagatran (the ethyl ester of melagatran, formed by reduction of the hydroxyl group) and hydroxy-melagatran (the hydroxyamidine of melagatran, formed by hydrolysis of the ethyl group).[1] The elimination of melagatran occurs predominantly via renal excretion.[1] The ester hydrolysis of ximelagatran is slow in human plasma[1] but is, presumably, catalysed by esterases that are present in most tissues. *In vitro* studies have shown that the reduction of the hydroxyl group in ximelagatran occurs in microsomal preparations from several tissues, including the liver, intestinal membrane and kidney.[19] The highest activity was found in liver mitochondria. No reduction of hydroxy-melagatran was found in preparations of nine different human cytochrome P450 (CYP) isoenzymes.[20] These results suggest that it is unlikely that CYP enzymes are involved in the bioconversion of ximelagatran.

The aim of the present studies was to investigate the potential for interactions between ximelagatran and drugs metabolised by the most common CYP isoenzymes. *In vitro* studies were performed in human liver microsomes and in heterologously expressed CYP isoenzymes. Guided by the findings of these *in vitro* studies, the influence of ximelagatran on the pharmacokinetics of diclofenac, diazepam, and nifedipine (selective substrates for the isoenzymes CYP2C9, CYP2C19 and CYP3A4, respectively), as well as the effects of these drugs on the pharmacokinetics of melagatran, were examined *in vivo* in healthy volunteers.

Methods

In Vitro Inhibition Studies

In vitro CYP inhibition studies were performed using two methods: heterologously expressed CYP

1 Use of tradenames is for product identification only and does not imply endorsement.

enzymes and human liver microsomes. The CYP inhibition assay with heterologously expressed enzymes used fluorescent substrates in a high-throughput screening format, which has been previously validated and described using well-established inhibitors[21] and human CYP enzymes expressed in yeast cells.[22] The substrates were 3-cyano-7-ethoxy-coumarin (CYP1A2), 7-methoxy--4-trifluoromethylcoumarin (CYP2C9 and CYP2C19), 7-methoxy-4-(aminomethyl)-coumarin (CYP2D6), and 7-benzyloxy-4-trifluoromethylcoumarin (CYP3A4). The substrate concentrations in the assay were equal to their respective Michaelis-Menten constant (Km) values. Ximelagatran, ethyl-melagatran, melagatran hydroxyamidine and melagatran were tested for CYP inhibition at eight different concentrations in the range 0.09–200 μmol/L. It was confirmed that the test compounds were not fluorescent at the respective emission wavelengths or metabolised to a fluorescent metabolite, and that the test compounds and the substrate did not produce a fluorescent complex. Selective inhibitors of CYP enzymes (α-naphthoflavone [CYP1A2], sulfaphenazole [CYP2C9], ticlopidine [CYP2C19], quinidine [CYP2D6] and ketoconazole [CYP3A4]) with known inhibitory potency toward each CYP enzyme were run in parallel as controls.

Human liver microsomes were prepared from liver samples (from excess material removed during liver surgery) from the Department of Surgery 1, Sahlgrenska University Hospital, Göteborg, Sweden. This procedure was approved by the local ethics committee. Small cubes (1–2 cm^3) were frozen in liquid nitrogen and stored at –70°C until preparation. The homogenate prepared from liver samples was centrifuged at 800*g* for 10 minutes. The supernatant was decanted, and the pellet was rehomogenised and centrifuged again at 800*g* for 10 minutes. The two supernatants were then combined and centrifuged at 10 000*g* for 20 minutes. The resulting supernatant was centrifuged at 100 000*g* for 60 minutes. The pellet containing the microsomal fraction was dissolved in 50 mmol/L Tris-HCl buffer (pH 7.4) containing 0.25 mol/L sucrose.

The CYP-selective enzyme activities 7-ethoxyresorufin-O-deethylase (CYP1A2), diclofenac 4-hydroxylase (CYP2C9), (*S*)-mephenytoin 4-hydroxylase (CYP2C19), chlorzoxazone 6-hydroxylase (CYP2E1), bufuralol 1-hydroxylase (CYP2D6) and testosterone 6β-hydroxylase (CYP3A4/5) were analysed as described previously.[22] Coumarin 7-hydroxylase activity (CYP2A6) was measured by incubating a mixture of 0.2 mg/mL human microsomal protein, 1 mmol/L NADPH, 25 mmol/L potassium phosphate buffer (pH 7.4) and 15 μmol/L coumarin in a total volume of 300μL. The samples were incubated for 1.5 minutes, and the reaction was stopped by adding 20μL of 40% acetic acid. The metabolite 7-hydroxycoumarin was analysed with a fluorescence detector (Shimadzu RF-551; Shimadzu GmbH, Duisberg, Germany) after separation of the product on a high-performance liquid chromatography (HPLC) system (Pharmacia LKB, Uppsala, Sweden). The substrate concentrations were equal to their respective Km values. Ximelagatran, ethyl-melagatran, melagatran hydroxyamidine or melagatran were added to the incubations at 10 or 50 μmol/L. Selective inhibitors (furafyllin [CYP1A2], sulfaphenazole [CYP2C9], quinidine [CYP2D6], diethyldithiocarbamate [CYP2E1] and ketoconazole [CYP3A4]) with known inhibitory potency towards each CYP enzyme were run in parallel. No specific inhibitors of CYP2A6 and CYP2C19 were run in parallel as controls.

Design of Clinical Studies

Potential interactions between ximelagatran and diclofenac, diazepam and nifedipine were investigated in three separate clinical studies. The studies with diclofenac and nifedipine were both nonblinded, randomised, three-way crossover studies, each consisting of 3 study days separated by washout periods of at least 7 days. Ximelagatran and diclofenac or nifedipine were administered once alone and once together. Ximelagatran was administered as a single 24mg immediate-release tablet. Diclofenac was administered as a single 50mg dose of enteric-coated tablet (Voltaren®; Novartis Sveri-

ge AB, Täby, Sweden). Nifedipine was administered as a single 60mg commercially available slow-release tablet (Adalat® OROS; Bayer AB, Göteborg, Sweden). Ximelagatran was coadministered with diclofenac, whereas in the nifedipine study ximelagatran was administered 4 hours after the nifedipine tablet was taken. In both studies, the order in which volunteers received the treatments was randomised according to a three-treatment, three-period crossover design. Venous blood samples, for the determination of melagatran plasma concentrations, were collected up to 12 hours after administration of ximelagatran. Blood samples were collected up to 24 hours after administration of diclofenac for determination of diclofenac plasma concentrations, and up to 48 hours after administration of nifedipine for determination of nifedipine plasma concentrations.

The diazepam study was a nonblinded, randomised, two-way crossover study, consisting of two treatment periods: one of 6 days duration (diazepam alone) and the second of 8 days duration (coadministration of diazepam and ximelagatran). The treatment periods were separated by a washout period of at least 3 weeks. On the first day of the 6-day treatment period, diazepam (Stesolid®; Novum, Dumex, Denmark) was administered as a 10-minute intravenous infusion at a total dose of 0.1 mg/kg, and repeated blood sampling was performed during the entire 6-day period for determination of plasma concentrations of diazepam and the metabolite *N*-desmethyl-diazepam. During the coadministration treatment period, ximelagatran 24mg was administered twice daily for 8 days as an immediate-release tablet, and a single dose of diazepam 0.1 mg/kg was administered as a 10-minute intravenous infusion on day 3, 2 hours after the morning dose of ximelagatran. Blood samples for the determination of plasma concentrations of melagatran were collected up to 12 hours after administration on days 2 and 3. Blood samples for determination of plasma concentrations of diazepam and *N*-desmethyl-diazepam were collected up to 6 days after diazepam administration, at the same times as when diazepam was administered alone (frequently on the first day and then at 24, 34, 46, 70, 94, 118 and 142 hours after administration of diazepam).

In all three studies, a follow-up visit took place 2–7 days after the last study day of the last study session. All volunteers were instructed to eat dinner no later than 21.00 and to fast from 22.00 on the evenings preceding the pre-entry visit, the study days and the follow-up visits, until either the examination at the study site was complete or a standardised meal was served. Use of tobacco and tobacco substitutes was not allowed during the fasting periods or during the study days at the study site. Alcohol use was not permitted during the 2 days preceding the pre-entry examination or from 2 days before the first study session until the follow-up visit was completed. No physical training was allowed from 2 days before the study sessions until the follow-up visit was completed.

Volunteers

Healthy volunteers were included in the studies. The baseline characteristics of the volunteers were similar in all three studies (age 26–29 years, weight 70–73 kg). The majority (>80%) of the volunteers were white Europeans. Informed consent was obtained prior to enrolment.

None of the volunteers had ingested any prescribed medication, aspirin or other nonsteroidal anti-inflammatory drugs within the 2 weeks prior to the first dose of study drug; any over-the-counter drugs other than paracetamol (acetaminophen) within the previous week; or any other investigational drugs within the previous 8 weeks. These studies were approved by the Comité Consultatif des Protection des Personnes dans la Recherche Biomédicale Paris, Pitié-Salpetrière, Paris, and were performed in compliance with the Declaration of Helsinki and good clinical practice.

Plasma Concentration Analyses

The method used for determination of plasma concentrations of melagatran has been described previously.[23] Briefly, the plasma concentration of melagatran was determined by liquid chromatography–mass spectrometry (LC-MS) using

electrospray ionisation and selected reaction monitoring (SRM) after solid-phase extraction (SPE) of melagatran from plasma. Concentrations of diazepam and *N*-desmethyl-diazepam in plasma were determined by LC-MS using atmospheric pressure chemical ionisation and SRM after SPE. Plasma concentrations of diclofenac were determined by LC with ultraviolet detection (LC-UV) after liquid-liquid partitioning and SPE of the compound from plasma. Plasma concentrations of nifedipine were determined by LC-UV after SPE and liquid-liquid extraction of the compound.

The lower limit of quantification (LOQ, coefficient of variation <20%) of melagatran was 10 nmol/L and the demonstrated linear range (defined as inaccuracy and imprecision for concentrations above LOQ of <15%) was 10–2000 nmol/L. Corresponding data for diazepam and *N*-desmethyl-diazepam were 3.5 nmol/L (range 3.5–3500 nmol/L), for nifedipine 5 nmol/L (range 5–1000 nmol/L) and for diclofenac 35 nmol/L (range 35–3500 nmol/L).

Pharmacokinetic Assessments

The area under the plasma concentration-time curve (AUC_{last}) up to the last measurable plasma concentration (C_{last}) was calculated using the trapezoidal rule and extrapolated to infinity by the formula $AUC_{last} + C_{last}/\lambda$ to give the total area under the plasma concentration-time curve (AUC), where λ, the terminal elimination rate constant, was estimated by linear regression of the logarithm of plasma concentration versus time in the terminal phase of the decline. The half-life ($t_{1/2z}$) was estimated as $0.693/\lambda$. The pharmacokinetic analyses were performed with WinNonlin Professional 1.5 (Pharsight Corporation, Mountain View, CA, USA) using the actual sampling times. For diclofenac and nifedipine, the data for some volunteers did not allow estimation of $t_{1/2z}$ and AUC. The AUC (extrapolated to infinity) was not calculated for *N*-desmethyl-diazepam and the AUC_{last} (C_{last} obtained at 142 hours) is therefore presented.

Statistical Analysis

Pharmacokinetic parameter values are presented as means ± SD. The estimates of AUC and the maximum plasma concentration (C_{max}) were analysed using an analysis of variance (ANOVA) model, with the main effects of volunteer, period and treatment. Logarithmically transformed values were used in the analysis. Least-squares estimates with 90% CIs for between-treatment ratios are presented. A 90% CI of 0.8–1.25 for AUC and of 0.7–1.43 for C_{max}, for between-treatment ratios was used to provide evidence that there was no interaction between ximelagatran and the other three compounds.

Results

In Vitro Inhibition Studies

The CYP inhibition studies, using heterologously expressed enzymes and fluorescent model substrates, showed that ximelagatran, the intermediates and melagatran did not inhibit CYP1A2, CYP2C9, CYP2C19 or CYP2D6, even at the highest concentration used (200 μmol/L) [figure 1]. For CYP3A4, the concentrations required for 50% inhibition (IC_{50} values) for ximelagatran, melagatran hydroxyamidine, and melagatran were higher than 100 μmol/L, whereas ethyl-melagatran was a weak inhibitor of CYP3A4, with an IC_{50} of 54 μmol/L. If competitive inhibition is assumed, and when the substrate concentration is equal to Km, the inhibition constant (K_i) is $IC_{50}/2$, which is 27 μmol/L.

The CYP inhibition studies in human liver microsomes, using CYP-selective substrates, showed no inhibition of CYP1A2, 2A6, 2D6 or 3A4 by 10 or 50 μmol/L of ximelagatran, the intermediates or melagatran (figure 2). Melagatran 50 μmol/L inhibited CYP2C9-mediated activity by 30%. Ethyl-melagatran 50 μmol/L and ximelagatran 10 and 50 μmol/L inhibited CYP2C9 activity by 23%, 32% and 36%, respectively. Melagatran 10 and 50 μmol/L showed a weak inhibition of CYP2C19 activity, by 25 and 35%, respectively. At a concentration of 10 μmol/L, ethyl-melagatran inhibited CYP2C19 activity by

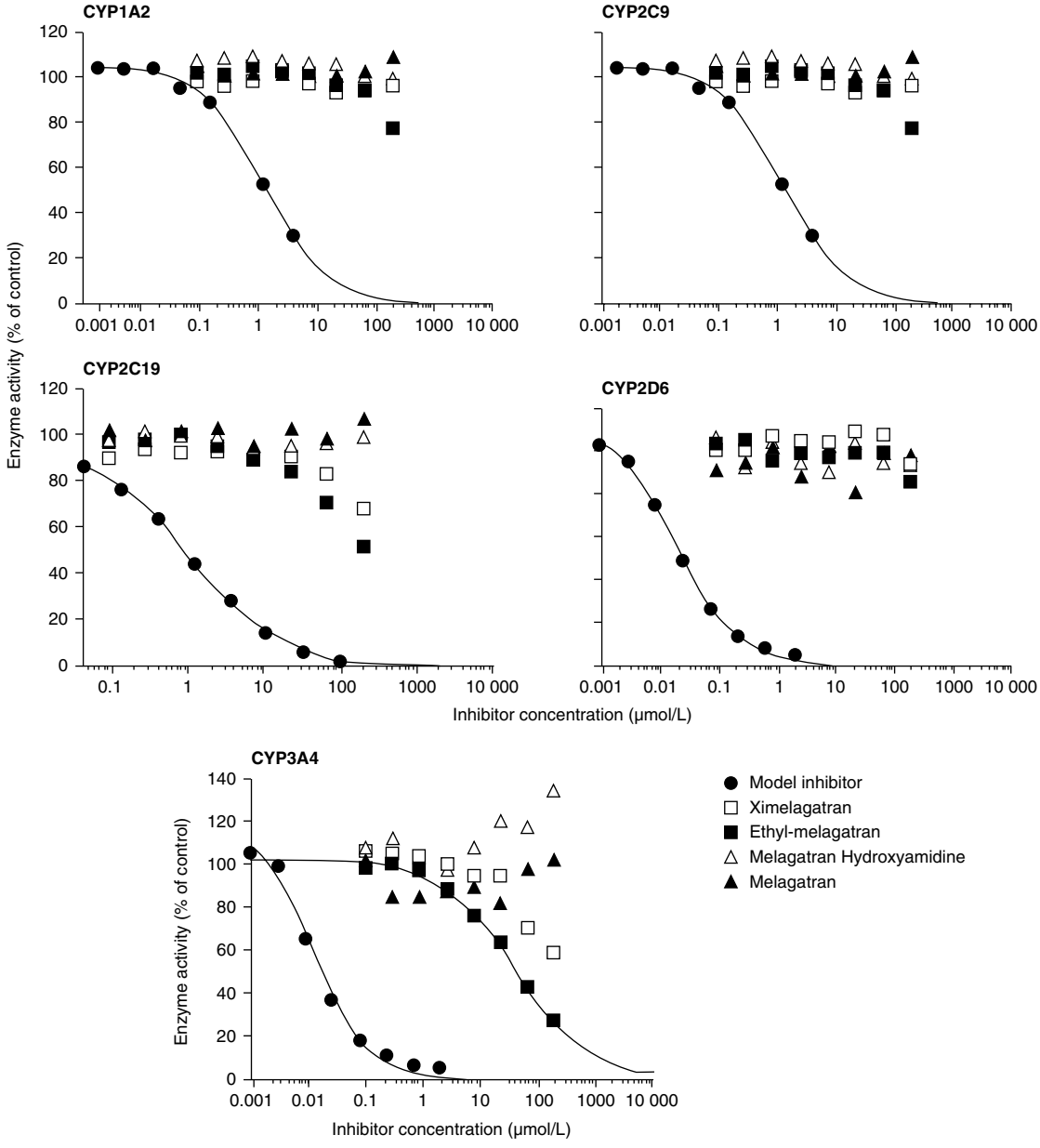

Fig. 1. Effects of ximelagatran, ethyl-melagatran, melagatran hydroxyamidine and melagatran on cytochrome P450 (CYP) metabolism using heterologously expressed enzymes and fluorescent substrates. The substrate concentrations were equal to their Michaelis-Menten constant (Km) values. The model inhibitors were α-naphthoflavone (CYP1A2), sulfaphenazole (CYP2C9), ticlopidine (CYP2C19), quinidine (CYP2D6) and ketoconazole (CYP3A4).

29%, whereas at 50 μmol/L it showed no inhibition. At concentrations of 10 and 50 μmol/L, melagatran hydroxyamidine inhibited CYP2C19 by 22 and 18%, respectively. With the exception of melagatran, the inhibition of CYP2C19 did not appear to be concentration-related.

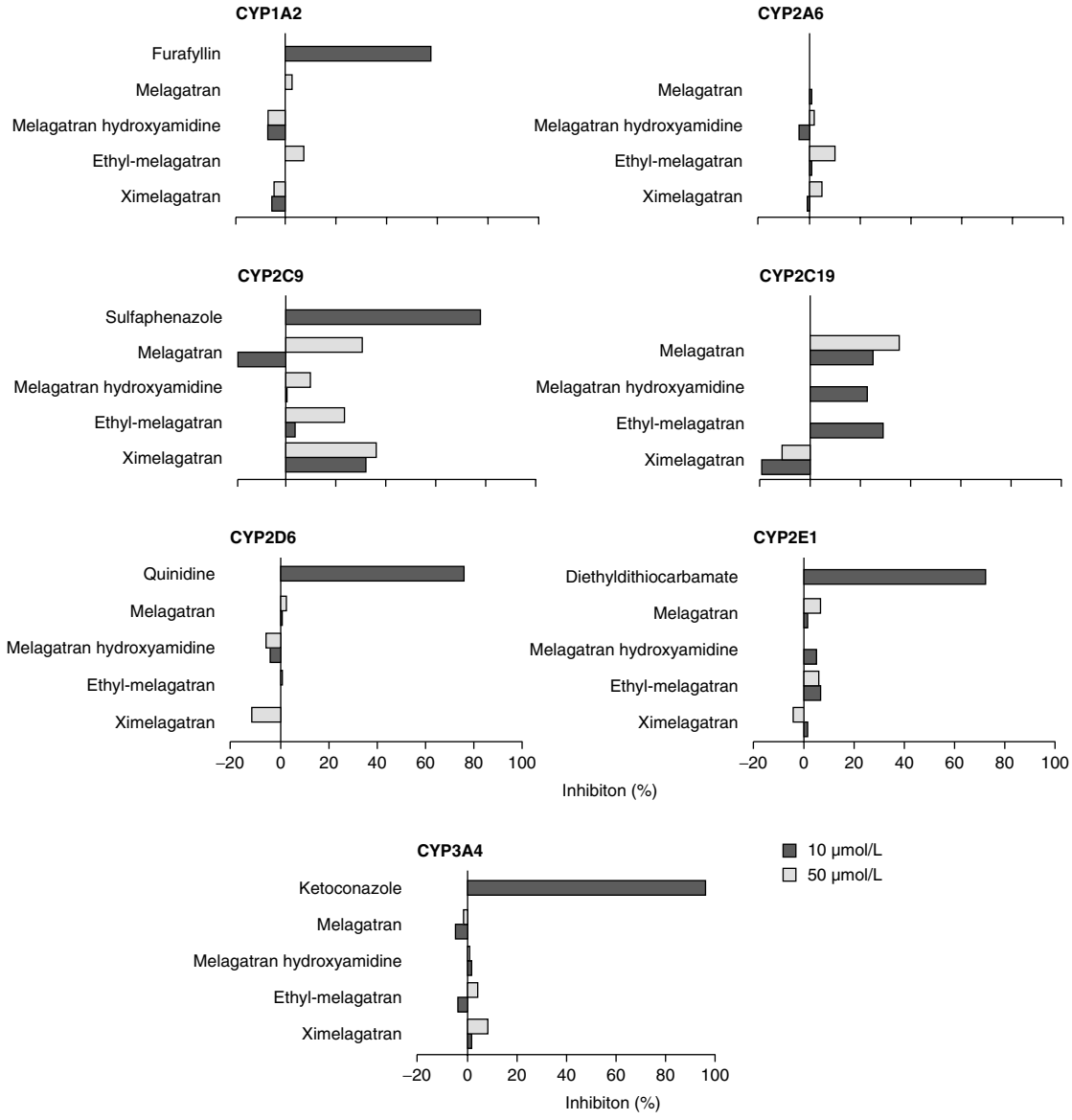

Fig. 2. Inhibition of cytochrome P450 (CYP)-selective substrates by ximelagatran, the intermediates and melagatran incubated at 10 or 50 µmol/L in human liver microsomes. The positive controls were incubated at 10 µmol/L. The substrates, incubated at their Michaelis-Menten constant (Km) values, were 7-ethoxyresorufin (CYP1A2), coumarin (CYP2A6), diclofenac (CYP2C9), (*S*)-mephenytoin (CYP2C19), bufuralol (CYP2D6), chlorzoxazone (CYP2E1) and testosterone (CYP3A4/5).

Clinical Studies

In the diclofenac and diazepam studies, 24 volunteers received the study drugs as per protocol and were included in the pharmacokinetic evaluation. In the nifedipine study, 36 volunteers received the study drugs; two discontinued prematurely and were not included in the pharmacokinetic evaluation. The mean plasma concentration-time curves for diclofenac, diazepam and its metabolite *N*-desmethyldiazepam, and nifedipine are shown in figure 3,

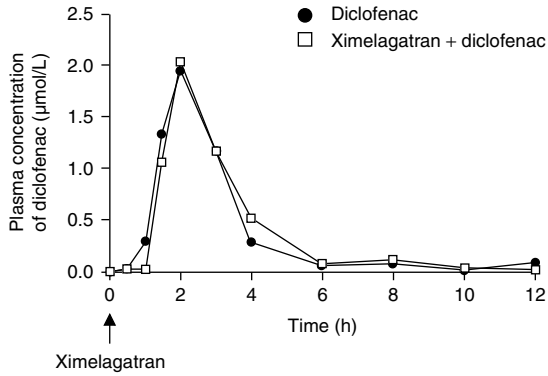

Fig. 3. Mean plasma concentrations of diclofenac versus time after administration of diclofenac alone or in combination with ximelagatran.

figure 4, and figure 5, respectively. Pharmacokinetic parameters for diclofenac, diazepam and nifedipine are presented in table I, table II and table III, respectively. It was difficult to estimate the $t_{½z}$ of nifedipine in 11 (nifedipine) and 9 (nifedipine plus ximelagatran) volunteers, and it was therefore not possible to calculate the extrapolated part of the AUC for these individuals. Hence, the numbers of volunteers included in the pharmacokinetic analysis were 23 (nifedipine) and 25 (nifedipine plus ximelagatran).

The least-squares estimates (with 90% CIs) of the ratios of AUC and C_{max} for the combined treatments versus the treatments with each drug alone are presented in table IV. There was no statistically significant effect of coadministration with ximelagatran on the AUC or C_{max} of diclofenac, diazepam, or nifedipine. For AUC, the 90% CIs were all within the predefined limits of 0.8–1.25. The least-squares estimates of ratios of C_{max} for diazepam, *N*-desmethyl-diazepam and nifedipine were also within the predefined limits of 0.7–1.43. For the C_{max} of diclofenac, the upper 90% CI was estimated to be at the limit of 1.43.

The mean plasma concentrations of melagatran as a function of time, following administration of ximelagatran alone and in combination with diclofenac, diazepam and nifedipine in the three studies, are shown in figure 6. These data show that diclofenac, diazepam and nifedipine had no effect on the plasma concentration of melagatran. Moreover, the pharmacokinetic parameter values of melagatran were similar whether ximelagatran was administered alone or in combination with any of the three drugs (tables I–III). The AUC and C_{max} of melagatran were unaffected by coadministration of diclofenac, diazepam or nifedipine (table IV).

Safety and Tolerability

No reported adverse events were considered to be related to the administration of ximelagatran in any of the studies. There were no bleeding events or

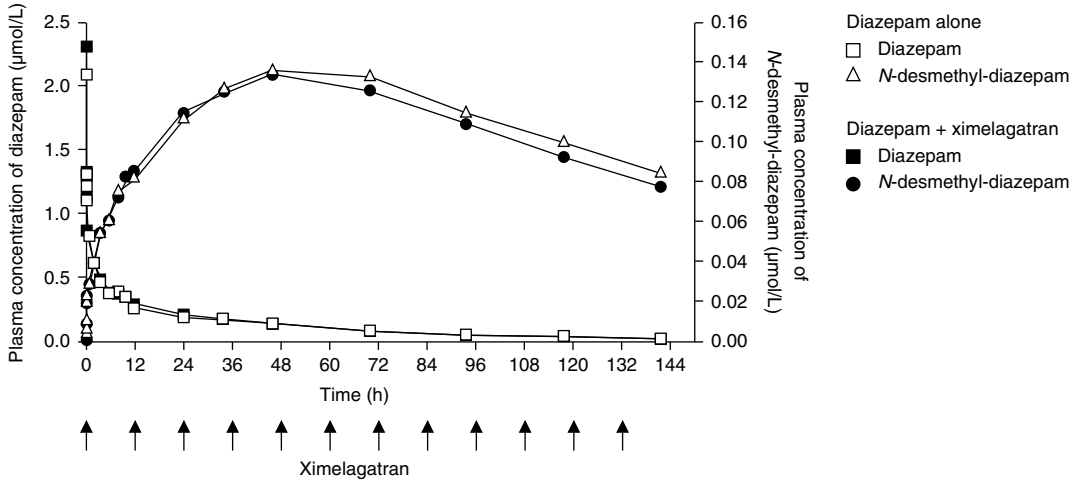

Fig. 4. Mean plasma concentrations of diazepam and *N*-desmethyl-diazepam versus time after administration of a single intravenous dose of diazepam alone or in combination with ximelagatran.

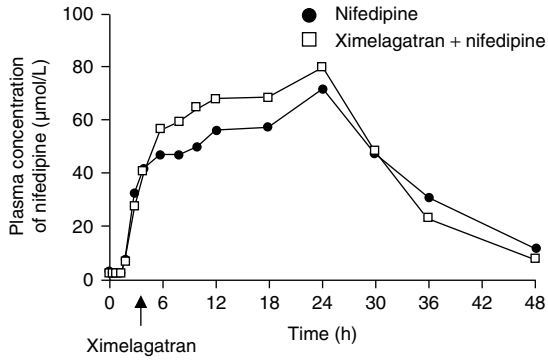

Fig. 5. Mean plasma concentration of nifedipine versus time after administration of nifedipine alone or in combination with ximelagatran.

clinically significant changes in laboratory variables or vital signs. As expected, there was a slight increase in pulse rate and a slight decrease in blood pressure when nifedipine plasma concentrations were highest (12–24 hours after administration).

Discussion

Pharmacokinetic interactions between drugs often arise because of changes in drug metabolism, most often through the CYP enzymes in the liver that are responsible for the oxidation of a large variety of drugs.[24] Variability in pharmacokinetics due to drug interactions can have serious consequences for an anticoagulant drug, as increased exposure may be associated with an increased risk for bleeding complications, whereas decreased exposure may be associated with an increased risk of thrombosis. The oral direct thrombin inhibitor ximelagatran is rapidly absorbed and bioconverted to its active form, melagatran, via ester hydrolysis and reduction. The results of the present *in vitro* and *in vivo* studies suggest that interactions between CYP enzymes and other drugs does not result in an interaction with ximelagatran.

No inhibition, or only minor inhibition, of the CYP enzymes studied was observed with ximelagatran, the two intermediates, ethyl-melagatran and melagatran hydroxyamidine, or melagatran when examined *in vitro* in human liver microsomes and with heterologously expressed enzymes. The seven CYP isoenzymes studied included the most common enzymes involved in the metabolism of drugs. As the substrate concentrations used in the incubations were equal to the Km values for their respective enzymes, and as inhibition is assumed to be competitive, the K_i can be estimated from equation 1:

$$K_i = V \bullet I / (V_{max} - 2V)$$

where V is activity at inhibitor concentration I and V_{max} is maximum activity.

The K_i values calculated for inhibition of CYP2C9 in human liver microsomes by ximelagatran, ethyl-melagatran, and melagatran are 10–46, 82 and 58 μmol/L, respectively. For CYP2C19, the K_i values for melagatran hydroxyamidine, ethyl-melagatran and melagatran are 15–47, 12 and 17–116 μmol/L. The K_i values for melagatran are more than 100-fold greater than the C_{max} of melagatran observed following oral administration of xime-

Table I. Mean (± SD) pharmacokinetic parameters of diclofenac and melagatran following administration of diclofenac and ximelagatran separately and in combination

Parameter and unit	Diclofenac		Melagatran	
	diclofenac alone (n = 24)[a]	diclofenac + ximelagatran (n = 24)[b]	ximelagatran alone (n = 24)	diclofenac + ximelagatran (n = 24)
AUC (μmol • h/L)	4.27 ± 1.38	4.19 ± 1.33	1.13 ± 0.24	1.13 ± 0.24
C_{max} (μmol/L)	3.56 ± 2.25	3.50 ± 1.92	0.23 ± 0.05	0.22 ± 0.05
t_{max} (h)	3.0 ± 2.5	3.0 ± 2.3	2.0 ± 0.4	1.9 ± 0.3
$t_{½z}$ (h)	0.9 ± 0.4	1.0 ± 0.6	2.9 ± 0.4	3.0 ± 0.4

a Except for AUC and $t_{½z}$ for which n = 21.

b Except for AUC and $t_{½z}$ for which n = 23.

AUC = area under the concentration-time curve from zero to infinity; **C_{max}** = peak plasma concentration; **t_{max}** = time to C_{max}; **$t_{½z}$** = terminal elimination half-life.

Table II. Mean (± SD) pharmacokinetic parameters of diazepam, *N*-desmethyl-diazepam and melagatran following administration of diazepam and ximelagatran separately and in combination

Parameter and unit	Diazepam		*N*-Desmethyl-diazepam		Melagatran	
	diazepam alone (n = 24)	diazepam + ximelagatran (n = 24)	diazepam alone (n = 24)	diazepam + ximelagatran (n = 24)	ximelagatran alone (n = 24)	diazepam + ximelagatran (n = 24)
AUC (μmol • h/L)	21.0 ± 8.6	20.5 ± 6.8	15.7 ± 3.2[a]	15.0 ± 3.0[a]	1.22 ± 0.27	1.20 ± 0.25
C_{max} (μmol/L)	2.12 ± 0.61	2.36 ± 0.88	0.15 ± 0.03	0.15 ± 0.04	0.22 ± 0.06	0.21 ± 0.06
t_{max} (h)	0.20 ± 0.08	0.20 ± 0.07	64.1 ± 28.9	56.3 ± 29.9	2.04 ± 0.63	1.82 ± 0.43
$t_{½z}$ (h)	35 ± 16	34 ± 13	ND	ND	3.09 ± 0.24	3.35 ± 0.31

a The AUC_{last} up to the last sampling time (142 hours) is reported.

AUC = area under the concentration-time curve from zero to infinity; **C_{max}** = peak plasma concentration; **ND** = not determined; **t_{max}** = time to C_{max}; **$t_{½z}$** = terminal elimination half-life.

lagatran 24mg (approximately 0.2 μmol/L). The CYP2C9- and CYP2C19-mediated activities of the heterologously expressed enzymes were not inhibited by ximelagatran, the intermediates or melagatran, suggesting a poor propensity to inhibit these enzymes. A weak inhibition of CYP3A4-mediated activity (K_i 27 μmol/L) by ethyl-melagatran was observed with heterologously expressed enzymes. Maximum plasma concentrations of ximelagatran are similar or slightly higher than those for melagatran, and maximum concentrations of melagatran hydroxyamidine and ethyl-melagatran are low, approximately 10 and 30% of those of melagatran, respectively,[2] and therefore much lower than the estimated K_i values. If plasma protein binding of ximelagatran, melagatran and its metabolites was to be taken into account the difference between C_{max} and estimated $K_{i:s}$ would be even greater. This finding suggests that the observed effects on CYP-mediated metabolism have no clinical relevance.

The results of studies in healthy volunteers receiving oral ximelagatran plus model substrates for CYP2C9, CYP2C19 or CYP3A4 confirmed that these CYP enzymes do not influence the bioconversion of ximelagatran to melagatran. The pharmacokinetics of the model substrates was also unchanged by coadministration with ximelagatran. In addition, the pharmacokinetics of melagatran were not influenced by the model substrates administered at therapeutic doses.

Many different CYP enzyme substrates are used as probes to investigate drug metabolism and drug-drug interactions. Diclofenac, a recommended model substrate of CYP2C9, is also of clinical relevance as it is a commonly used drug. In addition, diclofenac inhibits platelet aggregation and may therefore influence the pharmacodynamic effect of

Table III. Mean (± SD) pharmacokinetic parameters of nifedipine and melagatran following administration of nifedipine and ximelagatran separately and in combination

Parameter and unit	Nifedipine		Melagatran	
	nifedipine alone (n = 23)[a]	nifedipine + ximelagatran (n = 25)[a]	ximelagatran alone (n = 34)	nifedipine + ximelagatran (n = 34)
AUC (μmol • h/L)	2.10 ± 1.08	2.41 ± 1.01	1.03 ± 0.19	1.05 ± 0.19
C_{max} (μmol/L)[b]	ND	ND	0.20 ± 0.04	0.211 ± 0.04
t_{max} (h)[b]	ND	ND	2.1 ± 0.6	1.9 ± 0.5
$t_{½z}$ (h)	5.5 ± 2.7	5.3 ± 1.9	3.0 ± 0.4	3.0 ± 0.3

a The $t_{½z}$ of nifedipine could not be estimated for some volunteers, and AUC could not then be calculated for 11 and 9 volunteers in the nifedipine and nifedipine + ximelagatran groups, respectively.

b As nifedipine was administered as a slow-release tablet, C_{max} and t_{max} are not reported.

AUC = area under the concentration-time curve from zero to infinity; **C_{max}** = peak plasma concentration; **ND** = not determined; **t_{max}** = time to C_{max}; **$t_{½z}$** = terminal elimination half-life.

Table IV. Least-squares estimates (90% CI) of the ratios of AUC and C_{max} for combined treatment (diazepam, nifedipine or diclofenac plus ximelagatran) versus treatment with each drug separately

Treatment	AUC	C_{max}
Diclofenac + ximelagatran study		
Diclofenac	0.99 (0.85, 1.16)	1.05 (0.77, 1.43)
Melagatran	1.00 (0.93, 1.08)	0.94 (0.86, 1.03)
Diazepam + ximelagatran study		
Diazepam	0.99 (0.95, 1.04)	1.07 (0.92, 1.25)
N-Desmethyl-diazepam	0.96 (0.92, 1.01)	0.98 (0.92, 1.04)
Melagatran	0.99 (0.93, 1.06)	0.94 (0.83, 1.06)
Nifedipine + ximelagatran study		
Nifedipine	1.05 (0.94, 1.17)	ND
Melagatran	1.01 (0.97, 1.06)	1.04 (0.97, 1.10)

AUC = area under the concentration-time curve from zero to infinity; **C_{max}** = peak plasma concentration; **ND** = not determined.

melagatran. However, the anticoagulant effect of ximelagatran, measured in the present study as the activated partial thromboplastin time, an *ex vivo* coagulation time assay, was unchanged by diclofenac, and the effect of diclofenac on capillary bleeding time was not influenced by ximelagatran.[25] These findings suggest that ximelagatran can be safely coadministered with diclofenac. Diclofenac, at least in its enteric coated form, may not be optimal as a CYP2C9 probe in humans because of its highly variable intestinal absorption rate, although a different galenic form may be useful for quantifying CYP2C9 activity in humans, as was shown in a recent study by Morin et al.[26]

Diazepam is not an optimal probe for CYP2C19 inhibition, since diazepam is also metabolised by CYP3A4.[27] However, since concentrations of *N*-desmethyl-diazepam (which is formed by CYP2C19) were measured, any influence on CYP2C19 should be detected.

Nifedipine, which is primarily metabolised by CYP3A4,[24] is used in the treatment of hypertension and for the prevention and treatment of coronary heart disease. Thus, there is potential for concomitant use of nifedipine and ximelagatran in patients with cardiovascular disease. Therefore, the lack of interaction between these drugs is of great clinical importance.

Conclusion

The results of this study suggest that the oral direct thrombin inhibitor ximelagatran can be safely coadministered with diclofenac, diazepam and nifedipine in the clinical setting. Moreover, as ximelagatran does not exert a significant inhibitory effect on the CYP2C9, CYP2C19 and CYP3A4 hepatic isoenzymes responsible for the metabolism of diclofenac, diazepam and nifedipine, respectively, it is reasonable to expect that the CYP-mediated meta-

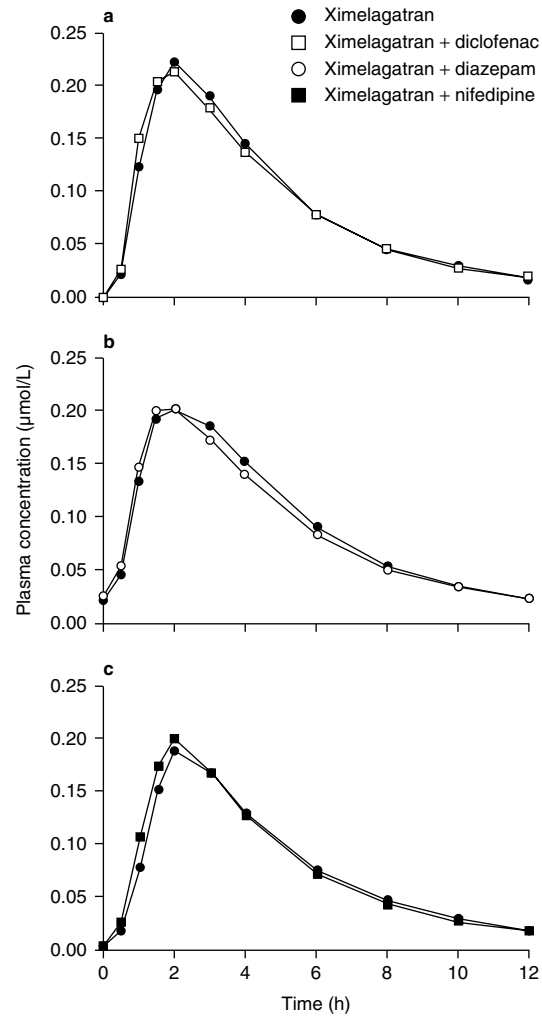

Fig. 6. Mean plasma concentration of melagatran versus time after administration of ximelagatran alone or in combination with (**a**) diclofenac, (**b**) diazepam and (**c**) nifedipine.

bolism of other drugs metabolised by these hepatic isoenzymes should also be unaffected. Furthermore, the pharmacokinetics of melagatran are not expected to be altered by inhibition or induction of CYP2C9, CYP2C19 or CYP3A4. Based on the results of the *in vitro* and *in vivo* studies, it could be concluded that metabolic drug-drug interactions involving the major human CYP enzymes should not be expected for ximelagatran.

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