INTRODUCTION

Australia is renowned as the home of the world’s most venomous snakes. Approximately 140 different snake species call Australia home. At least 81 of these species are venomous, with over 20 species classified as hazardous to animals and humans. Even though we have four families of snakes in Australia (Typhlopidae, Pythonidae, Colubridae and Elapidae), all the venomous snakes are restricted to the elapids, with the exception of the Brown Tree Snake (Boiga irregularis), a colubrid.

Elapid venom may be divided into the components of prothrombin activating enzymes, lipases and peptidic neurotoxins. Many species have prothrombin activating enzymes in their venoms, the vast majority contain phospholipase A2s (PLA2 - the major of the multiple lipases found in Australian elapid venoms) and all Australian elapid venoms are suspected to contain peptidic neurotoxins. The action of these venom components may result in neurotoxic, haemotoxic, cardiovascular, myotoxic and secondary nephrotoxic effects. Marked variability may occur in venom composition between and within snake species, resulting in varying toxicity between species and also potentially unreliable clinical syndromes developing subsequent to envenomation.

ELAPID VENOM COAGULATION EFFECTS

It has been estimated that over 6000 cases of snake bite occur in domestic animals each year in Australia alone. The value of the Australian veterinary elapid envenomation load is of extreme importance for obtaining further clinical data on this condition, as cases of snake envenomation in people continue to dwindle in number.

Coagulopathy is a significant cause of both morbidity and mortality in these patients, either directly or indirectly. The venom effects on coagulation parameters result from procoagulants and anticoagulant enzymes within the venom. The procoagulants in snake venom are prothrombin activators which ultimately lead to a consumptive coagulopathy recognized as snake venom-induced consumptive coagulopathy (SVICC). A massive intravascular coagulopathy ensues from the massive procoagulant effect, which clinically mimics disseminated intravascular coagulation (DIC). However, the syndrome is technically different from DIC as there is no evidence of platelet consumption. Multiple venom factors act on the coagulation pathways, inhibiting coagulation (anticoagulant effect). However in comparison to the SVICC, these anticoagulant factors produce relatively minor clinical effect, and mild elevations in clotting profile tests occur only in isolation.

AUSTRALIAN ELAPID SNAKE VENOM PROTHROMBIN ACTIVATING ENZYMES

A number of snake venoms contain an activator of prothrombin, with these enzymes differ markedly in their structure, properties and the cofactors they may or may not require for their maximal activity, resulting in four distinct groups, A-D. Australian elapid snake venom prothrombin activating enzymes are serine proteinases and therefore classified as group C and D prothrombin activators. Uniquely, Pseudonaja textilis venom appears to contain prothrombin activators from both groups, although some research suggests that Textarin D is simply the catalytic unit of Pseutarin C.

Group C prothrombin activators

Group C prothrombin activators are structurally and functionally similar to factor Xa-Va complex of the mammalian coagulation system. Like the factor Xa-Va complex, the group C prothrombin activators are comprised of two subunits, a catalytic serine protease resembling factor Xa and one non-enzymatic subunit with factor V like resemblance. Examples of Group C prothrombin activators include Oscutarin C and Pseutarin C, which have been isolated from the taipan (Oxyuranus scutellatus) and eastern brown snake (Pseudonaja textilis) respectively. The catalytic action of Pseutarin C is enhanced by but not completely dependent on the presence of phospholipids and calcium due to the presence of gamma-carboxyglutamic acid residues which enables Oscutarin C to bind to phospholipid. Pseutarin C, which was purified from Pseudonaja textilis, also induces a clot in FV and FX deficient plasma and the activity is enhanced fourfold in the presence of calcium.

Group D prothrombin activators

Group D prothrombin activators are factor Xa-like serine proteases whose activity is dependent on the presence of factor Va, phospholipids and calcium. Group D prothrombin activators have been isolated from the venom of the tiger snake family (Notecharin D and Notanarin D from Notechis scutatus scutatus and N. ater niger venom respectively), Tropidechis carinatus (Trocarin D), P. textilis (Textarin D), and Hoplocephalus stephensi (Hopsarin D). As research into the prothrombin activators expands, further characterisation of these
activators will continue to be revealed.

**SNAKE VENOM INDUCED CONSUMPTIVE COAGULOPATHY.**

The most important snake envenoming syndrome is venom-induced consumption coagulopathy (VICC). It is characterised by activation of the coagulation pathway by prothrombin activators in the venom of a number of different snakes. Snake venom induced consumptive coagulopathy (SVICC) develops early after envenoming due to components of elapid venoms, which activate prothrombin, or have a thrombin like action, which in turn leads to fibrinogen consumption, partial prothrombin consumption, as well as consumption of factor V and VIII. SVICC therefore results in marked prolongation of coagulation times and increase in fibrinogen degradation products. The onset of development of SVICC is rapid from moment snake envenomation occurs, with speed of onset varying between species.

The method and degree of fibrinogen and other coagulation factor consumption in cases of SVICC may vary between snake venoms. Many procoagulant toxins (e.g. thrombin-like enzymes) act directly on fibrinogen and consume it rather than converting it to fibrin to allow cross-linking. In contrast, procoagulant toxins which activate the coagulation pathway above thrombin cause conversion of fibrinogen to fibrin and its cross-linking. Due to the activation of the fibrinolytic pathways, large amounts of fibrin degradation products (FDP) are produced. In Australia, SVICC has been classified as complete VICC if there is complete fibrinogen consumption or partial VICC if the patients have low but detectable fibrinogen levels. In animals, SVICC has been shown to indicate evidence of a lethal envenomation, if it occurs in combination with preparalytic signs.

**SVICC VS ANTICOAGULANT EFFECTS**

A central feature of the clinical pathology produced by envenomations by members of the procoagulant group is SVICC (snake venom induced consumptive coagulopathy) produced by powerful coagulants present in the venoms. This feature of the clinical pathology is characterised by the disappearance of fibrinogen from the blood through the procoagulant process, prothrombin activation, which results in a net effect of incoagulable blood. In contrast, non-procoagulant species produce effects that are more along the lines of classical elapid envenomation and neurological effects from post- and pre-synaptic blockage may be severe. Some of these species, Pseudechis australis (Mulga snake) in particular, can also produce severe disruption of haemostasis but fibrinolysis is not a feature and the syndrome is purely anticoagulant in its action.

**OTHER COMPLICATIONS OF PROTHROMBIN ACTIVATION**

In addition to local bleeding from the bite site, venipuncture sites and from the gums, prothrombin activation may lead to gastrointestinal bleeding (haematemeses and melaena), ecchymoses, discoid haemorrhages, haematuria, haemoptysis and intracranial bleeding. Not all procoagulant coagulopathies caused by snake venoms, especially the Australian elapids, are so straightforward. Experiments in dogs have shown that for envenomation involving the eastern brown snake (Pseudonaja textilis), there can be a brief period of true coagulation, with thrombus formation, as the venom first reaches the circulation and before fibrinolysis is activated. The resultant thrombi can occlude critical vessels, notably coronary vessels, resulting in cardiac arrhythmias and arrest. These thrombi are quickly destroyed once fibrinolysis activates, but even a few minutes of such thrombotic complications can be devastating. This is likely the cause of early cardiac collapse previously documented in cases following brown snake bite, which is unpreventable by antivenom. Similar effects have been documented in tiger snake (Notechis) envenomation.

**CURRENT COAGULATION TESTS.**

Current tests utilised in the identification of SVICC include plasma based coagulation assays, D-dimer and fibrinogen degradation products. However these tests are limited in the information they provide to the clinician as they only examine part of the coagulation cascade, require multiple tests to be performed, do not include a platelet component, or cannot be analysed at the bed side.

**THROMBOELASTOGRAPHY.**

Traditional thromboelastography (TEG) allows for the assessment of the entire coagulation system from initiation of clot formation to fibrinolysis, using a very simple, automated process involving a small sample of whole blood. The blood sample is placed into the cup, the pin lowered into the sample and the sample cup is oscillated through an angle of 4°. As a clot begins to form, fibrin fibres begin to build between the pin and the cup, causing the pin to move. The degree and speed of movement is recorded and converted to an electrical output of clot quality and dynamics (a thromboelastogram) within 4-8 minutes.

Although a simple process in theory, the use of the traditional TEG is time consuming (2.5 hours using citrated blood to obtain all values) and requires skilled technicians to ensure minimal intraoperator and interoperator
TEG CHANGES IN DOGS WITH ELAPID SNAKE ENVENOMATION, SPECIFICALLY TIGER SNAKE ENVENOMATION.

TEG takes into account the interactions between numerous constituents of the coagulation processes and is thought to reproduce results similar to the in vivo situation. TEG has not previously been used to evaluate elapid snake envenomated veterinary patients. Two human studies, involving the coagulation effects of another Elapid, the King Brown Snake and Malayan Pit Vipers venom have previously been published. The aforementioned in vitro human elapid study found that the venom caused hypocoagulability and that TEG results paralleled the known in vivo effects of the venom. Therefore TEG has been shown to have the potential to provide information useful in classifying and monitoring coagulation effects in cases of snake envenomation.

Thromboelastography has been used to assess crotalid snake envenomation in dogs but information on the effects of elapid venom on viscoelastic features of clot formation in dogs is currently lacking in the veterinary literature. A prospective, observational study of tiger snake envenomation dogs is currently underway, with the overall objective to describe thromboelastographic coagulation abnormalities associated with tiger snake envenomation in dogs. The evidence obtained so far from the aforementioned study of tiger snake envenomated dogs has shown that thromboelastography is a promising method of monitoring the viscoelastic coagulation changes associated with SVICC. It has been shown that TEG readout from severely envenomated dogs (tiger snake) will display a flat line, due to the coagulation cascade being unable to activate in the defibrinated blood in SVICC affected patients and therefore failure of the TEG trace to move from baseline. This flat line is indicative of SVICC process having occurred in the envenomated patient. Repeated assessments of envenomated patients using TEG during hospitalisation then shows that SVICC resolves 12-18 hours after envenomation.

Concurrently, an associated bench top in vitro observational study, assessing various dilutions of tiger snake venom additions to citrated canine whole blood has commenced. Although this study is still in the development stages, it has been shown that tiger snake venom will cause citrated canine blood to clot within a few minutes. This initially hypercoagulable state is indicative of the extreme prothrombin activation that occurs in the initial stages of SVICC. This initial procoagulant state has been thought to occur with direct intravenous envenomation leading to cardiac arrhythmias and even sudden death as a result of embolic shower in cases of SVICC. Once these promising studies are completed, the findings may lead to the addition of thromboelastography to our coagulation assessment modalities in elapid snake envenomation. Furthermore, these findings may enable the veterinary field to develop a more uniform approach to the diagnosis, treatment and monitoring of patients presenting with elapid snake envenomation.

REFERENCES