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Review Article

Snake bites and hemostasis/thrombosis

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ABSTRACT

Snake venom toxins have evolved to affect many prey physiological systems including hemostasis and thrombosis. These toxins belong to a diverse array of protein families and can initiate or inhibit multiple stages of the coagulation pathway or platelet aggregation with incredible specificity. Such specificity toward vertebrate molecular targets has made them extremely useful for diagnosis of human diseases or as molecular scalpels in physiological studies. The large number of yet-to-be characterized venoms provides a vast potential source of novel toxins and subsequent cardiovascular therapeutics and diagnostic agents.

Contents

Introduction

Snakes are predatory animals that have evolved alongside their mainly vertebrate prey. As predators, they have developed multiple methods of capturing prey, but probably the most interesting of these is envenomation. The venom delivery system comprises the venom, venom glands and fangs of the snake, and it constitutes a highly adapted method for assuring food capture. While the venom contains the active toxins affecting prey hemostasis, the rest of the delivery system ensures their systemic administration. Snake venoms rapidly evolve through duplications of cognate (normally functioning in other tissue) genes that undergo neofunctionalization to become toxins [1] and venom gland-specific expression that results in relatively large quantities of protein being secreted within the venom [2].

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Although there are many different toxins and toxin families, resulting in a myriad of molecular targets, one of the major physiological systems affected by snake venoms is blood circulation [3]. Over approximately 50 million years [4], snake venom toxins have evolved to target multiple points along the blood coagulation and platelet aggregation pathways. These toxins can result in both pro- and anti-coagulation of blood or have both pro- and anti-platelet aggregation effects, thereby affecting normal thrombosis and hemostasis. As a result of these and other effects, snakebite is a major health factor in regions where humans routinely come into contact with potentially harmful snakes. Conversely, because toxins tend to exhibit high specificity for individual physiological targets, they can be useful for development into diagnostic tools or therapeutic drugs.

Pro-coagulant Toxins

Many toxins from snake venoms have a pro-coagulant effect on blood hemostasis, and they activate specific zymogens of endogenous coagulation factors in the blood. Among the known functional classes are activators of factor VII, factor X, factor V, and prothrombin as well as thrombin-like enzymes [5,6], all of which affect different steps in the coagulation pathway. Despite their pro-coagulant properties, due to subsequent defibrinogenation these toxins cause 'unclottable' blood in the prey/victim. However, the specificity of individual toxins allows their use as molecular scalpels for diagnostic or therapeutic treatment in human diseases (Fig. 1).

Factor X Activators

These molecules, which belong to either the snake venom metalloproteinase (SVMP) or snake venom serine protease (SVSP) toxin families, are components of venom from elapid and viperid snakes [6]. A very well studied example of a factor X activator is that from the Russell's viper (*Daboia russelii*), termed RVV-X. It is an SVMP that cleaves the Arg52-Ile53 bond of the heavy chain of human factor X, and is similar to factor X-activating SVMPs from other viperid snakes (for details, see [7,8]). RVV-X is a diagnostic tool that is most commonly used as a reagent in hematology laboratories for detecting anticoagulants associated with lupus [9].

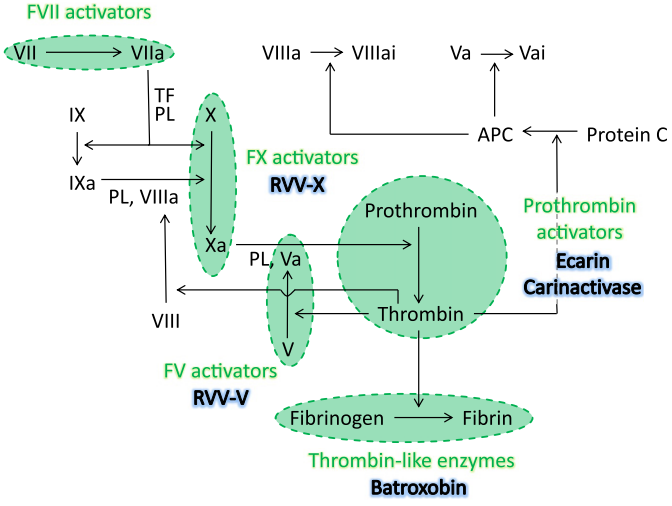

Fig. 1. Pro-coagulant toxins from snake venoms. Major factors of the blood clotting cascade are shown with specific activation parts of pathways highlighted in green and the general category name in green lettering. Diagnostic tools are highlighted in blue.

Prothrombin Activators

This large assemblage of pro-coagulant toxins from snake venoms is categorized into four different groups. Group A includes viperid SVMPs (such as ecarin from *Echis carinatus* venom) that can directly activate prothrombin; group B are SVMPs that further require the presence of Ca^{2+} to function, such as carinactivase-1 (also from *E. carinatus*); group C are SVSPs discovered in Australian elapid snakes that require Ca^{2+} and negatively-charged phospholipids for activity; and group D are SVSPs also from Australian elapid snakes that require Ca^{2+}, negatively charged phospholipids and factor Va for optimal activity. Ecarin (group A) converts prothrombin to meizothrombin, and is used in a clotting time assay to examine coagulation ability in patients treated with the anti-coagulant hirudin, because ecarin activity is halted in the presence of hirudin. By adjusting the concentrations of both ecarin and hirudin, the presence and concentrations of thrombin inhibitors are determined, and effective monitoring of clotting time is achieved [10]. Carinactivase (group B) is used as a diagnostic to determine prothrombin levels [11], and group C and D toxins (or subunits thereof) are being developed as therapeutic agents to control bleeding [12].

Factor V Activators

Activators of factor V have been isolated from various viperid and one elapid (*Naja oxiana*) snake species [13]. A highly specific factor V activator (RVV-V) from the venom of the Russell's viper (*D. russelli*) converts factor V into factor Va, with factor V being the only known substrate [14]. Therefore, it is used to examine levels of factor V in blood samples and is an important tool for diagnosis of certain blood diseases.

Thrombin-like Enzymes (SVTLEs)

These SVSPs are found in many different viperid and one colubrid (*Dispholidus typus*) species of snakes, and they generally release either fibrinopeptide A or B [15]. This is unlike endogenous thrombin, which releases both fibrinopeptide A and B [16]. Also, thrombin catalyzes the activation of other factors (factor XIII, factor V, factor VIII and protein C), but SVTLEs tend to degrade factor XIII and do not affect factor V or protein C. Many SVTLEs have been described so far, some rather recently [17,18]. SVTLEs, such as batroxobin (purified from *Bothrops atrox* venom), are excellent reagents that help to defibrinogenate blood without activating any other blood coagulation factors; being inhibited by exogenous factors such as antithrombin III, heparin or other direct thrombin inhibitors; or altering platelet function [15,19]. Some are being developed as therapeutic agents [20,21].

Factor VII Activator

Thus far, only one factor VII activator (from the venom of the taipan, *Oxyuranus scutellatus*) has been characterized [22]. Oscutarin, a prothrombin activator from this venom, is also able to activate factor VII. The resulting product is extremely similar to factor VIIa, indicating that the prothrombin activator is concomitantly an activator of factor VII.

Anti-coagulant Toxins

A number of snake venom toxins cause disruption of prey hemostasis through anti-coagulant functions. These include proteins that prevent the conversion of factor IX to IXa and factor X to Xa; inhibit the extrinsic tenase complex; disrupt the formation of the prothrombinase complex; activate protein C; or directly inhibit thrombin, thereby prolonging fibrin clot formation. These toxins also render the blood of the prey/victim 'unclottable' (Fig. 2).