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# Effectiveness of Snake Antivenom: Species and Regional Venom Variation and Its Clinical Impact

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## ABSTRACT

The ubiquity of venom variation in snakes poses special problems for the manufacture of antivenom and has undermined the commercial attractiveness of this class of therapeutic agent. In particular, it has been amply documented that both interspecific and intraspecific variation in venom composition can affect the neutralisation capacity of antivenoms. This may be exacerbated by the selective use of tests of venom toxicity

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and antivenom efficacy, such as the lethal dose and $ED_{50}$, resulting in inadequate neutralisation of time, rather than dose, dependent toxins, particularly enzymes involved in defibrinogenating, haemorrhagic and necrotising venom activities. The clinical consequences can be reduced efficacy against some important venom activities or even complete treatment failure in critical envenomations. All these factors, combined with the ongoing reduction in the number of antivenom manufacturers world-wide, and concomitant contraction in the range of available antivenoms, present significant challenges for the treatment of snakebite in the 21st century.

## INTRODUCTION

The debate about the clinical efficacy of snake antivenoms and the degree of variation in venom composition began soon after production of the first antivenoms in the late nineteenth century (Calmette, 1894; Frazer, 1895; Phisalix and Bertrand, 1894). The 'sérum antivenimeux', developed by Calmette using mainly monocled cobra (*Naja kaouthia*) venom, was initially promoted as a universal snake antivenom. This was a consequence of Calmette's belief in the single mode of snake venom action (Hawgood, 1992).

However, early attempts to neutralise two Australian snake venoms (that of the Red-bellied Black Snake, *Pseudechis porphyriacus*, and the Mainland Tiger Snake, *Notechis scutatus*) with this serum failed (Martin, 1897). Similarly, in 1898 serum from dogs immunised with Brazilian Pit Viper (*Bothrops jararaca*) venom was found to be ineffective at neutralising the venom of the South American Rattlesnake (*Crotalus durissus terrificus*) and vice-versa (Brazil, 1901). This and other work in India, Australia and North America (Lamb, 1904; Russell, 1988; Tidswell, 1902) provided clear evidence of antivenom specificity, undermining Calmette's 'universal' hypothesis. This ultimately led to the development of a series of regional antivenoms based on the venom of medically significant endemic species on the various continents.

Eventually Calmette accepted the concept of two modes of snake venom action (neurotoxic and haemorrhagic) emergent from this antivenom specificity work but he persisted in the view that his serum could neutralise all neurotoxic venoms (Calmette, 1908). The search for the minimal number of modes of ophidian venom action and its significance for antivenom manufacture and utility continues unabated (Mebs and Kornalík, 1981; Vogtman, 1950; Warrell, 1986; Warrell and Arnett, 1976). This review will discuss contemporary issues in interspecific and intraspecific variation in venom composition and their clinical significance.

## Molecular Basis of Venom Evolution

The mutational change of genes is the primary basis for evolution. Genetic drift or natural selection will cause the spread of nucleotide substitutions, insertions/deletions, recombinations or gene conversions through a population, until their fixation within that population. As a result, is well documented that significant venom variation can occur between closely related species or even within a species itself (Assakura et al., 1992; Daltry et al., 1996; Fry et al., 2002; Glenn et al., 1983; Jiménez-Porras, 1964; Yang et al., 1991).

Toxin-encoding genes undergo frequent gene duplication, sometimes followed by diversification into different functions and structures (Kordiš and Gubenšek, 2000; Slowinski et al., 1997). In contrast to mitochondrial protein endoding genes, toxin-encoding genes do not favor one codon for an amino acid over another (Fry, B.G. et al. unpublished results). Also in contrast to mitochondria, mutations in codons are more likely to occur in position 1 rather than positions 2 or 3. In addition non-synonymous substitutions are as likely to occur as synonymous substitutions. These factors combine to lead to a state where mutations are as likely as not to change the amino acid encoded by the codon. A change of as little as a single amino acid can have profound effects upon not only the specificity and potency of a molecule but also upon its antigenicity and thus relative neutralisation by antivenom. This situation is greatly complicated by frequent duplication of toxin genes, with each duplicate evolving rapidly and independently of each other (Afifiyan et al., 1999; Chang et al., 1999). Thus, the fundamental molecular basis of venom evolution favors a multiplicity of actions and consequently a multiplicity of toxins that need to be counteracted by antivenom.

## Snake Systematics

The problem of interspecific differences in venom composition can be overcome to some extent by paying scrupulous attention to the systematics of the snakes involved. Unfortunately, it is clear that many toxinologists do not pay sufficient attention to the systematic status of the snakes they are working with, and as a result, many venoms used in toxinology cannot be attributed to any known species (Wüster and McCarthy, 1996). In a clinical context, misidentification of physically similar species may result in the selection of the wrong antivenom type (Trinca, 1969; Winkel et al., 2001) and, consequently, a fatal outcome (Sutherland and Leonard, 1995).

Although attention to systematics can alleviate some of the problems caused by interspecific snake venom variation, the value of this is limited by our as yet inadequate or developing knowledge of the systematics of

many medically important groups of venomous snake. The systematics of many of the most medically significant groups of venomous snakes, such as Asian and African Cobras (*Naja*), Australian Death Adders, Brown snakes, and Black snakes (*Acanthophis*, *Pseudonaja* and *Pseudechis*), the South American Lanceheads (*Bothrops atrox* complex), Saw-scaled vipers (*Echis*) and Asian Green Pit vipers (*Trimeresurus* spp.) are either in a state of flux or remain poorly understood.

However, an understanding of the taxonomic status of different populations of venomous snakes alone cannot necessarily predict patterns of venom variation. There is ample evidence that venom composition can vary extensively even among populations which are unambiguously conspecific (e.g., *Crotalus scutulatus*—Wilkinson et al., 1991; *Daboia russelii*—Warrell, 1989; Wüster et al., 1992), perhaps as a result of natural selection for geographic differences in diet (Daltry et al., 1996). Such variation can have considerable implications for the effectiveness of antivenoms.

Even broad-spectrum polyvalent antivenoms may not be able to neutralize some of the venom variants present within species that are included in the manufacture of the antivenom: for instance, a broad-spectrum anti-*Bothrops* antivenom, raised from venoms of a number of species groups within the genus, was highly effective in neutralising the venoms of some populations of the *Bothrops atrox* species complex, but almost ineffective against that of other populations of the complex (Wüster et al., unpublished data).

## Venom Toxicity and Antivenom Efficacy Tests

An additional issue impinging on antivenom effectiveness in clinical practice is the definition of snake venom toxicity and antivenom efficacy. The presence of venom variation in this context means that the 'standard' definition of venom toxicity and antivenom efficacy used by commercial manufacturers can be suboptimal when applied in clinical situations. For example, the traditional measure of venom toxicity, the lethal dose (Trevan, 1927), has the disadvantage of being biased towards the most potent venom component and/or those toxins having a maximum effect within the predetermined time frame (Chippaux, 1998). Studies of antivenom effectiveness typically involve the determination of the median effective antivenom dose ($ED_{50}$) at which a sample of experimentally envenomed animals survive within a predetermined observation period after an otherwise lethal injection of venom through a specified route (Chippaux and Goyffon, 1998). In any case, it is becoming increasingly difficult to perform $LD_{50}$ studies for obvious ethical and regulatory requirements. However, venom toxicity and effectiveness of antivenom can be studied using in vitro (Barfaraz and Harvey, 1994; Fry et al., 2001) or insensate in vivo

(Sells et al., 2001) preparations. This enables the investigator to concentrate on a specific system (e.g. neuromuscular junction, blood, atria and blood vessels) that is targeted by a venom component. Furthermore, the effectiveness of antivenom against a toxic venom component acting at a specific system can be studied in detail.

Indeed, the overall toxicity of any venom is related to a variety of components interacting with a multitude of tissues, cells and receptors or substrates over time in a manner dependent on individual pharmacokinetics and dynamics. Consequently, in vitro toxicity and antivenom efficacy testing does not take these into account. In addition, such methods take no account of variable antivenom absorption and distribution, nor of the pharmacokinetics of different toxins, since the venom and antivenom are usually preincubated in vitro (Barfaraz and Harvey, 1994).

When compared to the reactions of the animals used in laboratory testing, it is worth noting the natural resistance of many animals to the venoms of the snakes that prey upon them. This classic tale of co-evolution has been well documented. Most notable of these studies was one that showed the resistance of eels to the venom of the sea kraits (Heatwole and Poran, 1995). The authors showed that moray eels of the genus *Gymnothorax* occuring sympatrically with sea kraits, and thus subject to predation from them, show dramatically more resistance to *Laticauda* venoms than populations from outside the range of the sea kraits. Comparative studies of ground squirrels (*Spermophilus beecheyi*) from areas with an without rattlesnakes yielded similar results (Poran et al., 1987). In the case of the mongoose, the resistance to the neurotoxicity comes down to a five residue difference in the acetylcholine receptor when compared to the equivalent receptor in the highly sensitive mouse (Barchan et al., 1992).

One example of the practical consequence of the focus on small animal survival experiments is the well-recognised limitation of the neutralising potential of the Australian brown snake (*Pseudonaja textilis*) antivenom (CSL Limited, Parkville). The efficacy of this antivenom is assessed in guinea pigs using an $ED_{50}$ assay that does not specifically examine its activity against the fibrinolytic effects of *Pseudonaja* prothrombin activators nor other cardiovascular effects (Sutherland and Tibballs, 2001; Tibballs and Sutherland, 1991). It is of concern that there is growing evidence of the slow, limited and interspecifically variable neutralisation of the cardiovascular and haematological effects of *Pseudonaja* venom by the existing brown snake antivenom (Masci et al., 1998; Sprivulus et al., 1996; Tibballs and Sutherland, 1991).

Similar limitations have been identified regarding the efficacy of Australian tiger snake, but not taipan (*O. scutellatus*), antivenom (Sprivulus et al., 1996). Interestingly, another problem, more related to interspecific venom variation, arises with this latter product. In vitro analysis of

neurotoxicity suggests that, although taipan antivenom effectively neutralises the presynaptic neurotoxins of both the coastal and inland (*O. microlepidotus*) species, it is less effective against the postsynaptic toxicity of the latter (Crachi et al., 1999). The clinical consequences of the aforementioned procedural limitations can therefore be reduced efficacy against some venom activities (Henderson et al., 1993) or even complete antivenom failure in critical envenomations (Gillissen et al., 1994).

### Global Antivenom Crisis

As the extent of clinically significant variation in snake venom composition undermined the possibility of a universal antivenom, so it erected a major barrier to the widespread availability of this product. The current global burden of snake bite is estimated at approximately 5 million bites and 100,000 deaths each year (Chippaux, 1998). Most of these are concentrated in the developing nations of the Indo-Pacific region and Africa, where there is little access to antivenom for the most at-risk populations (Cheng and Winkel, 2001a,b; Warrell, 1999). Even in affluent nations, the unattractive economics of antivenom development and production have resulted in pressure on existing manufacturers to withdraw from this market (Galli, 2001; Theakston and Warrell, 2000).

The need for further expenditure on procedural refinements to improve antivenom quality, as described here, will add to such pressures. This comes in the midst of an acute crisis in antivenom availability for Africa (Theakston and Reid, 1983) and a long-standing under-supply in Asia (McNamee, 2001). The ongoing reduction in the number of antivenom manufacturers world-wide, and concomitant contraction in the range of available antivenoms, present significant challenges for the treatment of snake bite. We therefore recently proposed a global strategy for snake bite control and procurement funding to overcome the inequality of antivenom supply (Cheng and Winkel, 2001a,b).

## RECOMMENDATIONS

All these factors put together result in a somewhat complex picture for antivenom producers and policy makers. The optimization of antivenom production and effectiveness requires a number of preconditions:

– An understanding of the medically important species in any given region. This appears trivial, but a several studies have shown that the medical importance of a number of species has been greatly

underestimated in the past (e.g., *Bungarus candidus* in Thailand—Looareesuwan et al., 1988; Viravan et al., 1992), whereas that of others has been overestimated (e.g., *Bungarus fasciatus* and *Ophiophagus hannah* in Thailand—Looareesuwan et al., 1988; Viravan et al., 1992), resulting in a lack of antivenom in the case of the former and the production of largely superfluous antivenoms in the case of the latter. Clearly, if resources are limited, antivenom production should concentrate on species of appreciable public health importance. This requires extensive epidemiological studies, particularly community surveys and pharmacological characterisation of venoms.

- Scrupulous attention to the systematics and identification of the snakes concerned. Antivenom should be produced against the species responsible for the majority of bites, wherever they may occur, and not against similar species that happen to be conveniently available, but are not in fact responsible for bites.
- Antivenoms should be raised from venoms collected across the entire range of each species across the target region. Due to intraspecific geographic variation in venom composition, an antivenom raised against the venom of one population may be less effective against the venom of another population of the same species (Fry et al., 2001, Wüster et al., in prep.). This concern is particularly applicable to venom producers who rely on captive-bred stocks of venomous snakes: often, the captive stocks originate from a few specimens from a single locality, and are thus likely to contain only a fraction of the total number of antigens present in the species as a whole.

At the very least, antivenoms should be tested for neutralising ability against the venoms of all major populations of any species, with special emphasis on those occurring in regions with a high incidence of snakebite.

The World Health Organisation recommendations (W.H.O., 1981) regarding the specific assessment of defibrinogenating, haemorrhagic and necrotising venom activities should form a routine part of snake venom toxicity testing and antivenom efficacy assessment (Gutiérrez et al., 1990; Theakston and Reid, 1983).

A global strategy for snakebite control and procurement funding is required to overcome the inequality of antivenom supply and to improve existing antivenom deficiencies in neutralising interspecific and intraspecific venom variation. This should form part of global initiatives to secure access to essential drugs through partnerships between donors, the public sector and pharmaceutical industry (Cheng and Winkel, 2001a,b; Scholtz, 1999).

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