Leeches, Snakes, Ticks, and Vampire Bats in Today’s Cardiovascular Drug Development

Nils U. Bang, MD

Through evolution, several animal species have been endowed with salivary anticoagulants, presumably to secure their nutritional requirements for fluid blood from their prey. It has been known for centuries that the bites of leeches, certain snakes, insects, and vampire bats can result in local and systemic bleeding. The use of the medicinal leech, Hirudo medicinalis, for therapeutic bloodletting goes back to the middle ages. The major anticoagulant from the saliva of the medicinal leech, hirudin, was purified two decades ago and found to be a highly specific inhibitor of thrombin. The complementary DNA for hirudin was later cloned and expressed, and recombinant hirudin has emerged today as an interesting candidate to replace heparin in acute anticoagulant therapy.

In recent years, recombinant DNA technology has made it relatively easy to produce large quantities of purified proteins from salivary gland complementary DNAs from leeches, snakes, insects, and bats. One group of proteins, now known as disintegrins, are found in many snake venoms. The disintegrins contain the sequence Arg-Gly-Asp-X (RGDX) also found in “sticky proteins” (e.g., fibrinogen, von Willebrand factor, and fibronectin), important in cell adhesion and aggregation (specifically platelet aggregation), through attachment to cell surface integrin binding proteins (glycoprotein IIb-IIIa is the platelet integrin). Small peptides of the RGDX sequence are competitive inhibitors of platelet aggregation. Disintegrins are low-molecular-weight, cysteine-rich peptides, and their potency as platelet function inhibitors is at least 500–2,000 times that of short RGDX peptides.

Highly specific and potent peptide inhibitors of clotting factor Xa have been isolated from the salivary glands of the leech Haemantheria ghilianii, the black fly Simulium vittatum, and the soft tick Omi-thodora moubata. The factor Xa–specific tick anticoagulant peptide has been cloned, expressed at high levels, and shown to be an effective adjunct in coronary thrombolysis in animal models.

The use of crystallography, nuclear magnetic resonance spectroscopy, and computer modeling have made possible the construction of small peptides incorporating the critical structural features of hirudin and the disintegrins, some of which (e.g., hirulog) are showing promise in arterial thrombosis animal models.

The paper by Gardell et al in this issue of Circulation presents elegant animal experimental studies on a new thrombolytic agent, a plasminogen activator (Bat-PA) extracted from the salivary glands of the vampire bat Desmodus rotundus. Three forms of the protein have been cloned and expressed by two groups. Bat-PAs bear a striking resemblance to human tissue-type plasminogen activator (t-PA), which has five distinct domains: finger (F), epidermal growth factor (EGF), kringle 1 (K1), kringle 2 (K2), and serine protease (SP). The domainal structures of the three forms of Bat-PA are F-EGF-K1-SP for the full-length form (45 kDa), EGF-K1-SP (41 kDa), and K1-SP (40 kDa). Thus, all three forms lack the K2 domain of t-PA. Within the conserved domains, all three forms of Bat-PA show 80–85% structural homology with t-PA. Like t-PA, all three forms of Bat-PA require fibrin to function optimally as plasminogen activators. However, the requirements for fibrin as a cofactor are far greater for Bat-PAs than for t-PA. In other words, Bat-PAs are far more fibrin-specific than t-PA. These findings present a serious challenge to the students of structure–function relations of the fibrinolytic enzyme system. It has been widely assumed that fibrin-dependent plasminogen activation by t-PA involves the formation of a trimolecular complex: t-PA–plasminogen–fibrin. The binding of t-PA to fibrin is supposed to involve the F and K2 domains; the binding of plasminogen to fibrin is supposed to involve kringle domains homologous to the t-PA K2. K2 in t-PA and plasminogen kringles share certain primary structural features not present in the t-PA K2 and not present in the single kringle in Bat-PA. The full-length 45-kDa form of Bat-PA binds to fibrin, presumably via the F domain; conversely, the shorter 41- and 40-kDa forms do not
bind. Yet fibrin strongly accelerates plasminogen activation by the 41- and 40-kDa forms of Bat-PA. Thus, the essential features of fibrin-specific plasminogen activation need to be thoroughly revised, and further studies of Bat-PA fibrin-dependent plasminogen activation should provide new insight into the structure–function relations of this essential fibrinolytic mechanism.

Gardell et al. provide ample evidence for the very high fibrin dependence of Bat-PA because it effectively dissolved arterial thrombin-induced thrombi in the rabbit with only negligible systemic plasminogen activation and fibrinogen depletion. In contrast, t-PA in doses effective for thrombolytic therapy also resulted in hyperplasminemia and substantial fibrinogen depletion. However, it is uncertain at this time whether these animal experiments herald an improved thrombolytic agent for clinical use. The following questions must be considered. First, as correctly pointed out by the authors, the association between hypofibrinogenemia and bleeding liability with thrombolytic therapy is weak at best. They do make the cogent argument, however, that Bat-PA, with its fastidious fibrin requirements, may be the tool to test such an association because t-PA not infrequently causes a systemic fibrinolytic state.

Second, it remains to be seen whether the fibrin specificity observed for Bat-PA in these animal experiments translates into fibrin specificity in clinical practice. It should be remembered that early animal experiments with t-PA demonstrated little, if any, evidence for systemic hyperplasminemia, a complication now frequently encountered in patients.

Third, the authors forward the theory that Bat-PA, with its extremely high fibrin dependence, may become a superior thrombolytic agent because the activation of the activator must be located in the vicinity of the thrombus, thereby avoiding the activation of circulating plasminogen. They argue that this mechanism could result in greater efficacy as well as safety. This theory is not new; proponents of t-PA have long forwarded similar arguments. However, opponents will argue that no plasminogen activator, no matter how fibrin specific, is capable of distinguishing between fibrin in a thrombus and fibrin in a hemostatic plug. In most clinical trials with thrombolytic agents, most bleeding episodes appear to arise from the dissolution of already-existing hemostatic plugs in diverse locations, for example, arterial and venous puncture sites, stress ulcers, and perhaps even hemostatic plugs sealing small lesions in the cerebral circulation.

Fourth, as correctly pointed out by the authors, the slower clearance of Bat-PA relative to t-PA convincingly demonstrated in their animal experiments may be of major significance in the greater efficacy of Bat-PA as assessed by the reperfusion incidence. This is particularly true because both agents were administered as a single bolus. The substantially shorter half-life of t-PA puts it at a distinct disadvantage with this experimental design. Several clinical studies have demonstrated that t-PA administered as a single bolus is largely ineffective in thrombolysis. It should also be pointed out that other experimental thrombolytic agents e.g., t-PA deletion mutants (Reference 20 inter alia) with prolonged half-life have demonstrated markedly improved thrombolytic properties vis à vis t-PA.

Fifth, as is the case with numerous PA mutants and hybrids, Bat-PA is potentially antigenic until proven otherwise, and clinically significant antigenicity would obviously exclude its use in man.

In spite of these concerns, Bat-PA represents an exciting new development. It is the first truly new thrombolytic agent since t-PA, single-chain urokinase, and APSAC were developed. In a broader sense, it is remarkable that Bat-PA, along with proteins or peptides from leeches, snakes, and insects—proteins which must have been here long before homo sapiens entered the picture—may come to assume major importance in cardiovascular drug development toward the end of the 20th century.

References


**KEY WORDS** • fibrinolysis • tissue-type plasminogen activator • vampire bat salivary plasminogen activator • thrombolysis • Editorial Comments
Leeches, snakes, ticks, and vampire bats in today’s cardiovascular drug development.

N U Bang

_Circulation_. 1991;84:436-438
doi: 10.1161/01.CIR.84.1.436

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/84/1/436.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/