Dabigatran: An Oral Novel Potent Reversible Nonpeptide Inhibitor of Thrombin

Wolfgang G. Eisert, Norbert Hauel, Joachim Stangier, Wolfgang Wienen, Andreas Clemens, Joanne van Ryn

Abstract—Dabigatran is a highly selective, reversible, and potent thrombin inhibitor and is orally available as the prodrug, dabigatran etexilate. It has shown antithrombotic efficacy in animal models of thrombosis, with a rapid onset of action and predictable pharmacodynamic response. Peak plasma concentrations of dabigatran occur 1 to 2 hours after ingestion of the prodrug. The terminal half-life of dabigatran is 12 to 14 hours in elderly volunteers. Dabigatran is not metabolized by cytochrome P450 isoenzymes and does not interact with food. Dabigatran has a low potential for drug-drug interactions and is predominantly renally excreted. Dabigatran etexilate as chronic therapy effectively prevents the recurrence of venous thromboembolism and cardioembolic stroke. For the first time, it has been demonstrated clinically that there may be an effective and safe alternative to warfarin. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: anticoagulants ▪ coagulation ▪ stroke ▪ thrombin inhibitors ▪ venous thrombosis ▪ cardioembolic stroke

Treatment with anticoagulants has shown significant benefit in a variety of acute as well as chronic thromboembolic disorders. However, particularly in long-term anticoagulation with vitamin K antagonists, it is important to carefully balance the level of thrombotic inhibition with the risk of bleeding. In particular, this is due to food and drug interactions, individual dosing, and the lack of fast onset or offset of anticoagulation, which require repeated monitoring in patients. Oral direct inhibition of thrombin offers an opportunity to overcome these limitations.

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The first oral direct thrombin inhibitor was ximelagatran, which showed antithrombotic efficacy and safety as compared with warfarin both preclinically and clinically (reviewed in Reference 1). It is a double prodrug, with the active principle melagatran shown to be a potent and selective thrombin inhibitor. It was developed in a wide range of clinical indications, including prevention of thrombosis after orthopedic surgery, treatment of established thrombosis, and prevention of stroke in patients with atrial fibrillation. However, liver toxicity associated with long-term dosing of ximelagatran (first seen ∼1 month after initiating therapy) resulted in a failed approval in the United States and withdrawal from the market in Europe.2

The direct thrombin inhibitor, dabigatran etexilate (Figures 1 and 2), is the next compound in this class to successfully achieve regulatory approval worldwide in >50 countries (excluding the United States and Japan). At the end of 2009, a submission for the indication of stroke prevention in patients with atrial fibrillation was filed in the United States and Europe. In contrast to ximelagatran, the recent clinical data with long-term treatment of dabigatran etexilate (6 months to 3 years) has shown all the benefits of antithrombotic efficacy and reduced bleeding seen with direct thrombin inhibition, without evidence of liver toxicity.3 Dabigatran etexilate effectively prevented stroke in patients with atrial fibrillation as compared with vitamin K antagonists with less bleeding complications at the lower dose and superior efficacy with the higher dose.4 Importantly, a ∼70% reduction in intracranial hemorrhage was seen in patients receiving both doses of dabigatran etexilate as compared with those receiving warfarin. Dabigatran etexilate also demonstrated equivalence to warfarin in the prevention of recurrent venous thrombosis in patients with established venous thrombosis.4 There was a tendency to less major bleeding and a significant reduction in all bleeding (major and minor) in dabigatran etexilate-treated patients.4 The preclinical development of dabigatran etexilate, including chemistry, pharmacology, and clinical pharmacokinetics, is reviewed here. Potential differences to ximelagatran to explain the difference in toxicity profiles will also be discussed.

Design of Dabigatran

Earlier approaches to design small molecule thrombin inhibitors were based on the structure of the tripeptide D-Phe-Pro-Arg.
However, the nonpeptide inhibitors with more favorable pharmacokinetic properties were preferred. Modern x-ray crystallography information of bovine thrombin bound to the peptide-like, benzamidine-based inhibitor N-α-naphthylsulphonylglycyl-4-amidinophenylalanine piperidine (NAPAP) was used in the search for novel structures. A number of cyclic scaffolds was designed that served as surrogates for the central glycine of the NAPAP molecule, omitting the hydrogen bonds formed by the glycine amino and carbonyl groups with the enzyme. During optimization of these molecules, a trisubstituted benzimidazole derivative was found that turned out to be an important lead compound for further improvements. In this compound class, a benzamidine moiety formed a salt bridge with the carboxylate of the enzyme aspartate residue Asp 189. The lipophilicity of the molecules was inversely correlated with the potency of inhibition when measured in the presence of blood plasma. Subsequently, polar groups were introduced to increase hydrophilicity, thereby reducing plasma protein binding. Adjusting the linker between the benzimidazole template and the benzamidine moiety and modifications of the peripheral substituents further increased activity and finally led to a compound with nanomolar activity. Dabigatran was the most potent thrombin inhibitor in this series and, due to the reduced plasma protein binding, showed excellent anticoagulant activity in human whole blood. Moreover, it exhibited a favorable selectivity profile with respect to other serine proteases (Table 1).

Dabigatran is a very polar, permanently charged molecule with a logP of −2.4 (n-octanol/buffer, pH 7.4) and therefore has no bioavailability after oral administration. Thus, a double prodrug, dabigatran etexilate was generated by masking the amidinium moiety as a carbamate ester and by turning the carboxylate into an ester group. It was then required that the hydrogen bonds formed by the glycine amino and carbonyl groups with the enzyme were omitted. Consequently, polar groups were introduced to increase hydrophilicity, thereby reducing plasma protein binding. Adjusting the amidinium moiety as a carbamate ester and by turning the carboxylate into an ester group, a trisubstituted benzimidazole derivative was found that turned out to be an important lead compound for further improvements. In this compound class, a benzamidine-based inhibitor N-α-naphthylsulphonylglycyl-4-amidinophenylalanine piperidine (NAPAP) was used in the search for novel structures.

Figure 1. Chemical structure of dabigatran (active principle).

Figure 2. Chemical structure of the double prodrug, dabigatran etexilate.

Table 1. Selectivity Profile of Dabigatran in Inhibiting Thrombin vs Other Serine Proteases

<table>
<thead>
<tr>
<th>Human Protease</th>
<th>( K_i ) (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Factor Xa</td>
<td>3760±20</td>
</tr>
<tr>
<td>Plasmin</td>
<td>1695±50</td>
</tr>
<tr>
<td>TPA</td>
<td>45 360±10</td>
</tr>
<tr>
<td>Trypsin</td>
<td>50.3±0.3</td>
</tr>
</tbody>
</table>

Table 2. Association and Dissociation Constants of Dabigatran and Melagatran Binding to Thrombin

<table>
<thead>
<tr>
<th></th>
<th>Association Constant ( (K_a) ) ( (M^{-1}) )</th>
<th>Dissociation Constant ( (K_d) ) ( (M) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran</td>
<td>1.45±0.05×10^6</td>
<td>7.0±0.26×10^{-10}</td>
</tr>
<tr>
<td>Melagatran</td>
<td>1.2±0.03×10^6</td>
<td>7.93±0.23×10^{-10}</td>
</tr>
</tbody>
</table>

In Vivo Experimental Thrombosis

The correlation between the anticoagulant and antithrombotic effects were investigated in a Wessler-type stasis model of venous thrombosis in rats by isolating a segment of the vena cava after systemic tissue factor injection and removal of the remaining clot. There was a dose-dependent prolongation of activated partial tissue thromboplastin time (aPTT) in conscious rats and rhesus monkeys. Five minutes after bolus administration of 0.3, 1.0, and 3.0 mg/kg in rats, the aPTT was increased to 29±1.6, 159±1.8, and 582±34 s. In rhesus monkeys, bolus doses of 0.15, 0.3, and 0.6 mg/kg led to an aPTT prolongation of 47.3, 70.1±1.3, and 98.9±8.8 s.

A single oral dose of 10, 20, or 50 mg/kg dabigatran etexilate in rats led to a significant prolongation of aPTT to 25.2±1.1, 38.4±3.9, and 78.3±9.5 s at its maximum 30 minutes after dosing and remained significantly prolonged for the first hour. Anticoagulant activity then normalized after 3 hours. In rhesus monkeys, oral doses of 1, 2.5, and 5 mg/kg showed increases from 19.4±0.4 s at baseline to 34.3±0.5, 44.0±5.7, and 63.0±2.5 s, respectively, 2 hours after administration. Eight hours after administration, the aPTT was still elevated to 25.5 and 35.2 s at the lowest and the highest doses, respectively.
Table 3. Species Dependency of Clotting Inhibition Expressed as Concentration of Dabigatran in Three Different Clotting Tests

<table>
<thead>
<tr>
<th>Species</th>
<th>aPTT (μM)</th>
<th>Prothrombin Time</th>
<th>Ecarin Clotting Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0.23±0.02</td>
<td>0.83±0.07</td>
<td>0.18±0.004</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>0.59±0.03</td>
<td>0.99±0.06</td>
<td>0.2±0.004</td>
</tr>
<tr>
<td>Rat</td>
<td>0.46±0.02</td>
<td>0.56±0.06</td>
<td>0.1±0.02</td>
</tr>
<tr>
<td>Dog</td>
<td>1.5±0.11</td>
<td>2.4±0.32</td>
<td>0.11±0.006</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.9±0.3</td>
<td>4.57±0.39</td>
<td>0.15±0.07</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±SE.

Bleeding time was measured in a well-established rat model. The effective antithrombotic dose of 0.1 mg/kg (ED_{100}) did not prolong the bleeding time. Bleeding was significantly prolonged only after administration of doses ≈5-fold higher than this dose (equivalent to doses ≈15-fold higher than the ED_{50}). Even bleeding resulting from the highest dose tested (1.0 mg/kg) returned to normal values after 90 minutes.

After oral administration of dabigatran etexilate, maximal inhibition of thrombus formation in this rat model was observed after 30 minutes of pretreatment, indicating a rapid onset of action. A low dose of 5 mg/kg dabigatran etexilate still showed significant reduction of thrombus formation after 2 hours, whereas higher doses (10, 20, and 30 mg/kg) showed a significant reduction of thrombus formation even 3 hours after administration.

Similarly, when tested in rabbits dabigatran resulted in a dose-dependent inhibition of thrombus weight after injection of increasing doses as an IV bolus, with an ED_{50} of 0.066 mg/kg. Also, in this species, this compares favorably with melagatran (ED_{50} of 0.058 mg/kg), unfractionated heparin (ED_{50} of 9.84 U/kg), and hirudin (ED_{50} of 0.016 mg/kg). Similarly, in rabbits, a dose-dependent increase in aPTT was observed in these studies. A correlation of clot weight and aPTT demonstrated that effective antithrombotic activity is already observed at moderate prolongation of aPTT (Figure 3).

Similar results were obtained after oral administration of dabigatran etexilate. There was an inverse correlation between thrombus formation and aPTT changes after oral application of 1, 3, 5, 10, and 20 mg/kg dabigatran etexilate. Thrombus growth measured ≈2.5 hours after oral dosing of 10 and 20 mg/kg fully inhibited thrombus formation. The ED_{50} for the reduction of clot weight was 4.7 mg/kg. aPTT was elevated ≈1.5-fold over control for doses of 1, 3, and 5 mg/kg, whereas 10 and 20 mg/kg showed ≈2.5- and ≈3-fold increase of the aPTT (Figure 4).

The investigation of the antithrombotic potency after a single oral dose of 10 mg/kg dabigatran etexilate revealed not only a rapid onset of activity but also showed a significant inhibition of clot formation even 7 hours after oral administration.

The transition to first-in-human studies is primarily driven by assuring safety, keeping an eye on the magnitude of desired effects. Subsequent dose-escalation studies in humans begin with the lowest dose that has been demonstrated safe by chronic toxicology studies. The target dose at this point is based on the level of inhibition demonstrated in animal studies to have the desired pharmacological effect. The fast onset of action after oral dosing in all species and the ≈2-fold elevation in aPTT seen in rhesus monkeys 1 to 2 hours after oral dosing were achieved with doses between 1 and 2.5 mg/kg. This is consistent with clinical dosing between 110 and 150 mg/d.

Metabolism, Pharmacokinetics, and Pharmacodynamics

Following IV administration of 14C-labeled dabigatran in healthy volunteers, the plasma concentration at the end of the infusion was found to be 258.2±43.1 ng-Eq/mL, which declined to 2.5 ng-Eq/mL 36 hours later.10 After oral administration of 200 mg 14C-labeled dabigatran etexilate, a plasma peak of 244.4±56.8 ng-Eq/mL was observed 1.5 hours after dosing. About 35% of dabigatran is bound to plasma protein independent of plasma concentration. Plasma clearance after IV administration of dabigatran was 149 mL/min, and the mean terminal half-life of dabigatran was 8.3 hours.

The absolute bioavailability following oral administration of dabigatran etexilate was found to be ≈7%.10,11 Dabigatran etexilate is more consistently absorbed in an acidic milieu in the gastrointestinal tract. Thus, to address potential problems with inconsistent absorption, a formulation was developed containing tartaric acid. A dabigatran etexilate coating is applied onto a tartaric acid core to form tiny pellets (≈1-mm diameter) that are placed in a capsule. A clinical capsule contains hundreds of these pellets, the exact number depending on the dose strength of the capsule. In this way dabigatran etexilate absorption is not dependent on the gastrointestinal
acidity of the patient but brings its optimal pH environment with it, reducing variability even with proton pump inhibitor coadministration.\textsuperscript{12}

The metabolite profile of dabigatran etexilate has been extensively analyzed and characterized by LC-MS/MS.\textsuperscript{10} After oral administration of dabigatran etexilate, there is rapid conversion into active dabigatran, which is the predominant compound in plasma. Maximal dabigatran plasma concentrations are dose dependent and observed \(\approx 1.5\) hours after oral dosing, and the mean terminal half-life of dabigatran after p.o. administration was 8.8 hours. In older healthy volunteers, the half-life was shown to be 13 hours, which is more typical of the patient population.\textsuperscript{12,13} Peak and trough plasma levels at steady state in patients with atrial fibrillation (150-mg bid) are \(\approx 180\) ng/mL at peak and \(\approx 90\) ng/mL at trough (ie, 11.5 hours after ingestion).\textsuperscript{14}

Unlike hydroxylamine-structured prodrugs of thrombin inhibitors, such as ximelagatran, dabigatran etexilate is not metabolized by the cytochrome P450 enzymes or other oxidoreductases. In contrast, ubiquitous plasma esterases are involved in the hydrolytic reactions that convert the double prodrug into the active drug dabigatran. The double prodrug and its single prodrug intermediates were just above the level of assay detection in plasma (\(\approx 0.5\) ng/mL) for \(\approx 2\) hours after ingestion. Dabigatran undergoes conjugation with activated glucuronic acid to yield pharmacologically active conjugates.

After a single oral dose of 24 mg ximelagatran, ximelagatran can be measured in plasma with peak levels of \(\approx 0.15\) \(\mu\)mol/L seen 30 to 60 minutes after ingestion. Melagatran attains a Cmax of \(\approx 0.15\) \(\mu\)mol/L with a time to maximum plasma concentrations, tmax, of 2 to 3 hours. At this point, circulating levels of ximelagatran have significantly diminished because of its conversion to melagatran.\textsuperscript{15} Thus, the prodrug ximelagatran attains peak concentrations similar to melagatran but has a much smaller area under the curve. This is in contrast to dabigatran etexilate, which is not detectable in plasma in significant amounts because of the more rapid conversion of the prodrug.

Renal excretion is the dominant elimination pathway in humans.\textsuperscript{10} Renal clearance (89 mL/min) of dabigatran accounts for \(\approx 80\%\) of the total clearance (112 mL/min) of absorbed drug. Primarily unchanged dabigatran and small amounts of dabigatran glucuronides were recovered in the urine. Thus, reduced kidney function results in elevated dabigatran plasma concentrations and prolonged half-life.\textsuperscript{12} The lower dose of dabigatran etexilate is recommended in patients with moderate renal impairment (creatinine clearance 30 to 50 mL/min), and dabigatran etexilate is contraindicated in patients with severe renal impairment (10 to 30 mL/min).\textsuperscript{12,13,16} In subjects with mild hepatic impairment, area under the curve after single oral dose of dabigatran etexilate was comparable with healthy controls, and the bioconversion of the prodrug was found to be only slightly slower.\textsuperscript{11,14,17}

There is a lack of interaction with the cytochrome P450 system. No important drug interactions with atorvastatin (CYP3A4) and its metabolites were measured.\textsuperscript{18} Likewise, the mean area under the curves and Cmaxs of dabigatran remained unchanged with a single dose of diclofenac (CYP2C9) and were slightly reduced with pantoprazole (CYP2C19).

Dabigatran etexilate (not dabigatran active principle) is an efflux P-glycoprotein substrate. Thus, any potential effects are only restricted to drug absorption. Because dabigatran etexilate does not inhibit P-glycoprotein, no relevant effect of digoxin was seen.\textsuperscript{16} Dabigatran plasma levels were elevated when given 1 hour following 2 known P-glycoprotein inhibitors, amiodarone and verapamil (in steady state), by \(\approx 1.5\)-fold (area under the curve) in drug interaction studies. Quinidine, a strong P-glycoprotein inhibitor, is contraindicated for use with dabigatran etexilate in the current approved indication. Two doses of dabigatran etexilate are approved for use in orthopedic indications, and thus with acute treatment (2 to 4 weeks), the lower dose of dabigatran etexilate is recommended in patients taking amiodarone and verapamil medications.

The prolongation of blood coagulation parameters, aPTT, prothrombin time, thrombin time, and ecarin clotting time paralleled the plasma concentration-time curve of dabigatran. This reflects the direct effect of dabigatran on thrombin in blood. The thrombin time was most sensitively prolonged by dabigatran, the ecarin clotting time, and aPTT less so, and the prothrombin time was least sensitive.\textsuperscript{14} Maximum prolongation of blood coagulation activity was observed at peak dabigatran plasma concentrations (\(\approx 2\) hours) and then declined. Twelve hours after administration, blood coagulation was still inhibited by \(\approx 50\%\) of the peak inhibition.\textsuperscript{19–23} This slow terminal phase indicates that therapeutic plasma concentrations may be achieved over a 24-hour period with once daily dosing. Steady state levels are achieved after 2 to 3 days of dosing. There is no unexpected accumulation of dabigatran after multiple dosing.

There is no specific antidote to reverse the anticoagulant effect of dabigatran. However, recommendations for treatment of patients in emergency situations have recently been published.\textsuperscript{14} Usually, drug discontinuation is sufficient to reverse anticoagulant activity. However, in emergency situations, several options are currently being considered. Due to its low protein binding, dabigatran can be dialyzed in patients with renal impairment.\textsuperscript{19} The feasibility of orally administered activated charcoal or active charcoal hemofiltration in case of potential overdose is currently being tested preclinically. Nonspecific prohemostatic agents, such as recombinant activated factor VII or prothrombinase complex concentrates, have reversed bleeding in a rat tail model.\textsuperscript{22} Dabigatran in plasma under emergency conditions can be most sensitively measured using the aPTT or a diluted thrombin time. The prothrombin time is not recommended, because it is very insensitive to dabigatran levels in plasma.\textsuperscript{14}

Because of the liver toxicity of ximelagatran, extensive liver function monitoring was performed in the phase III clinical trials with dabigatran etexilate.\textsuperscript{3,4,24–26} In the entire clinical trial program to date, including \(\approx 40\,000\) patients, with RE-LY patients (\(n=12\,000\)) treated for a mean of 2 years with dabigatran etexilate, no elevation in liver enzymes were measured. A possible explanation for this difference between these 2 compounds may be the metabolism of the prodrugs. Prodrug conversion of ximelagatran to the active drug differs very much from the conversion of dabigatran etexilate. The hydroxy-amidine group of ximelagatran is cleaved by oxidoreductases present mostly in the liver.\textsuperscript{26} In
contrast, the esterases required to convert dabigatran etexilate into dabigatran are general esterases located throughout the body, including plasma and also the liver. Thus chronic administration of ximelagatran results in short, spiked, high levels of ximelagatran in plasma. It has since been shown that ximelagatran (but not melagatran) increases membrane fluidity and changes membrane lipid composition in a human hepatocyte system. This may in part be an explanation for the toxic liver effects seen, because these in vitro effects could be observed with ximelagatran concentrations as low as 10 μmol/L. In contrast, the metabolism of dabigatran etexilate results in almost immediate conversion to the active principle, dabigatran, and levels of the prodrug in plasma are just above levels of detection, even at peak, and are not detectable after 2 hours.

Summary

Dabigatran etexilate represents the first synthetic oral, reversible, direct inhibitor of thrombin with a very favorable biochemical and pharmacological profile that translates into clinical efficacy and safety. The increasing amount of clinical data being obtained with dabigatran etexilate shows promise in introducing a new era in oral anticoagulation. Eventually, this may result in the replacement of vitamin K antagonists with a newer, safer, more convenient, and reliable anticoagulation by direct thrombin inhibition.

Disclosures

None.

References

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