Influence of Erythromycin on the Pharmacokinetics of Ximelagatran

May Involve Inhibition of P-glycoprotein-mediated Excretion*

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UGE, HD, JK, HF, K-JH, LO, TCS¹, UW and K-MS were all employees of AstraZeneca Ltd during this study.
Running Title

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Number of text pages: 25
Number of Figures: 4
Number of Tables: 5
Number of References: 29
Number of words in:

Abstract: 250
Introduction: 665
Discussion: 1,288

Non-standard abbreviations

ANOVA, analyses of variance;
APTT, activated partial thromboplatin time;
ATCC, American Type Culture Collection;
BMI, body mass index;
CI, confidence interval;
CYP450, cytochrome P450;
DMEM, Dulbecco’s Modified Eagle’s Medium;
HBSS, Hanks’ balanced salt solution
HEPES, N-2 hydroxyethyl-piperazine-N-2-ethanesulfonic acid;
HPLC, high pressure liquid chromatography;
IEC, Independent Ethics Committee;
LC-MS, liquid chromatography-mass spectrometry;
LOQ, limit of quantification;
MDCK, Madin-Darby canine kidney;
MDR1, multidrug resistance 1;
Oral DTI, oral direct thrombin inhibitor;
P-gp, P-glycoprotein;
TEER, transepithelial electrical resistance;
VTE, venous thromboembolic events;
Abstract

A pharmacokinetic interaction between erythromycin and ximelagatran, an oral direct thrombin inhibitor, was demonstrated in this study in healthy volunteers. To investigate possible interaction mechanisms, the effects of erythromycin on active transport mediated by P-glycoprotein (P-gp) in vitro in Caco-2 and P-gp overexpressing MDCK-MDR1 cell preparations and on biliary excretion of melagatran in rats were studied. In healthy volunteers (7 males and 9 females; mean age 24 years) receiving a single dose of ximelagatran 36 mg on Day 1, erythromycin 500 mg TID on Days 2–5, and a single dose of ximelagatran 36 mg plus erythromycin 500 mg on Day 6, the least-squares mean estimates (90% confidence intervals) for the ratio of ximelagatran with erythromycin to ximelagatran given alone were 1.82 (1.64–2.01) for the area under the concentration–time curve and 1.74 (1.52–2.00) for the maximum plasma concentration of melagatran, the active form of ximelagatran. Neither the slope nor the intercept of the melagatran plasma concentration–effect relationship for activated partial thromboplastin time (APTT) statistically significantly differed as a function of whether or not erythromycin was administered with ximelagatran. Ximelagatran was well tolerated regardless of whether it was administered with erythromycin. Erythromycin inhibited P-gp-mediated transport of both ximelagatran and melagatran in vitro and decreased the biliary excretion of melagatran in the rat. These results indicate that the mechanism of the pharmacokinetic interaction between oral ximelagatran and erythromycin may involve inhibition of transport proteins, possibly P-gp, resulting in decreased melagatran biliary excretion and increased bioavailability of melagatran.
Ximelagatran is an oral direct thrombin inhibitor (oral DTI) which is available for the prevention of venous thromboembolic events (VTE) in patients undergoing elective hip or knee replacement surgery and is being developed for the treatment of VTE, stroke prevention in patients with atrial fibrillation, and in the secondary prevention of cardiovascular events following myocardial infarction (Bergsrud and Gandhi, 2003; Eriksson H et al., 2003; Eriksson BI et al., 2003; Francis et al., 2003; Olsson et al., 2003; Schulman et al., 2003; Wallentin et al., 2003; Dahl et al., 2005). With anticoagulant efficacy comparable to that of warfarin – currently the primary option for oral anticoagulation – ximelagatran differs from warfarin: in having a predictable anticoagulant effect on coagulation assays that correlates closely with melagatran plasma concentrations; stable melagatran pharmacokinetics within and between patients; in lacking the requirement for coagulation monitoring; and lacking interactions with food, alcohol, and many commonly used medications (Bredberg et al., 2003; Eriksson UG et al., 2003a; Gustafsson and Elg, 2003; Johansson et al., 2003; Sarich et al., 2004a; Sarich et al., 2004b; Sarich et al., 2004c; Teng et al., 2004). On the basis of its efficacy and safety profiles, ximelagatran has been characterized as a possible alternative to warfarin which, although efficacious, is underutilized because of concerns about safety and tolerability (Boos and More, 2004; Donnan et al., 2004; Francis, 2004).

As most patients to be treated with oral ximelagatran will be taking other medications on an acute or chronic basis, its drug-interaction profile is being fully evaluated. Interactions involving cytochrome P450 (CYP450) metabolic pathways are not expected given that ximelagatran, its intermediate metabolites (ethyl-melagatran and hydroxy-melagatran), and melagatran (the predominant active form of ximelagatran) (Eriksson UG et al., 2003b) – are not substrates of, and do not inhibit any of, the major CYP450 isoenzymes (Bredberg et al., 2003). Consistent with
this expectation, studies in healthy volunteers have shown that co-administration of ximelagatran with amiodarone, atorvastatin, diazepam, diclofenac, digoxin, or nifedipine did not result in any relevant pharmacokinetic interactions for either of these drugs nor for melagatran (Bredberg et al., 2003; Gustafsson and Elg, 2003; Sarich et al., 2004b; Teng et al., 2004; Sarich et al., 2004c).

Drug effects on CYP450 metabolic pathways are thought to be a primary determinant of the majority of drug–drug interactions, whereas drug efflux mediated by the P-glycoprotein (P-gp) transporter is becoming increasingly recognized as an important contributor to pharmacokinetic interactions (Wacher et al., 1995; Yu, 1999; Dresser et al., 2000; Shang and Benet, 2001; Lin and Yamazaki, 2003). The P-gp transporter, which is widely distributed throughout the body in organs including the gut, brain, placenta, liver, and kidney, (Dresser et al., 2000; Lin and Yamazaki, 2003) prevents intracellular accumulation of drugs via active extrusion. Cytochrome P450 3A (CYP3A) is often co-localized intracellularly with P-gp, and substrates for, and inhibitors of, CYP3A often overlap with those of P-gp (Wacher et al., 1995; Yu, 1999; Dresser et al., 2000; Shang and Benet, 2001; Lin and Yamazaki, 2003). The clinical relevance of the CYP3A/P-gp overlaps in tissue distribution and substrate specificity is not yet established, but activity of the P-gp transporter can affect the disposition of drugs that also interact with CYP3A4 (Dresser et al., 2000; Zhang and Benet, 2001; Lin and Yamazaki, 2003).

The commonly used antibiotic erythromycin inhibits both CYP3A4 activity and P-gp transport (Schuetz et al., 1998; Zhang and Benet, 2001). This paper reports the results of studies conducted to elucidate the effect of erythromycin on the disposition of ximelagatran. In Study 1, the effects of erythromycin on melagatran pharmacokinetics and pharmacodynamics were assessed after
oral administration of ximelagatran to healthy volunteers. Coadministration of ximelagatran with erythromycin resulted in increased melagatran exposure with no influence on the melagatran plasma concentration–effect relationship for APTT. Studies 2 and 3 were conducted to identify possible mechanisms of the pharmacokinetic interaction observed in Study 1 by elucidating the effects of erythromycin on ximelagatran and melagatran P-gp-mediated transport in vitro and on biliary excretion of melagatran in rats in vivo.
Methods

Study 1: Erythromycin and Ximelagatran in Healthy Volunteers

Subjects

Healthy male and female volunteers aged 20–40 years with a body mass index (BMI) from 19–27 kg/m² were eligible. Reasons for exclusion included: any significant clinical illness within 2 weeks before the first dose of study medication; a history of bleeding or thrombotic disorder or of disease that might affect the rate and extent of absorption of study medication; use of prescription medicines except for contraceptives, nonsteroidal anti-inflammatory drugs, or aspirin within 2 weeks before the first dose of study medication; use of vitamins, herbals, minerals, or over-the-counter drugs except occasional acetaminophen within 1 week before the first dose of study medication; and the requirement for concomitant medication (except for contraceptives and occasional acetaminophen) through the duration of the study. In addition, females were excluded: if they were pregnant, lactating, or planning to become pregnant; if they were of childbearing potential but not using adequate birth control; or if they had insufficient iron depots as measured by plasma ferritin. All volunteers provided written informed consent.

Study Design and Treatments

The local Independent Ethics Committee (IEC) in Lund, Sweden, approved the protocol for this open-label, sequential study, which was conducted at a single centre in Sweden in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study comprised a screening visit occurring 14 or fewer days before the first administration of study medication, a 6-day treatment period during which volunteers who met eligibility criteria on the basis of
screening physical examinations and clinical laboratory tests received study medication and a follow-up visit occurring 2–7 days after the last dose of study medication. Treatment on Day 1 was ximelagatran 36 mg as a single oral dose at 8 AM. Treatment on Days 2–5 was erythromycin (Ery-Max®, AstraZeneca, Sweden) 500 mg three times daily (8 AM, 4 PM, and 12 midnight). Treatment on Day 6 was erythromycin 500 mg twice daily (8 AM and 4 PM) and ximelagatran 36 mg as a single oral dose with the morning dose of erythromycin. Medication was taken with meals. Volunteers remained in the clinic for 32 hours after the first dose of ximelagatran and began again before 4 PM on Treatment Day 5 through to 24 hours after the last ximelagatran dose.

Volunteers were instructed to fast and to abstain from drinking anything but water during the 3 hours before the screening and follow-up visits. Alcohol was prohibited during the 2 days before screening and from 2 days before the first administration of study medication through 2 days after the follow-up visit. Grapefruit and grapefruit juice were prohibited from the 3 days before the treatment period through to the end of the treatment period. Initiation of new physical training regimens and increases in the intensity of existing regimens were prohibited for the duration of the study.

**Pharmacokinetic Assessments**

Blood samples were taken from the forearm vein via an indwelling plastic cannula on Day 1 and Day 6 before dosing and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after the dose of ximelagatran for the determination of plasma concentrations of ximelagatran, melagatran, ethyl-melagatran, and hydroxy-melagatran. The latter two compounds are intermediary metabolites formed during
the bioconversion of ximelagatran to melagatran. Hydroxy-melagatran is formed by hydrolysis of the ethyl ester and ethyl-melagatran is formed by reduction of the hydroxyamidine in ximelagatran. These metabolites are subsequently converted to melagatran. Plasma concentrations were determined at DMPK & Bioanalytical Chemistry, AstraZeneca R&D Mölndal, Sweden, using liquid chromatography-mass spectrometry (LC-MS) with a limit of quantification (LOQ) of 0.010 µmol/L (Larsson et al., 2002). The plasma concentrations were adjusted by a factor of 1.185 to account for the dilution of blood with citrate buffer in the sampling tubes.

Pharmacokinetic parameters for melagatran included AUC, the area under the plasma concentration–time curve from time zero to infinity calculated using the log-linear trapezoidal rule to the last quantifiable plasma concentration (C_last) and then extrapolated to infinity by adding $C_{last}/\lambda$ ($\lambda$ = the elimination rate constant estimated from individual linear regression of the terminal part of the log concentration–time curve); $C_{max}$, the observed maximum plasma concentration; $t_{max}$, the time to $C_{max}$; and $t_{1/2}$, the elimination half-life, which was calculated as $\ln2/\lambda$. Actual sampling times were used to estimate pharmacokinetic parameters in a noncompartmental analysis performed with WinNonlin professional software (Pharsight Corporation, Mountain View, CA, USA). At time points before $C_{max}$, plasma concentrations below LOQ were taken as zero in the calculations. If more than one plasma concentration was below LOQ before $C_{max}$, the last one before the first quantifiable plasma concentration was calculated as LOQ/2.
Least-squares means and 95% confidence intervals (CI) were calculated for within-subjects data. Analyses of variance (ANOVA) with subject and treatment as factors were run on logarithmically transformed values of AUC and $C_{\text{max}}$ to establish 90% CI for the ratios between treatments. Least-squares estimates with 90% CI for mean ratio of with erythromycin (Day 6 data)/without erythromycin (Day 1 data) were calculated using the mean-square error from the ANOVA and quintiles from Student’s t-distribution. No clinically significant interaction was to be concluded if the 90% CI for the least-squares geometric mean treatment ratio of ximelagatran with erythromycin:ximelagatran without erythromycin fell within the intervals of 0.80 to 1.25 for AUC and 0.7 to 1.43 for $C_{\text{max}}$.

A sample size of 12 volunteers was estimated to provide 94% power to establish lack of a significant effect of erythromycin on melagatran AUC when ximelagatran tablets were administered with erythromycin versus without erythromycin.

**Pharmacodynamic Assessments**

Blood samples were taken predose and at 1, 2, 4, 6 and 10 hours after the ximelagatran dose on Day 1 and Day 6 for the determination of activated partial thromboplastin time (APTT) in plasma. The plasma samples were analysed using routine methods at the Laboratory of Clinical Chemistry, Lund University Hospital, Sweden. To evaluate a potential influence of erythromycin on the relationship between melagatran plasma concentration and APTT prolongation, a linear regression model with APTT ratio (i.e., prolongation of APTT relative to the predose value) as the dependent variable and the independent variables of erythromycin use, the square root of the melagatran plasma concentration, and the interaction between the two was fitted to investigate
the relationship between APTT and the plasma concentration of melagatran with erythromycin (Day 6 data) and without erythromycin (Day 1 data).

Safety Assessments
Adverse events, defined as any untoward medical occurrence developing or worsening after administration of study medication, were recorded from the first administration of study medication until the follow-up visit. All adverse events that volunteers reported spontaneously or in response to an open question, or that were revealed to the investigator by observation or clinical assessment were recorded regardless of their suspected cause. Adverse events were summarized descriptively, but no hypothesis testing was undertaken for these data. Likewise, results of standard clinical laboratory tests on blood and urine samples from the screening and follow-up visits were summarized descriptively.

Study 2: Pharmacokinetics and Biliary Excretion in Rats
The biliary excretion and pharmacokinetics of ximelagatran were investigated in anaesthetized male Sprague Dawley rats (SPRD, Harlan, The Netherlands). One group of four rats received a single intravenous dose of 18 µmol/kg [14C]-ximelagatran. A second group of four rats received erythromycin (Abboticin®, Abbott Scandinavia AB, Sweden) intraperitoneally (100 mg/kg) approximately one hour before intravenous administration of ximelagatran. The plasma concentration of ximelagatran, hydroxy-melagatran and melagatran were determined at DMPK & Bioanalytical Chemistry, AstraZeneca R&D Mölndal, Sweden, using the same method as described above for the human interaction study (Larsson et al., 2002). Concentrations of
ximelagatran, ethyl-melagatran, hydroxy-melagatran and melagatran were determined in plasma and in bile by liquid scintillation counting and high pressure liquid chromatography (HPLC) using mass-spectrometric and isotopic detection. Blood samples were collected from chronically implanted arterial cannula at 5, 15, 30, 60, 90, 120, 240 and 360 minutes after dosing. Bile was collected from a cannula implanted in the bile duct and samples were obtained in two time intervals from 0 to 4 and 4 to 6 hours after dosing.

**Study 3: In Vitro Transport in Caco-2 and MDCK cells**

*In vitro* experiments were done in Caco-2 and Madin-Darby Canine Kidney (MDCK) cell preparations to investigate the possible role of P-gp-mediated active transport of ximelagatran, melagatran, ethyl-melagatran, and hydroxy-melagatran.

**Chemicals**

Ximelagatran and [14C]-ximelagatran (2 GBq/mmol) were synthesized at AstraZeneca R&D Mölndal (Mölndal, Sweden). [3H]-digoxin (1369 GBq/mmol) was purchased from NEN Life Science Products Inc. (Boston, MA, USA). Erythromycin, verapamil and quinidine were obtained from Sigma-Aldrich Co. (Stockholm, Sweden). Cell culture media and supplements, as well as Hanks’ Balanced Salt Solution (HBSS) w/o phenol red and N-2hydroxyethyl-piperazine-N-2-ethanesulfonic acid (HEPES) were purchased from Gibco BRL, Life Technologies (Paisley, Scotland).
Cell cultures

The Caco-2 cell line was obtained from the ATCC at passage 18 (American Type Culture Collection, Rockville, MD, USA). The MDCK-wild-type cells (MDCK-WT) and MDCK cells transfected with human multidrug resistance-1 cDNA and stably expressing human MDR1 gene product P-glycoprotein (MDCK-MDR1), were obtained from The Netherlands Cancer Institute (Amsterdam, The Netherlands). Both cell lines were maintained at 37°C in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 9% heat-inactivated foetal bovine serum, 7 mM L-glutamine and 1% non-essential amino acids in an atmosphere of 95% air and 5% CO₂. The culture medium was replaced every second day (except for weekends) and split once a week. For transport studies, Caco-2 and MDCK cells were seeded onto 12 mm (1.13 cm² surface area, 0.4 μM pore size) Transwell® polycarbonate cell culture inserts (Corning Costar® Corporation, Cambridge, MA, USA). The Caco-2 cells were seeded at a density of 250 000 cells/filter insert and the MDCK cells at a density of 150 000 cells/filter insert. The culture medium was changed every other day until use of the Caco-2 cells 15–24 days after seeding and MDCK cells 3 days after seeding. Caco-2 cells used in this study were at passages 32–39 and the MDCK cells were used at an in-house passage number of 11. The integrity of the cell monolayers was checked by measuring the transepithelial electrical resistance (TEER) before and after the transport experiments. TEER was measured in Ω cm², at 37°C, using a WPI EVOM volt-ohmmeter fitted with STX2 chopstick electrodes (World Precision Instruments, Sarasota, FL, USA). The Caco-2, MDCK-WT and MDCK-MDR1 cell monolayers used in the present study had TEER values of approximately 300 Ω cm², 100 Ω cm² and 80 Ω cm², respectively. The TEER values of the cell monolayers were well maintained in the control cells and in the presence of the test compounds,
indicating that the integrity of the cell monolayers were not compromised at the concentrations of test compounds used.

Transport studies

All transport experiments were performed manually in 12-well plates (1.13 cm² filters) on a thermostatic shaker calibrated at 37°C and a stirring of 450 rpm (THERMOstar; BMG LABTECH, Durham, NC). Experiments were carried out in transport medium consisting of HBSS buffered to pH 7.4 with 25 mM HEPES. Drug transport was studied both in the apical-to-basolateral (A-to-B) and the basolateral-to-apical (B-to-A) directions in the absence or presence of inhibitor. Digoxin was used as a prototypical substrate for P-gp-mediated transport and to confirm the functional expression of P-gp in the test systems. Quinidine and verapamil were used as potent control inhibitors of P-gp. Before the start of the experiments, the cell monolayers were washed by replacing the culture medium with preheated (37°C) HBSS and then equilibrated at 37°C for approximately 15 minutes. The transport experiment was started by adding HBSS solution containing the test compound (ximelagatran or digoxin) to either the apical (520 µL) or the basolateral side (1,520 µL) of the monolayers and drug-free HBSS to the opposite side (1,500 or 500 µL), depending on the transport direction to be studied. Ximelagatran was added at 50 µM, [14C]-ximelagatran at 5 µM (0.28 µCi/ml) and digoxin at 27 nM (1 µCi/ml). For investigation of the inhibitory effect of erythromycin (10–500 µM), quinidine (1–100 µM) or verapamil (1–100 µM) on the transport of ximelagatran, the compounds were included in the transport medium at equal concentrations on the apical and basolateral side of the cell monolayers. Immediately after the start of the experiment a sample was removed from the donor compartment (20 µL) and subsequently samples were removed from the donor (20 µL) and
receiver (200–400 µL) compartment at predetermined time points up to 180 minutes. The sample volume removed from the receiver side was replaced with prewarmed (37°C) HBSS or HBSS containing appropriate concentration of inhibitor. The concentrations of ximelagatran, ethylmelagatran, hydroxy-melagatran and melagatran in samples removed from the donor and receiver compartment were determined at DMPK & Bioanalytical Chemistry, AstraZeneca R&D Mölndal, Sweden, using LC-MS with an LOQ of 0.020 µmol/L for all four analytes. Samples containing [³H]-digoxin and [¹⁴C]-ximelagatran were analysed by liquid scintillation counting.

Data analysis

The permeability coefficient (P_app) of the test compounds was calculated as (dQ/dt)* [1/(A*C_d)], where dQ/dt is the linear rate of appearance of drug in the receiver compartment, A is the surface area of the filter and C_d is the average drug concentration in the donor compartment over the time period which dQ/dt was determined. P_app is given in cm/s. The transporter-mediated net efflux of ximelagatran in the Caco-2 cell monolayers was obtained by subtracting the P_app value in the A-to-B direction from the P_app value in the B-to-A direction. A significantly higher P_app in the B-to-A direction than in the A- to-B direction, i.e. positive net efflux or high efflux ratio (P_app in B- to-A:P_app in A-to-B), is interpreted to indicate that the compound is a substrate for transport by efflux pumps such as P-gp. The decrease in net efflux transport of radiolabelled ximelagatran (measured as total radioactivity) in the presence of increasing concentrations of the putative inhibitors (erythromycin, quinidine and verapamil) was determined to assess their relative potency and to calculate 50% inhibitory concentrations (IC₅₀ values). An inhibitory concentration–effect model was fit to the net efflux data using nonlinear regression and the following equation: Net efflux = T_max * (1 – [Conc.⁹/(IC₅₀⁹ + Conc.⁹)], where T_max = net efflux
without inhibitor, Conc. = inhibitor concentration, IC_{50} = concentration of inhibitor resulting in 50\% of maximum inhibition and \gamma = sigmoidicity factor that determines the slope of the relationship. The nonlinear regression analysis was performed with WinNonlin professional software (Pharsight Corporation, Mountain View, CA, USA).
Results

Study 1: Erythromycin and Ximelagatran in Healthy Volunteers

Subjects

The number of volunteers randomized to treatment was 16 (7 men and 9 women; all white; mean ± SD age, weight and BMI of 24 ± 3.3 years, 69 ± 8.5 kg and 23±1.8 kg/m², respectively). One volunteer prematurely withdrew from the study because of adverse events described in the Safety and Tolerability section below. Data from the 15 volunteers who completed the study were included in the pharmacokinetic and pharmacodynamic analyses, and data from all 16 volunteers who were randomized to treatment were included in the safety and tolerability analyses.

Pharmacokinetics

Plasma concentrations of melagatran, ximelagatran, and the two intermediates ethyl-melagatran and hydroxy-melagatran were higher after administration of ximelagatran in combination with erythromycin (Day 6) compared with administration of ximelagatran alone (Day 1) (Figure 1). The increases were comparable for the two intermediates and melagatran, while a smaller increase was observed for ximelagatran The increases of both melagatran AUC and C_{max} suggests a clinically relevant interaction as the 90% CI and least-squares mean estimates for the ratio of ximelagatran with erythromycin:ximelagatran without erythromycin were higher than the predefined ranges for demonstrating no interaction (Table 1). There was no relevant change of t_{max} or t_{1/2} of melagatran following administration of ximelagatran with erythromycin compared with that observed when ximelagatran was given alone (Table 1).
Pharmacodynamics

A curvilinear relationship between APTT and plasma melagatran concentration was observed regardless of whether ximelagatran was administered with or without erythromycin (Figure 2). Neither the slope nor the intercept of the melagatran–APTT relationship statistically significantly differed (95% CI of the estimated differences included zero) as a function of whether or not erythromycin was administered with ximelagatran (Table 2). Because of the curvilinear concentration–effect relationship for APTT, only modestly higher APTT values were observed with ximelagatran administered with erythromycin (Day 6) than with ximelagatran administered without erythromycin (Day 1) (Figure 2). The erythromycin-associated elevation in plasma melagatran concentration increased the median (range) of the observed maximum APTT from 42 (35-54) seconds to 48 (35-52) seconds. The median (range) of the individual ratios for the maximum APTT observed after ximelagatran with erythromycin:ximelagatran without erythromycin was 1.12 (0.95-1.20), suggesting a consistent minor increase in the studied volunteers.

Safety and Tolerability

No serious adverse events or side effects of bleeding were reported. During treatment with ximelagatran alone (Day 1), the only adverse events were dysphonia (n = 1) and dysmenorrhoea (n = 1). Most of the adverse events occurred during treatment with erythromycin alone (Days 2–5), when gastrointestinal adverse events such as nausea (n = 10), abdominal pain (n = 4) and dyspepsia (n = 3) were the most common. During treatment with ximelagatran and erythromycin (Day 6), nausea, headache, pharyngitis, respiratory infection, and fungal infection were each reported in one patient. One female participant prematurely withdrew from the study on Day 3.
during erythromycin therapy because of chest pain, nausea and vomiting. Causal relationship with study drug(s) were not recorded by the investigator for non-serious adverse events. No clinically significant changes in results of clinical laboratory tests were observed during the study.

**Study 2: Pharmacokinetics and Biliary Excretion in Rats**

Ximelagatran was rapidly bioconverted and not detected in plasma. Hydroxy-melagatran, formed by esterhydrolysis of ximelagatran, achieved maximum plasma concentrations of about 25 µmol/L at 5 minutes after ximelagatran administration and was rapidly eliminated with no apparent changes in the concentration profile when erythromycin was given. Melagatran was rapidly formed and increased to mean plasma concentrations of 2 µmol/L and 7 µmol/L at 30 minutes after administration of ximelagatran alone and when combined with erythromycin, respectively. Melagatran concentrations remained approximately constant at these levels during the 6-hour sampling period. Bile was obtained during the 6-hour collection period for 3 of the 4 rats in each group. There was no change in the volume of bile (mean [SD]) collected during the 6-hour period after administration of ximelagatran alone (4.26 [0.21] mL) and combined with erythromycin (4.29 [0.31] mL). The mean (SD) cumulative excretion of total radioactivity in the bile accounted for 33.6% (4.9) and 14.4% (1.3) of the dose after administration of ximelagatran alone and when combined with erythromycin, respectively. Melagatran accounted for more than 90% of the radioactivity in bile for erythromycin-treated rats as well as for the rats receiving ximelagatran alone. Low amounts of hydroxy-melagatran but not the administered ximelagatran were observed in bile. Further, trace amounts were also detected of ethyl-melagatran. The metabolite profiles of the bile were similar between the groups.
Study 3: In Vitro Transport in Caco-2 and MDCK cells

Transport of ximelagatran in MDCK-MDR1 cells

In MDCK-MDR1 cells, transport of ximelagatran in the secretory basolateral-to-apical (B-to-A) direction was 33-fold greater than in the absorptive apical-to-basolateral (A-to-B) direction, and this efflux ratio decreased to 2.7 in the MDCK-WT cells (Table 3). The efflux ratio of ximelagatran was essentially abolished in the presence of quinidine (100 µM) or erythromycin (500 µM), which was caused by both a reduction of B-to-A transport and an increase of A-to-B transport. The greater efflux of ximelagatran in the P-gp overexpressing MDCK-MDR1 cells than in non-transfected MDCK-WT cells, and the inhibition effect by quinidine and erythromycin indicate that ximelagatran is a P-gp substrate. Bioconversion of ximelagatran and formation of its intermediates and melagatran was minor in the MDCK cells. The efflux ratio for the reference P-gp substrate digoxin was 19 in the MDCK-MDR1 cells, and this efflux ratio was decreased to 4.1 in the untransfected MDCK-WT cells (Table 3). The observed efflux in the MDCK-WT is due to the activity of endogenous canine P-gp in these cells.

Transport and bioconversion of ximelagatran in Caco-2 cell monolayers

In Caco-2 cells, the transport of ximelagatran was found to be 34-fold greater in the B-to-A direction than in the A-to-B direction, indicating active efflux of the compound (Table 4). This efflux ratio was reduced almost to unity in the presence of quinidine (100 µM) or erythromycin (500 µM), consistent with the results in the MDCK-MDR1 cells indicating that ximelagatran is a P-gp substrate. The P_app values for the total appearance rate of ximelagatran plus the intermediates (ethyl-melagatran, hydroxy-melagatran) and melagatran on the receiver side were
more than 2-fold higher compared with ximelagatran alone (Table 4). An efflux ratio of 23 was obtained for the total transport of ximelagatran plus intermediates and metabolites in the Caco-2 cells, and this efflux ratio decreased close to unity in the presence of quinidine or erythromycin, indicating that efflux transport for the formed intermediates and melagatran was also inhibited.

Ximelagatran was bioconverted into hydroxy-melagatran and melagatran during passage across the Caco-2 cell monolayers. Low levels of ethyl-melagatran could also be detected. Incubation of ximelagatran with transport buffer alone that had been exposed to the surface of Caco-2 cells did not produce any significant amounts of ximelagatran intermediates or melagatran (<3% of the dose in 3 h, data not shown), indicating that bioconversion occurred inside the cells. The fraction of ximelagatran bioconverted to intermediates and melagatran over 3 h of incubation was approximately 30% of the dose after an apical dose (25 nmol/0.5 mL) and approximately 20% of the dose after a basolateral dose (75 nmol/1.5 mL). The rate of appearance of hydroxy-melagatran and melagatran (following addition of ximelagatran) were greater in the B-to-A direction than in the A-to-B direction, indicating active net efflux transport across the apical membrane (Figure 3). The rate of appearance of hydroxy-melagatran and melagatran in the apical compartment following basolateral dosing of ximelagatran were significantly decreased in the presence of quinidine or erythromycin (Figure 3). These data suggest that substrates for efflux by P-gp may include intracellularly formed hydroxy-melagatran and melagatran, as well as ximelagatran.

Inhibition of efflux transport in Caco-2 cells by erythromycin, quinidine and verapamil
The concentration-dependent inhibitory effect of erythromycin, quinidine and verapamil on the A-to-B and B-to-A transport of ximelagatran across the Caco-2 cells was further characterized using radiolabelled ximelagatran. The transporter-mediated net efflux was obtained by subtracting the A-to-B transport from the B-to-A transport. Erythromycin, quinidine and verapamil all inhibited the net efflux transport of radioactive substances (ximelagatran plus ethyl-melagatran, hydroxy-melagatran and melagatran) added as [14C]-ximelagatran in the Caco-2 monolayers in a concentration-dependent manner (Figure 4). Erythromycin, quinidine and verapamil inhibited the net efflux transport in the Caco-2 monolayers with IC50 values of 119, 1.8 and 1.7 µM, respectively (Table 5).
Discussion

The results of this study in healthy volunteers reveal a pharmacokinetic interaction between the oral DTI ximelagatran and erythromycin, probably via a P-gp-mediated mechanism. Plasma concentrations of melagatran, and ethyl- and hydroxy-melagatran – the latter two being intermediates bioconverted to melagatran – were all increased with concomitant erythromycin administration. Melagatran AUC and C_{max} were increased by 82% and 74%, respectively, when ximelagatran was administered concurrently with erythromycin compared with when ximelagatran was administered alone. No relevant changes were observed for melagatran t_{max} and t_{1/2}. These findings suggest that erythromycin increases the bioavailability of melagatran but has no influence on the elimination of systemic melagatran, which occurs mainly by renal excretion (Eriksson UG et al., 2003b).

Single oral doses of 36 mg ximelagatran were given on two occasions to each subject, first alone and then together with erythromycin following a 4-day period of erythromycin treatment. Treatments were administered sequentially rather than in crossover fashion to avoid a possible carryover effect arising from the impact erythromycin has on gastrointestinal motility and gut bacteria, both of which can be disrupted for several weeks after a course of treatment. The clinically documented oral ximelagatran doses are 24 mg and 36 mg given twice daily (Bergsrud and Gandhi, 2003; Olsson et al., 2003). There are no time dependent pharmacokinetic changes and a low degree of accumulation with twice daily dosing of ximelagatran because the half-life of melagatran is 4-5 hours (Wolzt et al., 2003). Ximelagatran and the intermediary metabolites are rapidly eliminated and show no accumulation. Consequently, the plasma concentrations of ximelagatran, intermediary metabolites and melagatran following a single dose of ximelagatran...
are similar to that achieved after repeated twice daily dosing. The main objective of the present study was to determine changes in the pharmacokinetics of ximelagatran as a result of the co-administration with erythromycin, which was given according to a therapeutically used dose regimen.

Ximelagatran plasma concentrations appeared to be essentially unchanged suggesting that erythromycin had no influence on the rapid absorption and bioconversion of ximelagatran (Eriksson UG et al., 2003a). Inhibition of ximelagatran bioconversion is not a likely explanation for the effect of erythromycin as this would result in a decrease in plasma concentrations of melagatran and the two intermediary substances. Previous research also supports that erythromycin inhibition of CYP3A4-mediated drug metabolism does not explain the ximelagatran–erythromycin interaction. *In vitro* studies in human liver microsomes showed that ximelagatran and its intermediates did not inhibit, and were not substrates of, CYP3A4 (Bredberg et al., 2003). Furthermore, in studies in healthy volunteers, the pharmacokinetics of the CYP3A4 substrates diazepam and nifedipine were not influenced by the co-administration of ximelagatran (Bredberg et al., 2003).

In addition to inhibiting CYP3A4 activity, erythromycin has also been shown to inhibit P-gp-mediated drug efflux, which is increasingly recognized as an important contributor to pharmacokinetic interactions (Schuetz et al., 1998; Zhang and Benet, 2001; Lin and Yamazaki, 2003). P-gp is highly expressed on the canalicular membrane of hepatocytes and has been reported to be involved in the biliary excretion of many drugs (Lin and Yamazaki, 2003). Previous studies in rats, dogs and humans show an extensive first-pass metabolism of
ximelagatran with subsequent biliary excretion of some of the formed intermediates and melagatran (Eriksson et al., 2003b). The present study in rats demonstrated that erythromycin increased melagatran plasma concentrations, which is similar to the result of the study in humans, and inhibited the biliary excretion of melagatran while the bile flow and the metabolic pattern of bile was unchanged. These results are consistent with an inhibitory effect of erythromycin on the biliary excretion of melagatran and intermediates that may be mediated by P-gp.

The in vitro studies in MDCK and Caco-2 cell monolayers indicate that ximelagatran, and possibly also melagatran and hydroxy-melagatran are substrates for P-gp. Active efflux transport was observed for ximelagatran, intermediates and melagatran in the Caco-2 cells. In the MDCK-MDR1 cells, containing overexpressed P-gp, the transport of ximelagatran was 33-fold greater in the basolateral-to-apical direction than in the apical-to-basolateral direction, and this decreased to 2.7 in the wild-type MDCK cells. Erythromycin and quinidine, which are known inhibitors of P-gp-mediated active transport, inhibited the efflux of ximelagatran. Bioconversion of ximelagatran in MDCK cells was minor and it was therefore not possible to determine if active transport also occurs for the intermediates and melagatran following intracellular formation. As melagatran and the intermediary substances are polar molecules and the cellular uptake is limited by low membrane permeability, it was not possible to directly study the active secretion of these compounds. In the Caco-2 cells, ximelagatran was bioconverted to hydroxy-melagatran and melagatran while only low levels of ethyl-melagatran were detected. As for ximelagatran, the intermediates were preferentially excreted into the apical compartment and the rate of appearance of both hydroxy-melagatran and melagatran in the apical compartment were
significantly decreased in the presence of erythromycin and quinidine. These findings suggest that ximelagatran, and possibly also hydroxy-melagatran and melagatran are substrates for P-gp. However, as the Caco-2 cells have an array of transporters functionally expressed in their apical and basolateral membranes, the inhibitory effects of quinidine and erythromycin may also involve inhibition of other transporters than P-gp. Nevertheless, complete inhibition of total net efflux transport of ximelagatran, intermediates and melagatran was observed for erythromycin, quinidine and verapamil. Lower inhibitory potency was observed for erythromycin compared with quinidine and verapamil. This is consistent with previously published data for these compounds on their relative inhibitory potency of P-gp-mediated transport (Kim et al., 1999).

The pharmacokinetic interaction between ximelagatran and erythromycin, an inhibitor of the P-gp transporter, should not be extrapolated to predict similar interactions with all P-gp inhibitors. For example, neither amiodarone nor nifedipine, both of which are P-gp pump inhibitors (Zhang and Benet, 2001), had any relevant influence on melagatran pharmacokinetics in prospective interaction studies in healthy volunteers receiving oral ximelagatran (Bredberg et al., 2003; Teng et al., 2004). Likewise, the P-gp pump substrates digoxin (Zhang and Benet, 2001) and atorvastatin were also demonstrated not to interact with ximelagatran (Sarich et al., 2004c). Importantly, there was no relevant influence of ximelagatran on the pharmacokinetics of digoxin or atorvastatin. These findings suggest that at the studied dose of 36 mg given twice daily to healthy volunteers, ximelagatran does not inhibit the P-gp-mediated transport of digoxin or atorvastatin.
Co-administration of erythromycin and ximelagatran relative to administration of ximelagatran alone did not have a statistically significant effect on the relationship between APTT prolongation and melagatran plasma concentration in this study. Only a slight effect of erythromycin on melagatran pharmacodynamics was shown by the finding that erythromycin-associated elevation in plasma melagatran concentrations increased the maximum APTT by up to 20%.

Ximelagatran was well tolerated in this study regardless of whether it was administered with or without erythromycin. No serious adverse events or adverse events of bleeding were reported. Ximelagatran was not associated with any clinically relevant adverse events, a finding that corroborates previous studies showing ximelagatran to be well tolerated in both healthy volunteers and patients across a range of doses (Eriksson H et al., 2003; Eriksson BI et al., 2003; Francis et al., 2003; Olsson et al., 2003; Schulman et al., 2003; Wallentin et al., 2003; Bredberg et al., 2003; Eriksson UG et al., 2003a; Gustafsson and Elg, 2003; Johansson et al., 2003; Sarich et al., 2004a; Sarich et al., 2004b; Sarich et al., 2004c; Teng et al., 2004; Dahl et al., 2005).

Erythromycin was associated with gastrointestinal adverse events such as nausea and abdominal pain, a result consistent with previous findings (Blondeau, 2002).

In conclusion, the results of this study demonstrate an increased bioavailability of melagatran after oral administration of the oral DTI ximelagatran together with erythromycin. This pharmacokinetic interaction may be explained by erythromycin’s inhibition of P-gp-mediated transport resulting in inhibition of melagatran biliary excretion.
Acknowledgements

The authors would like to acknowledge the assistance of Jane Saiers in the writing and preparation of this manuscript.
References


Footnotes

* Financial support for this study was provided by AstraZeneca Pharmaceuticals, Mölndal, Sweden. Requests for reprints should be sent to Ulf G. Eriksson, AstraZeneca R&D’ Mölndal, S-431, 83 Mölndal, Sweden.

Some of the data described in this manuscript were presented in poster form at the Annual Meeting of the American Society of Clinical Pharmacology and Therapeutics (ASCPT) 2004 Congress, 24–27 March, Miami Beach FA, USA and at the 18th International Congress on Thrombosis, 20–24 June 2004, Ljubljana, Slovenia.

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1 TCS is now employed by Johnson & Johnson Pharmaceutical Research and Development
Figure Legends

Figure 1. Mean plasma concentrations of ximelagatran (A), melagatran (B), ethyl-melagatran (C), and hydroxy-melagatran (D) versus time after oral administration of the ximelagatran without erythromycin (Day 1) or with erythromycin (Day 6) (n = 15). The error bars show the SD.

Figure 2. APTT prolongations (relative to predose) versus plasma concentration of melagatran (A) and median APTT values (bars show the range of minimum and maximum) versus time (B) after oral administration of ximelagatran without erythromycin (Day 1) versus ximelagatran with erythromycin (Day 6). Regression lines for each treatment were fitted to values of APTT ratio and the square root of the plasma concentration of melagatran. $R^2 = 0.61$.

Figure 3. Effect of erythromycin and quinidine on the rate of appearance of ximelagatran (A), hydroxy-melagatran (B) and melagatran (C) on the opposite receiver side following addition of ximelagatran (50 µM) on the apical or basolateral side of Caco-2 monolayers.

Figure 4. Inhibitory effect of erythromycin, quinidine and verapamil on the net efflux transport of ximelagatran and metabolites in Caco-2 cells.
Table 1. Pharmacokinetic parameters of melagatran after oral administration of ximelagatran alone (Day 1) and in combination with erythromycin (Day 6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ximelagatran Alone (n = 15)</th>
<th>Ximelagatran + Erythromycin (n = 15)</th>
<th>Treatment Comparison Day 6/Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µmol·h/L)</td>
<td>Mean (SD) 1.52 (0.42)</td>
<td>Mean (SD) 2.76 (0.81)</td>
<td>1.82 (1.64, 2.01) a</td>
</tr>
<tr>
<td></td>
<td>Median (range) 1.39 (0.975-2.35)</td>
<td>Median (range) 2.51 (1.66-4.89)</td>
<td>1.93 (1.02, 2.42)</td>
</tr>
<tr>
<td></td>
<td>Cmax (µmol/L)</td>
<td>Mean (SD) 0.25 (0.06)</td>
<td>Mean (SD) 0.44 (0.11)</td>
</tr>
<tr>
<td></td>
<td>Median (range) 0.25 (0.15-0.35)</td>
<td>Median (range) 0.47 (0.22-0.69)</td>
<td>1.85 (0.81, 2.42)</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>Mean (SD) 2.2 (0.7)</td>
<td>Mean (SD) 2.5 (0.7)</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>Median (range) 2.0 (1.5-3.0)</td>
<td>Median (range) 3.0 (1.5-4.0)</td>
<td>1.0 (0.5, 2.0)</td>
</tr>
<tr>
<td>ts (h)</td>
<td>Mean (SD) 3.2 (0.3)</td>
<td>Mean (SD) 3.0 (0.4)</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>Median (range) 3.1 (2.9-4.0)</td>
<td>Median (range) 2.9 (2.5-3.7)</td>
<td>0.9 (0.8, 1.2)</td>
</tr>
</tbody>
</table>

a Least squares mean estimates (90% CI) for between-treatment comparison (Day 6/Day 1). NE not estimated.
Table 2. Parameter estimates (95% CI) for the regression lines associating APTT ratio with the square root of the plasma concentration of melagatran after oral administration of ximelagatran with erythromycin or without erythromycin (n = 15).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ximelagatran with erythromycin (n = 15)</th>
<th>Ximelagatran without erythromycin (n = 15)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.00 (0.84, 1.15)</td>
<td>0.97 (0.77, 1.17)</td>
<td>0.03 (–0.22, 0.27)</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.82 (0.73, 0.90)</td>
<td>0.82 (0.74, 0.91)</td>
<td>–0.01 (–0.13, 0.11)</td>
</tr>
</tbody>
</table>
Table 3. Transport of ximelagatran and digoxin across MDCK-WT and MDCK-MDR1 cell monolayers in the absence and presence of quinidine or erythromycin.

Values are mean and SD (n = 3) or the values for two replicates.

<table>
<thead>
<tr>
<th>Condition</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; A-B&lt;sup&gt;a&lt;/sup&gt; (x 10&lt;sup&gt;-6&lt;/sup&gt; cm/s)</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; B-A&lt;sup&gt;b&lt;/sup&gt; (x 10&lt;sup&gt;-6&lt;/sup&gt; cm/s)</th>
<th>Efflux ratio&lt;sup&gt;c&lt;/sup&gt; (P&lt;sub&gt;app&lt;/sub&gt; B-A / P&lt;sub&gt;app&lt;/sub&gt; A-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDCK-MDR1 cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ximelagatran alone (50 µM)</td>
<td>1.2 (0.2)</td>
<td>39 (3.7)</td>
<td>33</td>
</tr>
<tr>
<td>+ quinidine (100 µM)</td>
<td>4.9 (0.2)</td>
<td>7.0 (0.6)</td>
<td>1.4</td>
</tr>
<tr>
<td>+ erythromycin (500 µM)</td>
<td>5.3 (0.1)</td>
<td>12 (1.0)</td>
<td>2.3</td>
</tr>
<tr>
<td>[³H]-digoxin (27 nM)</td>
<td>2.0 (0.2)</td>
<td>37 (0.7)</td>
<td>19</td>
</tr>
<tr>
<td><strong>MDCK-WT cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ximelagatran alone (50 µM)</td>
<td>5.9, 5.2</td>
<td>15 (1.5)</td>
<td>2.7</td>
</tr>
<tr>
<td>+ quinidine (100 µM)</td>
<td>6.7 (1.7)</td>
<td>5.9 (0.3)</td>
<td>0.9</td>
</tr>
<tr>
<td>+ erythromycin (500 µM)</td>
<td>7.6 (1.7)</td>
<td>9.9 (3.7)</td>
<td>1.3</td>
</tr>
<tr>
<td>[³H]-digoxin (27 nM)</td>
<td>5.8 (0.8)</td>
<td>24 (9)</td>
<td>4.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Permeability in apical-to-basolateral direction.

<sup>b</sup> Permeability in basolateral-to-apical direction.

<sup>c</sup> Efflux ratio = (P<sub>app</sub> B-A)/(P<sub>app</sub> A-B).
Table 4. Transport of ximelagatran across Caco-2 cell monolayers in the absence and presence of quinidine or erythromycin.

Values are mean and SD (n = 3) or the values for two replicates.

<table>
<thead>
<tr>
<th>Condition</th>
<th>$P_{app, A-B}^a$ (x 10^{-6} cm/s)</th>
<th>$P_{app, B-A}^b$ (x 10^{-6} cm/s)</th>
<th>Efflux ratio$^c$ ($P_{app, B-A}/P_{app, A-B}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ximelagatran alone (50 µM)</td>
<td>0.57 (0.06)</td>
<td>19 (1.2)</td>
<td>34</td>
</tr>
<tr>
<td>+ quinidine (100 µM)</td>
<td>2.9 (0.3)</td>
<td>3.4, 3.3</td>
<td>1.2</td>
</tr>
<tr>
<td>+ erythromycin (100 µM)</td>
<td>1.4 (0.07)</td>
<td>16 (0.1)</td>
<td>11</td>
</tr>
<tr>
<td>+ erythromycin (500 µM)</td>
<td>6.4 (0.4)</td>
<td>7.9 (0.5)</td>
<td>1.2</td>
</tr>
<tr>
<td>X and M alone (50 µM)$^d$</td>
<td>2.0 (0.2)</td>
<td>45 (1.6)</td>
<td>23</td>
</tr>
<tr>
<td>+ quinidine (100 µM)</td>
<td>7.6 (0.3)</td>
<td>7.6, 7.5</td>
<td>1.0</td>
</tr>
<tr>
<td>+ erythromycin (100 µM)</td>
<td>3.3 (0.1)</td>
<td>38 (1.2)</td>
<td>12</td>
</tr>
<tr>
<td>+ erythromycin (500 µM)</td>
<td>8.3 (0.4)</td>
<td>13 (0.9)</td>
<td>1.6</td>
</tr>
<tr>
<td>$[^3]$H]-digoxin (27 nM)</td>
<td>1.5 (0.07)</td>
<td>39 (0.8)</td>
<td>26</td>
</tr>
<tr>
<td>+ verapamil (100 µM)</td>
<td>12 (0.9)</td>
<td>12 (0.5)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$^a$ Permeability in apical-to-basolateral direction.

$^b$ Permeability in basolateral-to-apical direction.

$^c$ Efflux ratio = ($P_{app, B-A}$)/($P_{app, A-B}$).

$^d$ X and M = total permeability for appearance of ximelagatran plus ethyl-melagatran, hydroxy-melagatran and melagatran on receiver side.
Table 5. Model parameters generated by fitting an inhibitory concentration–response model by nonlinear regression.

Parameters generated using the equation: Net efflux = $T_{\text{max}} \times (1 - \frac{\text{Conc.} \gamma}{IC_{50} \gamma + \text{Conc.}})$,

where $T_{\text{max}}$ = net efflux without inhibitor (x 10-6 cm/s), Conc. = inhibitor concentration (µM), IC50 = concentration of inhibitor resulting in 50% of maximum inhibition (µM), and $\gamma$ = sigmoidicity factor that determines the slope of the relationship. The standard errors (SEs) are estimates of the precision of the parameter estimates.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>$T_{\text{max}}$</td>
<td>41.3</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>IC50</td>
<td>119</td>
<td>6.66</td>
</tr>
<tr>
<td></td>
<td>$\gamma$</td>
<td>2.08</td>
<td>0.23</td>
</tr>
<tr>
<td>Quinidine</td>
<td>$T_{\text{max}}$</td>
<td>32.2</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>IC50</td>
<td>1.84</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>$\gamma$</td>
<td>1.02</td>
<td>0.10</td>
</tr>
<tr>
<td>Verapamil</td>
<td>$T_{\text{max}}$</td>
<td>33.6</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>IC50</td>
<td>1.74</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>$\gamma$</td>
<td>0.68</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Figure 1

1A. Ximelagatran

1B. Melagatran

1C. Ethyl-melagatran

1D. Hydroxy-melagatran

Plasma concentration (μmol/L) vs. Time (h)
Figure 4

The figure shows a graph plotting the net efflux (in cm/s) against the inhibitor concentration (in μM). The x-axis represents the inhibitor concentration ranging from 0.01 to 1000 μM, while the y-axis represents the net efflux ranging from -2 to 46 × 10^{-6} cm/s.

Data points for three different inhibitors are plotted: Erythromycin (○), Quinidine (△), and Verapamil (□). The data points are accompanied by error bars indicating the variability in the measurements.

The graph includes two curves: a dotted line for Erythromycin and a solid line for a combination of Quinidine and Verapamil. The curves demonstrate a decrease in net efflux as the inhibitor concentration increases.