The composition and actions of Papua New Guinean snake venoms

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Introduction

Snake venoms are complex mixtures of bioactive agents with diverse pharmacological activities against a wide range of physiological targets. Many of these agents are complex chemicals which have toxic effects upon the cells and cellular mechanisms that they target, and in some snakes the toxicity is sufficient to be extremely harmful to man. Understanding the composition and activities of different snake venoms forearms clinicians with a knowledge of the underlying physiological changes responsible for clinical envenomation syndromes. In some cases this knowledge may enable presumptive identification of the biting species and early selection of the most appropriate antivenom.

The biological roles of snake venoms

There are three natural uses for snake venom. The most obvious is the role of venom in subduing or killing other animals that the snake wants to eat. Snakes with toxic venom evolved in order to limit their exposure to the dangers that their own prey might present to them. If we consider that snakes already have a natural disadvantage through the lack of limbs with which to move, or to use for catching food, then the development of a venomous bite is a logical advantage. By quickly biting and injecting toxins into a prey animal, and then keeping a safe distance until the prey is immobilized or dead, snakes reduce the possibility that a prey animal (such as a large bush rat) might turn on them and use its own teeth to damage or kill the snake.

The second very important role of many types of venom is as an aid to the actual digestion of the prey that the snake kills and eats. Many of the toxins in snake venom are powerful hydrolysing agents that break down cellular tissue into more basic, easily absorbed and utilized nutrients. Snake venom myotoxins that break down muscle cells are one example of the types of toxin that can assist in the pre-digestion of food.

The role that brings snakes into so much conflict with humans, however, is the use of venom as a defensive tool against a snake's perceived enemies, and as a means of ensuring that the snake does not end up being eaten itself. Most snakes do not like to waste venom on their enemies, and this is the reason that its use is often reserved only for those occasions when the snake feels the most threatened or intimidated. Many snakes bite, but actually inject very little if any venom when they are defending themselves, and this is the reason that many people who are bitten by snakes do not become seriously ill.

The amount of venom that a snake uses when it bites is often determined by the reason for its use, and by other factors such as the temperament of the snake itself – some species are more

easily intimidated than others, and these species are most likely to inject venom when biting a potential enemy. Snakes that are hunting for food when they bite also tend to inject much more venom, than a snake that is defensive.

The difference between 'venoms' and 'poisons'

There are no 'poisonous' snakes, but many snakes are 'venomous'. The difference between being a 'venomous' snake as opposed to a 'poisonous' snake is related to the nature of venoms and poisons themselves.

Venoms are produced exclusively by animals, including spiders, scorpions, snakes, stingrays, stonefish, jellyfish, marine cone snails and invertebrates like ticks and centipedes. Venom is manufactured in specialized glands in the animal's body, and each animal has a specific mechanism for delivering the venom into the body of the animal it is used against. In the case of snakes, spiders, ticks and centipedes this mechanism is through biting, while stingrays and stonefish used special spines on their bodies to inject venom as a form of defence. Marine cone snails produce tiny spear-like teeth that they fire at their prey from their mouths, and jellyfish have special cells, called nematocysts that evert a long sharp tube into the skin of prey through which venom is injected.

Poisons on the other hand are passively acquired toxins that are produced by either animals or plants as a means of defence. These organisms do not have an active mechanism through which the poison is delivered. Many species of frogs defend themselves against enemies and predators by producing poison in glands on or under the skin; if they are picked up or eaten, the poison comes in contact with the enemy and may either kill it (preventing it from eating any more frogs), or taste so unpleasant that the animal spits out the frog and learns never to try and eat the same type of frog again. Poisonous plants use the same type of principle. The toxins produce an unpleasant experience for the animal eating them, and results in the plant being avoided and left alone.

Venoms are typically mixtures of proteins or peptides, while poisons may be much more diverse types of chemicals including steroids, alkaloids, amines and other substances.

Snake venom evolution and diversity

Not all snake venoms are the same. Although it is believed that the venoms of all venomous snakes evolved from common biological ancestors many millions of years ago, the reality today is that nearly every species of snake has different venom, with different types of components and different types of activity against different types of cellular targets.

This is a very important concept to understand when treating snakebites. Different venoms produce different clinical effects, and may require different treatments, including different types of antivenoms.

The vast majority of the world's 3,000 or so species of snakes belong to an evolutionary superfamily known as the Colubroidea, which includes all of the venomous species. Some of these snakes evolved specialized forms of saliva that served as aids to the digestion of food, and over time the glands producing this saliva diversified and developed further. Through a process of positive selection, proteins and peptides with specific toxic activity were conserved and retained. When these early snakes bit their prey the wounds made by their teeth allowed these toxic chemicals in their saliva to enter the bodies of the prey, speeding death and assisting in the breakdown of the food. This gave these snakes an important biological advantage that helped them to survive and colonize different environments.

Evolution also recognised that snakes with larger teeth made larger wounds in their prey, allowing more of the toxic saliva to enter, resulting in quick effects and even more success at obtaining food. Over millions of years three different large groups of snakes evolved very similar (but structurally different) teeth ('*fangs*') and jawbones that aided the injection of toxins. As we discussed in the previous chapter, the most primitive of these groups evolved fangs at the rear of the mouth that allowed venom to seep into a wounded prey animal along slight grooves; more advanced snakes had fangs at the front of the mouth, and the most advanced evolved very large fangs that folded against the roof of the mouth when not in use – but which rotated forwards to enable downward stabbing bites that injected venom deep into the prey for maximum digestive effect.

The process of evolution means that while most snakes started their development of venom with an arsenal of common components (many toxins can today be used to study the evolutionary relationships of different snakes), over the millions of years in which the species have developed and evolved into those that we see today, their venoms have also diversified and evolved. Studies of the toxic saliva found in some of the species of colubrid snake that we traditionally assumed to have been non-venomous have actually found that these snakes possess toxins that are remarkably like the very potent neurotoxins present in some sea snakes and many of the highly venomous land snakes, and this information tells us a lot about the origins of venom.

Many factors influence venom evolution and diversity. The diet of a snake is one important factor. A species that feeds on mammals is likely to have venom that acts more effectively against mammals than against fish or frogs. Another factor is the ability of the prey to defend itself against the snake. If the prey animal is potentially dangerous to the snake, there is a much greater chance of this species having extremely potent venom that acts quickly and lethally, than would be the case if the prey animal was unable to mount an effective defence.

Activity of Papuan snake venoms

The venoms of the medically important Papua New Guinean snakes contain many different types of toxins that act against different physiological targets.

Despite this diversity it is possible to broadly classify many components on the basis of just a handful of medically important clinical effects. What is important to remember, however, is that these effects may be produced by different toxins using different mechanisms of action at the cellular level. This has very important implications for the treatment of snakebite.

The venom components of Papuan snakes include:

(1) ***Neurotoxins*** – active against the nervous system
- (a) Presynaptic neurotoxins
- (b) Postsynaptic neurotoxins

(2) ***Haemotoxins*** – affecting normal coagulation and haemostasis
- (a) Procoagulants
- (b) Anticoagulants
- (c) Platelet toxins

(3) ***Myotoxins*** – destructive enzymes that cause skeletal muscle degeneration

Snake venoms also contain toxins which produce a variety of less medically obvious effects.

Snake venom neurotoxins

Papua New Guinean snakes produce neurotoxicity using toxins that target both presynaptic and postsynaptic neuromuscular junction targets. Depending on the species of snake involved, there may be more than toxin in the venom targeting the neuromuscular junction, and these may involve more than one mechanism, all with the common purpose of producing paralysis.

In order to understand how these toxins work, it is important to remember how the interface between the motor nerves and the skeletal muscle system operates (FIGURE 1):

? Nerve impulses generated by the body in response to either voluntary (i.e.: *a conscious decision to lift an arm*) or involuntary (i.e.: *the unconscious commands to relax and contract the diaphragm and other chest muscles that produces respiratory effort*) commands result in the depolarisation of nerve membranes.

? Depolarisation involves sodium (Na^+) and potassium (K^+) ions changing places across the nerve membrane via ion transport channels.

? At the axolemma of the nerve terminal this depolarisation opens calcium ($Ca2^+$) channels which produce the stimulus for the release of neurotransmitter, typically acetylcholine (ACh), from internal nerve cell structures known as presynaptic vesicles.

? The vesicles fuse with the nerve cell membrane allowing the acetylcholine inside to diffuse into the synaptic cleft.

? Acetylcholine binds to special protein complexes in the adjacent muscle cell membrane that are known as acetylcholine receptors (AChR), and in effect act like a key in a lock, opening the receptors to allow extracellular Ca^{2+} into the cell and K^+ to leave.

? This in turn activates the internal muscle cell components producing activation of the fibres, resulting in either contraction or relaxation.

? With its job finished, ACh dissociates from the AChR and is bound by another substance known as acetylcholinesterase (AChE) which breaks ACh down into acetate and choline.

? Acetate and choline are taken back up by the nerve cell, reconstituted to form more ACh and used to fill more vesicles so that the cycle can be repeated with the next impulse.

Snake venom neurotoxins break this cycle via several different mechanisms that have the same result: the ability of acetylcholine (ACh) to trigger muscle cell activation is interrupted and the muscle becomes paralysed.

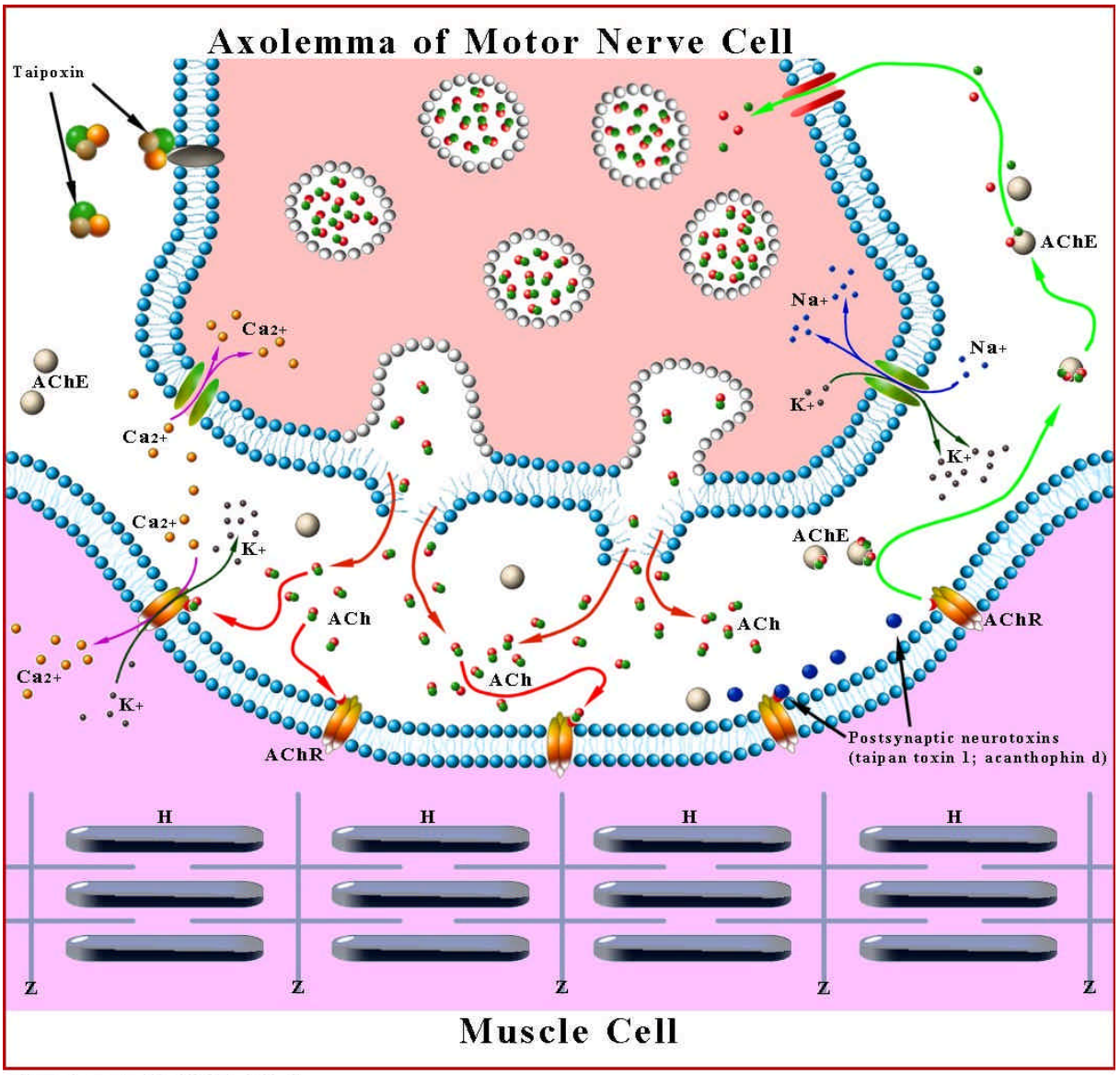
In Papua New Guinean snakes this is achieved by two main types of toxin that can be categorised simply according to the location of the site upon which they exert their effects:

? *Presynaptic* neurotoxins that directly target sites on the nerve cell; and,

? *Postsynaptic* neurotoxins that target the acetylcholine receptor (AChR) to prevent the binding of acetylcholine (ACh).

Some species such as the Papuan taipan (*Oxyuranus scutellatus canni*) have both types of neurotoxin in their venom, while others like death adders (*Acanthophis* spp.) possess only postsynaptic neurotoxins.

Although these neurotoxins all aim to produce the same result – paralysis of the victim, the means by which this occurs differs in different snakes and these different mechanisms mean that while the visible and detectable effects in the snakebite patient are *generally* the same, the type of treatment needed and the risks to patients can be *very* different. This is just one of the reasons it is so important to know how the venoms of different species work.



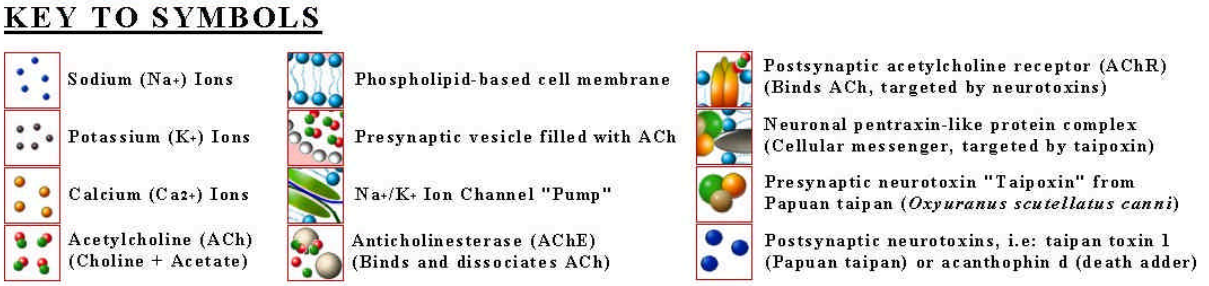


FIGURE 1: Representation of a neuromuscular junction showing normal ion transport and acetylcholine (neurotransmitter) dispersal, dissociation and reuptake. The target sites of some snake venom presynaptic and postsynaptic neurotoxins are also shown. ***Presynaptic***: The Papuan taipan (*Oxyuranus scutellatus canni*) toxin "taipoxin" is shown bound to presynaptic transmembrane protein complexes in the phospholipid cell wall of the neuronal axolemma; the consequence of binding is depletion of synaptic vesicles, cessation of acetylcholine release and physical destruction of the axolemma as a result of damage potentiated by massive Ca^{2+} influx into the neuron. ***Postsynaptic***: Long-chain neurotoxins such as taipan toxin 1 (Papuan taipan; *Oxyuranus scutellatus canni*) or acanthophin d (Papuan death adder; *Acanthophis* spp) compete with acetylcholine for postsynaptic receptor binding sites; neurotransmission is blocked by competition, but is easily reversed with specific antivenom and anticholinesterase drugs.

NOTE: These are not the only neurotoxins that act against these targets; there are other toxins in both the same species and in other snakes that target presynaptic and postsynaptic binding sites.

In Papuan New Guinean snakes with presynaptic neurotoxicity, such as the Papuan taipan (*Oxyuranus scutellatus canni*), the toxins belong to a class of very diverse proteins known as phospholipases A_2 (PLA_2) that under normal circumstances are responsible for a number of cellular activities including signal transduction and phospholipid metabolism through their ability to catalyse the 2-acyl ester bonds of 3-sn-phosphoglycerides. Not all PLA_2 are toxic, and many occur naturally in humans. Non-toxic human pancreatic PLA_2 are in fact very similar to extremely toxic snake venom PLA_2.

The venom PLA_2 are very small in size compared to some of the toxins that target haemostasis. In the Australo-papuan elapids these toxins are typically 13-15 kDa and are polypeptides comprised of 118-120 amino acids linked by 7 disulphide bonds that contribute to their three dimensional shape, and hence to their activity. These PLA_2 possess both toxic catalytic activity and non-toxic enzymatic activities at different locations within their structure; this means that they can often have more than one type of action. Some PLA_2 also bind to common receptors that may be located on both nerve cells and muscle cells. The result is that in addition to being neurotoxic, they are also powerful myotoxins which destroy muscle.

While many of the presynaptic PLA_2 neurotoxins are basic (alkaline) single polypeptide chain toxins, the most toxic are multi-chain proteins, such as the three subunit PLA_2 'taipoxin' from the venom of Australian coastal taipans (*Oxyuranus scutellatus scutellatus*) and Papuan taipans (*Oxyuranus scutellatus canni*), and 'paradoxin' from the inland taipan (*Oxyuranus microlepidotus*).

Postsynaptic neurotoxins that compete with ACh for AChR binding sites are a very diverse group that include some of the most primitive snake venom toxins. These very small polypeptides are usually 6-9 kDa in size with between 62-80 amino acids. Although some may be acidic, the majority are basic proteins. These postsynaptic neurotoxins typically target binding sites on the two a subunits that form the pentameric acetylcholine receptor (AChR), and this prevents ACh from binding, thereby inhibiting the activation of the ion channel and the exchange of Ca^{2+} and K^{+} that potentiates muscle contraction and relaxation.

Snake venom haemotoxins

The evolutionary recruitment of toxins that are active against haemostasis and coagulation is common in many of the world's snakes. The integrity of the circulatory system is crucial to the maintenance of life, and therefore presents itself as a logical target for venom-based toxins.

Haemostasis is a delicate balance between the two opposing forces of clot formation and dissolution. Appropriate timely clotting is essential at the site of a wound in order to maintain hemostasis and to prevent traumatic blood loss; however, the factors that produce clotting have to remain inactive away from the site of the wound in order to prevent life-threatening thrombotic events such as stroke. In a healthy person this balance is well maintained by a series of interconnected sequential reactions involving the proteins that initiate both clot formation and clot breakdown. Different snake venom toxins interfere with some of the specific biochemical reactions within coagulation pathways that are designed to ensure this balance (FIGURE 2), and the result is that they can produce both coagulopathy and thrombosis depending upon the mechanism that is activated or inhibited by the toxin.

From a Papua New Guinean perspective, the most important coagulation mechanism that is disrupted by snake venom toxins is the conversion of the zymogen, prothrombin, by activated Factor X (Factor X_a) to thrombin, which in turn drives the conversion of soluble fibrinogen to

insoluble fibrin – the necessary element, in conjunction with Factor $XIII_a$, for successful clot formation. Under normal conditions, this reaction is localized to the site of a vascular injury such as a break in a blood vessel wall, and is enhanced by the presence of activated platelets and the rate-increasing activated Factor V (Factor V_a). The result is the production of a stable cross-linked fibrin clot that stops the loss of blood, enabling the underlying vessel wall to heal and regenerate. As the wound heals, the body's fibrinolytic system converts plasminogen to plasmin via a fibrin bound tissue-type plasminogen-activating enzyme. Plasmin dissolves the fibrin clot, resulting in the production of fibrin degradation products – the clot remnants which are eventually completely reassimilated.

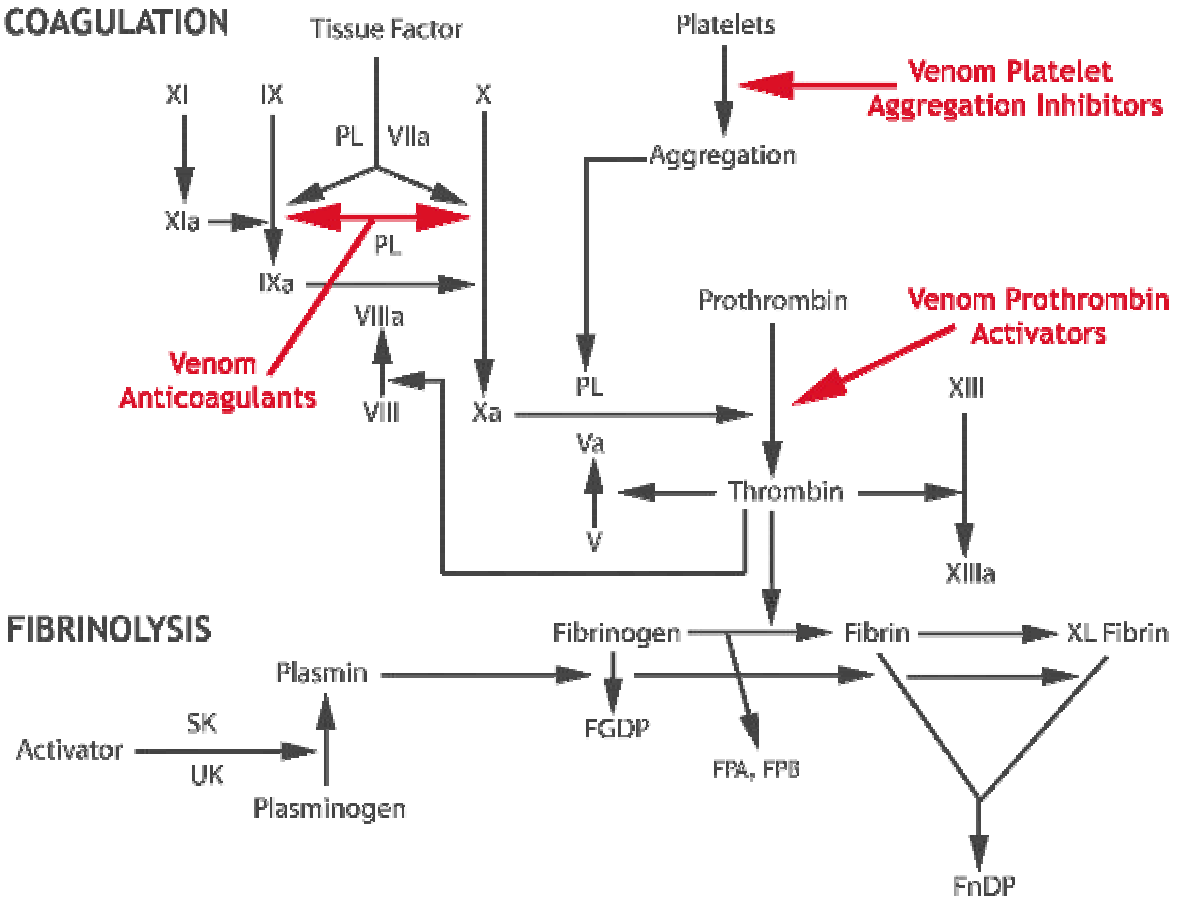


FIGURE 2: Simplified representation of coagulation and fibrinolytic pathways indicating the sites of action of the some important haemotoxic components in some Papua New Guinean snake venoms. Other toxins may also be present in PNG snake venoms that target additional reactions such as the conversion of plasminogen to plasmin, potentially inhibiting clot dissolution.

Procoagulants

There are four different classes of prothrombin-activating toxins that are defined by their need for circulating cofactors (i.e.: platelet phospholipids, Ca^{2+} and/or Factor V). These are not present in all snake venoms, however, and are notably absent from the venoms of Papua New Guinean death adders (*Acanthophis* spp.). The same is not true for the Papuan taipan (*Oxyuranus scutellatus canni*), whose venom contains the most medically significant toxin affecting coagulation in Papua New Guinean snakebite patients: a potent procoagulant that

converts prothrombin to thrombin in the presence of Ca^{2+} and circulating phospholipids without the need for Factor V (the toxins themselves contain a Factor V-like subunit). The consequence of abnormal prothrombin activation is a depletion of available fibrinogen resulting in consumption coagulopathy and incoagulable blood. Disseminated intravascular coagulopathy is the dominant clinical outcome and is the cause of the bleeding from mucosal membranes and venepuncture sites, prolonged clotting times and haematemesis.

Anticoagulants

The true anticoagulants bind Factor IX and Factor X and produce anticoagulation without concurrent fibrinolysis. These toxins are typically phospholipases A_2 and may also be involved in collagen-induced platelet aggregation. Bleeding may be a feature, but is usually not as significant as that seen following prothrombin activation by procoagulants.

Platelet Inhibitors & Activators

Platelets are abundant in blood and are small, discoid, phospholipid-rich entities with pivotal roles in maintenance of endothelial integrity and haemostasis. When blood vessel wall endothelium is damaged, platelets are immediately activated and will adhere and aggregate at the site of injury, effectively 'plugging the leak'. Platelets also stimulate circulating coagulation factors in plasma, and subsequently provide the phospholipid substrate for the cleavage of thrombin from prothrombin by Factor X_a and Factor V_a.

There are a number of toxins that act as either inhibitors or activators of platelet aggregation, a key process in the formation of normal clots. Platelet aggregation inhibition can be the result of either monophasic or biphasic mechanisms, with the latter involving initial arachidonic acid-induced platelet aggregation, followed by inhibition. In Papua New Guinean snakes, the inhibition of platelet aggregation is induced by PLA_2 toxins which also have anticoagulant effects.

Snake venom-induced myotoxicity

Many neurotoxic phospholipases A_2 are also potent myotoxins that are destructive to skeletal muscle tissue. Venom-induced myonecrosis, involving disruption of the plasma membrane and disorganization of the myofibrils, can result in rhabdomyolysis and myoglobinuria arising from elevated serum myoglobin levels, as tissue damage progresses. Affected muscle tissue may become heavily infiltrated with phagocytic cells. Oedema and muscle pain may be accompanied by gross elevation of serum creatine kinase, lactate dehydrogenase and or isoenzyme levels.

The destruction, while extensive, falls short of damaging basal lamina and myogenic stem cells in the muscle, and this enables muscle tissue regeneration, although the process may take several weeks. Patients affected by myotoxicity are at considerable risk of renal impairment due to tubular necrosis arising from the infiltration of myoglobin and other myolytic metabolites into the kidney nephrules.

Patients suffering from rhabdomyolysis frequently develop clinically evident myoglobinuria and will pass discoloured urine. In severe cases the urine will be deep brown in colour, and if a sample is allowed to stand it may take on a striated appearance, appearing 'stringy'. Many patients with myoglobinuria are mistakenly assumed to have haemoglobinuria, and it is important to distinguish between these two conditions as early as possible.

Venom Components of Medically Important Species

Papuan taipan *(Oxyuranus scutellatus canni)*

This species has the third most highly toxic snake venom in the world, with an experimental LD_{50} (lethal dose that kills 50% of test animals) of a mere 0.05 mg/kg body weight.

What makes Papuan taipans even more dangerous to humans is that they also have the most highly developed venom delivery system of any Australo-papuan snake; the fangs are well forward on a partially mobile maxilla, are extremely large compared to other elapids (up to 1.2 cm), and the venom yield of up to 600 mg is the highest of any Papuan snake. Taipans use a lightning-fast 'snap and release' biting strategy and may inflict multiple bites in rapid succession, with increasing quantities of venom being injected with each subsequent bite.

Combined with the relative abundance of the species throughout southern PNG in areas of high human population, and its nervous, excitable temperament, this large reptile is possibly the most dangerous snake in the world to encounter. In Central province it has been shown to cause more than 80% of serious snakebites.

Taipan venom is a complex mixture of many toxins; however, the clinical effects are probably dominated by just a few, and in particular:

? *Taipoxin*: a lethal phospholipase A_2 toxin with irreversible presynaptic neurotoxicity and myotoxicity.
? *OS-2*: a presynaptic inhibitor of K^+ transport that contributes to acetylcholine depletion by impairing vesicle recycling.
? *Taipan toxin 1*: a postsynaptic neurotoxin that competes with ACh for AChR binding sites on skeletal muscle cells, as well as several additional postsynaptic neurotoxins.
? *Oscutarin*: a powerful activator of prothrombin that produces rapid defibrination coagulopathy.
? *Taicatoxin*: a potent blocker of voltage-dependent myocardial Ca^{2+} channels and L-type Ca^{2+}-dependent neuronal K^+ channels in the brain.

Taipoxin, OS-2 and taipan-toxin 1

The severe neurotoxicity seen in taipan bite victims is a consequence of the destructive, irreversible neurotoxicity caused by taipoxin, in conjunction with additional neurotransmitter inhibition by taipan toxin 1 at the postsynaptic junction and the inhibition of acetylcholine vesicle recycling by OS-2.

Taipoxin is a multi-subunit neurotoxin that has an LD_{50} of a just 2µg/kg (in other words; only $1/500^{th}$ of 1 mg for each kg of the victim's body weight!), and the toxin may comprise more than 17% of the total protein in the venom. Taipoxin has a molecular weight of 45.6 kDa and binds to the neuronal integral membrane proteins NPR, NP1, NP2 and TCBP49. These are believed to form a novel pathway for the uptake of taipoxin into the synapse. The toxin consists of three polypeptide components:

? a neurotoxic basic a-subunit of 119 amino acids with an LD_{50} of 300 µg/kg;
? a neutral ß-subunit of 118 amino acids that lacks both toxicity and enzymatic activity;
? a 133 amino acid acidic ?-subunit with 133 amino acid that enhances the toxicity of the a-subunit and is believed to act as both a chaperone and an orientating partner, ensuring that the toxin is presented properly to its target receptor.

Perhaps the most important difference between taipoxin and other snake venom neurotoxins in Papua New Guinean species is that taipoxin does not only prevent the transmission of nerve impulses across the neuromuscular junction, but, more importantly, produces catastrophic physical destruction of nerve terminals.

The consequence of this is that once taipoxin has bound to the axolemma of the nerve terminal, the subsequent physical damage *cannot be treated with either antivenom or other drug therapies*.

For a patient whose nervous system has been physically damaged in this way the prognosis for survival is greatly reduced, and while the use of mechanical ventilation is of value in maintaining respiration while the nerve terminals recover and regenerate, there are numerous potential problems that can hinder recovery.

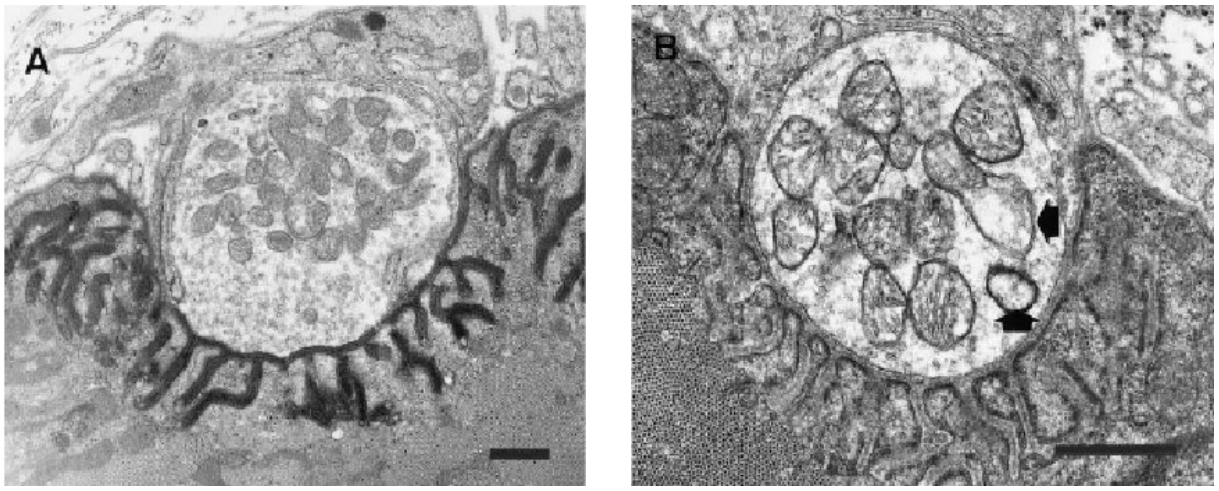


FIGURE 3: Damage to nerve terminals caused by exposure to the presynaptic snake venom neurotoxin taipoxin from the venom of the Papuan taipan (*Oxyuranus scutellatus canni*). **(A)** Normal neuromuscular junction on rat soleus muscle prior to exposure to taipoxin. **(B)** Rat soleus muscle neuromuscular junction one hour after the subcutaneous injection of 2 µg of taipoxin into the anterolateral aspect of the rat hind limb. The large arrows show the loss of cristae and depleted presynaptic vesicles within the axolemma of the nerve terminal.

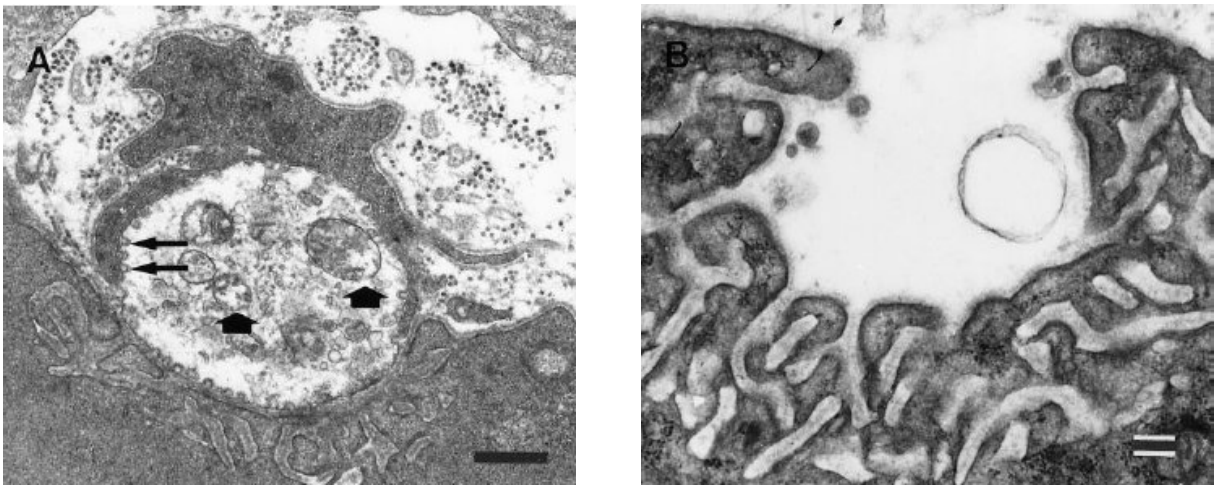


FIGURE 4: Damage to nerve terminals caused by exposure to the presynaptic snake venom neurotoxin taipoxin from the venom of the Papuan taipan (*Oxyuranus scutellatus canni*). **(A)** Rat soleus muscle neuromuscular junction 24 hours after exposure to taipoxin. Narrow arrows show the characteristic clathrin-coated ? shaped indentations on the nerve terminal membrane, while the larger arrows show damaged mitochondria. **(B)** The result is complete destruction of the nerve terminal.

SOURCE: HARRIS *et al* (2000)

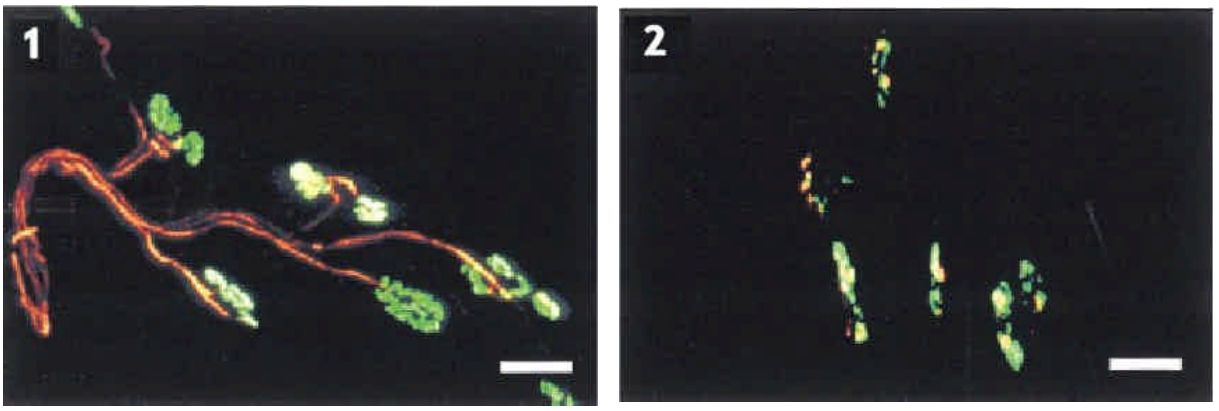


FIGURE 5: Nerve terminal damage caused by taipoxin, a presynaptic neurotoxin from Papuan taipan (*Oxyuranus scutellatus canni*) venom. Fluorescent antibody-labelled nerve fibre and fluorescein isothiocyanate-conjugated α-bungarotoxin-labelled acetylcholine receptors (AChR) are shown both **(1)** before and **(2)** after exposure to taipoxin. Loss of the nerve terminals is clearly seen in **(2)**.

SOURCE: HARRIS *et al* (2000)

Although there is still a lot to be learned about the actual mechanisms that produce the nerve terminal damage shown in figures 3-5, the sequence of events has been studied by Dr John Harris and his colleagues from the University of Newcastle in the United Kingdom; they determined that:

- within 1 hour of injection taipoxin produces depletion of ACh in synaptic vesicles and the emergence of clathrin-coated ?-shaped indentations at the synapse, as well as abnormalities in mitochondria and lysosomal structures;
- between 3-6 hours later the neuronal space may be completely empty of contents with significant mitochondrial damage, formation of large lysosome-like bodies, and invasion of junctional clefts with Schwann cell processes;
- at 24 hours more than 70% of muscle fibres are completely denervated; however junctional folds and segments of the plasma membrane may still be intact and numerous phagocytic cells are present;
- 88% of nerve terminals had regenerated and reinnervated within 5 days;
- total regeneration occurred within 28 days, although collateral innervation of the same fibres is common and was found to persist for more than 9 months.

It is worth noting that many of the patients who were ventilated in the Intensive Care Unit at Port Moresby General Hospital between 1992 and 2001 had a median ventilation duration of 4.5-5.0 days. This corresponds well to Dr John Harris' finding that reinnervation after the loss of nerve terminals following exposure to taipoxin occurred within 5 days.

In addition to its role as a presynaptic neurotoxin, taipoxin has also been shown to bind to integral membrane proteins on skeletal muscle cells and to produce clinically significant rhabdomyolysis. The basic a-subunit of taipoxin produces myonecrosis in both mammal and bird muscle fibres, and the myotoxicity is enhanced by the acidic taipoxin ?-subunit. The myotoxicity is the result of the hydrolysis of muscle plasma membranes and disruption of myofibril structure. Muscle necrosis results and may progress for more than 48 hours before the damaged tissue begins to recover and regenerate from the surviving basal lamina.

Common clinical features of myonecrosis are oedema, myoglobinuria and elevated serum levels of myoglobin, creatine kinase, lactate dehydrogenase and other isoenzymes. New myotubes begin to form within 3 days and small immature fibres are present after 5 days. Regeneration is usually complete within 21 days.

Among the other neurotoxins that contribute to clinical neurotoxicity is a presynaptic polypeptide PLA_2 named OS-2 that appears to be involved in the early depletion of ACh from presynaptic vesicles. The mechanism involves loss of outward K^+ ions transport from the nerve terminal due to putative K^+ channel blockade by the toxin. Like taipoxin, OS-2 was also found to bind to specific myotubule membrane proteins, and is probably a contributor to myonecrosis.

Not content with preventing the release of ACh and then physically destroying the nerve terminals at the neuromuscular junctions, taipan venom also contains several non-PLA_2 postsynaptic neurotoxins that bind to the nicotinic AChR. Only a couple of these toxins have been described in the scientific literature, including taipan-toxin 1, a 'short-chain' neurotoxin with a molecular weight of 6-7 kDa and a strong similarity to many of the other basic postsynaptic neurotoxins that are found in other land and sea snake venoms. As previously explained, the postsynaptic neurotoxins do not produce physical damage to the tissues that they bind with, and rapidly dissociate from AChR when exposed to specific antivenoms.

Oscutarin

Clinical defibrination coagulopathy seen in victims of taipan envenoming is due to the rapid cleavage of prothrombin to meizothrombin by Oscutarin, a potent Group III activator of prothrombin that is dependent on Ca^{2+} and phospholipids, but is Factor V independent. This is one of the largest components in taipan venom, being comprised of two large (110 kDa and 80 kDa) subunits that closely resemble Factor V_a. These are tightly bound to two disulphide-bonded Factor X_a-like serine proteinases of 57 kDa that contain the glycine-rich active site.

Oscutarin cleaves prothrombin randomly at either the ARG273-THR274 (thrombin-producing) site or the ARG322-ILE323 (meizothrombin-producing) site. The presence of phospholipid-rich platelets accelerates the cleavage of prothrombin. Coagulopathy may develop within less than 1 hour after envenoming and can be detected and monitored readily using the 20WBCT test.

In addition to gross prolongation of the blood clotting time, the clinical features of coagulopathy may include bleeding of the gingival sulci and other mucous membranes, haematemesis ('coffee-ground' stained vomit), haemoptysis, epistaxis, menorrhagia, haematuria, venepuncture site bruising and bleeding, and local bite site bleeding.

Disseminated intravascular coagulopathy is a characteristic indication of envenomation by the Papuan taipan (*Oxyuranus scutellatus canni*).

Taicatoxin

Taicatoxin is a 52 kDa oligomeric toxin comprised of a 16 kDa a-subunit, 8 kDa ß-subunit and four 7 kDa ?-subunits. The a-subunit is an extremely toxic PLA_2 that is essential to the activity of the complex. Taicatoxin was originally characterised as a specific voltage-dependent cardiac Ca^{2+} channel blocker, but further research demonstrated that it also blocks apamin-sensitive, small-conductance K^+ channels on chromaffin cells and in the brain. This makes taicatoxin unique in being the first small-conductance K^+ channel blocker to be described from snake venom.

Although it seems highly probable that this toxin might be one of the causes of some of the electrocardiographic disturbances (abnormalities in the 'electrical conductance' within the heart) seen in some victims of taipan envenoming, the actual clinical significance of the toxin remains unclear. Similarly, the potential effects on neurological function as a result of K^+ channel blockade in brain tissue are unknown.

New Guinean death adders (*Acanthophis* spp.)

The death adders are the most widely distributed species-group of highly venomous snakes in PNG and Papua. As explained in Chapter 2, the group is in urgent need of comprehensive research, not just to determine exactly how many different species do occur, but also to examine their venoms for specific variations that may result in variable clinical presentations.

Despite their fearsome common name, death adders are generally inoffensive snakes, and bites typically only occur when the snake is either stepped upon or accidentally handled by an unwary victim. These small snakes are largely nocturnal and are responsible for the majority of serious snakebites that occur at night. In fact, it would be very true to say that the majority of bites by death adders could be prevented if people used torches or kerosene lamps when walking around at night and took care to watch where they placed their feet. Death adders very rarely strike higher than the ankle so wearing shoes is also a very good means of preventing bites.

Unlike Papuan taipans (*Oxyuranus scutellatus canni*), the venom of death adders does not contain activators of prothrombin, and as a consequence incoagulable blood is not a feature of death adder envenomation. This is a very important distinction between the clinical presentations of bites by these two very different types of venomous snake.

Death adder venoms are rich in a diversity of 'short-chain' and 'long-chain' postsynaptic neurotoxins that bind to nicotinic AChR in skeletal muscle and produce facial and bulbar paralysis. Among these are the 'short-chain' neurotoxins acanthophin a and toxin Aa-c, and the 'long-chain' neurotoxins acanthophin d, toxin Aa-b and toxin Aa-e, all from the Australian common death adder (Acanthophis antarcticus). A postsynaptic neurotoxin called acantoxin IVa from the Seram population of *Acanthophis laevis* has also been isolated and characterized. Postsynaptic neurotoxicity can be effectively reversed with antivenom.

One of these neurotoxins (toxin Aa-c) is a basic (alkaline) 6.7 kDa 'short-chain' neurotoxin comprised of 62 amino acid residues cross-linked by four structurally supportive disulphide bridges that shape the toxin into an acceptable 'key' for the AChR 'lock'. Aa-c is very similar ('homologous') to the classic 'three-fingered erabutoxins' that are present in the venoms of the banded sea kraits (*Laticauda* spp.). The 'long-chain' neurotoxins in death adder venoms have molecular weights of 7.9-8.4 kDa, and comprise of 73-79 amino acid residues cross-linked by five disulphide bridges. Both the 'short-chain' and 'long-chain' neurotoxins compete with ACh for AChR binding sites and, once bound, these toxins prevent muscle contraction initiation, producing paralysis.

Several moderately toxic basic PLA_2 toxins which are presynaptically neurotoxic have recently been identified in death adder venoms, and were found to possess acceptor molecule recognition sites that enhance enzymatic activity once bound to their targets. These toxins may contribute to the potential myotoxicity in some death adder species. Research has shown that at least one race of New Guinean death adder (*Acanthophis rugosus*) from Papua possesses a PLA_2 toxin (Acanmyotoxin 1) capable of inducing *in vitro* myotoxicity.

While lacking prothrombin activators, death adder venoms have been found to contain several kunitz-type protease inhibitors of 6.6-7.2 kDa that may be potent inhibitors of coagulation factors. PLA_2 inhibitors of platelet aggregation known as acanthins are also present; however their activity is related more on enzyme isoelectric points than to PLA_2 enzymatic activity. These PLA_2 may be responsible for subclinical anticoagulant activity that prolongs PT and APTT in vitro without inducing fibrinogenolysis. There is however no clinical evidence of these activities.

New Guinean small-eyed snake (*Micropechis ikaheka*)

As is likely to be the case with the Papuan blacksnake (*Pseudechis papuanus*), the availability of live small-eyed snakes (*Micropechis ikaheka*) from wildlife dealers in Papua and Indonesia has resulted in the recent characterization of several major venom components by overseas researchers. The venom has strong neurotoxic, myotoxic, anticoagulant, platelet aggregation inhibiting and insulin-secretion stimulating activities. In patients bitten by this species neurotoxicity and myotoxicity appear to be the major clinical consequences.

Two cases in which patients had incoagulable blood have been attributed to powerful anticoagulant rather than procoagulant activity; however researchers in Singapore recently isolated Mikarin, a single-chain metalloproteinase of 47 kDa. Mikarin is unique compared to all other Australo-papuan snakes in being a Ca^{2+}-independent prothrombin activator, and is the first Group I prothrombin activator to be found in elapid venom.

A novel non-haemolytic, haemoglobinuria-inducing toxin (MiPLA-1), which is a 14 kDa, 124 amino acid residue PLA_2, has been identified, and shown to also strongly inhibit collagen-induced platelet aggregation, as well as being potently myotoxic and anticoagulant. It has been suggested that this toxin might produce haemoglobinuria by causing kidney leakage via either a direct or indirect nephrotoxic mechanism. MiPLA-1 is unique among snake venom PLA_2s in that it is one of only a few to possess a 'pancreatic loop' region which has a major role in toxin conformation and hydrolytic activity.

Several 'short-chain' and 'long-chain' postsynaptic neurotoxins with molecular weights between 6-8 kDa have also been isolated from *Micropechis ikaheka* venom. One of these, Mikatoxin, has been found to produce neuromuscular paralysis through irreversible nicotinic AChR antagonism. As well as these, an 11 kDa venom fraction containing a 'long-chain' neurotoxin also inhibited ADP-induced platelet aggregation. The anticoagulant activity of *Micropechis ikaheka* venom is underpinned by the presence of a 17 kDa PLA_2 toxin that inhibits both endothelial and platelet-induced procoagulation. Three additional PLA_2 toxins exhibit myotoxicity, anticoagulant activity and stimulate insulin secretion. While the myotoxicity and anticoagulant activity were induced by the enzymatic actions of the toxins, the stimulation of insulin secretion was independent of enzymatic activity.

Papuan blacksnake (*Pseudechis papuanus*)

Until very recently there was only 1 living specimen of this species in captivity anywhere in the world. As a consequence, very little is known about the actual venom composition of these rare, and possibly endangered, snakes. More specimens have become available during the last few years as a result of the wildlife trade in Papua, and a number of snakes are now held in collections in Europe and North America. Venom from Papuan blacksnakes is becoming available to researchers and during the next few years our knowledge may improve.

We do know that the main cause of death in experimental animals injected with Papuan blacksnake venom was cyanosis leading to sudden respiratory arrest, suggesting that neurotoxins are present. The venoms of other *Pseudechis* spp. have been shown to contain abundant PLA_2 toxins with neurotoxic, myotoxic and anticoagulant activity. Weak presynaptic PLA_2 neurotoxins are present in the venom of the Australian red-bellied black snake (*Pseudechis porphyriacus*), and that other species such as Collett's blacksnake (*Pseudechis colletti*) possess a potently myotoxic PLA_2, as well as a phospholipase B with haemolytic and erythrolytic activities. This latter species is closely related to the Papuan blacksnake (*Pseudechis papuanus*), which has been shown to be haemorrhagic in rats, with a minimum haemorrhagic dose (MHD) of 46-60 µg/rat, suggesting that Papuan blacksnake

venom may contain a weak non-zinc metalloproteinase haemorrhagin. Papuan blacksnake venom has a subcutaneous LD_{50} in experimental animals of 1.09 mg/kg and this is much less toxic than the 0.05 mg/kg subcutaneous LD_{50} of Papuan taipans (*Oxyuranus scutellatus canni*).

A 15 kDa neutral PLA_2 isolated by British researchers from Papuan blacksnake venom is a potent monophasic platelet aggregation inhibitor with strong anticoagulant activity, but lacks fibrinolytic activity. Snakebite victims, who were shown to have been bitten by this species using laboratory tests, typically developed reversible neurotoxicity, and had thrombocytopenia, mild defibrination with fibrinolysis, spontaneous bleeding and prolonged prothrombin (PT) and activated partial thromboplastin (APTT) times, suggesting that other venom components affecting coagulation are present in this snake venom. Platelets exposed to the PLA_2 toxin identified by British researchers lost their normal discoid shape, formed membranous projections and developed microfilament disruptions that impaired their ability to aggregate. The same toxin also has moderate myotoxic activity of 546 U/ml. At high concentrations, whole venom from Papuan blacksnakes does have mild procoagulant (prothrombin activating) activity, but this was not sufficiently powerful enough to prevent the clotting of blood within 20 minutes in envenomed animals. The clinical experience with patients bitten by Papuan blacksnakes is that they respond better to antivenom than victims of Papuan taipan (*Oxyuranus scutellatus canni*) bites and appeared to recover much more quickly.

Papuan mulga snake (*Pseudechis cf. australis*)

Nothing is currently known about the venom of the mulga snake species that occurs in Papua and the south-western corner of Western Province, and there are definite records of bites by these snakes. Reports from herpetologists in Europe who have captive specimens that were collected in the Merauke district of Papua suggest that this is a pugnacious snake that will bite with little provocation.

It is probable that the venom is very similar to that of Australian mulga snakes (*Pseudechis australis*), and while this snake has venom that contains many different toxins, the dominant clinical outcome is typically massive rhabdomyolysis with myoglobinuria and possible acute renal failure. There are several potent PLA_2 myotoxins in the venom including mulgatoxin a and Pa-5. Pa-5 has an LD_{50} of 0.25 mg/kg in laboratory mice while mulgatoxin a has an LD_{50} of 200 mg/kg in mice when given intraperitoneally. The serum creatine kinase levels of patient bitten by mulga snakes may rapidly reach levels of over 300,000 IU/L (the normal range is less then 200 IU/L), and myoglobinuria (dark brown 'stringy' urine) may persist for several days producing a high risk of nephrotic necrosis and renal failure.

Mulga snake venom also contains several presynaptic and postsynaptic neurotoxins, but, from a clinical perspective, neurotoxicity is usually either minor or non-existent. The neurotoxins include several 13.5-14.0 kDa PLA_2 toxins and at least one 'short-chain' (toxin Pa-a) and one 'long-chain' (toxin Pa-1D) postsynaptic neurotoxin. The toxin Pa-1D was found to be non-lethal in experimental studies despite binding to nicotinic AChR.

There are no known procoagulant toxins in mulga snake venom, but there are a number of haemolytic PLA_2 and possibly PLB toxins that may produce haemoglobinuria (although this is very likely to be overshadowed by myoglobinuria). Australian mulga snake venom also contains true anticoagulant PLA_2 toxins and has been shown to produce prolongation in PT and APTT, without fibrinolysis. The presence of inhibitors of platelet aggregation is unclear; however, these are present in other *Pseudechis* venoms, including *Pseudechis papuanus* and may therefore also occur in this snake.

New Guinean brown snake (*Pseudonaja cf. textilis*)

The common brown snake (*Pseudonaja textilis*) from eastern Australia, to which these Papua New Guinean snakes are the most closely related, is an extremely venomous species that has been implicated as the cause of most of Australia's snakebites and snakebite deaths over the last few decades. Venom from the Papua New Guinean species (*Pseudonaja cf. textilis*) has not been specifically studied, but it is very likely that it contains many of the same toxins and has similar clinical effects.

The most clinically important components in brown snake venom appear to be the prothrombin activator textarin and the plasmin inhibitor textilinin. Disseminated intravascular coagulopathy is the dominant clinical effect, vastly overshadowing neurotoxicity. The spontaneous bleeding from gingival sulci, mucous membranes and other sites that is seen after brown snake (*Pseudonaja cf. textilis*) envenomation is much more prominent than that seen in Papuan taipan (*Oxyuranus scutellatus canni*) envenomation. Textarin is a large molecule with a molecular weight of approximately 200 kDa and an LD50 of 0.023 mg/kg in laboratory rats. Defibrination coagulopathy and drops in the levels of Factor V, Factor VIII, plasminogen and Protein C are reported. In Australia, some patients have had to be treated with as many as 25 ampoules of monovalent brown snake antivenom in order to restore normal coagulation. Deaths are often due to cerebral haemorrhage.

Despite the fact that neurotoxicity is often considered to be relatively minor after bites by brown snakes, the common brown snake (*Pseudonaja textilis*) produces the most lethal presynaptic neurotoxin known from any snake venom. This toxin, known as textilon, is a multi-subunit PLA_2 toxin with some similarity to taipoxin from the venom of the Papuan taipan (*Oxyuranus scutellatus canni*). Like taipoxin, it causes irreversible presynaptic neurotoxicity and structural damage to nerve terminals including mitochondrial and organelle destruction through phospholipid hydrolysis. Myotoxicity and myoglobinuria have not been reported in this species, but there is evidence that there may be a directly nephrotoxic compound, as some Australian patients have experienced acute tubular necrosis in the kidneys.

A common occurrence following brown snake bites is early sudden collapse and transient loss of consciousness that may be due to a brief episode of hypotension brought about by the presence of vasoactive amines or other components.

Sea Kraits (*Laticauda* spp.) and true sea snakes (*Hydrophiinae*)

Remarkably, despite there being many different species of sea snakes with diverse dietary preferences, different evolutionary lineages and widespread distribution, the venoms of these snakes are unique in their relative simplicity. Sea snake venoms are predominantly comprised of 'short-chain' and 'long-chain' postsynaptic neurotoxins, most of which share a unique 'three-fingered' structural backbone designed to optimise the interaction of the toxin with the nicotinic AChR. Erabutoxins found in the venoms of sea kraits (*Laticauda* spp.) are classic three-fingered toxins that strongly bind to AChR, and this is reversible with antivenom.

Many sea snakes have powerfully myotoxic venom PLA_2 toxins. The beaked sea snake (*Enhydrina schistosa*), which is implicated in most sea snake bite fatalities, has a potent myotoxin and renal failure is a common outcome. In addition to a number of PLA2 toxins, this venom contains several postsynaptic neurotoxins including three enhydrotoxins. Bites by Stoke's sea snake (*Astrotia stokesii*) also produce extensive rhabdomyolysis and myoglobinuria. Lecithinases in some sea snake venoms may cause erythrolysis, and some species are also believed to have weak anticoagulants that may have clinical activity.

Summary Table: Venom components, clinical effects and antivenoms

Species	Toxin types	Typical clinical effects	Effective antivenoms
Papuan taipan (*Oxyuranus scutellatus canni*)	• Powerful procoagulant toxins • Irreversible, very destructive presynaptic neurotoxins • Postsynaptic neurotoxins • Myotoxic PLA_2 • Ca^{2+} channel toxins	• Incoagulable blood (20WBCT > 20 min.) and spontaneous bleeding • Irreversible destructive neurotoxicity leading to sustained facial, bulbar and respiratory paralysis • Rhabdomyolysis and myoglobinuria • Electrocardiographic disturbances	CSL taipan or CSL polyvalent
Death adders (*Acanthophis* spp.)	• Postsynaptic neurotoxins • Weak presynaptic neurotoxins • Weak anticoagulants • Possible myotoxins • Platelet aggregation inhibitors	• Reversible postsynaptic neurotoxicity • Normal 20WBCT (i.e. <20 min.)	CSL death adder or CSL polyvalent
Small-eyed snake (*Micropechis ikaheka*)	• Potent myotoxins • Postsynaptic neurotoxins • Platelet aggregation inhibitors • Haemotoxins ($MiPLA_2$)	• Significant rhabdomyolysis with myoglobinuria and potential renal failure • Reversible postsynaptic neurotoxicity • Thrombocytopenia • Haemoglobinuria	CSL polyvalent
Papuan blacksnake (*Pseudechis papuanus*)	• Presynaptic neurotoxins • Postsynaptic neurotoxins • Haemorrhagins • Platelet aggregation inhibitors	• Reversible neurotoxicity • Incoagulable blood (20WBCT > 20 min.) • Haemoglobinuria • Possibly rhabdomyolysis/myoglobinuria	CSL blacksnake or CSL polyvalent

Summary Table: Venom components, clinical effects and antivenoms *(Continued)*

Species	Toxin types	Typical clinical effects	Effective antivenoms
Papuan mulga snake (*Pseudechis cf. australis*)	• Powerful myotoxins • Weak presynaptic and post-synaptic neurotoxins • Anticoagulant PLA_2 toxins	• Local pain, oedema and ecchymosis • Rhabdomyolysis, myoglobinuria and subsequent acute renal failure • Subclinical neurotoxicity • Possible spontaneous bleeding due to anticoagulants rather then procoagulants	CSL blacksnake or CSL polyvalent
New Guinean brown snake (*Pseudonaja cf. textilis*)	• Dominant procoagulants • Irreversible and destructive presynaptic neurotoxin • Possible nephrotoxins • Plasmin inhibitors	• Sudden brief loss of consciousness • Incoagulable blood (20WBCT >20 min.) • Significant spontaneous bleeding • Moderate neurotoxicity • Possible renal failure	CSL brown snake or CSL polyvalent
Sea kraits (*Laticauda* spp.) True sea snakes (*Hydrophiinae*)	• Postsynaptic neurotoxins • Powerful myotoxins	• Reversible neurotoxicity • Massive rhabdomyolysis/myoglobinuria and subsequent renal failure	CSL seasnake or CSL polyvalent

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