INFLUENCE OF ERYTHROMYCIN ON THE PHARMACOKINETICS OF XIMELAGATRAN MAY INVOLVE INHIBITION OF P-GLYCOPEPTIDE-MEDIATED EXCRETION

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ABSTRACT:

A pharmacokinetic interaction between erythromycin and ximelagatan, 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 Madin-Darby canine kidney-human multidrug resistance-1 cell preparations and on biliary excretion of melagatan in rats were studied. In healthy volunteers (seven males and nine females; mean age 24 years) receiving a single dose of ximelagatan 36 mg on day 1, erythromycin 500 mg t.i.d. on days 2 to 5, and a single dose of ximelagatan 36 mg plus erythromycin 500 mg on day 6, the least-squares mean estimates (90% confidence intervals) for the ratio of ximelagatan with erythromycin to ximelagatan 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 melagatan, the active form of ximelagatan. Neither the slope nor the intercept of the melagatan plasma concentration-effect relationship for activated partial thromboplastin time statistically significantly differed as a function of whether or not erythromycin was administered with ximelagatan. Ximelagatan was well tolerated regardless of whether it was administered with erythromycin. Erythromycin inhibited P-gp-mediated transport of both ximelagatan and melagatan in vitro and decreased the biliary excretion of melagatan in the rat. These results indicate that the mechanism of the pharmacokinetic interaction between oral ximelagatan and erythromycin may involve inhibition of transport proteins, possibly P-gp, resulting in decreased melagatan biliary excretion and increased bioavailability of melagatan.

Ximelagatan is an oral direct thrombin inhibitor (oral DTI) which is available for the prevention of venous thromboembolic events in patients undergoing elective hip or knee replacement surgery and is being developed for the treatment of venous thromboembolic events, stroke prevention in patients with atrial fibrillation, and the secondary prevention of cardiovascular events after myocardial infarction (Bergstrud and Gandhi, 2003; Eriksson et al., 2003a,b; 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, ximelagatan differs from warfarin in having a predictable anticoagulant effect on coagulation assays that correlates closely with melagatan plasma concentrations and stable melagatan pharmacokinetics within and between patients, and in lacking the requirement for coagulation monitoring and lacking interactions with food, alcohol, and many commonly used medications (Bredberg et al., 2003; Eriksson et al., 2003c; Gustafsson and Elg, 2003; Johansson et al., 2003; Sarich et al, 2004a,b,c; Teng et al., 2004). On the basis of its efficacy and safety profiles, ximelagatan 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).

Because most patients to be treated with oral ximelagatan will be taking other medications on an acute or chronic basis, its drug-interaction profile is being fully evaluated. Interactions involving cytochrome P450 (P450) metabolic pathways are not expected, given that ximelagatan, its intermediate metabolites (ethyl-melagatan and hydroxy-melagatan), and melagatan (the predominant active form of ximelagatan) (Eriksson et al., 2003d) are not substrates of, and do not inhibit any of, the major P450 isoenzymes (Bredberg et al., 2003). Consistent with this expectation, studies in healthy volunteers have shown that coadministration of ximelagatan with amiodarone, atorvastatin, diazepam, diclofenac, digoxin, or nifedipine did not result in any relevant pharmacokinetic interactions for either of these drugs or for melagatan (Bredberg et al., 2003; Gustafsson and Elg, 2003; Sarich et al., 2004b,c; Teng et al., 2004). Drug effects on P450 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; Zhang 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 colocalized 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; Zhang and Benet, 2001; Lin and Yamazaki, 2003). The clinical relevance of the CYP3A/P-gp overlap 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 activated partial thromboplastin time (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.

Materials and Methods

Study 1: Erythromycin and Ximelagatran in Healthy Volunteers. Subjects. Healthy male and female volunteers aged 20 to 40 years with a body mass index from 19 to 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, herbs, 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 acetylsalicylic acid) during 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 in Lund, Sweden, approved the protocol for this open-label, sequential study, which was conducted at a single center 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 to 7 days after the last dose of study medication. Treatment on day 1 was ximelagatran 36 mg as a single oral dose at 8:00 AM. Treatment on days 2 to 5 was erythromycin (Ery-Max; AstraZeneca, Sweden) 500 mg three times daily (8:00 AM, 4:00 PM, and 12:00 midnight). Treatment on day 6 was erythromycin 500 mg twice daily (8:00 AM and 4:00 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 h after the first dose of ximelagatran and began again before 4:00 PM on treatment day 5 through to 24 h after the last ximelagatran dose.

Volunteers were instructed to fast and to abstain from drinking anything but water during the 3 h 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 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 h after the dose of ximelagatran for the determination of plasma concentrations of ximelagatran, melagatran, ethylmelagatran, and hydroxy-melagatran. The latter two compounds are intermediate 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 hydroxyamide in melagatran. These metabolites are subsequently converted to melagatran. Plasma concentrations were determined at DMPK and Bioanalytical Chemistry, Astrazeneca R&D (Mölndal, Sweden), using liquid chromatography-mass spectrometry with a limit of quantification (LOQ) of 0.010 μM (Larsson et al., 2003). 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 0 to infinity, calculated using the log-linear trapezoidal rule to the last quantifiable plasma concentration (Clast) and then extrapolated to infinity by adding Clast/(λ × elimination rate constant estimated from individual linear regression of the terminal part of the log concentration-time curve); Cmax, the observed maximum plasma concentration; Cmin, the time to Cmax and t1/2, the elimination half-life, which was calculated as ln 2/λ. Actual sampling times were used to estimate pharmacokinetic parameters in a noncompartmental analysis performed with WinNonlin professional software (Pharsight Corporation, Mountain View, CA). At time points before Cmax, plasma concentrations below LOQ were taken as zero in the calculations. If more than one plasma concentration was below LOQ before Cmax, the last one before the first quantifiable plasma concentration was calculated as LOQ/2.

Least-squares means and 95% confidence intervals (CIs) were calculated for within-subject data. Analyses of variance with subject and treatment as factors were run on logarithmically transformed values of AUC and Cmax to establish 90% CI for the ratios between treatments. Least-squares estimates with 90% CI for mean ratio with erythromycin (day 6 data)/without erythromycin (day 1 data) were calculated using the mean-square error from the analysis of variance 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 ratio with erythromycin fell within the intervals of 0.80 to 1.25 for AUC and 0.70 to 1.43 for Cmax. 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 melagatran tablets were administered with erythromycin versus without erythromycin.

Pharmacodynamic Assessments. Blood samples were taken predose and at 1, 2, 4, 6, and 10 h after the ximelagatran dose on day 1 and day 6 for the determination of APTT in plasma. The plasma samples were analyzed 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

776 ERIKSSON ET AL.
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 anesthetized male Sprague-Dawley rats (Harlan, Horst, 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 (Abbot; Abbott Scandinavia AB, Solna, Sweden) intraperitoneally (100 mg/kg) approximately 1 h before intravenous administration of ximelagatran. The plasma concentration of ximelagatran, hydroxy-melagatran, and melagatran were determined at DMPK and Bioanalytical Chemistry, AstraZeneca R&D (Mölndal, Sweden), using the same method as described above for the human interaction study (Larsson et al., 2003). 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 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 min 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 h after dosing.

**Study 3: In Vitro Transport in Caco-2 and MDCK Cells.** In vitro experiments were designed 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. [3H]Digoxin (1369 GBq/mmol) was purchased from NEN Life Science Products Inc. (Boston, MA). Erythromycin, verapamil, and quinidine were obtained from Sigma-Aldrich Co. (Stockholm, Sweden). Cell culture media and supplements, as well as Hank’s balanced salt solution (HBSS) without phenol red and sodium bicarbonate, were obtained from Scandiborn’s modified Eagle’s medium supplemented with 9% heat-inactivated bovine serum and 2% Hank’s balanced salt solution (HBSS) without phenol red and sodium bicarbonate. Cell culture media and supplements, as well as Hanks’ balanced salt solution (HBSS) without phenol red and sodium bicarbonate, were obtained from Scandiborn’s modified Eagle’s medium supplemented with 9% heat-inactivated bovine serum and 2% Hank’s balanced salt solution (HBSS) without phenol red and sodium bicarbonate.

**Cell Cultures.** The Caco-2 cell line was obtained from the American Type Culture Collection (Manassas, VA) at passage 18. 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 supplemented with 9% heat-inactivated fetal bovine serum, 7 mM l-glutamine, and 1% nonessential 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). 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 to 24 days after seeding, and MDCK cells, 3 days after seeding. Caco-2 cells used in this study were at passages 32 to 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). The Caco-2, MDCK-WT, and MDCK-MDR1 cell monolayers used in the present study had TEER values of approximately 200 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 transport compounds, indicating that the integrity of the cell monolayers was 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 min. 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 (1520 μl) of the monolayers and drug-free HBSS to the opposite side (1500 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 min. The sample volume removed from the receiver side was replaced with prewarmed (37°C) HBSS or HBSS containing appropriate concentrations of inhibitor. The concentrations of ximelagatran, erythromycin, hydroxy-melagatran, and melagatran in samples removed from the donor and receiver compartment were determined at DMPK and Bioanalytical Chemistry, AstraZeneca R&D Mölndal, using liquid chromatography-mass spectrometry with an LOQ of 0.020 μM for all four analytes. Samples containing [3H]digoxin and [14C]ximelagatran were analyzed by liquid scintillation counting.

**Data Analysis.** The permeability coefficient (Papp) of the test compounds was calculated as (dQ/dt) · [A] · C0, where dQ/dt is the linear rate of appearance of drug in the receiver compartment, A is the surface area of the filter, and C0 is the average drug concentration in the donor compartment over the time period for which dQ/dt was determined. Papp is given in centimeters per second. The transporter-mediated net efflux of ximelagatran in the Caco-2 cell monolayers was obtained by subtracting the Papp value in the A-to-B direction from the Papp value in the B-to-A direction. A significantly higher Papp in the B-to-A direction than in the A-to-B direction, i.e., positive net efflux or high efflux ratio (Papp in B-to-A/Papp 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 radiolabeled 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 = Tnet · [(1 − [Conc/IC50 + Conc])], where Tnet = net efflux without inhibitor, Conc = inhibitor concentration, and IC50 = concentration of inhibitor resulting in 50% of maximum inhibition. IC50 was determined by nonlinear regression analysis performed with WinNonlin professional software (Pharsight Corporation).

**Results**

**Study 1: Erythromycin and Ximelagatran in Healthy Volunteers.** Subjects. The number of volunteers randomized to treatment was 16 (seven men and nine women; all white; mean ± S.D. age, weight, and body mass index 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 under Safety and Tolerability, 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) (Fig. 1). The increases were comparable for the two intermediates and melagatran, whereas a smaller increase was observed for ximelagatran. The increases of both melagatran AUC and \( C_{\text{max}} \) suggest a clinically relevant interaction since 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 \( t_{\text{max}} \) or \( t_{1/2} \) of melagatran after 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 (Fig. 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) (Fig. 2). The erythromycin-associated elevation in plasma melagatran concentration increased the median (range) of the observed maximum APTT from 42 (35–54) s to 48 (35–52) s. 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 dysmenorrhea \( (n = 1) \). 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) was not recorded by the investigator for nonserious 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 approximately 25 \( \mu \text{M} \) at 5 min 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 \( \mu \text{M} \) and 7 \( \mu \text{M} \) at 30 min after administration of ximelagatran alone and combined with erythromycin, respectively. Melagatran concentrations remained approximately constant at these levels during the 6-h sampling period. Bile was obtained during the 6-h collection period for three of the four rats in each group. There was no change in the volume of bile [mean (S.D.)] collected during the 6-h period after administration of ximelagatran alone and combined with erythromycin, respectively. Melagatran concentrations remained approximately constant at these levels during the 6-h sampling period. Bile was obtained during the 6-h collection period for three of the four rats in each group. There was no change in the volume of bile [mean (S.D.)] collected during the 6-h period after administration of ximelagatran alone [4.26 (0.21) ml] and combined with erythromycin [4.29 (0.31) ml]. The mean (S.D.) 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 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. Furthermore, 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 B-to-A direction was 33-fold greater than in the absorptive A-to-B direction, and this efflux ratio decreased close to unity in the presence of quinidine or erythromycin, indicating that efflux transport in the MDCK-WT is due to the activity of endogenous canine P-gp in these cells.

The efflux ratio for the reference P-gp substrate digoxin to the receiver side were more than 2-fold higher compared with the total transport of ximelagatran plus intermediates and metabolites (ethyl-melagatran, hydroxy-melagatran) and melagatran formation of its intermediates and melagatran was also inhibited.

Table 1

<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>
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<tbody>
<tr>
<td>AUC (µmol · h/l) Mean (S.D.)</td>
<td>1.52 (0.42)</td>
<td>2.76 (0.81)</td>
<td>1.82 (1.64, 2.01)*</td>
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<tr>
<td>C_{max} (µmol/l) Mean (range)</td>
<td>1.39 (0.975–2.35)</td>
<td>2.51 (1.66–4.89)</td>
<td>1.93 (1.02, 2.42)</td>
</tr>
<tr>
<td>t_{max} (h) Mean (S.D.)</td>
<td>0.25 (0.06)</td>
<td>0.44 (0.11)</td>
<td>1.74 (1.52, 2.00)*</td>
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<tr>
<td>r (h) Mean (range)</td>
<td>0.25 (0.15–0.35)</td>
<td>0.47 (0.22–0.69)</td>
<td>1.85 (0.81, 2.42)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>2.2 (0.7)</td>
<td>2.5 (0.7)</td>
<td>N.E.</td>
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<tr>
<td>Median (range)</td>
<td>2.0 (1.5–3.0)</td>
<td>3.0 (1.5–4.0)</td>
<td>1.0 (0.5, 2.0)</td>
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<td>Median (range)</td>
<td>3.2 (0.3)</td>
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<td>N.E.</td>
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<tr>
<td>Median (range)</td>
<td>3.1 (2.9–4.0)</td>
<td>2.9 (2.5–3.7)</td>
<td>0.9 (0.8, 1.2)</td>
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N.E., not estimated.

* Least-squares mean estimates (90% CI) for between-treatment comparison (day 6/day 1).

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ximelagatran with Erythromycin (n = 15)</th>
<th>Ximelagatran without Erythromycin (n = 15)</th>
<th>Difference</th>
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<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>
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<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>
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</table>

Table 3

<table>
<thead>
<tr>
<th>Condition</th>
<th>P_{app, A-B}</th>
<th>P_{app, B-A}</th>
<th>Efflux Ratio ( \frac{P_{app, A-B}}{P_{app, B-A}} )</th>
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</thead>
<tbody>
<tr>
<td>MDCK-MDR1 Cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ximelagatran alone (50 µM)</td>
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<tr>
<td>+ Quinidine (100 µM)</td>
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<tr>
<td>+ Erythromycin (500 µM)</td>
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<tr>
<td>[3H]Digoxin (27 nM)</td>
<td></td>
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<tr>
<td>MDCK-WT Cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ximelagatran alone (50 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Quinidine (100 µM)</td>
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<tr>
<td>+ Erythromycin (500 µM)</td>
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<tr>
<td>[3H]Digoxin (27 nM)</td>
<td></td>
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</table>

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 rates of appearance of hydroxy-melagatran and melagatran (after 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 (Fig. 3). The rates of appearance of hydroxy-melagatran and melagatran in the apical compartment after basolateral dosing of ximelagatran were significantly decreased in the presence of quinidine or erythromycin (Fig. 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 radiolabeled 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 $[^{14}C]$ximelagatran in the Caco-2 monolayers in a concentration-dependent manner (Fig. 4). Erythromycin, quinidine, and verapamil inhibited the net efflux transport in the Caco-2 monolayers with IC$_{50}$ 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 $C_{\text{max}}$ were increased by 82% and 74%, respectively, when ximelagatran was administered concurrently with erythromycin compared with when melagatran was administered alone. No relevant changes were observed for melagatran $t_{\text{max}}$ 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 et al., 2003d).
The standard errors (S.E.) are estimates of the precision of the parameter estimates.

Concentration (IC50) and inhibition (IC50) are measured variables. The formed intermediates and melagatran (Eriksson et al., 2003d). The study was designed to determine the effect of erythromycin on ximelagatran pharmacokinetics in healthy volunteers receiving oral ximelagatran (Bredberg et al., 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 to 5 h (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 after 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 coadministration 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 et al., 2003c). Inhibition of ximelagatran bioconversion is not a likely explanation for the effect of erythromycin because this would result in a decrease in plasma concentrations of melagatran and the two intermediary substances. Previous research also supports the notion that the effect of 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 coadministration 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 early first-pass metabolism of ximelagatran with subsequent biliary excretion of some of the formed intermediates and melagatran (Eriksson et al., 2003d). 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, whereas the bile flow and the metabolic pattern of bile were 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 whether active transport also occurs for the intermediates and melagatran after intracellular formation. Because 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, whereas 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 was 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, since 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 transporters other 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 melagatran (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.

Coadministration 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%.

### Table 5

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Parameter</th>
<th>Estimate</th>
<th>S.E.</th>
</tr>
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<tr>
<td>Erythromycin</td>
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<tr>
<td></td>
<td>IC_{50}</td>
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</tr>
<tr>
<td></td>
<td>γ</td>
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</tr>
<tr>
<td>Quinidine</td>
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<td>IC_{50}</td>
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<td></td>
<td>γ</td>
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<tr>
<td>Verapamil</td>
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<td>IC_{50}</td>
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</tr>
<tr>
<td></td>
<td>γ</td>
<td>0.68</td>
<td>0.05</td>
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</table>
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 (Bredberg et al., 2003; Eriksson et al., 2003a,b,c; Francis et al., 2003; Gustafsson and Elg, 2004; Johansson et al., 2003; Olsson et al., 2004; Schultman et al., 2003; Wallentin et al., 2003; Sarich et al., 2004a,b; 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.

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References


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