

The kinetics of snake bite envenoming and therapy

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Introduction

The only scientifically and medically approved treatment of envenoming by snakes is the use of intravenously administered antivenom. This is usually prepared by hyperimmunisation of large animals, usually horses, and subsequent salt precipitation and enzyme (pepsin) digestion of the immunoglobulin G to produce an active neutralising F(ab)₂ fragment¹. The dose of antivenom, which may be either monospecific (active primarily against a single venom species) or polyspecific (active against a range of 2-5 different venom species), depends on the neutralising efficacy and also the route (usually intravenous) by which it is administered.

In the past, treatment of envenomed victims with antivenom has been carried out without any real scientific criteria as to the optimal dose. The amount of antivenom given to a patient currently depends on the individual clinician's experience in assessing the severity of envenoming based on clinical signs (eg local and systemic haemorrhage, incoagulable blood, neurotoxicity, myotoxicity, local swelling and necrosis and a range of other effects)².

This review considers the application of more objective methods of assessment involving the use of enzyme immunoassay for estimating the course of envenoming by medically important snakes in human victims, and assessing the effect of antivenom therapy, first aid and traditional methods in eliminating venom antigenaemia.

Some areas of the world where snake bite is of medical importance

In Nigeria, bites by the carpet viper (*Echis ocellatus*) constitute a major problem in farming communities^{3,4}. Envenoming by this species results in local and systemic haemorrhage, incoagulable blood and local necrosis. On the basis of these signs, two ampoules (20 ml) of a monospecific *Echis* antivenom (South African Institute for Medical Research) or four ampoules (40 ml) of either a French (Institute Pasteur) or German (Behringwerke) polyspecific antivenom have been recommended as a starting dose in the past^{5,6}.

In Sri Lanka, the incidence of snake bite is currently one of the highest in the world with a mortality of 6/100,000/year and an incidence exceeding 400/100,000/year⁷. The most important species is Russell's viper, *Daboia russelli pulchella*, and systemic envenoming involving haemorrhage, coagulopathy, neurotoxicity and myotoxicity, is normally treated with a starting dose of 5-15 (50-150 ml) of imported Indian (Haffkine or Serum Institute of India) polyspecific antivenom¹.

In Brazil, patients bitten by the Jararaca (*Bothrops jararaca*) are treated with a starting dose of 4 (40 ml), 8 (80 ml) or more ampoules of *Bothrops* polyspecific antivenom^{1,8}. The dose depends on the severity of envenoming as assessed by the individual clinician treating the patient².

Methodology used for investigating the problem

The development of enzyme immunoassay (EIA) for detection of specific venom antigen^{9,10} and for detection of therapeutic antivenom¹¹ has enabled a more effective assessment of antivenom dosage and efficacy to be made. It is now possible, using this method, to detect and quantify specific venom in the blood or body fluids at any time after the bite, as well as to calculate the amount of therapeutic antivenom circulating at any time after antivenom administration. Figure 1 shows how the kinetics of envenoming and therapy can be assessed when both an effective and a relatively ineffective antivenom is used.

Summary of results obtained

In northern Nigeria the best antivenom used in the past has proved to be the monospecific SAIMR antivenom raised against the venom of Kenyan *Echis*¹. This, in most cases, cleared venom from the circulation within six hours of intravenous administration of antivenom with simultaneous permanent resolution of the clinical signs of envenoming^{12,13} (Figure 1a). The polyspecific German and French antivenoms tested were less effective and, although their use resulted in an initial decrease in venom antigenemia and temporary resolution of the clinical signs after admission, this was often followed by an increase in levels of circulating venom and recurrence of clinical symptoms such as incoagulable blood, due presumably to further influx of venom from a depot area at the bite site^{5,6}. Owing to an unsustained and inadequate level of antivenom in the circulation, this additional venom was

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not neutralised and a further dose (or doses) of antivenom was required (Figure 1b). In a case of envenoming by a Tunisian snake of the *Echis pyramidium* complex, a total dose of over 300 ml of three different antivenoms (one monospecific and two polyspecific) with activity against *Echis* species failed to neutralise circulating venom in the patient demonstrating the lack of specificity of these antivenoms against this particular venom¹⁴.

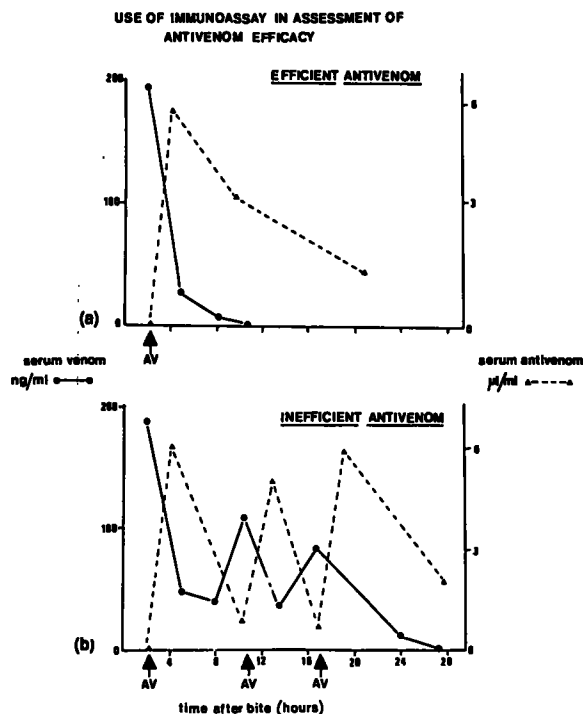


Figure 1. Typical curves demonstrating serum venom and antivenom levels in envenomed patients treated with an effective (a) and a relatively ineffective (b) antivenom.

A similar situation occurred in Sri Lanka where imported Indian (Haffkine or Serum Institute of India) polyspecific antivenom is used. These antivenoms are not very effective against the venom of Sri Lankan *D. russelii pulchella* because they are prepared against the venom of Indian *D. russelii russelii*^{1,7}. Sri Lankan Russell's viper contains some different components to the Indian venom such as a presynaptically-acting phospholipase A₂ (PLA₂) which is also myotoxic⁷. These PLA₂ activities are not neutralised by the imported antivenom⁷.

In Brazil, the three major polyspecific Bothrops antivenoms have been compared clinically in a randomised comparative trial^{2,11}. All the antivenoms produced a high degree of protection and a rapid rate of venom clearance after a single dose of the lowest amount of antivenom

(4 ampoules; 40 ml) recommended for the treatment of moderately envenomed patients². A more recent study demonstrated that even two ampoules (20 ml) was adequate in similarly envenomed patients⁹. The levels of circulating antivenom were maintained at a high level even after the venom antigenaemia had been abolished, and only finally cleared from the circulation after 37 days¹¹, this permitted immediate neutralisation of any additional venom entering the circulation from the depot at the bite site. The results of this particular study indicated both clinically and by immunoassay that the patients had received more antivenom than was actually necessary for neutralising the circulating venom especially in the higher dose ranges (4, 8 and more ampoules) in cases of moderate envenoming.

All these antivenoms are F(ab)₂ fragment preparations^{1,11,15}. More recently we have attempted to assess a novel ovine Fab antivenom, purified by papain digestion, raised against Nigerian *E. ocellatus* venom^{16,17}. Preliminary studies have yielded promising results. Such an antivenom, being a smaller fragment of the IgG molecule than F(ab)₂, has a larger volume of distribution and should theoretically also be less immunoreactive. Although it may have the disadvantage of being cleared more rapidly via the kidney, it may also have the advantage of being more potent than other available products and may also be more effective against local venom effects. Further studies, designed to fully assess this type of antivenom, are currently in progress.

Efficacy of antivenom in treating local effects of venom

The rationale for giving exceptionally high doses of antivenom in Brazil is that some clinicians consider that there may be some beneficial effect in reducing the extent of the local necrosis caused by the cytolytic enzymes present in Bothrops (and other) venoms. Both experimental¹⁸ and clinical (Theakston and Warrell, unpublished observations) studies, however, have shown that although a small amount of F(ab)₂ antivenom does gain eventual access to the area of local necrosis, this is well after the initial rapid and irreversible changes have occurred. In human victims, who usually arrive at hospital hours or even days after the bite, there appears to be no real effect on local lesions¹⁹.

First aid

Investigations in Burmese and Thai patients have attempted to assess the effectiveness of tourniquets as a first aid measure by estimating venom levels before and after tourniquet release²⁰, venom levels distal and proximal to the tourniquet²¹ and admission venom levels in patients admitted with and without tourniquets²². In

these cases tourniquets did not show any enhanced inhibition of spread of venom into the general circulation, but it should be borne in mind that, in the field, tourniquets are often not applied or managed correctly. In the Philippines, tourniquets properly applied have been shown to delay the spread of the major neurotoxin present in the venom of the Philippine cobra, *Naja philippinensis*, into the circulation until release in hospital²³. Likewise in Nigeria one patient, admitted to hospital with *E. ocellatus* bite, had no clinical signs and no detectable venom on admission but developed significant clinical signs (incoagulable blood) with associated venom antigenaemia immediately after release of the tourniquet²⁴. Another died, not because of envenoming, but due to pulmonary thromboembolus, preceded by thrombophlebitis, local necrosis and gas gangrene caused by the late removal of a tight tourniquet which had been in place for 48 hours after the bite²⁵.

The possibility that a smaller Fab IgG fragment, given by the intramuscular route, may be of use in prehospital treatment is currently under investigation. If this system works it could represent a major advance in early therapy. Although preliminary results have indicated that absorption even of an Fab fragment into the circulation by this route is still too slow to be useful in early neutralisation of venom, further more detailed studies are necessary.

Other proposed future studies will involve the objective assessment of so-called traditional and other untested remedies (such as the "black snake stone", high voltage/low current electric shock²⁶) and other somewhat suspect and unproven treatments, using EIA.

Conclusions

The first important stage in the evaluation of either a new or existing antivenom is its testing in an animal (usually rodent model) using assessment of protection against the lethal and other venom effects such as haemorrhagic, local necrotising, defibrinogenating and coagulant activities. WHO recommended tests are available for this purpose^{11,15,27,29}. However, it should be stressed that it is dangerous to extrapolate from animal results to the situation in man for a wide range of reasons (eg unrealistic routes of administration in animals of a preincubated mixture of venom and antivenom^{15,17,30}). The second stage, which is the only truly meaningful method, is therefore testing the experimentally approved antivenom in envenomed humans using both clinical observations and objective measurements of both venom and antivenom levels.

An effective antivenom is one in which a high level of active circulating antibody is maintained in the circula-

tion after initial venom clearance (eg Brazilian Bothrops antivenoms, SAIMR Echis antivenom in Nigeria) in human victims. This will be capable of neutralising any venom subsequently entering the circulation from a depot. Such conditions are usually obtained when the venom or venoms used for producing the antivenom is obtained from snakes present in the country in which it is proposed to use the antivenom. Venoms, even within the same species, vary dramatically in composition from region to region^{31,32}.

The study carried out in Brazil strongly suggests that patients were receiving more antivenom than was necessary, thus resulting in a high incidence (37-87%) of early anaphylactic reactions requiring urgent treatment with adrenalin and antihistamines². A lower but effective dose should decrease the extent of this problem and also result in a reduction in cost: the latter is of major importance in developing countries. There is no real evidence that a high antivenom dose is effective in decreasing or eliminating local venom effects such as necrosis^{8,18}. Both clinical observations and EIA results support these observations.

There is also no convincing general evidence that tourniquets are effective. As there are so many obvious variables, the results of studies using EIA are, not surprisingly, inconclusive^{20,25}. Likewise, further more detailed studies need to be performed on the possibilities of using lower molecular weight Fab fragments or even smaller antibody components, for early treatment following administration via the intramuscular route.

Using a combination of experimental, clinical and immunological assay systems, it is therefore possible to obtain a highly accurate assessment of the efficacy of antivenom and of current first aid procedures.

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