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Journal of Pharmacology & Clinical Toxicology

Research Article

The Role of the Lymphatic System in Systemic Toxicity of Snakebites

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Submitted: 16 April 2018

Accepted: 26 April 2018

Published: 29 April 2018

ISSN: 2333-7079

Keywords

- Snakebites
- lymphatic system
- lymphatic flow inhibitors

Abstract

Background: Toxic effects of snakebites consist of local toxicity at the site of the bite and systemic toxicity, with coagulopathy and neurotoxicity occurring, depending on the species and composition of the venom. Research has established that the lymph system is the mechanism of systemic spread of venom

Methods: PubMed searches were conducted for articles on snakebites and lymph system.

Results: The reviewed articles describe experimental studies of lymphatic inhibition on systemic toxicity of snakebites. Methods of inhibition include splints, ligation of peripheral lymphatic channels, compression methods to inhibit lymphatic flow, and pharmaceutical lymph inhibitors. All of these methods result in reduced systemic toxicity.

Conclusions: Experimental studies establish that inhibition of lymph flow by several mechanisms results in reduced systemic toxicity of snakebites.

INTRODUCTION

Toxic snakebites have both local effects and systemic effects. Of the two major classes of toxic snakes-vipers and elapids-the vipers have much greater local toxicity and produce coagulopathies. Elapids generally have neurotoxins that can cause paralysis and respiratory arrest. Local effects result from toxins in venom that damage tissues and cause local findings such as ecchymosis, blistering, swelling, and local pain. Local effects generally occur at the site of the bite and spread from that site, sometimes evolving an entire limp. Some vipers have neurotoxicity, for example the North American pit vipers Mohave A (*Crotalus scutulatus*) and Eastern diamondback (*Crotalus adamanteus*) rattlesnakes, and the Brazilian rattlesnake (*Crotalus durissus)*. Similarly, Asian cobra (*Naja naja*) bites can lead to local toxicity such as blistering.

To produce systemic toxicity, venom must reach the vascular system and be distributed throughout the body. Apart from unusual cases of direct injection of venom into a vein or artery that leads to immediate system toxicity [1,2], the lymphatic system is the major source of systemic spread of venom. This article will discuss the basic features of the lymph system and describe experimental studies that support the role of the lymph system in systemic toxicity of the snakebites.

METHODS

PubMed searches were conducted for articles on snakebite and lymph system, snakebites/physiopathology, snakebites/treatment, and snakebites/drug therapy. The searches contained articles from 1947 to February of 2018. 2,547 articles were returned. Combining these searches with lymph as a text word further screened articles. Experimental studies of techniques to inhibit lymph flow on systemic toxicity of snakebites are reviewed for their role in establishing the lymph system as a mechanism to spread venom to the systemic circulation. Techniques identified in the literature that have been studies in snake envenomations include lymphatic channel ligation, pharmaceuticals that inhibit lymph flow, splints, and devices to apply pressure significant to block lymph flow while preserving arterial flow and venous return.

The Lymph System

The lymph system is composed of lymph fluid, lymph vessels, and lymph nodes. Lymph fluid arises from blood capillaries. Fluid from the vascular system enters tissues and is either reabsorbed into blood capillaries or into lymph capillaries. Lymph fluid is transported proximally by lymph vessels of increasing size. It travels to lymph nodes where infectious agents are detected, then returned to the vascular system by entering subclavian veins. Entities injected into tissues, including chemicals and microorganisms, are transported to the system circulation by the lymph system. Several mechanisms propel lymph fluid from the periphery to the systemic circulation (Table 1) [3,4].

Lymphatic channel ligation

In a controlled study, Barnes and Trueta [5] ligated the femoral lymphatic channels of rabbits injected in a hind limb with the neurotoxic tiger snake (*Notechis scutatus*) venom. Toxic endpoint was fatal respiratory paralysis. Survival time was 10 minutes in the control group and one hour in the group that underwent blockage of lymphatic flow by ligation, thus demonstrating the role of lymphatic spread of venom through the lymph system.

Pharmaceutical inhibition of lymph flow

Topical agents reported in the literature to inhibit lymph flow include the L-type calcium channel antagonist nifedipine [6], the nitric oxide donor nitroprusside, the local anesthetic lignocaine [6], and the nitric oxide inhibitor glyceryl trinitrate ointment (GTNO) [7]. These agents have been studied in experimental snakebites. GTNO has been studies in a human study using and a human study using radiolabeled mock venom.

India ink was used to measure foot to groin lymph transit times after its injection into a hind foot of anesthetized rats. The hind limb was covered with 0.1 millimoles of nifedipine, 10% lignocaine, or the control solution of saline (1% percent dimethyl sulphoxide (DMSO) was added to improve skin wetting). Topical application occurred one minute after the injection of India ink. Groin lymphatic channels were exposed surgically in order to measure foot to groin transit time. Nifedipine increased transit time 500% relative to controls. Lignocaine increased transit time by 390%. Both results were highly significant with $p < 0.0001$ [6].

Nitric oxide inhibits the intrinsic lymphatic pump. Glyceryl trinitrate (GTNO) is a commercially available nitric oxide releasing agent (Rectogesic®, Care Pharmaceuticals)that has been studied as a topical agent to decrease toxicity in humans and injected with mock venom (50 microliters of sterile radiolabeled colloid). A crossover design was used in the human study (6 male, 9 female, age range 20 to 65 years), with subjects having the mock venom injected with and without topical application. Foot to groin transit time was measures. Treatment increased the transit time from 13 minutes (range 4 to 81 minutes) without treatment to 54 minutes (range 6.5 to 162 minutes) with treatment ($p < 0.0001$) [7 Saul].

In a rat study, GTNO was applied after injection with eastern brown snake venom (*Pseudonaja textilits*), an elapid that produces motor paralysis and respiratory arrest. The time to respiratory arrest increased from 65 ± 4 min in control rats to 96 ± 6 min in GTNO-treated rats ($P< 0.001$; log-rank test) [7].

Eastern brown snake toxicity was also studied for rats treated with nifedipine, lignocaine, and nitroprusside [6], all of which had efficacy. Topical application of 1millimole of nifedipine increased time to respiratory arrest by 61% ($p < 0.001$). Lignocaine (10%) increased time to respiratory arrest by 50% ($p < 0.01$). Nitroprusside (100mM) also demonstrated efficacy, with $p < 0.05$).

Table 1: Dynamic Mechanisms of Lymph Flow [scallan, Scanlon].

(a)	Contractions of lymphatic vesasel wall smooth muscle
(b)	Skeletal muscle contraction
(c)	Pressure gradients in the chest cavity from respirations
(d)	One-way valves to prevent retrograde flow.
(e)	Lymph endothelial cell contractive valves

Splints and compression

Immobilization of an extremity with a splint prevents muscle contraction, which reduces lymph flow. A variety of techniques have been studied that apply compression to an extremity that will retard lymph flow, including an elastic compression bandage (ace wrap), compression pad applied to the site of the envenomation, and a circumferential compression ring. A caveat for the use of these techniques is that for venoms with local toxicity, delaying systemic absorption is reasonably expected to sequester venom in the extremity and therefore increase local toxicity.

The combination of splint and elastic bandage suppresses lymph flow by reducing muscle contraction, applying sufficient pressure to inhibit lymphatic vessel wall smooth muscle contractions, and overcoming the proximal propulsion of lymph endothelial cell contractive valves. Delays of systemic toxicity by combinations of these methods have been demonstrated in a number of studies.

A mock venom study was performed in humans injected with the radioactive tracer 99mtechnetium antimony sulfur colloid to simulate snake and spider bites [8]. After injection, firm bandages and splints were applied. A large field of view gamma camera was used to follow the systemic absorption of the tracer. If tracer was sequestered in the extremity, the subjects were required to walk until the tracer was detected. This study found a mean periphery-to-systemic circulation transient time of 58 ± 7 minutes in controls. Prevention of systemic flow of venom was very effectively suppressed when pressure bandages were applied in the 40 mm to 70 mm Hg range in the upper extremity and 55 to 70 mm Hg in the lower extremity. Lower and higher pressures were ineffective. Walking for 10 minutes or more caused the systemic spread of venom in all subjects, emphasizing the importance of immobilization of the extremity to retard dissemination of venom.

A human study with a radiolabeled mock venom inhibited lymph flow by placing a pressure pad over the site of mock venom injection with an applied pressure of 70 mm Hg. Radioactivity in the serum was not detected by this inhibition of lymph flow. The authors also compared this technique to immobilizing the extremity with an air splint inflated to 55 mm Hg, and to a pressure bandage of 55 mm Hg with splint. Essentially no venom was detected in the serum by the 70 mm Hg pressure pad, but did reach the serum in the other two interventions [9]. Whether or not the difference was due to the techniques or the higher pressure with the pad was not determined.

Monkeys injected with the neurotoxic elapid tiger snake (*Notechis scutatus)* venom were treated with application of a splint and elastic bandage, with the bandage applied at the site of envenomation and extended peripheral to the entire limb. Another group had a tourniquet applied with a pediatric blood pressure cuff inflated to 155 mm/Hg, which was sufficient to block arterial flow. These interventions were compared to a

control group without immobilization. [10]. Endpoint was plasma venom measured to radioimmunoassay. The interventions demonstrated a delay in absorption of venom in the systemic circulation. Neither a splint applied without the elastic bandage nor the elastic bandage applied without a splint was found to delay systemic absorption of venom.

A study in anesthetized pigs demonstrated delays in systemic toxicity from the neurotoxic elapid eastern coral snake (*Micrurus fulvius fulvius*) venom by lymph flow retardation. The intervention group had a splint and elastic bandage applied in the manner of applying an ace bandage to a sprain, in an attempt to mimic field conditions. A distal extremity injection with a fatal dose of 10 mg of coral snake venom was given before application of the splint and ace bandage. A control group had an identical injection by no intervention. The endpoint was survival to eight hours [11]. Before the termination of the experiment, 5 subjects with no lymph flow retardation suffered respiratory arrest at 170.4 ± 33.3 minutes after the injection of venom. Four of 5 subjects in the treatment group were still breathing after 8 hours.

The use of prolonged lymphatic flow inhibition was studied in the porcine model of coral snake (*Micrurus fulvius fulvius*) envenomation [12]. Prevention of systemic toxicity could occur if the venom leaked from tissues very slowly or was degraded locally by hydrolysis. The authors conducted a randomized, controlled observational pilot study in ten anesthetized and incubated pigs. Ten mg of lyophilized coral snake (*Micrurus fulvius*) venom suspended in one ml of water was injected, followed immediately by application of a compression bandage and fiberglass cast. Endpoint was survival to 21 days. Control animals had a mean survival time of 307 minutes versus 1,172 minutes in treated animals (P = 0.10). Survival to 24 hours was 60% in the treatment group while no control group subjects met this endpoint (P =0.08). At 21 days, two of the treatment subjects were alive. When the fiberglass splint was removed from these two subjects at 21 days, necrotic lesions were found under the cast. The lesions may have been from mechanical trauma because coral snake venom has minimal local toxicity.

Local damage could be increased with lymphatic flow inhibition for venoms with tissue destruction at the bite site. None-the-less, studies have been conducted on the decrease in systemic toxicity. Monkeys were injected with 6 mg eastern diamondback (*Crotalus adamanteus*) venom [13]. Radioimmunoassay was used to monitor venom levels in urine and plasma. Firm pressure and splints were applied to the injection site. This intervention prevented systemic absorption of venom and reduced swelling at the injection site. The authors' recommended consideration of this technique for humans bitten by *Crotalus adamanteus.*

Further investigation of lymphatic flow inhibition for venom with serious local toxicity, the western diamondback rattlesnake (*Crotalus atrox*), was undertaken in the porcine model. Intervention was splints and pressure bandages, using ace wraps [14]. Both were applied immediately after subcutaneous administration of venom in a distal hind paw. Study design was to inhibit lymph flow for 24 hours, administer antivenom, then restore lymphatic flow. None of the control animals survived to 24 hours, with expiration between 191 and 305 minutes, while 100% of animals with lymphatic flow inhibition were alive at 24 hours. Local necrosis did occur and was treated with analgesia and antibiotics. Surviving subjects were walking on the extremity 7 days after the venom injection.

To inhibit lymphatic flow from the torso, a circumferential compression device was devised [15,16], consisting of a ring with hooks that was held in place by a strap around the torso. The porcine model was used to test this concept in eastern coral snake (*Micrurus fulvius fulvius)* [15], and eastern diamondback rattlesnake (*Crotalus adamanteus)* [16], experimental envenomations. The ring measured 8x5x3 cm. In the coral snake study, 5 of 6 treated subjects were alive at 8 hours, with one pig expiring after 293 minutes. 3 of 3 control group pigs suffered respiratory failure at a mean of 322 minutes (range 272 to 382 minutes). The result was significant with Fisher's exact test value of 0.04. For the eastern diamondback rattlesnake study, experimental subjects in the treatment group had a mean time to toxicity of 355 ± 65 min while the mean time to toxicity was 32 ± 3.5 minutes in the control group (paired t test p< 0.03).

In Myanmar, where snakebites by Russell's viper (*Daboia russelii siamensis*) are common, a prospective study of human victims treated with a compression pad was performed. Fifteen individuals envenomated by Russell's viper had serum levels monitored after a rubber pad (65x65x25 mm) was placed over the bite site attached with a cotton bandage (65 mm x 1.6 m). A smaller pad was used finger and toe bites. After application of the intervention, venom levels measured in the serum remained constant in 15 of 23 subjects. Proof that the pad retarded venom from reaching the central circulation was demonstrated because venom levels remained constant in 15 of 23 subjects, and then increased 10 to 40 ng/mL when the pad was removed. Serum venom levels were undetectable while the pad was in place in 7 of the 23 subjects [17].

Further support for lymphatic flow of venoms comes from observations of lymph node involvement. A human case series from Papua New Guinea found local tender lymph nodes in subjects envenomated by Papuan black snakes (*Pseudechis papuanus*), an observation that supports lymph flow of venom [18]. In the western diamondback rattlesnake (*Crotalus atrox*) study discussed above [14], necrosis of lymph nodes was found at necropsy of fatalities.

CONCLUSIONS

Different techniques to inhibit lymph flow from an extremity after a poisonous snakebite establish that systemic spread of venom occurs by lymph flow. Techniques include lymphatic channel ligation, pharmacological lymph flow inhibitors, splints, and mechanical pressure application at the bite site with sufficient pressure to inhibit lymph flow while preserving arterial and venous flow.

The extent to which this basic scientific knowledge transfers to human bites is a matter of discussion. Available of knowledge and materials in isolated regions where human snakebites are most common is limited. In Australia, where use of these techniques are recommended, in has been observed that efficacy in field use is compromised by inconsistent use of splints and applying the bandage loosely [19]. A study of the ability of both health professional and the general public to correctly use the

technique in a simulated setting also found that use was not optimal [20].

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Cite this article

Parker-Cote J, Meggs WJ (2018) The Role of the Lymphatic System in Systemic Toxicity of Snakebites. J Pharmacol Clin Toxicol 6(2):1105.