Coagulation Factor Xa Inhibition: Biological Background and Rationale

Robert J. Leadley, Jr.*

Pfizer Global Research and Development, Ann Arbor Laboratories, 2800 Plymouth Rd., Ann Arbor, MI 48105, USA

Abstract: Ischemic heart disease and cerebrovascular disease are the leading causes of death in the world. Surprisingly, these diseases are treated by relatively antiquated drugs. However, due to our improved understanding of the underlying pathology of these diseases, and a number of technological advances in tools for drug discovery and chemical optimization, an exciting new wave of antithrombotic compounds is beginning to emerge in clinical trials. These agents, referred to as direct coagulation factor Xa inhibitors, appear to provide an enhanced risk-benefit margin compared to conventional therapy. Preclinical and early clinical data gathered over the past few years suggests that direct fXa inhibitors will provide the necessary advancements in efficacy, safety, and ease of use required to displace conventional therapy. Whether or not these agents will succeed will be determined as this class of agents advances through clinical trials in the near future. This review describes some of the key studies that sparked an interest in fXa as a therapeutic target, highlighting the findings that provided important rationale for continuing the development of potent and selective direct fXa inhibitors.

INTRODUCTION

Many approaches to developing antithrombotic drugs that interfere with enzymes in the coagulation system have been pursued over the past few decades; however, currently approved drugs for thrombotic diseases have been around for quite some time. Heparin, discovered in 1916 [1] remains the intravenous anticoagulant of choice for acute thrombotic conditions. More recently, fractionated, or low-molecular-weight, heparins have demonstrated superiority over unfractionated heparin in several thrombotic indications [2, 3], but remain limited to intravenous and subcutaneous administration. And although the coumarin derivative warfarin was discovered in 1941 and used initially as a rodenticide [4] that provides systemic anticoagulation by indirect inactivation of vitamin-K dependent serine proteases, it remains the most frequently prescribed oral anticoagulant.

Since the principal components of the coagulation system have been known for some time, the lack of superior replacements for heparin and coumadin is rather surprising. On the other hand, the redundancy and complexity of the hemostasis system is extensive and has provided tremendous obstacles for discovering potent novel agents that are effective, yet safe. Fortunately, recent scientific findings and advances in technology and testing have provided tools to identify and develop new agents that will hopefully provide safer and more effective antithrombotic therapy than are currently available. Among these newer agents are direct thrombin inhibitors, tissue factor pathway inhibitors, antibodies against specific coagulation factors, synthetic indirect factor Xa (fXa) inhibitors, and possibly most promising, direct fXa inhibitors.

*Address correspondence to this author at the Cardiovascular Therapeutics, Pfizer Global Research and Development, Ann Arbor Laboratories, 2800 Plymouth Rd., Ann Arbor, MI 48105, USA; Tel: 734-622-1420; Fax: 734-622-1480; E-mail: ROBERT.LEADLEY@PFIZER.COM

FACTOR Xa BIOLOGICAL BACKGROUND

Factor X is the zymogen of fXa, a serine protease which occupies a pivitol position in the coagulation cascade. Factor X can be activated by the contact ("intrinsic tenase") pathway or by the tissue factor/VIIa ("extrinsic tenase") pathway of the coagulation system (Fig. **1**). Consequently, initiation of coagulation by either pathway in response to vascular injury activates factor X to fXa, making fXa inhibition a desirable intervention point when developing novel antithrombitics. Factor Xa and its cofactor, factor Va, combine on phospholipid membranes to form the "prothrombinase" complex, which activates prothrombin to thrombin. Thrombin, by cleaving fibrinogen to fibrin, by activating platelets, and by converting factor XIII to factor XIIIa, is the principal enzyme involved in thrombus generation, growth, and stabilization.

Factor X has long been recognized to play a significant role in hemostasis [5]. Factor X deficiency, while extremely rare, was first described in the 1950s in two families, Prower and Stuart (thus the original nomenclature of factor X as "Stuart" or "Stuart-Prower" factor [6, 7]). These patients had very low antigen and activity levels of factor X, which were manifested in severe bleeding diatheses. Also, in the 1950s, studies of serum from these patients led to the identification of a deficiency of a specific factor, factor X, that was responsible for the bleeding diatheses. Since then, a number of variants of factor X deficiency suggest that factor X activity levels must be less than 5% of normal to result in spontaneous bleeding tendencies [8]. This information suggests that factor X activity can be suppressed markedly

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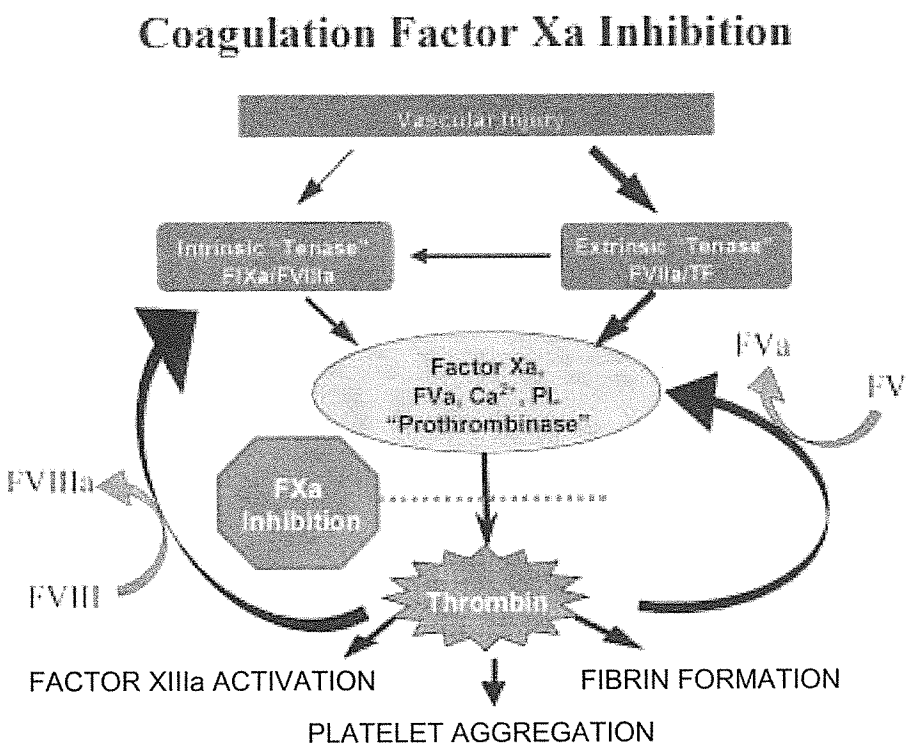

Fig. (1). Simplified schematic of the coagulation system and the intervention point for direct Factor Xa inhibitors.

without affecting hemostasis, a characteristic that is desirable for antithrombotic agents. That is, an ideal agent would prevent thrombosis without producing systemic hypocoagulation which would lead to bleeding complications at sites other than the intended target (e.g., intracerebral hemorrhage in patients treated for coronary artery disease). While this ideal approach was appealing, the hypothesis remained untested because no direct, selective inhibitors of fXa were available until fairly recently.

NATURALLY OCCURING INHIBITORS OF FACTOR Xa

In 1987, Tuszynski *et al*., published their discovery of an anticoagulant, antistasin, which they isolated from extracts of the Mexican leech, *Haementeria officinalis* [9]. The amino acid sequence of antistasin was determined soon thereafter [10], and enzyme kinetic analysis determined that antistasin is a slow, tight-binding, selective inhibitor of fXa with a Ki of 0.3-0.6 nM [11]. At approximately the same time, Waxman *et al*., reported the discovery of tick anticoagulant peptide (TAP), a single chain 60 amino acid peptide that was isolated from the extracts of the tick *Ornithodoros moubata* [12]. TAP is a reversible, slow, tight-binding inhibitor of fXa with an estimated Ki of 0.5 nM. Molecular biology techniques quickly allowed scientists to make recombinant forms of these peptides which, particularly in the case of TAP, were used to validate fXa as a viable drug target and to aid in our understanding of the significance of fXa in thrombosis.

VALIDATION OF FACTOR Xa AS A VIABLE DRUG TARGET

A number of animal models of thrombosis were used to compare the antithrombotic effect of peptide inhibitors of fXa to direct inhibitors of thrombin and to indirect inhibitors of thrombin and fXa, such as heparin and low-molecular weight heparins [e.g., 13, 14, 15, 16]. These studies provided a preponderance of evidence to support the concept that direct fXa inhibition may provide a more effective antithrombotic approach than other mechanistic inhibitors, and that there appears to be a larger safety margin for fXa inhibitors as well, particularly regarding primary hemostasis at wound sites. For example, in canine models of thrombosis and thrombolysis, recombinant TAP was shown to promote rapid and prolonged vascular reperfusion at doses which produced relatively minor elevations in prothrombin time (PT), activated partial thromboplastin time (APTT), and template bleeding time [13, 14, 16, 17]. Although TAP was apparently discontinued from development for undisclosed reasons, the preclinical studies demonstrated that fXa inhibition was, indeed, an attractive drug target for the development of small molecule inhibitors of fXa.

RATIONALE FOR DEVELOPING FACTOR Xa INHIBITORS

TAP also provided a valuable tool for defining the role of fXa in thrombus formation and growth, thereby providing

further rationale to support the concept of fXa inhibition as a desirable drug target. The following discussion highlights some of the important findings.

From experiments performed by Eisenberg, *et al* [18, 19], it became clear that Xa associated with thrombi is enzymatically active. For example, fXa in thrombi recovered from injured arteries *in vivo* is capable of activating prothrombin to thrombin [19]. Furthermore, fXa, in comparison to thrombin, appears to be primarily responsible for clot-associated procoagulant activity [20]. Specifically, TAP inhibited clot-associated generation of Fibrinopeptide A (a byproduct of thrombin-induced cleavage of fibrinogen to fibrin) to the same degree as the direct thrombin inhibitor, hirudin, suggesting that the procoagulant activity of thrombi is due to the *de novo* activation of prothrombin to thrombin, not merely to the presence of preexisting thrombin. These data indicate that fXa is active in thrombi and that direct inhibition of thrombus-associated fXa would be an effective, highly localized approach to prevention of thrombus growth.

One of the current problems associated with anti-thrombin therapy for acute coronary syndromes is the phenomenon of "rebound" ischemia shortly after the therapy is terminated. This phenomenon was observed with direct and indirect, antithrombin III-dependent inhibitors of thrombin (argatroban and heparin, respectively [21, 22] and is thought to occur because of continual generation of thrombin after therapy is discontinued. Presumably, these agents were able to block thrombin activity as long as the drug was present, but the generation of thrombin by thrombus-associated fXa continued after therapy was withdrawn, resulting in recurrence of thrombotic ischemic events.

Recent experiments indicate that short-term inhibition of fXa by TAP provides sustained inhibition of clot-associated procoagulant activity *in vitro* [23]. These results were validated *in vivo* by examining platelet deposition on dacron vascular grafts in baboons during and after a 2 hour intravenous infusion of rTAP [24]. TAP decreased platelet deposition during the infusion and for 2 and 53 hours after termination of low dose (10 µg/kg/min) and high dose (25 µg/kg/min) rTAP, respectively. In another experiment, **CI-1031 (ZK-807834)**, a small molecule, direct inhibitor of fXa, was administered as adjunctive treatment during and for 1 hr post fibrinolytic therapy to dogs that had experimentally-induced occlusive coronary artery thrombosis [25]. Compared to heparin plus aspirin, 24-hr vessel patency achieved with CI-1031 was markedly enhanced. These findings were also supported by a preliminary report on a study in which another small molecule inhibitor of fXa, **FXV673**, administered to canines over a few hours prevented thrombosis for three days following electrolytic injury-induced thrombosis in the carotid artery [26]. These data suggest that the sustained antithrombotic effect after short-term drug exposure is not a feature of TAP alone, but is a unique and highly desirable effect of direct fXa inhibition. This feature, sometimes referred to as "passivation," may be important to consider when designing clinical studies to evaluate novel fXa inhibitors in acute thrombotic diseases, especially in the current medical environment which mandates short hospital stays for interventions such a percuataneous coronary interventions.

Not only does fXa inhibition provide sustained antithrombotic protection, fXa inhibition also disaggregates preformed platelet thrombi *in vivo* [27]. In experiments using anesthetized pigs, the carotid artery was damaged by serial hemostat crushes and the resulting thrombus was allowed to grow for 30 min prior to drug administration. Despite the fact that TAP and hirudin have no intrinsic lytic activity, these agents not only prevented further platelet deposition, but they actually reduced radiolabelled platelet deposition at the site of vascular damage. Worth noting is the observation that with TAP, the "dissaggregation" or "dethrombosis" occurred at doses of TAP that yielded only modest changes in clotting time assays such as APTT. In summary, newly formed thrombi (<6 hours) can disaggregate if exposed to a hirudin or TAP. If proven to be true for small molecule direct inhibitors of fXa, this intriguing feature of "dethrombosis" may be an important therapeutic characteristic of direct fXa inhibitors, particularly for the acute treatment of thrombotic syndromes.

NEW INSIGHTS PROVIDED BY SYNTHETIC INHIBITORS OF FACTOR Xa

Based on the favorable preclinical evidence provided by recombinant forms of naturally occuring fXa inhibitors, many pharmaceutical companies quickly initiated chemistry programs that produced potent and selective small molecule inhibitors of fXa, of which **DX9065a** is the most widely characterized agent in the literature [e.g., 28, 29, 30, 31, 32, 33].

In addition, the success of indirect fXa inhibitor heparanoids, such as low-molecular-weight heparins, in venous [3] and arterial thrombosis [2] prompted interest in continuing the development of more selective indirect inhibitors of fXa that were designed based on the minimal saccharide sequence of heparin required for antithrombin-III binding [34]. **SR90107A/ORG31540** is the furthest-developed of a family of synthetic pentasaccharides that are selective, antithrombin-III dependent fXa inhibitors.

These two agents, **DX-9065a** and **SR90107A/ORG31540** have been extensively evaluated in *in vivo* models of thrombosis, exemplified best, perhaps, by their efficacy in a baboon model of shunt thrombosis. **SR90107A/ORG31540** dose-dependently inhibited platelet and fibrin deposition on shunts that represented conditions of venous thrombosis, but was relatively ineffective in preventing platelet deposition on shunts mimicing arterial, platelet-dependent thrombosis [35]. Likewise, intravenous or oral administration of **DX-9065a** significantly inhibited thrombus formation in a venous-type chamber but had no significant antithrombotic effect in an arterial-type chamber [30]. These results suggest that selective inhibition of fXa results in specific inhibition of fibrin formation with relatively less activity against platelet-dependent thrombi. These data appear to directly contradict the data from experiments mentioned earlier in which platelet deposition on injured porcine arteries was reversed by fXa inhibition [27]. Which experimental condition is more comparable to

the situation in patients with arterial thrombosis is still uncertain, especially in regard to how these new agents perform in the model versus the clinical setting. This apparent discrepancy is important to consider because it could have a direct impact on the indications targeted for a fXa inhibitor and on the design of clinical trials of novel fXa inhibitors (e.g., are antiplatelet agents required as concomitant therapy with fXa inhibitors?).

SEPARATION OF ANTITHROMBOTIC EFFICACY FROM BLEEDING EFFECTS

As alluded to previously, one of the most intriguing advantages of direct fXa inhibitors over conventional therapy is the relatively large therapeutic window between antithrombotic efficacy and bleeding tendency. Numerous studies have demonstrated antithrombotic efficacy at doses of fXa inhibitors that have little or no effect on markers of primary hemostasis such as template bleeding time, tail resection bleeding time, or cuticle bleeding time [32, 33, 36, 37, 38, 39]. Sato *et al.,* [36] demonstrated that the risk-benefit ratio (dose that doubled bleeding time/dose that produced 50% inhibition in a rat venous thrombosis model) for **YM-60828** was dramatically higher than the ratio measured with argatroban, heparin, and dalteparin (94 versus 7.4, 2.9, and 5.3, respectively). Similar risk-benefit analysis could be applied to the studies sited above, demonstrating a favorable safety profile for fXa inhibitors over other types of antithrombotic agents.

There are several mechanisms that have been postulated to explain the minimal bleeding observed during administration of antithrombotic doses of fXa inhibitors in animal models of thrombosis. First, fXa has no direct effect on platelet aggregation and only activates platelets indirectly via generation of thrombin when Xa is assembled into the prothrombinase complex. Consequently, unlike direct thrombin inhibitors or heparinoids, nearly all direct fXa inhibitors have no inhibitory effect on thrombin- or thrombin receptor activator peptide (TRAP)-induced platelet aggregation [28, 40, 41]. The only cited exception is **YM-60828**, which inhibits TRAP-induced platelet aggregation in plasma with an IC_{50} of 3.3 μM and thrombin-induced washed platelet aggregation with an IC_{50} of 23.4 μM [42]. Since thrombin is a potent activator of platelets, and its affinity for platelet receptors is 10,000-fold higher than for fibrinogen [43, 44] minimal amounts of thrombin may be adequate to activate platelets and allow for normal systemic hemostasis.

Minimal bleeding with fXa inhibitors may be attributed to reduction of thrombin generation, as opposed to blocking thrombin's activity once it is formed. Once incorporated into the prothrombinase complex, the reaction rate of fXa increases 300,000-fold compared to the rate of free fXa, thereby generating an explosive production of thrombin [45]. Inhibiting fXa directly with potent, selective agents therefore provides efficient reduction of thrombin generation at the thrombus, without the need for excessively high systemic plasma concentration of the antithrombotic drug.

SEPARATION OF ANTITHROMBOTIC EFFICACY FROM SYSTEMIC HYPOCOAGULATION

Several studies also demonstrated that, in comparison to other mechanisms of inhibition of thrombus formation (e.g., unfractionated heparin, low-molecular-weight heparin, and direct thrombin inhibition), fXa inhibitors produced their antithrombotic effect with only modest changes in markers of systemic anticoagulation, such as PT, APTT, and thrombin time [32, 33, 36, 37, 38, 39, 46) . For example, maximally-effective antithrombotic doses of the direct fXa inhibitor, **C921-78**, heparin, enoxaparin, and PPACK (a direct thrombin inhibitor) in a rabbit model of arteriovenous shunt thrombosis produced changes in cuticle bleeding times of approximately 2- (not significant from baseline), 5.5-, 3.5-, and 6-fold, and changes in APTT of 1.6-, >6-, 5-, and 1.3-fold over baseline, respectively [39]. Similarly, when **RPR208566** was compared to heparin and argatroban (a direct thrombin inhibitor), PT and APTT were not changed significantly (<1.5-fold), while maximally effective doses of argatroban and heparin increased PT by 2.5- and 2.5-fold and APTT by 3- and 6-fold, respectively [46]. These observations have been made with a number of compounds from different chemical series indicating that, although there are certainly differences in the pharmacodynamics between individual fXa inhibitors, the overall safety advantages of fXa inhibition are mechanism-related.

Interestingly, the effect of potent selective inhibitors of fXa on clotting time assays is highly variable. For example, some fXa inhibitors appear to be more potent in the APTT assay than in the PT assay (TAP [15]; **RPR120844**, [47]), some are more potent in the PT than in the APTT assay (**DX-9065a** [28]; **CI-1031** [38]), and others appear to be equally potent in both assays (**YM-60828** [42], **SK549** [48]). The sensitivity of these tests for fXa inhibitors appears to be compound-dependent and may reflect as yet undefined differences in enzyme kinetics. Regardless, the APTT, PT, and activated clotting time (ACT) assays, which are used routinely to monitor heparin and warfarin treatment, are not sensitive enough to accurately monitor fXa inhibitors when administered at antithrombotic doses in animal models of thrombosis. Consequently, other more specific assays such as chromogenic fXa activity assays, Russel's viper venom clotting assay, the Heptest®, or thrombin generation assays might prove to be more sensitive pharmacodynamic markers for fXa inhibitors.

Although the explanation for the ability of fXa inhibitors to provide antithrombotic protection without increasing systemic markers of anticoagulation is not yet clearly elucidated, the affinity of these agents for thrombus-associated fXa may provide a plausible explanation [49, 50]. By inhibiting thrombus-bound fXa with high affinity, lower plasma drug concentrations may be required to provide antithrombotic efficacy. Significant prolongation of APTT and PT can be achieved by high-dose administration of fXa inhibitors, indicating that systemic hypocoagulation, and the susceptibility for bleeding complications, is not avoided completely by fXa inhibitors. However, the doses required to reach potentially dangerous plasma levels are quite high compared to doses providing maximal antithrombotic efficacy.

OTHER ADVANTAGES OF FACTOR Xa INHIBITION OVER CONVENTIONAL THERAPY

Additional rationale for developing fXa inhibitors to replace heparin and warfarin are listed in (Table **I**). The considerations for dosing and monitoring heparin therapy have been extensively reviewed by Hirsh, *et al*. [51]. Briefly, heparin administration must be monitored carefully to maintain plasma drug concentrations within a safe and effective window. Monitoring is necessary because heparin binds to a number of plasma proteins and its activity is neutralized by platelet factor 4 released from activated platelets, resulting in highly variable anticoagulant responses. Also, heparin administration causes an immune reaction referred to as heparin-induced thrombocytopenia (HIT) in approximately 1-3% of patients; LMWHs also produce HIT, but at a lower incidence compared to unfractionated heparin. Low molecular weight heparins have eliminated the need for monitoring in most indications, but have not substantially replaced heparin in acute coronary syndromes due, in part, to the lack of a specific monitoring device that can provide rapid feedback for dose adjustment before, during, and after percutaneous coronary interventions. The limitations of indirect versus direct inhibition of coagulation enzymes has been highlighted in studies by Weitz *et al*. [52] and by Hérault *et al*., [49]. As mentioned above, fXa and thrombin are both enzymatically active when associated with the intravascular thrombus, so it is vitally important that an antithrombotic agent be able to inhibit this so-called "clot-bound" activity. Indirect inhibitors act by catalyzing the inactivation of thrombin or fXa by antithrombin-III, a large molecule that is not able to penetrate the thrombus. Consequently, heparin, LMWHs, or the synthetic pentasaccharides are not effective inhibitors of thrombus-associated fXa or thrombin. Direct fXa and thrombin inhibitors, however, are capable of inhibiting thrombus-associated fXa and thrombin, making these agents more attractive for medical interventions in thrombotic diseases. The other features of fXa inhibitors that were highlighted above, namely "dethrombosis" and vascular "passivation," are important characteristics for agents that will be administered over a brief period. Emergency treatment of acute coronary syndromes and percutaneous coronary intervention by intravenous administration of fXa inhibitors may provide ideal settings to evaluate whether the mechanistic advantages of fXa inhibition will translate into superior efficacy and safety over heparin.

Currently, heparinoids are limited to subcutaneous and intravenous administration because they are not absorbed adequately after oral administration. However, new carriers have been developed that have enhanced the bioavailability of heparin so that an oral form of this agent is now being evaluated in clinical trials [53]. Regardless, the oral formulation of heparin will still have the same limitations as i.v. heparin, so that by virtue of inherent interindividual differences in oral absorption, orally-administered heparin will likely be even more difficult to maintain in the safe and effective therapeutic plasma concentration range.

The limitations of oral anticoagulation with warfarin are well-recognized and have effectively restricted its use in the clinic [54]. Warfarin acts in the liver by antagonizing the vitamin K-dependent carboxylation of glutamic acid residues on the amino terminal of coagulation factors II, VII, IX, and X. These residues are essential for calcium-dependent binding of these enzymes into their appropriate enzyme complexes on phospholipid surfaces. The approximate half-life of factor II (prothrombin) is 50 hours, so several days are required before warfarin achieves its full antithrombotic effect. Likewise, the reversal of the effect of warfarin can take 24 hr or longer. In addition, warfarin has many drug and food interactions that complicate its dosing and require regular monitoring to maintain safe and effective plasma drug concentrations [54, 55]. The significant bleeding complications and difficulty maintaining plasma concentrations of warfarin within the targetted range has led to the labeling of warfarin (Coumadin®) as a "narrow therapeutic index drug."

Obviously, a fXa inhibitor that is orally bioavailable and does not have many of the drawbacks of warfarin would be

Table I. Comparison of Direct fXa Inhibitors to Currently Available Anticoagulants

Agent	Enzymes inhibited	Cofactor required?	Inhibition of clot-bound enzyme?	Monitoring required?	Heparin-induced thrombocytopenia?	Route of administration	Risk/Benefit +
Direct fXa	fXa	N	Y	?	N	i.v., oral	++++
Direct IIa	IIa	N	Y	Y	N	s.c., i.v.	+
Heparin	Xa=IIa	Y	N	Y	Y	i.v., s.c.	+
LMWH	Xa>IIa	Y	N	N**	Y	s.c., i.v.	++
Pentasaccharide*	Xa>>>IIa	Y	N	?	?	s.c., i.v.	+++
Warfarin	IIa, VIIa, IXa, Xa, PC, PS	Y	N	Y	N	Oral, i.v.	+

+ Risk/Benefit is based on a qualitative estimate using heparin as a comparator. The risk/benefit for fXa inhibitors is speculative, based on preclinical evidence and experience with indirect fXa inhibitors.

* The pentasaccharide is not currently approved for use, but is included because the positive results in Phase III clinical trials will likely lead to approval in the near future.

** In general, monitoring of LMWHs is not required. However, for indications such as percutaneous coronary intervention it is desirable to know the anticoagulation level of the patient before, during, and after the procedure.

PC: Protein C; PS: Protein S

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