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TOXICOLOGY INVESTIGATION

Efficacy of North American Crotalid Antivenom Against the African Viper *Bitis gabonica* (Gaboon Viper)

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Abstract Envenomations by exotic snakes occur from zoological collections and private individual collectors. Antivenoms to these snakes may not be readily available. The objective of this study is to determine the efficacy of North American crotalid antivenin in treating mice envenomated with venom of the African viper *Bitis gabonica* (Gaboon viper). The subjects of the study were Swiss Webster mice weighing approximately 30 g. The study was conducted in the University research laboratory. *B. gabonica* venom was obtained from Venom Supplies Pty Ltd (Tanunda, South Australia) and reconstituted in sterile water. North American Crotalid Fab2 antivenin (Anavyp®, Instituto Bioclon, Mexico) was donated by the manufacturer. The experimental groups were: Group I received two times an intraperitoneal LD_{50} dose of venom, 2.58 mg/kg. Group II received the same dose after incubation for 1 h with 10 mg of antivenin. Time to onset of toxicity defined as respiratory rate <10/min or absence of response to prodding. *t* test and Chi square with $p<0.05$ considered significant. Time to onset of toxicity was 7.040±4.334 h in group I, and 20.665±2.074 in group II ($p=0.0064$, 95% confidence interval of difference of means −22.694 to −4.556). Antivenin was efficacious to statistical significance at 4, 8, 12, and 16 h (*p* values of 0.062, 0.0067, 0.0067, and 0.0253, respectively). Improvement at 20 and 24 h (*p* values of 0.0673 and 0.0673, respectively) did not achieve statistical significance. North American Crotalid antivenin (Anavyp®, Instituto Bioclon, Mexico) demonstrated efficacy in increasing time to onset of distress in mice poisoned with *B. gabonica* (Gaboon viper) venom. Based on this result, treatment of humans envenomated with *B. gabonica* with North American Croatlid antivenin could be considered for severe envenomations if specific *B. gabonica* antivenin is unavailable.

Keywords Snakebite · Antivenom · Gaboon viper

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Introduction

Envenomation by exotic or non-native snakes is a worldwide problem [1–5]. In the USA, the number of exotic poisonous snake bites reported to the American Association of Poison Control Centers Toxic Exposure Surveillance system over the last 5 years ranged from 96 to 131 per year [6–10]. Species-specific antivenom, the definitive treatment of poisonous snake bites, may not be readily available for exotic species. When antivenom is available, it is often whole antibody serum derived from horse serum with a greater potential for anaphylactic reactions and serum sickness than the Fab products that are available for native species.

Mouse lethality models have been used to establish the efficacy of antivenoms between native and non-native snakes. Wisniewski and collaborators employed this model to demonstrate that Australian tiger snake (*Notechis scutatus)* and Mexican coral snake (*Micrurus* species) antivenoms are efficacious against a United States coral snake (*Micrurus fulvius fulvius*) [11]. Richardson and his collaborators demonstrated that North American coral snake antivenom is efficacious against the venom of non-native elapids such as cobras [12], and that North American crotalid antivenom is efficacious in treating South American vipers [13]. The efficacy of North American crotalid

antivenom against venom of vipers native to Africa has never been studied.

This study of the efficacy of a North American crotalid antivenom (Anavyp®, Instituto Bioclon, Mexico) against the Gaboon viper (*Bitis gabonica*) native to Africa was motivated by difficulties treating a Gaboon viper envenomation at our institution. Specific horse serum antivenin was obtained with difficulty and delays and resulted in anaphylactic reaction. Though exotic snakebites are rare, they do occur as illustrated by five other recent cases of Gaboon viper envenomations in North American [14].

The objective of this study was to use a mouse lethality model [11–13] to determine the efficacy of a North American crotalid antivenom (Anavyp®, Instituto Bioclon, Mexico) in treating mice given the venom of the African viper *B. gabonica* (Gaboon viper). It can be hypothesized that a North American crotalid $(Fab)_2$ antivenom would prolong the survival time of mice given the venom of the African viper *B. gabonica*.

Material and Methods

Materials Swiss Webster mice weighing approximately 30 g were used (Jackson Laboratories, Bar Harbor, Maine). They were housed in a standard mouse cage and fed standard mouse feed ad libitum. Gaboon viper venom was obtained from Venom Supplies Pty Ltd (Tanuda, South Australia). $(Fab)_2$ antivenom to North American rattlesnakes was donated by the manufacturer (Laboratorios Silanes S.A. de C.V., Mexico)

Study Design A randomized, placebo-controlled trial of the efficacy of a North American crotalid antivenom (Anavyp®, Instituto Bioclon, Mexico) in mice given Gaboon viper venom was performed. Mice were used because they have been previously used to study the cross-reactivity of species-nonspecific antivenom [11–13].

The study mice were divided into two groups. The control group received *B. gabonica* venom and 0.9% normal saline solution. The treatment group received *B. gabonica* venom and Fab_2. The control group consisted of five animals and the experimental group consisted of ten animals. All animals received the same injection volume and venom concentration.

Mice were given analgesia with a single dose of buprenorphine (0.01 mg/kg) by subcutaneous injection prior to administration of venom, using a No. 27 needle, 30 min prior to venom injection. The dose of venom used was two times the published LD_{50} in mice (1.58 mg/kg) [15]. Antivenom was received as a lyophilized powder that was reconstituted in 10-mL sterile water per vial. Mice in the treatment group received a venom–antivenom solution that was pre-mixed in a 3-mL syringe and incubated at 25°C for 1 h before intraperitoneal injection. The premixed syringes were diluted to a total volume of 1 mL to provide a uniform volume for intraperitoneal injection. All animals were weighed to determine the dose of venom to be administered. Normal saline solution was used for venom and antivenom dilutions.

Outcome Measures Primary outcome measures used were signs of distress and time to onset of distress for a 24-h period following the injections. Animals that showed signs of distress were euthanized by CO_2 inhalation. Distress was defined as respiratory rate less than ten breaths per minute or lack of response to a gentle tap on the back. Mice were observed continuously for the first 8 h. They were observed at 2-h intervals for the second 8 h. They were observed at 4-h intervals for the last 8 h of the study. At the conclusion of the 24-h study period, animals were euthanized by CO_2 inhalation. An independent observer, blinded to the group assignments, observed the animals and determined onset of distress.

Statistical Analysis *t* test and Chi square with $p<0.05$ considered significant.

Results Time to onset of distress or survival to 24 h was 7.040±4.334 h in the control group and 20.67±2.07 h in the treatment group ($p=0.0064$, 95% confidence interval of difference of means −22.69 to −4.56; Fig. 1). Seven of ten mice (70%) in the treatment group survived to 24 h of while only one of five mice (20%) in the control group survived the full 24 h. Antivenom improved survival without distress at 4, 8, 12, and 16 h (p values of 0.0062, 0.0067, 0.0067, and 0.0253, respectively). Survival was improved at 20 and 24 h (p values of 0.0673 and 0.0673, respectively) but statistical significance was not obtained.

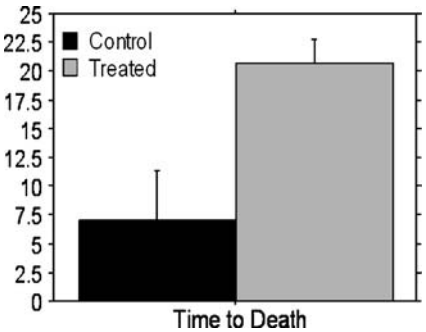

Fig. 1 Time to death in hours of the control vs. treated group. The mean time to death for the control group was significantly shorter than that of the treated group (7.04±4.3 h versus 20.67±2.1 h; $p=0.006$)

Discussion

This study evaluated the efficacy of North American crotalid $(Fab)_2$ antivenom (Anavyp®, Instituto Bioclon, Mexico) in treating mice injected with venom of the African viper *B. gabonica* (Gaboon viper). The North American antivenom was efficacious in this model. Other investigators have found that Australian tiger snake (*N. scutatus)* and Mexican coral snake (*Micrurus* species) antivenoms have efficacy against a United States coral snake (*M. fulvius fulvius*) [11]. A North American crotalid antivenom is efficacious in treating two South American crotaline snakes: *Crotalus durissus terrificus* (tropical rattlesnake) and *Bothrops atrox* (fer-de-lance) [12]. A North American coral snake antivenom is efficacious in treating two exotic elapid envenomations: *Naja naja* (Indian cobra) and *Dendroaspis polylepsis* (black mamba) [13].

While specific antivenoms are available in other countries to their native species, they are not readily available to emergency physicians in the USA. Zoos and other facilities that maintain collections of poisonous snakes often maintain stocks of antivenom against the venoms of snakes in their collections. Poison control centers maintain an Antivenom Index to assist in locating species-specific antivenom. If a patient is seriously ill after a non-native viper bite and specific antivenom is not available, using a native antivenom may be reasonable in light of these studies.

One of the limitations of this experiment is that the antivenom and the venom were mixed together prior to the injection into the mice. This does not accurately re-create what occurs in human snake envenomations in which the antivenom and venom enter the body separately and are mixed in vivo rather than in vitro. Another limitation in this study was that one of the mice in the control group survived the full 24 h of the experiment, while three of ten in the treatment group did not. That there were deaths in the treatment group most likely indicates an inadequate dose of antivenom. If a second or higher dose of antivenom were given, survival in the treatment group may have improved.

A dose–response curve was not performed for the venom used in the interest of limiting the number of animals used and not reproducing published results. A protein control was not used to determine that efficacy was from specificity of the antivenom rather than nonspecific binding of venom to protein, but this has been established for other venoms and antivenoms [11] and we have no reason to believe that nonspecific protein binding was responsible for the effect seen. In spite of these limitations, the study found a statistically significant improvement in survival with treatment.

This work extends earlier investigations demonstrating efficacy between venoms and antivenoms with similar toxicity [11–13] to an African viper. A clinician treating a severe poisonous snake bite can consider giving available elapid or viper antivenom while trying to find a species-specific antivenom. If species-specific antivenom is available but an intact horse serum antibody that is more likely to produce anaphylactic shock or serum sickness is not tolerated, a clinician could consider initiating treatment with Fab or Fab_2 fragments.

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