Cell. Mol. Life Sci. 63 (2006) 3030–3041
1420-682X/06/243030-12
DOI 10.1007/s00018-006-6315-0
© Birkhäuser Verlag, Basel, 2006

Cellular and Molecular Life Sciences

Review

Snake venom components and their applications in biomedicine

D. C. I. Koh., A. Armugam and K. Jeyaseelan*

Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 8 Medical Drive, Singapore 117597 (Singapore), Fax: +65 67791453, e-mail: bchjeya@nus.edu.sg

Received 6 July 2006; received after revision 14 August 2006; accepted 28 September 2006
Online First 13 November 2006

Abstract. Snake envenomation is a socio-medical problem of considerable magnitude. About 2.5 million people are bitten by snakes annually, more than 100,000 fatally. However, although bites can be deadly, snake venom is a natural biological resource that contains several components of potential therapeutic value. Venom has been used in the treatment of a variety of pathophysiological conditions in Ayurveda, homeopathy and folk medicine. With the advent of biotechnology, the efficacy of such treatments has been substantiated by purifying components of venom and delineating their therapeutic properties. This review will focus on certain snake venom components and their applications in health and disease.

Keywords. Venom, snake, neurotoxin, platelet aggregation, blood coagulation, receptor.

Introduction

Snakes have been a subject of fascination, fear and myths throughout history. In ancient Egypt, the cobra was worshipped and its replica was used to decorate the crowns of Roman emperors, while in the ancient Greek world, the god of medicine was depicted with a stick entwined with a snake, the symbol that is still used to represent the guilds of medicine and pharmacy. Snake venoms are complex mixtures of proteins, nucleotides and inorganic ions. These combinations confer a formidable array of toxic properties on the venom, the peptides and polypeptides being responsible for a variety of toxic properties. Annually, about 2.5 million people around the world are the victims to snake bites, of whom about 100,000 lose their lives. Most morbidity and mortality occurs in rural areas in the tropics [1, 2]. The populations of temperate western countries are not spared from wild snake bites, but they occur at a lower frequency. In addition, there are substantial incidents of envenomation by exotic captive snakes. The clinical manifestations of snakebite are dependent on two factors, the intrinsic toxicity and amount of venom injected. There are many signs and symptoms following envenomation by snakes, but the major ones with clinical significance can be divided into a few broad categories: (a) flaccid paralysis; (b) systemic myolysis; (c) coagulopathy and haemorrhage; (d) renal damage and failure; (e) cardiotoxicity and (f) local tissue injury at the bite site. The symptoms suggest that snake venoms affect various systems, particularly the central nervous system (CNS), cardiovascular system, muscular and vascular system. The aim of this review is to highlight some of the snake venom components that can be used as molecular probes in human health and disease.

Toxins affecting the CNS

The main toxins from snake venom that affect the CNS are neurotoxins (Table 1) and dendrotoxins. The general observation from neurotoxin envenomation is the development of cranial nerve palsies, which are characterized

* Corresponding author.

Table 1. Snake venom neurotoxins and their targets in the CNS.

Type of neurotoxin	Functional target	Source
Short-chain neurotoxins	post-synaptic toxin; high affinity to skeletal and Torpedo nAChR; bind to neuronal α7 nAChR with lower affinity or none.	elapids and hydrophids (cobras, sea snakes, kraits, Australian elapids)
Long-chain neurotoxins	post-synaptic toxin; comparatively higher affinity towards neuronal α7 nAChR than the skeletal receptors.	elapids and hydrophids (cobras, sea snakes, kraits, Australian elapids)
Weak neurotoxins	post-synaptic toxin; weak affinity to both the skeletal and neuronal nAChRs	elapids (cobras, kraits, Australian elapids)
Taipoxin	presynaptic toxin; binds specifically to neuronal plasma membranes especially at the neuromuscular junction.	Australian elapid (taipan)
β-Bungarotoxins	presynaptic toxin; presynaptic voltage dependent K^+ channel	elapids (kraits)
Muscarinic toxins	specific to mAChR subtype and bind with high affinity	elapids (mamba, kraits and cobras)

nAChR, nicotinic acetylcholine receptor; mAChR, muscarinic acetylcholine receptor.

by ptosis, blurred vision, difficulty in swallowing, slurred speech and weakness in facial muscle. Similarly, dendrotoxins have been demonstrated to block particular subtypes of voltage-dependent potassium channels in neurons.

Neurotoxins

Neurotoxins form one of the largest families of proteins with established primary structures. Among the best studied snake neurotoxins are the α-neurotoxins that bind to nicotinic acetylcholine receptors (nAChRs). They are capable of reversibly blocking nerve transmission by competitively binding to the nAChR located at the post-synaptic membranes of skeletal muscles and neurons, preventing neuromuscular transmission and thereby leading to death by asphyxiation [3]. The post-synaptic (α) neurotoxins are classified into four major groups: (a) short neurotoxin, (b) long neurotoxin, (c) κ-neurotoxins and (d) other unconventional neurotoxins called the weak neurotoxins.

The high specificity of neurotoxins for nAChRs has been utilized as a tool in understanding the structure and function of the nervous system. In particular, α-neurotoxins have been crucial for the isolation and characterization of the nAChR at the motor end plate [4]. Figure 1 shows the structure of α-neurotoxins binding to their molecular target nAChR. Figure 1a shows the binding between 5 α-neurotoxin to the acetylcholine receptor subunit while its interaction with a specific peptide of the nAChR isolated from *Torpedo californica* is shown in Figure 1b. The function of the neurotoxins of some elapids can be compared with curare or with the auto-antibodies detected in myasthenia gravis. A comprehensive review of neurotoxins can be found in Siew et al. [5] and Tsetlin and Hucho [3].

Activation of central cholinergic pathways by nicotine and nicotinic agonists has been shown to elicit anti-nociceptive effects in a variety of species and pain tests [6, 7]. Neurotoxins isolated from cobra venoms have shown significant analgesia in animal models. Cobrotoxin, a short-chain post-synaptic α-neurotoxin and α-cobratoxin, a long-chain α-neurotoxin isolated from *Naja atra*, have been reported to show analgesic activity. Cobrotoxin is a specific ligand for the muscle-based α1 nAChR and it produces strong, centrally mediated analgesic effects through an opiate-independent mechanism while the α-cobratoxin shows high affinity for the neuronal α7 nAChR and elicits an opiate-independent, anti-nociceptive effect [8]. Additionally, evidence is emerging that cobrotoxin can substitute for morphine and suppress the effects of morphine withdrawal [8]. Similarly, an α-neurotoxin from the king cobra, *Ophiophagus hannah*, has also been reported to exhibit potent analgesic activity [9].

Neurotoxins that recognize the muscarinic acetylcholine receptors (mAChRs) have been isolated from green mamba venom [10]. Due to their potency and selectivity, the muscarinic toxins (MT1–7) can be useful pharmacological tools for investigating the physiological roles of the muscarinic receptor subtypes [11–13]. These muscarinic receptors are of great interest in the treatment of neurodegenerative diseases, such as Alzheimer's and Parkinsons, and it is hoped that selective blocking of these receptors will greatly aid in restoring normal movement. In fact, the involvement of muscarinic receptors in Azheimer's disease was elucidated using these subtype-specific mamba toxins [14]. Potential sources of nicotinic and muscarinic neurotoxins are summarized in Table 1.

The β-neurotoxins, on the other hand, act presynaptically by affecting the release of acetylcholine via mechanisms

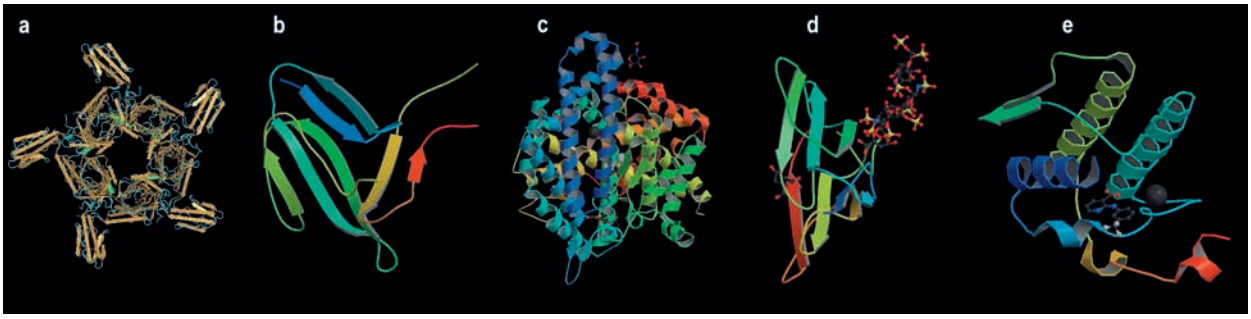

Figure 1. (*a*) Structure of the α-cobratoxin-AChBP complex (1YI5) [from ref. 88]. (*b*) Nuclear magnetic resonance structure of an AChR-peptide (*Torpedo californica*, alpha subunit residues 182–202) in complex with α-bungarotoxin (1L4W) [from ref. 89]. (*c*) Complex of the anti-hypertensive drug captopril and the human testicular angiotensin I-converting enzyme (1UZF) [from ref. 90]. (*d*) Structural Basis of venom citrate-dependent heparan sulphate-mediated cell surface retention of cobra cardiotoxin A3 (1XT3) [from ref. 91]. (*e*) Interactions of a specific non-steroidal anti-inflammatory drug with group I phospholipase A2 (PLA2): crystal structure of the complex formed between PLA_2 and niflumic acid at 2.5Å resolution (1TD7) [from ref. 92].

that differ for different β-neurotoxins. They are responsible for high toxicity and ultimately respiratory paralysis. They act by causing the disappearance of acetylcholine-containing vesicles, preventing the controlled release of acetylcholine and blocking impulse transmission. The pre-synaptic neurotoxins that have been thoroughly investigated to date are crotoxin (from *Crotalus durissus terrificus*), β-bungarotoxin (from *Bungarus multicinctus*), notexin (from *Notechis scutatus*) and taipoxin from the Australian taipan (*Oxyuranus scutellatus*) [15]. Crotoxin, a pre-synaptic neurotoxin with cytotoxic activity, is currently being used in phase 1 clinical trials. This toxin has been tried on advanced cancer patients, as an anti-cancer agent and is believed to act through a novel mechanism of action [16].

Dendrotoxins

Apart from the classical α-neurotoxins, another type of neurotoxin that has been identified in other snake venoms are the dendrotoxins isolated from the African mamba, *Dendroaspis* sp. The best-characterized dendrotoxins are highly potent blockers of $K_V1.1$, $K_V1.2$ and $K_V1.6$ potassium channels and are derived from the venom of the green mamba (*Dendroaspis angusticeps*). Mambas belong to the elapid family and have venom that contains toxins to potentiate acetylcholine release and subsequent synaptic transmission at the neuromuscular junction, leading to excessive muscular activity, trembling and fasciculation of the prey. This action has been ascribed to a small protein, dendrotoxin, that selectively blocked voltage-dependent potassium channels in neurons [17]. In the past 20 years, structural analogues of dendrotoxins have aided in defining the molecular recognition properties of different types of potassium channels, and radiolabelled dendrotoxins have been used to identify other toxins binding to potassium channels. They are also important markers for subtypes of potassium channels *in vivo*, and have been widely used as probes for studying the function of potassium channels both in physiological and pathophysiological conditions [17], and hence have been implicated in the development of new drugs for the treatment of neurodegenerative disorders, such as Alzheimer's disease (www.chemsoc.org/chembytes/ezine/1999/berressem-apr99.htm).

Toxins affecting the cardiovascular system

The first venom-based drug captopril discovered in 1975 also formed the first oral angiotensin-converting enzyme (ACE) inhibitor. This success story started with the observation of the toxic effect of venom from a Brazilian viper (*Bothrops jararaca*) that caused a sudden, massive drop in blood pressure. This piqued the interest of Nobel Prize winner Sir John Vane, who found that the viper venom was a potent inhibitor of ACE. Vane took this discovery to the pharmaceutical company Squibb where two scientists, David Cushman and Miguel Ondetti, created captopril, the first oral ACE inhibitor [18]. With the success of captopril, snake venoms have been explored for potential applications pertaining to the cardiovascular system. The binding of the anti-hypertensive drug captopril to its substrate ACE is shown in Figure 1c.

A toxin isolated from Indian cobra venom in the late 1940s was named cardiotoxin because it caused cardiac arrest when injected into experimental animals. Cardiotoxins, also known as cytotoxins are found exclusively in the venom of cobras and ringhals [19, 20], and are direct lytic factors and membrane-active polypeptides. They are single-chain, highly hydrophobic, basic, short polypeptides closely related to the α-neurotoxin that binds to nAChR, but cardiotoxins do not show any significant affinity for the receptors [21]. The main targets of cardiotoxins are on excitable cells. They cause depolarization and contracture of cardiac, skeletal and smooth muscles, and depolarization and loss of excitability of nerves.

These toxins are pore-forming agents [22] that lead to the depolarization and degradation of the plasma membrane of skeletal muscle cells. The mechanism of action of degeneration is most probably calcium dependent, involving the direct activation of calcium-dependent proteases and the eventual failure of mitochondrial respiration due to a calcium overload [23]. Cobra cardiotoxins may be useful probes in a number of cellular processes, including lipid metabolism and calcium ion regulation in skeletal as well as cardiac muscle [24]. Cardiotoxins have also been shown to be membrane-active proteins that recognize the proteoglycans of the membrane. Their cardiotoxicity is attributed to specific binding to the glycosaminoglycans, the sulphated carbohydrate moieties that occur abundantly in cells of cardiovascular tissues [25]. Figure 1d shows the binding of cardiotoxin to the heparan sulphate moiety. Cardiotoxin is currently being used in a phase 1 clinical trial for cancer treatment along with crotoxin (a pre-synaptic neurotoxin) in a combined therapy [26]. Cardiotoxin expression at higher levels (60% of the venom dry weigh) has been attributed to the difference in promoter activity [27]. The promoter activity of α-neurotoxin from *Naja sputatrix* was down-regulated by the presence of a 24 nucleotide (nt -678 to -655) silencer at the 5′-flanking region. These findings indicated that snakes produce venoms that contain highly lethal and specific toxins at lower levels than the multi-functional toxins like cardiotoxins. A study by Cher et al. [28] has revealed that the changes at the molecular level and physiological states in a victim upon cobra envenomation are initially due to cardiotoxins followed by the synergistic effects of all the other components including neurotoxins and phospholipase A_2 (PLA_2) and the spreading factor, hyaluronidase.

Toxins affecting the muscular system

There are three main classes of venom components that initiate cycles of degeneration and regeneration of skeletal muscle: (a) myotoxins, which are small polypeptides that can be isolated from the venoms of the New World viper subfamily Crotalinae, and which specifically act on skeletal muscles [29]; (b) cardiotoxins, polypeptides of 60–65 amino acids that can be isolated from venoms of cobras and which act on smooth muscles (see above) and (c) PLA_2, which can be isolated from venoms of a number of snake families, including Viperidae, Elapidae and Hydrophiidae. The PLA_2s can have either myotoxic, cardiotoxic or neurotoxic actions [30, 31].

Myotoxins

Myotoxins are also called myonecrotic toxins and are found in venoms from rattlesnakes and other pit vipers. One of the best-known myotoxins is myotoxin-a, isolated from the venom of the Prairie rattlesnake *C. viridis viridis* [29]. It is a small (4600 Da), basic protein devoid of enzymatic activity. Mytotoxin-a binds specifically to the sarcoplasmic reticulum of muscles, causing a change in ion permeability of the sarcoplasmic reticulum (an important calcium regulatory system) leading to swelling and disintegration of both the sarcoplasmic reticulum and muscle fibrils. Hence antibody to myotoxin-a has been used to treat myonecrosis resulting from Prairie rattlesnake venom poisoning [32].

Phospholipase A_2

PLA_2 enzymes may be single-chain polypeptides of around 120 amino acid residues or mixtures of two to five complementary polypeptides, and are Ca^{2+} enzymes. The structure of the complex formed between PLA_2 and the non-steroidal anti-inflammatory drug, niflumic acid, is shown in Figure 1e.

These enzymes catalyze the hydrolysis of phospholipids at the *sn-2* position of the glycerol backbone to release fatty acid and corresponding *1-acyl* lysophospholipid [33]. PLA_2s from snake venoms exhibit a wide variety of pharmacological effects by interfering in normal physiological processes [34].

PLA_2 triggers a cascade of inflammatory events characterized by increased microvascular permeability and oedema formation, leukocyte recruitment into tissues, nociception and release of inflammatory mediators which mimic a number of systemic and local inflammatory disorders in humans. These studies have helped to clarify the pathophysiological roles of these proteins in diverse inflammatory processes. In addition, knowledge of the mechanism of action of myotoxic PLA_2s has provided important clues for understanding snakebite envenomation and has formed a template for the design of new alternatives to conventional anti-venoms.

Anti-inflammatory and anti-neoplasmic activities of PLA_2. Naturally occurring anti-toxic factors that neutralize PLA_2 from the blood of both venomous and non-venomous animals have been isolated and studied [35–37]. Snake PLA_2 inhibitors (PLIs) are large multimeric, serum proteins that form soluble complexes with PLA_2 enzymes, thereby inhibiting their actions. The first PLIs were isolated from the serum of Habu snake, *Trimeresurus flavoviridis* (*Protobothrops flavoviridis;* EMBL Reptile Database) [38]. PLIs show specific affinities for various PLA_2 enzymes and some have been shown to have anti-enzymatic, anti-myotoxic, anti-oedema-inducing, anti-cytotoxic and anti-bacterial activities [39]. In addition to its role in various extra-neuronal (extra-cerebral) inflammatory processes, PLA_2 is also thought to be crucial in inflammatory processes present in numerous acute and chronic neurological disorders as-

sociated with neurodegenerative diseases, such as neural trauma, Alzheimer's disease and Parkinson's disease, and in some brain tumours [40]. Treatment of these disorders with non-specific inhibitors has met with limited success. Hence specific PLIs derived from animal sources might provide potentially more specific pharmacological tools. PLA_2 isolated from *Bothrops neweidii* venom and Indian cobra, *Naja naja* venom, was found to be cytotoxic towards B16 F10 melanoma and Ehrlich ascites tumour cells, respectively, suggesting its employment as an anti-cancer drug [41].

Toxins affecting the haemostatic system

The major symptoms from snakebite affecting the haemostatic system are (a) reduced coagulability of blood, resulting in an increased tendency to bleed, (b) bleeding due to the damage to blood vessels, (c) secondary effects of increased bleeding, ranging from hypovolaemic shock to secondary-organ damage, such as intracerebral haemorrhage, anterior pituitary haemorrhage or renal damage and (d) direct pathologic thrombosis and its sequelae, particularly pulmonary embolism [42]. More recently, snake venoms have been used in the development of platelet aggregation and blood-clotting inhibitors.

Venoms from vipers and some Australian snakes are rich sources of proteases that strongly affect the haemostatic mechanism [43]. Coagulant enzymes include activators of the blood coagulation factors II (prothrombin), V and X, while anti-coagulants include protein C activators, inhibitors of prothrombin complex formation and fibrinogenases (based on their specificity for the alpha, beta and gamma chains of fibrinogen). Intermediates between the true coagulants and true anti-coagulants are the thrombin-like enzymes which bring about clotting *in*

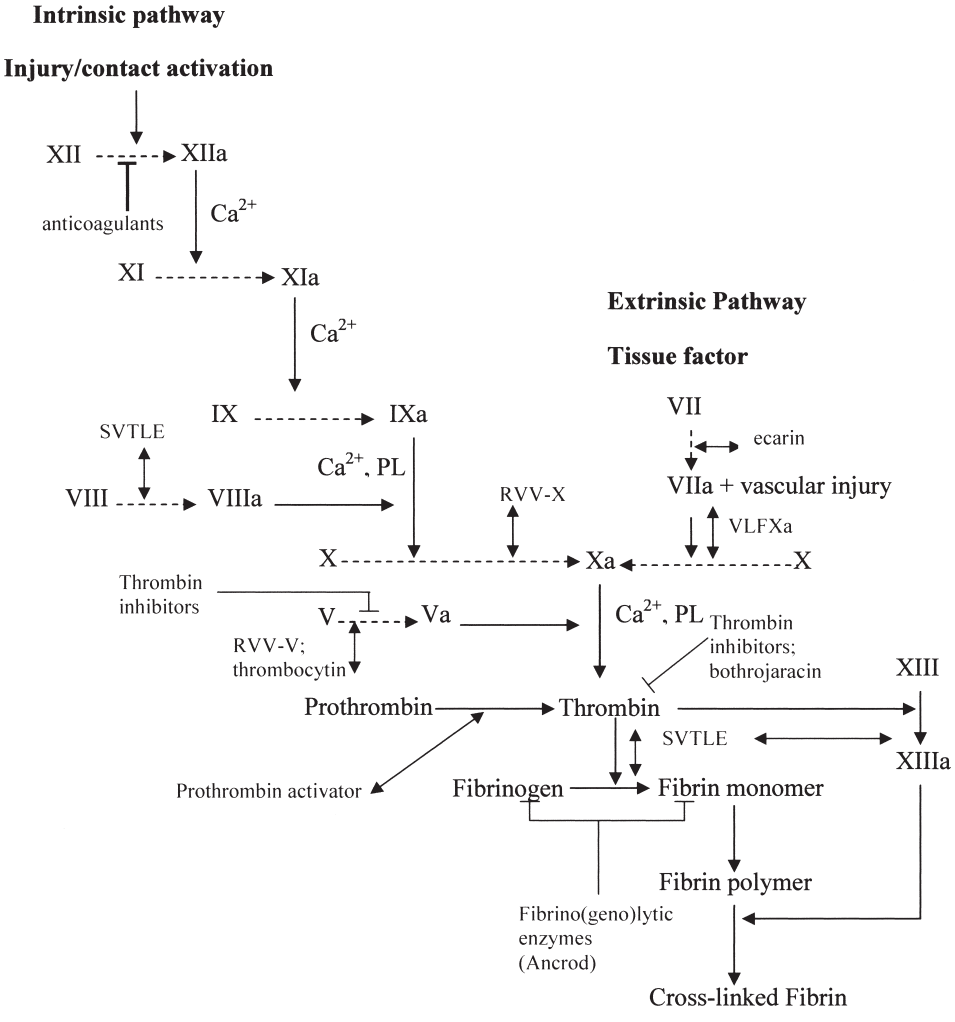

Figure 2. Blood coagulation pathways and the steps in which snake venom proteins interfere. SVTLE, snake venom thrombin-like enzymes; RVV-V, Russell's viper venom factor V activator; RVV-X, Russell's viper venom factor X activator; VLFXa, *Vipera lebetina* factor X activator. Activation by venom protein is denoted by ◄—► ; inhibition by ⊢—.

vitro but defribination (anti-coagulation) *in vivo*. Snake venoms (including the disintegrins, a group of RGD-containing proteins) also affect platelets by inducing or inhibiting platelet aggregation, fibrinolytic activators and haemorrhagins (alpha-fibrinogenases) to cause haemorrhage by acting via platelets or proteolysis of the blood vessel wall [44]. Snake venom proteins that affect the blood coagulation cascade are summarized in Figure 2. It appears that for every factor involved in the blood coagulation cascade, there is a counterpart among the snake venom compounds that could either activate or inactivate the factors. These activators or inhibitors usually belong to various families such as serine proteases, metalloproteinases, C-type lectins, disintegrins and phospholipases. The structure of a protein C activator from *Agkistrodon contortrix contortrix*, Protac, is shown in Figure 3a.

Thrombin-like enzymes and fibrinogen studies

Approximately 100 snake venom toxins have been identified as 'thrombin-like' enzymes [45]. Thrombin is able to cleave both fibrinopeptide A (FPA) and fibrinopeptide B (FPB) from fibrinogen and activating factor XIII (fibrin-stabilizing factor). While some actions of these snake venom thrombin-like enzymes (SVTLEs) mimic the effects of thrombin, they usually cleave FPA alone; only a few cleave FPB. Thus, without cleavage of both FPA and FPB, they are unable to activate factor XIII and the clots produced can easily be broken down. The failure of the clots to be cross-linked leads to a breakdown in the fibrinolytic system and effective removal of fibrinogen from the plasma. The most widely used SVTLEs are those from *Bothrops atrox* venom (Batroxobin, Reptilase) (Pentapharm, Basel, Switzerland) and from *Callosellasma rhodostoma* venom (Ancrod). Ancrod has been shown to be effective in limiting infarct volume [46]. The fibrinogenase family from snake venom that is able to cleave specifically one or more fibrinogen chains has received more attention in the last few years. The fibrin(ogen)olytic enzymes are either serine proteases or metalloproteinases. Though targeting a different factor (fibrin or fibrinogen), these proteins break down the fibrin-rich clots and prevent progression of clot formation. Fibrolase from southern copperhead (*A. contortrix*) snake venom can degrade both the α and β chains of fibrin and shows potential as a thrombolytic agent [47]. Other enzymes that dissolve blood clots both *in vitro* and *in vivo* include afaacytin from horned viper (*Cerastes cerastes*) venom [48], atroxase from western diamondback rattlesnake venom [49] and fibrinogenase from *Vipera lebetina* (*Microvipera lebetina*, EMBL Reptile Database) venom [50]. In 2001, alfimeprase, produced as a truncated recombinant form of fibrolase, was produced and introduced into clinical trials [51]. It is presently in a phase II clinical trial for two indications: (a) treatment of peripheral arterial occlusions and (b) clearance of occluded vascular excess catheters, in direct competition with plasminogen activators [52].

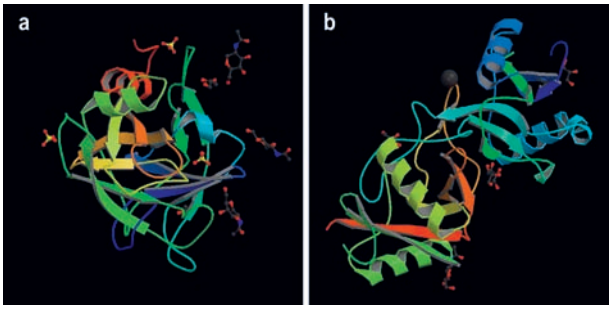

Figure 3. (*a*) Structure of native protein C activator from the venom of the copperhead snake *Agkistrodon contortrix contortrix* (2AIP) [from ref. 93]. (*b*) Structure of EMS16, an Antagonist of collagen receptor integrin $\alpha 2\beta 1$ (GPIa/IIa; 1UKM) [from ref. 94].

Prothrombin

Snake venoms are rich sources of prothrombin activators and hence are utilized in prothrombin assays, especially for studying dysprothrombinaemias and for preparing meizothrombin and non-enzymatic forms of prothrombin. Russell's viper (*Daboia russelli*) venom (RVV) contains toxins which have been used to assay blood clotting factors V, VII, X, platelet factor 3 and, importantly, lupus anti-coagulants (LAs). Other prothrombin activators (from the taipan, Australian brown snake and saw-scaled viper) have also been used to assay for LA. Protein C and activated protein C resistance can be measured by RVV and Protac, a fast-acting inhibitor from southern copperhead snake venom. A C-type lectin-like protein, botrocetin, isolated from *B. jararaca* venom can be used to study the von Willebrand factor [53]. These proteins are useful tools for elucidating the mechanisms involved in clotting and platelet activation as well as the structure-function relationships of both blood-clotting factors and platelet glycoproteins.

Platelet aggregation inhibitors

Many snake venom toxins affect platelet function [54]. They can be grouped into a few major families, such as enzymes like serine proteinases, zinc-dependent PI-PIV metalloproteinases of the reprolysin family and group II PLA_2 isoenzymes as well as proteins with no enzymatic activity, such as C-type lectins, CRISP and disintegrins [55]. Of these, disintegrins and C-type lectins [54, 56] have been considered as useful modulators of platelet function.

Disintegrins. Snake venom disintegrins are natural products that have been investigated as potent inhibitors of

various integrins, and major progress has been made in the functional characterization of disintegrins. Functionally, disintegrins can be divided into three groups according to their integrin selectivity and the presence of specific and active motifs. The specific adhesion molecule recognition motifs, their respective sequences and the specific targets are listed in Table 2. They are (a) those interacting with RGD motif-dependent integrins, (b) leukocyte integrin-binding disintegrins and (c) the $\alpha1\beta1$ integrin-binding disintegrins. The first group includes most of the monomeric disintegrins that contain the RGD motif, as well as disintegrins without the RGD motif but with inhibitory activity against RGD-dependent integrins such as KGD, MVD, MGD and WGD disintegrins. The second group is represented by MLD motif – containing disintegrins which interact with $\alpha4\beta1$, $\alpha4\beta7$ and $\alpha9\beta1$ integrins. The last group consists of the recently discovered, KTS motif-containing disintegrins, obtustatin and viperistatin. These disintegrins are potent and selective inhibitors of $\alpha1\beta1$, characterized as specific receptors for collagen IV [57].

Targeting and inhibiting RGD-dependent integrins is a major goal of the pharmacological research for many diseases. In thromboembolic disorders, the main aim is to block the fibrinogen receptor αIIb$\beta3$ integrin on the platelet. The structure of disintegrins has been used as a template to design compounds that bind to endogenous fibrinogen with higher affinity. This resulted in the introduction of two drugs, eptifibatide and tirofiban. Eptifibatide (integrelin) was modelled on the active site of barbuorin, (*Sistrurus m. barbouri*) and is, in fact, a KGD-containing protein [58], while tirofiban (Aggrastat) which was designed from echistatin (a disintegrin) is a synthetic compound that mimics RGD [59, 60]. Both drugs have been approved for the therapy of acute coronary ischaemic syndrome and the prevention of thrombotic complications in patients undergoing percutaneous coronary intervention such as balloon angioplasty and stenting [61, 62].

Blockers of platelet integrins and especially αIIb$\beta3$ could have applications in certain types of cancer treatment. It is well known that platelets contribute to tumor growth, angiogenesis and metastasis [63]. The calcium-dependent glycoprotein IIb/IIIa complex is the most prevalent platelet cell surface receptor and may combine with one of the four adhesive proteins, fibrinogen, fibronectin, von Willebrand factor and vitronectin, all containing the RGD-motif. The RGD-containing snake venom proteins interfere with platelet aggregation by reversibly blocking the GPIIb/IIIa receptor. The most studied disintegrins include trigramin [*Trimeresurus gramineus*; 64], rhodostomin [*C. rhodostoma*; 65] and triflavin [*T. flavoviridis (P. flavoviridis* (EMBL Reptile Database); 66].

Snake venom RGD-disintegrins have been the target for investigation of $\alpha v\beta3$ integrin as a potential target for the suppression of cancer. These disintegrins are useful tools to decipher the mechanisms that occur during $\alpha v\beta3$-dependent angiogenesis. Monomeric RGD-disintegrins, accutin [67], triflavin [68], salmosin [69], rhodostomin [70, 71], and the homodimeric RGD-disintegrin, contortrostatin [72], inhibit angiogenesis by binding to endothelial cells via $\alpha v\beta3$. It has been suggested that the binding of disintegrins to endothelial cells inhibits

Table 2. Adhesion molecule recognition motifs in snake venom disintergins.

Motifs recognized by adhesion molecules	Amino acid sequence	Physiological target	Examples/source
RGD	arginine-glycine-aspartate	blocks the GPIIb/IIIa receptor and binds to integrins αIIb$\beta3$, $\alpha8\beta1$, $\alpha v\beta3$, $\alpha v\beta5$ and/or $\alpha5\beta1$	trigramin (*Trimeresurus gramineus*); contortrostatin (*Agkistrodon contortrix contortrix*)
KGD	lysine-glycine-aspartate	binds specifically to integrin αIIb$\beta3$	barbuorin (*Sistrurus m. barbouri*)
MVD	methionine-valine-aspartate	potent inhibitor of both collagen- and ADP-stimulated platelet aggregation	atrolysin-E/D (*Crotalus atrox*)
MGD	methionine-glycine-aspartate	potent and selective inhibitor of $\alpha5\beta1$	EMF10 (*Eristocophis macmahoni*)
WGD	tryptophan-glycine-aspartate	potent inhibitor of the RGD-dependent integrins $\alpha5\beta1$, $\alpha v\beta3$, and αIIb$\beta3$.	CC8 (*Cerastes cerastes*)
MLD	methionine-leucine-aspartate	binds $\alpha4\beta1$, $\alpha4\beta7$, $\alpha9\beta1$, $\alpha5\beta1$ and αIIb$\beta3$ integrins	EC5 (*Echis carinatus sochureki*), VLO5 (*Vipera lebetina obtusa*)
KTS	lysine-tryptophan-serine	selective integrin $\alpha1\beta1$ inhibitors	obtustatin (*Vipera lebetina obtusa*)
RTS	arginine-tryptophan-serine	selectively blocks integrin $\alpha1\beta1$	jerdostatin (*Trimeresurus jerdonii*)

their motility and proliferation, possibly by induction of apoptosis in endothelial cells. Snake venom RGD-disintegrins showed direct interaction in several tumor cell lines. The blocking of $\alpha v\beta 3$ integrin in tumor cells inhibited their adhesion to the extracellular matrix and significantly reduced their motility, subsequently inhibiting metastasis. This effect was noted for monomeric medium-sized disintegrins, such as salmosin [69] and triflavin [68] and the homodimeric disintegrin, contortrostatin [73]. These drugs may be considered as alternatives to the humanized monoclonal antibodies used in cancer therapy.

Another RGD-dependent integrin that is targeted by certain snake venom disintegrins is fibronectin receptor, $\alpha 5\beta 1$ integrin. This cell surface receptor has been studied in the pathology of several diseases such as Alzheimer's disease [74], vascular diseases [75] and cancer [76]. This integrin is the target for regulation of angiogenesis [77, 78]. Most of the monomeric RGD-disintegrins are inhibitors of $\alpha 5\beta 1$ integrin. MLD- and KTS-disintegrins are specific for leukocyte integrins and collagen receptors, respectively, and are also being investigated as new areas in pharmaceutical research [57].

C-type lectin-like proteins. C-type lectins such as the mannose-binding proteins bind a sugar moiety in the presence of Ca^{2+} and contain the carbohydrate recognition domain (CRD). C-type lectins are a class of proteins widely distributed in nature that display various functions in important physiological processes. The C-type lectin-like proteins are an important group of proteins among the haemorrhagic components in snake venom. Most C-type lectin-like proteins in snake venom do not contain the classic calcium/sugar-binding loop and they have evolved to bind a wide range of physiologically important proteins and receptors [79]. Based on their structural and functional entities, these proteins in snake venom have been classified into the true C-type lectins (contains the CRD domain) that bind a sugar molecule and the C-type lectin-like proteins with CRD-related non-carbohydrate-binding C-type lectin-like domains (CTLDs) that do not bind a sugar moiety [80]. They are further divided into CRD-containing proteins, factor IX/X-binding proteins and those that bind to the platelet receptors [81].

Snake C-type lectin-like proteins bind to a wide range of coagulation factors that are important in haemostasis and to platelet receptors and display both anti-coagulant- and platelet-modulating activities. They activate platelets by binding to von Willebrand factor or specific receptors such as GPIb, $\alpha 2\beta 1$ and GPVI. Heterodimeric GPIb-binding molecules mainly inhibit platelet functions, while the multimeric binding molecules activate platelets. Some tetrameric snake venom C-type lectin-like proteins activate platelets by binding to GPVI, while others affect platelet function via integrin $\alpha 2\beta 1$. Some act by inducing von Willebrand factor to bind to GPIb as well, or activate platelets via $\alpha 2\beta 1$ and GPIb [81].

While the earliest described C-type lectin, botrocetin, clearly activates platelets by inducing interactions between GPIb and von Willebrand factor, many GPIb-binding C-type lectins are described as inhibitory [82]. In the last decade, numerous C-type lectin-like proteins, including IX/X-binding protein, CA-1, RVV-X, Convulxin, EMS16, botocetin and flavocetin-A, have been isolated from various snake venoms, sequenced and characterized. Echicetin, isolated from the *Echis carinatus*, spe-

Table 3. Drugs/clinical diagnostic kits from snake venoms.

Drug/trade name®	Target and function/treatment	Source
Captopril; enalapril	ACE inhibitor/high blood pressure	*Bothrops jaracusa* (Brazilian arrowhead viper)
Integrilin (eptifibatide)	platelet aggregation inhibitor/acute coronary syndrome	*Sisturus miliarus barbouri* (south-eastern pigmy rattlesnake)
Aggrastat (tirofiban)	GPIIb-IIIa inhibitor/myocardial infarct, refractory ischaemia	*Echis carinatus* (African saw-scaled viper)
Ancrod (Viprinex)	Fibrinogen inhibitor/stroke	*Agkistrodon rhodostoma* (Malayan pit viper)
Defibrase	thrombin and protrhombin inhibitor/acute cerebral infarction, unspecific angina pectoris	*Bothrops moojeni*
Hemocoagulase	thrombin-like effect and thromboplastin activity/ prevention and treatment of haemorrhage	*Bothrops atrox*
Protac/protein C activator	protein C activator/clinical diagnosis of haemostatic disorder	*Agkistrodon contortix contortix* (American copperhead)
Reptilase	diagnosis of blood coagulation disorder	*Bothrops jaraca* (South American lance adder)
Ecarin	prothrombin activator/diagnostic	*E. carinatus*
Exanta; ximelagatran	blood thinner/anti-coagulant, thrombin inhibitor	Cobra

cifically binds platelet GPIb and blocks platelet binding with von Willebrand factor and thrombin [83], while convulxin, isolated from *C. durissus terrificus*, activates platelets through interaction with GPVI [84]. The inhibitor protein isolated from *Echis multisquamatus*, EMS16, is a potent and selective inhibitor of integrin $\alpha 2\beta 1$ [85] while aggretin (*C. rhodostoma* [86] activates platelets by binding to $\alpha 2\beta 1$ and GPIb. The structure of EMS16 is shown in Figure 3b. Therefore, the C-type lectin-like proteins could be useful tools for elucidating the mechanisms involved in clotting and platelet activation as well as providing new possibilities in diagnosis and treatment through their interaction with platelets, plasma and the vascular wall [81, 87].

Conclusion

Nature has been a source of medicinal products for thousands of years, among which snake venoms form a rich source of bioactive molecules, such as peptides, proteins and enzymes with important pharmacological activities. Moreover, blood and bile duct from snakes have been widely used in Chinese traditional medicine. With the advent of protein fold structures, a rich source of peptides that interact specifically and with high affinity with human protein can be developed. This will help not only in understanding the implications of each interaction but will also lead to the development of effective drugs targeted to particular protein functions. Examples of drugs that have been derived from snake venom proteins and have progressed the clinic are listed in Table 3. From the initial discovery of captopril, the first oral ACE inhibitor to the recent application of disintegrins for the potential treatment of cancer, the various components of snake venoms have never failed to reveal amazing new properties. Snake venoms have been used in the coagulation laboratory for the routine assay of coagulation factors and as reagents to study both coagulopathy and haemostasis. A number of useful compounds have been identified; most notably, the disintegrins (eptifibatide and tirofiban) have been shown both *in vitro* and *in vivo* to be powerful anti-platelet aggregates. While the original native snake venom compounds are usually unsuitable as therapeutics, interventions by medicinal chemists as well as scientists and clinicians in pharmaceutical R & D have made it possible to use the snake venom proteins as therapeutics for multiple disorders based on the available structural and functional information. Snake venoms, with their cocktail of individual components, have great potential as therapeutic agents for human diseases.

Acknowledgements. D. K. C. I. was in receipt of a research scholarship from the NUS. This work was supported by a research grant (R183-000-162-112) from the Academic Research Fund, NUS, Singapore.

1 Lalloo, D., Trevett, A. J., Saweri, A., Naraqui, S., Theakston R. D. G. and Warrell, D. A. (1995) The epidemiology of snake bite in Central Province and National Capitol District. Papau New Guinea. Trans. R. Soc. Trop. Med. Hyg. 89, 178–182.
2 Warrell, D. A., Bhetwal, B. B., Chugh, K. S., Lallo, D. G., Looareesuwan, S., Win, M. M., Sjostrom, L., Theakston R. D. G., Watt, G. and White, J. (1999) Asian snakes and snakebite. Southeast Asian J. Trop. Med. Public Health 30, 1–85.
3 Tsetlin, V. I. and Hucho, F. (2004) Snake and snail toxins acting on nicotinic acetylcholine receptors: fundamental aspects and medical applications. FEBS Lett. 557, 9–13.
4 Silverira, R. and Dajas, F. (1994) Neurotoxins in neurobiology. In: Neurotoxins in Neurobiology: Their Actions and Applications, pp. 3–26, K. S. Tipton, F. Dajas (eds), Horwood, New York.
5 Siew, J. P., Khan, A. M., Tan, P. T., Koh, J. L., Seah, S. H., Koo, C. Y., Chai, S. C., Armugam, A., Brusic, V. and Jeyaseelan, K. (2004) Systematic analysis of snake neurotoxins' functional classification using a data warehousing approach. Bioinformatics 20, 3466–3480.
6 Damaj, M. I., Meyer, E. M. and Martin, B. R. (2000) The antinociceptive effects of alpha7 nicotinic agonists in an acute pain model. Neuropharmacology 39, 2785–2791.
7 Decker, M. W., Meyer, M. D. and Sullivan, J. P. (2001) The therapeutic potential of nicotinic acetylcholine receptor agonists for pain control. Expert Opin. Investig. Drugs 10, 1819–1830.
8 Chen, Z. X., Zhang, H. L., Gu, Z. L., Chen, B. W., Han, R., Reid, P. F., Raymond, L. N. and Qin, Z. H. (2006) A long-form alpha-neurotoxin from cobra venom produces potent opioid-independent analgesia. Acta Pharmacol. Sin. 27, 402–408.
9 Pu, X. C., Wong, P. T. and Gopalakrishnakone, P. (1995) A novel analgesic toxin (hannalgesin) from the venom of king cobra (*Ophiophagus hannah*). Toxicon 33, 1425–1431.
10 Harvey, A. L., Kornisiuk, E., Bradley, K. N., Cervenansky, C., Duran, R., Adrover, M., Sanchez, G. and Jerusalinsky, D. (2002) Effects of muscarinic toxins MT1 and MT2 from green mamba on different muscarinic cholinoceptors. Neurochem. Res. 11, 1543–1554.
11 Bradley K. N. (2000) Muscarinic toxins from the green mamba. Pharmacol. Ther. 85, 87–109.
12 Potter, L. T. (2001) Snake toxins that bind specifically to individual subtypes of muscarinic receptors. Life Sci. 68, 2541–2547.
13 Jerusalinsky, D., Alfaro, P., Kornisiuk, E., Quillfeldt, J., Alonso, M., Rial Verde, E., Durán, R. and Cerveñansky, C. (2000) Muscarinic toxins: novel pharmacological tools for the muscarinic cholinergic system. Toxicon 38, 747–761.
14 Mulugeta, E., Karlsson, E., Islam, A., Kalaria, R., Mangat, H., Winblad, B. and Adem, A. (2003) Loss of muscarinic M4 receptors in hippocampus of Alzheimer patients. Brain Res. 960, 259–262.
15 Rossetto, O., Rigoni, M. and Montecucco, C. (2004) Different mechanism of blockade of neuroexocytosis by presynaptic neurotoxins. Toxicol. Lett. 149, 91–101.
16 Cura, J. E., Blanzaco, D. P., Brisson, C., Cura, M. A., Cabrol, R., Larrateguy, L., Mendez, C., Sechi, J. C., Silveira, J. S., Theiller, E., de Roodt, A. R. and Vidal, J. C. (2002) Phase I and pharmacokinetics study of crotoxin (cytotoxic PLA(2), NSC-624244) in patients with advanced cancer. Clin. Cancer Res. 8, 1033–1041.
17 Harvey, A. L. and Robertson, B. (2004) Dendrotoxins: structure-activity relationships and effects on potassium ion channels. Curr. Med. Chem. 11, 3065–3072.
18 Patlak, M. (2003) From viper's venom to drug design: treating hypertension. FASEB J. 18, 421.
19 Jeyaseelan, K., Armugam, A., Lachumanan, R., Tan, C. H. and Tan, N. H. (1998) Six isoforms of cardiotoxin in malayan spit-

ting cobra (*Naja naja sputatrix*) venom: cloning and characterization of cDNAs. Biochim. Biophys. Acta 1380, 209–222.
20 Chang, L. S., Huang, H. B. and Lin, S. R. (2000) The multiplicity of cardiotoxins from *Naja naja atra* (Taiwan cobra) venom. Toxicon 38, 1065–1076.
21 Dufton, M. J. and Hider, R. C. (1991) The structure and pharmacology of elapid cytotoxins. In: Snake Toxins, pp. 259–302, A. L. Harvey (ed.), Pergamon, New York.
22 Forouhar, F., Huang, W. N., Liu, J. H., Chien, K. Y., Wu, W. G. and Hsiao, C. D. (2003) Structural basis of membrane-induced cardiotoxin A3 oligomerization. J. Biol. Chem. 278, 21980–21988.
23 Harris, J. B. (2003) Myotoxic phospholipases A2 and the regeneration of skeletal muscles. Toxicon 42, 933–945.
24 Fletcher, J. E. and Jiang, M. S. (1993) Possible mechanism of action of snake venom cardiotoxins and bee venom melittin. Toxicon 31, 669–695.
25 Wang, C. H., Liu, J. H., Lee, S. C., Hsiao, C. D. and Wu, W. G. (2006) Glycosphingolipid-facilitated membrane insertion and internalization of cobra cardiotoxin: the sulfatide.cardiotoxin complex structure in a membrane-like environment suggests a lipid-dependent cell-penetrating mechanism for membrane binding polypeptides. J. Biol. Chem. 281, 656–667.
26 Costa, L. A., Miles, H., Araujo, C. E., Gonzalez, S. and Villarrubia, V. G. (1998) Cardiotoxin in therapy: immunopharmacology Immunotoxicology 20, 15–25.
27 Ma, D., Armugam, A. and Jeyaseelan, K. (2001) Expression of cardiotoxin-2 gene: cloning, characterization and deletion analysis of the promoter. Eur. J. Biochem. 268, 1844–1850.
28 Cher, C. D., Armugam, A., Zhu, Y. Z. and Jeyaseelan, K. (2005) Molecular basis of cardiotoxicity upon cobra envenomation. Cell. Mol. Life Sci. 62, 105–118.
29 Ownby, C. L., Cameron, D. and Ju, A. T. (1976) Isolation of mytotoxic component from rattlesnake (*Crotalus viridis viridis*) venom. electron microscopic analysis of muscle damage. Am. J. Pathol. 85, 149–166.
30 Harris, J. B., Johnson, M. A. and Karlsson, E. (1975) Pathological responses of rat skeletal muscle to a single subcutaneous injection of a toxin isolated from the venom of Australian tiger snake, *Notechis scutatus scutatus*. Clin. Exp. Pharmacol. Physiol 2, 383–404.
31 Gutiérrez, J. M., Ownby, C. L. and Odell, G. V. (1984) Skeletal muscle regeneration after myonecrosis induced by crude venom and a myotoxin from the snake *Bothrops asper* (Fer-De-Lance). Toxicon 22, 719–731.
32 Ownby, C. L., Colberg, T. R. and Odell, G. V. (1986) *In vivo* ability of antimyotoxin a serum plus polyvalent (Crotalidae) antivenom to neutralize Prairie rattlesnake (*Crotalus viridus viridus*) venom. Toxicon 24, 197–200.
33 Verheij, H. M., Boffa, M. C., Rothen, C., Bryckert, M. C., Verger, R. and de Hass, G. H. (1980) Correlation of enzymatic activity and anticoagulant properties of phospholipase A2. Eur. J. Biochem. 112, 25–32.
34 Valentin, E. and Lambeau, G. (2000) Increasing molecular diversity of secreted phospholipases A(2) and their receptors and binding proteins. Biochim. Biophys. Acta 1488, 59–70.
35 Thwin, M. M., Gopalakrishnakone, P., Kini, R. M., Armugam, A. and Jeyaseelan, K. (2000) Recombinant antitoxic and anti-inflammatory factor from the non-venomous snake *Python reticulatus*: phospholipase A_2 inhibition and venom neutralizing potential. Biochemistry 39, 9604–9611.
36 Dunn, R. D. and Broady, K. W. (2001) Snake inhibitors of phospholipase A_2 enzymes. Biochim. Biophys. Acta 1533, 29–37.
37 Faure G (2000) Natural inhibitors of toxic phospholipases A(2). Biochimie 82, 833–840.
38 Kihara, H. (1976) Studies on phospholipase A in *Trimeresurus flaoviridis* venom. III. Purification and some properties of phospholipase A inhibitor in Habu serum. J. Biochem. (Tokyo) 80, 341–349.
39 Soares, A. M., Marcussi, S., Stabeli, R. G., Franca, S. C., Giglio, J. R., Ward, R. J. and Arantes, E. C. (2003) Structural and functional analysis of BmjMIP, a phospholipase A_2 myotoxin inhibitor protein from *Bothrops moojeni* snake plasma. Biochem. Biophys. Res. Commun. 302, 193–200.
40 Farooqui, A. A., Litsky, M. L., Farooqui, T. and Horrocks, L. A. (1999) Inhibitors of intracellular phospholipase A2 activity: their neurochemical effects and therapeutical importance for neurological disorders. Brain Res. Bull. 49, 139–153.
41 Basavarajappa, B. S. and Gowda, T. V. (1992) Comparative characterization of two toxic phospholipases A_2 from Indian cobra (*Naja naja naja*) venom. Toxicon 30, 1227–1238.
42 Numeric, P., Moravie, V., Didier, M., Chatot-Henrey, D., Cirille, S., Bucher, B. and Thomas, L. (2002) Multiple cerebral infarctions following snakebite by *Bothrops carribbaeus*. Am. J. Trop. Med. Hyg. 67, 287–288.
43 White, J. (2005) Snake venom and coagulopathy. Toxicon 45, 951–967.
44 Marsh, N. A. (1994) Inventory of haemorrhagic factors from the snake venoms. Thromb. Haemost. 71, 793–797.
45 Pirkle, H. (1998) Thrombin-like enzymes from snake venoms: an updated inventory. Thromb. Haemost. 79, 675–683.
46 Samsa, G. P., Matchar, D. B., Williams, G. R. and Levy, D. E. (2002) Cost effectiveness of ancrod treatment of acute ischaemic stroke: results from the Stroke Treatment with Ancrod Trial (STAT). J. Eval. Clin. Prac. 8, 61–70.
47 Markland, F. S. (1998) Snake venom fibrinogenolytic and fibrinolytic enzymes: a updated inventory. Thromb. Haemost. 79, 668–674.
48 Laraba-Djebari, F., Martin-Eauclaire, M. F., Mauco, G. and Marchot, P. (1995) Afaacytin, an alpha beta-fibrinogenase from *Cerastes cerastes* (horned viper) venom, activates purified factor X and induces serotonin release from human blood platelets. Eur. J. Biochem. 233, 756–765.
49 Baker, B. J. and Tu, A. T. (1996) Atroxase: a fibrinolytic enzyme isolated from the venom of Western diamondback rattlesnake: isolation, characterization and cloning. Adv. Exp. Med. Biol. 391, 203–211.
50 Gasmi, A., Chabchoub, A., Guermazi, S., Karoui, H., Elayeb, M. and Dellagi, K. (1997) Further characterization and thrombolytic activity of a rat model of a fibrinogenase from *Vipera lebetina* venom. Thromb. Haemost. 86, 233–242.
51 Toombs, C. F. (2001) Alfimeprase: pharmacology of a novel fibrinolytic metalloproteinase for thrombolysis. Haemostasis 31, 141–147.
52 Swenson, S., Toombs, C. F., Pena, L., Johansson J. and Markland, F. S. Jr. (2004) Alpha-fibrinogenases. Curr. Drug Targets Cardiovasc. Haematol. Disord. 4, 417–435.
53 Marsh, N. and Williams, V. (2005) Practical applications of snake venom toxins in haemostasis. Toxicon 45, 1171–1181.
54 Andrews, R. K. and Berndt, M. C. (2000) Snake venom modulators of platelet adhesion receptors and their ligands. Toxicon 38, 775–791.
55 Juárez, P., Sanz, L. and Calvete, J. J. (2004) Snake venomics: characterization of protein families in *Sistrurus barbouri* venom by cysteine mapping, N-terminal sequencing, and MS/MS analysis. Proteomics 4, 327–338.
56 Wisner, A., Leduc, M. and Bon, C. (2002) C-type lectins from snake venoms: new tools for research in thrombosis and haemostasis. In: Perspectives In Molecular Toxinology, pp. 357–375, Ménez, A. (ed.) Wiley, Chichester, UK.
57 Marcinkiewicz, C. (2005) Functional characterization of snake venom disintegrins: potential therapeutic implication. Curr. Pharm. Des. 11, 815–827.
58 Scarborough, R. M., Naughton, M. A., Teng, W., Rose, J. W., Philips, D. R. and Nannizzi, L. (1991) Design of potent and specific integrin antagonists. peptide antagonists with high specificity for glycoprotein IIb-IIIa. J. Biol. Chem. 268, 1066–1073.

59 Marwick, C. (1998) Nature's agents help heal humans – some now take steps to reciprocate. JAMA 279, 1679–1681.
60 Hantgan, R. R., Stahle, M. C., Connor, J. H, Lyles, D. S., Horita, D. A., Rocco, M., Nagaswami, C., Weisel, J. W. and McLane, M. A. (2004) The disintegrin echistatin stabilizes integrin alphaIIbbeta3's open conformation and promotes its oligomerization. J. Mol. Biol. 342, 1625–1636.
61 Pang, J. T., Fort, S., Della Siega, A. and Cohen, E. A. (2002) Emergency coronary artery bypasses surgery in the era of glycoprotein IIb/IIIa receptor antagonist use. J. Card. Surg. 17, 425–431.
62 Gilchrist, I. C. (2003) Platelet glycoprotein IIb/IIIa inhibitors in percutaneous coronary intervention: focus on the pharmacokinetic-pharmacodynamic relationship of eptifibatide. Clin. Pharmacokinet. 42, 703–720.
63 Trikha, M. and Nakada, M. T. (2002) Platelets and cancer: implications for antiangiogenic therapy. Semin. Thromb. Hemost. 28, 39–44.
64 Huang, T. F., Holt, J. C., Lukasiewicz, H. and Niewiarowski, S. (1987) Trigramin: a low molecular weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein IIb/IIIa complex. J. Biol. Chem. 262, 16157–16163.
65 Huang, T. F., Wu, Y. J. and Ouyang, C. (1987) Characterization of a potent platelet aggregation inhibitor from *Agkistrodon rhodostoma* snake venom. Biochim. Biophys. Acta 925, 248–257.
66 Huang, T. F., Sheu, J. R. and Teng, C. M. (1991) A potent antiplatelet peptide, triflavin from *Trimeresurus flavoviridis* snake venom. Biochem. J. 277, 351–357.
67 Yeh, C. H., Peng, H. C. and Huang, T. F. (1998) Accutin, a new disintegrin inhibits angiogenesis *in vitro* and *in vivo* by acting as integrin alphabeta3 antagonist and inducing apoptosis. Blood 92, 3268–3276.
68 Sheu, J. R., Yen, M. H., Kan, Y. C., Hung, W. C., Chang, P. T. and Luk, H. N. (1997) Inhibition of angiogenesis *in vitro* and *in vivo*: comparison of the relative activities of triflavin, an Arg-Gly-Asp- containing peptide and anti-alpha(v)beta3 integrin monoclonal antibody. Biochim. Biophys. Acta 1336, 445–454.
69 Kim, S. I., Kim, K. S., Kim, H. S., Choi, M. M., Kim, D. S. and Chung, K. H. (2004) Inhibition of angiogenesis by salmosin expressed *in vitro*. Oncol. Res. 14, 227–233.
70 Yeh, C. H., Peng, H. C., Yang, R. S. and Huang, T. F. (2001) Rhodostomin, a snake venom disintegrin, inhibits angiogenesis elicited by basic fibroblast growth factor and suppresses tumor growth by a selective alpha(v)beta(3) blockade of endothelial cells. Mol. Pharmacol. 59, 1333–1342.
71 Huang, T. F., Yeh, C. H. and Wu, W. B. (2001) Viper venom components affecting angiogenesis. Haemostasis 31, 192–206.
72 Markland, F. S., Shieh, K., Zhou, Q., Golubkov, V., Sherwin, R. P. and Richters, V. (2001) A novel snake venom disintegrin that inhibits human ovarian cancer dissemination and angiogenesis in an orthotopic nude mouse model. Haemostasis 31, 183–191.
73 Zhou, Q., Sherwin, R. P., Parrish, C., Richters, V., Groshen, S. G. and Tsao-Wei, D. (2000) Contortrostatin, a dimeric disintegrin from *Agkistrodon contortrix contortrix*, inhibits breast cancer progression. Breast Cancer Res. Treat. 61, 249–260.
74 Matter, M. L., Zhang, A., Nordstedt, C. and Ruoslahti, E. (1998) The alpha5beta1 integrin mediates elimination of amyloid-beta peptide and protects against apoptosis. J. Cell. Biol. 141, 1019–1030.
75 Hein, T. W., Platts, S. H., Waitkus-Edwards, K. R., Kuo, L., Mousa, S. A. and Meininger, G. A. (2001) Integrin-binding peptides containing RGD produce coronary arteriolar dilation via cyclooxygenase activation. Am. J. Physiol. Heart Circ. Physiol. 281, H2378–H2384.
76 Adachi, M., Taki, T., Higashiyama, M., Kohno, N., Inufusa, H. and Miyake, M. (2000) Significance of integrin alpha5 gene expression as a prognostic factor in node-negative non-small cell lung cancer. Clin. Cancer Res. 6, 96–101.
77 Kim, S., Bell, K., Mousa, S. A. and Varner, J. A. (2000) Regulation of angiogenesis *in vivo* by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. Am. J. Pathol. 156, 1345–1362.
78 Wickstrom, S. A., Alitalo, K. and Keski-Oja, J. (2002) Endostatin associates with integrin alpha5beta1 and caveolin-1 and activates Src via tyrosyl phosphatase-dependent pathway in human endothelial cells. Cancer Res. 62, 5580–5589.
79 Lu, Q., Navdaev, A., Clemetson, J. M. and Clemetson, K. J. (2005) Snake venom C-type lectins interacting with platelet receptors: structure-function relationships and effects on haemostasis. Toxicon 45, 1089–1098.
80 Drickamer, K. (1999) C-type lectin-like domains. Curr. Opin. Struct. Biol. 9, 585–590.
81 Clemetson, K. J., Lu, Q. and Clemetson J. M. (2005) Snake C-type lectin-like proteins and platelet receptors. Pathophysiol. Hemost. Thromb. 34, 150–155.
82 Fukuda, K., Doggett, T. A., Bankston, L. A., Cruz, M. A., Diacovo, T. G. and Liddington, R. C. (2002) Structural basis of von Willebrand factor activation by the snake toxin botrocetin. Structure 10, 943–950.
83 Navdaev, A., Dormann, D., Clemetson, J. M. and Clemetson, K. J. (2001) Echicetin, a GPIb-binding snake C-type lectin from *Echis carinatus*, also contains a binding site for IgMκ responsible for platelet agglutination in plasma and inducing signal transduction. Blood 97, 2333–2341.
84 Polgar, J., Clemetson, J. M., Kehrel, B. E., Wiedemann, M., Magnenat, E. M., Wells, T. N. and Clemetson, K. J. (1997) Platelet activation and signal transduction by convulxin, a C-type lectin from *Crotalus durissus terrificus* (tropical rattlesnake) venom via the p62/GPVI collagen receptor. J. Biol. Chem. 272, 13576–13583.
85 Horii, K., Okuda, D., Morita, T. and Mizuno, H. (2004) Crystal structure of EMS16 in complex with the integrin α_2-I domain. J. Mol. Biol. 341, 519–527.
86 Chung, C. H., Peng, H. C. and Huang, T. F. (2001) Aggretin, a C-type lectin protein, induces platelet aggregation via integrin alpha(2)beta(1) and GPIb in a phosphatidylinositol 3-kinase independent pathway. Biochem. Biophys. Res. Commun. 285, 689–695.
87 Morita, T. (2005) Structures and functions of snake venom CLPs (C-type lectin-like proteins) with anticoagulant-, procoagulant-, and platelet-modulating activities. Toxicon 45, 1099–1114.
88 Bourne, Y., Talley, T. T., Hansen, S. B., Taylor, P. and Marchot, P. (2005) Crystal structure of a Cbtx-AChBP complex reveals essential interactions between snake alpha-neurotoxins and nicotinic receptors. EMBO J. 24, 1512–1522.
89 Samson, A., Scherf, T., Eisenstein, M., Chill, J. and Anglister, J. (2002) The mechanism for acetylcholine receptor inhibition by alpha-neurotoxins and species-specific resistance to α-bungarotoxin revealed by NMR. Neuron 35, 319–332.
90 Natesh, R., Schwager, S. L. U., Evans, H. R., Sturrock, E. D. and Acharya, K. R. (2004) Structural details on the binding of antihypertensive drugs captopril and enalaprilat to human testicular angiotensin I-converting enzyme. Biochemistry 43, 8718.
91 Lee, S.-C., Guan, H.-H., Wang, C.-H., Huang, W.-N., Tjong, S.-C., Chen, C.-J. and Wu, W.-G. (2005) Structural basis of citrate-dependent and heparan sulfate-mediated cell surface retention of cobra cardiotoxin A3. J. Biol. Chem. 280, 9567–9577.
92 Jabeen, T., Singh, N., Singh, R. K., Sharma, S., Somvanshi, R. K., Dey, S. and Singh, T. P. (2005) Non-steroidal anti-inflammatory drugs as potent inhibitors of phospholipase A(2): structure of the complex of phospholipase A(2) with niflumic

acid at 2.5 Å resolution. Acta Crystallogr. Sect. D. 61, 1579–1586.

93 Murakami, M. T. and Arni, R. K. (2005) Thrombomodulin-independent activation of protein C and specificity of hemostatically active snake venom serine proteinases: crystal structures of native and inhibited A*gkistrodon contortrix* *contortrix* protein C activator. J. Biol. Chem. 280, 39309–39315.

94 Horii, K., Okuda, D., Morita, T. and Mizuno, H. (2003) Structural characterization of EMS16, an Antagonist of collagen receptor (GPIa/IIa) from the venom of *Echis multisquamatus*. Biochemistry 42, 12497–12502.


To access this journal online:
http://www.birkhauser.ch