Review Article

Local tissue damage treatment of snake venom

Suchitra Khunsap[1]*

Abstract

Local tissue damage caused by snake envenomation was a medical problem that caused morbidity and mortality for more than a decade, with no defined treatment guidelines. Nowadays, antivenom is the primary treatment for snakebite, which substantially reduces systemic venom but does not significantly reduce local symptoms. We compiled a list of local damage therapies, including antivenom medication, surgery and skin grafting, small molecule medicines, and alternative therapies. Although each was not approved as a standard treatment, it may be a treatment option for tissue damage caused by snake venom. However, antivenoms and most resistant compounds may not be the best strategy to treat snake envenoming-related local tissue damage. The route of substance delivery may be the optimum regimen for treatment.

Keywords: Antivenom and Small molecules, local tissue damage, snake venom

[1]*Research and Development Department, Queen Saovabha Memorial Institute*
***Correspondence**: sthaithumnas@gmail.com* (S. Khunsap)
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Introduction

Snakebite envenomation is a medically problematic cause of mortality and morbidity in people. The World Health Organization (WHO) reports more than millions of cases of snakebite envenoming people around the world annually, approximately 100,000 deaths, and 400,000 cases of morbidity lifelong (Williams *et al., 2019*; Lewin *et al., 2022*). WHO has planned to reduce snakebites by one-haft by 2030 (Albulescu *et al., 2019*; Hall *et al., 2023*). Antivenom is an effective strategy for the remedy of snakebite. Unfortunately, it fails in neutralizing local effects such as pain, edema, bleeding, and myonecrosis (Sheeja, 2017; Salama *et al., 2018*; Lauria *et al., 2021*). Local tissue damage at the bite site has recently been treated with herbal medications (Félix-Silva *et al., 2017*), surgical treatments, and broad-spectrum antibiotics (Sadeghi *et al., 2021*; Wang *et al., 2022*). However, many patients of snakebite were irregular and long-suffering of livelihood. Therefore, surgery and skin graft are the choice of methods, in some cases, as the wound site was detected and severely damaged (Sheeja, *2017*). There was no better specific remedy yet.

Envenoming by snake venoms which contain a lot of proteins that have various harmful actions to systemic morbidity and on the muscles around the bite site. Immediately, in snakebite, the tissues were rapidly destroyed from the amount of toxin leading to damage the muscle and the capillary blood at the bite site. The severity of the wound depends on the amount of toxin concentration detected injected by the snake (Seifert *et al., 2022*; Wang *et al., 2023*). Considering that it is a therapeutic gap for snakebite envenoming. Therefore, researchers have pursued to developed novel substances for local wounds by snakebite which have properties that include portability, heat resistance, less expensive, easy to use that no need to rely on an expert (Gutiérrez *et al., 2020*).

Due to technological advances and interest in venom molecules, the composition and structure of snake venom components have been widely revealed. As such, understanding the venomous molecules leads to pharmaceutical innovations to develop novel drugs for venomous snakebite. An innovative idea has been focusing on the basis structural characterization and functions of the toxin components (Castro-Amorim *et al., 2013*). Since then, many drugs have been developed that target specific enzymes in the venoms, especially the highly toxic components (Williams *et al., 2019*). Utilization of technology for snake bite treatment, identification of snake venom components, and assessment of snake venom target specificity for medical purposes. (Smith *et al., 2024*). Therefore, the necrotic wounds caused by snake venom, which do not yet have an unclear cure, have a bright future.

The major components of the snake venom affected on local tissue damage

Snake venom consists of abundant proteins, enzymes, and other substances that cause local and systemic effects in humans leading to mortality and permanent morbidity (Bittenbinder *et al., 2024*). The venomous snakes are classified into the three major families as following the Elapidae, the Viperidae, and the Colubridae Hydrophidae (Willams *et al., 2019*). However, the major components that were affected to local tissue damage such as phospholipase A_2 (PLA_2), snake venom metalloproteinase (SVMP), snake venom serine protease (SVSP), L-amino acid oxidase (LAAO) and the cytotoxic three-finger toxins (3FTXs) (Tiwari *et al., 2022*; Bartlett *et al., 2024*). Herein, we focus on two key enzymes that degrade and disrupt cell membrane integrity, causing local pathological effects (Castro-Amorim *et al., 2023*).

Snake venom-secreted phospholipase A_2 ($svPLA_2$) was found in all venomous snakes with various concentrations. It contained both catalytic function and inactive forms, which can induce a wildly pharmacological effect. The PLA_2 acts at the sn-2 position of glycerophospholipids and hydrolyzes the ester bond releasing a lysophospholipid and a free fatty acid (Castro-Amorim *et al., 2023*). The hydrolyzed reaction of the enzyme is caused of membrane permeabilization and disruption inducing influx of extracellular molecules lead to cell death. The resulting enzyme activity is often toxic to cells and muscles and enhances the activity of other toxic components (Tiwari *et al., 2022*; Werner and Soffa, 2023). However, the mechanism of $svPLA_2$ enzyme activity is still poorly understood.

Snake venom-metalloproteinases (svMPs) consist of a large group of zinc-dependent proteinases of varying domain composition. It is an important enzyme across multiple venomous snake species that induces both local and systemic toxicities (Albulescu *et al., 2019*; Smith *et al., 2024*). The enzyme has been concerned with hemorrhagic and extracellular matrix degrading activities (Bittenbinder *et al., 2024*; Williams *et al., 2019*). The most recent version of svMP, released in 2008, was divided into three major classes: P-I, P-II, and P-III, with sub-classes of P-II and P-III that feature a disintegrin domain attached to the C-terminus of the metalloproteinase domain. It was abundant in the Viperidae snake family and less common in the Elapidae, Atractaspididae, and Colubridae snake families (Preciado *et al., 2018*; Olaoba *et al., 2020*). The pathogenic activities of svMP-induced systemic indicated the cleavage of basement membrane (BM) proteins, signifying an enzymatic disruption of the extracellular matrix structure, resulting in an increase in protein concentration and an inflammatory response (Castro *et al., 2021*).

The treatments of local tissue damage by snake venom:

Snake bites have commonly happened on farmers or in remote places, resulting in delayed hospitalization and severe systemic and local tissue damage symptoms. The treatment gap may be insufficient of antivenom or the other drugs, and the mode of delivery. The solutions for treating local tissue damage are listed here.

Surgical procedure: Surgical treatment for snakebite venom aims to minimize venom load, prevent local tissue harm, and cure wound necrosis and its consequences (Mohsin *et al., 2023*). At this point, many patients have had severe symptoms, including pain

from peeling off damaged wounds or having their organs removed. As a result, some patients are suffering and seek folk medicine doctors for help. However, it may be more severe than previously.

Antivenom and supplementary drugs: Antivenom is a major treatment for snakebite venom. The antivenoms were derived from animal serum, such as horse serum, which was injected with the whole venom. The number of foreign proteins in animal serum, poor manufacturing, and quality control cause adverse reactions to antivenom in the patients (de Silva *et al., 2015*). Much evidence has shown that antivenoms only neutralize the venom in the systemic, limiting local tissue damage (Salama *et al., 2018*; *Lauria et al., 2021*). The limitation is not simply its ineffectiveness. The reason is that snake venom is not neutralized by antivenom at the site of the bite. The movement of ineffective distribution of antivenom from the blood flow to various tissues was interrupted by obstacles in the circulation and the properties of the substance (Werner *et al., 2023*). Meanwhile, snake venom rapidly destroys the muscles around the wound, and dermonecrotic conditions occur immediately after exposure to the venom (Lui *et al., 2020*). Therefore, although the patient received the antivenom, the severity of wound damage was developed. However, some studies showed that the *Bo*throps antivenom combined with dexamethasone enhanced local damage by *Bothrops atrox* envenomation (Santos Barreto et al., 2017). This study might be the guideline for local damaged solutions of snakebite further.

Similarly, anti-snake venom blocking therapy or local blocking treatment with anti-snake venom serum was applied directly to the bite site. The ideal of this method is to solve the limitation of antivenom via intramuscular or intravenous administration. Results showed that anti-venom can stop the spread of the venom and reduce tissue degeneration. Based on the study, anti-venom could neutralize the snake venom at the bite site via venom blocking therapy (Zeng *et al., 2022*). Moreover, the study of small incisions combined with negative pressure wound therapy proved effective for reducing injured limb swelling and systemic inflammatory reactions (*Zeng et al., 2019*). Therefore, it is possible that, the anti-venom has effectively prevented the snakebite, both systemic and local, dependent on the routes of administrations.

Small molecule therapeutics: The synthetic molecule therapeutics (SMTs) are the new agents to relieve the necrotic wound of snake envenomation. Almost all SMTs are repurposed drugs that have the potential to reduce snake venom toxin effects (Preciado *et al., 2019*; Layfield *et al., 2020*). The efficiency of the molecule was dependent on the species of snake, which has a different composition. Herein, we discussed the SMTs, which are the potential treatment for necrotic wounds caused by snakebite venomous (Williams *et al., 2019*). For instance, Marimastat, Varespladib, and 2,3-dimercapto-1-propanesulfonic acid (DMPS), which were the drugs for cancer treatments, as then repurposed to prevent morbidity-inducing dermonecrotic by snake venomous snakes (Hall *et al., 2023*).

Marimastat is a low molecular weight mimic peptide of the matrix metalloproteinase substrate. It is specific to the metal ion of the binding pocket in the active site, which inhibits a broad spectrum of MMPs. It acted to prevent the degradation of the basement membrane by these proteases (Williams *et al., 2019*). The first-generation Marimastat was designed to inhibit the MMPs, which are involved in the proliferation of various cancer types. Nevertheless, it was withdrawn from the clinical trial phase due to unforeseen toxicity or unacceptable pharmacokinetics in the long-term treatment (Brown and Giavazzi, 1995; Denis and Verweij, 1997). Due to the structure of snake venom MMP, which is like Marimastat substrate. Therefore, Marimastat was adopted as a drug candidate against SVMP toxins in various snake venom both *in vitro* and *in vivo* (Hall *et al., 2023*; Williams *et al., 2019*; Xie *et al., 2020*). Such as, Menzies and the team had investigated the *in vitro* and *in vivo* preclinical efficacy of SVMP inhibitors: marimastat, prinomastat, and the metal chelators dimercaprol and DMPS on the *Dispholidus typus* venom. The result found that Marimastat and DMPS showed equivalent inhibition to SVMP of the venom, which led to partial protection against venom lethality and prolonged survival times of experiment animals (Menzies *et al., 2022*).

Varespladib is a synthetic molecule that blocks inflammatory cascades of several diseases associated with elevated levels of secreted phospholipase A_2 ($sPLA_2$). Nevertheless, the phase II clinical trial was hated due to inefficient efficacy-causing elevated serum levels of $sPLA_2$ in sickle-cell patients or in patients with respiratory diseases (Gutiérrez *et al., 2020*). Due to $sPLA_2$, it was also found in snake venoms, which plays a role in the morbidity and mortality from snakebite envenomation. Therefore, it was used to treat snake bites caused by the action of the enzyme PLA_2 and the other enzymes. There are numerous reports showing that Varespladib can prevent both systemic and local dermal tissue damage in various snake venoms (Bartlett *et al., 2024*). The following is a compilation of reports on the action of Varespladib, focusing on the inhibition of dermal tissue necrosis.

2,3-Dimercapto-1-propanesulfonic acid (DMPS) is a derivative of dimercaprol that has the properties to form complexes with various heavy metals called chelating agents. In 2019, it was found to potently antagonize the activity of Zn^{2+}-dependent snake venom metalloproteinases *in vitro*. Moreover, DMPS prolonged or conferred complete survival in murine preclinical models of envenoming against a variety of saw-scaled viper venoms (Albulescu *et al., 2019*).

Alternative therapy: The wildly alternative substance is focusing on medicinal plants, which have the effect of neutralizing venom enzymes without side effects (Singh *et al., 2017*). There are many reports proving that herbs are effective for systemic effects. However, there is evidence indicating hypersensitivity reactions and limitations in the treatment of skin necrosis (Liaqat *et al., 2022*). The plant has been used to heal the local damage, such as Rosemary leaf extract. Rosmarinic acid is the major component that neutralizes the

enzymatic activities of *Egyptian Cerastes cerastes* venom leading to reduced lethality, hemorrhage, edema, and liver toxicities (Salama *et al., 2018*). The popular medicinal plants for local tissue damage induced by snake venom were accumulated and discussed in pharmacologic assays (Félix-Silva *et al., 2017*). A few medicinal plants have the potential to be snake bite antidotes, which are strictly conserved among tribes through generations without recorded data (Sulochana *et al., 2015*). However, the other method in the experiment phase such as, the low-intensity laser called photobiomodeling therapy (PBMT), which affects the local actions induced by *B. leucurus* venom in mice. The laser reduced paw edema formation, whether irradiation was performed immediately or half an hour after venom injection but did not prevent myotoxicity and hemorrhage (Lauria *et al., 2021*). The mechanism of PBMT is poorly understood; further study would be needed.

Conclusion

Antivenom is still a major strategy for snakebite treatment. Many reports show that antivenom is only effective in neutralizing the toxic components in the systemic circulation, but poorly relieves the local tissue damage at the bite site (Hall *et al., 2023*). The therapeutic gaps of antivenom for snakebite treatment include the properties of antivenom molecules, storage, and transportation. The small molecule drugs (SMTs), Marimastat and Varespladib, have been reported to have a potent effect on relieving the local wound (Lewin *et al., 2022*). However, the administration of SMTs is still performed by the physician or the medical expert, which causes a delayed period of treatment.

In fact, the new molecule was not the priority for solving the problem. The antivenoms and SMTs have the potential for both systemic (Santos Barreto *et al., 2017*) and local damage snake envenomation (Liu *et al., 2020*; Preciado *et al., 2020*). It should be better to consider how to inject or deliver the antivenom to the wound at the bite site. The intramuscular or intravenous routes have always been the delivery of antivenoms and drugs. The delivery of antivenoms has always been the blood circulation passing through the tissue targets. At the same time, the enzyme components of snake venom have immediately and severely destroyed the tissues around the bite site. Neither antivenom nor medicinal drugs can stop and repair wounds that have already occurred at the bite site. Therefore, several administrative strategies for the quick delivery of antidotes or medicinal drugs should be considered.

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