Editorial

Charming the Snake
Venom-Derived Peptides Show Surprising Efficacy as Glycoprotein VI–Targeting Antithrombotic Agents

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Snake venoms are potent cocktails containing a diverse array of proteins that typically target neuromuscular junctions or the hemostatic system of the snake’s prey. Many of the venom proteins that target hemostasis adopt a C-type lectin-like fold and are therefore termed snaclexes (ie, snake venom C-type lectins).1 Despite the structural similarity to functional lectins, the snaclexes do not exhibit carbohydrate- or calcium-binding properties. Instead, the typical calcium-stabilized carbohydrate-binding loop found in functional lectins adopts a highly extended loop that forms a domain swap between α and β subunits in the snaclexes, forming an αβ heterodimer that interacts with critical proteins that control hemostasis. The resulting effect can be pro- or anticoagulant (eg, snaclexes that target factor IX, factor X, prothrombin, or α-thrombin) or pro- or antithrombotic (eg, snaclexes that target platelet receptors, such as glycoprotein Ibα, glycoprotein IIa/IIIa, or GPVI [glycoprotein VI]).1,2 The structures of several snaclexes have been determined, revealing conserved features of the fold: each forms a disulfide-linked heterodimeric subunit of α and β chains that may comprise the mature protein (eg, factor X–binding protein3 or EMS16)4 or may further assemble into higher order structures, such as the (αβ)4 rings of convulxin5,6 or flavocetin.7 Several structures have been reported for complexes between snaclexes and their targets, revealing that a concave surface between the α and β subunits interacts with the target protein to form an extensive binding site.5,6,8,9

See accompanying article on page 1307

Snaclexes have been incredibly useful tools for deciphering pathways of platelet activation, given their potent activities and high affinity for target receptors.1,2 For example, convulxin is widely used as a standard agonist in platelet activation assays. It is particularly effective at activating GPVI signaling because of a high affinity for GPVI (picomolar to low nanomolar Kd) and high avidity (4 binding sites per (αβ) ring).10 These same general characteristics for snaclexes could make them attractive starting points for the design of new therapeutic agents. However, recombinant expression of snaclexes is challenging because of their disulfide-linked heterodimeric nature. Even when heterologous expression is successful, the biological activity of the recombinant snaclex may be significantly lower than that of the venom-purified natural protein, as seen for convulxin.11 An intriguing new study from Chang et al12 in this issue of ATVB reports that short peptides derived from the snaclex trowaglerix is surprisingly effective at inhibiting GPVI-mediated platelet activation in vitro and in vivo, without inducing thrombocytopenia or significantly prolonging the bleeding time.

Trowaglerix is a snaclex from Wagler’s pit viper that forms a higher-order oligomeric assembly of αβ subunits, similar to convulxin or flavocetin. It binds specifically to GPVI and induces phosphorylation of Fyn, Src, and LAT in platelets at a 10-fold lower dose than convulxin.13 Unlike convulxin that can bind to both GPVI and glycoprotein Ibα,14 trowaglerix is specific for GPVI. Chang et al12 tested the inhibitory activity of short synthetic peptides (6 or 10 residues long) from the C-terminal portion of trowaglerix α subunit (named Troα6 or Troα10, respectively) along with similar hexapeptides from convulxin, aggretin/rhodocytin, and agkistin. Remarkably, they found that Troα6 in particular was effective at inhibiting collagen-induced platelet aggregation. By extending the peptide sequence to a decapptide (Tro10), they achieved nearly complete inhibition of collagen-induced platelet activation. They confirmed that Troα10 binds specifically to GPVI with micromolar affinity. Interestingly, Troα10 does not inhibit GPVI binding to collagen, suggesting that it interacts with a distinct region of GPVI. The authors propose that Troα10 may bind in the cleft between the 2 Ig-like domains of GPVI15 although this will need future confirmation by site-directed mutagenesis or structural characterization. However, the proposed binding site does highlight the point that there is no reason to assume that a free, unconstrained Troα10 peptide would engage GPVI in the same conformation or at the same site as the corresponding sequence in the folded trowaglerix protein. The ability of Troα10 to bind GPVI and inhibit platelet activation is, therefore, a rather unexpected and exciting development (Figure).

Most importantly, Chang et al12 demonstrate that Troα10 exhibits antithrombotic activity in both ex vivo and in vivo mouse models. Platelet-rich plasma from mice treated with either 30 mg/kg Troα6 or 8 mg/kg Troα10 showed significant inhibition of collagen-induced platelet activation, with no apparent thrombocytopenia. In addition, they showed that both Troα6 and Troα10 delayed thrombus formation and prolonged the time to occlusion of mesenteric venules or the carotid artery in 2 mouse models of thrombosis. Furthermore, neither Troα6 nor Troα10 significantly increased bleeding time in a mouse tail transection model. Taken together, these
data suggest that targeting GPVI with Troα6 or Troα10 peptides would be safe and effective.

This study by Chang et al is a fascinating new development that could provide effective new avenues for the design of novel antithrombotic agents. The use of simple linear peptides from trowaglerix will likely be much more tractable than recombinant expression of full-length active protein. Such peptides could be developed into useful therapeutic agents, either as the unmodified native peptide or through design of peptidomimetic inhibitors with improved stability and favorable pharmacokinetic properties.

Disclosures
None.

References


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