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 snaclecs (i.e., snake venom C-type lectins).\(^1\) Despite the structural similarity to functional lectins, the snaclecs 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 \(\alpha\) and \(\beta\) subunits in the snaclecs, forming an \(\alpha\beta\) heterodimer that interacts with critical proteins that control hemostasis. The resulting effect can be pro- or anticoagulant (e.g., snaclecs that target factor IX, factor X, prothrombin, or \(\alpha\)-thrombin) or pro- or antithrombotic (e.g., snaclecs that target platelet receptors, such as glycoprotein Ib\(x\), glycoprotein Ia/IIa, or GPVI [glycoprotein VI]).\(^1,2\) The structures of several snaclecs have been determined, revealing conserved features of the fold: each forms a disulfide-linked heterodimeric subunit of \(\alpha\) and \(\beta\) chains that may comprise the mature protein (e.g., factor X–binding protein\(^3\) or EMS16\(^4\)) or may further assemble into higher order structures, such as the (\(\alpha\beta\))\(_4\) rings of convulxin\(^5\) or flavocetin.\(^7\) Several structures have been reported for complexes between snaclecs and their targets, revealing that a concave surface between the \(\alpha\) and \(\beta\) subunits interacts with the target protein to form an extensive binding site.\(^1,3,4,8,9\)

Snaclecs 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 \(K_a\)) and high avidity (4 binding sites per (\(\alpha\beta\))\(_4\) ring).\(^10\) These same general characteristics for snaclecs could make them attractive starting points for the design of new therapeutic agents. However, recombinant expression of snaclecs is challenging because of their disulfide-linked heterodimeric nature. Even when heterologous expression is successful, the biological activity of the recombinant snaclec may be significantly lower than that of the venom-purified natural protein, as seen for convulxin.\(^11\) An intriguing new study from Chang et al\(^12\) in this issue of \(ATVB\) reports that short peptides derived from the snaclec trowaglerix are 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 snaclec from Wagler’s pit vipers that forms a higher-order oligomeric assembly of \(\alpha\beta\) 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 Ib\(x\),\(^14\) trowaglerix is specific for GPVI. Chang et al\(^12\) tested the inhibitory activity of short synthetic peptides (6 or 10 residues long) from the C-terminal portion of trowaglerix \(\alpha\) subunit (named Trow6 or Trow10, respectively) along with similar hexapeptides from convulxin, aggrekin/rhodocytin, and agkistin. Remarkably, they found that Trow6 in particular was effective at inhibiting collagen-induced platelet aggregation. By extending the peptide sequence to a decapeptide (Trow10), they achieved nearly complete inhibition of collagen-induced platelet activation. They confirmed that Trow10 binds specifically to GPVI with micromolar affinity. Interestingly, Trow10 does not inhibit GPVI binding to collagen, suggesting that it interacts with a distinct region of GPVI. The authors propose that Trow10 may bind in the cleft between the 2 Ig-like domains of GPVI\(^15\) 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 Trow10 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 Trow10 to bind GPVI and inhibit platelet activation is, therefore, a rather unexpected and exciting development (Figure).

Most importantly, Chang et al\(^12\) demonstrate that Trow10 exhibits antithrombotic activity in both ex vivo and in vivo mouse models. Platelet-rich plasma from mice treated with either 30 mg/kg Trow6 or 8 mg/kg Trow10 showed significant inhibition of collagen-induced platelet activation, with no apparent thrombocytopenia. In addition, they showed that both Trow6 and Trow10 delayed thrombus formation and prolonged the time to occlusion of mesenteric venules or the carotid artery in 2 mouse models of thrombosis. Furthermore, neither Trow6 nor Trow10 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


**Key Words:** Editorials, anticoagulant, blood platelets, hemostasis, snake venoms, thrombosis
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VI–Targeting Antithrombotic Agents
Andrew B. Herr

doi: 10.1161/ATVBAHA.117.309627
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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