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Review

Protease-activated receptor-1 antagonists in long-term antiplatelet therapy. Current state of evidence and future perspectives

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ABSTRACT

Atherothrombosis and its clinical manifestations are among the leading causes of death in the developed world. The current standard-of-care antiplatelet therapy for the treatment of such events comprises aspirin and a thienopyridine or ticagrelor. However, recurrent ischemic events due to residual cardiovascular risk are a common phenomenon in these patients. It is believed that this residual risk is caused, at least in part, by thrombin, which signals through protease-activated receptors (PARs) and especially PAR-1. Thus, PAR-1 antagonism could represent an effective approach in the treatment of atherothrombotic disease. In this context, two potent and selective agents have been developed, vorapaxar and atopaxar. However, only vorapaxar has completed phase 3 clinical trials. In the present review, the main pharmacodynamic and pharmacokinetic properties of the PAR-1 antagonists are briefly described and the latest clinical data on vorapaxar are presented.

1. Introduction

Atherothrombosis is the leading cause of death worldwide [1]. The currently used standard-of-care antiplatelet therapy for the treatment of atherothrombotic disease consists of aspirin in combination with a platelet receptor $P2Y_{12}$ antagonist (thienopyridine or ticagrelor). Despite dual antiplatelet therapy, residual cardiovascular risk remains relatively high and patients often experience a recurrent ischemic event [2]. Intensive research work over the last years has suggested that this residual risk may be attributed, at least partially, to thrombin-mediated platelet activation through protease-activated receptors (PARs) and especially PAR-1. In this context, PAR-1-targeted antagonists have been developed and this has led to a substantial improvement of our understanding of the functionality of PAR-1 as well as its role in platelet activation. Among the various PAR-1 antagonists tested in vitro and in animal models in vivo [3], two potent and specific antagonists, namely atopaxar and vorapaxar have been proceeded in phase 2 clinical studies and only vorapaxar has completed phase 3 clinical trials. In the present review, the main pharmacodynamic and pharmacokinetic properties of the PAR-1 antagonists are briefly described and the latest clinical data on vorapaxar are presented.

1.1. Thrombin-mediated platelet activation

Thrombin is a multifunctional serine protease that plays an important role in the coagulation cascade, being primarily responsible for cleaving fibrinogen and generating fibrin. In addition to this role, thrombin can signal to multiple cell types through PARs, a subset of G protein-coupled receptors (GPCRs). Mammals express all four types of PARs, designated PAR-1 to -4 in order of their discovery [4–8]. PAR-1 and -3 are activated mainly by thrombin [4,6], PAR-2 is activated mainly by trypsin and trypsin-like proteases (but not thrombin) [5] and PAR-4 is activated mainly by thrombin and trypsin [7]. Thrombin is the most potent agonist of human platelets [9] and mediates platelet activation mostly, if not entirely, through PAR-1 and PAR-4. Furthermore, it has been reported that thrombin also binds to glycoprotein Ibα (GPIbα) of the glycoprotein complex Ib-IX-V (GPIb-IX-V), which may have two distinct consequences in platelets: a direct intracellular signaling effect [10] or the use of GPIbα as a co-factor for PAR-1 activation [11].

PAR-1 is a high-affinity thrombin receptor, whereas PAR-4 is a low-affinity thrombin receptor. Certain studies demonstrated that in transfected COS7 fibroblasts PAR-4 requires 25- to 100-fold higher enzyme concentrations to be stimulated than PAR-1 [7,12]. However, when the two receptors are expressed in the same cell, such as in platelets, PAR-4 requires only about 2-fold higher thrombin concentration to be activated compared to PAR-1/-4 [13–15]. This may be due to the formation of a stable, heterodimeric complex [15,16], which allows PAR-1 to act as a co-factor for PAR-4 activation, while thrombin is still bound to PAR-1 [15].

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PARs are stimulated by a unique "self-activation", irreversible mechanism that requires prior proteolysis of the extracellular *N*-terminal domain. Thrombin cleaves PAR-1 at the site $LDPR^{41}\downarrow S^{42}FLLRN^{47}$ (downwards arrow indicates the peptide bond being hydrolyzed) [4] and generates a new *N*-terminus ($S^{42}FLLRN^{47}$) which acts as a "tethered-ligand" and interacts intramolecularly with other portions of this domain [17,18]. Additionally, the unmasked *N*-terminus may interact with the receptor's second and third intracellular loops [19–22]. The overall result is a conformational change that spreads throughout the entire receptor and initiates the signaling cascade. Alternatively, synthetic peptides that mimic the newly formed *N*-terminus can activate PARs (but probably not PAR-3) independently of proteolysis [4,5,7,8]. Downstream of the cleavage site reside regions that are crucial for high-affinity thrombin binding to PAR-1 and thus, proper receptor activation. These regions are a "hirudin-like domain" ($K^{51}YEPF^{55}$) and an anionic cluster ($E^{57}DEE^{60}$) that interplay electrostatically with the thrombin's

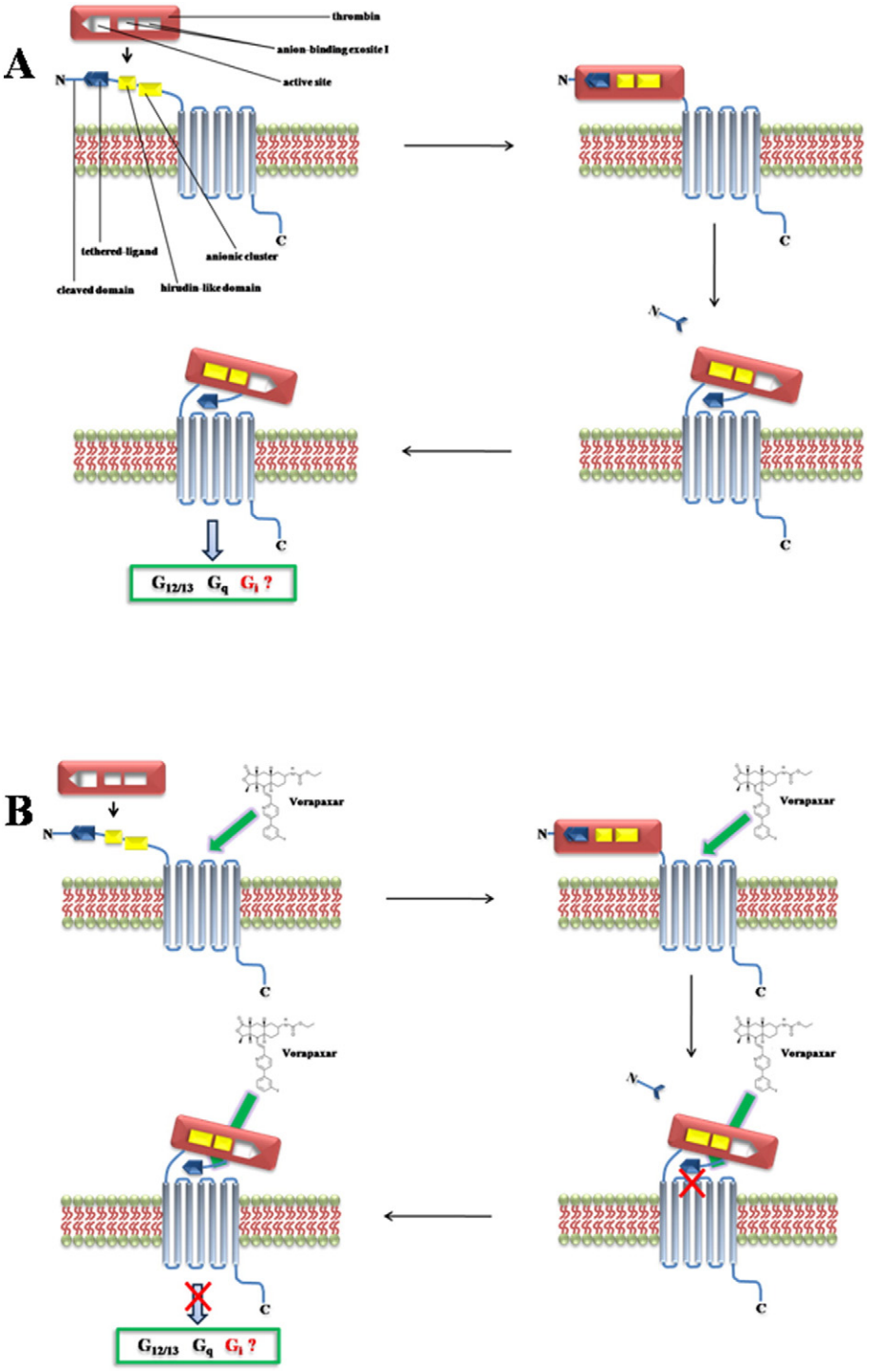

Fig. 1. A. Thrombin-induced activation of the PAR-1 receptor. Thrombin uses its positive-charged anion-binding exosite I to dock with high affinity to the PAR-1 hirudin-like domain and an anionic cluster, two regions that range in the receptor's *N*-terminal domain. Subsequently, thrombin cleaves the receptor's *N*-terminus and exposes a new terminus, which begins with the sequence $S^{42}FLLRN^{47}$ and is termed "tethered-ligand". Then, the tethered-ligand interacts intramolecularly with PAR-1 and induces the signaling cascade. B. Vorapaxar inhibition of the thrombin-induced PAR-1 activation. Vorapaxar binds to PAR-1 with high affinity and abolishes the tethered-ligand intramolecular interactions and the subsequent signaling events.