New Issues in Oral Anticoagulants

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Polymorphisms in CYP2C9, a critical cytochrome P-450 enzyme in the metabolism of warfarin, alters its clearance and affects dosing. CYP*1 has higher activity than either the *2 or *3 variants, and patients with the *2 or *3 variants require a lower dose. VKORC1 is the enzyme inhibited by warfarin, and its levels are affected by several polymorphisms that can be divided into high or low level haplotypes, and patients with high level haplotypes require higher warfarin doses. The use of algorithms for dosing that incorporate pharmacogenomic information perform better than those using clinical data alone. Considerable effort is ongoing to develop new oral anticoagulants as alternatives to warfarin, and three agents are in advanced development. Dabigatran is an oral direct thrombin inhibitor that has been compared with enoxaparin for prevention of VTE following hip or knee replacement. Based on non-inferiority results in European trials, it has now been approved for marketing in Europe. Phase III trials with a new oral Xa inhibitor, rivaroxaban, have been completed in hip or knee replacement, and rivaroxaban was superior to enoxaparin in prevention of VTE with no increase in bleeding complications. Phase III studies with apixaban, another oral Xa inhibitor, are in progress. These agents are also being evaluated in large studies for prevention of stroke in atrial fibrillation and for VTE treatment.

Table 1. Common variations in CYP2C9 and VKORC1 that affect warfarin dose.

<table>
<thead>
<tr>
<th>Name</th>
<th>Variation</th>
<th>Caucasian Prevalence, %</th>
<th>Effect on warfarin dose, %</th>
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<tr>
<td>CYP2C9</td>
<td>*1</td>
<td>Wild-type</td>
<td>81 94 98</td>
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<tr>
<td></td>
<td>*2</td>
<td>R144C</td>
<td>13 3 0</td>
</tr>
<tr>
<td></td>
<td>*3</td>
<td>I359L</td>
<td>7 2 2</td>
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<tr>
<td>VKORC1</td>
<td>Group A</td>
<td>Haplotypes 1,2†</td>
<td>37 14 89</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>Haplotypes 7,8,9</td>
<td>58 49 10</td>
</tr>
</tbody>
</table>

† Data approximate from references ¹³,¹⁴,¹⁶,¹⁷
‡ From reference ¹²
§ From references ¹²,¹⁴

Warfarin and Pharmacogenomics

The anticoagulant response to warfarin is affected by both environmental influences such as vitamin K in the diet and also by genetically determined levels of critical enzymes. These include the cytochrome P-450 2C9 (CYP2C9) enzyme that is involved in the metabolism of warfarin, and also vitamin K epoxide reductase (VKORC1), which is the enzyme inhibited by warfarin. The clearance of warfarin in almost entirely the result of hepatic metabolism, and CYP2C9 is the most important enzyme mediating its clearance.¹² A number of polymorphisms in CYP2C9 have been identified, but the most important are CYP2C9*2 and CYP2C9*3. The *2 allele (R144C) and the *3 allele (I359L) are found in approximately 11% and 7% of patients and result in reductions of enzymatic activity of about 30% and 80%, respectively.⁵⁻⁷ (Table 1). The reduced metabolic clearance leads to increased drug levels and an increased anticoagulant effect.
The second key enzyme in warfarin pharmacogenomics is VKORC1, the enzyme that converts oxidized vitamin K to the active reduced form that is required for the post-translational (gamma) carboxylation of the vitamin K–dependent coagulation factors.8-10 VKORC1 is the target of warfarin and other coumarin anticoagulants that function as competitive inhibitors. Numerous coding and noncoding polymorphisms in VKORC1 have been identified that can affect the response to warfarin.11,12 There include 5 major haplotypes that can be separated into low (A) and high (B) dose groups that differ in sensitivity to warfarin.

Evidence is clear that polymorphisms in either CYP2C9 or VKORC1 affect warfarin sensitivity. Typical studies have performed genotyping in patients on stable anticoagulation and then related genotype to warfarin dose and outcomes. For example, Higashi et al conducted a retrospective cohort study of 200 at two anticoagulation clinics.13 At least one variant of the CYP2C9 allele was found in 31% of patients, whereas 69% had the wild-type (*1/*1) genotype. The mean maintenance warfarin dose varied significantly among the six possible genotype groups (P < .001). The highest dose of 5.63 mg/day was required in the *1/*1 group and the lowest in the *3/*3 group (1.60 mg/day). Patients with at least one variant allele had an increased risk of INRs over the desired range, and the variant groups also required more time to achieve stable dosing compared to patients with the wild-type allele. A serious or life-threatening bleeding event was also more common in patients with a variant genotype.

In a similar study, Sconce et al determined CYP2C9 and VKORC1 genotypes in 297 patients with stable anticoagulation.14 The mean daily dose was highest in CYP2C9 homozygous wild-type patients compared with those with variant *2 or *3 alleles (P < .001). It was also higher in patients with the VKORC1 GG genotype compared with those with either GA or AA (P < .001). A multivariate regression model that included age, height, and CYP2C9 genotypes explained approximately 55% of the variation in warfarin dosing. Carliquist et al studied 213 patients with a stable INR between 2.0 and 3.0.15 Warfarin dose correlated with CYP2C9 genotype and was 33.3 mg/week for wild-type CYP2C9 but reduced by 18% to 31% for carriers of a single variant and by 82% for double variant carriers. Similarly, for VKORC1 wild-type patients the average weekly warfarin dose was 38.4 mg compared with 21.0 mg/week for homozygous variant carriers. Genotype was the dominant predictor of warfarin dose; weaker predictors were age, weight, and gender in modeling studies that explained approximately 50% of the dose variation.

Although CYP2C9 and VKORC1 genotypes clearly affect warfarin sensitivity, the clinical value derives from the potential to use genotyping as a guide in warfarin dosing, and recent studies indicate that this may be useful. For example, Gage and colleagues developed a pharmacoge-
roxaban and apixaban are factor Xa inhibitors. They are orally active and directly inhibit their target enzyme, whereas rivaroxaban and apixaban. All three agents are approved in Europe and continue in Phase III studies. Other agents in advanced Phase III studies in-clude rivaroxaban and apixaban. The need for new oral anticoagu-lants is great, but the search has been more elusive. Warfarin is the only currently available oral anticoagulant, and it represents the mainstay of treatment for a large number of patients needing long-term anticoagulation for common conditions including atrial fibrillation, prosthetic heart valves, VTE and coronary artery disease. Several new oral anticoagulants have entered clinical trials, and one, ximelagatran, was approved in Europe and then withdrawn from the market after several cases of hepatotoxicity appeared. A second new oral agent, dabigatran, has recently been approved in Europe and continues in Phase III studies. Other agents in advanced Phase III studies include rivaroxaban and apixaban. All three agents are orally active and directly inhibit their target enzyme with no intermediate such as antithrombin. (Table 2). Dabigatran inhibits thrombin, whereas rivaroxaban and apixaban are factor Xa inhibitors. They are administered once or twice daily, and they have followed a similar clinical development strategy starting with prophylaxis of VTE after major orthopedic surgery and then expanding to long-term treatment of atrial fibrillation and/or VTE.

Dabigatran etexilate is a small molecule direct-acting thrombin inhibitor that was recently approved (April 2008) by the European Medicines Agency for use in Europe in prophylaxis of VTE following total hip and total knee arthroplasty. It is a prodrug that is rapidly converted by esterases to the active form, dabigatran, a reversible, high affinity (K_s = 4.0 nm) thrombin inhibitor. Its absorption is pH sensitive, and it is formulated in tartaric acid–containing capsules to facilitate absorption, which is decreased by approximately 30% in the presence of proton pump inhibitors. The metabolism is not dependent on cytochrome P-450 enzymes, reducing the risk of drug interactions. Its bioavailability is low (approximately 6%), and it reaches a peak concentration approximately 2 to 3 hours after ingestion. The terminal plasma half-life is approximately 12 to 17 hours, with about 80% excreted renally. Therefore, drug accumu-lation can occur with renal failure. Although dabigatran prolongs the PTT, the effects are not dose dependent. There is little effect on the PT, but the ecarin clotting time prolonga-tion appears to reflect plasma concentration.

Dabigatran was studied in Phase II studies in prevention of VTE following total hip or total knee replacement and also in prevention of stroke and systemic embolization in patients with atrial fibrillation. In a multicenter, double-blind study, 1973 patients undergoing total hip or knee replacement were randomized to receive 6 to 10 days of oral dabigatran or enoxaparin. Dabigatran was given in doses of 50, 150, or 225 mg twice daily or 300 mg once daily. The first dose of dabigatran was administered 1 to 4 hours after surgery; and enoxaparin 40 mg was started 12 hours prior to surgery and administered once daily. VTE

| Table 2. Properties and status of dabigatran, rivaroxaban, and apixaban. |
|-------------------|-----------------|-----------------|
| Target            | Thrombin        | Xa              | Xa              |
| Time to peak level, hr | 2-3             | 3               | 3               |
| Half-life, hr     | 12-17           | 5-9             | 9-14            |
| Bioavailability, % | 6               | 80              | >50             |
| Renal excretion, %| 80              | 66              | 25              |
| Dosing            | Oral, once or twice daily | Oral, once or twice daily | Oral, once or twice daily |
| Status            | Approved in Europe for prophylaxis after TKR and THR. | Phase III development | Phase III development |
|                   | Phase III studies ongoing |                 |                 |
occurred in 28.5%, 17.4%, 16.6%, 13.1%, and 24% of patients assigned to dabigatran 50 or 150 mg twice daily, 300 mg daily, 225 mg twice daily and enoxaparin, respectively. There was a significant dose-dependent decrease in VTE with increasing doses of dabigatran ($P < .0001$). VTE was significantly lower in patients receiving 150 mg or 225 mg twice daily or 300 mg once daily than with enoxaparin. Compared to enoxaparin, major bleeding was significantly lower with 50 mg twice daily but increased at higher doses.

These results formed a basis for subsequent Phase III trials.

A second Phase II study was conducted in patients with nonvalvular atrial fibrillation. A total of 502 patients were randomized to 1 of 10 treatment groups that included 3 doses of dabigatran (50, 150, and 300 mg twice daily) combined in a $3 \times 3$ factorial fashion with no aspirin or 81 or 325 mg once daily. A comparator group received warfarin alone with an INR target range of 2.0 to 3.0. Participants were monitored as outpatients for up to 12 weeks. Bleeding events were more frequent in the 300 mg (23%) and 150 mg (18%) dabigatran groups compared to the 50 mg groups (7%). The only thromboembolic events occurred in the 50 mg dabigatran dose groups (2%). Dabigatran was well tolerated and there were no major unexpected adverse events.

Phase III studies have been completed with dabigatran for prevention of VTE after total hip or knee replacement. The RE-NOVATE trial was a double-blind study that included 3494 patients undergoing total hip replacement who were randomized to receive dabigatran 220 mg or 150 mg once daily, starting with a half dose 1 to 4 hours after surgery or enoxaparin 40 mg once daily starting the evening before surgery. Treatment was continued for 28 to 35 days until bilateral venography. The primary efficacy outcome was a composite of total venous thromboembolic events (including both symptomatic VTE and asymptomatic DVT detected by venography) and all-cause mortality. This occurred in 60/897 (6.7%) of patients receiving enoxaparin versus 53/880 (6.0%) of patients in the dabigatran 220 mg group and 75/874 (8.6%) of those in the 150 mg group. The results in both dabigatran groups were non-inferior to enoxaparin based on pre-specified criteria, and there was no significant difference in the rates of VTE or bleeding among the groups.

A similar trial (RE-MODEL) was conducted in patients undergoing total knee replacement. A total of 2076 patients were randomized in a double-blind study to receive dabigatran, 150 mg or 220 mg once daily starting with a half dose 1 to 4 hours after surgery or enoxaparin 40 mg daily starting the evening before surgery for 6 to 10 days of treatment. The primary efficacy outcome occurred in 193/512 (37.7%) evaluable patients in the enoxaparin group compared with 183/503 (36.4%) dabigatran 220 mg group and 213/526 (40.5%) in the 150 mg group. Both doses of dabigatran were non-inferior to enoxaparin on the basis of prespecified criteria, and there was no significant difference in bleeding events, most of which occurred at the surgical site.

A third Phase III orthopedic study (RE-MOBILIZE) was conducted in North America. This double-blind study included 2615 patients undergoing total knee replacement who were randomized to receive 12-15 days of treatment with dabigatran 150 mg daily, dabigatran 220 mg daily or enoxaparin 30 mg twice daily. Dabigatran was given as a half dose 6 to 12 hours postoperatively, and enoxaparin was started 12 to 24 hours after surgery. As in the prior studies, the primary efficacy end point was the composite of symptomatic VTE, all-cause mortality and asymptomatic DVT detected by screening venography. This end point was reached in 188 of 604 (31.1%) patients in the dabigatran 220 mg group, 219 of 649 (33.7%) in the 150 mg group and 163 of 643 (25.3%) in the enoxaparin group. Both dabigatran dose regimens failed to show non-inferiority to enoxaparin based on the prespecified margin of 9.2%. The rate of the primary end point was significantly higher in the 220 mg group ($P = .02$) and in the 150 mg group ($P = .0009$) compared with enoxaparin. Major bleeding events were uncommon in all three groups and did not differ significantly. As in the other studies, dabigatran was well tolerated, and there was no evidence of excessive hepatic, cardiac or other toxicities.

The results in the North American knee replacement trial were clearly different from those in the studies reported earlier. Although this may have been due to chance, significant differences in study design could explain the different results. An important difference was in the enoxaparin regimens. In the European studies, enoxaparin was started the evening before surgery in a dose of 40 mg once daily and continued for a mean of 7 days. In the North American study, the enoxaparin dose was 50% higher (30 mg twice daily), it was started 12 to 24 hours after surgery, and treatment was continued for a mean of 13 days. The higher enoxaparin dose and longer treatment may have contributed to the lower frequency of VTE in the enoxaparin group in the North American study (25% vs 38%). The dabigatran regimens also differed; the start time was 1 to 4 hours after surgery in the European studies and 6 to 12 hours in the North American study. Overall, the results of these three studies indicate that dabigatran appears safe following major orthopedic surgery and is effective in reducing the rate of VTE. Thus, it confirms the overall findings with ximelagatran and supports the potential value of an oral direct thrombin inhibitor as a new antithrombotic agent. Based on the findings of the European studies in hip and knee replacement, the European Medicines Agency approved dabigatran for prevention of VTE following total hip or knee replacement in April 2008. Additional large Phase III studies are ongoing with dabigatran in prevention of stroke and systemic embolism in patients with atrial fibrillation and in long-term treatment of patients with VTE.
Rivaroxaban (BAY59-7939) is an oral, direct factor Xa inhibitor in advanced Phase III trials for prevention and treatment of thromboembolic disease. It binds tightly but reversibly to the active site of factor Xa (K = 0.4 nm), and it inhibits factor Xa either in solution or associated with prothrombinase activity in a clot. It has good bioavailability after oral administration with approximately 80% absorbed, and plasma levels peak at approximately 3 hours. The terminal half-life is between 5 and 9 hours but somewhat longer in elderly subjects. The majority of excretion is through the kidneys, with a smaller portion through biliary/fecal elimination. Rivaroxaban prolongs both the PT and aPTT, and levels can be estimated using factor Xa inhibition assays.

In three Phase II double-blind studies, 2236 patients undergoing hip replacement were randomized to a wide range of rivaroxaban doses once or twice daily starting 6 to 8 hours after surgery or to enoxaparin 40 mg once daily starting the evening before surgery. Treatment was continued until venography 5 to 9 days after surgery. The primary efficacy endpoint of any VTE and all-cause mortality did not demonstrate any significant dose response relation for rivaroxaban, but there was a significant increase in the frequency of major bleeding. A companion study was conducted in total knee replacement and included 621 patients randomized to 2.5, 10, 20, and 30 mg of rivaroxaban twice daily initiated 6 to 8 hours after surgery or to enoxaparin in a dose of 30 mg twice daily initiated 12 to 24 hours after surgery. Treatment was continued for 5 to 9 days postoperatively until mandatory venography. The primary composite efficacy endpoint of any DVT, confirmed pulmonary embolism and all-cause mortality occurred in 23% and 40% of patients receiving rivaroxaban compared with 44% in the enoxaparin group. There was no statistically significant dose trend for efficacy, but major bleeding increased with increasing doses of rivaroxaban.

Based on this extensive Phase II program, a rivaroxaban dose of 10 mg once daily beginning 6 to 8 hours postoperatively was selected for the Phase III studies in total hip and knee replacement. All three studies were randomized and double-blind, employed bilateral venography at the end of treatment and used the composite end point of all DVT, pulmonary embolism, and all-cause mortality. The RECORD1 study randomized 4541 patients having hip replacement to either rivaroxaban or enoxaparin 40 mg once daily beginning the evening before surgery and continued for 35 days. The primary end point was reached in 166 of 878 (18.9%) of those receiving enoxaparin, demonstrating superiority of rivaroxaban (P < .001). No difference in bleeding rates was observed. The RECORD3 trial compared treatment with the same doses of enoxaparin and rivaroxaban for 10 to 14 days in patients undergoing knee replacement. This study randomized 2531 patients, and the primary efficacy endpoint occurred in 79 of 824 (9.6%) of patients receiving rivaroxaban compared to 166 of 878 (18.9%) of those receiving enoxaparin, demonstrating superiority of rivaroxaban (P < .001). As in the prior studies, there was no significant difference in bleeding.

This large program clearly demonstrates the effectiveness of rivaroxaban in prevention of VTE after hip or knee replacement. The rates of thrombosis with the 10 mg dose were very low, and rates of bleeding were not higher than with the enoxaparin comparator. In contrast to studies with dabigatran, however, there was no comparison with a group receiving 30 mg enoxaparin dose given twice daily, which is the standard in North America and which may be more effective. Based on these results, rivaroxaban has been submitted to the European regulatory authorities for market approval.

Two Phase II studies have examined rivaroxaban treatment in acute symptomatic DVT. The ODIXa-DVT Study randomized 613 patients to twice daily doses of 10, 20, or 30 mg, or a once daily dose of 40 mg or to standard treatment with enoxaparin and a vitamin K antagonist for 12 weeks. The primary end point of thrombus regression as assessed by ultrasonography did not differ significantly among the rivaroxaban groups or in comparison with standard treatment. Rates of symptomatic recurrence and of major bleeding were low. A second Phase II study in treatment of symptomatic DVT has been reported in abstract form. The Einstein-DVT study included 543 patients randomized to receive rivaroxaban 20, 30, or 40 mg once daily or standard treatment for 3 months. As in the first study, there was no significant difference in the primary outcome of thrombus burden as measured by quantitative ultrasound in the four treatment groups. Phase III studies of rivaroxaban for treatment of VTE and also for stroke prevention in patients with atrial fibrillation are ongoing.

Apixaban is another small molecule, high affinity (K = 0.8 nM), selective, reversible inhibitor of factor Xa, that is active against both free enzyme and also that within the prothrombinase complex. The bioavailability after oral absorption is over 50%, and peak plasma levels are observed after 3 hours. The half-life is between 9 and 14 hours, and the primary elimination route appears to be fecal. Apixaban has little effect on the PT or PTT at therapeutic concentrations, but plasma levels can be assessed using a factor Xa inhibition assay.

A Phase II double-blind trial included 1238 patients undergoing knee replacement who were randomized to 1
of 6 doses of apixaban or to enoxaparin 30 mg twice daily or to warfarin (INR 1.8-3.0). Treatment was begun 12 to 24 hours after surgery with apixaban or enoxaparin and on the evening of surgery with warfarin and continued for 10 to 14 days when venography was performed. Total VTE rates ranged from 5% to 13% in the apixaban groups without a significant dose-dependent effect. The rates were, however, lower than with enoxaparin (17%) or warfarin (29%). There was a significant dose-related increase in the incidence of bleeding with apixaban.

The results of a Phase II trial evaluating treatment with apixaban in patients with acute symptomatic DVT has been reported in abstract form. In a double-blind study, 520 patients were randomized to receive one of three regimens of apixaban (5 mg BID, 10 mg BID, or 20 mg QD) or to standard treatment for up to 91 days. The response was evaluated as a composite of symptomatic response and change in the thrombotic burden as assessed by repeat ultrasound or perfusion lung scanning. The primary outcome rates varied from 2.6% to 6% in various groups with no significant difference, and rates of bleeding were also comparable. Studies to evaluate the efficacy and safety of apixaban in patients with coronary artery disease, prevention of thromboembolic events in patients with cancer, and prevention of stroke and systemic embolism in patients with atrial fibrillation are ongoing.

Disclosures
Conflict-of-interest disclosure: The author serves as a consultant for and has received honoraria from Boehringer-Ingelheim and Eisai, and is also on the speakers’ bureau for Eisai.
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