Peptidomimetic Thrombin Inhibitors

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Key Words

Thrombin inhibitor · Peptidomimetic · Hirulog · Bivalirudin · Argatroban · Ximelagatran · Dabigatran

bivalirudin, argatroban, ximelagatran and dabigatran is presented.

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Abstract

The central position of thrombin in the coagulation cascade has made it a popular target for discovery of novel antithrombotic agents. Starting with hirudin, a natural peptide isolated from the medicinal leech, its shorter synthetic analogue hirulog, and argatroban, the first therapeutically used synthetic small-molecule thrombin active site inhibitor, hundreds of direct thrombin inhibitors have been discovered over the last 20 years. Most of them are peptidomimetic compounds, based on the amino acid sequence of fibrinogen which binds into the thrombin active site. Since elucidation of the crystal structure of human thrombin in 1989, the structure-based design of low-molecular-weight peptidomimetic thrombin inhibitors has been greatly aided by the use of x-ray crystallographic analysis of thrombin-inhibitor complexes. The ultimate goal of most research programmes and drug optimization strategies is to develop an orally bioavailable, small-molecule, direct thrombin inhibitor that would be suitable for once or twice daily dosing. An overview of the most advanced peptidomimetic direct thrombin inhibitors

Introduction

Thromboembolic diseases are the leading cause of mortality and morbidity worldwide [1,2]. Despite the well established use of antithrombotic drugs, which include anticoagulants, antiplatelet drugs and thrombolytic drugs, there is an urgent need for more efficacious and safer compounds for the prevention and treatment of ischaemic events. Among novel approaches to the treatment of thrombosis, the past decade has seen major progress in the development of thrombin inhibitors, which are forecast to take an important share of the global antithrombotics market by the end of the decade [3,4].

Thrombin is a trypsin-like serine protease formed through the cleavage of prothrombin by the serine protease factor Xa. Thrombin is the central enzyme that controls the balance between hemostasis and fibrinolysis. It catalyzes the conversion of fibrinogen to fibrin by cleaving the peptide bond between arginine and glycine in a fibrinogen sequence Gly-Val-Arg-Gly-Pro-Arg. Thrombin also cleaves other substrates such as factors V, VIII and XIII. It also activates the platelet thrombin receptor, being a potent stimulant of platelet aggregation [5]. As revealed by sequencing and

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later confirmed by x-ray crystallography [6,7], human thrombin contains an A chain of 36 amino acids and a B chain of 259 amino acids, which are connected by a disulfide bridge. It contains the characteristic catalytic triad Asp 102, His 57 and Ser 195 and an insertion loop formed by Tyr 60A - Pro 60B - Pro 60C - Trp 60D, which restricts access of substrates and inhibitors to the active site region. The S1 site is a deep pocket with Asp 189 at the bottom, providing a specificity for P1 arginine residues. The S2 site, defined by the YPPW insertion loop, and the distal pocket, which is separated from the S2 site by the side chain of Leu 99, are hydrophobic in nature. In addition to the active site itself, thrombin contains the fibrinogen-binding and heparin binding exosites that are composed of several basic arginine and lysine side chains.

The three-dimensional structure of human thrombin inspired intensive structure-based design of thrombin inhibitors which resulted in several hundreds of thrombin

inhibitors over the last 15 years. Apart from natural thrombin inhibitors such as hirudin, bothrojaclin, rhodiin, triabin and dipetalin, most inhibitors are peptidomimetic compounds mimicking the fibrinogen sequence which interacts with the thrombin active site. Peptidomimetics are defined as designed compounds whose pharmacophoric elements mimic a peptide's pharmacophoric elements in 3D space, mimicking the binding and the biological activity of the natural peptide ligand [8], with equivalent or superior desirable properties and lacking undesirable properties of the natural peptides [9]. Several reviews covering the advances in the discovery of thrombin inhibitors have been published [5, 10-17]. This paper will deal only with peptidomimetic thrombin inhibitors that are already used in therapy (argatroban, bivalirudin, ximelagatran) or which are most advanced in their development (dabigatran etexilate).

Figure 1. Structures of bivalirudin, argatroban, melagatran, ximelagatran, dabigatran and dabigatran etexilate.

argatroban

bivalirudin

melagatran

ximelagatran

dabigatran

dabigatran etexilate

From Hirudin to Bivalirudin

Hirudin is a 65 residue polypeptide from the leech *Hirudo medicinalis* having a highly anionic C-terminus containing a large number of acidic amino acid residues [18]. It is a potent anticoagulant that forms a 1:1 non-covalent complex with thrombin ($K_i = 20$ fM) by binding with the C-terminus to the fibrinogen-binding exosite and covering the thrombin active site with its N-terminus. The structure of hirudin bound to thrombin determined by X-ray crystallography [19] revealed that hirudin does not bind into the S1 specificity pocket. In natural hirudin a tyrosine residue in its C-terminus is sulphated, whereas this is not the case in a recombinant hirudin which therefore binds less tightly to thrombin [20]. Recombinant hirudins, lepirudin [21] and desirudin [22] are produced in yeast cells. They are used for preventing deep vein thrombosis in patients undergoing elective hip or knee replacement surgery. However, clinical trials comparing r-hirudins (lepirudin, desirudin) with heparin in patients with coronary syndromes either failed to show sustained efficacy or showed excessive bleeding compared with heparin [23].

Hirulogs are bivalent peptide inhibitors of thrombin that were conceived as combined active site and fibrinogen-binding exosite inhibitors. Bivalirudin was the first hirulog to reach the market, being approved by FDA in December 2002 as an anticoagulant in patients with unstable angina undergoing percutaneous transluminal coronary angioplasty [24]. It is a 20 amino acid peptide, containing an active site thrombin inhibitor D-Phe-Pro-Arg linked via a Pro-(Gly)₄ chain to a dodecapeptide analogue of the carboxy-terminus of hirudin. Like hirudin, bivalirudin also forms a 1:1 stoichiometric complex with thrombin. Although in bivalirudin a scissile Arg-Gly bond is replaced by Arg-Pro bond, thrombin can slowly cleave bivalirudin between arginine and proline, thereby reactivating its own active site and allowing for subsequent hemostasis. This would prevent major bleeding events observed with hirudin. Unlike hirudin, however, bivalirudin causes only temporary inhibition of the active site of thrombin with a half-life of 20 to 30 minutes. Because of its peptidic nature, bivalirudin must be given parenterally [25-27]. Several other peptidomimetic modifications have been implemented in the design of hirulogs. Thus, hirulog 3 - a potent, non-cleavable hirudin-based peptide of the "hirulog" class contains a beta-homoarginine at the scissile bond [28].

Argatroban

Argatroban is a potent synthetic thrombin inhibitor with an inhibitory constant (K_i) of 40 nM. It is derived from L-

arginine and was developed by Mitsubishi in Japan before the crystal structure of human thrombin was published. It resulted from a rational step by step modification of the lead compound N α -tosyl-L-arginine methyl ester which included exchange of the ester group for tertiary amides and optimization of the arylsulfonamide moiety [29]. Although at first glance argatroban may appear as a mimetic of Arg-Gly dipeptide, the X-ray crystal structure of an argatroban - human thrombin complex [7] revealed that the piperidine ring occupies the S2 binding pocket, being almost parallel to the arylsulfonamide moiety which is positioned in the distal hydrophobic pocket of the thrombin active site. The guanidino group of arginine binds into the S1 binding pocket where it interacts via hydrogen bonds with Asp 189. Argatroban has 4 asymmetric carbons; with respect to the stereogenic centre at the partially saturated quinoline ring, it is a mixture of two diastereomers at a ratio of approximately 65:35 [30]. Argatroban was first introduced in Japan in 1990 for treating peripheral vascular disorders. It was approved by FDA in June 2000 as an anticoagulant for prophylaxis or treatment of thrombosis in patients with heparin-induced thrombocytopenia and in April 2002 as an anticoagulant in patients with or at risk of heparin-induced thrombocytopenia while undergoing percutaneous coronary intervention [30-33]. Due to the presence of a highly basic guanidine moiety ($pK_a = 13$) [11] which prevents absorption from the gastrointestinal tract, argatroban must be applied parenterally.

Melagatran and Ximelagatran

Ximelagatran is the first peroral thrombin inhibitor to reach the pharmaceutical market. It was first approved in France in December 2003 for the indication of prevention of venous thromboembolic events in patients undergoing major elective orthopaedic surgery, i.e., hip or knee replacement, and in May 2004 it successfully completed the mutual recognition procedure in Europe for registration for short-term usage for this purpose. Filings for two other indications - prevention of stroke and other thromboembolic complications associated with atrial fibrillation - have been made in Europe and in the USA. Ximelagatran is the first oral anticoagulant since the development of warfarin more than 50 years ago. Ximelagatran is a double prodrug of a small-molecule non-covalent peptide thrombin inhibitor melagatran, which exhibits poor bioavailability and absorption upon oral dosing. It is obtained by esterification of the carboxylic group of melagatran with ethanol and conversion of the amidine to an amidoxime moiety. Following oral administration, the prodrug ximelagatran is metabolized to the active thrombin inhibitor melagatran [34, 35]. Melagatran is a

tripeptidomimetic thrombin inhibitor mimicking the D-Phe-Pro-Arg sequence, which has served as a lead compound for numerous peptidomimetic thrombin inhibitors [11]. This sequence resulted as a promising lead compound from systematic studies of tripeptide aldehydes containing P3-P1 amino acids of the fibrinogen sequence Gly-Val-Arg-Gly-Pro-Arg in which the binding affinity of the tripeptide aldehyde D-Phe-Pro-ArgH to thrombin was found to be 7370 times higher than that for Gly-Val-ArgH [20, 36]. Melagatran contains a benzamidine arginine side chain mimetic and azetidine 2-carboxylic acid as proline mimetic. The non-natural amino acid D-cyclohexylglycine mimics P3 D-phenylalanine. The carboxymethyl group in connection with a basic benzamidine moiety might indicate a specific transport mechanism, possibly a charge compensation phenomenon or a carrier mediated form. It has also been shown that neutralization of amidine or guanidine groups by a carboxylate results in less toxic compounds [11]. The binding mode of melagatran in the thrombin active site has been studied by x-ray crystallography of a complex of melagatran with human thrombin [37].

Dabigatran

Dabigatran etexilate, an orally active double prodrug of the potent small molecule direct thrombin inhibitor dabigatran ($K_i = 4.5$ nM) has recently been evaluated in phase II clinical trials in patients undergoing total hip replacement [23]. Orally administered dabigatran etexilate is rapidly converted to its active form. Dabigatran is a peptidomimetic thrombin inhibitor, containing tri-substituted benzimida-

zole as a central scaffold. It was conceived as a sterically constrained analogue of NAPAP [11], which contains 4-amidinophenylalanine as a mimic for arginine. A polar carboxyethyl group was built into the molecule in order to increase its hydrophilicity, thereby reducing protein binding and optimizing in vivo activity. As revealed by X-ray structure analysis of the inhibitor in complex with thrombin, the carboxylate is located outside the active site and does not interfere with inhibitor binding. One of the most remarkable characteristics of dabigatran is that, in complex with thrombin, it does not make use of the canonical hydrogen bonding pattern with the enzyme which is characteristic for almost all other potent thrombin inhibitors. Apart from the salt bridge with Asp 189, the binding of dabigatran in the thrombin active site results solely from hydrophobic interactions [38].

Conclusion

After two decades of intensive search for therapeutically useful thrombin inhibitors, five direct thrombin inhibitors have reached the pharmaceutical market. Among them, the peptidic recombinant hirudins desirudin and lepirudin, a hirulog bivalirudin and the highly basic argatroban require parenteral application. The overriding medical need for an oral anticoagulant that overcomes the interactions, safety concerns and need for monitoring that limit the use of warfarin will probably be fulfilled by ximelagatran, the first approved orally active direct thrombin inhibitor.

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