BRIEF REPORT

Regional vs Systemic Antivenom Administration in the Treatment of Snake Venom Poisoning in a Rabbit Model: A Pilot Study

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Objective.—To develop a model that compares 2 different routes of antivenom administration (standard intravenous [IV] administration vs regional administration below a tourniquet) to assess their ability to limit muscle necrosis in a rabbit model of rattlesnake venom poisoning.

Methods.—New Zealand white rabbits were randomly assigned to 4 groups. All animals underwent general anesthesia and were then injected intramuscularly (IM) with a sublethal dose of western diamond-back rattlesnake (*Crotalus atrox*) venom in the right thigh and a similar volume of normal saline (NS) control in the left thigh. Thirty minutes later, standard treatment group animals ($n = 4$) received 1 vial of reconstituted Antivenin (Crotalidae) Polyvalent (ACP) and 10 mL of NS through an ear vein. Experimental treatment group animals ($n = 4$) had their lower extremities exsanguinated and isolated by arterial tourniquets. One vial of ACP was then given through a distal IV in the envenomed extremity, and 10 mL of NS was given through an IV in the sham extremity. Tourniquets were removed 30 minutes later. Positive control group animals ($n = 2$) similarly had their lower extremities exsanguinated and isolated by tourniquets. They then received 10 mL of NS through distal IVs in each lower extremity. Tourniquets were again removed after 30 minutes. Negative control group animals ($n = 2$) received 2 doses of NS only (10 mL each) through an ear vein. Serum creatinine phosphokinase (CPK) levels were drawn at baseline and 48 hours following venom injection. At 48 hours, the animals were injected with technetium pyrophosphate. Two hours later, they were euthanized, and the lower extremities were scanned to determine levels of radionucleotide uptake in envenomed muscles compared to contralateral sham-injected muscles. The anterior thigh muscle groups were then removed, fixed, stained, sectioned, and analyzed in a blinded fashion by a veterinary pathologist for muscle necrosis grading.

Results.—There was no evidence of statistically significant differences in changes in serum CPK levels (from baseline to 48 hours), technetium pyrophosphate uptake ratios (right leg/left leg), or muscle necrosis indices in any 2-group analysis.

Conclusions.—Results of this pilot study do not suggest any beneficial effect of ACP, in the dose and routes used, in limiting local muscle necrosis following IM rattlesnake venom poisoning in the rabbit model.

Key words: snakebite, antivenom, necrosis, myonecrosis

Introduction

A poster summarizing this study was presented at the 2001 American College of Emergency Physicians Scientific Symposium Research Forum, Chicago, IL, October 2001.

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Bites by pit vipers in the United States can cause significant systemic abnormalities and local tissue damage. It has been estimated that 32% of pit viper bites to the upper extremity and 12% to the lower extremity result in long-term functional impairment in victims.¹ It is likely that these are very conservative estimates, as no one

has yet performed an appropriate study truly evaluating bitten limbs (especially digits) for long-term, reduced function (eg, range of motion, sensation).²

The mainstay of treatment of significant pit viper bites is the early administration of an appropriate antivenom. Although North American pit viper antivenoms have been demonstrated to reverse systemic abnormalities such as coagulopathy, cardiovascular instability, gastrointestinal symptomatology, and neurologic dysfunction,³⁻⁵ they have not been definitively proven to have a significant effect on local tissue necrosis. The literature on this point is somewhat mixed. Some authors have found no protective effect from antivenom on necrosis in animal models,⁶⁻⁹ whereas others have suggested some degree of benefit in this regard.¹⁰⁻¹²

Bite site tissue necrosis is thought to be largely due to local hemorrhage and cell membrane disruption^{13,14}—venom effects that occur quickly and are poorly responsive to systemic antivenom administration. In addition, there may be venom myotoxins that are only poorly covered by antibodies or antibody fragments in currently available antivenoms.¹⁵ It may also be true that once venom constituents bind to their target sites, current antivenoms have difficulty limiting their effects.¹⁶ Because venom proteins and low-molecular-weight polypeptides bind very quickly to target cells at the bite site, antivenom administered after the victim arrives at the hospital may be too late to protect against local wound necrosis. As Lindsey¹⁷ has pointed out, the manufacturer of Antivenin (Crotalidae) Polyvalent (ACP; Wyeth-Ayerst Laboratories, Philadelphia, PA) has never claimed any benefit for this drug on limiting wound necrosis. Others have suggested that antivenom must be given within minutes of the bite if it is to be effective in preventing wound necrosis.¹⁸ Christensen¹⁹ found that South African Medical Institute of Research polyvalent antivenom, administered within 10 to 15 minutes of *Bitis arietans* (puff adder) venom injection in guinea pigs, did reduce necrosis. Ownby et al²⁰ noted a protective effect against myonecrosis in mice injected with *Crotalus viridis viridis* venom only when antivenom was given 5 minutes before venom injection or 5 or 30 minutes after venom injection. There was no protection when antivenom was given at 60 minutes or 3 hours. Similarly, Russell et al²¹ noted a decrease in necrosis caused by *Crotalus viridis helleri* venom only when antivenom was given within 20 minutes of venom injection in a rat model. Such times to treatment are clearly impossible goals for most human cases, given delays in getting to medical care facilities and the time required to reconstitute lyophilized antivenoms.

The obstacle of time to administration of antivenom may be insurmountable until we have the ability to ad-

minister antivenom quickly, safely, and effectively in the field. Therefore, methods of increasing its efficacy in terms of wound necrosis when given after the victim arrives at the hospital are desirable. Although some authors have attempted to find ways to maximize antivenom exposure to venom components at the bite site in the hopes of limiting necrosis, none, to our knowledge, have met with significant success to date. Bania et al²² studied intra-arterial administration of ACP in a porcine model of rattlesnake venom poisoning and found a “modest” reduction in soft tissue swelling, but they did not measure or evaluate necrosis. McCollough and Genaro²³ used radiolabeled antivenom in a dog model that compared intravenous (IV), intramuscular (IM), and subcutaneous (SQ) administration and found that more antivenom got to the venom injection site by the IV route (84.9% compared to 1.43% and 5.6%, respectively). Minton²⁴ compared intraperitoneal administration of an early form of American pit viper antivenom in mice with local infiltration and found direct injection to be ineffective. The study by Reid²⁵ suggests no beneficial effects for antivenom given by any route in terms of local tissue damage.

Another benefit that might be realized from regional antivenom administration, if effective at limiting necrosis, is a reduction in the total antivenom dose required, resulting in the preservation of a valuable resource (particularly important in areas of the world where antivenom availability is limited) as well as a reduction in the cost of treatment.²⁶

The purpose of this pilot study was to evaluate a novel approach by administering antivenom in a regional fashion to determine whether there was any evidence of reduced myonecrosis compared to standard IV administration.

Materials and methods

Large New Zealand white rabbits (ranging from 2.6 to 3.2 kg) were randomly assigned to 1 of 4 treatment groups. All animals underwent general anesthesia using glycopyrrolate 0.02 mg/kg SQ, ketamine 30 mg/kg IM, and xylazine 4 mg/kg SQ (these and all postprocedure medications were injected at sites remote from the lower extremities). The animals were endotracheally intubated, and anesthesia was maintained with inhalational halothane (titration was from 0.25% to 2.0%). Once anesthetized, each rabbit had its lower legs shaved with electric hair clippers from hip to foot. Each rabbit was then injected IM with a sublethal dose (1 mg/kg) of reconstituted, lyophilized western diamondback rattlesnake (*Crotalus atrox*) venom in the right anterior thigh and a similar volume of normal saline (NS) control in the left

thigh. Thirty minutes later, group A animals (standard treatment group, $n = 4$) received 1 vial (10 mL) of reconstituted ACP and 10 mL of NS slow IV push through a 25-gauge butterfly needle in an ear vein. Group B animals (experimental treatment group, $n = 4$), following venom injection, had their lower extremities exsanguinated (using self-adhesive, elastic wraps) and isolated by arterial tourniquets. Femoral artery occlusion was confirmed by Doppler. After removal of the wraps, 1 vial of ACP was given IV through a 22-gauge catheter in the distal envenomed extremity, and 10 mL of NS was given through a similar IV catheter in the sham-injected extremity (with the tourniquets in place). The thigh muscles of the extremities were gently massaged for 30 minutes, after which the tourniquets were released. Group C animals (positive control group, $n = 2$) similarly had their lower extremities exsanguinated and isolated by tourniquets. They then received 10 mL of NS through a distal 22-gauge IV catheter in the envenomed extremity and 10 mL of NS through a similar IV catheter in the sham-injected extremity. Massage again took place, and the tourniquets were removed after 30 minutes. Group D animals (negative control group, $n = 2$) received 2 doses of NS (10 mL each) through a 25-gauge butterfly catheter in an ear vein (no tourniquets). Following the procedure, animals were awakened, returned to their individual pens, given food and water ad lib, and medicated with buprenorphine 0.05 mg/kg SQ q8h as needed for pain.

Serum creatinine phosphokinase (CPK) levels were drawn at time 0 (baseline, before any manipulation) and at 48 hours postvenom injection. At 48 hours, the animals were sedated with acepromazine 2 mg SQ and ketamine 20 mg/kg SQ and were taken to the nuclear medicine laboratory, where they were injected by an ear vein catheter with a dose of technetium pyrophosphate (approximately 1 mCi). Two hours later, they were euthanized with a 1-mL IV bolus of euthanasia solution (390 mg of sodium pentobarbital per milliliter) (Euthasol, Delmarva Laboratories Inc, Des Moines, IA), and ventral scans of the lower extremities were obtained. The anterior thigh muscle groups were then removed and fixed in 10% buffered formalin. Sections were taken from each thigh group at 3 locations (at the injection site and 1 cm above and below the injection site). Sections were mounted and prepared with hematoxylin-eosin stains and with Mallory trichrome stain.

The nuclear scans (Figure) were read by a radiologist, blinded to the treatment groups, who compared technetium pyrophosphate uptake between the envenomed and sham-injected extremities of each animal. The muscle sections were examined under light microscopy in a blinded fashion by a single veterinary pathologist to de-

A representative radionuclide scan (taken of a group B animal, treated with regional antivenom). Increased tracer uptake can be seen in the envenomed right thigh muscles compared to the left.

termine a muscle necrosis index on the basis of the severity of cell damage (mild = 1; mild-moderate = 1.5; moderate = 2; moderate-marked = 2.5; marked = 3; marked-severe = 3.5; and severe = 4).

Group comparisons of measures of change in serum CPK level, tracer uptake, and muscle necrosis index were performed by the Wilcoxon 2-sample test.

This study was approved by the Stanford University Institutional Review Board and the Animal Care Committee.

Results

Group means (with SE) for changes in CPK levels from baseline to 48 hours postvenom poisoning, CPK ratios (change in CPK levels from baseline to 48 hours divided by the baseline level), radionuclide uptake levels, and necrosis indices are listed in the Table. There were no differences between the 4 groups in terms of muscle necrosis by any measure (none of the dependent measures between any of the groups achieved statistical significance), nor was there any suggestion on gross examination at the time of necropsy of any beneficial antivenom effect in any of the treated animals. All animals demonstrated severe necrosis.

Discussion

This pilot study failed to demonstrate any statistically significant protective effects of ACP on local muscle necrosis, whether given by a standard IV route or by regional administration. Although there was a trend toward reduced total increases in CPK levels in those rabbits that received antivenom (most pronounced in the stan-

Comparison of CPK levels, CPK ratios, radionuclide uptake levels, and necrosis indices between treatment groups*†

	Group A standard AV treatment	Group B regional AV treatment	Group C positive control	Group D negative control
n	4	4	2	2
CPK ₄₈ - CPK ₀	12 959 (8113)	38 262 (27 388)	55 590 (14 360)	50 033 (8484)
(CPK ₄₈ - CPK ₀)/CPK ₀	22.84 (15.38)	104.29 (80.0)	113.89 (41.56)	89.78 (35.75)
Tc uptake ratio (right leg/left leg)	1.40 (0.10)	1.54 (0.12)	1.39 (0.33)	1.97 (0.05)
Necrosis index	3.62 (0.375)	3.88 (0.125)	2.75 (0.25)	2.25 (0.25)

* AV indicates antivenom; CPK, creatinine phosphokinase; and Tc, technetium pyrophosphate.

† All values given are group means, with standard error in parentheses. No values achieved statistical significance.

dard IV-treated group), radionuclide scanning and microscopic analysis suggested an equivalent degree of necrosis in all groups.

It is obviously possible that, given the pilot study nature of this project, our sample sizes were inadequate to demonstrate any treatment effect. It may also be that the dose of antivenom chosen was too low. It is possible, however, that antivenom does not have any appreciable effect on local necrosis when given 30 minutes postenvenoming in the rabbit model. There are, without doubt, interspecific differences in response to snake venoms²⁷ and antivenoms, and it may well be that the rabbit is not an appropriate model for pit viper venom or antivenom research.

It is unlikely that the batch of ACP we used lacked protective antibodies against the snake venom we chose to study (*C atrox*), as this species is specifically used in its production. There are, however, intraspecific venom differences between snakes based on geographic location (among other factors),²⁸ and this variability cannot be completely ruled out as a cause for our failure to demonstrate any local protective effect by ACP.

In an effort to find ways to limit the most common complication following pit viper bites—local tissue necrosis—it is important for research in this area to continue. Future studies should include the use of the new ovine Fab antivenom (CroFab, Savage Laboratories, Melville, NY). Although evidence does not yet suggest an ability of this product to reduce local wound damage,¹³ it may be that the smaller molecular weight of the Fab fragments and the larger volume of distribution allow better access to venom components at the bite site.²⁹ Likewise, varying the dose of antivenom should be studied, particularly with CroFab, to determine whether higher doses would show more local benefit. Russell⁵ anecdotally noted an apparent reduction in necrosis in clinical cases of rattlesnake envenomation after he in-

creased the doses of ACP he administered to patients after 1980.

To overcome the significant obstacle of time to administration of antivenom, it will be important to systematically study the potential benefits of giving antivenom in the field in terms of effect on local tissue necrosis as well as systemic effects. Now that North America has an environmentally durable antivenom,¹³ CroFab, that appears relatively safe to administer,⁴ it may be possible to equip prehospital providers or well-funded wilderness expeditions with this product for administration prior to hospital arrival, particularly in areas where transport times are prolonged.

Finally, the optimal animal model for snake venom-induced necrosis should be established. This may involve a porcine, canine, or even primate model. Venom research of this sort will continue to be significantly limited until we arrive at a standard model that produces data that can be confidently extrapolated to humans.

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