Effects of taipan (*Oxyuranus scutellatus*) venom on erythrocyte morphology and blood viscosity in a human victim *in vivo* and *in vitro*

Christopher K. Arthur, Dugald McCallum, Diane J. Loveday, Anne Collins, James P. Isbister and Malcolm McD. Fisher *Departments of Haematology and Intensive Care, Royal North Shore Hospital, St Leonards, Sydney, NSW 2065, Australia*

Abstract

The case of a snake handler with envenoming due to Australian taipan (*Oxyuranus scutellatus*) showing marked morphological changes in his red blood cells is presented. The red cells underwent sphero-echinocytic transformation and in subsequent experiments *in vitro* the effects of taipan venom on red cells were further characterized. Taipan venom induced sphero-echinocytic transformation at nanogram/ml concentrations and led to a marked increase in whole blood viscosity. These changes have not been featured in previous reports of taipan envenomation and are reported to highlight the diagnostic value of blood film examination in cases of suspected envenomation. The significance of the hyperviscosity, and consequent reduction in blood fluidity, is unknown and requires further investigation.

Introduction

The Australian taipan (*Oxyuranus scutellatus*) ranks with the world's most venomous snakes and numerous fatalities have occurred following its bite. Taipan venom contains several components including neurotoxins, myotoxin, pro-coagulants, and phospholipases. The case of a snake handler envenomed by a small taipan is reported to highlight the changes that occur in red blood cell morphology and blood viscosity.

Case report

A 43-year-old zoo snake handler presented 90 min after being bitten by an Australian taipan about 25 cm in length. The patient believed he had been only lightly 'scratched' by the snake's fangs and delayed seeking medical attention until after one hour, when he developed pain in the 2nd web space of the right hand with systemic symptoms. He complained of peri-oral paresthesiae, blurred vision, vomiting, dyspnoea, and severe headache.

On examination he was distressed, muscle fasciculations were present and tender right axillary lymph nodes were palpable. Blood pressure was 140/85 mm Hg, respiratory rate 20/min, pulse 80/min and regular. The urine was dark and positive for haemoglobin (using a benzidine reagent). The results of biochemical and haematological investigations are shown in Table 1. The blood film was grossly abnormal, showing sphero-echinocytes, small spherocytes and fragmented cells (Fig. 1).

Because of a history of previous reactions to antivenom the patient was pretreated with intravenous saline, methylprednisolone, adrenaline, and promethazine before taipan antivenom (12 000 units intravenously). Three units of fresh frozen plasma and 5 g of fibrinogen concentrate were also given. Mannitol was added to the saline infusion because of the possibility that the dark urine was the result of rhabdomyolysis or intravascular haemolysis.

Within 10 min of completing the antivenom infusion the patient was symptomatically improved, and

Table 1. Case of envenoming due to *Oxyuranus scutellatus*: haematology and biochemistry

	Day 1 (1615 h)	Day 2 (0630 h)
Haemoglobin (g/litre)	173	147 g/litre
Leucocytes (per litre)	11×10^9	Not done
Platelets (per litre)	92×10^9	200×10^9/litre
Total bilirubin (μmol/litre)	16 μmol	35 μmol/litre
Conjugated bilirubin (μmol/litre)	3 μmol	5 μmol/litre
Creatinine (mmol/litre)	0·13	0·11 mmol/litre
Urea (mmol/litre)	6·8	6·8 mmol/litre
Creatine phosphokinase (units/litre) (<150 units/litre)[a]	145	255 units/litre
Whole blood clotting time (5–11 min)[a]	>4 h	Not done
Prothrombin time (12–16 s)[a]	>120 s	16 s
PTTK (40 s)[a]	>240 s	44 s
Thrombin time (14 s)[a]	>120 s	20 s
Reptilase time (14 s)[a]	>120 s	Not done
Fibrinogen (g/litre) (2–4 g/litre)[a]	<0·1	1·7
Fibrinogen products degradation (μg/dl) (<10 μg/dl)[a]	>20 000	1280–2560

[a]Normal or (for PTTK, thrombin and reptilase) control values; PTTK = partial thromboplastin time (kaolin).

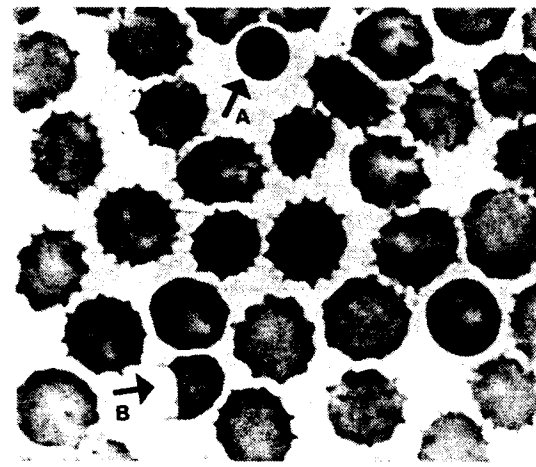


Fig. 1. Peripheral blood film showing sphero-echinocytic change of most red cells. A small spherocytic red cell is shown at A and a fragmented red cell at B, indicating that some cells are damaged in the circulation. (May-Grunwald Giemsa stain, magnification×500.)

within a few hours of treatment symptoms resolved apart from tenderness in the right axilla. The dark urine cleared following the initiation of a diuresis. No complication was encountered and the patient was discharged 24 h after admission.

Experimental Materials and Methods

Full blood examination was performed by an automated cell counter (Coulter Counter, Coulter Diagnostics). Blood films were made on washed glass slides with freshly collected blood samples and stained by an automatic staining machine using May-Grunwald-Giemsa stain. Staining solutions were made up daily and regular quality control avoided artefactual abnormalities in the preparation of blood films. Biochemical estimations were performed on a SMAC II® Multi-channel autoanalyser (Technicon Industries). Coagulation studies were performed manually by standard methods (DACIE & LEWIS, 1984). Fibrin degradation products were measured by a latex agglutination method (Wellcome Diagnostics).

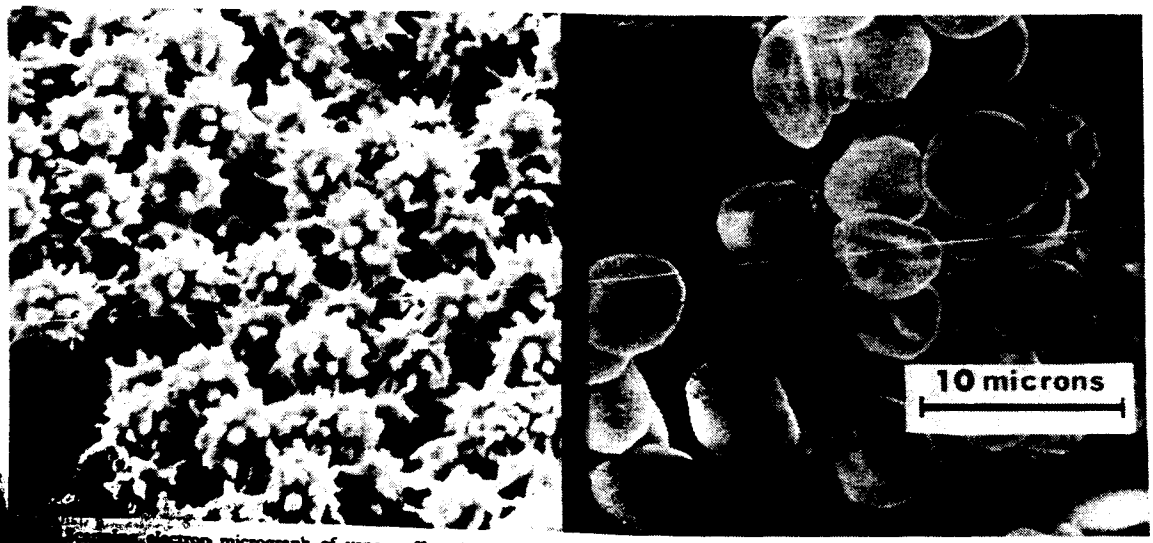
Studies of venom *in vitro*

Studies *in vitro* were performed by incubating normal donor defibrinated blood with taipan venom (kindly supplied by Dr Schumack, Macquarie University, Sydney). The venom was diluted to a concentration of 1 μg/100 μl, and 100 μl were added to 5 ml of defibrinated blood to give a final concentration in blood of 200 ng/ml. This concentration was chosen because it approximates to the expected blood levels from 1 mg of venom injected intravenously. We believe this is a conservative assumption on the following grounds. The average venom yield from milking taipans is 120 mg, and probably much less than this is injected into the tissues following a bite. Much of the venom remains in the tissues and only a fraction enters the circulation. One mg is less than 1 % of the average milking yield, but even if this is an overestimate in the systemic circulation it is likely that red cells would still be exposed to relatively high concentrations in the regional circulation of the bite area.

The blood was incubated with venom for 5 min and 20 min and then whole blood viscosity and plasma viscosity were measured. A small aliquot was removed for blood film examination and for fixation in glutaraldehyde for electron microscopy. Plasma viscosity was measured using a capillary viscometer comparing the values against water. Whole blood viscosity was measured using a concentric cylinder rotational viscometer as previously described (PALMER & DENBY, 1981). A sample of defibrinated blood to which no venom was added served as a control. The results are summarized in Table 2. Whole blood

Table 2. Effects *in vitro* of *Oxyuranus scutellatus* venom on defibrinated blood

Test	Venom samples 5 min	20 min	Control
Whole blood viscosity at shear rate 100 sec^{-1} (3·6–5·1 mPas)[a]	4·6	9·1	4·2
at shear rate 0·1 sec^{-1} (38–121 mPas)[a]	122	170	42
Plasma viscosity (1·6–1·9)[a]	1·7	1·7	1·7
Spun haematocrit (%)	48	48	46·5
Coulter haematocrit (%)	43·8	43·4	45·3
Mean corpuscular volume (fl) (76–96 fl)[a]	84	84·1	84·3
Fibrin/fibrinogen degradation products (μg/dl) (<10 μg/dl)[a]	<10	<10	<10
Osmotic fragility (MCF) (g/litre) (4·0–4·45 g/litre)[a]	–	5·5	4·25
Red cell filterability (% cells filtered)	–	14·5	32



...electron micrograph of venom-affected cells after 20 minutes incubation (left), control cells (right).

viscosity was markedly elevated for both high ($100\ sec^{-1}$) and low shear rates ($0{\cdot}1\ sec^{-1}$) in the 20 min venom sample at 9·1 milliPascal seconds (mPa.s) and 170 mPa.s respectively compared with the control value of 4·2 mPa.s (high shear) and 42 mPa.s (low shear). The blood was examined macroscopically and microscopically for fibrin strands that might have caused an increase in viscosity, but none was found. Furthermore fibrin/fibrinogen degradation products were undetectable in the sample, indicating that soluble or insoluble fibrin complexes were not present. The blood viscosity change was due to reduced red cell deformability, as demonstrated by the fall in the percentage of cells passing through a 1·2 μm Millipore® filter. The rigidity of venom-treated red cells was also reflected in their increased osmotic fragility (see Table 2).

Discussion

It has been estimated from animal toxicity studies that the maximum single venom yield from a taipan (400 mg) could kill 50–70 people (CAMPBELL, 1967; O'NEILL, 1980). It is not surprising therefore that this patient developed significant envenoming from the bite of a young taipan that was barely noticed by the patient, although it is part of Australian folklore that significant envenomation will not occur from a snake smaller than a man's finger (S. K. Sutherland, personal communication). The case reported by BRIGDEN & SUTHERLAND (1981) has already highlighted the fact that a bite may go unnoticed by the patient.

The coagulation abnormalities caused by taipan venom have been well described (DENSON, 1969; MARSHALL & HERRMAN, 1983), and are due to the prothrombin activating action of the venom leading to defibrination. Despite the fact that the blood is unclottable after significant envenoming, bleeding is usually not a problem, being manifest only from sites of defective vascular integrity. The treatment of the coagulation defects is primarily with antivenom. Once the cause of the defibrination syndrome is reversed a normal liver will rapidly replenish lost coagulation factors. Coagulation factor replacement should be reserved for patients with bleeding. In this case fresh frozen plasma and fibrinogen were probably not necessary.

Another interesting feature of this case that is rarely mentioned in case reports is the abnormal red cell morphology. Sphero-echinocytes represent a reversible change in red cell membranes that can be induced by several agents including fatty acids and lysolecithin. The microspherocytes probably represent an irreversibly damaged cell that has lost membrane and is destined for early destruction. Taipan venom contains phospholipases A and B (DOERY & PEARSON, 1961, 1964) and this could account for the red cell changes seen since the phospholipase can attack cell membranes, releasing lysolecithin. The red cell shape changes due to snake venoms have been reviewed by CONDREA (1979). The increased whole blood viscosity in the sample containing venom is expected because blood viscosity is dependent on red cell deformability. Spherocytic and fragmented red cells are less deformable than normal red cells. The clinical relevance of the viscosity changes demonstrated *in vitro* is not known. Viscosity studies were not performed on the patient's blood during the admission, so it is not possible to comment on the viscosity changes following envenoming. However, it is likely that some increased viscosity occurred because the red cell changes in the blood film were so marked. It is possible that hyperviscosity could be detrimental and influence the clinical features of snake bite by compromising the microcirculation and contributing to neurological impairment and other organ damage. This subject requires further investigation.

Our patient also had laboratory features of a mild haemolytic reaction. A haemolysin of relatively low activity has been found in taipan venom (DOERY & PEARSON, 1961).

The finding of abnormal coagulation results in severely ill unconscious patients of unknown cause has been useful as a diagnostic pointer towards snake bite (e.g., in the case reported by BRIGDEN & SUTHERLAND, 1981) and is also useful as a marker of systemic envenoming. We also advocate review of the blood film and red cell morphology as another indicator of envenoming.

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