Randomized controlled trial of intravenous antivenom versus placebo for latrodectism: the second redback antivenom evaluation (RAVE- II) study.

Abstract

Objective: Latrodectism is the most important spider envenomation syndrome worldwide. There remains considerable controversy over antivenom treatment. We aimed to investigate whether antivenom resulted in resolution of pain and systemic effects in patients with latrodectism given standardized analgesia.

Methods: In a multicentre randomized placebo-controlled trial of redback spider antivenom for latrodectism, 224 patients (>7yr) with a redback spider-bite and severe pain with or without systemic effects were randomized to receive normal saline (placebo) or antivenom, after receiving standardized analgesia. The primary outcome was a clinically significant reduction in pain 2 hours after trial medication compared to baseline. A second primary outcome for the subgroup with systemic features of envenomation was resolution of systemic features at 2 hours. Secondary outcomes were improved pain at 4 and 24 hours, resolution of systemic features at 4 hours, administration of opioid analgesics or unblinded antivenom after 2 hours and adverse reactions.

Results: Two hours after treatment, 26/112 patients (23%) from the placebo arm had a clinically significant improvement in pain versus 38/112 (34%) from the antivenom arm (difference in favor of antivenom 10.7%;95%CI:–1.1% to +22.6%;p=0.10). Systemic

effects resolved after two hours in 9/41 patients (22%) in the placebo arm and 9/35 (26%) in the antivenom arm (difference 3.8%;95%CI:−15% to +23%;p=0.79). There was no significant difference in any secondary outcome between antivenom and placebo. Acute systemic hypersensitivity reactions occurred in 4/112 (3.6%) patients given antivenom.

Conclusions: The addition of antivenom to standardized analgesia in patients with latrodectism, did not significantly improve pain or systemic effects.

Introduction

Spider bite is a common problem worldwide.[1] Latrodectism is the most common severe spider envenoming syndrome and is caused by widow spider (*Latrodectus* spp.) bites, particularly in warmer parts of America, Europe, and Australia.[2-4] In Australia there are 3000 to 5000 cases every year.[3] Widow spiders are medium sized black spiders that vary in appearance and include 30 species on most continents.[5] Envenoming is characterized by local, regional or generalized pain associated with systemic symptoms and autonomic effects. The severity and features of latrodectism appears to vary for different widow spiders from different regions,[2-4,6-9] but pain is the most prominent feature in all cases.

Despite the medical importance of latrodectism, treatment continues to be problematic with wide variations in clinical practice worldwide.[1] Antivenom is only available in some countries,[10] and there is controversy regarding its effectiveness and safety.[1] In the United States there has been limited use of antivenom due to the perceived risks of adverse reactions following a death attributed to antivenom.[4] In Australia, a highly purified equine antivenom raised against the local species *Latrodectus hasselti* (redback spider) has been widely used for 60 years, mainly by the intramuscular route. Its introduction was prior to the era of randomized controlled clinical trials. Numerous other treatments with little evidence for effectiveness have been used, including benzodiazepines, calcium, magnesium and combinations of non-opioid and opioid oral analgesia.[1]

Three randomized controlled trials of antivenom for latrodectism have been published.[2,11,12] Two previous trials in Australia both reported no difference between intravenous and intramuscular antivenom.[2,11] These were unexpected outcomes because it

was assumed that intravenous antivenom would be more effective. In addition, a pharmacokinetic analysis of a subgroup of patients from one trial found that antivenom was only detectable in serum after intravenous administration.[13] A small phase II study of black widow spider antivenom versus placebo found no significant benefit of antivenom over placebo.[12] Taken together, these results suggested that antivenom might be no more effective than placebo and provided sufficient doubt to warrant a placebo-controlled trial.

The aim of this study was therefore to determine whether the administration of redback spider antivenom is superior to placebo for treating the pain and systemic effects of latrodectism in patients already receiving standardized analgesic treatment.

Methods

This was a placebo-randomized controlled trial of red-back spider antivenom in patients with moderate to severe latrodectism (redback spider envenoming) with a primary outcome of a clinically significant reduction in pain two hours after the trial medication (compared to baseline). A second primary outcome for the subgroup with systemic features of envenoming was the resolution of these features also at two hours. Two primary endpoints were chosen because both types of response are clinically important but not necessarily linked. All participants received analgesia according to a standardized protocol, commenced prior to the administration of trial drug or placebo. The study was approved by seven Human Research Ethics Committees to cover all hospital sites. The trial was registered with the Australian New Zealand Clinical Trials Registry, http://www.anzctr.org.au/, ACTRN12609000063213.

Study Patients

Patients were recruited from 20 emergency departments around Australia between January 2009 and June 2013 if they had a redback spider bite and the treating clinician would normally administer antivenom or analgesia for the pain, or systemic envenoming. A redback spider bite was defined as either a bite by a spider which was clearly identified as a redback spider (by the patient or clinician) or a clinical syndrome consistent with typical redback spider envenoming, that is the sensation of a bite followed by two or more of (increasing pain over the first hour, radiating, regional or generalized pain, local or regional diaphoresis).

Local envenoming was defined as severe local pain, for which the patient was requesting analgesia, or that was preventing sleep. Systemic envenoming was defined as the presence of ≥3 of the following: nausea, vomiting, headache, lethargy, malaise and abdominal pain.

Exclusion criteria were age less than 8 years (due to the unreliability of the Verbal Numerical Rating Scale (VNRS) for assessment of pain in this group), prior administration of antivenom and presentation to hospital more than 36 hours after the bite.

Treatment Protocol

Patients were identified by nursing or medical staff on or soon after admission. It was not possible to keep a record of patients with redback spider bites not recruited to the study because the investigators were only contacted for patients that met the inclusion criteria. The study was explained and written informed consent was obtained from the patient or the parent/guardian of the patient. The treating doctor then contacted a national free-call telephone number to enroll the patient and receive a randomization code. The patient was put in an acute observation area with cardiac monitoring, pulse oximetry and automated blood pressure measurements, and an intravenous cannula was inserted.

All patients received a standard analgesia protocol prior to receiving the study intervention with oral paracetamol 1g (20mg/kg up to a maximum of 1g in children), ibuprofen 800mg (10mg/kg up to a maximum of 800mg in children) and oxycodone 5mg (0.1mg/kg up to a maximum of 5mg in children).

The Calvary Mater Newcastle pharmacy in conjunction with Richard Stenlake Compounding Chemist produced pre-packed kits for the trial. Each treatment kit contained two vials of either redback spider antivenom or normal saline. Normal redback spider antivenom vials (equine $F[ab']_2$ antivenom, 500U/vial, raised against *L. hasselti*) as well as identical empty vials were purchased from CSL Ltd. The compounding chemist filled the identical (empty) vials with normal saline, which was visually indistinguishable from antivenom. Labels were removed from vials and the central pharmacy re-labeled the vials with study numbers. Each kit was then randomized to contain either two vials of antivenom (active) or two vials of normal saline (placebo).

Block randomization was used (with variable block-sizes of 2 and 4) with stratification between local and systemic envenoming. Block sizes of 2 and 4 meant that each pack of four randomized treatments provided to the hospital might feasibly contain any combination of placebo and antivenom (including four antivenom or four placebo), making it impossible to predict the last kit in each pack. During the study each site was kept stocked with 2 packs each containing 4 treatment kits, one pack of 4 for local envenoming and the other for systemic envenoming.

Using a pre-randomized list of blocks, the chief investigators and on-call research assistants allocated study codes to patients in sequential order based on the hospital site and whether the patient had systemic effects or not. The study code was then used to identify the correct trial pack stored at the site. The content of each treatment pack (active or placebo) was only known by the centralized pharmacy so that treating clinicians, patients and investigators were blind to the study intervention.

The trial drug was administered (2 vials of placebo or two vials of redback spider antivenom) mixed in 200 mL normal saline over 20 minutes. The patient had continuous monitoring (electrocardiogram, pulse oximetry and non-invasive blood pressure) during the infusion of the study drug and for 30 minutes after completion. Study observations were performed at 2 hours and 4 hours after infusion commencement and patients remained in hospital until at least 4 hours after the study drug. Further study observations were done immediately prior to discharge if kept in hospital for longer than 4 hours.

After measurement of the primary endpoint 2 hours from study commencement, parenteral opioid analgesia (e.g. morphine) and (unblinded) doses of redback spider antivenom were permitted and their use determined by the treating doctor. All patients were given regular ibuprofen (400mg three times daily) for 24 to 48 hours and oxycodone as required following the 4-hour study period.

Data Collection

Data collected on case report forms included patient demographics, details of the bite (spider identification, circumstances of the bite), baseline clinical effects, serial clinical effects during the study, additional treatment (analgesia and unblinded redback spider antivenom) and adverse reactions. Pain was assessed using a verbal numeric rating scale (VNRS) which required the patient to verbally provide a score between 0 and 10 inclusive where 0 represented “no pain” and 10 “worst pain possible”. Immediate-type hypersensitivity reactions were recorded according to a previously published grading scale as skin only systemic hypersensitivity reactions, anaphylaxis or severe anaphylaxis (hypotension or hypoxia).[14]

Patients were followed up by telephone by a research assistant at 24 hours from the time of administration of the study treatment, 7 to 10 days and 6 weeks to assess for effectiveness and adverse events including symptoms of serum sickness. The research assistant used a proforma to ask the patient pre-defined questions, including the VNRS, if any further analgesia was used, whether the patient was re-admitted to the emergency department/hospital or visited their local doctor. In addition, they would ask about each of the symptoms of serum sickness at the 7 to 10 day and 6 week follow up.

Data Analysis

The first primary outcome was a clinically significant reduction in the severity of pain 2 hours after the commencement of the study treatment using the VNRS. This was dependent on the baseline starting point – a reduction of 2 or greater was required for baseline score of 0 to 3, 3 or greater for a baseline score of 4 or 5, 4 or greater for a baseline score of 6 or 7, and 5 or greater for a baseline score of 8 to 10. This approach was similar to that previously used in the RAVE I study (see Appendix).[2] The required reduction for each baseline score was modified slightly after trial registration but prior to unblinding of the data, because of an inconsistency in the registered method that would have given inconsistent results in patients with similar scores (see Appendix). The second primary outcome was a resolution of systemic features of envenoming within 2 hours, in the subgroup of patients with systemic envenoming. Resolution of systemic envenoming was defined as not having more than one remaining systemic symptom/feature. Both primary outcomes were analyzed by intention to treat.

Secondary outcomes were pre-defined as – clinically significant reduction in pain and resolution of systemic features (if present) at 4 hours (same definition of resolution as at 2 hours), administration of opioid analgesics (oral or parenteral) or further doses of antivenom after 2 hours, a clinically significant reduction in pain at 24 hours, use of opioid analgesia after discharge, re-presentations for medical care, acute systemic hypersensitivity reactions and serum sickness defined as 3 or more characteristic symptoms (fever, malaise, rash, itchiness, myalgia, arthralgia). Predefined subgroup analyses were planned for patients with systemic envenoming.

A sample size of 240 (including 94 patients with systemic effects) was calculated to give 80% power to detect a 20% difference in the primary outcome of clinically significant pain reduction (regarded to be a clinically important difference by clinicians[15]) and/or a 30% difference in the primary outcome of resolution of systemic effects. The study was stopped early (16 patients short of the sample size) because there was no further funding to re-supply all 20 hospitals with antivenom and placebo trial packs which expired annually and had to be replaced prior to the next bite season.

Once the study was finished and all data entered into the study database, the chief clinical investigator (GI) remained blinded to the allocation and audited all primary and secondary outcomes against original datasheets and clinical notes. If an inconsistency was identified, a second investigator (SGAB or CP) adjudicated. During this stage only the study numbers, not group allocation, were known to these investigators. One investigator, not involved with the day-to-day conduct of the study (NB) was then supplied with the blinded data and separately with the group allocation as either “A” or “B” by the central pharmacy. He undertook the study analysis independently and

presented this to the writing group. The final analysis was then approved before the central pharmacy revealed whether “A” or “B” was antivenom.

Statistical methods.

Continuous variables were presented as medians with interquartile ranges (IQR) and proportions were given with 95% confidence intervals (CIs). Dichotomous primary outcomes were analyzed using a two-tailed Fisher’s exact test ($p<0.05$ to be significant). Continuous secondary outcomes were analyzed by either a t-test (parametric) or Mann-Whitney test (non-parametric). A post-hoc analysis was performed with the primary pain outcome as a continuous variable according to percent pain reduction. This also explored whether there was any effect of time from the bite to antivenom on response in the antivenom arm using linear regression. All analyses and graphs were done with GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

Results

Of 227 patients recruited to the study, 224 were randomized and received their allocated treatment. Two patients were inadvertently given unblinded antivenom rather than the trial drug and one did not have the trial drug code recorded. One hundred and twelve patients were randomized to receive normal saline (placebo arm) and 112 to receive redback spider antivenom (Figure 1). The two study arms had similar baseline characteristics although the placebo group tended to present earlier, had more patients with an initial pain score of 8 to 10, and fewer patients were given prior analgesia (Table 1). There were 76 patients (34%) with systemic effects and 176 patients (79%) developed diaphoresis (local [111, 50%], regional [31, 14%] and generalized [34, 15%]).

Primary Outcomes

Two hours after treatment, 26 of 112 patients (23%) from the placebo arm had clinically improved pain versus 38 of 112 patients (34%) from the antivenom arm (difference in favor of antivenom 10.7%; 95% CI: -1.1% to +22.6%; p=0.10)(Table 2). The change in pain score for individual patients comparing placebo to antivenom in shown in Figure 2 and the percentage reduction in pain scores in Figure 3 and **Table 3**. Additional analyses of the primary outcome using absolute (non-weighted) and relative measures of changes in pain had no significant difference between placebo and antivenom (See Appendix and **Table 3**). Systemic effects resolved after two hours in 9 of 41 patients (22%) patients in the placebo arm compared to 9 of 35 patients (26%) in the antivenom arm (difference 3.8%; 95% CI: -15% to +23%; p=0.79).

Secondary Outcomes

There was no significant difference between placebo and antivenom for the improvement in pain at 4 hours and 24 hours (Table 2). Figure 4 shows the change in pain over the study period including pain on follow up at 7 to 10 days and at 6 weeks. There was also no difference in the resolution of systemic effects between placebo and antivenom.

In total 135 patients required rescue opioid analgesia, 77 receiving an oral opioid (oxycodone, codeine or tramadol), 29 receiving parenteral opioids (morphine, fentanyl) and 29 receiving both. Fifty nine patients were given unblinded antivenom 2 or more hours after the study treatment. More patients in the placebo group were given rescue opioid analgesia and unblinded antivenom, although these differences were not statistically significant (Table 2).

Post-hoc analysis of effectiveness data

Combining the primary outcomes to measure those with either a significant reduction in pain OR systemic features did not alter conclusions about the lack of effectiveness (30/112 [27%] in the placebo arm versus 41/112 (37%) in the antivenom arm; (difference 9.8%, 95%CI: –2.4% to +22%; p=0.15). Using the registered primary pain outcome (with the statistical inconsistency) also did not change conclusions (21/112 [19%] in the placebo arm versus 31/112 (28%) in the antivenom arm; (difference 9.8%, 95%CI: –2.4% to +22%; p=15). The median percent pain reduction was 25% in placebo arm compared to 33% in antivenom arm, (Figure 3, Mann Whitney P=0.0517). However, there was no relationship between percent pain reduction and time from bite to antivenom in the antivenom arm (Supplementary Figure 1). Response rates were equally poor when

comparing confirmed (spider identified) and non-confirmed bites (see **Table 3 and** Appendix).

Adverse Reactions

Acute skin only (mild) hypersensitivity reactions occurred in 4 of the 112 patients (3.6%) given antivenom and none in the placebo group. Twenty one patients (9%) developed symptoms consistent with serum sickness and five of these presented to the emergency department or local doctor. There was no difference in reports of 'serum sickness' between those randomized to antivenom and placebo, although slightly fewer cases occurred once the use of unblinded antivenom was accounted for (Table 2). Eighteen (8%) patients represented to either the emergency department (11) or their local doctor (7), 12 of these for ongoing pain for symptoms of the bite. This did not differ between placebo and antivenom arms.

Limitations

A potential limitation of the study was the sample size being too small to completely exclude a small benefit. It is possible that a larger study might find a beneficial treatment effect of antivenom, but the effect is very likely to be small. This study was powered to detect a difference of 20% (NNT $\leq$ 5). It is important to consider that redback spider antivenom is used as an analgesic and not to save lives. The NNT in trials of effective analgesics range from 2 to 4 in meta-analyses.[16] Much higher NNT are not clinically significant for studies of analgesia. In other words, if the study was larger and showed a statistically significant and similar absolute difference in the order of 10%, (NNT = 10), ten patients would need to be given antivenom for one patient to get significant pain

relief. This is a very poor result for pain relief and also must be balanced against the risk of a hypersensitivity reaction of around 4% in this and previous studies (i.e. numbers needed to harm = 25).[2,17] It is important to note that neither our standard analgesia nor analgesia and antivenom resulted in pain resolution in three quarters of the patients so further trials are needed to investigate alternate and more effective treatments, whether or not antivenom has a marginal effect.

Another limitation of the study was that some cases were included based on a clinical diagnosis of latrodectism rather than a definite bite. However, our clinical definition has been validated by a prospective study of identified spider bites[18] and re-analysis of the primary outcomes including only cases where the spider was identified resulted in the same outcomes.

Finally, the outcomes we used have not been fully validated which may have resulted in either under-estimation or over-estimation of the measured effect for both pain and systemic effects. However, this would have affected both arms of the study. A similar primary pain outcome has been used in two previous studies, one positive and one negative.[2,19]

Discussion

This trial demonstrates that the addition of redback spider antivenom to a standardized analgesic treatment protocol in patients with latrodectism (redback spider envenoming), did not significantly improve pain or systemic effects. Patients responded poorly to analgesia alone or analgesia plus antivenom, with only a quarter of patients on average having an improvement at 2 hours. There were also no differences in secondary outcomes between the placebo group and the antivenom group.

The use of antivenom in latrodectism has always been contentious because it is not a life-threatening envenoming syndrome. Therefore, clear benefits of antivenom over standard care are required to balance against the known but small risk of anaphylaxis to the antivenom. In our study, more patients had an improvement in pain in the antivenom group at 2 hours, but this did not reach statistical significance and there was no evident treatment effect on systemic envenoming. Furthermore, there was also a slight imbalance between the placebo and antivenom arms of the study with the antivenom group presenting somewhat later and with less patients having high initial pain scores (Table 1). This imbalance may favor the pain response in the antivenom group, because later-presenting patients are further down the pathway of spontaneous recovery by the time of randomization. Later presentation of the antivenom group could alternatively favor the placebo group if antivenom has a time-dependent effect, but there was no relationship between the time to antivenom and the pain response in the antivenom group (Supplementary Figure 1). This indicates that redback spider antivenom is not more likely to be effective if given earlier, which is not consistent with the expected effect of antivenoms.

A number of *in vitro* studies have demonstrated that redback spider antivenom is able to neutralize the effects of the venom.[20-22] In addition, Graudins et al showed that redback spider antivenom was able to prevent *in vitro* neurotoxicity in a chick isolated biventer cervicis nerve-muscle preparation and lethality in mice, from a range of widow spider venoms.[21] Daly et al also showed that redback spider antivenom prevented the lethal effects of *L. Hesperus* and *L. mactans*.[22] It is therefore highly unlikely that poor clinical effectiveness in our study is due to the antivenom having poor binding or neutralization properties. The likely reason for treatment failure is that *in vitro* antivenom efficacy does not always translate into clinical effectiveness. The antivenom has not been shown to distribute to the peripheral site of the envenoming syndrome. Nor has it been shown to bind to the toxin at its site of action and reverse pathophysiological changes. To be clinically effective an antivenom must be capable of doing both, and there are several previous examples of antivenoms that cannot reverse pathophysiology and are therefore not capable of significantly improving clinical outcomes.[23]

A better understanding of the pathophysiology of latrodectism is required so that effective treatments can be developed. This study and previous work suggests that standard analgesic treatment is also unable to provide relief for patients. Other treatments such as calcium, magnesium and muscle relaxants have never been tested in a controlled way in patients. Potentially treatments used for neuropathic pain, such as gabapentin, could also be trialed.

The study cannot be immediately generalized to other widow spider antivenoms.[10] All widow spiders are closely related and a recent study has shown that the redback spider (*L. hasselti*) and both American black widow spiders (*L. mactans, L. hesperus*), contain

similar neurotoxins to α-latrotoxin, originally identified in the European widow spider (*L. tredecimguttatus*).[24] In addition, animal studies have demonstrated cross-neutralisation of black widow spider venoms by redback spider antivenom.[21,22] The study therefore provides some support for the idea that widow spider antivenoms may not be effective. The only other placebo randomized controlled trial of widow spider antivenom was also a negative trial.[12] However, further and larger studies are required for different widow spiders and antivenoms.

References

1. Isbister GK, Fan HW. Spider bite. Lancet 2011;378:2039-47.

2. Isbister GK, Brown SG, Miller M, et al. A randomised controlled trial of intramuscular vs. intravenous antivenom for latrodectism--the RAVE study. QJM 2008;101:557-65.

3. Isbister GK, Gray MR. Latrodectism: a prospective cohort study of bites by formally identified redback spiders. MedJ Aust 2003;179:88-91.

4. Clark RF, Wethern-Kestner S, Vance MV, Gerkin R. Clinical presentation and treatment of black widow spider envenomation: a review of 163 cases. Annals of Emergency Medicine 1992;21:782-7.

5. Garb JE, Gonzalez A, Gillespie RG. The black widow spider genus Latrodectus (Araneae: Theridiidae): phylogeny, biogeography, and invasion history. MolPhylogenetEvol 2004;31:1127-42.

6. Afshari R, Khadem-Rezaiyan M, Balali-Mood M. Spider bite (latrodectism) in Mashhad, Iran. Human and Experimental Toxicology 2009;28:697-702.

7. Maretic Z. Latrodectism: variations in clinical manifestations provoked by Latrodectus species of spiders. Toxicon 1983;21:457-66.

8. Sari I, Zengin S, Davutoglu V, Yildirim C, Gunay N. Myocarditis after black widow spider envenomation. American Journal of Emergency Medicine 2008;26:630-3.

9. Artaza O, Fuentes J, Schindler R. [Latrodectism: clinico-therapeutical evaluation of 89 cases]. [Spanish]. Revista Medica de Chile 1982;110:1101-5.

10. Isbister GK, Graudins A, White J, Warrell D. Antivenom treatment in arachnidism. Journal of Toxicology - Clinical Toxicology 2003;41:291-300.

11. Ellis RM, Sprivulis PC, Jelinek GA, et al. A double-blind, randomized trial of intravenous versus intramuscular antivenom for Red-back spider envenoming. EmergMedAustralas 2005;17:152-6.

12. Dart RC, Bogdan G, Heard K, et al. A randomized, double-blind, placebo-controlled trial of a highly purified equine F(ab)2 antibody black widow spider antivenom. Ann Emerg Med 2013;61:458-67.

13. Isbister GK, O'Leary M, Miller M, et al. A comparison of serum antivenom concentrations after intravenous and intramuscular administration of redback (widow) spider antivenom. British Journal of Clinical Pharmacology 2008;65:139-43.

14. Brown SG. Clinical features and severity grading of anaphylaxis. Journal of Allergy and Clinical Immunology 2004;114:371-6.

15. Brown SG, Isbister GK, Stokes B. Route of administration of redback spider bite antivenom: Determining clinician beliefs to facilitate Bayesian analysis of a clinical trial. Emerg Med Australas 2007;19:458-63.

16. Moore A, McQuay H, Gavaghan D. Deriving dichotomous outcome measures from continuous data in randomised controlled trials of analgesics. Pain 1996;66:229-37.

17. Isbister GK. Safety of i.v. administration of redback spider antivenom. InternMed J 2007;37:820-2.

18. Isbister GK, Gray MR. A prospective study of 750 definite spider bites, with expert spider identification. QJM 2002;95:723-31.

19. Loten C, Stokes B, Worsley D, Seymour JE, Jiang S, Isbister GK. A randomised controlled trial of hot water (45 degrees C) immersion versus ice packs for pain relief in bluebottle stings. Med J Aust 2006;184:329-33.

20. Wiener S. Red back spider antivenene. Medical Journal of Australia 1961:44-9.

21. Graudins A, Padula M, Broady K, Nicholson GM. Red-back spider (Latrodectus hasselti) antivenom prevents the toxicity of widow spider venoms. Annals of Emergency Medicine 2001;37:154-60.

22. Daly FF, Hill RE, Bogdan GM, Dart RC. Neutralization of Latrodectus mactans and L. hesperus venom by redback spider (L. hasseltii) antivenom. Journal of Toxicology - Clinical Toxicology 2001;39:119-23.

23. Isbister GK. Antivenom efficacy or effectiveness: the Australian experience. Toxicology 2010;268:148-54.

24. Graudins A, Little MJ, Pineda SS, et al. Cloning and activity of a novel alpha-latrotoxin from red-back spider venom. Biochem Pharmacol 2012;83:170-83.

25. Bird SB, Dickson EW. Clinically significant changes in pain along the visual analog scale. Annals of Emergency Medicine 2001;38:639-43.

26. Holdgate A, Asha S, Craig J, Thompson J. Comparison of a verbal numeric rating scale with the visual analogue scale for the measurement of acute pain. EmergMed(Fremantle) 2003;15:441-6.

Figure Legends

***Figure 1*:** Flow chart of all persons recruited to the study, their allocation to placebo or antivenom and the two primary outcomes.

Figure 2: A waterfall plot showing all individual changes in pain score between baseline and 2 hours in each group ordered according to baseline score and then response. Baseline score is shown as small black bar and the vertical bars indicate the movement with worsening pain shown as a checkered pattern and improvements shown in grey, with the darker grey indicating they met criteria for a significant change in pain scores (pre-specified primary outcome).

***Figure 3*:** Scatter plot of the percentage change in the pain score from baseline to the 2 hour pain score comparing placebo versus antivenom, including lines marking the median and interquartile range.

***Figure 4*:** Box plots of the pain score for all time points including on follow up at 7 to 10 days and at 6 weeks. Patients given placebo are in white and those given antivenom in grey. The boxes are the 25^{th} to 75^{th} percentile and the whiskers are 5 to 95 percentiles.

Supplementary Figure 1: A linear logarithmic plot of the percent reduction in pain for the antivenom arm versus the time from bite to antivenom (top panel) and the placebo arm versus the time from bite to antivenom (bottom panel). The solid line and shaded area represent the line of best fit by linear regression and the 95% confidence band.

Table 1: Baseline characteristics

Baseline parameters	Placebo n=112		Antivenom n=112	
Age (Median;IQR)	40.0	(26.0-54.0)	39.0	(31.8-54.0)
Male	55	49%	59	53%
Baseline Pain score[1]				
2-3	7	6%	10	9%
4-5	20	18%	22	20%
6-7	36	32%	42	38%
8-10	49	44%	37	33%
Spider Identified	87	78%	84	75%
Bite site (n=109, 111)				
Distal limb	58	53%	70	63%
Proximal Limb	26	24%	28	25%
Trunk/head/neck	25	23%	13	12%
Time to Study Treatment (Median;IQR)	2.0	(1.1-9.5)	2.7	(1.0-14.8)
Time from Analgesia to Treatment (Median;IQR)	0.5	(0.23-1.06)	0.5	(0.17-0.99)
Prior Analgesia				
Yes	47	42%	58	52%
No	52	46%	49	44%
Not recorded	13	12%	5	4%

Diaphoresis				
Nil	24	21%	24	21%
Local	56	50%	55	49%
Regional	14	13%	17	15%
Generalized	18	16%	16	14%
Systemic Effects	41	37%	35	31%

IQR – interquartile range; [1] one patient in the antivenom group had a baseline pain score of zero because there was a delay with the trial drug.

Table 2: The primary and secondary outcomes for the study.

Outcomes	Number[2]	Placebo	%	Antivenom	%	Difference	(95%CI)	P value
Primary Outcome (Pain 2h)		26	23%	38	34%	10.7%	-1.1% to +22.6%	0.1034
Primary Outcome (Systemic 2h)	(n=41, 35)	9	22%	9	26%	3.8%	-15% to +23%	0.7894
4hr Pain Reduction	(n=105, 106)	46	44%	56	53%	9.0%	-4.5% to +23%	0.2159
24hr Pain Reduction	(n=105, 107)	57	54%	67	63%	8.3%	-5% to +22%	0.2649
4 hr Systemic Resolved	(n=41, 34)	23	56%	21	62%	5.7%	-17% to +28%	0.6455
Rescue Opiate		73	65%	62	55%	-9.8%	-3% to +23%	0.172
Unblinded Antivenom		36	32%	23	21%	-11.6%	-23% to 0%	0.0682
PRN Analgesia		25	22%	29	26%	3.6%	-8% to +15%	0.6396
Serum Sickness		9	8%	12	11%	2.7%	-5% to +10%	0.6476
Serum Sickness(No unblind AV)[1]	(n=76, 89)	5	7%	9	10%	3.5%	-‡	0.5770
Acute Reactions		0	0%	4	3.6%	3.6%	-	0.1216
Repeat Presentations		11	10%	7	6%	-3.6%	-11% to +3%	0.4619

[1] Serum sickness occurring in patients who were not also given additional labeled antivenom; AV – antivenom; [2] 112 unless otherwise stated

Table 3: Additional primary outcome analyses and sensitivity analyses of the primary outcome.

Primary Outcome (pain at 2h)	Placebo	%	Antivenom	%	Diff.	(95%CI)	P value
Final definition (as per study)	26	23%	38	34%	10.7%	-1.1% to +22.6%	0.103
Original registered definition	21	19%	31	28%	8.9%	-2.1% to +20%	0.154
Median absolute change VNRS (IQR)	2	0 to 3	2	1 to 4	0	0 to 1	0.064
Median relative change (%) VNRS (IQR)	25%	0 to 50%	33%	12 to 66%	8.3%	0% to +20%	0.052
Bites with definite spider ID	N=87		N= 84				
Final definition (as per study)	22	25%	29	35%	9.2%	-4.5% to +23%	0.240
Median absolute change VNRS (IQR)	2	1 to 3	2	1 to 4	0	0 to 1	0.193
Median relative change (%) VNRS (IQR)	25%	11 to 57%	38%	13 to 63%	12.5%	0% to 20%	0.15
Absolute change VNRS > 2	**39**	**35%**	**51**	**46%**	**10.7%**	**-2.1% to +23.6%**	**0.133**
Baseline Pain (4 or greater)	N=105		N=101				
Final definition (as per study)	23	22%	33	33%	10.8%	-1.4% to +23%	0.088

Appendix – supplementary methods and results

The definition of the primary outcome for pain severity involved a reduction in the VNRS that was dependent on the baseline starting point. Initially this was defined based on a study done by Bird and Dickson on the effect of the baseline visual analogue score (VAS) on a clinically important detectable change in the VAS [25] We have previously defined primary outcomes based on Bird and Dickson that use the VAS, including both a positive and a negative randomized controlled trial.[2,19] In this study a VNRS was used to measure pain because in the previous RAVE-I study it was recognized that a VNRS was more feasible than a VAS in a study in multiple busy emergency departments. Previous research had demonstrated that there is good correlation between the two scores.[26] To allow for the categorical nature of the VNRS when using the baseline approach we had developed from Bird and Dickson, the change in pain score needed to be a whole number (i.e. 16mm became 2 or greater, 33mm became greater than 3, and 45mm became 5 or greater). The registered primary outcome therefore had a clinically significant reduction in pain defined as: 2 or greater for baseline scores of 0 to 3, greater than 3 for baseline scores of 4 to 6, and 5 or greater for baseline scores of 7 to 10. However, prior to unblinding of the study is was recognized that this would produce inconsistent results for some pain scores – e.g. a person scoring 3 only needed a change of 2 (3 to 1), whereas a person scoring 4 needed a change of 4 (4 to 0). A slight modification was made so that a clinically significant reduction in pain was defined as: 2 or greater for baseline scores of 0 to 3, 3 or greater for baseline scores of 4 or 5, 4 or greater for baseline scores of 6 or 7, and 5 or greater for baseline scores of 8 to 10.

In addition to the pre-defined definition of a clinically significant reduction in pain the change in pain was also analysed as an absolute change in the VNRS and a relative change (%) in the VNRS as sensitivity analysis. Further sensitivity analyses were done that only included cases where the spider was definitely identified and also for patients with baseline VNRS of 4 or greater. The additional outcomes and sensitivity analyses are included in table 1 and none were significant. The absolute difference in the median absolute change in the VNRS was 0 with 95%CI of 0 to 1 (table 1), which does not cross 1.3/1.4 (equivalent to a minimally clinically significant difference defined as 13mm or 1.4cm)[25,26] so there is not a detectable minimally clinically significant difference between the two groups.