Pressure Immobilization Delays Mortality and Increases Intracompartmental Pressure After Artificial Intramuscular Rattlesnake Envenomation in a Porcine Model

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Study objectives: We determine the effect of pressure immobilization on mortality and intracompartmental pressure after artificial intramuscular *Crotalus atrox* envenomation in a porcine model.

Methods: We prospectively studied 20 pigs using a randomized, controlled design. After anesthesia, *C atrox* venom (20 mg/kg) was injected with a 22-gauge needle 10 mm deep into the tibialis anterior muscle of the hind leg. Pigs were randomized to receive either pressure immobilization (applied 1 minute after envenomation and maintained throughout the duration of the experiment) or no pressure immobilization. We measured time to death, intracompartmental pressure before venom injection and at 2 hours after injection, and leg circumference at a standardized location before injection and immediately postmortem. Duration of survival was compared using Kaplan-Meier survival analysis.

Results: The dose of venom resulted in 100% mortality. The median survival was longer in the pressure immobilization group (191 minutes, range 140 to 240 minutes) than in the control group (median 155 minutes, range 119 to 187 minutes). The difference between the groups was 36 minutes (95% confidence interval [CI] 2 to 64 minutes; P=.0122). The mean intracompartmental pressures were 67±13 mm Hg±SD with pressure immobilization and 24±5 mm Hg without pressure immobilization. The difference between groups was 43 mm Hg (95% CI 32 to 53 mm Hg). The mean circumferences were 14.3 cm in the pressure immobilization group and 19.1 cm in the control group. The difference between groups was –4.8 cm (95% CI –5.7 to –3.9 cm).

Conclusion: Compared with control animals without treatment, the pressure immobilization group had longer survival, less swelling, and higher intracompartmental pressures after artificial, intramuscular *C atrox* envenomation in our porcine model.

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INTRODUCTION

Background

After a bite by a large snake such as Viperidae, there is little that an individual can do in the field to mitigate the effects of the bite. No first aid technique has been shown to reduce

Editor's Capsule Summary

What is already known on this topic

Although well established for the treatment of elapid snakebites, the pressure immobilization bandage has been questioned for use on pit viper bites, which cause more local injury.

What question this study addressed

This study attempts to determine the effect of pressure immobilization on mortality and intracompartmental pressure after artificial, intramuscular *Crotalus atrox* envenomation in a porcine model.

What this study adds to our knowledge

In the 20 pigs studied, application of the pressure immobilization bandage prolonged survival slightly, but increased compartmental pressure greatly.

How this might change clinical practice

Pressure immobilization should be discouraged for bites that involve venoms that produce substantial local injury, such as the rattlesnake, copperhead, and cottonmouth snakes of the United States.

mortality or otherwise improve outcome after severe viper envenomation. On the contrary, many historically recommended first aid techniques (eg, incision and suction, cryotherapy, electroshock) have been shown to worsen envenomation sequelae or even result in injury independent of the bite.

Pressure immobilization is recommended for first aid field treatment of venomous snakebites in Australia. The technique involves wrapping the entire extremity, starting at the bite site, with an elastic or compressive bandage and immobilizing it with a splint. When properly applied, this technique has been shown to slow systemic spread of venom.[1-3] Whether slowing the spread of venom is beneficial after viper envenomation has been the subject of intense debate.[4] Pressure immobilization has not gained wide acceptance for treatment of snakebites by species capable of causing serious tissue destruction, particularly in species or regions of the world where mortality is low. In general, Australian snakes are not known for causing serious morbidity from tissue injury. In contrast, morbidity after pit viper envenomation in the United States is reported to be around 10%, although mortality is rare.[5] Most vipers possess highly proteolytic venom, which is composed of many enzymatic components and essentially results in digestion of tissue.[6] Because pressure immobilization sequesters venom near the bite site, some have argued that it may worsen morbidity by augmenting the venom's locally destructive effects, resulting in more extensive tissue necrosis. For this reason, the technique is currently not recommended for first aid field treatment of venomous snakebites in the United States. The question of whether to use pressure immobilization is even more difficult to answer in other parts of the world, where large vipers can cause severe tissue destruction but mortality is higher.

Importance

Access to emergency medical care and antivenom may be exceedingly delayed in some parts of the world (ie, deep in the wilderness or in resource-challenged countries). Delays in treatment have been reported to contribute to fatalities.[7] Perhaps pressure immobilization could increase the time a victim has to get to medical care, although this gain may be at the expense of increased local tissue injury. Therefore, the goal of our study is to determine the effect of pressure immobilization on mortality and intracompartmental pressure after artificial intramuscular *Crotalus atrox* envenomation in a porcine model.

MATERIALS AND METHODS

We performed a randomized, controlled trial using a porcine model.

The Animal Research Committee at our institution approved this study. All procedures involving animals complied with the regulations and guidelines of the Animal Welfare Act, the National Institutes of Health *Guide for the Care and Use of Laboratory Animals,* and the American Association for Accreditation of Laboratory Animal Care. The housing of animals and the performance of the study took place in the Animal Care Facility at our institution.

Female domestic pigs were selected because of the similarities between porcine and human tissues.[8-12] Additionally, we believed that the pig was the best animal model to simulate the human response to envenomation under the conditions of our experiment. Pigs have been used successfully in previous envenomation research.[8,9]

According to previous research,[8] the mortality in control pigs treated with *C atrox* venom at 20 mg/kg was estimated to be 100%. We were unable to perform a sample size calculation because of the absence of baseline data on time to mortality and compartment pressures. Therefore, we selected 20 pigs as a reasonable sample size for this project.

Interventions

We wished to simulate a severe snakebite inflicted by a large viper to a victim who was far away from any sort of

medical care. In our scenario, the needle represented a long viper fang, which penetrated a muscle compartment. We chose intracompartmental deposition of venom because it could produce the most serious local sequelae after viper envenomation, such as the loss of a limb or digit or the loss of function at a joint.

Needle size was chosen on the basis of a previous report,[8] comparison of previously published measurements of fang length,[13] and the authors' visual comparison of actual rattlesnake fangs. Depth of injection was based on illustrations from an anatomy text and previous studies.[14]

Although there are no studies confirming this hypothesis, scientific literature suggests that venom is usually injected subcutaneously after snakebites.[15] However, large vipers possess long fangs relative to other families of snakes and certainly may deposit venom intramuscularly, which could quickly and easily lead to compartment syndrome. Although unusual, compartment syndrome has been documented to occur after some pit viper envenomations.[16,17]

We also wished to contrive a wilderness situation in which a layperson might manually apply pressure immobilization. Therefore, no pressure-measuring device (ie, a manometer) was used while the wrap was applied to our subjects. The tension placed in the wrap was similar to that used for a typical human ankle sprain. Additionally, we used only Ace wraps and splints to represent items that could easily be carried in a backpack.

Twenty pigs were randomized into experimental and control groups containing 10 animals in each group. It was impossible to blind the investigators conducting the experiment, but the investigator who analyzed the data was blinded. Each procedure was performed in the same fashion by a single investigator. Animals were anesthetized with 20 mg/kg of ketamine intramuscularly and 5 mg/kg of xylazine intramuscularly and then given 1.5 mg/kg of meperidine intramuscularly for analgesia before venom injection and as needed thereafter. Isoflurane was administered by mask at injection and when compartment pressures were measured. All injections into the tibialis anterior muscle of the left hind limb were performed using a 22-gauge needle. The site of injection was 3 cm proximal to the metatarsophalangeal joint. The dose was 20 mg/kg of *C atrox* venom. All experiments used lyophilized *C atrox* venom from a single lot (Sigma Chemical, St. Louis, MO), which was reconstituted by mixing 1,000 mg in 10 mL of normal saline solution for a final concentration of 100 mg/mL. Before injection, the investigator aspirated for blood to avoid intravascular administration of venom. In the experimental group, pressure immobilization (ie, the independent variable) was applied within 1 minute using 2 Ace wraps and a splint according to previously published recommendations[1,4,18] and left in place for the duration of the experiment. In the control group, there was no pressure immobilization applied. After recovery from anesthesia, the pigs were placed in individual cages, where they were allowed to ambulate ad libitum under constant observation.

Methods of Measurement

The dosage of venom was extrapolated from a dosage that resulted in lethal envenomation in a pilot study using a similar model.[8] The dosage was also based on findings from venom expenditure studies.[19] The venom quantities used in our experiment paralleled the amount of venom that could be delivered by a large *C atrox* during a defensive bite.

The time to death was measured in minutes. Death was confirmed by absence of spontaneous respiration and heart sounds on auscultation. The pressure within the anterior tibialis compartment was measured using a handheld device (Stryker Instruments, Kalamazoo, MI) in both groups before venom injection and at 2 hours afterwards. Circumference was measured at a site marked in permanent ink 2 cm proximal to the metatarsophalangeal joint before venom injection and immediately postmortem after removal of the wrap.

Data Collection and Processing

All data were hand collected by 1 investigator onto a standardized data sheet. A second investigator double checked the primary investigator's data collection for outcomes other than compartment pressure in a convenience sample of about one third of the animals, and their measures were consistently within 1 minute for time to death and 1 mm for circumference.

Outcome Measures

The main outcome variables were the duration of survival and the increase in measured compartment pressure compared with baseline. A secondary outcome variable was the maximal increase in measured leg circumference compared with baseline. We were unable to assess long-term morbidity in our model (because the animals died within hours); compartment pressures were used as a proxy for potential tissue damage. We wanted to assess immediate threats to life or limb associated with pressure immobilization use after a massive viper envenomation.

Primary Data Analysis

Duration of survival was compared using Kaplan-Meier survival analysis techniques. Confidence intervals (CIs) of the difference between medians were calculated by using methods described by Daniel.[20] All other analyses were performed using Stata statistical software (version 7.0; Stata Corporation, College Station, TX). Statistical significance was accepted at *P* value less than .05.

RESULTS

The median weight in the control group was 13.0 kg (range 10.1 to 17.7 kg) versus 11.2 kg (range 9.8 to 16.4 kg) in the treatment group. The median baseline circumference in the control group was 15.0 cm (range 13.8 to 16.5 cm) versus 14.5 cm (range 13.0 to 16.2 cm) in the treatment group.

One animal in the control group was excluded because it unexpectedly died immediately after venom injection (Figure 1).

Main Results

The dose of venom resulted in 100% mortality. The median survival was longer in the pressure immobilization group (191 minutes, range 140 to 240 minutes) than in the control group (median 155 minutes, range 119 to 187 minutes). The effect size was 36 minutes (95% CI 2 to 64 minutes). Survival analysis confirmed that the difference was statistically significant (*P*=.0122; Figure 2). The mean intracompartmental pressure in the pressure immobilization group was 67±13 mm Hg (mean±SD) with pressure immobilization and 24±5 mm Hg without pressure immobilization (effect size: 43 mm Hg, 95% CI 32 to 53 mm Hg). The mean leg circumference was 14.3±0.8 cm with pressure immobilization and 19.1±1.0 without pressure immobilization (effect size: –4.8 cm, 95% CI –5.7 to –3.9 cm).

LIMITATIONS

Our study is limited to the general degree by which conclusions from an animal model can be extrapolated to

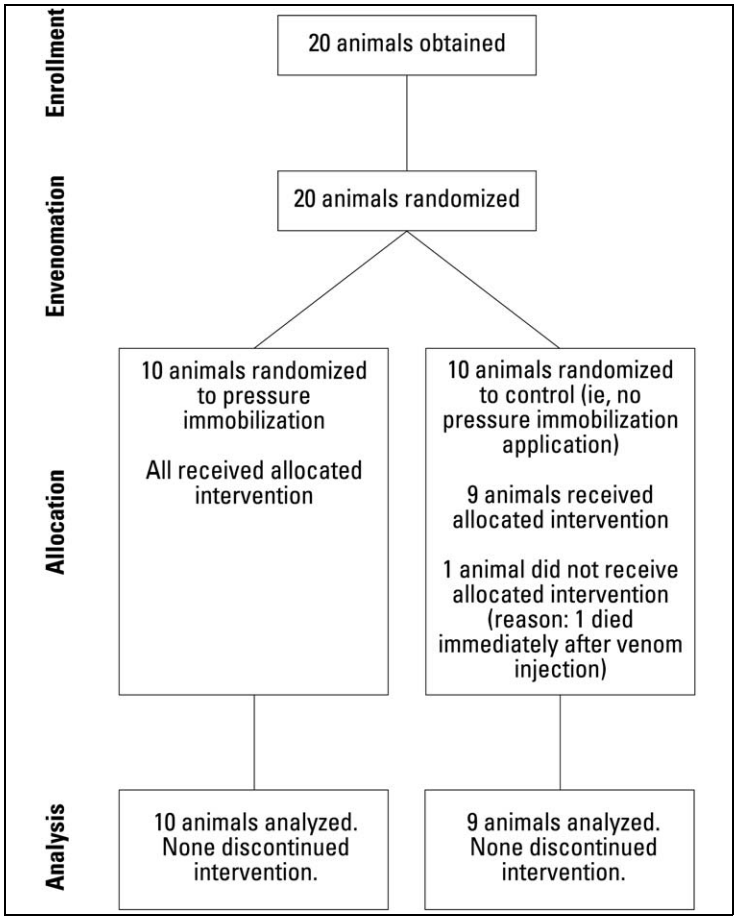

Figure 1.
The CONSORT diagram showing the flow of participants through each stage of the randomized trial.

human beings. However, this method of investigation would not be possible in humans.

The venom load injected was large, and our design did not evaluate the effects of pressure immobilization on less severe envenomations or throughout a longer period of follow-up. Necrosis was difficult to quantify in our model. It is not known exactly how permanent injury relates to elevated compartment pressures that are compounded by effects induced by proteolytic venom.

Finally, there may be limitations to our study because it was impossible to blind the investigators conducting the experiment.

DISCUSSION

In our study, pressure immobilization resulted in significantly longer survival, less swelling, and higher intracompartmental pressures. The median time to death after a fatal envenomation was delayed by 23%, which has statistical, as well as clinical, significance.

However, pressure immobilization increased compartment pressure by 179%. Persistent compartment pressures of 35 to 40 mm Hg are generally considered grounds for emergency fasciotomy.[21] Therefore, pressure immobilization increased compartment pressures from the nonsurgical range to the surgical range. It is possible that the application of pressure immobilization in our subjects created a surgical emergency where there was not one before. In the scenario of intramuscular venom deposition, a prolonged application of pressure immobilization could contribute significantly to compartment syndrome, which could ultimately lead to limb-threatening complications, fasciotomy, further loss of tissue and function, and additional scarring.

The use of pressure immobilization decreased swelling by 25% in our model. Whether this might lead to less loss of tissue or function is unknown.

Other studies support our findings. Pressure immobilization was evaluated using monkeys injected subcutaneously in the lower leg with *C adamanteus* venom.[2] This investigation revealed that the systemic absorption of venom was slowed. Local venom effects appeared to be more severe in the animals in which pressure immobilization was used. The 2 animals that did not receive any first aid died. However, this study included only 4 animals. Because of the small sample size, it is impossible to know whether these deaths were caused by chance alone.

Another study similarly showed increased survival times with pressure immobilization but also increased local tissue damage in a mouse tail model after injection with several species of viper's venoms.[3]

One clinical study found that venom absorption was retarded after a Russell's viper (*Daboia russelii siamensis*) bite with a variant of pressure immobilization ("compression immobilization," in which a rubber pad is wrapped with a "hand-tight" bandage and a splint is applied) but states that "the incidence of local necrosis 3/42 (8%) following use of the pad was comparable to that of the systemic cases without the pad."[22] The reason for this is unclear but could be related to the small number of patients with local necrosis. Only 3 of 42 patients had local necrosis in the study by Pe et al.[22] The study may not have been large enough to detect a difference in local necrosis between groups. Of note, local pain was twice as common in the pad-treated cases, although not intense enough "to demand for removal of the pad."

The assertion that pressure immobilization slows the systemic spread of venom is almost universally undis-

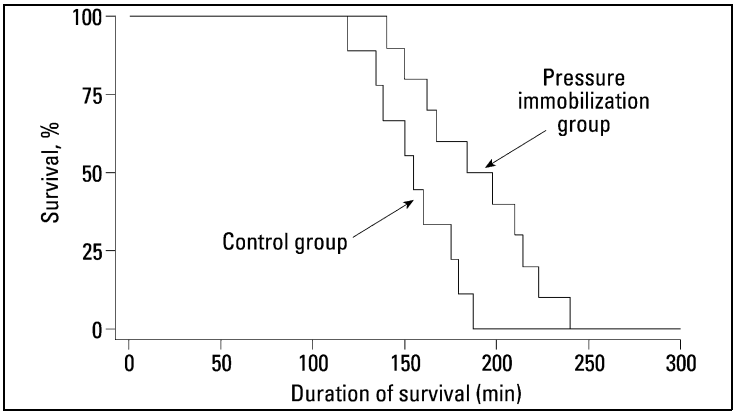

Figure 2.
Kaplan-Meier survival plot. Log-rank test for equality of survivor functions (P=.0122).

puted, and our data confirm this effect as well. The point of contention is whether it increases long-term local tissue sequelae when applied after viper envenomation. Our study supplies objective confirmation that pressure immobilization significantly delays death but could worsen local tissue complications. However, many questions still remain. On the basis of our findings, we cannot recommend pressure immobilization widely for viper envenomation, although specific scenarios may warrant its use. Individuals who chose to consider pressure immobilization will still have to assess risks versus benefits versus alternatives on a case-by-case basis. An informed decision should take into consideration factors such as the size and species of snake, the patient's size, duration and location of fang contact, previous exposures to snake venom, and time and accessibility to medical care and antivenom.

We propose that the next steps in resolving this dilemma might involve longer-term observation followed by an evaluation of functionality after a pressure immobilization–treated viper envenomation, which might yield more information pertaining to the effect of pressure immobilization on local sequelae. Less severe envenomations could be examined. Concomitantly, factors that predict envenomation severity should be explored further. The threshold to use pressure immobilization might be much lower if an envenomation were more likely to be fatal. Conversely, a bite that was more likely to result in mild envenomation might not warrant the risk to tissue associated with pressure immobilization.

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Author contributions: SPB conceived and designed the study, supervised the conduct of the study, coordinated data collection, managed the data (including quality control), and drafted the manuscript. SMG analyzed the data. SPB, TAL, WKH, MDC, and DAT assisted in animal handling on the days of the experiments. All authors contributed substantially to the revision of the manuscript before submission for publication. SPB takes responsibility for the paper as a whole.

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