COAGULOPATHY FOLLOWING PAPUA NEW GUINEAN TAIPAN (*Oxyuranus scutellatus canni*) ENVENOMING

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SUMMARY

The mechanisms of coagulopathy were studied in 13 patients with incoagulable blood following bites by the Papua New Guinean taipan (*Oxyuranus scutellatus canni*). Standard coagulation assays demonstrated profound defibrination and reduced factor II levels consistent with a prothrombin activator in taipan venom. Moderate deficiencies of factors IX, XI and XII were also demonstrated, although these factors were also low in the control population suggesting a possible racial variation. Reduction in Factor XIII subunits, plasminogen and alpha-2 antiplasmin, together with elevated cross-linked FDPs indicated activated fibrinolysis, although the much higher total FDP indicated that fibrinogenolysis is also occurring. The results indicate that a consumption coagulopathy, due in part to the action of the prothrombin activator, is induced by taipan envenoming. There is also evidence that taipan venom may contain other haemostatically active components which contribute to the clinical picture.

Introduction

Snake bite is a considerable problem in the low-lying regions of Papua New Guinea. Over 100 systemically envenomed patients are admitted to Port Moresby General Hospital each year and enzyme immuno-assay indicates that 88% of these are due to envenoming by the taipan (Currie et al 1990, Lalloo et al 1991). The predominant picture in these patients is of incoagulable blood, bleeding from the bite site, venepuncture sites and gingival sulci and neurotoxicity which necessitates ventilation in approximately 25 % of these patients. (Campbell 1967, Lalloo, unpublished observations). This preliminary study was performed as part of a larger on-going prospective study of the clinical pattern and laboratory features of envenoming following snakebite in the Central Province of Papua New Guinea.

Methods

Patients

13 patients (10 males, 3 females, aged 7-45 years) admitted to Port Moresby General Hospital with incoagulable blood following snake bite were studied. Envenoming by *Oxyuranus scutellatus canni* was confirmed by detection of venom antigen in the patients serum. Full clinical details are available for 10 of the patients. All patients were treated with 1 ampoule of polyvalent Australia-New Guinea antivenom (CSL, Australia); one

patient received an additional ampoule of Black snake antivenom (CSL). All patients recovered following the administration of antivenom. Control samples were taken from 32 healthy Papua New Guinea controls matched for socio-economic status. For the factor assays, the reference was a pool of plasma derived from 20 healthy subjects in London, UK.

Blood samples

Samples were taken upon admission prior to antivenom and at six hourly intervals following the administration of antivenom. 1) 2ml of blood was placed in a glass test tube for determination of the whole blood clotting test (Warrell et al 1977). The serum was subsequently allowed to separate, centrifuged and stored at -70° C until assayed for venom antigen. 2) 9 ml of blood was added to 1ml of tri-sodium citrate (31.3g/l). This was immediately centrifuged and the plasma was stored in 1.5 ml aliquots for coagulation assays. 3) 2.5 ml of blood was placed in an EDTA tube for determination of platelet count. 4) A 2ml sample was collected into a FDP tube (Wellcome Diagnostics) containing thrombin/soy bean trypsin inhibitor, incubated for 30 min at 37°C and the serum stored.

Whole blood clotting test

Blood was left undisturbed for 20 min. The test was considered positive if the blood was incoagulable at this time.

Coagulation assays

Screening tests of haemostasis were performed using standard methods (Denson 1972). Fibrinogen was measured by the Clauss method (Clauss 1957). Other coagulation factors assays were carried out by one-stage assays using the coagulation channel of the ACL 300R coagulometer (Instrumentation Laboratories). Anti-thrombin III, plasminogen and alpha-2-antiplasmin were assayed using Chromostrate kits (Organon-Teknika) and Protein C by the method of Bertina (1984), all using the chromogenic channel of the ACL 300R. Von Willebrand factor antigen (vWF:ag) and plasminogen activator inhibitor type 1 (PAI-1) were measured by ELISA using reagents obtained from DAKO Ltd and a kit from Biopool Ltd, respectively. Factor XIIIA and S subunits and C1 esterase inhibitor were assayed by Laurell electroimmunoassay (Laurell 1966). Kits containing antibody coated latex beads were used to measure fibrin(ogen) degradation products (FDP, ThromboWellcotest, Wellcome Diagnostics) and cross-linked fibrin degradation products (XDP, Dimertest, Baxter Ltd).

Venom antigen detection

Serum was tested for the presence of *Oxyuranus scutellatus canni venom* by enzyme immunoassay using the method of Theakston et al (1977) with the modification described by Ho (1986). Microsorp F96 microtitre plates (Nunc Gibco Ltd, UK) were coated with 100µg/ml rabbit anti-whole *Oxyuranus scutellatus canni* venom IgG (protein

A-derived) in coating buffer at 4°C overnight. At all stages 100µl volumes were used in each well. Following washing, plates were post-coated with 1% bovine serum albumin (Sigma Chemical Co, UK) for 1 hr as a blocking step, and subsequently rewashed. Test samples, together with a series of venom standards (500-0.1ng/ml) and appropriate positive and negative controls diluted 1:10 in incubation buffer were then added in duplicate to the wells. To each of these was also added 1% normal rabbit serum (10µl/ml) incubation buffer to prevent non-specific reactions. Following incubation at room temperature for 4 hr, plates were rewashed. A 1:500 dilution of rabbit anti-*Oxyuranus scutellatus canni* venom IgG alkaline phosphatase (Sigma Ltd, UK) conjugate in incubation buffer was added and the plates were incubated at 4°C overnight. They were subsequently rewashed and the substrate (p-nitrophenyl phosphate disodium, Sigma Ltd., UK; 1mg/ml in diethanolamine buffer) was added. Following generation of colour, the optical densities were read on a Titertek Mulitskan ELISA 'through-the-plate' reader (Flow Laboratories, UK) after 30 min substrate incubation. Venom levels in the test samples were estimated by reference to the standard curve. The baseline for the venom assay was established by assaying 96 serum samples from individuals who had never been exposed to snakebite and who were from the same socio-economic background as the patients. After 30 min substrate incubation, the mean optical density was 3.0 (0.1 SEM) ng/ml.

Platelet count

Platelets were counted manually using a modified Neubauer counting chamber.

Results

All of the patients had a positive whole blood clotting test (incoagulable blood at 20 min). Two out of ten patients had bleeding from the gingival sulci, one was bleeding from a knife wound over the bite site, one was bleeding from a venepuncture site, and one was bleeding from both the gingival sulci and the bite site. Other clinical finding are detailed in figure 1. Sequential whole blood clotting tests in eight of the patients showed that all but one had coagulable blood six hours after the administration of antivenom; the exception demonstrated coagulable blood at twelve hours. The change in activated partial thromboplastin time following antivenom is shown in six patients in figure 2. The single patient whose APTT was not immediately corrected by antivenom had bleeding from the gingival sulci upon admission, which had stopped by six hours and had no further haemorrhagic episodes.

Platelet counts were less than 100,000 in four out of ten patients; none of these demonstrated systemic or local bleeding.

The results of coagulation assays are shown in table 1. All of the 13 patients showed profound defibrination and factor II levels were invariable below those in the controls. Factor V and VII levels were markedly reduced, and Protein C, the physiological inhibitor of these factors, was also below normal in every case. Factors IX, XI and particularly factor XII levels were also deficient in many cases but the interpretation of this is confounded by the fact that many of the healthy Papua New Guinean controls demonstrated levels of these factors below that of the reference plasma from 20 healthy UK subjects. There was a reduction in factor XIII sub-units, plasminogen and alpha-2 antiplasmin, whereas von Willebrand factor antigen was above the "local" normal range in many patients. Both FDPs and XDPs were elevated, the former more markedly so.

Oxyuranus scutellatus canni venom was detected in the serum of all of the patients, with admission venom levels ranging from 10-251 ng/ml (geometric mean 32.8).

Discussion

Taipan venom has been shown to contain a prothrombin activator, the action of which is enhanced by the addition of phospholipids and Ca^{2+} but not by activated factor V (Denson 1968, Pirkle et al 1972, Speiger et al

1986). It thus appears to consist of a factor XA-like enzyme and a factor VA-like co-factor (Speiger 1986). In the presence of platelets which provide phospholipids, this is analagous to the prothrombinase produced during normal haemostasis. It would convert prothrombin to thrombin and activate factor XIII, leading to formation of cross-linked fibrin. The thrombin, either directly or by activating the Protein C pathway, would also result in the proteolysis of factors V and VIII. The profound defibrination, the reduction of factor II, V, VIII and Protein C levels and the reduction in factor XIIIA and XIIIS subunits seen in envenomed patients in the present study, are all consistent with this mechanism.

The low plasminogen and alpha-2 antiplasmin levels and the elevated XDP concentration support the contention that physiological activation of fibrinolysis i.e. secondary to fibrin deposition is occurring. However, that this is not the sole or even the primary cause of the coagulopathy, is suggested by the observation that most of the FDPs which are detected after taipan envenoming are not cross-linked and presumably are thus derived from lysis of either non-cross-linked fibrin or of fibrinogen. Taipan venom does indeed clot fibrinogen directly, albeit less effectively than many crotalid venoms, and it also appears to contain a weak directly-fibrinolytic component (Marshall and Herrman 1998). Further studies of these potentially important mechanisms are underway in our Unit.

The observation that the average level of several coagulation factors, notably factors IX, XI and XII, was reduced in the controls from Papua New Guinea in comparison with the UK reference plasma suggests that there may be an ethnic variability which needs to be substantiated. Notwithstanding these possible physiological differences, envenomed patients showed even lower levels of factors IX,XI and particularly factor XII. One possible mechanism for this would be the activation by taipan venom of the complement and/or kinin generating systems, since these systems are intimately linked with the early (contact) stages of the coagulation cascade. Elucidation of these interactions forms part of our ongoing studies. However, these changes are not likely to be of great importance with regard to the coagulopathy, since even severe factor XII deficiency is not normally associated with haemorrhagic complications and in the majority of our cases, the extent of the deficiency in factors IX and XI was insufficient to cause major bleeding problems.

The high levels of Von Willebrand factor (vWF) may be a consequence of venom-induced damage to the vessel wall since this factor is synthesised and released from endothelial cells. However, in this preliminary study, we did not observe elevated levels of PAI-2, another endothelial cell-derived factor. Moreover, the venom component usually implicated in vessel wall damage is a

haemorrhagin, which few elapids have been found to possess (Ohsaka 1979) Further studies on the action of taipan venom on vessel wall components and on vWF structure and function are currently underway.

These preliminary studies confirm the clinical importance of the prothrombin activator in taipan venom and suggest potentially important additional mechanisms for the coagulopathy occurring in many envenomed subjects. We have found no correlation to date between any of the haemostatic factors and the presence of local or systemic bleeding, apart from the association with defibrination. A further 70 cases are being currently analysed with a view to defining laboratory markers of defibrination and potential haemorrhagic complications.

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