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SNAKE VENOM
The Amazing Poison

Profesor Tan Nget Hong

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SNAKE VENOM: THE AMAZING POISON

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Synopsis

Snakebite is a serious medical problem in Malaysia. During the period 1958 to 1980, as many as 55000 cases of snakebites were admitted to the hospitals of Malaysia. In Malaysia there are more than 40 different species of venomous snakes, belonging to three families: the crotalids (pit viper), elapids (cobras and kraits) and the sea snakes.

Dried snake venom contains mainly proteins (70-90%) and small amounts of metals, amino acids, peptides, nucleotides, carbohydrates, lipids and biogenic amines. The major toxins are proteins. Investigations of the biochemistry and toxinology of snake venoms have led to a better understanding of the pathophysiology of snake venom poisonings and improvement in the treatment of snakebites as well as the discovery of novel therapeutic agents and biomedical tools.

Elapid venoms generally exhibit neurotoxicity and cardiotoxicity. The major toxins of the elapid venoms include polypeptide postsynaptic neurotoxins, polypeptide cardiotoxins and phospholipases A. These toxins are valuable molecular probes and pharmacological tools to investigate the functional biology of receptors and ion channels as well as providing lead compounds for the design of clinically useful drugs. Postynaptic neurotoxins, for example, play an important role in understanding of the pathogenesis of auto-immune neuromuscular diseases such as Myasthenia gravis. Candoxin, a neurotoxin from Malayan krait, is a useful tool in the investigation of molecular basis of Alheimer's disease as well as a muscle relaxant.

The crotalid venoms generally cause hemorrhages. For example, the victims of Malayan pit viper bite usually develop blood clotting defect due mainly to defibrination and thrombocytopenia. Defibrination is due to the action of the venom thrombin-like enzymes on the fibrinogen. The major thrombin-like enzyme, ancrod, has been used in anticoagulation therapy. Thrombocytopenia is presumably due to the combined actions of the platelet aggregation inducer, aggretin, and the anti-platelet L-amino acid oxidases and phospholipases A. Aggretin, a non-enzymatic protein, is an endothelial integrin $\alpha_2\beta_1$ agonist which also induces angiogenesis. Other venom constituents of the Malayan pit viper that also contribute to the hemorrhagic actions of the venom include hemorrhagins and platelet aggregation inhibitors. The major hemorrhagin is rhodostoxin, a metalloproteinase which presumably degrades capillary basement membrane. Two platelet aggregation inhibitors, rhodostomin and rhodocetin, have been isolated from the venom. Rhodostomin is an RDG-containing polypeptide that blocks the binding of fibrinogen to the integrin $\alpha_{IIb}\beta_3$ of platelet whereas Rhodocetin acts as $\alpha_2\beta_1$ integrin inhibiting disintegrin and may be a valuable tool to manipulate other $\alpha_2\beta_1$ integrin mediated functions.

Venomous snakes of Malaysia

Snakebite is a serious medical problem in Malaysia. During the period 1958 to 1980, as many as 55000 cases of snakebites were admitted to the hospitals of Malaysia (Lim and Ibrahim, 1970; Lim, 1982). While the mortality rate is only 0.3 per 100000 population, snake venom poisoning can cause prolonged morbidity or even crippling deformity.

In Malaysia there are at least 18 different species of venomous front fanged land snakes, and more than 22 different species of venomous sea snakes (Tweedie, 1983). These venomous snakes belong to the crotalid (pit viper) family (the genus *Calloselasma* and *Trimeresurus*), elapid family (the genus *Naja, Bungarus* and *Ophiophagus*) and sea snake family (the subfamilies *Laticaudinae, Hydrophiini* and *Ephalophiini*). Only a few of the Malaysian venomous snakes can be regarded as of medical importance, and these include the pit vipers *Calloselasma rhodostoma* (Malayan pit viper), *Trimeresurus purpuromaculatus*

(shore pit viper), *Trimeresurus wagleri* (Wagler's pit viper) *Trimeresurus sumatranus* (Sumatran pit viper), the elapids *Naja naja sputatrix* (Malayan cobra), *Naja naja kaouthia* (Monocellate cobra), *Ophiophagus Hannah* (king cobra), *Bungarus candidus* (Malayan krait), *Bungarus fasciatus* (banded krait) and the sea snake *Enhydrina schistosa* (beaked sea snake).

Table 1 summarizes the reports on snakebites from monthly statistics of 28 hospitals throughout Malaysia during the six-years period 1965-1971 (Sawai *et al*, 1972).

Table 1: Snakebites in West Malaysia (1965-1971)

Snake Species	Total Cases	Fatal Cases
Malayan pit viper (*Calloselasma rhodostoma*)	1136	4
Sea snake	158	5
Asian common cobra (*Naja naja*)	112	3
Asian lance-headed viper (*Trimeresurus*)	25	0
King cobra (*Ophiophagus hannah*)	6	0
Krait (*Bungarus*)	1	0
Unidentified	3765	6
Nonpoisonous	184	0

Composition of snake venom

Dried snake venom contains mainly proteins (70-90%) and small amounts of metals, amino acids, peptides, nucleotides, carbohydrates, lipids and biogenic amines. The protein components include both enzymes and non-enzymatic proteins.

Venoms of many elapid snakes (cobra, krait and sea snakes) produce flaccid paralysis and respiratory failure in animals. These effects are due to the

polypeptide neurotoxins and certain phospholipases A. Elapid venoms also contain various pharmacologically active basic polypeptides. In cobra, the major basic polypeptides are polypeptide cardiotoxins.

The major toxic principles in pit viper venoms are thrombin-like enzymes, hemorrhagic proteases, platelet-aggregation inducers and inhibitors, as well as other enzymes that interfere with the blood coagulation pathway.

Snake venoms consist of many other enzymes, including protease, L-amino acid oxidase, hyaluronidase, phosphodiesterase, alkaline phosphatase and 5-nucleotidase. Most of these enzymes play a role in the digestion of the prey but some also exhibit toxic properties.

Elapid Venom Poisoning

Elapid venoms (cobra, kraits and sea snakes) generally exhibit neurotoxicity and cardiotoxicity (Lee, 1972; Reid, 1964). The earliest symptom of systemic elapid poisoning is a feeling of drowsiness intoxication, difficulty in opening the eyes (bilateral ptosis). In severe poisoning, respiratory failure sets in rapidly. In cobra, these neurotoxic symptoms are caused by the polypeptide neurotoxins. These are postsynaptic neurotoxins, generally basic polypeptides containing 60-70 amino acid residues with an intravenous LD_{50} (median lethal dose) of approximately 0.1 μg/g in mouse. Postsynaptic neurotoxins isolated from cobra, krait and sea snake venoms are very similar in their amino acid sequence.

Toxinology of the Malayan cobra venom

In Malayan cobra (*Naja naja sputatrix*) venom, postsynaptic neurotoxins account for approximately 5% of the venom dry weight. Two major neurotoxins, with 62 and 63 amino acid residues respectively, have been purified (Tan, 1983). The amino acid sequences of the two toxins have been elucidated (Chung *et al*, 1994) as follows:

LECHDQQSSQTPTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSV
KNGIEINCCTTDRCNN

LECHNQQSSQAPTTKTCSGETNCYKKWWSDHRGTHERGCGCPKV
KPGVKLNCCTTDRCNN

The neurotoxin genes have also been cloned and expressed (Afifiyan *et al*, 1998)

Cobra venom cardiotoxicity is caused by polypeptide cardiotoxin that impairs the structure and function of various cells, contributing to muscle paralysis and leading to circulatory failure and systolic arrest (Lee, 1972). Polypeptide cardiotoxins account for 60% of the Malayan cobra venom by weight. Three polypeptide cardiotoxins, sputa-cardiotoxin A, B and C have been isolated from the venom (Tan 1982a). They are basic polypeptides with 59-60 amino acid residue with intravenous LD_{50}'s of 1.0-1.1 µg/g mouse.

Jeyaseelan *et al* (1998) reported the cDNA cloning of the cardiotoxins and the deduced amino acid sequences of the cardiotoxins. The sequences are homologous to other cobra venom cardiotoxins.

Malayan cobra venom contains substantial quantity of phospholipases A, which account for 15% of the venom dry weight. Three lethal phospholipases A, sputatrix PLA-1, sputatrix PLA-2 and sputatrix PLA-3 have been isolated (Tan, 1982b; Tan and Arunmozhiarasi, 1989). The three enzymes have intravenous LD_{50}'s of between 0.28-0.86 µg/g mouse. Sputatrix PLA-3 alone accounts for 10% of the venom protein. It exhibits weak anticoagulant activity and induces contracture of muscle in a chick biventer cervicis nerve-musclepreparation (Geh and Tan, 1988). Sputatrix PLA-2 and PLA-3 exhibit potent anticoagulant activity and are the major anticoagulants of the venom. Following is the sequence of PLA-2:

NLYQFKNMIQCTVPNRSWWHFADYGCYCGRGGSGTPVDDLD
RCCQVHDNCYGEAEKCWPYFKTYSYECSQGTLTCKGGNNACAAAVC
DCDRLAAICFAGAPYNNNNYNIDLK

Jeyaseelan *et al* (2000) reported the cloning of the phospholipase A genes from Malayan cobra venom. Comparison of the deduced amino acid sequences shows that the signal peptide sequences are conserved, and the three PLA's are highly homologous with slight variations only.

Malayan cobra venom also causes extensive local necrosis. Our investigations indicated that the local necrosis is presumably caused by the synergistic action of cardiotoxin and phospholipase A (Tan and Arunmozhiarasi, 1990)

Sea snake venom poisoning and the biochemistry of sea snake venom

The commonest species of sea snake is the *Enhydrina schistosa* (beaked sea snake). In rat, its venom is neurotoxic acting mainly at the neuromuscular junction. In human, however, the venom is primarily myotoxic (Reid, 1979). The myonecrosis causes generalized muscle movement pains and myoglobinuria. Postsynaptic neurotoxins account for 70% of the venom dry weight. The two major neurotoxins are polypeptides with 60 amino acid residues and the amino acid sequences are homologous to the cobra neurotoxins. The venom contains more than 9 phospholipases A. The major basic phospholipase A which accounts for 7% of the venom dry weight has been isolated and demonstrate to exhibit strong myotoxic activity. Its sequence is similar to the PLA from other venom sources. Two acidic phospholipasess A have also been isolated (Tan, 1982c) and they are able to depress muscle excitability due to direct stimulation as well as the response of muscle to nerve stimulation.

Venoms of King cobra and Malaysian kraits

King cobra is one of the world's most dangerous snakes. The snake, however, is generally not aggressive and king cobra bite in man appears to be infrequent. King cobra venom has a much greater enzyme content than other cobra venoms The major lethal toxins are also polypeptide neurotoxins, and the main systemic effect of king cobra venom poisoning appears to be neurotoxic poisoning. The

major hemorrhagin, the L-amino acid oxidase and two acidic phospholipases A have been isolated (Tan and Saifuddin, 1989a,b, 1990)

There are three species of krait in Malaysia: *Bungarus fasciatus* (banded krait), *Bungarus candidus* (Malayan krait) and *Bungarus flaviceps* (Red-headed krait). The toxinology of the banded krait has been investigated (Lu and Lo, 1981). We have isolated two highly lethal phospholipases A from the Malayan krait (Tan *et al*, 1989). Little is known about the toxinology of the Red-headed krait.

Elapid toxins as biomedical tools

Knowledge about snake venom toxins may lead to improvement of treatment of snake venom poisoning and development of effective antivenoms. Besides, the toxins are also valuable molecular probes and pharmacological tools, as well as providing lead compounds for the design of clinically useful drugs.

For example, postsynaptic neurotoxins from cobra and krait venoms play an important role in the understanding of the pathogenesis of auto-immune neuromuscular diseases such as Myasthenia gravis. Candoxin, a neurotoxin isolated from Malayan krait, is a useful tool in the investigation of the molecular basis of Alzheimer's disease. It is also a muscle relaxant. Cobra cardiotoxins, on the other hand, can act as inhibitors of coagulation and platelet aggregationa and as research tool in neurobiology because of its ion channel activity.

Pit Vipers in Malaysia

Pit viper bites usually result in swelling and sometime, local necrosis. The principal characteristic of systemic pit viper poisoning in Malaysia is systemic bleeding, characterized by defibrination and thrombocytopenia (Reid, 1968; Warrell, 1986).

The Malayan pit viper (*Calloselasma rhodostoma*) previously known as *Agkistrodon rhodostoma,* is the commonest cause of snakebite in Peninsular Malaysia. It is a bad tempered snake, quick to strike if disturbed. Fortunately,

only 10% of those bitten developed severe poisoning and death rate is less than 2%.

Clinical effects of Malayan pit viper Venom

The intravenous LD_{50} for Malayan pit viper venom is 6.1 µg/g mouse, which is considerably less lethal than cobra venom. This partially explains the low mortality rate of Malayan pit viper bites.

Local swelling begins minutes after the bite and may continue to increase from 24 to 72 hours. The swelling, which is due to the extravasations of plasma and red blood cells into tissue, results in discoloration. If large amount of venom has been injected, blisters may form around the bite followed by superficial necrosis.

Systemic poisoning is characterized by systemic bleeding, which may be slight with only a prolonged clotting time or when severe, present as hemorrhagic syndrome with or without shock (Chan 1979). The earliest manifestation of severe systemic poisoning is hemoptysis. Other signs that followed are continued oozing of blood from the wound site, gum bleeding, tooth sockets or ulcers, discoid hemorrhage. In severe systemic poisoning, the blood may remain incoagulable for prolonged period. Reid *et al.* (1963) reported that the systemic bleeding is characterized by defibrination and thrombocytopenia. The major toxins of the Malayan pit viper venoms include thrombin-like enzymes, hemorrhagins, platelet aggregation inducer, platelet aggregation inhibitors, L-amino acid oxidases and phospholipases A (Tan and Ponnudurai, 1996).

(i) Thrombin-like enzymes

The venom contains several thrombin-like enzymes. The major form, ancrod, accounts for approximately 7.5% of the venom dry weight (Esnouf and Tunnah, 1967). The enzyme coagulates fibrinogen solution by catalyzing the release of fibrinopeptide A, AP and AY. Clots that formed with ancrod are not cross-linked and are susceptible to rapid lysis by plasmin. When injected into human/animals, it causes continual microcoagulation of fibrinogen but the resulting fibrin is

virtually simultaneously disposed off. Thus, although ancrod is a 'coagulant' *in vitro*, the *in vivo* effect is non-clotting blood because of the very low fibrinogen level. The amino acid sequence (Au *et al.*, 1993) exhibits a high degree of sequence similarity to those of mammalian serine proteinases as well as reptilian fibrinogenases.

The use of ancrod in anticoagulation therapy has been examined in many clinical trials. Ancrod provides optimal therapy for patients suspected of having heparin associated thrombocytopenia and thrombosis (HATT). Depletion of fibrinogen with ancrod results in anticoagulation comparable to therapy with heparin within 12 hours (Cole *et al.*, 1990). Ancrod has also been used in the treatment of acute ischaemic stroke (Sherman *et al.*, 2000)

(ii) Hemorrhagins

Snake venom hemorrhagins are generally metalloproteinases that probably act by destruction of the collagenous basement membrane and other connective tissue collagens with consequent weakening of the blood vessel wall causing hemorrhagic effect.

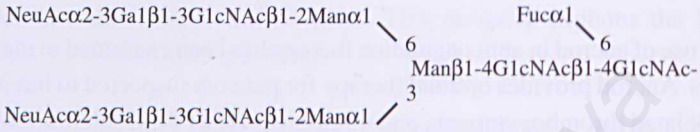
The major Malayan pit viper venom hemorrhagin, termed rhodostoxin, has been isolated and its amino acid sequence is homologous to other venom hemorrhagins (Chung et al., 1996). It is a zinc-metalloproteinase with 203 amino acid residues (25 kDa) belonging to class P-I of snake venom hemorrhagin:

NHEIKRHVDIVVVVDSRFCTKHSNDLEVIRKFVHEVVNAIIESYKYMHFGISL
VNLETWCNGDLINVQEDSYETLKAFGKWRESDLIKHVNHSNAQFLTDMKF
IKNIIGKAYLDSICDPERSVGIVQNYHGITLNVAAIMAHEMGHMLGVRHDGE
YCTCYGSSECIMSSHISDPPSKYFSNCSYYQFWKYIENQNPQCILNKP

Rhodostoxin is a potent hemorrhagin with MHD comparable to the venom hemorrhagins but was not lethal to mice at a dose of 6 µg/g (i.v.), indicating that it is not a major lethal factor of the venom as the LD_{50} (*i.v.*) of Malayan pit viper venom is around 6 µg/g in mice.

Glycobiology of rhodostoxin

Rhodostoxin is glycosylated with two N-linked glycopeptides attached to residues 91 and 181 (Chung *et al.*, 1996). The glycan chain is a complex type of carbohydrate structure with novel 2,3-linked sialic acid and Galβ(1,3)GlcNAc linkages:



Deglycosylation of rhodostoxin results in an apparent increase in the stability of the hemorrhagin as well as an increased specificity (Tan *et al.*, 1997). It appears that the glycol moieties of rhodostoxin may play a role in the interactions between rhodostoxin and the relevant substrate in the membrane.

Rhodostoxin is also known as kistomin. It inhibited angiogenesis in a doe-dependent manner and induced apoptosis of human endothelial cells by degrading extracellular matrix. (Hsu and Huang, 2004). It may provide a potential strategy in exploring the apoptosis induction of endothelial cells and the drug development in angiogenesis therapy.

(iii) Platelet aggregation inducer

Platelet aggregation occurs during vascular injury, and coverage of the exposed subendothelium by platelets depends on the recognition of adhesive proteins (fibrinogen, collagen, vWF etc) by specific platelet membrane glycoproteins, many of which are integrins. Among them, $\alpha_2\beta_1$(GPIa/IIa) and $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) are known to play important roles in mediating adhesion and aggregation of platelets. Snake venom platelet aggregation inducers are known to interact with the integrins.

Malayan pit viper venom exhibits strong platelet aggregation inducing activity which contributes to the thromocytopenia in the victim. Huang *et al.* (1995) reported the isolation of a potent platelet aggregation inducer, aggretin, from

the venom. It is a heterodimeric protein (29 kDa) and is devoid of enzymatic activity. It has been suggested that aggretin is an endothelial integrin $\alpha_2\beta_1$ agonist (Chung *et al.*, 2001). Study on the interaction of platelet aggregation inducer and collagen would increase our knowledge of platelet-collagen interaction at the molecular level and may provide a new avenue for developing inhibitors to prevent detrimental effects of collagen.

(iv) Platelet aggregation inhibitors

Many crotalid snake venoms contain polypeptide platelet aggregation inhibitors known as disintegrins. Generally, the polypeptide disintegrins contain Arg-Gly-Asp (RGD) tripeptide sequence near its carboxyl terminus. The tripeptide RGD plays an essential role in mediating the binding of $\alpha_{IIb}\beta_3$.

Teng and Huang (1991) reported the isolation of a disintegrin from Malayan pit viper venom. Termed rhodostomin, it is a 68 amino acid residue polypeptide and inhibits platelet aggregation by blocking the binding of fibrinogen to the integrin $\alpha_{IIb}\beta_3$ of platelet. The amino acid sequence deduced from the cDNA sequence (Au *et al.*, 1991) indicates that the 68-amino acid sequence of rhodostomin is located at the carboxyl terminus of the precursor protein, which also contains the sequence of rhodostoxin, the major hemorrhagin. It appears that rhodostomin and rhodostoxin share a common gene sequence, suggesting that these proteins may be synergistic in function. Presumably, the metalloprotease degrades capillary basement membrane and the soluble rhodostomin then binds to platelet integrins, inhibiting platelet aggregation, resulting in hemorrhages.

Extensive studies of disintegrins have revealed some potential uses of these peptides in the design of antithrombotic agents, the diagnosis of cardiovascular diseases and as novel tools for the study of cell-adhesion, cell migration, angiogenesis and other integrin-related disorders (Huang, 1998). Yeh *et al.* (2001) reported that rhodostomin inhibits angiogenesis elicited by basic fibroblast growth factor and suppresses tumor growth by a selective $\alpha_V\beta_3$ blockade of endothelial cells.

Wang *et al* (1999) reported the isolation of a different, RGD-independent platelet aggregation inhibitor from Malayan pit viper venom. Termed rhodocetin, it is a CLP (C-type lectin-like protein) that blocks $\alpha_2\beta_1$ integrin and is responsible for hampering collagen-induced, $\alpha_2\beta_1$ integrin-mediated platelet activation, leading to hemorrhages and bleeding disorders of the snakebite victims. Because of its high affinity to $\alpha_2\beta_1$ integrin, rhodocetin may be a useful agent to study and influence $\alpha_2\beta_1$ integrin-triggered cell function, like cell adhesion, cell migration or secretion of matrix metalloproteases. Thus, rhodocetin may help not only in treating thrombosis but also in treatments aimed to prevent tumor invasion and metastasis.

(v) L-Amino acid oxidases

L-Amino acid oxidase (LAAO) is a flavoprotein which catalyses the transformation of L-amino acids to the corresponding α-keto acids, with the concomitant release of hydrogen peroxide and ammonia. It is found in the venoms of most venomous snakes and contributes to the yellowish colour of the dry venom powder. LAAO is a major constituent of the Malayan pit viper venom, constituting 30% of the venom dried weight. The enzyme is an acidic glycoprotein with a molecular weight of 132000 (Ponnudurai *et al.*, 1994). The cDNA-deduced amino acid sequence of the enzyme shows 83% identity to LAAOs from Eastern and Western diamondback rattlesnake (Macheroux *et al.*, 2001).

LAAO is thought to contribute to the venom's toxicity, possibly through generation of hydrogen peroxide formed as a result of the reaction it catalyzes. The hydrogen peroxide generate may cause impairment of platelet aggregation. The enzyme was not lethal but exhibited strong edema-inducing activity in rat.

(vi) Phospholipase A_2 enzymes

Preliminary studies indicated the presence of four acidic phospholipase A2 enzymes in the venom (Tan *et al.*,1986). Tsai *et al.* (2000) reported the isolation

of three acidic PLA_2 and one basic PLA_2-homolog from the venom Some of these phospholipases A exhibit anti-platelet activity.

(vii) Other venom constituents

The venom contains at least four different basic proteases with molecular weight of approximately 25000, all of which exhibit moderate edema inducing activity It also contains more than nine different forms of arginine ester hydrolases, five of which exhibit thrombin-like enzyme activity. Two minor forms of arginine ester hydrolase also exhibit arginine amidase activity (Tan, 1991).

The venom also contains autopharmacological factors that may cause local swelling. The factors may release bradykinin which eventually can lead to shock.

Like all other snake venoms, Malayan pit viper venom contains hyaluronidases which are hydrolytic enzymes that function as 'spreading factor' of the venom.

Malayan pit viper venom contains high level of 5'-nucleotidase acitivity but moderate to low levels of phosphodiesterase and alkaline phosphomonoesterase activities. Little is known about the toxicological properties of these enzymes.

Antivenom therapy of Snake Venom Poisoning

Antivenom therapy is the only specific treatment of snake venom poisoning that is of proven value. If used correctly, it can reverse most systemic poisoning even when given hours and days after the bite, in the case of pit viper bites. Antivenom, however, is generally ineffective in preventing or lessening local necrotic effects of snake venom poisoning.

The specific, monovalent antivenoms are more effective antidote in snakebite. At present, however, only a few monospecific anivenoms are available for snakebites in Malaysia, and these include anti-Malayan pit viper, anti-Cobra, anti-Sea Snake and anti-King cobra. Our studies suggest that the Polyvalent

Trimeresurus Antivenom (National Institute of Prventive Medicine, ROC) is effective against most Malaysian *Trimeresurus* venoms, and the Polyvalent Elapid Antivenom produced by the same institute is effective against Malaysian cobras and kraits (Tan, 1998).

Application of Enzyme-linked Immunosorbent Assay (ELSA) in the diagnosis of the biting species

The identification of the biting species is important so that the victim can be given the right type of monospecific antivenom. Diagnosis of the biting species can be ascertained by identification of the snake if the patient brings the snake to the hospital. In the absence of the snake, diagnosis can be made by clinical observations. Accurate diagnosis of the biting species, however, can be made by ELISA method.

Double sandwich ELISA has been developed for the diagnosis of bites caused by most Malaysian poisonous snakes (Tan et al., 1992). The ELISA method is very specific and the venom concentration can be readily quantitated.

Application of ELISA in snake venom research

Information on serum kinetics of envenoming is important in the development of a personalized modality of treatment of snakebite, where the amount and frequency of antivenom administration is to be based on the amount of venom injected, and the kinetics of venom distribution in the body. For example, in experimental king cobra venom envenomation, it was found that the level of the major lethal neurotoxin reached peak level 72 hours after the bite (Tan *et al*, 1994).

Prphylactic action of *Mucuna pruriens* against snakebite

Seed of the Nigerian traditional medicinal plant *Mucuna pruriens* exhibits prophylactic action against snakebite in Nigeria, and it has also been used locally for treatment against snakebites. Preliminary investigation indicated that the

prophylactic action of the seed is due to its ability to elicit antibodies that neutralize certain toxic components of Nigerian snake venoms, including phospholipase A.

The immuoreactivity of mouse anti-Mucuna pruriens extract (MPE) IgG against the medically important Malaysian snake venoms was examined using Western blot analysis. Anti-MPE IgG reacted against some proteins from venoms of King cobra, Malayan cobra, Malayan krait and Malayan pit viper, but not that of shore pit viper.

The reactive proteins in most of the venoms appeared to be phospholipases A, indicating that MPE seed extract shared common epitopes with many snake venom phospholipase A. However, several high molecular weight proteins in Malayan pit viper venom were also reactive. The preliminary study suggested that *Mucuna pruriens* may also offer prophylactic protection against Malaysian snake venom poisoning (Marinello *et al.*, 2004).

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