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[Intervention Protocol]

Antivenom for snake venom-induced neuromuscular paralysis

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To assess the effects of antivenom on neuromuscular paralysis in people with neurotoxic snake envenoming.

BACKGROUND

Description of the condition

Snakebite leads to significant morbidity and mortality globally, with an estimated burden of 421,000 to 1,841,000 envenomings and 20,000 to 94,000 deaths per year. Of this, more than 90% of envenomings are reported from tropical Asia, sub-Saharan Africa, and Latin America (Kasturiratne 2008). Venom-induced neuromuscular paralysis is one of the major clinical manifestations of envenoming, predominately by elapid snakes. In some neurotoxic snakebites, such as by kraits (genus *Bungarus*) in Asia, life-threatening paralysis occurs in more than 50% of patients (Kularatne 2002; Hung 2009).

Neurotoxic snake venoms primarily affect the neuromuscular junction causing a disruption of neurotransmission, resulting in paralysis of the skeletal muscles (Harris 2009; Ranawaka 2013). Snake venom neurotoxins target multiple sites in the neuromuscular junction. The majority of the snake venom neurotoxins either

act on the motor nerve terminals (presynaptic) or the nicotinic acetylcholine receptor on the motor end-plate (postsynaptic).

Presynaptic toxins initially lead to a depletion of the synaptic vesicles and ultimately cause structural damage to the motor nerve terminals (Logonder 2008; Prasarnpun 2005). This type of insult is most likely to be treatment resistant, and recovery depends on the natural regeneration of the nerve terminal, as shown from experimental studies using presynaptic toxins isolated from krait and viper venoms (Dixon 1999; Logonder 2008; Prasarnpun 2004; Prasarnpun 2005). Snake venom postsynaptic neurotoxins competitively bind to the agonist-binding sites of the nicotinic acetylcholine receptors on the motor end-plate with high affinity and poor reversibility, blocking neuromuscular transmission (Ishikawa 1985; Vincent 1998). Some neurotoxic snake venoms, as in kraits, contain both types of toxins (Rusmili 2014). Several snake venom toxins act on specific ion channels or affect acetylcholinesterase activity in the neuromuscular junction (Harris 2009).

Whatever the mechanism, all of these toxins result in the same clinical effect: neuromuscular weakness, which can range from a

mild weakness of the eyelid and facial muscles to fatal paralysis of bulbar and respiratory muscles (Connolly 1995; Isbister 2012; Johnston 2012; Kularatne 2000; Kularatne 2002; Silva 2016). In extreme cases, complete neuromuscular paralysis involving all skeletal muscles of the body can occur (Silva 2016). To sustain life, mechanical ventilation is essential in people with respiratory paralysis. Depending on the snake species involved, neuromuscular paralysis can co-exist with other clinical manifestations of envenoming, such as local tissue necrosis seen in cobras, Kularatne 2009, and venom-induced consumptive coagulopathy in vipers, Sano-Martins 2001, and some Australasian elapids (Isbister 2012). The detection and monitoring of the neuromuscular paralysis in people with snakebite in the clinical setting as well as for research purposes are almost entirely dependent on the clinical examination. For this, patients are constantly monitored for clinical features of neurotoxicity such as ptosis, ophthalmoplegia, and facial, neck, bulbar, respiratory, and limb weakness (Isbister 2012; Johnston 2012; Kularatne 2000; Kularatne 2002). Neurophysiological tests such as single-fibre electromyography have also been used for this purpose (Silva 2016). However, such tests require equipment and skills beyond the reach of rural settings, where snakebites are mostly prevalent.

Description of the intervention

Antivenoms have been used for the treatment of snakebite for more than a century (Gutiérrez 2011; WHO 2010). They are polyclonal whole immunoglobulin (IgG) or immunoglobulin fractions (Fab or F(ab')₂) raised against venom from one (monovalent) or several (polyvalent) snake species in other animals, most commonly horses. The immunised animals are periodically bled and the immunoglobulins are separated from the blood using ammonium sulphate or caprylic acid to produce whole IgG antivenom. During the production of many commercial antivenoms, the whole immunoglobulins are fractionated by papain or pepsin digestion to make Fab or F(ab')₂, respectively (Chippaux 2006; Gutiérrez 2011; WHO 2010). Depending on the production protocol, the immunoglobulins or fractions may be subject to further purification involving chromatographic steps and pasteurisation (León 2013). Antivenoms are available in freeze-dried powdered form (where the powder is reconstituted with sterile water prior to use) or liquid form. Snake antivenoms are almost always delivered to the patients via the intravenous route. Antivenom therapy is associated with adverse reactions, and frequent life-threatening reactions are a major problem associated with some antivenoms (de Silva 2011; de Silva 2015; León 2013).

How the intervention might work

In doses used in the clinical setting, antivenom molecules (polyclonal antibodies) likely outnumber the venom molecules (toxins)

in the circulation (Allen 2012; Isbister 2015). The polyclonal nature of the antivenoms means that they contain a range of antibodies or antibody fractions against a range of neurotoxins (both pre- and postsynaptic), relevant to this review, as well as non-neurotoxic toxins. These antivenom molecules bind with circulating toxins, forming large venom-antivenom complexes, trapping the venom molecules in the circulation (O'Leary 2006; O'Leary 2014). The antibodies likely act via a number of mechanisms, including blocking the active site of the neurotoxin molecules, preventing the toxins from interacting with the target site (neuromuscular junction) by restricting the movement of the neurotoxins to the extravascular target sites, and also increasing the elimination of the toxins (Maduwage 2015). In addition, if the antivenom molecules are able to distribute from the circulation, they might be able to reach the neuromuscular junctions and neutralise the neurotoxins at their target site. However, it is unclear how effectively the whole IgG, F(ab')₂, or Fab molecules in the antivenoms can distribute to the neuromuscular junctions.

Presynaptic neurotoxins result in structural damage to the motor nerve terminals that is irreversible (in the short term). Antivenom is therefore unlikely to be able to reverse already established presynaptic neurotoxic injury (Harris 2013; Logonder 2008; Prasarnpun 2005). In contrast, postsynaptic neurotoxins act in a similar way to reversible non-depolarising type neuromuscular blockers or muscle relaxants. The reversibility of the binding of postsynaptic toxins to the nicotinic acetylcholine receptor varies based on the structural properties of the individual toxins (Barber 2013). Experimental evidence suggests that specific immunoglobulins are able to increase the recovery of the neuromuscular junctions from postsynaptic toxin-mediated neuromuscular block (Gatineau 1988).

Why it is important to do this review

Although antivenom therapy is commonly utilised for neurotoxic snake envenoming, its effectiveness in preventing or reversing neurotoxicity is less clear and has been questioned in several studies conducted in different regions (Johnston 2012; Richardson 2007; Theakston 1990; Silva 2016). Recovery of the neurotoxicity in snake envenoming without antivenom has also been reported (Hung 2009; Pochanugool 1997). In practice, it is doubtful whether the antivenom could be delivered early enough to prevent the neurotoxins from reaching neuromuscular junctions. Furthermore, it is unclear whether the antivenoms can speed recovery of already established neurotoxicity. A recent study of common krait envenoming demonstrated that even in the patients who received early antivenom (median 3.5 hours postbite) in an adequate dose to bind with all circulating venom antigens, antivenom was unable to prevent the subsequent development of life-threatening paralysis (Silva 2016). In contrast, a study of taipan bites in Papua New Guinea found that early administering of antivenom prevented intubation in a proportion of patients (Connolly 1995).

OBJECTIVES

To assess the effects of antivenom on neuromuscular paralysis in people with neurotoxic snake envenoming.

METHODS

Criteria for considering studies for this review

Types of studies

We will consider randomised controlled trials in humans for inclusion in this review. Of the randomised controlled trials published after 2010, we will exclude those without an accessible, registered protocol. We will not consider cluster trials due to issues related to the unit of analysis.

Types of participants

People of any age, who were bitten or envenomed by neurotoxic snakes, and have either developed venom-induced neuromuscular paralysis or have not yet developed venom-induced neuromuscular paralysis. We will base diagnosis of venom-induced neuromuscular paralysis on clinical features of neuromuscular paralysis such as ptosis, ophthalmoplegia, and facial, neck, bulbar, respiratory, and limb weakness.

Types of interventions

Intravenous administration of snake antivenom regardless of the type or dose of antivenom. The comparison group will be people who were not treated with antivenom.

Types of outcome measures

Primary outcomes

1. Mortality as a direct result of neuromuscular paralysis within 14 days of the snakebite.

Secondary outcomes

1. Incidence of life-threatening paralysis that requires intubation or mechanical ventilation, or both within 24 hours of the snakebite.
2. Duration of mechanical ventilation.
3. Incidence of any of the following clinical effects of neuromuscular paralysis*: ptosis, ophthalmoplegia, weakness of facial, neck, bulbar, or limb muscles within 48 hours of the snakebite.

4. Incidence of immediate systemic hypersensitivity reactions within four hours of antivenom administration.

5. Incidence of serum sickness within 14 days of the administration of antivenom.

*Neuromuscular paralysis is defined here as presence of at least a single clinical feature of clinically detectable paralysis (e.g. ptosis, ophthalmoplegia, facial muscle weakness, neck muscle weakness, bulbar palsy, respiratory muscle weakness, weakness in upper and lower limbs).

Information size calculation

To our knowledge, an estimate of the mortality rates due to venom-induced neuromuscular paralysis is unavailable. However, the prevalence of life-threatening paralysis (that required intubation and mechanical ventilation) in krait and taipan envenomings is 49% to 51% (Trevett 1995; Silva 2016; Kularatne 2002). In a previous study, antivenom therapy improved the outcome of 3 out of 6 people with paralysis due to Papuan death adder envenoming (Laloo 1996). Based on this, we can assume that the mortality rate for untreated patients (i.e. no antivenom given) due to venom-induced neuromuscular paralysis in neurotoxic snake envenoming is 50%. Conservatively we expect the antivenom will lower the mortality by 25% (i.e. half the mortality). Based on the above assumptions, with a statistical power of 90% and alpha of 0.05, the information size required is a total of 168 participants (84 participants with antivenom treatment and 84 participants without antivenom treatment).

Search methods for identification of studies

In order to reduce publication and retrieval bias, we will not restrict our search by language, date, or publication status.

Electronic searches

The Cochrane Injuries Group's Information Specialist will search the following databases:

1. Cochrane Injuries Group Specialised Register (present version);
2. The Cochrane Library (www.cochranelibrary.com) (latest issue);
3. Ovid MEDLINE(R), Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid OLDMEDLINE(R) (1946 to present);
4. Embase Classic + Embase (OvidSP) (1947 to present);
5. ISI Web of Science: Science Citation Index Expanded (SCI-EXPANDED) (1970 to present);
6. ISI Web of Science: Conference Proceedings Citation Index-Science (CPCI-S) (1990 to present);
7. ISI BIOSIS Citation Index (1969 to present);
8. KoreaMed (www.koreamed.org) (all available dates);

9. IndMed (indmed.nic.in) (all available dates);
 10. LILACS (Latin American and Caribbean Center on Health Sciences Database) (lilacs.bvsalud.org/en/) (all available dates);
 11. ClinicalTrials.gov (www.clinicaltrials.gov);
 12. World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (apps.who.int/trialsearch/).
- We will adapt the MEDLINE search strategy provided in [Appendix 1](#) as required for the other databases.

Searching other resources

We will search the reference lists of all relevant studies and contact experts in the field in order to identify ongoing and completed studies. We will also run a search on regional databases and journals from South and Southeast Asia, sub-Saharan Africa, and Latin America, and search the guidelines, conference proceedings, theses, and other sources of grey literature.

Data collection and analysis

We will perform a systematic review following the instructions in the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2011](#)).

Selection of studies

Two review authors (AS and GKI) will independently scan the titles and abstracts of all articles identified by the search strategy. If either or both review authors identify an article as possibly meeting the inclusion criteria, we will obtain the full text of the published article. Both review authors will review the full text of each article to determine if it meets the inclusion criteria. Disagreements between the two review authors will be resolved by a third review author (NB). We will provide details of the included studies in the appropriate tables within the review. We will report studies not meeting the inclusion criteria in the 'Characteristics of excluded studies' section of the review and the reasons for exclusion in the 'Characteristics of excluded studies' table. In the event of disagreement between the review authors, we will seek the opinion of a third review author (NB). For ambiguous studies and where there are insufficient data, we will attempt to contact the authors of the articles for further clarification and more information. We will grade the studies for quality, using the instructions in the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2011](#)).

Data extraction and management

Two review authors will independently review each article that meets inclusion criteria, and extract data from the article onto a standard data extraction form for the following items.

- General information about the article: title of the article, source, publication year, years the study was conducted, language of publication.
- Clinical trial characteristics: design, diagnostic ascertainment, standard care provided, randomisation, allocation concealment, interventions, dropouts and lost to follow-up, definitions of outcomes, and methods of outcome assessment.
- Participants: inclusion and exclusion criteria, sample size, baseline characteristics (participant age, past history of neuromuscular disorders, clinical severity on enrolment).
- Interventions: type of antivenom (polyvalent or monovalent), manufacturer, dose of antivenom (number of vials or milligrams), time administered postbite and duration of administration.
- Outcomes: mortality*, duration of clinical features of neuromuscular paralysis including ptosis, ophthalmoplegia, facial, neck, bulbar, respiratory, and limb weakness, duration of mechanical ventilation, length of hospital stay, immediate systemic hypersensitivity reactions, serum sickness.

*In instances where other clinical manifestations of envenoming, such as coagulopathy and local effects, coexisted and are a likely cause of mortality rather than neurotoxicity, we will exclude such cases from the analysis.

Assessment of risk of bias in included studies

Two review authors (AS and GKI) will independently assess the included studies for risk of bias using the suggested domains and guidance provided in the Cochrane 'Risk of bias' tool as detailed in section 8.5 of the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2011](#)). We will assess random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other sources of bias (in particular funding source). If information to make a judgement is insufficient, we will initially assess domains as 'unclear risk' and will attempt to clarify the risk of bias by contacting the study authors. We plan to include all studies irrespective of the risk of bias. However, we plan to perform a sensitivity analysis; if the sensitivity analysis shows substantial differences, we will present alternative estimates that exclude studies with high or unclear risk of bias.

We will categorise the overall risk of bias of individual studies as follows:

- low risk of bias (plausible bias unlikely to seriously alter the results) if all domains were at low risk of bias;
- unclear risk of bias (plausible bias that raises some doubt about the results) if one or more domains had an unclear risk of bias; or
- high risk of bias (plausible bias that seriously weakens confidence in the results) if one or more domains were at high

risk of bias.

Measures of treatment effect

We will define measures of treatment effects as follows.

Dichotomous data

We will present dichotomous data outcomes as risk ratios (RR) with 95% confidence intervals (CI) for individual trials.

Continuous data

We will present continuous data outcomes with mean difference (MD) and 95% CI. As mean differences are easier for clinicians and readers to interpret, we will calculate mean difference where possible; we will use standardised mean difference when different scales are used in the trials.

Ordinal data

We will report ordinal data outcomes such as types of adverse events and complications depending on the length of the scales used. If the scale is longer (> 5), we will treat the data as continuous; if the scale is short (5 or less), we will combine adjacent categories to produce dichotomous data.

Unit of analysis issues

The unit of analysis will be the individual participant. To answer our primary question (does antivenom change mortality due to neuromuscular paralysis compared to no antivenom treatment), we will in the first instance simply combine all active intervention groups of the study into a single group and compare their outcomes to the control groups(s) not receiving antivenom, whilst acknowledging the limitations related to the heterogeneity of the data.

We have excluded cluster randomised trials from the review. We do not expect to identify cross-over trials as they are an inappropriate study design for this type of treatment.

Dealing with missing data

We will contact the authors of the original studies if essential data are missing from their trial reports. If we receive no reply after eight weeks, we will extract the available data from the published reports. We will assess the missing data and attrition rates for each of the included studies and report the number of participants who are included in the final analysis as a proportion of all participants in the study.

Assessment of heterogeneity

We will evaluate statistical heterogeneity using the Chi² test, and the I² statistics for quantifying heterogeneity across studies. The importance of the observed value of I² depends on (i) magnitude and direction of effects and (ii) strength of evidence for heterogeneity (e.g. P value from the Chi² test, or a confidence interval for I² as outlined in [Higgins 2011](#)). We expect high levels of heterogeneity due to considerable variation across trials in setting, snake, intervention, and outcomes; we will consider I² values of more than 85% as considerable heterogeneity. The possible elements of heterogeneity will be included for exploration in a subgroup analysis, as mentioned in [Subgroup analysis and investigation of heterogeneity](#). We intend to use the random-effects model to account for this heterogeneity in any summary estimates of effect. We will discuss the implications of heterogeneity and how they relate to external validity in the Discussion.

Assessment of reporting biases

We will refer to systematic differences between reported and unreported findings as reporting bias. We will include selective-outcome reporting assessment as part of the 'Risk of bias' table and also under intention-to-treat analysis.

We will assess publication biases by using funnel plots when at least 10 studies are included in the meta-analysis.

Data synthesis

We will pool dichotomous outcomes such as mortality, risk of immediate-type hypersensitivity reactions, and risk of serum sickness, and report the RR with 95% CIs. We will use a Mantel-Haenszel random-effects model for dichotomous data meta-analysis. For continuous outcomes (duration of mechanical ventilation), we will use an inverse-variance, random-effects model for the analysis and the mean difference, or the standardised mean difference if outcomes were measured using different scales. We will perform meta-analysis if we find two or more studies assessing the same outcome. If a meta-analysis is not possible, we will write a narrative summary of the study findings and follow alternative methods as described in the *Cochrane Handbook* ([Higgins 2011](#)).

Subgroup analysis and investigation of heterogeneity

Where possible (if sufficient data and information are available) we will perform subgroup analysis based on the following factors, which are thought to affect outcomes after neuromuscular paralysis.

- Presynaptic versus postsynaptic versus mixed mechanism of neurotoxic snake envenoming
- Each specific species of snake envenoming
- Type of snake antivenom
- Dose of antivenom

The immediate hypersensitivity reactions and serum sickness (secondary outcome measures) are likely to be strongly affected by the type of snake antivenom, hence we will analyse these based on the different types of antivenom.

Sensitivity analysis

We will restrict sensitivity analyses to include studies with both (1) allocation concealment carrying low risk of bias and (2) having blinded outcome assessment. Different batches of the same antivenom used for the same study may show interbatch variation of efficacy (leading to variation in effectiveness). Strict implementation of the random allocation is therefore important in minimising bias. Some secondary outcomes such as the duration of the clinical features of paralysis and the duration of mechanical ventilation are purely based on the clinical decision-making of the treating staff, hence implementing the blinded outcome assessment is important in minimising bias. Furthermore, since immediate hypersensitivity reactions may be affected by pretreatment with epinephrine, we will carry out sensitivity analysis excluding those participants treated with epinephrine.

Summarising findings and assessing the quality of the evidence

We will generate a 'Summary of findings' table for comparing antivenom versus no antivenom. We will report the following outcomes in the 'Summary of findings' table.

1. Mortality as a direct result of neuromuscular paralysis within 14 days of the snakebite.
2. Incidence of life-threatening paralysis that requires intubation or mechanical ventilation, or both within 24 hours of the snakebite.
3. Duration of mechanical ventilation.
4. Incidence of immediate systemic hypersensitivity reactions within four hours of antivenom administration.
5. Incidence of serum sickness within 14 days of the administration of antivenom.

We will grade the quality of the evidence in the studies as high, moderate, low, or very low according to the section 11.5 of Higgins 2011 using GRADE methods and GRADEpro software (GRADE 2004; GRADEpro 2015). We will assess the body of evidence based on the risk of bias of the included studies, directness of the evidence, inconsistency in results, imprecision of the measure of effects, and publication bias.

We will provide citations and a rationale for the figures on which the calculation of assumed and corresponding risks in the 'Summary of findings' table are based.

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* Indicates the major publication for the study

APPENDICES

Appendix I. Search strategy

Ovid MEDLINE (databases) will be searched using the following terms:

1. Snake Bites/
2. (snakebit* or ((snake* or rattlesnake* or viper* or cobra* or asp or asps or mamba* or krait* or adder* or Vipirid* or Vipirin* or Elapid* or Colubrid* or Hydrophiin* or Laticaudin* or Crotalid* or Crotalin* or Bitis* or Vipera* or Ophiophagus* or Bungarus* or Crotalus* or Daboia* or Micrurus* or Micruroides* or Adenorhinos* or Atheris* or Cerastes* or Echis* or Eristicophis* or Macrovipera* or Montatheris* or Proatheris* or Pseudocerastes*) adj3 bit*)).ti,ab,kf.
3. 1 or 2
4. exp Snakes/

5. (snake* or rattlesnake* or viper* or cobra* or asp or asps or mamba* or krait* or adder* or Vipirid* or Vipirin* or Elapid* or Colubrid* or Hydrophiin* or Laticaudin* or Crotalid* or Crotalin* or Bitis* or Vipera* or Ophiophagus* or Bungarus* or Crotalus* or Daboia* or Micrurus* or Micruroides* Adenorhinos* or Atheris* or Cerastes* or Echis* or Eristicophis* or Macrovipera* or Montatheris* or Proatheris* or Pseudocerastes*).ti,ab,kf,hw.
6. Poisoning/
7. (poison* or toxin* or venom* or envenom* or antivenom*).ti,ab,kf,hw.
8. (neurotoxin* or phospholipase A2*).ti,ab,kf,hw.
9. (4 or 5) and (6 or 7 or 8)
10. exp Snake Venoms/
11. (fasciculid* or dendrotoxin* or alpha-neurotoxin* or crotamine* or bungarotoxin*).ti,ab,kf,hw.
12. Antivenins/
13. or/9-12
14. (neuro* or paralys*).ti,ab,kf.
15. (ptosis* or weakness* or flaccid* or palsy*).ti,ab,kf.
16. exp Paralysis/
17. or/14-16
18. 13 and 17
19. 3 or 18
20. randomized controlled trial.pt.
21. controlled clinical trial.pt.
22. placebo.ti,ab,kf.
23. trial.ti,ab,kf.
24. (RCT or random*).ti,ab,kf.
25. clinical trials as topic.sh.
26. (animals not (humans and animals)).sh.
27. (or/20-25) not 26
28. 19 and 27

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GKI and AS conceived the idea. AS, KM, and GKI developed the initial draft of the protocol, which was improved by HJdeS, NAB, and DGL.

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Anjana Silva: No conflicts of interest

Kalana Maduwage: No conflicts of interest

Nick A Buckley: No conflicts of interest

David G Lalloo: No conflicts of interest

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