Hirudin and Hirulog*: Advances in antithrombotic therapy

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SUMMARY

Over the past two decades, advances in the prevention and treatment of coronary artery disease, e.g., coronary angioplasty and coronary thrombolysis, have been coupled with the need for improved antithrombotic therapy to prevent acute, life-threatening thrombotic complications. Concurrently, studies on the pathophysiologic mechanisms for the arterial thrombotic process have demonstrated a central, mediating role for thrombin. Brought forward from its identification in the late 19th century, the leech protein hirudin emerged as a model for recombinant DNA engineering and 'protein-mimetic' drug design. Recombinant hirudin (r-hirudin) and Hirulog are the resulting drug candidates which, as direct thrombin inhibitors, offer several potential advances in the management of acute thrombotic disorders. Clinical trials of these drug candidates have already provided evidence for clinical activity and tolerability in thromboembolic disease. Definitive evidence for efficacy is currently sought in large, controlled studies in the settings of coronary angioplasty, coronary thrombolysis and unstable angina.

INTRODUCTION

Thrombin is a serine proteinase which acts in the thrombotic process by catalyzing cleavages of the A-alpha and B-beta chains in fibrinogen, yielding fibrin monomer [1], and cleavage of the Arg\(^{41}\)-Ser\(^{42}\) peptide bond in its specific platelet receptor, resulting in platelet activation [2]. Thrombin also acts in thrombotic processes by activation of factors V and VIII [3], a positive feedback amplification reaction promoting further thrombin generation, and in activation of factor XIII [4], a plasma transglutaminase which stabilizes polymerized fibrin monomer. Endogenous mechanisms for control of thrombin action include its neutralization by the plasma antiproteinases antithrombin III (ATIII) and heparin cofactor II [5,6]. In addition, thrombin bound to

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the endothelial cell-surface glycoprotein thrombomodulin can catalyze efficiently the activation of protein C, which, in turn, promotes negative feedback control of coagulation by inactivation of factors Va and VIIIa [7]. Thus, thrombin plays a central regulatory role in the thrombotic process.

Most proteinases of the blood coagulation cascade undergo zymogen activation, resulting in maintenance, via interchain disulfide bridges, of key structural elements in the ‘activation fragment’ which participate in enzymic specificity (e.g., the Gla-domain in vitamin K-dependent coagulation factors). The exception is prothrombin, whose activation results in generation of a short A-chain (with no known functional relevance) and a B-chain proteinase domain, generally homologous with trypsin. Thrombin has thus evolved its specificity for macromolecular substrates, cofactors and inhibitors within the framework of the proteinase domain, and this appears to be elaborated through certain surface loops. These loops generally represent insertions based on topological alignment of thrombin amino acids with corresponding residues in chymotrypsin [8].

Studies on thrombin structure–function relationships have indicated the existence of an interaction area, capable of binding anionic ligands, distant from the catalytic center [9]. This ‘anion-binding exosite’ appears to be involved centrally in thrombin interactions with and specificity for fibrinogen, thrombin receptor, thrombomodulin, and, as discussed further below, hirudin. A second highly electronegative [10] exosite exists on the opposite surface of thrombin, and has been shown to represent a site for heparin binding by chemical modification [11] and for binding of the second prothrombin kringle, fragment 2 by X-ray crystallographic analysis [12]. Ligand occupancy of the anion-binding exosite appears to modify the catalytic reactivity of thrombin toward small tripeptidyl substrates [13], indicating that exosite structures serve as both an ‘anchoring site’, to achieve high-affinity complexes, and a ‘trigger site’, to assist in efficient and specific catalysis. The structural basis for exosite binding serving as an enzymatic ‘trigger’ remains to be established.

As addressed in part above, X-ray crystallographic studies on thrombin and its complexes with inhibitors and proteins [8,12,14–18] have been particularly instructive in delineating the structural basis for thrombin action. Furthermore, data from these studies have served as the basis for drug design. These studies have solidified an understanding of interaction areas, such as the anion-binding exosite, as the paradigm for thrombin physiologic actions.

THROMBIN AND THE ARTERIAL THROMBOTIC PROCESS

As the ‘terminal’ step in the blood coagulation cascade, thrombin action toward fibrinogen, resulting in generation of the fibrin clot, is critical for development of venous thrombosis, where thrombi principally comprise fibrin with entrapped erythrocytes. In arterial thrombosis, the role of thrombin has been controversial. Here, in addition to fibrin, thrombi comprise activated platelets, and thrombin is but one of many potential agonists for platelet activation and recruitment during thrombus formation. However, as discussed below, studies on the pathophysiologic mechanisms for arterial thrombus formation indicate a mediating role for thrombin.

The arterial thrombotic process occurs in association with deep arterial injury, such as that occurring spontaneously from rupture of atherosclerotic plaques and that occurring from mechanical injury during revascularization (e.g., coronary angioplasty). The exposure of subendothelial