In vivo Pharmacokinetics of Two New Thrombin Inhibitor Prodrugs

Emphasis on Intestinal and Hepatobiliary Disposition and the Influence of Interacting Drugs

ELIN MATSSON
Dissertation presented at Uppsala University to be publicly examined in B41, Biomedical Centre (BMC), Husargatan 3, Uppsala, Friday, June 11, 2010 at 13:15 for the degree of Doctor of Philosophy (Faculty of Pharmacy). The examination will be conducted in English.

Abstract

Biliary excretion is an important elimination route for many drugs and metabolites. For such compounds, it is important to know the extent of excretion and drug exposure in the bile, e.g., for the risk assessment of drug interactions, liver toxicity and the effects of genetic variants. In this thesis, duodenal aspiration of bile was performed in healthy volunteers and complemented with experiments in an in vivo model in pigs to increase the understanding of the intestinal and hepatobiliary disposition of two direct thrombin inhibitors.

The compounds investigated, ximelagatran and AZD0837, are both prodrugs that require bioactivation to exert their pharmacological effect. Upon co-administration with erythromycin and ketoconazole, respectively, altered plasma exposure to ximelagatran and AZD0837 and their respective metabolites has been observed. The main objective of this thesis was to characterize the biliary excretion of the compounds, and investigate whether this elimination route explains the observed drug-drug interactions.

High plasma-to-bile AUC ratios were observed, in particular for ximelagatran, its active metabolite melagatran, and AR-H067637, the active metabolite of AZD0837. These high ratios indicate the involvement of active transporters in the biliary excretion of the compounds, which is important since transporters constitute possible sites for drug interactions. The effects of erythromycin and ketoconazole on the plasma exposure of the prodrugs and metabolites were confirmed in both the pig and the clinical studies. The changes seen in plasma for ximelagatran and its metabolites were partly explained by reduced biliary clearance. Inhibited CYP3A4 metabolism likely caused the elevated plasma levels of AZD0837, whereas reduced biliary clearance was seen for AR-H067637 suggesting an effect on its excretion into bile. In summary, the studies led to mechanistic insights in the hepatobiliary disposition of ximelagatran and AZD0837, and demonstrate the value of combined clinical and animal studies for the investigation of the biliary drug excretion.

Keywords: Direct thrombin inhibitors, prodrugs, ximelagatran, AZD0837, erythromycin, ketoconazole, drug-drug interactions, hepatobiliary transport, biliary clearance, ATP-binding cassette, ABC transporters, solute carriers, SLC transporters, CYP3A4

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List of Papers

This thesis is based on the following papers, which are referred to in the text by the Roman numerals assigned here.


Reprints of Papers I and II were made with permission from the respective publishers.
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>A&lt;sub&gt;e&lt;/sub&gt;&lt;sub&gt;bile/intestine&lt;/sub&gt;</td>
<td>amount excreted into bile/intestine</td>
</tr>
<tr>
<td>APTT</td>
<td>activated partial thrombin time</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the plasma/bile concentration-time curve</td>
</tr>
<tr>
<td>CES</td>
<td>carboxylesterase</td>
</tr>
<tr>
<td>C</td>
<td>concentration</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximum concentration</td>
</tr>
<tr>
<td>CL</td>
<td>clearance</td>
</tr>
<tr>
<td>D</td>
<td>dose</td>
</tr>
<tr>
<td>DDI</td>
<td>drug-drug interaction</td>
</tr>
<tr>
<td>E&lt;sub&gt;G&lt;/sub&gt;</td>
<td>extraction ratio in the gut wall</td>
</tr>
<tr>
<td>E&lt;sub&gt;H&lt;/sub&gt;</td>
<td>extraction ratio in the liver</td>
</tr>
<tr>
<td>f&lt;sub&gt;abs&lt;/sub&gt;</td>
<td>fraction of dose absorbed</td>
</tr>
<tr>
<td>F</td>
<td>bioavailability</td>
</tr>
<tr>
<td>FV</td>
<td>coagulation factor V</td>
</tr>
<tr>
<td>FVIII</td>
<td>coagulation factor VIII</td>
</tr>
<tr>
<td>FXI</td>
<td>coagulation factor XI</td>
</tr>
<tr>
<td>hCE-1</td>
<td>human CES1 isoenzyme</td>
</tr>
<tr>
<td>hCE-2</td>
<td>human CES2 isoenzyme</td>
</tr>
<tr>
<td>iv</td>
<td>intravenous</td>
</tr>
<tr>
<td>k&lt;sub&gt;a&lt;/sub&gt;</td>
<td>absorption rate constant</td>
</tr>
<tr>
<td>k&lt;sub&gt;e&lt;/sub&gt;</td>
<td>terminal rate constant</td>
</tr>
<tr>
<td>K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>substrate concentration at half the theoretical maximum depletion rate</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>liquid chromatography followed by tandem mass spectrometry</td>
</tr>
<tr>
<td>MATE1</td>
<td>multidrug and toxin extrusion protein 1</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MS/MS</td>
<td>tandem mass spectrometry</td>
</tr>
<tr>
<td>N.A.</td>
<td>not applicable</td>
</tr>
<tr>
<td>OAT</td>
<td>organic anion transporter</td>
</tr>
<tr>
<td>OATP</td>
<td>organic anion transporting polypeptide</td>
</tr>
<tr>
<td>OCT</td>
<td>organic cation transporter</td>
</tr>
<tr>
<td>OCTN</td>
<td>organic cation/carnitine transporter</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>OH-melagatan</td>
<td>hydroxy-melagatan</td>
</tr>
<tr>
<td>P450</td>
<td>cytochrome P450 isoenzyme</td>
</tr>
<tr>
<td>QH</td>
<td>hepatic blood flow</td>
</tr>
<tr>
<td>SLC</td>
<td>solute carrier</td>
</tr>
<tr>
<td>t½</td>
<td>terminal half life</td>
</tr>
<tr>
<td>t_max</td>
<td>time to reach C_max</td>
</tr>
<tr>
<td>UDP</td>
<td>uridine 5’-diphosphate</td>
</tr>
<tr>
<td>V</td>
<td>apparent volume of distribution</td>
</tr>
<tr>
<td>VF</td>
<td>femoral vein</td>
</tr>
<tr>
<td>VH</td>
<td>hepatic vein</td>
</tr>
<tr>
<td>V_max</td>
<td>theoretical maximum depletion rate</td>
</tr>
<tr>
<td>VP</td>
<td>portal vein</td>
</tr>
</tbody>
</table>
1. Introduction

The pharmacological and toxicological effects of drugs are profoundly influenced by the concentration levels of the drug compound attained in the body. These, in turn, are determined by the extent to which the drug is absorbed, distributed to target tissues, and eliminated through metabolism or excretion into bile or urine, and the rate at which this takes place. Consequently, alterations in any of these pathways can affect plasma and tissue drug levels, and may ultimately lead to the loss of pharmacological effect or to drug-induced toxicity. Such alterations can, for example, occur in disease or as a result of food-drug or drug-drug interactions.

This thesis describes research conducted to clarify and elucidate two pharmacokinetic interactions that affect plasma levels of the direct thrombin inhibitor prodrugs, ximelagatran and AZD0837. The interactions were brought about by erythromycin and ketoconazole, respectively; these are compounds that are known to inhibit metabolizing enzymes as well as various transport proteins. The work presented here has focused, in particular, on the effect of the interacting drugs on the disposition of ximelagatran and AZD0837 in the intestine and in the liver. Investigation of the interactions has also served to improve the knowledge of these processes for the prodrugs and their respective metabolites. In a broader picture, the work conducted emphasizes the importance of understanding the intestinal and hepatobiliary disposition and exposure of drugs. Such knowledge is valuable for the assessment of the risk of drug-induced toxicity and the impact of pharmacokinetic drug-drug interactions.

1.1 Drug disposition in the intestine

The gastrointestinal tract is exposed to a considerable variety of substances and microorganisms, and needs to be able to distinguish between those that are essential for the body and those that the body needs to be protected against. For pharmaceutical compounds, the protective function of the gut must be circumvented so that orally administered drugs will reach the blood and the target tissue. In addition to its importance in the absorption of drug molecules, there are also reports that the intestine may contribute to the elimination of some drugs.1-3
1.1.1 Basic physiology of the intestine

The intestine is divided into two primary regions, the small and the large intestine, which differ in terms of anatomy and function.\(^4\,^6\) The small intestine is crucial for digesting food and absorbing nutrients; to accomplish this, it contains a range of digestive enzymes and is shaped to provide a large absorptive area. The small intestine is further divided into the sections duodenum, jejunum and ileum. In the lower part of the duodenum, the common bile duct and the pancreatic duct merge at the papilla of Vater, where they empty into the intestine. The large intestine is of importance for the absorption of ions and water. It also contains a vast number of different bacteria with the capacity to ferment carbohydrates and proteins, and to metabolize drugs in a reductive and hydrolytic manner.\(^7\,^9\) This may be of importance for drugs that, for various reasons (e.g. enterohepatic circulation or modified release systems), reach the colon.

The gastrointestinal motility depends on whether the body is in the fed or fasted state. However, irrespective of its state, the transit through the small intestine is consistently 3±1 hours with low intra- and interindividual variation.\(^10\) In contrast, the transit in the large intestine is highly variable, but always dominates the total time of the dosage form spent in the gastrointestinal tract.\(^11\) During the fasted state the peristalsis is governed by a cycle of movements that originates in the stomach and descends through the intestine.\(^12\) This interdigestive activity is referred to as the migrating motor complex and consists of four phases:

- Phase I: a resting phase (45-60 min) with rare contractions.
- Phase II: a phase (~40 min) with irregular contractions increasing in amplitude and frequency with time.
- Phase III: a phase (5-15 min) with intense contractions.
- Phase IV: a short phase when the contractions subside and lead to the quiescent state of phase I.

These movements in the gastrointestinal tract are of importance for the work conducted for this thesis, since they affect the emptying of the gall bladder, and hence, the volume of bile sampled from the individuals in the four clinical studies.

1.1.2 The enterocyte

The absorptive cells lining the gut lumen are called enterocytes. These cells are derived from stem cells located at the base of the villus and differentiate into highly polarized cells during their migration to the villus tip (Figure 1).\(^4\,\,^{13},^{14}\) Like epithelial cells in other organs, enterocytes mature to form two distinct cell surfaces, which differ in structure and membrane composition.\(^15\,\,^{16}\) The tight junctions that connect adherent cells are crucial
for maintaining this asymmetric distribution of membrane lipids and proteins.\textsuperscript{15}

Characteristic of the structure of the apical membrane, \textit{i.e.}, the one in contact with the gut lumen, are the microvilli, which function to increase the absorptive surface area of the gut (Figure 1).\textsuperscript{4} The basolateral membrane, which faces the underlying blood vessels, is considered to have a higher permeability than the apical membrane.\textsuperscript{17} Apart from differing in their membrane composition, and thereby affecting the simple passive diffusion of drugs through the membrane, they also exhibit different expression of the proteins that are important for carrier-mediated transport of drug molecules.\textsuperscript{18-22} A selection of such transport proteins of possible importance for the drug-drug interactions investigated in this thesis are described in the context of the hepatocyte (see Section 1.2.2., The hepatocyte).

\section*{1.2 Drug disposition in the hepatobiliary tract}

The liver plays an important role in the first-pass extraction, which occurs during the absorption phase, and in the subsequent elimination of drugs. These processes consist of metabolism, \textit{i.e.} the conversion of hydrophobic compounds into more water soluble forms, and excretion into bile.\textsuperscript{23-25} The metabolic reactions can be further divided into phase I and II reactions.\textsuperscript{26} Phase I metabolism includes reactions such as reduction, oxidation and hydrolysis, which add functional groups to the compound being metabolized. For drugs, the majority of these reactions are catalyzed by cytochrome P450 (P450) proteins, of which CYP3A4/5, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 dominate the metabolism of drugs currently on the market.\textsuperscript{27-30} Phase II enzymes catalyze the conjugation of hydrophilic functional groups
to the drugs, and include various glutathione S-transferases, UDP-glucuronosyltransferases and sulfotransferases.26, 31-33

The liver also has the capacity to eliminate drugs through excretion into the bile.25, 34-36 Compounds eliminated this pathway reach the intestine and can be either re-absorbed or eliminated with feces.23 The molecular properties associated with biliary excretion of a compound are a high molecular weight and the presence of polar groups.37-39 However, most biliary excretion data in the literature are based on investigations in rats or dogs.40-42 Consequently, care must be taken when extrapolating such results to humans, since there are important differences between the species with regard to, for example, the expression of transport proteins and the rate of bile flow.39, 43

1.2.1 Basic physiology of the liver

In addition to its high content of metabolizing enzymes, the importance of the liver for metabolic and excretion processes is greatly influenced by that venous blood flows directly to it from the gastrointestinal tract.44 The liver receives oxygenated blood from the hepatic artery, which diverges throughout the liver alongside the portal vein and the bile duct, and together these form the so called portal triads. The blood flow from the portal vein and hepatic artery merge into the sinusoidal space, which is filled with hepatocytes, and continues to the central veins (Figure 2).45 To further optimize the exchange of nutrients, xenobiotics and endogenous compounds between the blood and the liver, the endothelial cells separating the blood from the hepatocytes form a discontinuous layer with intercellular gaps. Through these gaps, compounds can come in direct contact with the lateral membrane of the hepatocyte.44

The bile canaliculi are formed between adjacent hepatocytes and separated from other extracellular space by the tight junctions between the cells. These canicular tubes merge to form bile ductules, and subsequently, ducts, leading to the gall bladder.45 The bile flow into the canaliculi is driven by the excretion of bile acids and other solutes from the hepatocytes, creating an osmotic gradient across the canicular membrane.46 About one third of the hepatic bile is directly diverted into the duodenum,47, 48 while the majority first enters the gall bladder, where it is concentrated and stored. The contraction and emptying of the gall bladder depends on the feeding state. In the fed state, nutrients reaching the duodenum trigger a contraction of the gall bladder, leading to a maximum emptying of approximately 60-75% of the gall bladder volume.49, 50 The emptying of the gall bladder in the fasted state is controlled by the migrating motor complex (see Section 1.1.1., Basic physiology of the intestine). The contraction of the gall bladder occurs at the end of phase II, and ultrasonographic measurements have shown that the volume of the gall bladder is reduced by 20-35% during this phase.51
Figure 2. Schematic picture of the liver and the gall bladder (A), the functional unit formed between the portal triad and the central vein (B), and two hepatocytes that transport drug compounds either into the blood or into the bile canaliculi. The arrows indicate the possible directions of drug transport across the hepatocyte membranes.

1.2.2 The hepatocyte

The hepatocyte is a polarized cell with three distinct membrane domains: (1) the canalicular (apical) membrane, which is rich in microvilli and faces the biliary channels (10-15% of the surface area); (2) the lateral membrane, which forms a smooth contact area between adjacent hepatocytes (15% of the surface area); and (3) the sinusoidal membrane, which has irregular microvilli that increase the surface area for efficient exchange of endogenous and exogenous substances with the blood.

Similar to the enterocyte, the uptake and efflux processes for drug molecules into and out of the cells are governed by a combination of passive and active transport mechanisms. Passive transport is often a result of diffusion through the lipid bilayer, but may also be facilitated by carrier proteins. In contrast, the involvement of transport proteins is a prerequisite for active transport. In the human body, a vast number of such transport proteins exists and these are primarily classified into two superfamilies: the ATP-Binding Cassette (ABC) transporters and the Solute Carriers (SLCs).

The main difference between these two superfamilies lies in the way they utilize energy. Substrate transport by ABC proteins is directly coupled to ATP hydrolysis, whereas SLCs include facilitative transporters, ion-coupled transporters, and exchangers, and substrate transport is driven through concentration gradients of the substrate itself or of a co- or counter-transported molecule.

A brief description of the transporters that may be of relevance for the drug-drug interactions investigated in this thesis follows:

**ABCB1.** This protein is expressed at the apical membrane in several tissues, where it plays pivotal roles in the body’s defense against toxins: in the intestine, it prevents the entry of xenobiotics into the body; in the liver and kidney, it facilitates the elimination of toxins from the body; and in
barrier tissues such as the blood-brain barrier and the placental trophoblasts it protects vital organs from exposure to foreign compounds.53, 55

ABCB1 belongs to the ABCB subfamily and is often referred to as P-glycoprotein or multidrug resistance protein 1. It was the first human ABC transporter to be discovered and cloned,62 and thus, has been extensively investigated with regard to, for example, substrate specificity, transport mechanisms, involvement in drug-drug interactions and the effects of genetic variants. The protein has a very broad substrate specificity, transporting numerous structurally diverse drug compounds, from different therapeutic classes. Most of its substrates are hydrophobic and either uncharged or cationic at physiological pH.56 Notably, a significant substrate overlap exists between ABCB1 and CYP3A4, which has been interpreted as a functional interplay between these proteins in detoxification processes.63

OATP1B1/3. OATP1B1 and OATP1B3 both belong to the organic anion transporting polypeptide (OATP) superfamily.60 Both are primarily expressed at the basolateral membrane of the hepatocytes,64, 65 where they mediate the uptake of mainly negatively compounds into the cell. OATP1B1 has a broad substrate specificity, transporting both endogenous and exogenous compounds such as bile acids, bilirubin, methotrexate, various statins and many conjugated compounds.60 It has also been extensively investigated with regard to genetic polymorphism, with a few genetic variants having been associated with increased systemic exposure of statin drugs, including pravastatin and pitavastatin.66, 67 OATP1B3 is sometimes referred to as a liver-specific transporter, owing to its seemingly exclusive expression at the basolateral membrane of the hepatocyte. The protein has a distinct but overlapping substrate specificity to that of OATP1B1.60

OAT2. This transporter is a member of the SLC22 family, which comprises the organic cation transporters (OCTs), the organic cation/carnitine transporters (OCTNs) and the organic anion transporters (OATs).58, 59 OAT2 transports small anionic compounds into the hepatocyte across the basolateral membrane, and is also expressed to a lesser extent in the kidney.68 Its involvement in drug transport has so far not been extensively investigated.

MATE1. The recently identified multidrug and toxin extrusion protein 1 (MATE1) is expressed at the apical membrane of renal tubule cells and hepatocytes, where it functions as a cation-proton exchanger.69, 70 Metformin, fexofenadine and tetraethylammonium are examples of those compounds that, so far, have been found to be substrates for this transporter. In addition to this, there are also reports of genetic variants of MATE1 that can affect drug disposition.71
Box 1. Drug-drug interactions

When the disposition or effect of two or more drugs is different after concomitant administration in comparison to when administered alone, they are said to interact with each other. Such interactions can result in the absence of a therapeutic effect or in severe side-effects, which in turn may lead to the termination of the drug’s development, prescribing restrictions or even withdrawal from the market.\textsuperscript{72, 73} When the alterations of the drug are caused by changes in the absorption, distribution, metabolism or elimination processes, they are referred to as pharmacokinetic interactions. In this thesis, two drug-drug interactions were investigated and hypothesized to occur at the level of metabolizing enzymes and transport proteins.

Interactions involving metabolizing enzymes. Most reported drug-drug interactions are caused by the inhibition of metabolizing enzymes, in particular the P450 isoenzymes.\textsuperscript{28} This overrepresentation compared to other mechanisms potentially involved in pharmacokinetic drug-drug interactions, such as inhibition of transport proteins, may be a result of the relatively long time that these enzymes have been known, and that a rather limited number of P450s are involved in the majority of drug metabolism. The available data and the long experience of P450 interactions have allowed the regulatory agencies to establish guidelines for the pharmaceutical industry supporting their drug development programs.\textsuperscript{72, 74} These guidelines include recommendations on suitable in vitro and in vivo studies to perform in order to discover effects arising from induced or inhibited enzymes as early as possible in drug development.

Interactions involving transport proteins. Transport proteins are expressed in most tissues in the body and may, therefore, influence the absorption and disposition of drugs. However, as a potential site for drug-drug interactions, transporters are difficult to investigate owing to the deficiency of specific inhibitors, antibodies and substrates for many of the transporters.\textsuperscript{18, 75} The efflux pump ABCB1 is probably the most well characterized transporter, yet the numbers of clinically relevant interactions reported that have been attributed exclusively to ABCB1 are few.\textsuperscript{76} The large overlap in substrate specificity with CYP3A4 and the lack of specific probes makes it difficult to determine the relative contributions of the different mechanisms. So far, regulatory guidelines for transporter studies, such as those established for P450s, are only available for ABCB1.\textsuperscript{74} To fill this gap, an international transporter consortium uniting academic, industrial and regulatory expertise was recently formed, and has published advice for decision-making related to transporter interaction studies.\textsuperscript{18}
1.3 Direct thrombin inhibitors

Ximelagatran and AZD0837, the drugs investigated in this thesis, both belong to the pharmacological class of direct thrombin inhibitors. Thrombin plays a pivotal role in the coagulation cascade through its procoagulant activities, such as the formation of fibrin and the activation of platelets and coagulation factor V (FV), FVIII, FXI, and FXIII. As a result of this, thrombin is one of the key target proteins for anticoagulants, for those available on the market and for those undergoing clinical development. The thrombin inhibitors are classified into indirect or direct thrombin inhibitors, depending on the mechanism by which they affect the activity of the protein. Indirect thrombin inhibitors (e.g. heparins) exert their inhibitory effect by binding to antithrombin and increasing its affinity for thrombin, whereas direct thrombin inhibitors specifically bind to and block the active site of thrombin.

At present, the first choice for anticoagulant treatment is warfarin, which has the great advantage over other current treatments of being administered orally. However, the use of warfarin is complicated by safety issues arising from its narrow therapeutic window, in combination with its unpredictable pharmacokinetics and numerous food and drug-drug interactions. An effective and safe oral anticoagulant that can be prescribed and taken without the need for plasma concentration monitoring, would therefore be of great value for the improvement of current cardiovascular therapy.

The early design of direct thrombin inhibitors was based on the interaction between thrombin and its substrates, hirudin and fibrinopeptide A, with a particular interest being placed on the potent tripeptide sequences Phe-Val-Arg and Phe-Pro-Arg. Highly basic and polar functional groups are characteristic for compounds in this substance class. The amidine group seems to be important for the affinity to the active site of thrombin, but being a polar and positively charged (at physiological pH) group it also complicates the oral absorption. One means of managing this delicate balance has been to convert the active compound into a more lipophilic prodrug and thereby increase its intestinal permeability. In doing this, the compounds can be given orally, and this is usually the preferred administration route by the patients and the manufacturer alike. Such a prodrug approach was successfully used in the development of the active compound of both ximelagatran and AZD0837. Developing a prodrug from a promising drug candidate makes it possible to improve the pharmacokinetic profile of the drug, but is also a very challenging approach in that it requires efficient absorption of the prodrug, rapid formation of the active metabolite and sufficient concentrations of the active compound at the site of its pharmacological effect before it is eliminated.
1.3.1 Ximelagatran

Ximelagatran was the first oral anticoagulant to be launched on the market after the introduction of warfarin about 60 years ago.\textsuperscript{92} It was approved in 2004 in several countries for short-term usage after elective hip or knee replacement surgery, and showed that it is possible to develop effective oral anticoagulants without the need for coagulant monitoring.\textsuperscript{94-96} However, after only two years on the market, ximelagatran was withdrawn out of concern that long-term exposure (>35 days) to the compound might be associated with a risk of adverse effects on the liver.\textsuperscript{97-100}

The formation of the active metabolite of ximelagatran involves two metabolic reactions: ester-hydrolysis (by unspecific carboxylesterases) and reduction (by the N-hydroxylamine reductase enzyme system).\textsuperscript{101-103} Two intermediate metabolites are formed during the biotransformation: OH-melagatran and ethyl-melagatran (Figure 3). In fact, ethyl-melagatran is also a potent inhibitor of thrombin, but it is present at too low plasma concentrations in humans to contribute to the efficacy of the drug.\textsuperscript{104}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{ximelagatran_biotransformation.png}
\caption{The biotransformation of ximelagatran to its active metabolite melagatran requires two metabolic steps: hydrolysis of the ethyl group on the carboxylic acid and reduction of the hydroxyl group on the amidine.}
\end{figure}

During the clinical development of ximelagatran, several drug-drug interaction studies were conducted.\textsuperscript{105-109} These revealed that the well-studied CYP3A4 inhibitor erythromycin increased the plasma exposure of OH-melagatran, ethyl-melagatran and melagatran.\textsuperscript{109} This had not been expected, since no P450 isoenzymes are involved in the biotransformation of the prodrug.\textsuperscript{103} However, erythromycin is also known to inhibit the efflux transporter ABCB1\textsuperscript{110, 111} and results obtained from in vitro experiments suggested that ximelagatran, OH-melagatran and melagatran were substrates for ABCB1.\textsuperscript{109} Taken together with mass balance data indicating biliary
excretion as one of the elimination routes for melagatran, it was hypothesized that erythromycin could exert its effect on ximelagatran disposition via inhibition of this transporter. These results led to the conduction of two of the investigations presented here (Papers I and II).

1.3.2 AZD0837

AZD0837 is a direct thrombin inhibitor under clinical development for the prevention of venous thromboembolic events. In both healthy volunteers and patients with atrial fibrillation, the compound has shown good tolerability and safety with low to moderate variability in the pharmacokinetic exposure and anticoagulant effect.

Two metabolic steps are required to biotransform AZD0837 to its active metabolite AR-H067637: the first step is a demethylation catalyzed by CYP3A4, CYP2C9 and CYP2C19, and the second is a reduction step, mediated by the same enzyme system as is known for the reduction of ximelagatran (Figure 4).

![Figure 4. The biotransformation of AZD0837 involves two reactions: (1) demethylation and (2) reduction.](image)

The involvement of P450 isoenzymes in the metabolism of AZD0837 may make the compound susceptible to drug-drug interactions, and consequently, the potential for this has been investigated in a number of clinical trials. In a single-dose interaction study, ketoconazole was shown to elevate the plasma exposure of AZD0837 and its active metabolite approximately two-fold. The effect on the prodrug was explained by ketoconazole being a potent inhibitor of CYP3A4, whereas the increased plasma concentrations of AR-H067637 were unexpected. Similar to the case with ximelagatran, it was hypothesized that inhibited biliary excretion of the active compound causes the interaction, and this was the focus of two of the papers included in this thesis (Papers III and IV). The hypothesis was based on the ability of ketoconazole to inhibit several hepatic transport proteins, and clinical data suggesting the contribution of biliary excretion to the pharmacokinetics of AZD0837 and its metabolites.
Box 2: Pharmacokinetics

The focus of the investigations in this thesis was to study ximelagatran and AZD0837 from a pharmacokinetic perspective; 
i.e., the aspect of interest has been to investigate what the body does to the drugs. Several important parameters are needed to describe the pharmacokinetics of a drug. A selection of the pharmacokinetic parameters used in the work conducted for this thesis is introduced here and the terms are explained briefly:

**Bioavailability, F:** The fraction of the dose that reaches the systemic circulation in its unchanged form. The oral bioavailability is determined by the absorption of the drug \( f_{abs} \) and its extraction during passage through the intestine \( (E_G) \) and the liver \( (E_H) \) in accordance with:

\[
F = f_{abs} \cdot (1 - E_G) \cdot (1 - E_H)
\]

**Fraction of dose absorbed, \( f_{abs} \):** The fraction of an oral dose that permeates into the enterocytes.

**Hepatic extraction ratio, \( E_H \):** The fraction of drug that is extracted by the liver during its passage through the organ. It is dependent upon three factors: the blood flow in the liver, the intrinsic clearance and the fraction of the drug that is unbound in blood.

**Clearance, \( CL \):** The proportionality constant between the rate of drug elimination and the plasma concentration. It reflects the rate of removal from the systemic circulation and can also be determined for a specific eliminating organ.

**The apparent volume of distribution, \( V \):** The proportionality constant between the amount of the drug in the body and the plasma concentration. It is determined by the size of physical distribution phases and by the interaction of the drug with body components in plasma and tissue.

**The area under the concentration-time curve, \( AUC \):** This term describes the exposure to the drug in different compartments, for example, plasma and bile.

**The half-life, \( t_{1/2} \):** This secondary parameter is determined by the volume of distribution and clearance of the drug, and describes how long it takes to eliminate 50% of the drug from the body.
2. Aims of the thesis

The general aim of this thesis was to elucidate the in vivo mechanisms responsible for two clinical pharmacokinetic interactions affecting the thrombin inhibitor prodrugs ximelagatran and AZD0837, and thereby improve the understanding about the processes that are important for the disposition of the prodrugs and their respective metabolites.

The specific aims were:

- To investigate the intestinal and hepatobiliary disposition of ximelagatran and AZD0837 and their respective metabolites using an advanced pig model.

- To use the pig model to evaluate the influence of concomitantly administered drugs, known to affect the pharmacokinetics of ximelagatran and AZD0837, on the intestinal and hepatobiliary disposition of the prodrugs.

- To characterize the biliary excretion of $[^{14}\text{C}]$ximelagatran and $[^{3}\text{H}]$AZD0837, and of their known and possible unknown metabolites, in healthy volunteers.

- To estimate the biliary clearance of ximelagatran and AZD0837 and their respective metabolites in healthy volunteers, and to determine if the interactions under investigation occur at the level of this elimination route.
3. Methods

3.1 Investigations in pigs

Pigs were used as the in vivo model to study the biotransformation, the hepatobiliary disposition and the intestinal secretion of the prodrugs ximelagatran and AZD0837 and the respective metabolites (Papers I and III). Specifically, these investigations focused on the interactions between ximelagatran and erythromycin, and between AZD0837 and ketoconazole. The pharmacokinetics of the compounds were assessed in the bile duct, the portal (VP), hepatic (VH) and femoral (VF) veins and in a perfused intestinal segment after enteral dosing of the drugs under investigation (Figure 5). The prodrugs and their active metabolites were also administered intravenously as reference doses for the pharmacokinetic analysis.

Figure 5. The experimental model in pigs in which catheters have been inserted into the bile duct, the portal (VP), the hepatic (VH) and the femoral (VF) veins, and a jejunal segment has been isolated to allow controlled perfusion of drug solution.

Animals. The pigs (Hampshire, Yorkshire and Swedish Landrace) included in the studies were 10-12 weeks old and weighed on average 27 (±3) kg. The handling of the animals followed national regulations and the protocols for the investigations were approved by the local ethics committee for animal experiments (C 33/5, C 257/6, C 276/9).
Study design and investigational drugs. The interaction studies in pigs were performed using a parallel study design: one group of animals received the prodrug (ximelagatran or AZD0837) alone and the other group was given the prodrug together with the interacting drug (erythromycin or ketoconazole). Up to 6 h after administration of the investigational drugs, samples were taken from three venous blood vessels, the bile duct and from a perfused intestinal segment.

The drugs were given intra-intestinally via a multi-channel tube (Loc-I-Gut®, Synectics Medical, Stockholm, Sweden) positioned in the proximal jejunum of the animals (Figure 5). Both of the doses given in Paper I (200 mg ximelagatran, 250 mg erythromycin) were prepared by dissolving the respective substance in isotonic sodium chloride (pH 4 for ximelagatran). Erythromycin was administered in the form of erythromycin lactobionate (Abboticin®, Abbot, Sweden) 20 min prior to the ximelagatran dose being given. In Paper III, AZD0837 (500 mg) was dissolved in polyethylene glycol 400/ethanol/water (v/v/v, 20/5/75) with 5 mM citric acid, and the ketoconazole dose (600 mg) was prepared by dispersing tablets (Fungoral®, Janssen-Cilag, Sweden) in water. Ketoconazole was given to the animals 30 min before administration of AZD0837. Ximelagatran and AZD0837 were provided by AstraZeneca R&D Mölndal, Sweden.

Additional animals were included in each study to receive the prodrugs and the active metabolites intravenously (iv) as reference doses: ximelagatran (15 mg), melagatran (15 mg), AZD0837 (200 mg) and AR-H067637 (100 mg).

Surgical procedure. The anesthesia and surgical procedure are described in detail in Paper I. Briefly, the animals were kept anesthetized, sedated and mechanically ventilated throughout the experiment. Their physiological state was satisfactory as controlled for by monitoring the body temperature, blood gases, heart rate, and arterial and central venous pressures. The abdominal cavity was opened with a midline incision to enable cannulation of the bile duct and the portal vein, and to insert the perfusion tube into the proximal jejunum. Catheters for blood sampling were also positioned in the hepatic and femoral veins. At the end of the experiment the animals received a lethal iv dose of potassium chloride when still under anesthesia.

Collection of biological samples. Blood samples were withdrawn from the portal (VP), hepatic (VH) and femoral (VF) veins prior to dosing, and 10, 30, 50, 70, 90, 110, 130, 150, 170, 190, 240, 300, and 360 min after the administration of investigational drugs. The bile was sampled continuously in 20 min fractions throughout the 6 h long experiments. Perfusion of an intestinal segment (of length 10 cm) with isotonic phosphate buffer (pH 6.5) was performed during 100 or 360 min with a fraction sampling time of 10 or 20 min depending on the length of the perfusion.
**Bioanalysis.** The concentrations of ximelagatran and AZD0837 and the respective metabolites in plasma, bile and perfusate samples were determined at AstraZeneca R&D Mölndal, Sweden. The analytical system used consisted of liquid chromatography followed by mass spectrometry (LC-MS/MS) and is described in Papers I and III. Determination of the ketoconazole concentration in the plasma samples was performed at Uppsala University using the LC system coupled with ultraviolet spectrophotometric detection (Paper III).

### 3.2 Investigations in healthy volunteers

The clinical trials included in the thesis were performed with the primary objective of assessing if biliary clearance-related effects could explain the interactions between ximelagatran and erythromycin, and between AZD0837 and ketoconazole (Papers II and IV). For this purpose, the thrombin inhibitor prodrugs were first administered as a radiolabeled compound to healthy volunteers, to characterize the biliary excretion of each prodrug and of their respective metabolites (radioactive studies). These studies were followed by randomized cross-over interaction trials (drug-drug interaction studies). In all of the investigations, bile was collected by duodenal aspiration via the multi-channel Loc-I-Gut® catheter, which was also used for administration of the drugs under study (Figure 6).

![Figure 6](image)

*Figure 6.* The multi-channel Loc-I-Gut® tube was located in the proximal jejunum of the healthy volunteers and its position was verified by fluoroscopy. The catheter was used for administration of the study drugs and duodenal aspiration of bile. The site of administration and of aspiration were separated by a balloon attached to the tube.
The investigations were conducted at the Clinical Research Department, Uppsala University Hospital, Sweden. The protocols were approved by the Swedish Medical Products Agency and the local independent research ethics committee in Uppsala, and the investigations were performed in accordance with good clinical practice guidelines and the Declaration of Helsinki. The healthy subjects included in the studies all provided informed consent to their participation.

The volunteers were ascertained to be healthy from their medical history, a physical examination and routine laboratory tests. The exclusion criteria and the restrictions ensured, for example, that the subjects included did not use any drugs (other than occasional use of acetaminophen), St. John’s wort, grapefruit juice, nicotine or caffeine. The subjects were also instructed to have fasted since the evening before the day of the study and were given standardized meals following the removal of the tube. The subjects were not allowed to leave the site on which the study was conducted until the coagulation parameter APTT was below the predose value +10 sec.

3.2.1 Radioactive studies

Number of subjects. In Paper II, four healthy male volunteers were enrolled for \([14C]ximelagatran\) administration and all of them successfully completed the study. In Paper IV, when \([3H]AZD0837\) was administered, eight healthy male subjects were enrolled, six of whom were included in the final data analysis as one subject was excluded because the administration of the investigational product deviated from the protocol and another because the volume of bile obtained during the experiment was insufficient (<3 ml after 1 h of sampling).

Study design and investigational products. Both trials consisted of 1) a pre-entry visit, taking the form of a clinical examination, 2) the day when the study was conducted and on which the investigational product was administered, and 3) a follow-up visit to ensure the wellness of the subjects.

The drugs were administered intra-intestinally through the Loc-I-Gut® perfusion tube positioned in the duodenum/proximal jejunum of the subjects. This multi-channel catheter was also used for aspirating bile from the intestine at a site completely separated from the site of drug administration by an inflated balloon. Bile was aspirated continuously from the intestine during the three hours immediately subsequent to dosing of the respective prodrug. Blood samples were collected for up to 12 h and 24 h following administration of \([14C]ximelagatran\) and \([3H]AZD0837\), respectively.

The drugs being examined in the study were provided in the form of solutions by AstraZeneca R&D Mölndal, Sweden. The doses were dispensed at the clinical site: 36 mg \([14C]ximelagatran\) (1 MBq) and 350 mg \([3H]AZD0837\) (7.4 MBq). To ensure that the full dose was given the syringe
and catheter used for administration were rinsed with water after each drug dosing.

**Bioanalysis.** The concentrations of ximelagatran and AZD0837 and the respective metabolites in plasma and bile were determined by LC-MS/MS as detailed in Papers II and IV. These analyses were either outsourced or performed by AstraZeneca R&D Mölndal, Sweden. The total amount of radioactivity was determined in each bile sample by scintillation counting. The samples containing sufficient amounts of radioactivity were further analyzed by LC and transferred for fraction collection into deep-well microplates using a fraction collection time of 0.08 min ([14C]ximelagatran) or 0.13 min ([3H]AZD0837). The radiochromatograms obtained were used to prioritize the collected fractions for structure elucidation by MS.

3.2.2 Drug-drug interaction studies

**Number of subjects.** In the ximelagatran and AZD0837 interaction studies 16 and 19 volunteers, respectively, were randomized for the treatment. The number of volunteers who successfully completed the trials was 13 (ximelagatran) and 17 (AZD0837). The reasons for the premature withdrawal from the studies were either non-compliance with the technique used for the study or with the inclusion criteria.

**Study design and investigational products.** An open-labeled, cross-over design was used in both studies. The trials comprised a pre-visit for physical examination of the subjects, two study periods separated by a wash-out period of 14-28 days (ximelagatran trial) or 7-21 days (AZD0837 trial), and a follow-up visit for physical examination and safety laboratory tests. In a randomized order, the subjects received the prodrug given alone and in combination with the interacting drug. The drug administration and collection of plasma and bile were performed as described above for the radioactive studies.

In Paper II, ximelagatran (36 mg) and erythromycin (500 mg) were administered as single doses, with erythromycin being administered 15 min before ximelagatran. In Paper IV, repeated dosing was used for ketoconazole, which was administered once daily on four successive days (4×400 mg) and co-administered with AZD0837 (175 mg) on Day 4. All drugs included in the study were provided by AstraZeneca R&D Mölndal, Sweden, and were dispensed at the clinical site. The ximelagatran dose was prepared by dispersing tablets in sterile water, and erythromycin, supplied in the form of erythromycin lactobionate powder (Abbotacin®, Abbot Scandinavia AB, Solna, Sweden) was dissolved in sterile water. AZD0837 was provided in solution, whereas ketoconazole was administered as tablets (Days 1-3) and tablets dispersed in sterile water (Day 4). A solution of non-
absorbable $[^{14}\text{C}]$-labeled polyethylene glycol 4000 ($[^{14}\text{C}]\text{PEG} \ 4000$) containing 18.5 kBq was added to the ximelagatran and AZD0837 dispersions to monitor possible leakage of the investigational drugs from the site of administration to the site of bile sampling. Following each dosing the beaker, syringe and catheter used for administration were rinsed with water to ensure that the entire dose reached the intestine.

**Bioanalysis.** The bioanalysis was performed using LC-MS/MS for the determination of all compounds in plasma and bile, and is described in detail in Papers II and IV. The analyses were either outsourced by or performed by AstraZenca R&D Mölndal, Sweden.

### 3.3 Pharmacokinetic analysis

The pharmacokinetic parameters area under the plasma/bile curve (AUC), maximum plasma/bile concentration ($C_{\text{max}}$) and time to reach $C_{\text{max}}$ ($t_{\text{max}}$) were determined by non-compartmental analysis using WinNonlin (Pharsight Corp., Mountain View, USA). Regression analysis of the last 3-5 data points in the terminal part of the log concentration-time curve yielded the terminal rate constant, $k_e$, which was used to calculate the terminal half-life ($t_{1/2}$) and to extrapolate the AUC to infinity. The AUC was derived by means of the linear/logarithmic trapezoidal rule (for the up and down portions of the curve, respectively).

The amount of the compounds recovered in bile ($A_{\text{bile}}$) was calculated and expressed as a percentage of the administered dose. The mean biliary flow was determined for each collected fraction.

**Parameters specific to the pig model.** In the animal studies, the prodrugs were given both intra-intestinally and iv, and hence, the bioavailability (F) and fraction of dose absorbed ($f_{\text{abs}}$) could be calculated. The $f_{\text{abs}}$ was calculated from the biliary excretion data (amount excreted in bile, $A_e$) assuming that the clearance (CL) of the compounds was unaffected by the administration route:

$$f_{\text{abs}} = \frac{A_{e,\text{bile,enteral}}}{A_{e,\text{bile,iv}}} \cdot \frac{dose_{\text{iv}}}{dose_{\text{enteral}}}$$

The hepatic extraction ratio ($E_{\text{H}}$) was determined from the difference in exposure to the compound in the portal and hepatic veins, and was used to obtain the hepatic clearance (CLH):
\[ E_H = \frac{AUC_{VP} - AUV_{VH}}{AUC_{VP}} \]

\[ CL_{HH} = E_H \cdot Q_H \cdot \frac{C_{blood}}{C_{plasma}} \]

where \( Q_H \) is the hepatic blood flow. The biliary clearance (\( CL_{bile} \)) and intestinal clearance (\( CL_{intestine} \)) were also determined:

\[ CL_{bile} = \frac{Ae_{bile}}{AUC_{VP}} \]

\[ CL_{intestine} = \frac{Ae_{intestine}}{AUC_{VF}} \]

The \( CL_{bile} \) is a complex term since it includes several processes; uptake into the hepatocytes, metabolism, formation (for the metabolites) and efflux into the bile canaliculi. The parameter is therefore referred to as the apparent \( CL_{bile} \) throughout the thesis.

**Parameters specific to the clinical trials.** In humans, the apparent \( CL_{bile} \) was calculated based on the exposure to the compound in a peripheral vein. The parameter is, therefore, more accurately described as \( CL_{bile}/F_H \). Notably, if an interacting drug affects the \( CL_{bile} \) of a compound, it may also change the \( F_H \). This means that interactions caused by a changed \( CL_{bile} \) can be confounded when comparing \( CL_{bile}/F_H \)-values obtained with and without co-administration of an interacting drug.

In Paper II, the concentration of erythromycin (\( C_{max,portal} \)) entering the liver was estimated from its systemic \( C_{max} \) in plasma by adding the contribution from the intestinal absorption. This concentration of erythromycin was corrected for the fraction bound to plasma proteins and then used for comparison with reported IC\(_{50}\)-values, to assess the potential for the in vivo inhibition of different transport proteins by erythromycin.

\[ C_{max,portal} = C_{max,systemic} + \frac{k_a \times D \times f_{abs} \times F_G}{Q_H} \times \frac{C_{plasma}}{C_{blood}} \]

where \( k_a \), \( D \), and \( F_G \) are the absorption rate constant, the dose and the fraction of dose entering the enterocytes that escapes gut wall metabolism, respectively.
3.4 In vitro metabolism study

In Paper III, we investigated whether AR-H067637, the active metabolite of AZD0837, was metabolized in pig liver microsomes and, if so, whether this process was affected by ketoconazole. AR-H067637 was incubated at several concentrations (200, 50, 10, 2, and 0.5 μM) with and without the addition of ketoconazole, and its depletion was measured over a time period of 60 min. The multiple depletion curves method was then used to estimate the intrinsic clearance (CL\text{int}) of AR-H067637, by simultaneous non-linear fitting of the concentration-time profiles for all concentration levels (WinNonlin, Pharsight Corp., Mountain View, USA) through\textsuperscript{122}:

\[
- \frac{d[C]}{dt} = v = \frac{V_{\text{max}} \cdot [C]}{K_m + [C]}
\]

where [C] is the substrate concentration, \(V_{\text{max}}\) is the theoretical maximum depletion rate and \(K_m\) is the substrate concentration at half the theoretical maximum depletion rate. The CL\text{int} was obtained by dividing \(V_{\text{max}}\) by \(K_m\) and was normalized to the protein concentration used in the incubation.
4. Results and discussion

The primary aim of the clinical and pre-clinical studies included in this thesis was to elucidate two pharmacokinetic interactions and thereby increase the understanding of the biotransformation and the hepatobiliary disposition of the prodrugs under investigation.

4.1 The biotransformation of ximelagatran and AZD0837

4.1.1 Plasma concentration-time profiles of the prodrugs and metabolites in humans

The two thrombin inhibitors investigated in this thesis, ximelagatran and AZD0837, require bioactivation to exert their pharmacological effect. Both prodrugs are designed to rapidly form the active metabolites following administration, as observed from their plasma concentration-time profiles in humans (Figure 7A and 8A).

Figure 7. The plasma concentration-time profiles (mean and SEM) for ximelagatran, OH-melagatran, ethyl-melagatran, and melagatran in (A) healthy volunteers and (B) pigs.
Ximelagatran peaked early \( (t_{\text{max}}=0.5 \text{ h}) \) following intra-intestinal administration of ximelagatran dispersion (36 mg) to healthy volunteers (Table 1). Its active metabolite, melagatran, formed via either of the intermediate metabolites, reached \( C_{\text{max}} \) approximately two hours after dosing and was eliminated with a terminal \( t_{1/2} \) of 3.1(±0.9) h. Melagatran was the predominant compound in plasma and constituted 80(±1)% of the total plasma AUC\(_{0-12h}\).

The \( C_{\text{max}} \) in plasma of AZD0837, its intermediate and active metabolite, all appeared within less than one hour of the intra-intestinal dose of AZD0837 being given in solution (175 mg) (Table 2). AR-H067637, the active compound, was eliminated more slowly than the prodrug and intermediate, and had a terminal \( t_{1/2} \) of 7.0(±0.9) h. The major compounds in plasma were AZD0837 and AR-H067637, which had a plasma exposure of 63(±5)% and 21(±4)% of the total AUC\(_{0-\infty}\), respectively.

The biotransformation of ximelagatran and AZD0837 both include two metabolic steps (Figures 3 and 4). One of the reactions, the reduction of the N-hydroxylamine, is catalyzed by the same three-component enzyme system for both compounds. The other step deviates between the prodrugs: the ester-hydrolysis of ximelagatran is mediated by carboxylesterases, whereas the demethylation of AZD0837 involves CYP3A4, CYP2C9 and CYP2C19.\(^{102,114,115}\)

### 4.1.2 Plasma concentration-time profiles of the prodrugs and metabolites in pigs

The plasma concentration-time profiles following enteral ximelagatran administration were similar in healthy volunteers and pigs (Figure 7 and Table 1). Melagatran was the major compound circulating in the plasma in both species, but the intermediate, OH-melagatran, contributed to a greater...
extent to the sum of the total plasma exposure for ximelagatran and its metabolites in pigs than in humans.

Table 1. Parameters (mean and sd or median and range) describing the plasma pharmacokinetics of ximelagatran and its metabolites in humans\(^a\) and pigs\(^b\).

<table>
<thead>
<tr>
<th></th>
<th>ximelagatran</th>
<th>OH-melagatran</th>
<th>ethyl-melagatran</th>
<th>melagatran</th>
</tr>
</thead>
<tbody>
<tr>
<td>(t_{\text{max}}) (h)(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humans</td>
<td>0.2 (0.2-0.2)</td>
<td>0.7 (0.5-1.2)</td>
<td>1.2 (0.8-1.2)</td>
<td>1.5 (1.2-2.5)</td>
</tr>
<tr>
<td>pigs</td>
<td>0.2 (0.2-0.5)</td>
<td>0.9 (0.5-1.9)</td>
<td>0.9 (0.5-1.8)</td>
<td>1.7 (1.2-2.2)</td>
</tr>
<tr>
<td>(t_{\frac{1}{2}}) (h)(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humans</td>
<td>0.4 (0.3)</td>
<td>0.9 (0.06)</td>
<td>1.1 (0.03)</td>
<td>2.9 (0.1)</td>
</tr>
<tr>
<td>pigs</td>
<td>0.2 (0.1)</td>
<td>1.4 (0.4)</td>
<td>1.9 (0.3)</td>
<td>2.5 (1.1)</td>
</tr>
<tr>
<td>AUC (%)(^d,)(^e)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humans</td>
<td>13 (2)</td>
<td>5 (1)</td>
<td>2 (1)</td>
<td>80 (1)</td>
</tr>
<tr>
<td>pigs</td>
<td>6 (2)</td>
<td>32 (7)</td>
<td>4 (1)</td>
<td>58 (6)</td>
</tr>
</tbody>
</table>

\(^a\) Investigation in healthy volunteers when 36 mg \([^{14}\text{C}]\)ximelagatran was administered intraintestinally (n=4) (Paper II). \(^b\) Investigation when 200 mg ximelagatran was given intraintestinally to pigs (n=6) (Paper I). \(^c\) \(t_{\text{max}}\) is presented as median and range. \(^d\) Expressed as mean and sd. \(^e\) Expressed as a percentage of the sum of the plasma AUC\(_{0-\infty}\) for ximelagatran, OH-melagatran, ethyl-melagatran, and melagatran.

In pigs, the metabolite pattern in plasma depended on whether ximelagatran was introduced enterally or intravenously. This was evident from the exposure to the intermediate metabolites. Iv administration resulted in a dramatically decreased presence of OH-melagatran in plasma compared to enteral administration; instead of OH-melagatran, ethyl-melagatran was the major circulating intermediate, constituting about 20% of the sum of the AUC\(_{0-\infty}\) for ximelagatran and its metabolites. Route-dependent metabolism of ximelagatran has not been observed in humans or rats.

The higher plasma concentrations of OH-melagatran after enteral administration in comparison to those obtained intravenously suggested that the formation of this intermediate occurred primarily during the passage of ximelagatran through the intestinal epithelium. This metabolic reaction is mediated by unspecific carboxylesterases (CESs), of which there are two major forms in humans, hCE-1 and hCE-2 (the human CES1 and CES2 isoenzymes, respectively).\(^{123, 124}\) These isoenzymes differ in terms of their substrate specificity: hCE-1 preferentially hydrolyzes ester-containing compounds, which, like ximelagatran, contain a small alcohol and a large acyl group, whereas substrates for hCE-2 have a large alcohol and a small acyl moiety.\(^{124}\) The intestinal and liver CES isoforms present in pigs share a high amino acid sequence identity with hCE-1 (76% and 77% identity), but are relatively more divergent from hCE-2 (46% and 47% identity).\(^{125}\) Assuming that similar substrate specificity follows with high sequence homology, the hydrolysis of ximelagatran in pigs may indeed commence immediately upon absorption into the enterocytes. This is in contrast to the
human intestine, where the hydrolase activity is catalyzed by hCE-2. The difference in the tissue distribution of the enzymes capable of efficiently hydrolyzing ximelagatran explains why the route-dependent metabolism of the compound was observed specifically in the pigs.

Table 2. Parameters (mean and sd or median and range) describing the plasma pharmacokinetics of AZD0837 and its metabolites in humans<sup>a</sup> and pigs<sup>b</sup>.

<table>
<thead>
<tr>
<th></th>
<th>AZD0837</th>
<th>AR-H069927</th>
<th>AR-H067637</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>t&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humans</td>
<td>0.3 (0.3-0.3)</td>
<td>0.3 (0.3-0.5)</td>
<td>0.5 (0.5-2.5)</td>
</tr>
<tr>
<td>pigs</td>
<td>1.2 (0.9-1.5)</td>
<td>1.5 (0.5-2.5)</td>
<td>2.2 (1.9-3.2)</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;1/2&lt;/sub&gt; (h)&lt;sup&gt;d&lt;/sup&gt;</strong></td>
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</tr>
<tr>
<td>humans</td>
<td>1.5 (0.6)</td>
<td>1.4 (0.1)</td>
<td>7.0 (0.9)</td>
</tr>
<tr>
<td>pigs</td>
<td>0.7 (0.04)</td>
<td>1.0 (0.1)</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td><strong>AUC (%)&lt;sup&gt;d&lt;/sup&gt;&lt;sup&gt;e&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humans</td>
<td>63 (5)</td>
<td>16 (3)</td>
<td>21 (3)</td>
</tr>
<tr>
<td>pigs</td>
<td>70 (16)</td>
<td>5 (4)</td>
<td>25 (14)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Investigation in healthy volunteers when 350 mg [3H]AZD0837 was administered intra-intestinally (n=7). <sup>b</sup>Investigation when 500 mg AZD0837 was given intra-intestinally to pigs (n=5). Data presented from the femoral vein. <sup>c</sup>t<sub>max</sub> is presented as median and range. <sup>d</sup>Expressed as mean and sd. <sup>e</sup>Expressed as a percentage of the sum of the total plasma AUC<sub>0-146</sub> for AZD0837, AR-H069927, and AR-H067637. <sup>f</sup>Expressed as a percentage of the sum of the plasma AUC<sub>0-6h</sub> for AZD0837, AR-H069927, and AR-H067637.

The plasma pharmacokinetics of AZD0837 and its metabolites displayed some differences between humans and pigs that had not been observed for ximelagatran (Figure 8 and Table 2). In spite of the shorter terminal half-lives of the prodrug and its metabolites, their respective t<sub>max</sub> were longer in pigs than in healthy volunteers. This suggests that the pigs absorbed the prodrug at a slower rate than humans. Notably, the inter-species differences were most pronounced for the active metabolite, which had a significantly shorter terminal t<sub>1/2</sub> in pigs than in humans (Table 2). Furthermore, concomitantly administered ketoconazole increased the t<sub>1/2</sub> of AR-H067637, an effect that was not seen in healthy volunteers. Taken together with the results from the depletion experiments with AR-H067637 in pig liver microsomes, we suggested that, unlike in humans, metabolism may contribute to the elimination of the active compound.

The presence of AR-H067637 in plasma varied greatly from one pig to another, with the exposure ranging from 9 to 43% of the total AUC<sub>0-6h</sub>. This has not been observed in clinical use, where the prodrug strategy has proven to be successful with low inter-individual variation in plasma levels.<sup>79, 112</sup> In pigs, the variation in plasma AUC was observed also for the prodrug and the intermediate. Hence, we reasoned that the absorption of AZD0837 or the capacity of the animals to biotransform the compound may have differed between individuals. Since the estimated fraction absorbed displayed low variability (fabs=0.76±0.08), the variation was suggested to be caused at the
level of bioconversion. The results obtained earlier with ximelagatran in pigs showed that the bioactivation of that prodrug was not subject to any inter-individual differences. This suggests that the variation was caused by the CYP3A activity rather than the reduction step, since the later reaction is catalyzed by the same enzyme system for both ximelagatran and AZD0837. Furthermore, a similarly large inter-individual variability in pharmacokinetic parameters has been observed in other investigations in pigs performed by our group with the CYP3A4 substrates verapamil and finasteride. This might be associated with the age of the animals included in our studies (10-12 weeks), since mRNA levels of CYP3A29 (corresponding to human CYP3A4) increase rapidly during this stage of their lives.

4.2 The metabolite profile in plasma and bile

Most pharmacokinetic investigations do not sample the biliary compartment explicitly, but instead use plasma data together with complementary in vitro experiments to extrapolate the exposure of the parent drug and metabolites in the hepatic tissue. In this thesis, the metabolite patterns of both ximelagatran and AZD0837 and the exposure of the compounds in plasma differed substantially from that in bile. This information about the biliary excretion may help in assessing the potential for different important aspects of drug efficacy and safety, for example, drug-drug interactions, drug-induced toxicity and for effects related to pharmacogenetics.

4.2.1 The metabolite pattern in humans

Following administration of ximelagatran to healthy volunteers, the bile collected by duodenal aspiration primarily contained ximelagatran and melagatran. Since ximelagatran is subject to degradation during passage through the intestinal lumen, this biliary excretion of the parent compound was not detected by traditional mass-balance studies. The structure elucidation of the compounds recovered in bile after dosing with \[^{14}\text{C}\]ximelagatran resulted in the identification of four hydroxylated forms of ximelagatran at low concentrations (<0.1% of the administered dose). The concentrations of ximelagatran and melagatran were higher in bile than in plasma, with AUC bile-to-plasma ratios for ximelagatran and melagatran of 260 and 70, respectively (Figure 9A and 9B). The AUC bile-to-plasma ratio is valuable in that it gives an easily interpreted estimate of the exposure of the compounds to the bile duct and gall bladder. In addition, high ratios, such as the ones obtained for ximelagatran and melagatran, indicate that carrier proteins mediate the transport of the compounds into bile. When
evaluating possible involvement of transporters, it should be noted that the comparisons between bile and plasma made in the thesis are based on the total plasma concentrations. Furthermore, bile sampled from the duodenum differs from the primary bile excreted into the canaliculi in that it is concentrated in the gall bladder and, once expelled into gut lumen, it is diluted with fluid from the pancreas and the intestine.

**Figure 9.** The plasma and bile concentration-time profiles of the most prominent compounds excreted in bile following enteral administration of ximelagatran and AZD0837, respectively. The concentrations shown are as follows: (A) ximelagatran and melagatran in humans, (B) ethyl-melagatran and melagatran in pigs, (C) AR-H067637 in humans, (D) AR-H067637 in pigs.

The compounds detected in bile following AZD0837 dosing of healthy volunteers were almost exclusively in the form of the active metabolite; the amounts of the prodrug and the intermediate recovered were negligible. Hence, the metabolite pattern in bile differed significantly from what was observed in plasma. The exposure of AR-H067637 in bile during the three hour-long sampling time was approximately 75 times higher than in plasma, suggesting the involvement of transport proteins in excreting this compound.
into bile (Figure 9C and 9D). In contrast to the metabolite identification study for ximelagatran, the radiochromatogram obtained with bile sampled after administration of \[^{3}\text{H}\]AZD0837 revealed no other peaks than the known metabolites AR-H067637 and AR-H069927.

4.2.2 The metabolite pattern in pigs

The major compounds excreted into bile in pigs following enteral administration of ximelagatran were ethyl-melagatran and melagatran with AUC bile-to-plasma ratios of 1200 and 160, respectively. These results deviated from the ones obtained in the clinical studies, where ximelagatran and melagatran predominated in bile. The dissimilarity between the two species likely arose from differences either in the metabolic or the transport processes that ximelagatran is exposed to in vivo. Interestingly however, co-administration with erythromycin reduced the biliary clearance of the compounds in both humans and pigs (Table 3).

The active metabolite of AZD0837 dominated the biliary excretion following enteral administration of the prodrug in pigs as well as in humans. In pigs, the exposure to AR-H067637 in bile was 1700-fold higher than in plasma. Even though both species excreted the active metabolite, the effect of concomitantly administering ketoconazole on the biliary excretion differed. There was a tendency towards reduced biliary clearance of AR-H067637 by ketoconazole in humans, whereas the process seemed to be unaffected in pigs (Table 4). Hence, these results differed from the ones obtained for ximelagatran where different compounds were excreted, but erythromycin exerted a similar inhibitory effect on the process.

4.3 The biliary excretion in humans and pigs

The total amount of ximelagatran and its metabolites excreted into bile following administration of the prodrug to healthy volunteers and pigs were 3.8(\(\pm\)3.2)\% and 13.6(\(\pm\)3.2)\% of the enteral dose, respectively (Figures 10A and 10B). When comparing these numbers it should be noted that two distinct techniques were used when sampling bile: in pigs, bile was collected directly from the bile duct during the six hours immediately following the dosing by the study drug, whereas, in the clinical trials, a sampling time of three hours was used out of concern for the comfort of the healthy volunteers. In addition to the difference in sampling time, the duodenal aspiration of bile applied in the human subjects relies on the expulsion of bile from the gall bladder, a process that couldn’t be controlled for in the studies.

For AZD0837, the same sampling techniques were used as in the ximelagatran studies. However, an additional difference between pigs and
humans affected the detected biliary excretion. The compound excreted the most into bile, AR-H067637, exhibited a significantly different terminal $t_{1/2}$ in the two species (1.2 h vs 7.0 h). The shorter $t_{1/2}$ of the active metabolite in pigs led to that the bile sampling time covered five $t_{1/2}$ for the compound, and that complete recovery was obtained in bile. In contrast, a much smaller part of the biliary concentration-time curve was covered in humans. Consequently, the total amount of AZD0837 and its metabolites detected in bile in pigs (53.5%±5.6% of an enteral dose) was very much higher than in humans (4.8%±4.5% of an enteral dose) (Figures 10C and 10D).

**Figure 10.** The cumulative amount excreted in bile (% of enteral dose) during the time bile was collected. (A) Ximelagatran and its metabolites excreted in humans (n=13) (B) Ximelagatran and its metabolites excreted in pigs (n=6) (C) AZD0837 and its metabolites excreted in humans (n=15) (D) AZD0837 and its metabolites excreted in pigs (n=5).

### 4.4 The intestinal excretion and clearance

In the pig studies, we were able to measure the intestinal excretion of the prodrugs and metabolites, since the experimental model enabled perfusion of
a 10 cm segment positioned in the proximal jejunum (Figure 5). The samples leaving the perfused segment were analyzed to determine the amounts of AZD0837, ximelagatran and the respective metabolites excreted. To estimate the total excretion throughout the entire small intestine (5 m), the amounts recovered in the perfusate samples were scaled assuming no changes in transport processes along the intestinal path.

Following administration of ximelagatran, neither the parent drug nor its metabolites were detected in the perfusate samples. A similar situation was observed for AZD0837, where the scaled intestinal clearance values of AZD0837, AR-H069927, and AR-H067637 were 0.4, 2.8, and 1.1 ml/min/kg, respectively. These values were concluded to be negligible in comparison to the other elimination routes: the prodrug and intermediate are believed to be eliminated primarily via metabolism and the active compound was subject to biliary excretion and further metabolism in pigs.

4.5 Pharmacokinetic drug-drug interactions with ximelagatran and AZD0837

The two drug-drug interactions investigated in this thesis were both discovered in the clinical development of ximelagatran and AZD0837. The mechanisms responsible for the interactions were not fully understood from the first interaction trials, which led to the present studies being conducted in humans and pigs, with the focus on the hepatobiliary disposition of the compounds.

4.5.1 Ximelagatran and erythromycin

A single dose of erythromycin co-administered with ximelagatran to healthy volunteers increased the plasma exposure of ximelagatran, OH-melagatran and melagatran. The effect on ethyl-melagatran could not be assessed since the majority of plasma concentrations of ethyl-melagatran were below the lower limit of quantification. The changes in the plasma pharmacokinetics were manifested as elevations in the AUC and Cmax of the compounds, whereas their terminal t1/2 remained unchanged by erythromycin (Table 3).

The primary aim of this interaction study was to evaluate a possible effect of erythromycin on the biliary excretion of ximelagatran and its metabolites. There was a tendency towards a reduction in the apparent CLbile/FH for the compounds detected in bile as seen in the geometric mean ratios for ximelagatran, OH-melagatran and melagatran (Table 3). Comparisons of the CLbile/FH between the treatments were complicated by intra-individual differences in the biliary metabolite pattern. The variability could be caused by differences in the timing of the gall bladder contraction: a contraction
occurring shortly after administration of the drug, when ximelagatran is circulating in the plasma and the metabolites are still being formed, would result in dominance of ximelagatran in the bile collected. Hence, the effect of erythromycin on the apparent total CL_{bile}/F_{H} for all the compounds summed together (total CL_{bile}/F_{H}) was also evaluated and shown to represent a 35% reduction (p=0.09) (Table 3).

In pigs, only the melagatran plasma parameters were affected by erythromycin (Table 3). As observed in humans, the increased plasma

Table 3. The effect of erythromycin on certain pharmacokinetic parameters of ximelagatran and its metabolites in humans (n=13) and pigs (n=6).

<table>
<thead>
<tr>
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<th>Humans</th>
<th></th>
<th></th>
<th>Pigs</th>
<th></th>
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</tr>
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<td></td>
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<td>TII^b</td>
<td>ratio^c</td>
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<td>TI^a</td>
<td>TII^b</td>
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<td>90 (30)</td>
<td>170 (60)</td>
<td>1.78</td>
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<td>910 (260)</td>
<td>850 (380)</td>
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<td>1.89</td>
<td></td>
<td>4270 (1550)</td>
<td>3760 (720)</td>
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<td>860 (950)</td>
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<td>1234 (533)</td>
<td>858 (323)</td>
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<td></td>
<td>2015 (697)</td>
<td>2352 (868)</td>
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<td>372 (364)</td>
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<tr>
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<td>260 (70)</td>
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<td>1807 (407)</td>
<td>2748 (862)</td>
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<tr>
<td>t_{1/2, plasma} (h)</td>
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<td>N.A.</td>
<td></td>
<td></td>
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<td>1.4 (0.4)</td>
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<tr>
<td>A_{e bile} (% of dose)</td>
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<td>0.2 (0.1)</td>
<td>1.40</td>
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<td>1.22</td>
<td></td>
<td>13.6 (3.3)</td>
<td>9.4 (4.7)</td>
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<tr>
<td>CL_{bile} (ml/min/kg)</td>
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<td>2.3 (2.7)</td>
<td>0.11</td>
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<td>0.03 (0.01)</td>
<td>0.02 (0.01)</td>
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<td>0.7 (0.3)</td>
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<td></td>
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<td>Bile flow (ml/min/kg)</td>
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<td>(0.012)</td>
<td></td>
<td></td>
<td>(0.007)</td>
<td>(0.009)</td>
</tr>
</tbody>
</table>

^a TI (treatment I), the control group receiving ximelagatran alone. The data are presented as arithmetic means. ^b TII (treatment II), the group receiving ximelagatran together with erythromycin. The data are presented as arithmetic means. ^c The geometric mean ratios between the two treatments (TII/TI).
exposure to melagatran was accompanied by a reduced apparent CL_{bile} of the compound. It is possible that the absence of an erythromycin-induced effect on the plasma AUC of the prodrug and the intermediates in pigs was caused by the inter-species difference in the pattern of compounds excreted into bile.

Importantly, the bile flow in both species was unaffected by the enteral erythromycin dose. More likely, the pharmacokinetic interaction between ximelagatran and erythromycin occurred at the level of transport proteins involved in the biliary excretion of ximelagatran and its metabolites. This was seen in vivo by the reduced AUC bile-to-plasma ratios and the apparent CL_{bile}-values. Together with in vitro data showing that ximelagatran, OH-melagatran and melagatran are substrates for the efflux protein ABCB1, we conclude that inhibition of this transporter is the most likely cause of the altered pharmacokinetics. Hence, concomitant treatment with drugs that are potent inhibitors of ABCB1 may result in pharmacokinetic drug-drug interactions for ximelagatran that increase the exposure of the active form by approximately two-fold.

4.5.2 AZD0837 and ketoconazole

Dosing of AZD0837 during repeated oral dosing of ketoconazole to healthy volunteers increased the plasma exposure of AZD0837 and the active metabolite, AR-H067637 (Table 4). Ketoconazole prolonged the terminal t_{1/2} of the prodrug and an increased AUC and C_{max} was observed for both compounds. The prodrug is eliminated primarily via CYP3A4 mediated metabolism, which explains its interaction with the potent CYP3A4 inhibitor ketoconazole. For the active metabolite, the changes seen in plasma were likely caused by a reduced apparent CL_{bile}/F_{H} (Table 4). The effect was not as clear as for the ximelagatran-erythromycin interaction, possibly owing to the longer terminal t_{1/2} of AR-H067637 compared to that of melagatran lead to that the bile sampling time did not cover the plasma exposure to the same extent.

Similar trends in the plasma pharmacokinetics of AZD0837 and its metabolites were observed in pigs, with the difference that in this species ketoconazole increased the terminal t_{1/2} of the active metabolite. The total amount of compounds recovered in bile, which were primarily the active metabolite AR-H067637, was large and represented a high fraction of the administered dose. In spite of the high biliary concentrations and the fact that bile sampling time covered the total plasma exposure, no reduction in the apparent CL_{bile} was observed in the pigs. This suggested that the ketoconazole effect was on the level of metabolism rather than biliary excretion. Depletion experiments in pig liver microsomes showed that AR-H067637 was indeed metabolized at a slow rate, and ketoconazole reduced its intrinsic clearance from 12.2(±2.9) to 2.4(±3.7) ml/min/g protein.
In conjunction with these in vitro experiments, the data led us to conclude that the active metabolite was further metabolized in pigs and that ketoconazole had the potential of affecting this pathway.

Table 4. The effect of ketoconazole on pharmacokinetic parameters of AZD0837 and its metabolites in humans (n=15) and pigs (n=5).

<table>
<thead>
<tr>
<th></th>
<th>Humans</th>
<th>Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>TII&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>AUC&lt;sub&gt;0-t, plasma (h·μmol/l)&lt;/sub&gt;</strong></td>
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<tr>
<td>AZD0837</td>
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<td>28.5 (7.8)</td>
</tr>
<tr>
<td>AR-H069927</td>
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<td>3.4 (1.4)</td>
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<tr>
<td>AR-H067637</td>
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<td><strong>C&lt;sub&gt;max, plasma (μmol/l)&lt;/sub&gt;</strong></td>
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<td>AZD0837</td>
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<td>14.9 (2.7)</td>
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<td>1.5 (0.5)</td>
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<td>AR-H067637</td>
<td>0.6 (0.2)</td>
<td>1.0 (0.3)</td>
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<td><strong>t&lt;sub&gt;1/2,plasma (h)&lt;/sub&gt;</strong></td>
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<tr>
<td>AZD0837</td>
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<td>2.1 (0.6)</td>
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<td>AR-H069927</td>
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<td>AR-H067637</td>
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<td><strong>A.cellbile (% of dose)</strong></td>
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<tr>
<td>AR-H067637</td>
<td>0.99 (1.15)</td>
<td>0.64 (0.72)</td>
</tr>
<tr>
<td><strong>Bile flow (ml/min/kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(TI&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>0.008</td>
<td>0.006</td>
</tr>
<tr>
<td>(TII&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>(0.010)</td>
<td>(0.008)</td>
</tr>
</tbody>
</table>

<sup>a</sup> TI (treatment I), the control group receiving AZD0837 alone. The data are presented as arithmetic means. <sup>b</sup> TII (treatment II), the group receiving AZD0837 together with ketoconazole. The data are presented as arithmetic means. <sup>c</sup> The geometric mean ratios between the two treatments (TII/TI). <sup>d</sup> AUC<sub>0-24h</sub> in humans and AUC<sub>0-6h</sub> in pigs.
5. Conclusions and future outlook

The in vivo pharmacokinetics of the thrombin inhibitor prodrugs ximelagatran and AZD0837 were investigated with a specific focus on two pharmacokinetic drug-drug interactions that have been hypothesized to occur at the level of hepatobiliary transport. The use of an advanced in vivo model in pigs and a novel method for the determination of biliary excretion in healthy volunteers allowed the mechanisms triggering the interactions to be studied in more detail than before. The bioactivation of both prodrugs was followed in plasma and the formation of the respective active metabolites was observed immediately upon administration. The interactions studied, ximelagatran-erythromycin and AZD0837-ketoconazole, were both discovered during the clinical development of the drug candidates and the results obtained from those trials were confirmed by the work included in this thesis. The major conclusions drawn were:

- The metabolite pattern in bile following administration of both ximelagatran and AZD037 differed from that observed in plasma. In addition, the exposure to the excreted compounds was several-fold higher in bile than in plasma. This indicates that the involvement of hepatic transport proteins mediating the excretion into bile should be considered when evaluating possible pharmacokinetic interactions.

- The compounds detected in bile after administration of ximelagatran differed from those recovered in fecal samples in previous mass balance studies. This shows the advantage of duodenal aspiration of bile over fecal recovery when investigating metabolites excreted into bile.

- Erythromycin reduced the apparent $\text{CL}_{\text{bile}}/F_H$ after ximelagatran dosing in humans. This was also observed in pigs receiving ximelagatran alone or together with erythromycin. Thus, inhibited biliary clearance of ximelagatran and its metabolites is likely to explain the interaction with erythromycin, at least in part.

- Co-administration of ketoconazole resulted in a moderately reduced apparent $\text{CL}_{\text{bile}}/F_H$ for AR-H067637, the active metabolite of AZD0837, in healthy volunteers. However, the apparent $\text{CL}_{\text{bile}}$ of the compound in pigs seemed to be unaffected by the inhibitor. Complementary in vitro
depletion experiments in pig liver microsomes suggested that the AZD0837-ketoconazole interaction in pigs could instead be explained by reduced metabolism of the active compound.

- The intestinal secretion determined from a perfused jejunal segment in pigs was minor following administration of ximelagatran and AZD0837. Hence, it was concluded that this potential elimination route for the two prodrugs and their metabolites was insignificant for the overall pharmacokinetics of the compounds.

- The importance of clinical trials when confirming the clinical relevance of results obtained from animal studies was highlighted in the papers included in this thesis as a few significant species-related differences were observed: ximelagatran displayed route-dependent metabolism in pigs; the compounds recovered in bile after ximelagatran dosing differed between humans and pigs; and metabolism seemed to contribute to the elimination of the active metabolite of AZD0837 to a greater extent in pigs than in humans.

Reports in the literature describing the biliary clearance of drugs in healthy volunteers are rare owing to the inevitable complexity of the studies. In the clinical trials included in this thesis, we used duodenal aspiration to sample bile. The great advantage of this method over others is that it enables direct characterization of biliary excretion in healthy volunteers. Using the technique for evaluating biliary excretion as a possible site for drug-drug interactions is, however, more challenging. The bile sampling time is limited (~ three hours) and the amounts of parent drug and metabolites remaining in the gall bladder at the end of the study are not readily controllable. Imaging techniques to determine the ejection fraction of the gall bladder during the bile sampling time may be utilized in the future to further improve this aspect of the studies.

Reliable in vitro-in vivo correlations for CL_{bile} would provide a valuable tool with which to predict the biliary clearance and exposure of drug candidates and their metabolites at an early stage of drug development. This would in turn aid in the assessment of risks for drug-induced toxicity and pharmacokinetic drug-drug interactions. Biliary clearance values determined in healthy volunteers, for drugs with differing chemical structures, are crucial for establishing such correlations. The clinical studies included in this thesis provide such data, but additional inquiries like them are needed.
Kroppen har ett effektivt försvar mot främmande ämnen. Detta skydd har till uppgift att göra det svårt för icke önskvärda ämnen att nå blodet och att snabbt eliminera dem därifrån. För att lyckas ta fram ett läkemedel, som ska nå sitt målorgan i tillräckligt hög koncentration och under tillräckligt lång tid för att vara verksamt, måste man ta hänsyn till dessa försvarsmekanismer. Ett läkemedel som intas genom att sväljas måste, för att kunna nå kroppens blodcirkulation, bland annat vara 1) stabilt i mag- och tarmkanalen, 2) lösas upp till enskilda molekyler i tarmen för att kunna passera tarmväggen, 3) undgå alltför hög nedbrytning (metabolism) i tarm och lever, och 4) undvika utsöndring från levern via gallan.

De arbeten som presenteras i den här avhandlingen är främst inriktade på att studera kroppens förmåga att utsöndra läkemedel från levern till gallan. Det är ett område som är svårt att utforska på människa eftersom prover från galla - till skillnad från blod, urin och feces - är svåra att ta. Den metod som användes innebar att försökspersoner svalde en kateter, som leddes genom magsäcken till övre delen av tarmen där gallblåsan tömmer sig (Figur 6). Katetern kopplades till en pump med vilken galla sögs upp från tarmen efter det att försökspersonen fått de läkemedel som studerades. Genom att komplettera med blodprover kunde vi på så sätt följa vad som händer med läkemedlen och deras metaboliter i galla och i blod. För att få en än mer detaljerad bild av hur kroppen hanterar läkemedlen utfördes även studier på försöksdjur (gris), där prover togs direkt från gallgången, från ett tarmsegment, från ett perifert blodkärl samt från de blodkärl som går till och från levern (Figur 5). Detta gav en större förståelse för de processer som styr läkemedlen i tarm och i lever.

De läkemedel som studerades i avhandlingen är två trombinhämmare, ximelagatran och AZD0837. Dessa är så kallade prodrugs, det vill säga de måste omvandlas till sin respektive aktiva metabolit för att kunna ge effekt i kroppen. Substanserna var av särskilt intresse, eftersom de har visat sig interagera med erytromycin (ximelagatran) och ketoconazol (AZD0837). Läkemedel sågs interagera om de ändrar varandras nivåer i kroppen då de tas samtidigt, jämfört när de tas vart och ett för sig. Erytromycin ökar koncentrationerna i blod av ximelagatran och dess metaboliter, och motsvarande sker då ketoconazol ges tillsammans med AZD0837.
Interaktionerna misstänks bero på att erytromycin och ketoconazol hindrar respektive läkemedel från att utsändras till gallan, vilket undersöktes i avhandlingens delarbeten.

I galla från försökspersoner uppmättes höga koncentrationer av ximelagatran och dess aktiva metabolit, och för AZD0837 återfanns höga koncentrationer av dess aktiva metabolit. Koncentrationerna i galla var betydligt högre än i blodet, vilket visar att substanserna pumpats ut i galla med hjälp av specialiserade transportproteiner på levercellernas yta. Erytromycin och ketoconazol minskade denna utsöndring av substanserna i galla, vilket kan förklara varför nivåerna av trombinhämmarna i blodet påverkades. Resultaten från försöken på gris stärkte bilden av att det som sker i levern är av betydelse för läkemedelsinteraktionerna. Samtidigt upptäcktes dock skillnader i resultat från försökspersoner och gris, vilket visar på svårigheten av att använda djur som modell för människa.

Genom dessa interaktionsstudier mellan ximelagatran och erytromycin respektive AZD0837 och ketoconazol, har kunskapen om de processer som styr trombinhämmarna och deras metaboliter i tarm och i lever ökats. Sådan information är värdefull, inte bara för dessa interaktioner, utan även vid riskbedömning av andra läkemedelsinteraktioner, toxicitet och tänkbara effekter av genetiska skillnader mellan individer.
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