

REVIEW ARTICLE

Antivenom for local effects of snake envenoming

Umesha Madhushani, Geoffrey K. Isbister, Wayne C. Hodgson, Sisira Siribaddana and Anjana Silva*



Highlights

- Efficacy and effectiveness of antivenoms for local effects of snake envenoming need assessment.
- Most current tests of antivenom efficacy test is clinically less relevant in preventing local effects.
- Clinically relevant experimental models of testing antivenom efficacy for local effects are required.
- The current clinical literature on antivenom effectiveness for local effects is limited.
- Future research needs to focus on early antivenom to prevent local effects of envenoming.

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Antivenom for local effects of snake envenoming

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Abstract: Snake envenoming is a significant public health issue that disproportionately affects the poorest communities in the tropical regions. There is a spectrum of local effects following snakebite, including pain, swelling, bluish discolouration, haemorrhagic blistering, local tissue necrosis and gangrene at the bite site. In severe local envenomings, significant tissue loss and impaired function can occur and may result in permanent disability in snakebite survivors. Although the mainstay of hospital treatment for snake envenoming is antivenom, its effectiveness for local effects remains contentious.

The preclinical efficacy of antivenoms against the local effects of envenoming is examined with a range of in-vivo and in-vitro tests. Most of these tests are only capable of examining the ability of the antivenom to prevent, rather than reverse, the local effect. A limitation of the above tests is that they do not consider venom pharmacokinetics or the time course of irreversible effects in envenomed humans. Therefore, more clinically relevant experimental models of antivenom efficacy are required.

We searched MEDLINE for studies on the local effects of snakebite. The current clinical literature on the effectiveness of antivenom for local effects appears to be limited. We identified only two randomised trials that compared antivenom with placebo and six randomised trials that tested the effectiveness of one antivenom or one dosage regimen of an antivenom compared to another antivenom or different dosage of antivenom for preventing or reversing the local effects. All these studies were on viperine envenomings. In addition, several studies without a control/comparative group have commented on antivenom effectiveness, although they invariably have significant bias. The existing studies had contrasting conclusions, including no effect of antivenom, antivenom halting the progression of local effects, early antivenom preventing the occurrence of severe local effects including necrosis, early antivenom leading to faster functional improvement, antivenom accelerating the resolution of local effects, or no conclusion. Future research needs to focus on well-designed studies investigating whether the early administration of antivenom will prevent severe local effects.

Keywords: snakebite; envenoming; antivenom; efficacy; effectiveness

INTRODUCTION

Snake envenoming is a significant public health issue in

the tropics. Although literature-based estimates suggest 0.4-1.8 million envenomings and 20,000- 94,000 deaths occur due to snakebite each year globally, actual figures are considered to be even higher (Gutiérrez *et al.*, 2017; Kasturiratne *et al.*, 2008). Hospital statistics-based estimates may not show the actual disease burden because some snakebite victims in rural tropics may not present to hospitals due to inaccessibility as well as seeking treatment from traditional practitioners (Gutiérrez *et al.*, 2013). Snakebite disproportionately affects the poorest of the poor in these tropical regions, making it a neglected tropical disease (WHO, 2007).

Snake envenoming leads to diverse clinical manifestations in victims, including a spectrum of local effects and systemic effects. Local effects include pain, swelling, bluish discolouration, haemorrhagic blistering, local tissue necrosis and gangrene around the bite site. Severe local effects following envenoming by some elapids and viperids may result in significant tissue loss and impaired function, leading to permanent disability (Gutiérrez *et al.*, 2006). Furthermore, severe local tissue damage in snake envenoming may require surgical interventions such as amputation, resulting in permanent disability in snakebite survivors (Jorge *et al.*, 1999; Ribeiro. *et al.*, 2001; Waiddyanatha *et al.*, 2019).

Antivenom is the mainstay of hospital treatment for snake envenoming (Silva and Isbister, 2020). Although some experimental and clinical studies support antivenom therapy for local effects of snake envenoming, other studies have challenged the above, based on the nature of the irreversible pathophysiology of the tissue damage (Silva and Isbister, 2020). We aimed to review the experimental methods of testing antivenom efficacy and the clinical evidence for the effectiveness of snake antivenoms on local effects of snake envenoming.

LOCAL ENVENOMING

The term 'local envenoming' has been widely used in snakebite literature to describe a range of clinical manifestations resulting from the effects of venom toxins

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on the tissues at the site of snakebite and the response of the host tissue to the toxic effects. This includes fang marks, pain, swelling, erythema, haemorrhagic blistering, induration, bluish discolouration, skin and muscle necrosis, and gangrene.

A number of elapids and vipers cause local envenoming effects worldwide. In the Americas, envenoming by the pit viper genera such as *Bothrops* and *Crotalus* frequently cause local manifestations (Heise *et al.*, 2018; Lasoff *et al.*, 2016; Severyns *et al.*, 2018), while in Asia, the pit-viper genera such as *Hypnale*, *Trimeresurus* (Maduwage *et al.*, 2013; Rojnuckarin *et al.*, 2006) and *Callocellasma* (Wongtongkam *et al.*, 2005), true vipers such as *Daboia* (Ariaratnam *et al.*, 2001) and elapid genera such as *Naja* (Kularatne *et al.*, 2009; Wong *et al.*, 2010) also cause local effects. In Africa, the true viper genera such as *Bitis* (Warrell, 1975), *Echis* (Warrell *et al.*, 1974) and most importantly, several species of *Naja* (Kandiwa *et al.*, 2018; Tilbury, 1982; Warrell *et al.*, 1976), cause local manifestations. In Oceania, *Acanthopis* spp and mulga snake (*Pseudechis australis*) envenoming may cause local effects (Isbister *et al.*, 2010). While in Europe, the genus *Vipera* is responsible for local manifestation (Dopfer *et al.*, 2010; Karlson-Stiber & Persson, 1994). In addition to elapids and viperids, atractaspidids (borrowing asps, *Actractaspis* spp.) can cause local effects, including necrosis and amputations. (Pallett *et al.*, 2022; Warrell *et al.*, 1976). Although rare, some colubrids have also caused considerable local effects following envenoming (Pinto *et al.*, 1991), including oedema and local haemorrhage.

A venomous snakebite often leaves one or two clear puncture marks from the fangs. Non-venomous snakes may leave teeth marks due to the four rows of teeth (two rows of maxillary and two rows of palatine teeth). Venomous snake bites result in varying degrees of pain at the bite site, developing within a variable time frame (Ward-Smith *et al.*, 2020). Different snake venom components such as phospholipases A₂ (PLA₂), serine proteases (SVSP), snake venom metalloproteinases (SVMP) and fasciculins act

directly and indirectly to induce envenoming-associated pain (Ferraz *et al.*, 2019). The reporting of local pain at the bite site in most clinical studies is subjective, and a pain scale is rarely used. Oedema at the bite site is common and may be more severe in viper bites (Ariaratnam *et al.*, 2001; Bush *et al.*, 2002; Karlson-Stiber *et al.*, 1997; Lasoff *et al.*, 2016; Mazer-Amirshahi *et al.*, 2014; Mong & Tan, 2016; Ruha *et al.*, 2002; Schier *et al.*, 2003) (Figures 1 A and B). Both catalytically active (Asp49) and inactive (Lys49) classes of PLA₂s and SVMP (haemorrhagins) can destroy the vascular wall, resulting in fluid leakage into the interstitial space causing oedema (Rojnuckarin *et al.*, 2006).

In more severe cases, local envenoming can result in haemorrhagic blistering (Figure 1 E-H), skin necrosis and subcutaneous tissue gangrene (Figure 2). Compartment syndrome may develop, mainly in Asian viper, Asian cobra and rattlesnake bites (Bucaretschi *et al.*, 2014; Heise *et al.*, 2018; Mao *et al.*, 2018; Mazer-Amirshahi *et al.*, 2014; Pochanugool *et al.*, 1997; Rathnayaka *et al.* 2022; Severyns *et al.*, 2018; Valenta, 2010; Wong *et al.*, 2010). PLA₂, SVMP, SVSP and cytotoxic three-finger toxins act synergistically to induce tissue necrosis in snakebite victims. Usually, PLA₂ and SVMP cause skin necrosis in viper envenoming and three-finger toxin cytotoxins are mostly responsible for necrosis in cobra envenoming. (Harris, 2003; Harris & Cullen, 1990; Kularatne *et al.*, 2009). Blistering is likely to result from the direct proteolysis at the dermal-epidermal junction caused by SVMPs (Severyns *et al.*, 2018). Tissue necrosis can also occur from tissue ischemia secondary to increased intracompartmental pressure (Gutiérrez *et al.*, 2007). Secondary infections at the bite site, such as gangrene, necrotic fasciitis and abscesses, could further complicate the local effects, with the oral bacteria of snakes may also play a role (Resiere *et al.*, 2020)

Acute local effects may progress to chronic effects in some individuals, including disability due to amputations, contracture formation, chronic ulcers with some leading to malignant changes, chronic pain and swelling, and



Figure 1: Some local effects caused by Merrem's hump-nosed pit vipers (*Hypnale hypnale*) in Sri Lanka: A and B, swelling around the bite site; C and D, bleeding from fang marks; E-H, haemorrhagic blistering (Images: Anjana Silva).



Figure 2: Moderate to severe acute local effects: Sri Lankan cobra (*Naja polyocellata*) bite-induced induration and blistering (A and C) progressing into dermonecrosis (B and D); Sri Lankan cobra bite-induced local swelling (E) progressing to dermonecrosis requiring surgical debridement (F); Merrem's hump-nosed viper (*Hypnale hypnale*) bites in Sri Lanka resulting in the amputation of digits (G and H) (Images: Anjana Silva).

blindness. This mainly results from envenoming by African and Asian cobras, and central and South American pit vipers (Waiddyanatha *et al.*, 2019).

LOCAL EFFECTS AND ANTIVENOMS

Snake antivenoms are polyclonal, whole immunoglobulin (IgG) or immunoglobulin fractions (Fab or F(ab')₂) raised against venom from one (monovalent) or more than one (polyvalent) snake species in other animals, most commonly horses (Silva and Isbister, 2020). In most settings, polyvalent antivenoms are used due to the geographical presence of several medically important snakes and the difficulty in precisely identifying the snake that caused the bite (Chippaux, 2006; WHO, 2010).

Following a snakebite, some toxins in the snake venom gradually distribute locally at the bite site and exert harmful actions directly on the surrounding tissues, while other toxins are absorbed and distributed systemically. After intravenous administration of antivenom antibodies/fragments rapidly distribute in blood and extracellular interstitial fluid in tissue compartments and bind to toxins present in the blood and tissues. However, the ability of antibodies to reach target sites outside the circulation depends on the tissue-penetrating potential of the antibodies or antibody fragments (Silva and Isbister, 2020). Even if the antibodies/fragments reach the tissues at the site of the bite and are able to bind with the toxins, the irreversible action of some toxins, such as PLA₂ and SVMPs, directly on local tissue are likely to have already been initiated. Hence, reversing these effects is unlikely. Nonetheless, different antivenoms have been used in different regions both in clinical settings as well as experimentally.

EFFICACY OF ANTIVENOMS RELATED TO LOCAL EFFECTS OF SNAKE ENVENOMING

The 'efficacy' of antivenom is determined by the ability of antibodies and their fragments to bind venom toxins under

ideal conditions (i.e. in-vitro), which, while important, is not the only determinant of the clinical 'effectiveness' of an antivenom (Isbister, 2010). Antibodies block the action of the venom toxins through different mechanisms, which can prevent the action of the toxin or, in some cases, reverse already initiated toxin action. In prevention, paratopes of the antibodies directly bind and occupy the pharmacological site of the toxin and preventing the toxin from reaching the target site. Secondly, antibodies can bind to epitopes situated in the vicinity of the pharmacological site of the toxin, and the therapeutic activity is expected to be achieved by steric hindrance with the consequent inability of the toxin to reach the target tissue site. To induce reversal of toxic effects, the antibody/fragment binds to an epitope at a distance from the pharmacological site of toxins and makes conformational changes at the pharmacological site of the toxin, leading to a decrease of the affinity of the toxin to their tissue target (Gutiérrez *et al.*, 2003; Silva and Isbister, 2020).

Rodent skin patch model for necrosis and haemorrhage

The WHO-recommended mouse skin patch model (Ferreira *et al.*, 1992; Leon *et al.*, 1997; Santos Barreto *et al.*, 2017) is the preferred test for hemorrhagic and necrotising activity by measuring the MHD-median effective dose (MHD₅₀) & MND-median effective dose (MND₅₀). The rat skin patch model (Collaço *et al.*, 2017), and, rarely, the rabbit skin patch model (Sánchez *et al.*, 2003) have also been used to measure hemorrhagic activity.

In the skin patch model, venom solutions are injected intradermally into the shaved skin of lightly anesthetised mice or rats. After a defined time interval (usually 2-3 hours), mice are sacrificed, the area of the injected skin is removed, and the size of the haemorrhagic lesion in the inner side of the skin is measured using callipers in two directions with background illumination. For measuring local necrotising effects, the same protocol is followed,

except that the skin is examined three days after the intradermal injection of the venom (WHO, 2016).

Rodent footpad model for oedema

The mouse footpad (paw) model (Galvão Nascimento *et al.*, 2010; Gutiérrez *et al.*, 1987; Prezotto-Neto *et al.*, 2016; Rocha *et al.*, 2006) and rat paw model (Collaço *et al.*, 2017; Moraes *et al.*, 2003) are used to measure the oedema-forming activity of snake venoms and the efficacy of antivenoms in neutralising this activity. In the footpad model, Minimum oedema forming Dose (MOD) is defined as the amount of venom which induces 30% oedema 6 hours after injection. For neutralisation assays, six minimum oedema-forming venom doses (in 50 µl) are injected into the right footpad, while the left footpad is injected with physiological saline solution. For prevention experiments, antivenom is given intravenously 5 min before venom. In reversal experiments, antivenom is administered intravenously at different time intervals after venom (Gutiérrez *et al.*, 1987).

Rodent footpad model for local pain

The rodent footpad model is used to measure local pain. Rats are injected into the subplantar surface of one hind footpad with venom dissolved in a sterile saline solution. The control group is injected with the same volume of sterile saline. The contralateral footpad is not injected. The pain threshold is measured at 1 and 4 h or 2 and 4 h after injection using an Ugo-Basile pressure apparatus. The force required to induce the rat to withdraw its footpad is recorded and represents the pain threshold (Picolo *et al.*, 2002).

Assays for local myotoxicity

Local muscle damage (local myotoxicity) can be measured by the injection of venom into the gastrocnemius muscle of rodents (Gutiérrez *et al.*, 2008; Rojas *et al.*, 2001). and the quantification of muscle injury by means of plasma creatine kinase (CK) (Moraes *et al.*, 2003; Otero *et al.*, 1995; Rojas *et al.*, 2005). Alternatively, the chick biventer nerve-muscle preparation has also been used to assess myotoxicity. In this in vitro skeletal muscle preparation, the effects of venom/toxins on direct (muscle) twitches are measured, with myotoxicity indicated by inhibition of the twitches and, usually, an increase in baseline tension (Madhushani *et al.*, 2021; Thakshila *et al.*, 2022).

Cytotoxicity assays

Cell viability assays measure the viability of cells in response to extracellular stimuli, chemical agents or therapeutic treatments. For examining snake venoms, the rat aorta smooth muscle cell line (A7r5) and rat skeletal muscle myoblast cell line (L6) have also been used to investigate the efficacy of antivenom in preventing cytotoxicity. (Kalam *et al.*, 2011).

Issues with experimental models for testing antivenom efficacy for local effects

Although mimicking the venom toxin activity on human tissues, as well as the tissue response in humans, it is difficult to replicate in experimental models the real situation. They are useful in providing an initial assessment of the efficacy of antivenom for neutralising the local

effects of snake venoms. However, the mere presence of antibodies capable of neutralising the toxins responsible for inducing the local effects does not assure the effectiveness of antivenom in the clinical setting. This is due to the unique pathophysiological processes of the tissue injury at the bite site, as described above, which are difficult to reverse once initiated. All the above tests, except for the in-vitro chick biventer model and the in-vivo rodent gastrocnemius myotoxicity model, are only capable of demonstrating the ability of the antivenom to prevent the tested local effect and not the clinically more important, reversal of the effect.

In addition, there are limitations of the above tests due to problems in reproducing the pharmacokinetics in an envenomed human, in which the antibodies in the circulation are expected to reach the tissues around the bite site (Silva *et al.*, 2022; Silva & Isbister, 2020). Furthermore, models of local envenoming may be practically impossible to conduct for certain venoms, if the experimental animal dies due to other effects of venom, such as neurotoxicity or cardiovascular collapse, before observations related to local envenoming are made (Madhushani *et al.*, 2021). In addition, in-vivo tests require the live experimental animals to be subjected to venom injection and kept for hours or days, which may have significant animal ethics concerns.

EFFECTIVENESS OF ANTIVENOM RELATED TO LOCAL EFFECTS OF SNAKE ENVENOMING

The clinical effectiveness of antivenom for the local effects of snake envenoming could be viewed as the ability of antivenom to either prevent the occurrence of local effects or, prevent the local effects from becoming severe, or reversing established local effects, in a clinically detectable manner.

To identify clinical studies on the effectiveness of antivenom for the local effects of snake envenoming, we carried out a search in MEDLINE from 1st January 1945 to 31st December 2022 and included studies in the English language on snakebites that describe local effects. We used the search terms “snake envenoming” AND “antivenom”, “snake envenomation” AND “antivenom”, “snakebite” AND “antivenom”, “snake” AND “antivenom” in combination with the terms “necrosis”, “swelling”, “blistering”, “oedema”, “discolouration” and “induration”. This search yielded 349 studies. After reviewing the abstracts and removing experimental studies and reviews, we identified a total of 165 studies for further review and the full articles of these were read. From these, we excluded 19 studies because of insufficient information on the local effects and the remaining 146 studies were included (Figure 3).

A range of clinical studies, including randomised placebo-controlled trials, randomised comparative trials, case-control studies, and case series and observational studies, have commented on the effectiveness or ineffectiveness of antivenom for local effects of snake envenoming. Placebo-controlled, randomised clinical trials provide the highest-level evidence for the effectiveness of antivenom for local effects of snake envenoming (Table 1).

Placebo-controlled randomised trials

There were two placebo-controlled randomised trials that

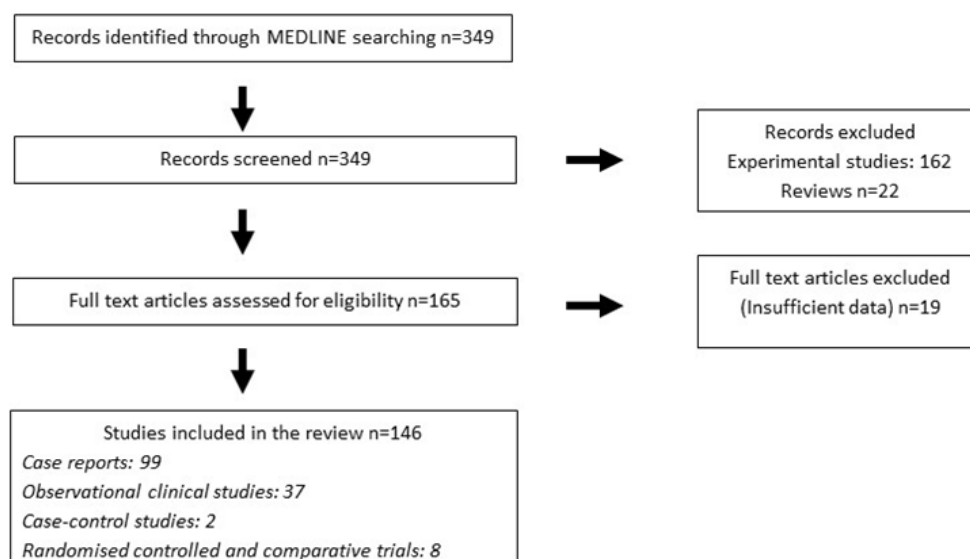


Figure 3. Selection of clinical studies for the review

investigated the effectiveness of antivenoms for local effects of pit-viper envenoming (Rojnuckarin *et al.*, 2006; Sellaheewa *et al.*, 1995). The first was a double-blind (patients and the investigators), randomised placebo-controlled trial, Rojnuckarin *et al.* that compared 14 patients who received Thai monovalent green-pit F(ab')₂ antivenom and 14 patients who received a placebo, following *Trimeresurus albolabris* and *T. macrops* envenoming (Rojnuckarin *et al.*, 2006). They found that the percentage reduction in limb circumferences on first two days post-bite, were significantly better in the antivenom group compared with the placebo group, with no difference in the pain scores and the functional activity. However, the authors did not recommend the use of antivenom for swelling in pit-viper envenoming in Thailand, due to the uncertainty of the clinical significance of the findings (Rojnuckarin *et al.*, 2006). In addition, this study had only one patient with local necrosis, who was in the placebo group. However, the smaller number of patients with severe local effects and the greater bite-to-intervention time in the antivenom group may limit the conclusions.

The second was a single-blind (patients) randomised controlled trial, Sellaheewa *et al.* compared the resolution of local pain and induration in 29 patients, who received Indian polyvalent antivenom (Haffkine) with 31 patients who received placebo, following envenoming by Merrem's hump-nosed viper (*Hypnale hypnale*) (Sellaheewa *et al.*, 1995). In this study, the outcome measure was the time for complete resolution of the local pain and induration in days. The induration at the site of the bite was quantified, while the severity of local pain was not quantified in this study. The study found no difference in the outcome between the two groups. Nonetheless, the antivenom used in this study is not raised against the Hump-nosed vipers; hence would not be relevant in investigating the effectiveness of a homologous antivenom for local effects of snakebite in general.

Randomised comparative trials.

Several randomised comparative trials (Table 1) have tested

the effectiveness of one antivenom or one dosage regimen of an antivenom compared to another antivenom or another dosage regimen of antivenom for preventing or reversing local swelling (Otero, 1996), necrosis (Cardoso *et al.*, 1993; de Oliveira Pardal *et al.*, 2004; Otero, 1996; Warrell *et al.*, 1986) and oedema (Ariaratnam *et al.*, 2001; Otero, 1996; Warrell *et al.*, 1986) following antivenom therapy for viperine bites. The outcome measures commonly included oedema in most studies, while pain, local haemorrhage, blistering and necrosis were included in some studies. These studies did not conclude a clear benefit of one antivenom/ regime over another in terms of resolving the local effects of envenoming (Table 1).

Comparative studies with historical controls

In a study that compared 30 patients treated with two European equine F(ab')₂ antivenoms, with a historical control (n=16) who required but did not receive any antivenom following envenoming by *Vipera berus*, only 23% of the antivenom group had extensive oedema while 88% of the non-treated group displayed extensive oedema (Karlson-Stiber & Persson, 1994). In a subsequent study, the same two groups (historical) were compared with 30 patients who received a specific sheep antivenom following *Vipera berus* envenoming. This study showed only 23% of the group receiving sheep antivenom group developed extensive oedema as opposed to 88% of the historical non-treated group (Karlson-Stiber *et al.*, 1997). However, 'extensive oedema' in both these studies was qualitatively defined as swelling involving not only the bitten extremity but also parts of the trunk, without quantification.

Observational studies

In determining the role of antivenom in preventing the development of severe local effects and reversing the established local effects such as swelling, it is critical to understand, with evidence, whether the antivenom has altered the natural course of the local effects. As such, this is impossible without a comparison group of patients. Therefore, although these studies commonly comment

on the 'effectiveness of antivenom', studies such as case reports, case series and any interventional or observational study with only one arm, are unlikely to provide a strong conclusion on effectiveness due to the considerable bias.

These studies have investigated or commented on the effects of antivenom on local effects caused by pit vipers such including *Crotalus* species (Caiaffa *et al.*, 1994; Grace and Omer, 1980; Heise *et al.*, 2018; Ruha *et al.*, 2002), *Bothrops* species (Caiaffa *et al.*, 1994), *Bothrops alternatus* (Bauab 1994), *Crotalus helleri* (Bush *et al.*, 2002), *Agkistrodon contortrix* (Lavonas *et al.*, 2004); *Protobothrops mucrosquamatus* and *Viridovipera stejnegeri* (Lin *et al.*, 2022), *Trimeresurus albolabris* and *T. macrops* (Chotenimitkhun and Rojnuckarin, 2008); and *Hypnale hypnale* (Ariaratnam *et al.*, 2008); true vipers such as *Echis carinatus* (Warrell *et al.*, 1977), *Echis ocellatus* (Chippaux *et al.*, 1998; Monzavi *et al.*, 2019), *Echis coloratus* (Glatstein *et al.*, 2022), *Daboia palaestinae* (Bentur, 1997; Pivko-Levy *et al.*, 2017), *Vipera berus* (Karlson-Stiber & Persson, 1994), *V. aspis* (Boels *et al.*, 2012), *Vipera ammodytes* (Brvar *et al.*, 2017), *Bitis arietans* (Warrell, 1975), and *Bitis gabonica* (Marsh *et al.*, 2007), Asiatic cobras such as *Naja polyocelata* (formerly Sri Lankan *Naja naja*) (Kularatne *et al.*, 2009; Phillips *et al.*, 1988), *Naja kaouthia* (Faiz *et al.*, 2017; Pochanugool *et al.*, 1997), and *Naja atra* (Mao *et al.*, 2018; Wong *et al.*, 2010), African cobras such as *Naja nigricollis* (Warrell *et al.*, 1976), *Naja mossambica* (Tilbury, 1982) and *Atractaspis dahomeyensis* and *A. microlepidota* (Warrell *et al.*, 1976) and in Australia, death adders (*Acanthophis* sp) (Isbister *et al.*, 2010). The case reports, due to their high reporting bias, are not discussed here.

These observational studies either suggested (1) no effect of antivenom or failure of antivenom to prevent local effects (Chotenimitkhun and Rojnuckarin, 2008; Heise *et al.*, 2018; Kularatne *et al.*, 2009; Phillips *et al.*, 1988; Warrell *et al.*, 1977; Warrell *et al.*, 1976), (2) antivenom halting the progression of local effects (Lavonas *et al.*, 2004; Ruha *et al.*, 2002), (3) early antivenom preventing the occurrence of severe local effects including necrosis (Warrell, 1975), (4) early antivenom leading to faster functional improvement (Boels *et al.*, 2012), (5) antivenom accelerating the resolution of local effects (Brvar *et al.*, 2017; Glatstein *et al.*, 2022) or (6) no conclusion (Bauab 1994; Bush *et al.*, 2002; Caiaffa *et al.*, 1994; Chippaux *et al.*, 1998; Lin *et al.*, 2022; Mao *et al.*, 2018; Pochanugool *et al.*, 1997).

In clinical studies that concluded the effectiveness of antivenom for local effects, resolution of oedema following antivenom have been more frequently described, especially in viper bite patients (Bentur, 1997; Brvar *et al.*, 2017; Karlson-Stiber and Persson, 1994; Schier *et al.*, 2003). However, the role of antivenom in managing compartment syndrome without surgical decompression has been disputed (Mazer-Amirshahi *et al.*, 2014; Severyns *et al.*, 2018).

Compared to those who receive IgG and F(ab')₂ antivenoms, patients who received Fab antivenoms have frequently developed recurrence of envenoming effects, including the

local effects, requiring repeated antivenom doses (Lasoff *et al.*, 2016; Lavonas *et al.*, 2004; Ruha *et al.*, 2002). This phenomenon occurs due to the significant mismatch between venom and antivenom kinetics with the dynamics of Fab antivenoms. More rapid clearance of unbound Fab compared to F(ab')₂ may result in the recurrence of local and systemic venom effects (Seifert and Boyer, 2000).

CONCLUSION AND FUTURE DIRECTIONS

This review found that the F(ab')₂ and Fab' antivenoms have commonly been in use in different regions of the world to treat the local effects of snakebite such as pain, swelling, bluish discolouration, blistering, necrosis and gangrene. The preclinical efficacy of antivenoms for different local effects is currently being tested using different in-vivo and in-vitro tests, although the venom toxin activity on the human tissues, as well as tissue response by the human, are difficult to be replicated in experimental models. Moreover, most of these tests are only capable of demonstrating the ability of the antivenom to prevent the tested local effect and not the clinically more important, reversal of effect. In addition, there are limitations of the above tests due to the problems in accurately reproducing the pharmacokinetics of an envenomed human, in which the antibodies in the circulation are expected to reach the tissues around the bite site. Therefore, more clinically relevant experimental models of antivenom efficacy are needed.

The current clinical literature on the effectiveness of antivenom for local effects appears to be limited. We found only two randomised trials that compared the treatment arm with a placebo, and among non-randomised studies, only two compared the antivenom-treated patients with an untreated historical group of patients. All randomised studies were on viperid envenoming. The existing studies had contrasting conclusions, including no effect of antivenom, antivenom halting the progression of local effects, early antivenom preventing the occurrence of severe local effects including necrosis, early antivenom leading to faster functional improvement, antivenom accelerating the resolution of local effects, or no conclusion. Apart from a few studies, all the available randomised trials, comparative studies and observational studies had deficiencies either in the case definition, study design, small sample size or in the objectivity of the measures of paralysis.

Future research needs to focus on well-designed studies investigating whether the early administration of antivenom will prevent severe local effects, with better case definition by using venom-specific enzyme immunoassays to confirm envenoming and improved objective measures of acute local effects in terms of the quantification, and the long term sequelae.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

Table 1: Randomised trials on antivenom for local effects of snake envenoming.

Study	Number in each arm	Snake species	Arms	Blinded	Randomisation method specified	Allocation concealed	AVS dose defined	AVS route	Primary outcome defined	Local effects measured	Conclusion
<i>Randomised, placebo-controlled trials</i>											
Rojnukarin et al. (2006)	14/14	<i>Trimeresurus Albolabris T. macrops</i>	Monovalent green pit-viper antivenom vs placebo	Patients and investigators	No	Yes	Yes	i.v.	Yes	Oedema Pain	Antivenom accelerates local oedema resolution, but not the local pain resolution compared to placebo
Sellahewa et al. (1995)	29/31	<i>Hypnale</i> species	Indian Polyvalent antivenom (Haffkine) – This is a heterologous antivenom	Patients	No	Yes	Yes	i.v.	Yes	Pain and induration	There was no significant difference between the antivenom and placebo groups in the time taken for complete resolution of the local envenomation
<i>Randomised comparative trials</i>											
Otero et al. (1996)	20/19	<i>Bothrops atrox</i>	Monovalant anti-B. atrox antivenom vs Polyvalent Crotaline antivenom	Patients and investigators	No	Yes	Yes	i.v.	No	Oedema Local haemorrhage Blistering Necrosis	No significant difference two antivenoms on resolution of haemorrhage and oedema progression.

Ariaratnam et al. (2001)	23/20	<i>Daboia russelii</i>	Monovalent ovine new antivenom (PolangaTab) vs Haffkine polyvalent	No	Yes	Yes	Yes	i.v.	No	Oedema	Tendency towards more rapid resolution of local swelling in the Haffkine group
de Oliveira Parda et al. (2004)	36/38	<i>Bothrops</i> and <i>Lachesis</i> snakes	Standard <i>Bothrops-Lachesis</i> antivenom vs Specific <i>B.atrox-Lachesis</i> antivenom	Patients and investigators	No	No	No	i.v.	No	Oedema, bruising, blistering and signs of infection and local necrosis.	No difference between two antivenoms.
Cardoso et al. (1993)	39/41/41	<i>Bothrops species</i>	polyspecific Bothrops antivenoms: Instituto Butantan vs Instituto Vital Brazil vs Fundacao Ezequiel Dias (FUNED)	Patients and investigators	No	No	Yes	i.v.	No	Oedema, local haemorrhage, bruising, Blistering, necrosis	No difference between three antivenoms in resolution of local effects
Warrell et al. (1996)	15/15/16	<i>Calloselasma rhodostoma</i>	Monospecific antivenoms for Malayan pit-viper: Thai Red Cross (TRC) vs Thai Government Pharmaceutical Organization (GPO) vs Twyford Pharmaceutical	No	No	No	Yes	i.v.	No	Oedema blistering necrosis	No significant difference between the groups on maximum extent and degree of local swelling and change in these variables after antivenom.

Dart et al. (2001)	15/16	North American Crotaline snakes	A single dose of crotaline Fab (ovine) vs an initial dose plus repeated doses	No	Yes	Yes	Yes	i.v.	Yes	A severity score that included pain, oedema, blistering, necrosis, in addition to systemic effects	No difference in the treatment schedules
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