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Brief Reports

A new F(ab')₂ antivenom for the treatment of crotaline envenomation in childrenDaniel R. Lasoff, MD^{a,b}, Anne-Michelle Ruha, MD^c, Steven C. Curry, MD^c, Cynthia Koh, MD^{a,b}, Richard F. Clark, MD^{b,*}^a VA Medical Center, San Diego, CA^b UCSD Division of Medical Toxicology, Department of Emergency Medicine, San Diego, CA^c Department of Medical Toxicology, Banner University Medical Center, Phoenix, AZ

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ABSTRACT

Study objective: To evaluate the efficacy and safety of a new F(ab')₂ antivenom preparation in the treatment of Crotalinae envenomation in children.**Methods:** We present a case series of children younger than 16 years who suffered Crotalinae envenomation and were treated with a new F(ab')₂ antivenom. Envenomated children treated with the new antivenom were assessed for efficacy of the product, defined as improvement of any hemotoxicity (hypofibrinogenemia, defined as fibrinogen <150 mg/dL, or thrombocytopenia, defined as platelets <150 000/mm³), and the cessation of the advancement of swelling. Safety was also evaluated by monitoring for adverse events. A secondary parameter assessed in these children was recurrent hemotoxicity after initial control of signs and symptoms with follow-up visits and laboratory testing on posttreatment days 5, 8, and 15.**Results:** Twenty-one children received the F(ab')₂ antivenom. Efficacy was achieved in all children receiving the product with initial control of swelling and improvement in those with hemotoxicity. No patients suffered anaphylaxis or any other serious adverse events from the F(ab')₂ treatment. There were no cases of recurrent hemotoxicity recorded in the study between time of initial control and postinfusion day 15.**Conclusions:** In this series of children, the F(ab')₂ antivenom appeared to be both safe and effective in the treatment of hemotoxicity and local tissue toxicity (swelling) from Crotalinae envenomation.

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1. Introduction

More than 6000 envenomations from snakebites were reported to US Poison Control Centers in 2013, many of which received antivenom therapy [1]. Despite the high number of snakebites occurring each year, there are only a handful of deaths reported annually, likely in part due to antivenom therapy.

North American crotaline snake venom contains diverse components that vary depending on many factors. Two of the most important clinical effects seen in humans after envenomation include the following: (1) hemotoxicity, characterized by hypofibrinogenemia and thrombocytopenia, and (2) tissue toxicity or swelling of the bitten extremity [2]. In patients treated in the United States with the presently approved crotaline Fab antivenom, the hemotoxicity and swelling can usually be stabilized or reversed with varying doses of antivenom [3].

Initial antivenoms were developed in domestic animals by inoculating them with small nonlethal doses of venom. The animals then produce antibodies to the venom that were collected and somewhat purified before human administration. Because some of these first antivenoms were poorly purified horse serum with whole-IgG antibodies that included the immunogenic Fc portion, their use was often complicated by immunologic or allergic/anaphylactoid reactions. Acute reactions such as hypotension, bronchospasm, and urticaria were common, and delayed reactions such as serum sickness were reported in many cases [4,5]. The more recent crotaline Fab antivenom (CroFab) used in the United States is formed by separating antibodies from other serum components and then cleaving off the Fc portion of the antibodies with papain, using only the remaining Fab fragments. These Fab fragments were found to be less immunogenic when compared with whole-IgG products, and effective in both adults and children [6,7]. However, extended experience with this Fab antivenom has revealed the unanticipated problem of recurrence of hemotoxicity and other signs of envenomation after initial improvement, hours or days after initial control of toxicity associated with envenomation, likely due to the different pharmacokinetics of Fab fragments [8].

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There have been recent promising data that this recurrence phenomenon may be attenuated using a $F(ab')_2$ antivenom, in which most of the Fc portion is cleaved and removed, but the Fab fragments of the antibody remained joined [11,18]. $F(ab')_2$ antivenoms directed at other venomous snake species are available in other parts of the world and are used successfully [9,10]. In a recently published study by Bush et al [11] in the United States, the presently approved crotaline Fab antivenom was compared with a new $F(ab')_2$ crotaline antivenom for treatment of Crotalinae envenomation. The goal of that trial was to compare the efficacy of each antivenom to prevent recurrence or late hemotoxicity after treatment of the envenomation. The overall findings of the study showed that management with $F(ab')_2$ antivenom may reduce the risk of posttreatment hemotoxicity when compared with management with Fab [11]. We present in more detail a series of children from this study bitten by crotaline snakes who were treated with a new $F(ab')_2$ antivenom preparation.

2. Methods

2.1. Patients

Patients were boys and girls aged 2 through 15 years who presented for treatment of a crotaline envenomation at tertiary care centers in multiple states. Signs of envenomation were judged by on-site physicians and included swelling, ecchymosis, blebs, fall in platelet counts, fall in plasma fibrinogen concentration, hypotension, refractory bleeding, vomiting, diarrhea, and neurotoxicity.

Patients were excluded from study entry if they had allergies to horse or sheep serum, or had any known underlying bleeding or clotting disorders. Enrolled patients were randomized to receive either Fab antivenom or $F(ab')_2$ antivenom [11]. This report concerns the group of children who received the $F(ab')_2$ antivenom. The $F(ab')_2$ antivenom group was further subdivided into a group that received maintenance dosing of antivenom or placebo. Patients were treated between May 2008 and September 2011. All patients were followed up for at least 22 days after their initial event. Patients had in-person follow-up visits on days 5, 8, and 15 after envenomation, with an additional phone interview on the 22nd day after treatment. The study was approved by the investigational review boards of each participating institution.

2.2. Measurements

The measurements of initial efficacy of antivenom treatment were tissue toxicity or swelling, and hemotoxicity, specifically *thrombocytopenia* (defined for the purposes of this study as platelets $<150\,000/\text{mm}^3$) and *hypofibrinogenemia* (defined for purposes of this study as fibrinogen concentration $<150\text{ mg/dL}$). Platelet counts and fibrinogen concentrations were drawn before administration of antivenom. Repeat studies were drawn 2 hours after initial antivenom treatment. Patients received additional doses of antivenom to treat progressing symptoms (continued progression of swelling, continued hemotoxicity, or other venom-related signs and symptoms based on the clinical judgment of the treating clinician). Patients receiving additional doses of antivenom had additional laboratory studies drawn 2 hours after each additional antivenom administration. At follow-up visits, platelet counts and fibrinogen concentrations were rechecked, and swelling was reassessed for evidence of recurrence of toxicity. Tissue toxicity or swelling was assessed in all patients by measuring the leading edge of the proximal progression of edema and limb circumference, if the bite was in an extremity. A torso bite would have swelling measured by the leading edge of edema only.

2.3. End points

Each child was evaluated by the treating clinician for antivenom efficacy against hemotoxicity, swelling, and any other systemic

symptoms. *Efficacy against hemotoxicity* was defined as correction toward normal of initial hypofibrinogenemia or thrombocytopenia. *Efficacy in treating swelling* was defined as cessation of advancement of swelling in both leading edge of edema and limb circumference. Safety of the $F(ab')_2$ was assessed through the reporting of adverse events after antivenom infusion. *Significant adverse side effects* were defined as development of hypotension (defined by age-appropriate normal values), anaphylaxis (defined as the development of clinical bronchospasm and hypotension), or airway compromise after administration of the study drug. Rashes, nausea, vomiting, and other mild adverse events were also recorded.

Each child was also evaluated for the development of recurrent hemotoxicity at follow-up visits on days 5, 8, and 15 after the envenomation, defined as the redevelopment of thrombocytopenia or hypofibrinogenemia during that period. Our definitions of thrombocytopenia and hypofibrinogenemia were the same for recurrence as for initial measurements. Adverse events were also recorded from these follow-up visits.

2.4. Antivenom

The $F(ab')_2$ preparation used in all patients was equine derived. It is currently approved for use in the United States but will not be marketed until 2018. The product was created by immunizing horses against venom from *Bothrops asper* and *Crotalus simus*. Whole immunoglobulin molecules were isolated and treated with pepsin to cleave them into $F(ab')_2$ units. Each vial produced contained approximately 10 mg of purified antivenom. The dosing protocol for the study called for 10 vials of antivenom administered for 1 hour, followed by up to 10 additional vials every 2 hours until the treating physician felt initial control was obtained, specifically relating to tissue toxicity or swelling, hemotoxicity, or other venom-related signs or symptoms. After initial control was obtained, treating physicians could provide additional 4 vial doses as the treating physician felt necessary based on progression or return of signs or symptoms.

Patients were randomized for purposes of the initial comparative study [11] where 12 patients received “maintenance dosing” of 4 additional vials of $F(ab')_2$ antivenom every 6 hours for 3 to 5 doses determined by the treating physician, similar to the maintenance dosing described in the package insert that can be used with the presently approved crotaline Fab antivenom product. Nine patients received no maintenance dosing.

3. Results

A total of 21 enrolled children received $F(ab')_2$ antivenom, and none were excluded. Demographics are included in Table 1. Thirteen participants were boys and 8 were girls. Participants ranged in age from 2 to 15 years. “All bites occurred on the extremities, with the majority (14) on lower extremities. Table 2 shows the geographic breakdown of envenomations by state. Six children presented with initial hypofibrinogenemia and 6 children presented with initial thrombocytopenia.

3.1. Efficacy

Initial control of swelling and hemotoxicity was obtained in all participants with administration of the antivenom. All participants experienced a halt of progression of swelling. Total antivenom dosing ranged between 10 and 46 vials per patient. The median treatment was 22 vials. Two children presented with platelets less than $100\,000/\text{mm}^3$, and both of these patients' platelet counts and those of 4 others presenting with thrombocytopenia rose to a normal range with treatment. In children presenting with hypofibrinogenemia, all children stabilized or improved their fibrinogen concentrations after the initial 10 vial infusion of antivenom. Children with initial thrombocytopenia tended to require more antivenom for initial efficacy, with 4 children requiring 20

Table 1
Patient demographics

Age (y)	
Median	8
Mean	7.6
Range	2–15
Sex	
Male	13 (61.9%)
Female	8 (38.1%)
Ethnicity	
White	12
Black	1
Hispanic	7
Native American	1
Initial platelets (thousand/mm ³)	
Mean	225.9
Median	208
Range	51–400
Initial fibrinogen (mg/dL)	
Mean	248.1
Median	253
Range	60–646
Time from bite to antivenom (min)	
Mean	396
Median	288
Range	180–1116
Bite location	
Upper extremity	7 (33.33%)
Lower extremity	14 (66.67%)
Face	0
Trunk	0

vials of antivenom and 2 requiring 30 vials of antivenom for improvement in platelet counts.

After initial control was obtained with F(ab')₂ antivenom in all children, 12 of 21 participants also received maintenance or extra doses between 12 and 20 additional vials as part of the study protocol. In the group receiving maintenance dosing, no cases of delayed hemotoxicity were encountered at follow-up. Similarly, in the 9 participants who received no maintenance dosing, there were no episodes of delayed hemotoxicity at follow-up (Table 3). There was no recurrent swelling or other tissue toxicity noted at follow-up.

3.2. Adverse events

Three participants experienced adverse events. One child experienced pruritus and hives soon after administration of F(ab')₂ antivenom that resolved with diphenhydramine. Another child developed nausea after administration of antivenom but required no intervention. A third child developed pruritus without rash during treatment. All adverse events resolved without long-term sequelae, and all participants completed antivenom treatment. No cases of anaphylaxis or delayed serum sickness occurred in this case series. No episodes of hypotension or airway complications occurred with treatment. There were no episodes of vomiting, diarrhea, or abdominal pain.

4. Discussion

In this case series, we examined a group of children from a previous study treated with a new F(ab')₂ antivenom for crotaline snake

Table 3
Treatment results of follow-up visits for patients.

Cases of delayed hemotoxicity	0
Platelets day 5 (n = 21; thousand/mm ³)	
Mean	291.2
Median	268
Range	170–427
Platelets day 8 (n = 20; thousand/mm ³)	
Mean	306.5
Median	308
Range	181–403
Platelets day 15 (n = 19; thousand/mm ³)	
Mean	366.4
Median	373
Range	209–639
Fibrinogen day 5 (n = 20; mg/dL)	
Mean	301.75
Median	301
Range	173–412
Fibrinogen day 8 (n = 18; mg/dL)	
Mean	309.22
Median	310
Range	195–441
Fibrinogen day 15 (n = 18; mg/dL)	
Mean	298.22
Median	270.5
Range	183–761

Laboratory results were not obtained in some children on some follow-up days. n = the number of children who had laboratory results recorded on corresponding follow-up day.

envenomation, focusing on efficacy and safety. All children in this case series were successfully treated using the F(ab')₂ preparation. Furthermore, there were no life-threatening or severe adverse events. There were also no episodes of delayed hypersensitivity encountered. This is consistent with previously demonstrated safety profiles of F(ab')₂ fragment antivenoms that are used in other parts of the world [9,10]. In the United States, the only Food and Drug Administration–approved antivenom for envenomation by *Centruroides* scorpions is a F(ab')₂ preparation, which likewise has proven to be clinically efficacious and safe [12,13].

One significant finding in this case series was that no patients developed a delayed or recurrent hemotoxicity. This is in contrast to patients treated with crotaline Fab snake antivenom in this country, of whom as many as 53% may develop a recurrence of hemotoxicity [14]. On rare occasions, this recurrent hemotoxicity may be fatal [15]. Delayed hemotoxicity can also occur in children bitten by crotaline snakes treated with Fab antivenom [16,17]. A previous adult case series of crotaline envenomations comparing recurrence in crotaline-envenomated participants treated with either Fab or F(ab')₂ antivenom showed much less recurrent hemotoxicity when F(ab')₂ product was administered [18]. The higher incidence of delayed or recurrent hemotoxicity with Fab antivenom compared with the F(ab')₂ has been postulated to result from pharmacokinetic differences between the molecules. Because Fab fragments generally have a molecular weight around 50 kDa compared with that of F(ab')₂ at about 110 kDa, this may result in a more rapid renal clearance of smaller Fab fragments than circulating snake venom [19]. Fab fragments have been shown to have an elimination half-life of around 12 to 23 hours [20]. This would correspond temporally with the reappearance of free venom in some cases of recurrence. The larger F(ab')₂ molecules may be able to avoid renal excretion for a longer period and thus exhibit a longer therapeutic effect [21].

5. Limitations

Limitations of our study include its small sample size case series and geographical variations in snakes and their venom. In addition, as in most clinical cases of Crotalinae bites in the United States, the decision on giving antivenom both initially and as the case progressed was somewhat dependent on the clinician's subjectivity. However, using predefined hemotoxicity parameters allowed for objective measurements of recurrent

Table 2
Geographic location of envenomations in this series

State	Cases
Arizona	6
California	5
New Mexico	1
North Carolina	2
Texas	7

effects. In the parent study, only 1 child received the Fab antivenom, so making comparisons between the study groups with regard to recurrent hemotoxicity was not possible.

6. Conclusions

Although the number of patients in this series is small, this new crotaline F(ab')₂ antivenom appears to be effective and safe in children. Efficacy was obtained in all children with initial control of hemotoxicity and swelling after antivenom administration. No significant adverse events were encountered. Finally, there were no cases of delayed or recurrent hemotoxicity noted on follow-up. When this product is available in the United States, it should provide an alternative treatment of crotaline envenomation that will likely be effective and safe in the initial control of venom toxicity. It is possible that the F(ab')₂ antivenom may decrease the rate of recurrent hemotoxicity, but larger studies in children are needed.

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