

Snake Venom Toxins: Clinical use and as Diagnostic Agents

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OPEN ACCESS

Volume: 8

Special Issue: 1

Month: February

Year: 2021

P-ISSN: 2321-788X

E-ISSN: 2582-0397

Impact Factor: 3.025

Citation:

Kumar, MS. "Snake Venom Toxins: Clinical Use and as Diagnostic Agents." *Shanlax International Journal of Arts, Science and Humanities*, vol. 8, no. S1, 2021, pp. 1–5.

DOI:

<https://doi.org/10.34293/sijash.v8iS1-Feb.3923>

Abstract

Snakes are fascinating creatures and were inhabitants of this world well before the earth was populated by ancient humans. Snakes with a lethal secretion known as venom were endowed by nature. Snake venom is a very poisonous mixture consisting of a number of molecules such as carbohydrates, nucleosides, amino acids, lipids, proteins and peptides, making it a cocktail of diversified molecules. Snake envenomation is responsible for the disruption in the envenomed victim's fundamental physiological processes contributing to serious health problems. Millions of snakebites are recorded annually, and due to snake venom poisoning, a significant number of individuals are injured and die. However, through technical developments, many fatal snake venom toxins have found potential applications as diagnostic agents, medicinal agents, or drug leads. From the development of Captopril, the first drug derived from *Bothrops jarararaca*'s bradykinin potentiating peptide, to the disintegrins that have potent activity against some forms of cancers. Therefore, components of snake venom have shown tremendous potential for the development of lead compounds for new drugs. Complementary tools and techniques are currently being used to isolate and characterize peptides and to study their potential uses as molecular probes and templates for drug development and design investigation models.

Keywords: Snake venom, Snake venom toxins, Toxins, Clinical use, Diagnostic agents

Introduction

Snakes, the objects of terror, myths and fascination, cause 2.5 million cases of poisoning and at least 100,000 deaths worldwide each year. Only a very small number, approximately 3000 known snake species are venomous, belonging primarily to the Viperidae, Elapidae Colubridae and Atractaspididae families. Numerous different proteins, peptides, toxins, inorganic cations (Na^+ , K^+ , Ca^{+2} , Mg^{+2} , Mn^{+2} , Ni^{+2} , Zn^{+2} , Fe^{+2} , Co^{+2}), lipids, carbohydrates, free amino acids and biogenic amines are concocted into snake venom. The proteins and peptides have toxico-logical properties and/or enzymatic activity. In various combinations and amounts, snake venoms contain at least 25 different enzymes and 30 to 100 different toxins, but no single venom contains all of them. Acetylcholinesterases, L-amino acid oxidases, phospholipases A₂, proteases (metallo-, serine), hyaluronidases, fibrogenases, collagenases, elastases, catalases, etc. are the most popular snake venom enzymes (1). With affinity and selectivity, they can target membrane, coagulation proteins, and cell

surface receptors. These have been used in the diagnosis of hemostatic disorders and function as biochemical tools for the study of cellular mechanisms. Some of which have been used as hypotensive, antithrombotic, anticoagulant, antiviral, analgesic, neuromodulatory, fibrinolytic agents, for the stabilization of hemostasis and wound healing and for the treatment of various disorders in both clinical or in clinical trials. They also act as scaffolds on which new medicines can be produced. Captopril, Tirofiban, and Eptifibatide, mimetics of peptides extracted from venom, are the best examples.

Drugs in Clinical Usage obtained from Snake Venom

Since antiquity, the use of snake venom has been documented in battles as a biological weapon. For the treatment of gastrointestinal diseases and arthritis, and for longevity, its use as a medicine has been recorded in Ayurveda since the 7th century (2). The philosophers and physicians of ancient Greece wrote about the pharmacological behavior of snake venom. It was commonly used in the middle ages until the 19th century as a remedy. Since 1853, heart diseases, measles, multiple sclerosis, asthma, pain, cancer and rheumatism have been treated with crude cobra venom. In patients bitten by rattlesnake, epileptic seizures were found to be stopped. The powerful analgesic action of tiny doses of cobra venom was recorded in 1934. Without inducing addiction, the activity was greater than morphine. The development of antihypertensive ‘Captopril’, a peptidomimetic bradykinin potentiating peptide (BPP), isolated from the venom of *Bothrops jararaca* (Brazilian pit viper) (2), began the modern age of venom-derived drugs. For the past 400-500 years, the toxicological properties of snake venom have been studied. However, substantial improvement in the structural function and mechanism of snake venom components has been made with the advent of modern research techniques (1, 2). Despite its long historical significance, only a few of the components of venom are actually used. Following are some of the diseases which are treated by using snake venom components or they have been derived from them as therapeutics and diagnostics.

Treatment of High Blood Pressure by using Captopril

High blood pressure (hypertension) is a common condition which can damage the brain, heart, blood vessels, kidneys, and other parts of the body when not treated. Heart disease, heart attack, heart failure, stroke, renal failure, vision loss, and other complications can be caused by damage to these organs. Normally, the peptide called Bradykinin produced in the body, controls high blood pressure. The cleavage product of endogenous protein high molecular weight kininogen is called Bradykinin. By improving capillary permeability and vasodilation, Bradykinin induces a hypotensive effect. Whereas Angiotensin II is a strong vasoconstrictor formed by the action of Angiotensin Converting Enzyme (ACE) on Angiotensin I. Bradykinin is also broken down by ACE. Therefore, the action of bradykinin is potentiated by a class of ACE inhibitor drugs that tend to increase bradykinin levels by preventing its degradation. The bradykinin-potentiating factor (BPF) of the *Bothrops jararaca* (Brazilian pit viper) venom was isolated. It induces a hypotensive effect through increased bradykinin activity and inhibition of ACE (3). Sir John Vane took this BPF to Squibb, a pharmaceutical firm, where he customized and modified it to create an orally accessible peptidomimetic, “Captopril.” On April 6, 1981, Captopril received approval from the Food and Drug Administration (FDA) and it became a generic medication in the United States in February 1996. The first blockbuster drug that derived from an animal toxin was Captopril. It is used to treat high blood pressure and heart failure alone or in conjunction with other drugs. In patients with a heart disease called left ventricular hypertrophy, it is often used to improve survival and reduce the risk of heart failure after a heart attack. Captopril is also used for the treatment of

diabetes-induced kidney failure (nephropathy) in patients with type 1 diabetes and retinopathy (eye disease) which was licensed for clinical use on April 6, 1981 (4).

Treatment of Coronary Artery Disease by using Tirofiban and Eptifibatide

In the usual phase, platelets are bound to the exposed collagen at the injury site if there is an injury. Following adhesion of platelets to collagen, platelets are activated by a number of agonists such as adenosine diphosphate (ADP), thrombin, and also collagen. The conformational shift in the fibrinogen receptor ($\alpha\text{IIb}\beta\text{3}$) present on the surface of the platelets occurs in the activated platelets. Fibrinogen binds to these fibrinogen receptors, thereby interlinking the neighboring platelets to form a platelet clump. Along with the mesh like fibrin structure which is formed by the cleavage of fibrinogen, the platelet aggregates limit the ooze out of the blood at the injury site. If there are any defects in platelet aggregation leads to bleeding and thrombotic disorders. Atherosclerotic plaque ruptures cause the adhesion and accumulation of platelets in arterial thrombosis (myocardial infarction and stroke), contributing to the development of clots in the arteries and restricting the supply of blood to the brain and heart. Echistatin is a 5.4 kDa (49 residue) peptide isolated from the *Echis carinatus* (Saw-scaled viper) venom, containing an RGD (Arg-Gly-Asp) sequence, showing inhibition of fibrinogen, thrombin, and collagen depended platelet aggregation (3). Tirofiban was created with an RGD sequence that is a mimic of Echistatin. It is a non-peptide substance (Aggrastat [N-(butylsul—)Fonyl]-O-[4-(4-piperidiny) butyl]-L-tyrosine monohydrochloride monohydrate]) is a fast-acting and highly selective competitive $\alpha\text{-IIb}\beta\text{3}$ antagonist which inhibits the formation of clots.

Similarly, peptide/disintegrin isolated from the venom of *Sistrurus miliarius barbouri* (Florida ground rattlesnake), named 'Barbourin' (74 residues), acts as a specific $\alpha\text{IIb}\beta\text{3}$ antagonist. It contains the sequence of KGD (Lys-Gly-Asp), as opposed to the typical sequence of RGD (Arg-Gly-Asp). The development of the drug Eptifibatide (Integrilin® [N6-(aminoiminomethyl)-N2(3-mercapto-1-oxopropyl-L-lysylglycyl-L-aspartyl-L-tryptophyl-L-prolyl-L-cysteinamide, cyclic (106)-disulfide)], a synthetic cyclic heptapeptide (5), resulted from this high specificity of Barbourin. Both eptifibatide and Tirofiban suppress $\alpha\text{-IIb}\beta\text{3}$ integrins and have been approved for acute coronary artery disease treatment and anti-thrombotic therapy.

Both Tirofiban and Eptifibatide are the "blood thinners" used to prevent blood clots that may cause a heart attack or other severe issues with blood flow. Both are used to open up blood vessels in the heart before such operations (e.g., balloon angioplasty, coronary stent placement, percutaneous coronary intervention-PCI, coronary artery bypass graft-CABG). It is used for other drugs as well. Either one of these are used with other drugs (e.g., nitrates, beta-blockers) to avoid or prevent heart attacks when the above treatments are not possible in people with ongoing chest pain. Usually, heparin and aspirin are used for it.

Treatment of Thrombotic Disease by using Batroxobin

Snake venom Thrombin-like enzymes (svTLEs) are serine proteases which catalyze the cleavage of the fibrinogen chains of either αA or βB , but sometimes both chains (6). Thrombin inhibitors do not inhibit them. They do not activate other factors of coagulation and do not degrade factor FXIII of coagulation. Thus, the amounts of plasma fibrinogen are depleted and the clots that are produced is fragile and can be easily solubilized. Therefore, they also show anticoagulant effect, along with having procoagulant activity, and are therefore used as defibrinogenating agents. Batroxobin (Reptilase®) is a thrombin-like protease (mol wt. of 25.5 kDa deglycosylated protein, 231 residues) that has demonstrated anticoagulant activity from the *Bothrops atrox* (Common lancehead) venom. It catalyzes the cleavage of Arg16-Gly17 bond in $\text{A}\alpha$ -chain of fibrinogen in transforming

spontaneously into loose fibrin clots. Similarly, the defibrinogenating metalloproteinase (45 kDa), 'Moojenin' (Defibrase®), was isolated from the venom of *Bothrops moojeni* (Brazilian lancehead). It showed anticoagulant activity by cleavage of the fibrinogen A α or B β chains, thus inhibiting the stable clot formation (6). Without affecting normal platelet activity, both act directly on fibrinogen, platelet-rich plasma clot, and are insensitive to thrombin inhibitors. By using batroxobin from snake venom (*Bothrops moojeni*) and patient plasma, the Vivostat® system provides autologous fibrin sealant. This system relies solely on blood from the patient's own fibrinogen and thrombin. Batroxobin is only necessary to start the process of clotting by inducing fibrinogen activation in the patient. In different procedures, it is very effective in preserving hemostasis, such as hepatic, neuro, cardiac, plastic, head and neck surgery, skin grafting, etc. The process of preparing autologous fibrin sealant based on thrombin and fibrinogen was time consuming, complicated and laborious. This process becomes quick and uncomplicated with the Vivostat® method.

Treatment of Chronic Wounds by using Plateltext®

Thrombin activates platelets shortly after the development of the lesion. Growth factors present in platelets are released after their activation at the site of injury and aid in tissue repair (7). In semisolid content, platelets are embedded, and can be applied topically to speed up tissue healing. However, platelet thrombin activation results in a loss of the proportion of growth factors in the medium around it. Unlike thrombin, a separate mechanism occurs with Batroxobin-mediated conversion of fibrinogen to fibrin, which does not require platelet activation. It yielded soft clots / gel that could be added to the lesion when platelet rich plasma (PRP) was treated with Batroxobin. The platelets have retained all their growth factors that assist in the healing of local tissue. This process was led to the development of Plateltext® (platelet gel) by Sacchi and Bellanda, 2001 and they were patented. Human bovine or autologous thrombin is used in some other commercialized goods. Human thrombin, however, is costly; bovine sources have different risks associated with it, and autologous thrombin has issues with concentration. Finally, Batroxobin (thrombin-like protease), a fast-acting and reproducible gel that enables slower release of growth factors from platelets as they are not activated by batroxobin, has solved these problems. Plateltext® has been found to be used in the treatment of chronic wounds and has numerous surgical and medical applications (8).

Diagnostic Agents from Snake Venom

The diagnostic methods currently used are four Viperid snake venom enzymes. Coagulopathies are associated with Viperid snakes envenoming, so these enzymes are commonly used in blood component based studies. A thrombin-like serine proteinase fibrinogenase isolated from the *Bothrops atrox* venom was identified early as being in production for use in cerebral infarction. Reptilas® (batroxobin). It is also used to test the components of blood fibrinogen and fibrinogen degradation (6). Protac® is also an *Agkistrodon contortix* venom serine proteinase that activates plasma protein C and is thus used in the determination of blood levels of protein C and protein S. Botrocetin® is a platelet-aggregating *Bothrops jarararaca* venom protein and acts by improving the von Willebrand Factor A1 domain's affinity for the GPIb alpha platelet receptor. Viper Russell venom serine proteinase RVV-V is a factor V activator. Activated factor V is not stable and RVV-V can therefore be used to selectively inactivate factor V in plasma and thus help to determine levels of factor V. RVV-X is also a V. russelli P-IV metalloproteinase that is a factor X proteolytic activator and is used to quantitatively transform pro-factor X into factor Xa in diagnostics (9).

Summary

The decades of toxicological research is now translated into meaningful and has practical applications as evidenced by toxins and toxin structure-based drugs in clinical use and production pipelines. Therefore, the production of medicinal, diagnostic, and cosmetic agents from molecules extracted from snake venom is increasing. Snake venom research will continue to contribute to applied science and to the development of promising drug candidates, and is expected to gain substantial market share from the pharmaceutical industry.

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