The scientific community’s quest to identify optimal targets for anticoagulant pharmacotherapy must be soundly based on fundamental constructs of vascular biology and coagulation. Although this tenet, which has served as a platform for drug development during the past 50 years, is incontrovertible, one should also ask, “What experimental model of coagulation is most applicable and directly translatable to mammalian coagulation in general and to human thrombotic disorders in particular?” Is it the “waterfall-cascade” or “autoprosthenin model” of blood coagulation first described by Macfarlane and Davie and Ratnoff that so elegantly described inactive precursors being converted to active proteases in a sequential series of biosynthesis steps? Or is it a cell-based model of coagulation that portrays an integrated and functional representation of complex biochemical events occurring on cellular surfaces in lieu of functionally independent cascades that principally reflect clot formation in static fluid systems rather than the dynamic interplay of tissue factor (TF)-bearing cells, platelets, and their respective protein intermediaries? Or is it a cumulative 4-component model based on numerical approximations of TF-mediated thrombin generation, TF activation of the blood coagulation proteome, TF-activated and contact pathway–inhibited whole blood in vitro, and blood shed from standardized microvascular wounds in vivo as an interactive paradigm of real-time events? Clearly, each model honestly represents, with increasing biochemical rigor, authenticity, and translatability, blood coagulation under normal and pathological conditions. The remaining step, which provides a personalized stamp toward translating experimental models to human biology with concerted effort and safe, effective, and patient- and disease-specific care, is applied genomics, wherein gene expression profiles and pharmacogenomics identify, through predictive models, individuals at risk for thrombosis and those likely to experience treatment failure and/or complications from anticoagulant pharmacotherapy. On the basis of a contemporary understanding of vascular biology and hemostasis as they apply to thrombotic disorders and on previously summarized models depicting the inherently dynamic properties of coagulation, we will consider the merits of factor Xa as a target for anticoagulant pharmacotherapy.

Factor X (Stuart factor) (reviewed by Graham) is a vitamin K–dependent glycoprotein (15% carbohydrate) that is hepatically synthesized and secreted into plasma as a precursor to a serine protease. In blood coagulation (eg, waterfall-cascade model), the conversion of factor (f) X to fXa is catalyzed by the fIXa–fVIIIa complex in the presence of calcium and phospholipids. The prothrombinase complex (fXa, fVa calcium, and phospholipids) that rapidly converts prothrombin to thrombin is a membrane-dependent process (eg, cell-based model) that requires several binding steps. The cell-surface dependency of serine proteases, including fXa, which is bound to TF-bearing cells and platelets through specific binding sites and receptors, limits the degrees of freedom for interacting molecules, restricts them to biochemical reactions in 2 dimensions (rather than 3 in the fluid phase), and offers protection from circulating inhibitors such as tissue factor pathway inhibitor and protein Z–dependent protease inhibitor (eg, integrated model).

The binding of fXa to vascular endothelial cells, an event that occurs independently of thrombin and/or fVa, is associated with a wide range of prothrombotic, proinflammatory, and promitogenic responses that include TF expression, plasminogen activator inhibitor-1 release, interleukin-1 and -8 release, expression of E-selectin, and intercellular adhesion molecule-1 and protease activated receptor-1 and -2 activation. The discriminating expression patterns of individuals with venous thromboembolism, including the fX gene, close the biological loop on fXa as a worthy target for pharmacological inhibition; coupled with a wealth of favorable clinical data accrued with indirect and nonselective, indirect and semiselective (reviewed by Geerts et al), and indirect and selective (reviewed by Bauersachs) fXa antagonists, and administered subcutaneously, these patterns set the stage to develop and enthusiastically investigate a direct and selective drug given orally.

**Trial Design**

Ericksson and colleagues, a highly experienced investigative group, are to be acknowledged for completing an important double-blind trial, published in this issue of Circulation, that evaluates a novel therapeutic option for preventing venous thrombotic complications among patients after total hip arthroplasty. Comparison of an oral anticoagulant with another agent administered subcutaneously presents operational challenges that the investigators had to overcome.
With more than 870 randomized patients, the Oral, Direct Factor Xa Inhibitor, BAY 59-7939, Given Once Daily in Patients Undergoing Total Hip Replacement (ODIXa-HIP) study represents a relatively large phase II trial that provides requisite clinical information about rivaroxaban at several doses, ranging from 5 to 40 mg given once daily. Unfortunately, more than 250 patients (29% of those randomized) did not contribute to the primary efficacy analysis, and these data were not presented as a pure intention cohort. In contrast, 845 of the 873 patients enrolled were included in the safety analysis. Although similar dropout rates for an efficacy assessment have been reported in prior trials, this high number is concerning for this particular phase II study evaluating once-daily dosing, wherein a tendency toward a lower incidence of the primary efficacy end point was observed with increasing doses of rivaroxaban.

On the basis of the available data, the investigators conclude that a 10-mg once-daily dose should be studied in future trials of orthopedic thromboprophylaxis. We agree fully that deciding on a drug dose for phase III trials is a complex process and represents a major challenge of unparalleled relevance for clinical trialists and sponsors alike; however, although there was a numeric excess of bleeding and thrombotic events in the 5-mg group (compared with the 10-mg group), the small number of events may not provide a solid foundation for dose selection. Accordingly, further investigation of both the 5- and 10-mg doses may be prudent, especially if a practical design that includes patients with renal insufficiency and/or other comorbid factors influencing drug clearance, and a more prolonged period of preventive administration, is chosen.

The clinical consequences of bleeding and blood product transfusion in patients with critical illness, end-stage renal disease, and acute coronary syndromes has received heightened attention and underscores ongoing efforts to develop anticoagulants with improved safety profiles and more favorable therapeutic indices. Despite acceptable major bleeding rates of approximately 4% to 5%, nearly 60% of patients in ODIXa-HIP were transfused after treatment. Although allogeneic (in contrast to autologous) blood products are used frequently in elective orthopedic surgery, one must consider the potential impact of transfusions, including their proinflammatory, immunomodulating, and prothrombotic effects (reviewed by Twomey et al17).

The study of a direct Fxa inhibitor rivaroxaban by Eriksson and colleagues provides an opportunity to discuss several questions raised frequently by clinical investigators and practicing clinicians concerning coagulation measurement tools and the importance of contact activation (and its subsequent pharmacological inhibition) in the pathogenesis (and prevention) of artificial surface thrombosis. The development of coagulation-dependent clotting assays played an important role in diagnosing both inherited and acquired bleeding disorders associated with factor deficiencies and inhibitors, respectively. Early laboratory tests such as the partial thromboplastin time, activated partial thromboplastin time (an activator such as kaolin, celite, or powdered glass (photometric assay) of 20% to 60%, respectively, with a lower incidence of the primary efficacy end point was observed with increasing doses of rivaroxaban. PT) were subsequently adapted to gauge and define safety and, to a lesser degree, efficacy for anticoagulant pharmacotherapy. Variability in PT values after the introduction of numerous commercial thromboplastin reagents led to marked inconsistencies of dose response and bleeding complications with warfarin administration, prompting laboratory standardization in the form of the International Normalized Ratio. The concerted, albeit protracted, response undertaken by the World Health Organization to a laboratory-based problem that was directly compromising patient care is illustrative for several reasons: First, it underscores the impact of test reagents and instrumentation on clot-based coagulation assays; and second, it emphasizes the importance of interpreting coagulation tests in the context of specific drugs, their mechanism of action, target protease kinetics, and applied laboratory substrates. Accordingly, each anticoagulant, even those within the same general class of inhibitors (including FXa inhibitors), may influence clot-based assays differently, precluding extrapolation of results across agents with the assumption that they honestly reflect parameters of safety or efficacy. Similarly, methodologies for individual coagulation protease assays (chromogenic assays, ELISAs), although theoretically favored over clot-based assays for their ability to quantitate activity of a specific protein being inhibited, are based on the ability of serine proteases to cleave one or more peptide bonds of synthetic substrates. In reality, an enzyme’s ability to cleave synthetic substrate does not necessarily reflect functional activity in vivo.

The previous point is highlighted by our group’s observations in the Xa Neutralization for Atherosclerotic Disease Understanding trial with DX-9065a, a parenterally administered, direct, selective FXa inhibitor. All pharmacodynamic measurements, including PT, activated partial thromboplastin time, and anti-FXa activity, increased with increasing drug concentration (measured directly with liquid chromatography/mass spectrometry); however, the correlations differed 2- to 3-fold and varied according to time of measurement (reflecting different sensitivities to plasma drug concentrations), and the absolute values for both clot-based and chromogenic tests were markedly different than those reported previously with other FXa inhibitors (both direct and indirect, selective or semiselective).19 Even emerging methods for coagulation measurement, such as high-performance liquid chromatography and flow cytometry, may not be able to standardize assays across or within drug classes.

Single-dose pharmacokinetic and pharmacodynamics studies with rivaroxaban (5 to 80 mg) in healthy male volunteers (ages 19 to 45 years) revealed inhibition of FXa activity (photometric assay) of 20% to 60%, respectively, with a maximum inhibition of 75% after an 80-mg dose. PT and activated partial thromboplastin time prolongation was approximately twice that of baseline at the highest dose and correlated strongly with plasma drug concentrations.20 The highly distinct drug–coagulation measurement relationships for most anticoagulants, although considered by some to introduce unwanted complexity in drug development and integration to patient care, may in fact represent a critical opportunity to define requisite (for benefit) and safe levels of
antiplatelet agents, and they can damage the surrounding endothelium during insertion and cause recurring injury if left in place, impairing the synthesis and release of nitric oxide, ADPase, prostacycline, heparan sulfate, antithrombin III, plasminogen activators, and antinflammatory cytokines. Recurrent contact activation on artificial surfaces may also deplete vital thromboregulatory proteins. Flow conditions take on a particularly important role in determining the overall thrombogenicity of artificial surfaces, wherein the maintenance of laminar flow and avoidance of stasis and eddy currents reduce the accumulation of activated platelets and coagulation factors.

Reports of guide-catheter thrombosis and coronary arterial complications, including abrupt closure, new angiographic thrombus, and no-reflow among patients receiving fondaparinux in the Organization to Assess Strategies for Ischemic Syndromes (OASIS)-5 and OASIS-6 studies suggest that anticoagulants preventing contact activation are preferable in this environment and provide thromboprophylaxis. As it turns out, inactivation of IXa and kallikrein by heparin compounds parallels their anti-Xa rather than anti-IIa effects and is potentiated (through antithrombin III) by low-molecular-weight fractions that have little direct activity on thrombin. Further, the rate constant for IXa inhibition by antithrombin (in the presence of heparin) is comparatively slow compared with IXa and thrombin. Thus, it seems much more likely that the ability of heparin, particularly unfractionated molecules, to prevent thrombosis on artificial surfaces relates predominantly to locally achieved drug concentrations, kinetics (Vmax-substrate concentration relationship) of thrombin inhibition, and, perhaps of equal importance, their profound inhibitory effect on protein adsorption, a rapidly occurring process known as “conditioning of the surface” that is characterized by a high degree of thrombogenicity.

Potential for Rivaroxiban

Novel anticoagulant pharmacotherapies such as rivaroxaban, developed toward carefully chosen targets with attractive characteristics (oral administration, once-daily dosing, rapid onset of action, an intermediate offset rate, and no requirement for routine coagulation monitoring), herald an exciting time in cardiovascular research. Thrombotic disorders of the venous and arterial circulatory systems, including deep vein thrombosis/pulmonary embolism, atrial fibrillation, and acute coronary syndromes, are representative examples of prime areas of investigation for both short- and long-term administration.

Development of drugs across broad indications requires careful consideration on the part of the pharmaceutical, academic, and regulatory communities. Reliance on a traditional paradigm of investigation that focuses on one disease state at a time is costly, inefficient, and time consuming. Carefully coordinated activities within national and possibly international research networks, coupled with large-scale consortium development through National Institutes of Health Clinical and Translational Science Awards, may create the necessary infrastructure to accelerate and realize in our lifetime the global objective of “Bench to Bedside to Community” benefit.

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References


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