ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF XIMELAGATRAN, AN ORAL DIRECT THROMBIN INHIBITOR, IN RATS, DOGS, AND HUMANS

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ABSTRACT:

The absorption, metabolism, and excretion of the oral direct thrombin inhibitor, ximelagatran, and its active form, melagatran, were separately investigated in rats, dogs, and healthy male human subjects after administration of oral and intravenous (i.v.) single doses. Ximelagatran was rapidly absorbed and metabolized following oral administration, with melagatran as the predominant compound in plasma. Two intermediates (ethyl-melagatran and OH-melagatran) that were subsequently metabolized to melagatran were also identified in plasma and were rapidly eliminated. Melagatran given i.v. had relatively low plasma clearance, small volume of distribution, and short elimination half-life. The oral absorption of melagatran was low and highly variable. It was primarily renally cleared, and the renal clearance agreed well with the glomerular filtration rate. Ximelagatran was extensively metabolized, and only trace amounts were renally excreted. Melagatran was the major compound in urine and feces after administration of ximelagatran. Appreciable quantities of ethyl-melagatran were also recovered in rat, dog, and human feces after oral administration, suggesting reduction of the hydroxyamidine group of ximelagatran in the gastrointestinal tract, as demonstrated when ximelagatran was incubated with feces homogenate. Polar metabolites in urine and feces (all species) accounted for a relatively small fraction of the dose. The bioavailability of melagatran following oral administration of ximelagatran was 5 to 10% in rats, 10 to 50% in dogs, and about 20% in humans, with low between-subject variation. The fraction of ximelagatran absorbed was at least 40 to 70% in all species. First-pass metabolism of ximelagatran with subsequent biliary excretion of the formed metabolites account for the lower bioavailability of melagatran.

Thrombin plays a central role in the processes of hemostasis and thrombus formation. Among its numerous functions are the catalysis of the transformation of fibrinogen to fibrin monomers producing the fibrin network that reinforces the fragile, platelet-rich plug and the stimulation of platelet aggregation. A number of agents, including warfarin and low-molecular-weight heparins, are available for the prevention and treatment of thromboembolic complications. Indeed, warfarin and other vitamin K antagonists are the only oral anticoagulants available today. However, patients receiving these agents require frequent monitoring of prothrombin time because of the narrow therapeutic window and slow on- and offset of action of these agents (Hirsh et al., 2001), characteristics that make it difficult to predict their anticoagulant effect. Warfarin is also subject to large variability in its pharmacokinetic (PK) properties due to drug and food interactions (Hirsh et al., 2001). Despite monitoring, these agents are associated with a high incidence of bleeding and drug-related deaths (Anonymous, 1993). Low-molecular-weight heparins can be administered without routine coagulation monitoring (Hull and Pineo, 1995), but the fact that they can only be administered parenterally limits their use. Currently available anticoagulants therefore have a number of drawbacks, and there is an ongoing quest for a new anticoagulant that is effective, safe, and can be administered orally without routine coagulation monitoring.

Melagatran is a novel, direct thrombin inhibitor that binds rapidly, reversibly, and competitively to the active site of thrombin with a $K_i$ of 0.002 μM (Gustafsson et al., 1998). It has shown promise as an antithrombotic drug, both in animal models of experimental thrombosis (Elg et al., 1997, 1999; Eriksson et al., 1997; Mattsson et al., 1997; Gustafsson et al., 1998; Metha et al., 1998) and in clinical trials in orthopedic surgery patients (Heit et al., 2001; Eriksson et al., 2002a,b,c) and patients with deep venous thrombosis (Eriksson et al., 1999a, 2003). Because of low membrane permeability, melagatran is not suited to oral administration; however, its favorable pharmacodynamic properties were the impetus for the development of ximelagatan, a novel, direct thrombin inhibitor that can be administered orally, whereupon it is rapidly transformed to melagatran, its active form (Gustafsson et al., 2001). Melagatran may be formed from ximelagatran via two different pathways, as shown in Fig. 1. The primary compounds formed during the bioconversion of ximelagatran to melagatran are OH-melagatran (formed by hydrolysis of the ethyl ester) and ethyl-melagatran (formed by reduction of the hydroxyamidine), which are both subsequently converted to melagatran. The thrombin-inhibiting activity of ximelagatran and OH-melagatran is about 1% of
that of melagatran, whereas ethyl-melagatran has about the same inhibitory potency as melagatran (Gustafsson et al., 2001).

The aim of the studies described here was to examine the metabolism and excretion patterns and the PK properties of ximelagatran and melagatran following the administration of single oral and i.v. doses to animals and humans. The major focus was on evaluating the bioavailability of melagatran, the active thrombin inhibitor, following oral administration of ximelagatran.

Materials and Methods

Ximelagatran and melagatran were synthesized by AstraZeneca (Mölndal, Sweden), using methods analogous to those described previously (Antonsson et al., 1994, 1997). Ethyl-melagatran and OH-melagatran, used as reference compounds for metabolite identification in biological samples, and radiolabeled compounds were prepared at the Department of Medicinal Chemistry, AstraZeneca. The chemical structures of these compounds are shown in Fig. 1. The radiolabeled compounds (specific radioactivity and radiochemical purity given in parentheses) used were [14C]ximelagatran (2.0 kBq/nmol, >97%), [3H]melagatran (55 kBq/nmol, >97%), [3H]melagatran (521 kBq/nmol, 97%), OH-melagatran-[3H] (55 kBq/nmol, >95%), and ethyl-melagatran-[3H] (900 kBq/nmol, >98%). In essentially all studies, aqueous solutions (pH adjusted to 3–5) of melagatran (chemical purity 99.3%) and ximelagatran (chemical purity >97%) were used for dosing to animals and humans. In the food interaction study for melagatran in human subjects, an immediate-release tablet of melagatran was administered. All other chemicals used were of analytical grade.

Design of Studies in Animals

Sprague-Dawley rats and beagle dogs were used in studies to examine the mass balance and PK properties of melagatran and ximelagatran after i.v. and oral (by gastric gavage) administration. All animal studies described were approved by the regional ethics committee.

Mass Balance and Pharmacokinetics in Rats. The rats were starved overnight until after drug administration but retained free access to drinking water at all times. In mass balance studies, the rats were placed in all-glass metabolic cages that allowed separate collection of urine and faeces. PK studies were performed in separate groups of rats. Two days before dosing, these rats were anesthetized, and a catheter was placed in the carotid artery for collection of blood samples. For i.v. dosing, a catheter was also inserted in the right jugular vein. The catheters were exteriorized at the nape of the neck and sealed. After surgery, the rats were housed individually and had free access to water and food.

Mass balance studies. Single, 2 μmol/kg (3.5 MBq/kg) i.v. doses of [3H]melagatran were administered (via a catheter placed in the right vena jugularis) to one group of four rats, and single, 30 μmol/kg (2 MBq/kg), oral doses of [3H]melagatran were administered by gavage to a separate group of four rats. The rats used were 12 to 14 weeks old and weighed 339 to 355 g. Urine and feces were collected during the 19-h period before dosing and at 24-h intervals for 7 days after dosing. Urine samples collected during the 0- to 24-h time period and feces collected during the 0- to 24- and 24- to 48-h time periods postdosing were used in the analysis of metabolic patterns of melagatran.

Single, 40 μmol/kg (19.4 MBq/kg), oral doses of [14C]ximelagatran were administered to one group of eight rats (four male and four female) and single, 10 μmol/kg (21.9 MBq/kg), i.v. doses of [14C]ximelagatran were administered by bolus injection in the tail vein to a separate group of eight rats (four male and four female). The rats used were 6- to 7-weeks old. At the time of dosing, the females weighed from 173 to 255 g and the males from 145 to 218 g. Urine and feces were collected at 24-h intervals for 7 days postdosing. Urine samples were also collected during the 0- to 6- and 6- to 24-h time periods postdosing. Metabolic patterns were determined in the urine samples collected during the 0- to 6- and 6- to 24-h time periods postdosing and in fecal samples collected during the 0- to 24- and 24- to 48-h time periods postdosing.

Excretion in bile was determined in bile-duct-fistulated rats (three male and three female rats, starved overnight), each of which received a single, 40 μmol/kg (20 MBq/kg), oral dose of [3H]melagatran. The rats were housed individually in metabolic cages, and bile was collected during the 0- to 2-, 2- to 4-, 4- to 8-, and 8- to 24-h time periods postdosing.

PK studies. Unlabeled melagatran was administered as an i.v. bolus dose (2 μmol/kg) or orally (30 μmol/kg) to separate groups of four male or four female rats. A higher oral dose of 100 μmol/kg was also given to another group of four male rats. The rats used were 12- to 14-weeks old. At the time of dosing, the females weighed from 209 to 240 g and the males from 338 to 365 g. Blood samples (0.2 ml) were collected from all rats 60 min or less before and at regular intervals until 240 min after i.v. dosing and 480 min after oral dosing.

PK properties of ximelagatran in rats were examined following oral and i.v. administration of [14C]ximelagatran. Oral administration involved a single dose of 10 μmol/kg (8.89 MBq/kg) ximelagatran, administered by gavage to five male and five female rats, and a single dose of 40 μmol/kg (35.6 MBq/kg) ximelagatran administered to a separate group of five male rats. Administration (i.v.) involved a single, 5 μmol/kg (4.39 MBq/kg), dose of ximelagatran administered by bolus injection to three male and four female rats and a single, 10 μmol/kg (8.78 MBq/kg) dose of ximelagatran administered to a separate group of four male rats. Each rat received only one dose of drug. The rats used

FIG. 1. The proposed metabolic pathway of ximelagatran showing the formation of melagatran via two intermediate compounds, ethyl-melagatran, and OH-melagatran, by reduction of the OH group and hydrolysis of the ethyl ester.

The 14C- and tritium (T)-labeled positions are indicated in the figure.
were 12-week-old males and females weighing 200 to 450 g. Blood samples (250 μl) were collected from the carotid artery before and up to 24 h after oral and i.v. administration. After oral administration, nine blood samples were taken from each rat. After i.v. administration, 12 samples were taken from each male rat and six from each female rat.

**Mass Balance and Pharmacokinetics in Dogs.** Male and female dogs that were 2- to 6-years old and weighing 10 to 17 kg were used in the experiments. Two groups of four dogs (two males and two females) were administered single doses of [3H]melagatran either as an i.v. infusion over 1 min in a front leg vein (2 μmol/kg, 2MBq/kg), or orally by gavage (30 μmol/kg, 6 MBq/kg). Just before administration of both the oral and i.v. doses of melagatran, the dogs were placed in metabolic cages. Blood samples (5 ml) were collected from a front leg vein before and then at frequent intervals until 300 min after i.v. dosing and 360 min after oral dosing. Additional blood samples were then collected every 24 h for 5 days. Urine was collected in the cage during the 0- to 12- and 12- to 24-h time periods postdosing and thereafter at 24-h intervals for 5 days. Feces were collected at 24-h intervals for 5 days postdosing.

The influence of food on the absorption of melagatran was the subject of a second study in four dogs who were administered a single oral dose of 30 μmol/kg melagatran on two study days separated by a washout period of 5 days. On one study day, the dogs were administered melagatran under fasting conditions, whereas on the other study day, they were administered the drug 1 h after food intake in a crossover design. Blood samples were collected before dosing and then frequently until 6 h postdosing. In both studies, the dogs were fasted from approximately 3:00 PM on the day before dosing until approximately 6 h after dosing but retained free access to water throughout the experiments.

Two male and two female dogs received a single, 40 μmol/kg (5.16 MBq/kg) oral dose of [14C]ximelagatran by gavage. At a washout period of 4.5 weeks, the same dogs were each given a single, 10 μmol/kg (5.09 MBq/kg), i.v. dose of [14C]ximelagatran, by bolus injection into the cephalic vein over a period of approximately 1 min. The dogs were housed individually in metabolic cages immediately following each dose of drug. Venous blood samples (approximately 8 ml) were collected from a leg vein into lithium heparin tubes before and up to 48 h after oral and i.v. administration. Urine was collected before and 6 and 24 h after both oral and i.v. drug administration, and then at 24-h intervals until 168 h post drug administration. Feces were collected predose and at 24-h intervals for 7 days postdosing. In addition, two male and two female dogs were given a 10 μmol/kg oral dose of unlabeled ximelagatran. After a washout period of 2 weeks, the same dogs received a 5 μmol/kg i.v. dose of ximelagatran. The dogs were starved overnight before dosing and then until approximately 5 h post dosing but had free access to water at all other times. Blood samples (5 ml) from a front leg vein were collected predose and up to 24 h post dosing.

Excretion in bile was determined in a chronically bile-duct canulated female fasted dog (15.3 kg) administered a single, 40 μmol/kg (5.28 MBq/kg), oral dose of [1H]melagatran. Bile was collected during the 0- to 2-, 2- to 4-, 4- to 8-, and 8- to 24-h time periods postdosing.

**Design of Studies in Humans**

**Subjects.** Young healthy white male subjects, from whom informed consent was obtained prior to enrollment, were included in the studies that were performed in compliance with current good clinical practice guidelines and the Declaration of Helsinki. The subjects included in three melagatran studies were 20- to 40-years old and weighed 66 to 86 kg. These studies were performed in Sweden and approved by the Medical Products Agency of Sweden and the Göteborg University Ethics Committee. For the ximelagatran study, the subjects were 31- to 50-years old and weighed 76 to 86 kg. This study was performed in Scotland and approved by the local independent ethics committee.

For all studies, eligibility for entry was assessed at a screening visit that took place within 14 days of the start of the study. No medication, including aspirin, other nonsteroidal anti-inflammatory drugs, or prescribed medication, were allowed within the 2 weeks prior to the first dose of study drug and until the end of the study period. None of the subjects had received another investigational drug within 2 to 3 months prior to the first treatment day.

**Melagatran Studies.** *Mass balance and pharmacokinetics.* Fixed doses of [3H]melagatran were administered to 12 healthy male volunteers in an open-label, crossover study. Each subject was to receive two single doses of [3H]melagatran, one as an i.v. infusion and the second by an oral solution, with at least 2 weeks between doses. The i.v. dose consisted of 5.3 μmol (2.3 mg) melagatran containing 5.2 MBq of [3H]melagatran in a volume of 15 ml infused over 10 min. The oral dose consisted of 256 μmol (110 mg) melagatran containing 7.4 MBq of [3H]melagatran in a volume of 40 ml followed by 60 ml of water to rinse the vial. On the evening before each investigational day, subjects were instructed to have dinner no later than 7:00 PM and to abstain from food and drink from 10:00 PM until a standardized meal (lunch) was served 3 h after drug administration. Other standardized meals were served 6 h (snack), 10 h (dinner) and 13 h (snack) after drug administration. Venous blood samples were drawn by repeated venipuncture (Venoject; Terumo Europe N.V., Leuven, Belgium) from a forearm (the forearm contralateral to that of the infusion for subjects receiving i.v. melagatran) before and then frequently during the 24-h period after administration of both the i.v. and the oral doses of melagatran. Urine was collected predose, during the 0- to 4-, 4- to 12-, and 12- to 24-h time periods postdosing and thereafter at 24-h intervals for 5 days postdosing. Feces were collected at 24-h intervals over the 5-day period postdosing. The amounts of urine and feces collected were determined by weighing.

**Dose linearity.** This was examined in two studies as follows: 1) an open-label, dose-escalation study in 26 healthy male volunteers, in which melagatran was administered as gradually increasing single i.v. doses (administered as a constant infusion over 10 min) from 1.7 to 82 μg/kg (0.004-0.19 μmol/kg) to groups of two or four subjects per dosing level; 2) an open-label, dose-escalation study in 23 healthy, male volunteers, in which melagatran was administered as gradually increasing single oral doses (in solution) from 0.02 to 3.28 μg/kg (0.05-7.6 μmol/kg) to groups of two or four subjects per dosing level. Each subject received a maximum of two doses of melagatran separated by at least 1 week. On the evening before each investigational day, subjects were instructed to have dinner no later than 7:00 PM and to abstain from food and drink from 10:00 PM until a standardized meal was served postdrug administration. In the i.v.-dosing study, standardized meals were served 1 h (breakfast) and 4 h (lunch) after the start of the i.v. infusion, whereas in the oral dosing study, they were served 3 h (lunch), 6 h (snack), 10 h (dinner), and 13 h (snack) after drug administration. In the i.v.-dosing study, blood samples were collected immediately before and then frequently until 4 h after dosing for doses up to 50 μmol/kg, and additionally at 6 and 8 h after dosing for doses of 50 μmol/kg and above. In the oral dosing study, blood samples were drawn before and at frequent intervals until 24 h after dosing.

**Effect of food.** Six healthy human volunteers received melagatran as a 50 mg (105 μmol) oral dose on the investigational day separated by washout periods of at least 1 week, in an open-label, three-way, randomized crossover study. Administration of melagatran occurred under fasting conditions, together with breakfast, or 2 h after breakfast, in randomized order.

**Ximelagatran study.** The mass balance and pharmacokinetics of ximelagatran was examined in an open-label, sequential, nonrandomized study, in which each subject received a single, 50 mg (105 μmol), oral dose of [13C]ximelagatran (2.52 MBq), at a concentration of 1.25 mg/ml. This was followed 20 days later by a single, 10 mg (21 μmol), i.v. dose of unlabeled ximelagatran infused over 10 min with the aid of an infusion pump. Subjects were required to remain at the study site for 7 to 9 days following drug administration in the first study session and for 24 h following drug administration in the second study session. Subjects were required to fast from 11:00 PM on the evenings before drug administration until a standardized lunch was served 4 h postdosage. Subjects also abstained from food for 2 h before each laboratory investigation.

Blood (10-12 ml), urine, and feces samples for PK analysis were collected before and up to 168 h post oral administration, by which time excretion of drug was essentially complete. Blood (5 ml) and urine samples were collected before and up to 12 h and 24 h post i.v. administration, respectively. Whole blood samples were collected via a venous catheter or by venopuncture.

**Plasma Protein Binding and Blood–Plasma Partitioning.**

The plasma protein binding and the partitioning between blood and plasma were determined in vitro in freshly collected blood and plasma. For ximelagatran and the two intermediates, ethyl-melagatran and OH-melagatran, blood from four dogs and four healthy human subjects (two male and two female
animals/subjects) were used in the experiments. For melagatran, pooled blood from three male dogs, ten male rats, and individually in four healthy human subjects (two male and two female) was used. The blood samples from the different species were treated separately, and all experiments were carried out in triplicate. Blood and plasma were incubated separately with $^3$Hmelagatran at concentrations of 0.1, 1, and 10 nM, and with $^3$H-labeled ethyl-

melagatran and OH-melagatran at concentrations of 0.05, 0.5, and 5 nM. $^3$Hmelagatran was incubated at the following concentrations: 0.05, 0.5, and 5 μM in humans; 0.05, 5, and 100 μM in dogs; and 0.05, 5, and 30 μM in rats. Plasma protein binding, determined using ultrafiltration, was given as the ratio of concentrations in ultrafiltrate and plasma. No adsorption to the membrane or the ultrafiltration device was observed for any of the compounds. The whole blood to plasma concentration ratio was calculated as the concentration of radioactivity in whole blood divided by the concentration of radioactivity in the corresponding plasma samples.

**Sample Analysis**

**Handling and Stability of Biological Samples.** All urine and feces samples were directly frozen and stored at −20°C until analysis. Blood samples were collected in heparinized test tubes, kept on ice until plasma was separated by centrifugation (within 1 h of collection), and then kept frozen at −20°C until analysis. The stability of ximelagatan in freshly collected dog and human blood was evaluated by incubation for 1 h at 37°C. In these incubation experiments, more than 90% of the initial ximelagatan concentrations were remaining, supporting that the stability of ximelagatan was satisfactory when the blood samples were handled as described above.

The stability of ximelagatan in human feces was studied by addition of $^3$Hximelagatan (50 nM) to feces homogenate from blank human feces. Incubation of the samples was performed under anaerobic conditions at 37°C. At selected incubation times (5, 15, 30, and 60 min, 24 h, and 14 days), the feces slurry was centrifuged at 15,000g for 10 min and the supernatant analyzed using the reversed-phase gradient liquid chromatographic (LC) system with on-line radioactivity and mass spectrometry (MS) detection described below.

**Concentration of Total Radioactivity.** In the melagatran studies, the concentration of total radioactivity in the biological samples was determined by liquid scintillation counting after mixing the samples with liquid scintillation fluid. Fecal samples were homogenized in approximately twice their volume in tap water. For ximelagatan studies, all samples except whole blood and bile were analyzed as described for melagatan studies. Whole blood, feces homogenate, and bile were combusted using a Packard Tri-Carb Automatic Sample Oxidizer (PerkinElmer Life Sciences, Boston, MA). For mass balance studies, the amounts of radioactivity in urine and feces were expressed as a percentage of the administered dose.

**Metabolic Patterns in Urine, Bile, and Feces.** For both melagatran and ximelagatan studies, a reversed-phase gradient LC system with on-line radioactivity detection was used to determine the metabolic pattern in urine and feces. Urine samples were injected directly onto the LC column. Bile samples were diluted with mobile phase A and centrifuged at 15,000g for 10 min. Feces homogenates (100-μg aliquot from samples containing >3% of the dose) were mixed with 0.5 ml of phosphoric acid (1 M), shaken for 5 min, and centrifuged at 10,000g. The result supernatant was injected onto the LC column. Complete recovery of radioactivity for the extraction from fecal samples was demonstrated by liquid scintillation counting.

The LC system consisted of a high-pressure LC pump (Pharmacia LKB 2248; Pharmacia AB, Uppsala, Sweden) connected to an autosampler (CMA/ 200, 200-μl loop volume) and a Symmetry C18 (150 × 3.9 mm) analytical column protected by a precolumn (Symmetry C18, 20 × 3.9 mm; Waters, Milford, MA). A gradient of two mobile phases was pumped at a flow rate of 1 ml/min. The mobile phases A and B consisted of 10% and 50% acetonitrile in 0.05 M phosphate buffer (pH 7). After sample injection (100–150 μl), the mobile phase B was increased linearly from 0 to 30% in 15 min and then to 100% in 10 min. An equilibration time of at least 6 min with 0% mobile phase B was allowed before injection of the next sample. The radioactivity in the eluate was continuously measured using a Radiomatic FLO-ONE (PerkinElmer Life Sciences) detector with a 1-m1 flow cell and a scintillation fluid (Ultima-FLO AP) at a flow rate of 3 ml/min. The radioactivity signal was stored in a PC-based evaluation system (Radiomatic 500TR FLO-ONE). The
A bi-exponential model was fit to the data obtained after i.v. dosing in the human melagatran study whereas noncompartmental analysis was used for all other data. Weighted least-squares nonlinear regression using PCNONLIN (version 4.2; Statistical Consultants Inc., Lexington, KY) was used to fit the bi-exponential model to the data for i.v. melagatran in human subjects. The weights used were \((Y_{\text{pred}})^{-2}\) or \((Y_{\text{pred}})^{-1}\), where \(Y_{\text{pred}}\) is the model-predicted plasma concentration. Basic PK parameters were derived from the bi-exponential model in a standard manner. The \(C_{\text{max}}\) was the highest plasma concentration observed, and \(t_{\text{max}}\) was the time at which \(C_{\text{max}}\) occurred. The area under the plasma concentration–time curve (AUC) was calculated using the log-linear trapezoidal rule from time 0 to \(t_{\text{last}}\) for all measurable plasma concentration, and extrapolated to infinity by addition of the quantity \(C_{\text{inf}}/k\), where \(C_{\text{inf}}\) was the predicted plasma concentration at \(t_{\text{inf}}\), and \(k\) was the elimination rate constant. \(C_{\text{inf}}\) and \(k\) were estimated by linear least-squares regression of log plasma concentration–time data in the terminal phase of the decline. The \(t_{1/2}\) was calculated as \(\ln2/k\). The area under the first moment curve (the curve of the product of concentration and time versus time; \(\text{AUMC}\)) was calculated using the linear trapezoidal rule and extrapolated to infinity by adding the quantity \((C_{\text{last}} \times t_{\text{last}})/k + (C_{\text{inf}}/k^2)\). Plasma clearance (CL) and volume of distribution at steady state (\(V_{\text{ss}}\)) were also calculated following i.v. dosing: CL as dose\(\times\text{AUC}_{\text{ss}}\) and \(V_{\text{ss}} = \text{CL} \times \text{MRT}\), where MRT is the mean residence time calculated as \(\text{AUMC}_{\text{ss}}/\text{AUC}_{\text{ss}}\).

Oral bioavailability was calculated as \(100 \times (\text{AUC}_{\text{oral}}/\text{AUC}_{\text{iv}})/(\text{dose}_{\text{oral}}/\text{dose}_{\text{iv}})\), where the oral and i.v. suffixes denote the quantities for oral and i.v. dosing, respectively. After both oral and i.v. administration of ximelagatran, the bioavailability of melagatran \((F_{\text{Mel}})\) was calculated as \((\text{AUC} \times \text{CL}_{\text{Mel}})/\text{dose}\), where \(\text{CL}_{\text{Mel}}\) is the mean value of melagatran clearance. Renal clearance \((\text{CL}_{\text{R}})\) of melagatran was estimated as \(A_{\text{f}}/\text{AUC}\), where \(A_{\text{f}}\) was the amount of melagatran excreted in urine.

In the food interaction study in dogs, differences in the parameters \(C_{\text{max}}\), AUC, and \(t_{1/2}\) between the two treatments (administration with and without food) were tested by the Student’s paired \(t\) test. Log-transformed values were used for AUC and \(C_{\text{max}}\). In the human study, analysis of variance analysis was used to test for between-treatment differences in \(C_{\text{max}}\) and AUC using log-transformed values. Differences were considered significant at \(p < 0.05\).

### Results

**Mass Balance and Metabolic Profile of Melagatran.**

The mean cumulative recovery of total radioactivity excreted in urine and feces, following i.v. and oral administration of \(^{3}H\)melagatran to rats, dogs, and human subjects, is shown in Table 1. In rats and dogs, the major portion of radioactivity excreted in feces and urine following both oral and i.v. administration of melagatran was identified as unchanged melagatran. However, a few minor peaks in the chromatogram eluted from the reversed-phase LC system with a shorter retention time than melagatran, suggesting that metabolites with a more polar nature than melagatran were formed. The concentration of these metabolites was low in relation to melagatran, except in the urine of dogs, where they were present in somewhat larger amounts. The structure of these polar metabolites remains to be identified.

As in animals, LC-analysis of samples of human urine and feces showed that most of both the oral and the i.v. doses were excreted as unchanged melagatran. However, in contrast with the animal studies, no peak other than that belonging to unchanged melagatran was detected in urine after both oral and i.v. administration, showing that there were no metabolites of melagatran excreted in human urine. After i.v. administration, the amount of radioactivity in samples of feces was too low to allow detection of any metabolites. For both human and animals, the LC analysis of urine and feces showed no evidence of formation of tritiated water indicating that the tritium-labeled position of melagatran was stable in vivo.

**Mass Balance and Metabolic Profile of Ximelagatran.**

The mean cumulative recovery of total radioactivity in urine and feces, following oral and i.v. administration of \(^{14}C\)ximelagatran is shown in Table 2. The excretion of radioactivity was rapid and essentially complete for both animals and human subjects.

LC analysis of samples of urine from male and female rats and dogs showed that most of the radioactivity in urine was melagatran (Fig. 2), whereas ximelagatran, ethyl-melagatran, and OH-melagatran accounted for only small fractions of the administered dose excreted. No ximelagatran was detected in the urine of male or female rats after either oral or i.v. administration, and only trace amounts were found in the urine of dogs. Melagatran was also the dominant compound recovered in feces (Table 3). Only small amounts of metabolites with retention times other than that of ximelagatran or its three main products were observed in the chromatograms of urine and feces. Most of these eluted earlier than melagatran, with retention times ranging from 2.1 to 3.8 min, suggesting that they are more polar than melagatran. In urine, the fraction of the dose accounted for by these unknown metabolites after i.v. administration of ximelagatran to male rats, female rats, and dogs was 10.6, 3.1, and 9.5%, respectively. The corresponding fractions in urine after oral administration of ximelagatran were 1.7, 1.8, and 9.1%, respectively. The amounts of unknown metabolites in feces are given in Table 3.

The amount of radioactivity excreted in bile (mean ± S.D.) expressed as a percentage of the administered dose of \(^{3}H\)ximelagatran was 16.4 ± 3.3% for male rats, 7.2 ± 0.7% for female rats, and 33.5% for one female dog. LC-MS analysis with radiochemical detection of the collected bile samples showed that most of the radioactivity was melagatran. For male and female rats, 80 and 88% of the radioactivity in bile was melagatran, respectively. Trace amounts of OH-melagatran and ethyl-melagatran (only for male rats) accounted for the remainder of the radioactivity in rat bile. In bile from the dog, 51% of the radioactivity was melagatran, and 41% was ethyl-melagatran.

The compounds in human urine were positively identified as ximelagatran, ethyl-melagatran, OH-melagatran, and melagatran using LC-MS analysis, based on comparison with the chromatographic retention times, molecular ions, and product ion spectra of available synthetic compounds. The predominant compound in urine after both oral and i.v. administration of ximelagatran was melagatran (Fig. 2). Melagatran was excreted rapidly, with maximal concentrations in urine samples collected 4 h postdosing. However, by 24 to 48 h postdosing, melagatran was either present in urine in very small quantities or was below LOQ. Low levels of the parent compound, ximelagatran, and the two intermediates, ethyl-melagatran and OH-melagatran, were also detected, with maximal concentrations also observed 4 h post oral and i.v. dosing.

The metabolic pattern in urine determined by radiochemical detection methods supported the results from the LC-MS measurements shown in Fig. 2 and also revealed unknown metabolites in the urine.
and feces of human subjects administered oral ximelagatran. These accounted for 1.8% of the oral ximelagatran dose excreted in the urine collected up to 24 h postdosing. As in the animal experiments, melagatran was the dominant compound in feces (Table 3).

In vitro, the disappearance of ximelagatran and the formation of ethyl-melagatran and melagatran in human feces homogenate were rapid under anaerobic conditions. The half-life for the reduction of ximelagatran to ethyl-melagatran was about 30 min. The subsequent ester-hydrolysis with formation of melagatran was slower, with detectable concentrations (about 10% of the radioactivity) after 24 h of anaerobic incubation. Polar metabolites, with about the same retention times as the unknown metabolites found in feces samples collected from the human subjects who had been administered oral ximelagatran, were also detected and accounted for about 50% of the radioactivity after 14 days of anaerobic incubation. The remaining radioactivity had retention times corresponding to ethyl-melagatran (26%) and melagatran (22%).

### Pharmacokinetics of Melagatran

The mean plasma concentration–time profiles in rats, dogs, and human subjects, after oral and i.v. administration of melagatran, are shown in Fig. 3. The PK parameters of melagatran are presented in Tables 4 and 5. For rats, the apparent t_{1/2} of melagatran after oral administration (mean values of 1.1–1.6 h) was longer than after i.v. administration indicating absorption-rate limited elimination. Renal clearance of melagatran was estimated to 8.6 ml/min/kg for male rats and 119 ± 10 ml/min for human subjects.

In the ascending dose study in humans, both C_{max} and AUC increased linearly with dose for i.v. dosing (Fig. 4). PK parameters

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### Table 2

**Mean (S.D.) percentage of administered dose of [14C]ximelagatran excreted in urine and feces following oral and i.v. administration to rats, dogs and humans**

<table>
<thead>
<tr>
<th></th>
<th>Rats</th>
<th>Rats</th>
<th>Dogs</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>n; gender</td>
<td>4; M</td>
<td>4; F</td>
<td>4; 2 M, 2 F</td>
<td>5 M</td>
</tr>
<tr>
<td>Oral dose</td>
<td>40 μmol/kg</td>
<td>40 μmol/kg</td>
<td>40 μmol/kg</td>
<td>105 μmol</td>
</tr>
<tr>
<td>Urine</td>
<td>21.3 (1.9)</td>
<td>16.5 (1.7)</td>
<td>22.6 (2.4)</td>
<td>25.2 (4.3)</td>
</tr>
<tr>
<td>Feces</td>
<td>71.3 (1.1)</td>
<td>77.0 (2.0)</td>
<td>66.9 (3.1)</td>
<td>71.1 (4.5)</td>
</tr>
<tr>
<td>Total</td>
<td>94.9 (1.5)</td>
<td>96.6 (1.0)</td>
<td>90.5 (2.2)</td>
<td>96.3 (0.8)</td>
</tr>
<tr>
<td>i.v. dose</td>
<td>10 μmol/kg</td>
<td>10 μmol/kg</td>
<td>10 μmol/kg</td>
<td>N.D.</td>
</tr>
<tr>
<td>Urine</td>
<td>45.8 (3.5)</td>
<td>34.6 (15.1)</td>
<td>32.3 (1.3)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Feces</td>
<td>37.4 (4.4)</td>
<td>54.9 (8.7)</td>
<td>57.9 (2.8)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Total</td>
<td>88.8 (0.9)</td>
<td>96.0 (6.1)</td>
<td>91.3 (1.6)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

M, male; F, female; N.D., not determined as only unlabeled compound was given.

*a* Includes cage-wash.

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**Fig. 2.** Cumulative amounts of ximelagatran, melagatran, ethyl-melagatran, and OH-melagatran excreted in urine in the 0- to 24-h period following oral and i.v. administration of ximelagatran to rats, dogs, and humans (as determined using radiochemical detection methods in rats and dogs and LC-MS in humans).

Results are expressed as mean percentages of administered dose.
estimated for dose levels with sufficient number of measurable plasma concentrations (16–191 nmol/kg) were consistent and showed low interindividual variability (CV of melagatran CL was 13%). In contrast, the PK variability for oral dosing was large. The mean (±S.D.) estimates for melagatran bioavailability in the ascending oral dose study ranged from 3.9 ± 1.8% at the lowest evaluable dose (1.61 μmol/kg) to 7.6 ± 2.4% at the 6.1 μmol/kg dose, with a mean value across all doses of 5.8 ± 2.3%. In addition, a tendency for dose dependence was observed in both Cmax and AUC as shown by the fact that there was a slight increase in the dose-normalized values of both Cmax and AUC with dose (Fig. 4). There was a linear correlation between Cmax and AUC (r2 = 0.96).

Food markedly decreased the oral bioavailability of melagatran in both dogs and humans. AUC and Cmax of melagatran (6.9 ± 2.7 μmol·h/l and 2.2 ± 1.0 μM) was significantly decreased (p < 0.05) when dogs received the oral dose with food compared with under fasting conditions (39.7 ± 18 μmol·h/l and 21.6 ± 13 μM). In humans, the median (range) of AUC and Cmax was decreased from 1.02 (0.31–1.38) μmol·h/l and 0.18 (0.06–0.27) μmol/l under fasting conditions to 0.14 (0.01–0.31) μmol·h/l and 0.03 (0.02–0.06) μmol/l when the tablet of melagatran was given together with food. The bioavailability of melagatran, calculated using the median value of AUC to be 7.6% when melagatran was administered to fasting subjects, was reduced to 1.1% when it was administered with breakfast. Likewise, when melagatran was administered 2 h after breakfast, its median AUC and Cmax were both decreased approximately 3-fold, compared with fasting conditions, and the bioavailability was estimated to 2.4%.

Pharmacokinetics of Ximelagatran. The mean plasma concentration–time profiles for those compounds with measurable plasma concentrations in rats, dogs, and human subjects are shown in Fig. 5. Melagatran was formed rapidly and was the predominant compound in plasma after both oral and i.v. administration. Consequently, the PK analysis was focused on melagatran. The PK parameters of melagatran following oral and i.v. administration of ximelagatran are presented in Tables 6 and 7. CLR of melagatran after i.v. administration of ximelagatran was 23.1 and 4.37 ml/min per kg body weight for rats and dogs, respectively, and 120 ml/min for humans.

In rats, the metabolism of ximelagatran was so rapid that no measurable concentrations of ximelagatran or ethyl-melagatran were detected in any of the rat plasma samples (Fig. 5). Besides melagatran, only OH-melagatran was detectable in rat plasma. After oral administration of ximelagatran to rats, the AUC of OH-melagatran was about 30% of that of melagatran. Following i.v. administration of ximelagatran, the AUC of OH-melagatran was higher than for melagatran, but the concentration of OH-melagatran declined rapidly and was below LOQ 30 min postdosing.

In the plasma of dogs, tmax of ximelagatran occurred 15 to 30 min post oral administration, and both intermediates were measurable (Fig. 5). The concentration of ximelagatran decayed faster in dogs after i.v. than after oral administration (t1/2 of 1.9 and 3.9 min for the 5 and 10 μmol/kg i.v. doses, respectively, compared with 32 min for the 40 μmol/kg oral dose), indicating absorption-limited kinetics for orally administered ximelagatran. Fximel = 37% after administration of the 40 μmol/kg oral dose. The exposure of ximelagatran was approximately 30 and 8% of that of melagatran after i.v. and oral administration of ximelagatran, respectively. The corresponding figures for the oral exposure of ethyl-melagatran were 12 to 21% and 17 to 22%, and for OH-melagatran were 23 to 25% and 7.5 to 13% of that of melagatran after i.v. and oral administration of ximelagatran, respectively.

In both rats and dogs, the mean plasma concentration of total radioactivity peaked at the same level and at the same time as did the sum of the plasma concentrations of those compounds that were detectable using LC-MS (ximelagatran, ethyl-melagatran, OH-melagatran, and melagatran). However, the total radioactivity in plasma declined with a longer t1/2 than did the total concentration of metabolites (14 to 24 h and 45 h in rats and dogs, respectively, after oral administration; 13 to 27 h and 54 h, respectively, after i.v. administration).

As in rats and dogs, ximelagatran was rapidly absorbed (mean tmax of 0.33 h postdosing) and metabolized (mean t1/2 of ximelagatran, 0.34 and 0.18 h post oral and i.v. administration, respectively) in the human subjects. Ximelagatran and both intermediates were observed in plasma in humans, although their exposure was low relative to that of melagatran. At sampling times where concentrations were <LOQ for some subjects, these were set to LOQ/2 for calculation of mean plasma concentrations shown in Fig. 5. The mean value of Fximel = 21.9 ± 1.7%. The exposure of ximelagatran was 18.3 and 36.1% of that of melagatran after oral and i.v. administration of ximelagatran, respectively. After oral administration the mean AUC estimates for ethyl-melagatran and OH-melagatran were 3.2 and 10.6% of that of melagatran, respectively. Likewise after i.v. administration, the mean AUC estimates for ethyl-melagatran and OH-melagatran were 4.2 and 12.8% of that of melagatran, respectively.

Following oral administration of [14C]ximelagatran, maximal total radioactivity in plasma reached a peak of 0.88 μL, 40 min postdosing, and declined with a t1/2 of 4.2 h in the 5 min to 8 h time period post drug administration. The plasma concentration of total radioactivity at tmax and times after tmax was consistently 0.1 to 0.2 μM higher than the sum of the plasma concentrations of ximelagatran, the intermediates, and melagatran. The total radioactivity in whole blood mirrored that in plasma but at lower levels.

Plasma Protein Binding and Blood–Plasma Partitioning. The degree of plasma protein binding and the partitioning between blood and plasma was low and concentration-independent for all species and compounds. The mean values for the plasma protein binding of melagatran were 10.6 and 10.0% in dogs and rats, respectively, with low variability. Of the human subjects, one male and one female had essentially no plasma protein binding of melagatran, whereas the other two had values of 13.3 and 15.3%. For ximelagatran, the mean percentage binding was 79% in humans and 76% in dogs. For ethyl-melagatran, the corresponding estimates were 44 and 63%, and for OH-melagatran they were 10 and 12% in humans and dogs, respectively. The mean blood-to-plasma concentration ratios of melagatran were 0.55 in dogs and 0.62 in rats. The individual mean values in humans ranged from 0.56 to 0.62. The mean
ratios of ximelagatran, ethyl-melagatran, and OH-melagatran ranged from 0.54 to 0.67 in dogs and humans.

**Discussion**

The PK properties of the oral direct thrombin inhibitor, ximelagatran, and in particular the formation and bioavailability of its active form, melagatran, were examined in animals and healthy male human subjects. Ximelagatran was rapidly absorbed and metabolized following oral administration to rats, dogs, and humans. Melagatran was the dominant compound in the plasma of all three species. It was demonstrated that the bioconversion of ximelagatran to its active form,
Melagatran, occurring by ester hydrolysis and reduction via two intermediates, ethyl-melagatran and OH-melagatran (Fig. 1). Ethyl-melagatran is formed by reduction of the hydroxyamidine and OH-melagatran by ester hydrolysis. In vivo reduction of an amidoxime derivative, pentamidoxime, to its corresponding amidine has been demonstrated previously (Clement, 1998). The PK properties of melagatran, following oral and i.v. administration, were also consistent between the animal species and humans. Melagatran has a relatively low plasma clearance, negligible plasma protein binding, a small volume of distribution, and a short elimination half-life. Renal excretion of unchanged melagatran was the main route of elimination. In humans, linear PK of melagatran were observed after i.v. dosing over the entire dose range studied with low interindividual variability. The calculated renal clearance of melagatran in rats and humans was similar to the glomerular filtration rate (Davies and Morris, 1993). The polar nature of melagatran, which prevents passive reabsorption from the proximal and distal tubules, and the fact that plasma protein binding is negligible, suggests that glomerular filtration is the mechanism of elimination in the kidneys. After i.v. administration of ximelagatran to rats, the renal clearance of melagatran was higher than that determined after administration of melagatran and higher than the glomerular filtration rate. This may be due to metabolism of ximelagatran and the formation of melagatran in the kidneys. In dogs and humans, Cl_{re} of melagatran was after i.v. administration of ximelagatran approximately equal to the glomerular filtration rate.

The low and dose-dependent oral bioavailability of melagatran in rats and humans is consistent with its polar properties and poor membrane permeability determined in the Caco-2 cell line (Gustafsson et al., 2001). The markedly higher bioavailability observed in dogs compared with rats and humans is probably the result of greater absorption across the gut wall in this species. This is consistent with a recent report reviewing the absorption data for a large number of compounds, which concluded that the fraction absorbed was markedly higher in dogs than in humans, whereas the absorption data obtained in rats were in much better agreement with data in humans (Chiu et al., 2000). The interindividual variability in melagatran exposure following its oral administration to humans was also large. The combination of low interindividual variability after i.v. administration and the strong correlation between C_{max} and AUC after oral administration suggest that the variability in plasma levels after oral administration is mainly due to variability in the extent of absorption. In the presence of food, the bioavailability of melagatran was significantly reduced in both dogs and humans.

Ximelagatran, for which the membrane permeability is 80 times higher than for melagatran (Gustafsson et al., 2001), was rapidly absorbed and metabolized following oral administration. In rats, the bioconversion of ximelagatran was so rapid that no detectable concentrations of ximelagatran were found in plasma after either i.v. or oral administration. Ethyl-melagatran was also undetectable in rat plasma. However, by using sodium dodecyl sulfate as an esterase inhibitor in the blood collection test tube (Holm et al., 1985), it was shown that both male and female rats had measurable concentrations of ximelagatran and ethyl-melagatran. This finding indicates that ester hydrolysis of ximelagatran occurred to some extent ex vivo in blood after collection of the sample. The levels of ximelagatran and ethyl-melagatran were much lower than that of melagatran, and the clearance of ximelagatran exceeded cardiac output (data on file), suggesting a high esterase activity in blood and tissues of rats. Also, OH-melagatran was present in rat plasma with high peak concentrations after both i.v. and oral administration but declined rapidly. In dogs and humans, the plasma concentrations of the intermediates were low compared with that of melagatran. The plasma concentration of OH-melagatran appeared to be higher than that of ethyl-melagatran, suggesting that the ester hydrolysis occurred more readily than the reduction.

In dogs and rats, there was a dose-dependent increase in the bioavailability of melagatran after both oral and i.v. administration of ximelagatran. In a previous study in which healthy male human subjects received escalating single oral doses of ximelagatran ranging from 5 to 98 mg, the bioavailability of melagatran was dose-independent and estimated to be about 20% (Eriksson et al., 1999b). This is consistent with the value of 19% observed at the dose of 50 mg.
(equivalent to approximately 0.3 \mu mol/kg) given to the male human subjects in the present study. Consequently, the dose-dependent increase in \( F_{\text{Mel}} \) observed in the animal experiments is likely due to the higher doses (40 \mu mol/kg) that were given to the rats and dogs. Possible reasons for the observed dose-dependent increase in \( F_{\text{Mel}} \) are an increase in the fraction of ximelagatran absorbed, increased metabolism of ximelagatran to melagatran, or decreased elimination of melagatran at higher doses. Dose proportional PK for i.v. melagatran was shown in humans and has also been demonstrated in rats and dogs for a wide range of doses (data on file), which supports that the elimination of melagatran is independent of dose.

The bioavailability of melagatran and the amount of melagatran
recovered in urine was higher after i.v. than after oral administration of ximelagatran, which suggests incomplete absorption. The relative bioavailability of melagatran after oral versus i.v. administration of ximelagatran, obtained as the ratio of the AUC estimates of melagatran and corrected for difference in dose, was 40 to 70% at the 10 μmol/kg dose given to rats and dogs. In humans, the relative bioavailability of melagatran was 43%. Similar estimates were obtained for the relative bioavailability of the intermediates, ethyl-melagatran (34%) and OH-melagatran (37%). Assuming that the metabolism of ximelagatran is the same after oral and i.v. administration, this means that 40 to 70% of the oral dose of ximelagatran is absorbed. As $F_{\text{ximel}}$ after oral administration to humans was only 21.9%, this suggests that first-pass metabolism of ximelagatran during absorption reduces its oral bioavailability. If the absorption of ximelagatran is incomplete, there is a possibility that it may be increased at the higher doses that were given to animals.

The estimate of the fraction of oral ximelagatran absorbed is uncertain because presystemic metabolism and sequential biliary excretion of ximelagatran or its products appeared to occur. It is therefore likely that the fraction absorbed is even higher than 40 to 70%. The principal route of excretion of the orally administered dose of ximelagatran was fecal in rats, dogs, and humans. High recovery of radioactivity in the feces was also observed after i.v. administration to rats and dogs, suggesting biliary excretion of ximelagatran and/or formed metabolites. In fact, this was demonstrated by the substantial fraction of the dose excreted in bile collected after oral administration of ximelagatran to rats and one dog. The predominant compound in the bile of rats was melagatran whereas about equal amounts of melagatran and ethyl-melagatran were found in bile from the dog. The dose-dependent increase in $F_{\text{Mel}}$ observed in the animal experiments could therefore be caused by saturation of the biliary excretion of melagatran and ethyl-melagatran.

In addition to being the major circulating compound in the plasma of all three species following oral and i.v. administration of ximelagatran, melagatran was also the major product found in urine and feces collected after oral and i.v. administration. Appreciable quantities of ethyl-melagatran were also recovered in fecal samples, whereas neither OH-melagatran nor ximelagatran were detected. Polar metabolites were also found that in general were present in larger amounts in feces than in urine. This may be due to instability of ximelagatran in the gastrointestinal tract. Anaerobic incubation of ximelagatran in human feces homogenate showed rapid disappearance of ximelagatran and formation of melagatran, ethyl-melagatran, and unknown polar metabolites. In the human subjects, the concentration of total radioactivity in plasma was higher than the sum of the plasma concentrations of ximelagatran, the intermediates, and melagatran. This suggests the premise that unknown metabolites were present in plasma, but it is also possible that this is an artifact, as the two assay methods may give slightly different results.

The plasma protein binding of ximelagatran, ethyl-melagatran, OH-melagatran, and melagatran was relatively low in both human and dog plasma, although that of ximelagatran and ethyl-melagatran was higher than that of OH-melagatran, the binding of which was similar to that of melagatran. The low blood-to-plasma concentration ratios for ximelagatran, ethyl-melagatran, OH-melagatran, and melagatran suggest a low affinity for, and a low penetration into, red blood cells.

In conclusion, ximelagatran was rapidly absorbed and converted to melagatran, the predominant compound in plasma and the active form of ximelagatran, following oral administration. The bioavailability of melagatran in humans was about 20%, presumably because of incomplete oral absorption of ximelagatran, but also first-pass metabolism of ximelagatran with subsequent biliary excretion of the formed metabolites.

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**References**


