Venom and antivenom

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ABSTRACT
The fauna of Saudi Arabia comprises a large number of venomous species of snakes, fish, scorpions, insects, coelenterates and molluscs. The venoms of these animals show a remarkable diversity of actions on human tissues. Neurotoxins (for example, from the Arabian cobra Naja haje arabica) interrupt transmission at peripheral neuromuscular junctions or (for example scorpion neurotoxins) stimulate voltage sensitive synaptic sodium and potassium channels with release of acetylcholine and catecholamines. The venoms of Red Sea carnivorous marine snails (genus Conus) contain "conotoxins" which have effects on voltage sensitive calcium and sodium channels, acetylcholine receptors, the vasopressin receptor and N-methyl-D-aspartate receptors. Of particular interest are sarafotoxins from the venom of the burrowing asp (Atractaspis engaddensis, Atractaspidae), which cause coronary artery vasoconstriction and atrioventricular block. They are homologous with human endogenous endothelins. Human victims of bites by this species may die very rapidly after developing anaphylactic/autonomic symptoms, shock and atrioventricular conduction abnormalities.

Improved ovine Fab fragment antivenoms are being developed for treatment of envenoming by Saudi Arabian snakes and scorpions. These Fab antivenoms have the pharmacological advantage of more rapid tissue distribution, a larger apparent volume of distribution and less risk of Fc or aggregate-induced complement activation than conventional F(ab')2 antivenoms.

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Venoms are noxious substances secreted by specialized glands in some animals that are actively injected through or squirted onto the skin or absorbent mucous membranes (such as the conjunctivae) of the animals' prey or enemies. The venom injecting apparatus varies in sophistication from crude, ungrooved teeth, in the case of some colubrid snakes, shrews and vampire bats, to the cannulated fangs of some venomous snakes. Venoms also vary in their complexity from single small molecules (for example hydrogen cyanide and methoxybenzoquinones in the secretions of millipedes - Diploda, and the aliphatic hydrocarbons of ants - Formicidae) to snake venoms, which are mixtures of 20 or more constituents - enzymes, non-enzymatic proteins, polypeptide toxins, oligopeptides, amino acids, carbohydrates (as glyco-protein), biogenic amines such as 5-hydroxytryptamine, metals and non-metals.

The biological function of venom is to immobilize and digest relatively large and vigorous prey species. Some snakes (spitting cobras of Africa and Asia, genus Naja; South African rinkhals - Hemachatus), toads, scorpions and other arthropods squirt or "spit" their venom defensively into the eyes of aggressors. In "bloodsucking" species such as vampire bats, ticks and leeches, venomous saliva promotes bleeding. Some animals use their venom in defence and in fights with other members of the same species. Immobilization of the prey is achieved by venom components which cause paralysis and pathophysiological processes leading rapidly to unconsciousness and death, such as the massive intravascular coagulation caused by snake venom procoagulant enzymes, circulatory collapse by many different mechanisms and even severe pain and fear.

Complex venoms, such as those of snakes, often show a wide range of lethal effects ("overkill") as well as synergism between different components. Thus, elapid snake venoms may contain several different neurotoxins active against a variety of tissue receptors (acceptors) and many viper venoms contain components acting on the hemostatic system as well as causing shock (fall in blood pressure) by different mechanisms. Distinctive "warning" coloration or markings of venomous species may have a protective value, as shown by the evolutionary selection of non-venomous species with an almost identical appearance. Remarkable examples of this Batesian mimicry are Sinnophis rhinostoma and the Latin American coral snake, Micrurus frontalis; and Dryocalamus davisonii and the Malayan krait, Bungarus candidus.

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The exercise of evolutionary pressures over millions of years has resulted in an enormous diversity of animal toxins that are specifically agonistic/antagonistic to physiological receptors. As a result, venoms have proved to be powerful tools and reagents for investigating physiological functions such as blood coagulation, neuromuscular transmission, central nervous system function, blood pressure control and the complement system.

Uses of venoms in science and medicine.
Snake venoms and the control of blood pressure.

Rocha e Silva and his colleagues in Brazil discovered bradykinin while studying in dogs the physiological action of the venom of a locally important pit viper, the jararaca (Bothrops jararaca) (Fig. 1).1 Bradykinin, a nonapeptide, was released from its α-globulin precursor (bradykininogen) in the dog’s blood by a venom component, causing a fall in blood pressure and a delayed (hence brady-) contraction of isolated guinea pig ileum. Jararaca venom also contains a number of peptides (bradykinin-potentiating factors) which inhibit both kininase II, responsible for inactivating bradykinin, and angiotensin-converting enzyme (ACE).1,2 Synthetic ACE inhibitors, modelled on the structure of the B jararaca venom component, have become an important new class of hypotensive agents (including captopril, cilazapril, enalapril, oxisopril, lisinopril etc.). The development of the ACE inhibitors is a striking justification for the study of animal venoms. “The discovery of potent inhibitors of converting enzyme provides one of many illustrations of the utility of seemingly esoteric pharmacological enquiry on the properties of poisons of plant or animal origin”.1

Snake venoms and the function of endothelins.

The burrowing asps, also known as burrowing or mole vipers/adders or stiletto snakes, belong to the genus Atractaspis of the family Atractaspidae. This genus is represented in Saudi Arabia by A microlepidota (see below).3 Rapid fatalities have been described following bites by several species of Atractaspis in Africa and the Middle East.4 Some of the victims developed profound cardiovascular disturbances, including atrioventricular conduction defects and hypotension. In the past few years, the venom of the Israeli burrowing asp (A engaddensis) has been found to contain four 21-aminoacid isotoxins, named sarafotoxins (SRTXa-d). These toxins show approximately 60% sequence homology with the endothelins which are potent vasoconstrictor peptides from vascular endothelium (Fig. 2).5,6 Their actions include coronary artery vasoconstriction, atrioventricular block and a positive inotropic action on the heart. The close similarity in structure and function of the sarafotoxins and endothelins suggest a common evolutionary origin. Endothelins and sarafotoxins bind to different receptors in the brain, atrium and smooth muscle, causing hydrolysis of phosphoinositides and immobilization of intracellular calcium ions. Competitive binding studies with labelled endothelins and sarafotoxins have allowed the identification of endothelin receptor subtypes.

Snake venoms and the investigation of hemostasis.

Felice Fontana, in the 1760s, was one of the first to report the paradoxical effects of snake venoms on blood coagulability; large doses of viper venom increased coagulability and yet the blood of animals dying of viper bite was incoagulable. Early work on Russell’s viper (Daboia russellii) venom in India at the turn of the century demonstrated its ability to clot citrated plasma, to cause intravascular thrombosis in animals and, in survivors, to induce a state of blood incoagulability.7,8 These observations were pursued by Gwyn MacFarlane in 1934 when, in collaboration with Burgess Barnett the Curator of Reptiles at the London Zoo, he studied the coagulant properties of the venom of a large Russell’s viper, probably from Thailand (Fig. 3).9,10 The venom, at a dilution of 1 in a million, clotted hemophilic blood in less than a minute. It was first used as a local hemostatic in a hemophilic patient in 1934 and was later marketed by Burroughs Wellcome as “Stypven”. MacFarlane’s work, employing the procoagulant enzymes from Russell’s viper venom, contributed to the elucidation of the blood clotting cascade.9 Many of these enzymes and other venom components which affect other clotting factors, fibrinolysis and platelet function, are currently used in experimental and clinical hemostasis laboratories.11 Batroxobin (“Reptilase”) from the venom of Brazilian pit vipers (Bothrops atrox and B moojeni) is a fibrinogen-clotting enzyme, used to monitor the fibrinogen-fibrin reaction in patients being treated with heparin and other protase inhibitors such as hirudin, e-aminocaproic acid and aprotinin. Russell’s viper venom (“Stypven” RVV) contains a Factor X activator, employed in the “Stypven time” to distinguish Factor VII and Factor X deficiencies and for characterizing Factor X dysproteinemias. It is also used in Factor X assays and as a test for platelet phospholipid availability (platelet procoagulant activity). The dilute Russell’s viper venom time detects lupus anticoagulant and antiphospholipid coagulation inhibitor. Assays employing other snake venoms, such as those of the eastern brown snake (Pseudonaja textilis) and Taipan (Oxyuranus scutellatus) have also been used for the detection of lupus anticoagulant. A standard assay in patients with thrombophilia, employs a specific protein C activator (Protac C) from the venom of the southern copperhead snake (Agkistrodon contortrix).12

Platelet aggregating and inhibiting factors.11

Many snake venoms contain components which either induce platelet aggregation (for example crotalcytin from the venom of the timber rattlesnake, Crotalus horridus) or inhibition (such as bitistatin from the venom of the puff adder, Bitis arietans). Bothocetin (from Bothrops venoms) is used, together with ristocetin, to distinguish the molecular variants of von Willebrand’s disease. Fibrinogen receptor antagonists such as echistatin from the venom of the saw-scaled viper (Echis species), trigamin from the venom of the Taiwanese bamboo vipers (Trimeresurus stejnegeri) and kistrin from the venom of the Malayan pit vipers (Calloselasma rhodostoma) are of special interest because of their potential therapeutic use. They possess an Arg-Gly-Asp (RGD) sequence which allows them to recognize specific integrin subtypes.
Table 1 - Neurotoxic peptides found in the venom of the marine snail, *Conus geographus*.18

<table>
<thead>
<tr>
<th>Class</th>
<th>Mode of action</th>
<th>No. isolated</th>
<th>No. of amino-acids in peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Conotoxin</td>
<td>Inhibits acetylcholine receptors</td>
<td>3</td>
<td>13-15</td>
</tr>
<tr>
<td>μ-Conotoxin</td>
<td>Blocks muscle sodium channel</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>ω-Conotoxin</td>
<td>Blocks neuronal calcium channel (analgesic SNX-III)</td>
<td>5</td>
<td>27-30</td>
</tr>
<tr>
<td>Conantokin</td>
<td>Inhibits glutamate receptors of the N-Methyl-D-aspartate subtype</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Conopressin</td>
<td>Agonist of vasopressin receptor</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Others</td>
<td>Excitotoxins, α-adrenergic and cholinomimetic toxins</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

glycoprotein GPIIb-IIIa, blocking the interaction of platelets with adhesive proteins such as fibrinogen and inhibiting platelet aggregation.

*Treatment of thrombosis and thrombotic states with venom enzymes.*

**Snake venom procoagulant enzymes.**

Ancrod and batroxobin are fibrinogen-clotting enzymes which have been used to induce therapeutic defibrinogenation. This prevents thrombus formation or the extension of pre-existing thrombi, promotes fibrinolysis and, by reducing blood viscosity, improves blood flow through diseased and narrowed vessels. Snake venom procoagulants cause fewer hemorrhagic, thromboembolic and allergic effects than heparin or streptokinase and their effects can be rapidly reversed by the use of specific antivenoms.13,14 However, they are more expensive than heparin or streptokinase, are less effective than streptokinase and no more effective than heparin. Antibody formation can be expected to cause resistance to treatment during prolonged therapy. Ancrod or batroxobin are indicated in patients in whom heparin is contraindicated, such as those with heparin-induced thrombocytopenia and thrombosis, those with heparin antibodies and those with protamine hypersensitivity in whom anticoagulation cannot be rapidly reversed using this drug. In heparin-induced thrombosis, low molecular weight heparin or hirudin is now preferred by some authorities. Efficacy of ancrod or batroxobin has also been claimed in acute or progressive ischemic cerebral infarction, thrombotic lupus glomerulonephritis, thrombo-angiitis obliterans, central retinal vein thrombosis, priapism and hyperviscosity syndrome.14,15

**Therapeutic agents from leech venoms.**

Hirudin is a direct thrombin and factor IXa inhibitor, independent of antithrombin III, from the saliva of the medicinal leech, *Hirudo medicinalis*.16 Recombinant hirudin has proved superior to heparin in the treatment of post-operative thromboembolism and acute coronary syndromes. Other leech toxins of medical interest are hementin from *Hementeria ghilianii* which is directly fibrinolytic, and hementerin from *H. depressa*, a plasminogen activator.

**Venom neurotoxins in experimental neurophysiology and neuropharmacology.**

Venom neurotoxins have been identified which are agonists or antagonists of almost every known class of receptor/ion channel in the excitable tissues of animals.

**Snake venom neurotoxins.**

Post-synaptic toxins (α neurotoxins) bind to the α component of the nicotinic acetylcholine receptors of vertebrate neuromuscular junctions. They are polypeptides consisting of either 60-62 (short) or 70-74 (long) amino-acid residues. α neurotoxins are found in the venoms of two Saudi Arabian species - *Naja haje arabica* and *Walterinnesia aegyptia*, but the best known example of this class of toxin is α-bungarotoxin from the venom of the Chinese krait (*Bungarbus multicinctus*). This toxin has been used to study the structure and function of the mammalian neuromuscular junction. β-bungarotoxins, such as κ-flavotoxin from the venom of the red-headed krait (*B. flaviceps*), bind to neuronal nicotinic acid receptors. The venoms of the African mambas (genus *Dendroaspis*) contain some unusual pre-synaptic neurotoxins: the dendrotoxins, which facilitate acetylcholine release by blocking voltage dependent potassium channels, fasciculins which inhibit cholinesterases and muscarinic toxins.17 Phospholipase A2 toxins are pre-synaptic in action, initially releasing acetylcholine from the nerve terminals and then damaging nerve and muscle, inhibiting neuromuscular transmission. These β-neurotoxins occur in the venoms of some cobras, kraits (for example, β-bungarotoxin from the venom of *B. multicinctus*), Australasian elapid snakes (for example, taipoxin from the venom of the taipan, *Oxyuranus scutellatus*) and vipers (for example, crototoxin from the venom of the tropical ratel snake, *Crotalus durissus terrificus*).

**Invertebrate neurotoxins.**

Neurotoxins from the venoms of scorpions and spiders are polypeptides which bind to sodium, potassium and calcium channels.

**Table 2 - Efficacy of antivenom: changes in the case fatality of snake bite after the introduction of antivenom**

<table>
<thead>
<tr>
<th>Country</th>
<th>Species</th>
<th>Before</th>
<th>After</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td><em>Oxyuranus scutellatus</em></td>
<td>~100</td>
<td>&lt; 1%</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Death adders <em>Acanthophis sp</em></td>
<td>50</td>
<td>&lt; 1%</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Tiger snake <em>Notechis scutatus</em></td>
<td>45.5</td>
<td>&lt; 1%</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Eastern brown snake <em>Pseudonaja textilis</em></td>
<td>18.7</td>
<td>&lt; 1%</td>
<td>20</td>
</tr>
<tr>
<td>South America</td>
<td></td>
<td>8</td>
<td>2.4%</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Lance-headed vipers <em>Bothrops sp</em></td>
<td>74</td>
<td>12%</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Tropical ratel snake <em>Crotalus durissus terrificus</em></td>
<td>20</td>
<td>&lt; 3%</td>
<td>23</td>
</tr>
</tbody>
</table>

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Fig. 1 - Jararaca (*Bothrops jararaca*). The Brazilian lance-headed viper. The prototype ACE inhibitor was identified in its venom.1,2

Fig. 4 - Enzyme refinement of antivenoms.

calcium channels of excitable tissues, causing release of neurotransmitters such as acetylcholine and catecholamines.

Conotoxins.

More than 500 species of carnivorous marine snails, the coneshells (genus Conus, class Gastropoda, Phylum Mollusca) occur from Hawaii and Australia across the Pacific and Indian Oceans and the Red Sea to East Africa. They immobilize their prey, polychaete worms, other gastropods, pelecypods, octopuses and small fishes, by injecting venom by means of a detachable dart-like radular tooth which is implanted by the harpoon-like proboscis. The rapidly acting conotoxins are peptides, 9-30 amino acids in length, which have a wide range of neuromuscular targets (Table 1).18 The α-conotoxins block neuronal calcium channels. Some of them have analgesic properties which have led to the production of a synthetic α-conotoxin, SNX-III, which is undergoing trials in patients with malignant disease who are suffering intractable pain.

Animal venoms are rich sources of enzymes for biochemists (for example phospholipases, endopeptidases and L-aminooxidases). Cobra venom factor, a C3b-like protein found in elapid venoms, activates the alternative complement pathway by binding to Factor B and has been used to explore the complement system and to depress complement activity in patients with immunological diseases.

Fig. 2 - Comparison of sarafotoxins (from the venom of the burrowing asp, *Acarasps enigmatis*) and endoelatis, showing 60% sequence homology.3

Antivenom.

Antivenoms and antitoxins, made by immunizing animals such as horses, mules and sheep, with particular venoms, have been used to treat victims of snake bite for over 100 years. They remain the only specific antidotes for the treatment of envenoming. Until quite recently, completely unrefined hyperimmune horse serum/plasma was still used in some countries, but, increasingly since the 1930s, most manufacturers have concentrated the immunoglobulin (IgG) fraction by ammonium sulphate precipitation and have removed the opsonizing complement activating Fc fragment by pepsin digestion (Fig. 2). Antivenoms are now available for the treatment of bites and stings by snakes, fish, box jellyfish, spiders, scorpions and ticks.

Although the first formal randomized comparative clinical trial of antivenom was not carried out until 1973,24
evidence has now accumulated that antivenoms can reduce the case fatality of snake bite (Table 2) and reverse anti-hemostatic, cardiovascular, post-synaptic, neurotoxic and rhabdomyolytic effects of snake venoms. Whether local tissue necrosis, pre-synaptic toxicity and nephrotoxicity can be prevented by antivenom, remains controversial.25

The standard methods by which antivenom manufacturers assess the efficacy of their products do not seem to be appropriate. Venom and antivenom are incubated together before being injected into a rodent. This method will demonstrate neutralization of the venom in vitro, but ignores the inevitable delay between envenoming and administration of antivenom, diffusion barriers between antivenom and toxins which have been distributed into the tissues and the possibility of reversal of envenoming after toxins have been bound to their tissue acceptors. Other problems associated with antivenoms are their high cost and consequently their limited supply in developing countries, their distribution to rural areas of countries where snake bite tends to be most frequent and their limited shelf life. Conventional antivenoms carry a considerable risk of inducing early anaphylactoid, pyrogenic and late serum sickness type reactions which are potentially fatal. It is now clear that the vast majority of these reactions are not strictly "allergic" or "hypersensitivity reactions" as they are not predicted by skin tests and do not result from prior sensitization to the same foreign protein. The mechanism of most early anaphylactoid reactions to antivenoms is probably complement activation by IgG aggregates or the presence of residual Fc fragments.

In an attempt to improve the efficacy and safety of antivenoms, one manufacturer is now using sheep rather than horses as they produce higher and more persistent antibody titers. Instead of conventional peptic digestion of concentrated IgG to produce F(ab)2 fragments, papain is used to yield Fab fragments (Fig. 4). Specific Fab can be isolated by passing the total Fab down columns coated with venom antigens. Residual Fc fragments can be removed by simple ion exchange. The first antivenoms produced in this way have been raised against venoms of the European adder (Vipera berus),26 North American rattlesnakes,27 the Nigerian saw-scaled viper (Echis ocellatus)28-29 and the Sri Lankan Russell's viper (Daboia russelli). Theoretical advantages of Fab, compared to F (ab)2, antivenoms are their more rapid tissue penetration and larger apparent volume of distribution. Disadvantages include more rapid clearance, requiring repeated doses, and lower binding capacity. Fab fragments have proved effective in treating drug overdose with digoxin and colchicine and in neutralizing tumor necrosis factor-α in acute inflammatory states.30

**Venomous bites and stings in Saudi Arabia.**

Saudi Arabia and its adjacent seas, the Arabian Gulf and the Red Sea, are home to at least 10 species of terrestrial venomous snake, as many species of sea snake, about 14 species of scorpion and a variety of coleterates (corals, sea wasps), molluscs (coneshells) and fish (sting rays, zebra fish and stonefish). The clinical aspects of bites and stings by these animals were reviewed recently.31

Considerable taxonomic confusion surrounds two genera of Saudi Arabian venomous snakes. The Israeli burrowing asp (*Atractaspis engaddensis*) which is distributed in Israel and Sinai may be conspecific with *A. micrlepidota andersoni* of Saudi Arabia.32 This is of pure academic interest as the venom of *A. engaddensis* contains potent cardiovascular toxins, the sarafotoxins (see above). No specific antivenom for treatment of *Atractaspis* envenoming is commercially available. The speed of development of severe and fatal envenoming in reported cases suggests that effective first aid treatment, such as pressure immobilization to delay systemic uptake of the venom, may be crucial.

It is generally recognised that saw-scaled vipers (*Echis*) are responsible for most cases of severe and fatal envenoming in Saudi Arabia. Cherlin's classification recognises 12 full species, four of which occur in the Arabian Peninsula.33 The composition and antigenicity of *Echis* venoms varies, both between species and within a single species throughout its geographical range.34 It is important that *Echis* species in this region should be defined morphologically so that they can be reliably identified as a source of venom for antivenom production. Pooled venom from specimens of these species collected throughout the geographical range should be used for immunization.

A regional polyspecific antivenom is needed for the Arabian Peninsula to cover venoms of the relevant species of *Echis*, *Cerastes*, *Naja haje arabica*, *Walterinnesia aegyptia* and *Atractaspis*. To ensure optimal specificity, the venoms used for production of this new antivenom should be obtained from specimens caught in the region.

**Scorpion envenoming.**

The treatment of scorpion envenoming remains controversial.35 Many clinicians and pharmacologists are now convinced that antivenom is effective, especially if given early after the sting, before the development of profound cardiovascular complications.36,37 However, some authorities maintain that only ancillary pharmacological agents such as α-1 blockers (prazosin) or other vasodilators are effective. Well designed clinical trials are needed. The pharmacokinetics of Fab fragment antivenoms make them particularly promising for the treatment of scorpion envenoming in view of the rapid distribution and tissue binding of scorpion toxins.36

**References**


