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Visions & Reflections

Factor Xa – a promising target for drug development

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Background

Based on their pivotal role in the blood coagulation cascade, the serine proteases thrombin and factor Xa (FXa) have become a focus of interest for the development of new anticoagulant/antithrombotic drugs. Direct inhibition of these key enzymes may offer new ways to affect the blood clotting process as well as other actions of coagulation enzymes which may be important for the pathogenesis of various cardiovascular disorders.

The trypsin-like serine protease FXa is an essential component of the prothrombinase complex which is assembled on phospholipid surfaces involving the calcium-dependent association of FXa and FVa. FX, which is a two-chain glycoprotein synthesised in the liver and secreted into the blood as a zymogen, is converted to the active protease by a complex of either tissue factor/FVIIa or FIXa/FVIIIa/phospholipid/calcium. The catalytic activity of free FXa is extremely low, but upon formation of the prothrombinase complex it is strongly enhanced so that prothrombin can be rapidly cleaved and sufficient amounts of thrombin can be generated at sites of vascular injury and thrombus growth [1]. Under physiological conditions, the activity of clotting enzymes is controlled by endogenous inhibitors such as antithrombin III (AT III) which effectively inactivates thrombin and FXa as well as other serine proteases by forming very stable complexes that block the active site of the enzymes. The reaction between AT III and its target enzymes is very strongly accelerated by interaction with glycosaminoglycans, especially the binding of a sequence-specific heparin pentasaccharide, which causes conformational changes in AT III [2, 3].

Besides its important role in the plasmatic coagulation system with the catalytic conversion of prothrombin to thrombin, FXa induces cellular responses implicated in cardiovascular and inflammatory diseases. FXa has been shown to be a strong mitogen and it exerts mitogenic actions via high-affinity binding sites on vascular smooth muscle cells. Although effector cell protease receptor-1 (EPR-1) was identified and cloned as a cellular receptor for FXa [4, 5], whether EPR-1 is really involved in the cellular effects of FXa or whether alternative cellular receptors for FXa exist such as members of the protease-activated receptor family remains a controversial issue [6–9]. The inhibition of FXa-mediated mitogenic effects by specific inhibitors found in experimental studies suggests that this class of drugs might be effective to reduce neointimal hyperplasia in vivo either by preventing the mitogenic effects of FXa and/or by inhibiting the generation of thrombin which is also known to be a potent mitogen [10, 11].

Commonly used anticoagulant/antithrombotic drugs such as heparin have limited efficacy, e. g. because of the requirement for endogenous cofactors, their inactivation by platelet factor 4 or the induction of various side effects such as heparin-induced thrombocytopaenia. Oral anticoagulants such as warfarin have a slow onset of action, may cause a paradoxical increase in coagulation activity due to the early inhibition of protein C and S, and require individual treatment with continuous laboratory monitoring as well as dose adjustment. Alternative antithrombotics would be highly desirable. An important aspect for the development of small-molecule inhibitors of FXa as well as thrombin is their ability to inactivate the clotting enzymes not only in plasma but also when they are bound to fibrin within a clot, an effect which is not seen with AT III and AT III-dependent inhibitors. Because FXa and active prothrombinase complexes are main determinants for the procoagulant activity of intravascular thrombi, effec-

tive inactivation of the clot-bound enzyme is of particular importance [12, 13].

The important role of FXa in the coagulation network at the stage of the common pathway of both the tissue factor-activated extrinsic and the surface-activated intrinsic system as well as the amplification of the procoagulant action of FXa by prothrombinase complex formation leads to the expectation that inhibitors with a high affinity and selectivity towards the enzyme will be potentially valuable therapeutic agents for various cardiovascular indications. Compared to thrombin, FXa acts at an earlier level in the coagulation system and is not such a multifunctional protein, i.e. it exerts its action mainly on the substrate prothrombin. Inactivation of FXa by specific inhibitors does not influence preformed thrombin but effectively prevents the generation of thrombin whereas in the presence of thrombin inhibitor, considerable thrombin formation was demonstrated. The increased thrombin activity observed after cessation of therapy with thrombin inhibitors might be responsible for the continuation of the thrombotic process and contribute to early rethrombosis. The apparently incomplete and only temporary suppression of thrombin generation by these drugs is not expected with FXa inhibitors because of their mechanism and site of action in the coagulation cascade [14, 15]. In addition, with the different mechanism of action, FXa inhibitors might have a better efficacy/safety profile than specific thrombin inhibitors for which clinical trials showed a relatively narrow safety/efficacy margin which can result in bleeding complications at drug overdosage [16].

Present state of development of FXa inhibitors

Due to its critical role in the clotting cascade, FXa presents one of the most popular coagulation enzymes for the design of new oral directly acting anticoagulants/antithrombotics. Based on the specific mechanism of action of FXa, an effective inhibitor must have an extremely high affinity for the enzyme. Numerous inhibitors of FXa have been described in the literature and investigated in mainly preclinical studies. Besides naturally occurring FXa inhibitors such as antistasin and tick anticoagulant peptide, a variety of peptide, peptidomimetic and nonpeptide small-molecule FXa inhibitors with a high affinity and selectivity for the enzyme have been synthesised by several pharmaceutical companies and characterised biochemically and pharmacologically [for reviews see refs 17–21]. Structure-activity relationship studies have led to selective inhibitors of FXa that are active at subnanomolar concentrations [22, 23]. However, many of the small-molecule FXa inhibitors are highly basic moieties leading to poor pharmacokinetic properties and, especially, limited oral bioavailability. More recent developments have focused on compounds with less basic groups which may represent potent orally available FXa inhibitors for prophylactic and/or therapeutic use in clinical states.

Effective indirect inhibition of FXa can be achieved by heparin pentasaccharide which represents the minimum saccharide sequence in the heparin molecule required for an antithrombotic activity as well as by synthetic, structurally modified analogues [24–26]. The first and at present only available representative of this class of FXa inhibitors is the synthetic heparin pentasaccharide Org31540/SR90107A which provides potent anti-FXa activity through selective inhibition of the clotting enzyme by high-affinity binding to AT III [25].

Pharmacological profile

In vitro experiments and the first clinical studies have shown that FXa inhibitors exert strong anticoagulant, antithrombotic and even antiproliferative actions [10, 11, 14, 15, 27]. Due to the central position of FXa in the coagulation cascade, the inactivation of this serine protease offers a more global control of clotting. FXa inhibitors strongly inhibit the generation of thrombin and, thus, also thrombin-mediated positive feedback reactions such as the activation of factors V and VIII, as well as thrombin-mediated platelet reactions. Small-molecule FXa inhibitors can inactivate both free and especially clot-bound FXa. Because of the presence of FXa and active prothrombinase complexes in intravascular and mural thrombi [12, 13], the inactivation of FXa and the resulting inhibition of thrombin formation may be an effective way to control clot-associated procoagulant activity. Results from experimental studies indicate a role for FXa in the complex pathogenesis of restenosis and atherosclerosis. Both thrombin and FXa most likely contribute to vascular smooth muscle cell proliferation in vivo, although the precise role of the serine proteases and especially the significance of their mitogenic activities for restenosis have still to be clarified.

Clinical studies

At present, there are no direct FXa inhibitors marketed as drugs, but several compounds are in early phase clinical trials. Published clinical data are available for DX-9065a, a small-molecule, direct FXa inhibitor, and for the synthetic pentasaccharide Org31540/SR90107A which provides potent antithrombotic activity through selective inhibition of FXa by high-affinity binding to AT III. Both drugs offer safe and predictable pharmacokinetic and pharmacodynamic profiles after subcutaneous (s.c.) and intravenous (i.v.) administration in healthy volunteers [28].

The number of clinical studies published on the direct FXa inhibitor DX-9065a is rather limited. Studies on the pharmacokinetic and pharmacodynamic profile of DX-9065a after i.v. and s.c. administration in healthy volunteers or in patients with stable coronary artery disease showed a strong correlation between doses and plasma concentrations of the drug [29–31]. Given intravenously to healthy male volunteers, DX-9065a reduced platelet thrombus formation ex vivo using a perfusion chamber model [32].

For the synthetic pentasaccharide Org31540/SR90107A in phase II and III clinical trials, a significant efficacy and safety was demonstrated in orthopaedic patients undergoing total hip replacement [33–35], elective major knee surgery [36] or hip fracture surgery [37]. Furthermore, there are promising preliminary results with this drug in cardiology trials in patients undergoing percutaneous transluminal coronary angioplasty or with unstable angina or acute myocardial infarction (AMI) [38]. In patients with AMI treated with aspirin and alteplase, cotherapy with Org31540/SR90107A was as safe and effective as unfractionated heparin in restoring coronary artery patency [39].

Concluding remarks

The FXa inhibitors which are presently under development are structurally diverse compounds with different biochemical and pharmacological characteristics. Despite the anticoagulant and antithrombotic effectiveness of direct and indirect FXa inhibitors demonstrated in comprehensive experimental studies, a general assessment of the therapeutic potential of this new class of drugs has to consider various additional aspects. Pharmacokinetic characteristics such as oral bioavailability, biological half-life, metabolic transformations or excretory routes are important factors for the clinical use of a given drug. Interactions with other drugs or endogenous factors as well as additional mechanisms of action have to be taken into consideration. A particular FXa inhibitor might be useful for a specific clinical indication and one drug might not be the optimum treatment for all thrombotic situations. FXa inhibitors inhibit the generation of thrombin very effectively but are expected to be ineffective in clinical conditions where thrombin has already been formed. The complex pathogenesis of thromboembolic processes which involves both plasmatic and cellular reactions requires a careful analysis of the usefulness of a combination of FXa inhibitors with other drugs showing different mechanisms and sites of action in order to enhance the therapeutic effect. Simultaneous direct inhibition of thrombin and FXa by synthetic proteinase inhibitors might represent a novel approach to develop antithrombotics with improved pharmacological properties [40]. Although the efficacy/safety profile of FXa inhibitors may be better than that for heparin or antithrombin agents, these agents may also cause undesired side effects such as bleeding complications. Furthermore, combination of FXa inhibitors with other drugs such as thrombin inhibitors or platelet function inhibitors might not only show synergistic therapeutic effects, but could also enhance side effects. Other important points to consider and problems to be solved with the use of FXa inhibitors are whether and how to monitor the effect of a given FXa inhibitor in clinical practice, i.e. is there an easy and reproducible assay, and how can the effect of the drug be neutralised in case of overdose or occurrence of undesired side effects?

In conclusion, FXa inhibitors represent promising drugs for the prophylaxis and/or therapy of various thromboembolic disorders. However, additional experimental studies and comprehensive clinical trials are required to demonstrate the inhibitory profile of FXa inhibitors, their effectiveness and especially their superiority over other commonly used drug regimens for cardiovascular indications.

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