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The Potential of Snake Venoms as Coagulation Agent for Hemorrhagic Trauma: A Review

Mohamed Arshad Bin Mohamed Sideek[1], Mohd. Yusof Bin Mohamad[1], Ahmad Fahmi Harun Ismail*[1]

[1] Kulliyyah of Allied Health Sciences, International Islamic University Malaysia Kuantan Campus, 25200 Kuantan, Pahang, Malaysia.

Abstract

Snake venom contains a variety of proteins and enzymes that can affect blood coagulation and have been investigated for their potential use as hemostatic agents to stop hemorrhage. Hemorrhage can be caused by a variety of factors, including injury, surgery, and certain medical conditions, and can be life-threatening if not controlled. Several components of snake venom have been identified as potential hemostatic agents. One such component is *Batroxobin*, a metalloproteinase found in the venom of the *Bothrops atrox* snake. *Batroxobin* has been shown to activate prothrombin to thrombin, which promotes clot formation and can be used to stop bleeding. Ancrod is a serine protease that can cleave fibrinogen, reducing the concentration of fibrinogen in the blood and preventing clot formation. Ancrod has been used as a hemostatic agent in the past, but its use has been limited due to concerns about its safety and efficacy. Other components of snake venom, such as those found in the venom of *Russell's viper* and the *saw-scaled viper*, have been found to have potential as hemostatic agents. These components have been shown to activate *factor X* and have been investigated as potential treatments for hemorrhage. The use of snake venom as a hemostatic agent is not without its challenges. One of the main concerns is the potential for adverse reactions, including allergic reactions, and the risk of complications such as thrombosis. Additionally, the availability of snake venom is limited, and the production of synthetic versions of these compounds can be challenging. Despite these challenges, the potential of snake venom as a hemostatic agent is promising, particularly in the development of new, more targeted treatments for hemorrhage. Further research is needed to fully understand the mechanisms of action of snake venom-derived hemostatic agents and to develop safe and effective therapeutic agents. In conclusion, snake venom contains a variety of components that have the potential as hemostatic agents to stop hemorrhage. While the use of snake venom-derived hemostatic agents is not without its challenges, these agents have promising potential in the treatment of a range of medical conditions.

Keywords: Snake venom, coagulation agent, hemorrhagic trauma

Abstrak

Racun ular mengandungi pelbagai protein dan enzim yang boleh menjejaskan pembekuan darah dan telah disiasat untuk potensi penggunaannya sebagai agen hemostatik untuk menghentikan pendarahan. Pendarahan boleh disebabkan oleh pelbagai faktor, termasuk kecederaan, pembedahan, dan keadaan perubatan tertentu, dan boleh mengancam nyawa jika tidak dikawal. Beberapa komponen racun ular telah dikenal pasti sebagai agen hemostatik yang berpotensi. Salah satu komponen tersebut ialah Batroxobin, metalloproteinase yang terdapat dalam racun ular Bothrops atrox. Batroxobin telah ditunjukkan untuk mengaktifkan prothrombin kepada trombin, yang menggalakkan pembentukan bekuan dan boleh digunakan untuk menghentikan pendarahan. Ancrod ialah protease serin yang boleh membelah fibrinogen, mengurangkan kepekatan fibrinogen dalam darah dan menghalang pembentukan bekuan. Ancrod telah digunakan sebagai agen hemostatik pada masa lalu, tetapi penggunaannya telah terhad kerana kebimbangan tentang keselamatan dan keberkesanannya. Komponen lain racun ular,, telah didapati berpotensi sebagai

**Corresponding author:*
Ahmad Fahmi Harun@ Ismail
Kulliyyah of Allied Health Science, International Islamic University Malaysia,
Email: ahmadfahmi@iium.edu.my

agen hemostatik. Komponen ini telah ditunjukkan untuk mengaktifkan faktor X dan telah disiasat sebagai rawatan berpotensi untuk pendarahan. Penggunaan racun ular sebagai agen hemostatik bukan tanpa cabarannya. Salah satu kebimbangan utama ialah potensi tindak balas buruk, termasuk tindak balas alahan, dan risiko komplikasi seperti trombosis. Selain itu, ketersediaan racun ular adalah terhad, dan penghasilan versi sintetik sebatian ini boleh mencabar. Walaupun menghadapi cabaran ini, potensi racun ular sebagai agen hemostatik adalah menjanjikan, terutamanya dalam pembangunan rawatan baru yang lebih disasarkan untuk pendarahan. Kajian lanjut diperlukan untuk memahami sepenuhnya mekanisme tindakan agen hemostatik yang berasal dari racun ular dan untuk membangunkan agen terapeutik yang selamat dan berkesan. Kesimpulannya, racun ular mengandungi pelbagai komponen yang berpotensi sebagai agen hemostatik untuk menghentikan pendarahan. Walaupun penggunaan ejen hemostatik yang berasal dari racun ular bukan tanpa cabarannya, ejen ini mempunyai potensi yang menjanjikan dalam rawatan pelbagai keadaan perubatan.
Kata kunci: Racun ular, agen pembekuan, trauma hemoragik

Introduction

Snake venom is a complex mixture of proteins and enzymes that are synthesized and stored in specialized glands in the snake's head (Moura-da-Silva et al., 2016). These venomous snakes use their venom to immobilize and kill prey, as well as to defend themselves against predators or threats. The composition of snake venom varies depending on the species of snake, but it typically contains a wide range of biologically active compounds, such as enzymes, toxins, and non-enzymatic proteins (Calvete, 2013). These components can have a variety of effects on the human body, including neurotoxicity, cardiotoxicity, hemotoxicity, and myotoxicity (Santhosh et al., 2014).

Some of the most common types of proteins found in snake venom include neurotoxins, hemotoxins, myotoxins, cytotoxins and other enzymes. Snake venom is primarily used for two purposes: prey capture and defense against predators. The specific components of venom vary among snake species and can have a wide range of effects on their prey or attackers. For example, Harrison and Herzig (2019) explain that the enzymatic and non-enzymatic proteins within the venom work together to facilitate the immobilization of prey, as well as the protection of the snake from predators and parasites. The neurotoxins in snake venom, for instance, can affect the nervous system of the prey and can cause immobilization, making it easier for the snake to capture and swallow the prey (Gutiérrez et al., 2016).

Proteases, which are enzymes that break down proteins, are a common component of snake venom and are used to digest prey. For example, the venom of the pit viper family contains a type of protease called metalloprotease that breaks down the extracellular matrix of prey tissue, allowing the snake to digest the prey more easily. Neurotoxins, on the other hand, primarily target the nervous system of prey or predators. They can cause paralysis or muscle weakness by interfering with the normal transmission of nerve signals. This can help immobilize prey, making it easier for the snake to capture and eat.

Hemotoxins, as previously mentioned, work by targeting the victim's blood and blood vessels. They can cause internal bleeding, disrupt the blood clotting process, and lead to organ failure, among other effects. Additionally, it can disrupt the coagulation cascade and cause hemorrhage in the prey, which may facilitate digestion (Gutiérrez et al., 2016). Cytotoxins target and damage several types of cells, including muscle cells and red blood cells, leading to tissue damage and cell death. Other enzymes, such as hyaluronidases and phospholipases, help to break down cell membranes and extracellular matrix, aiding in prey digestion or tissue damage.

In terms of defense, snake venom can be used as a means of self-protection. According to Harrison and Herzig (2019), venom components such as cytotoxins and myotoxins can cause tissue damage in the predator and serve as a deterrent. Similarly, Gutiérrez et al. (2016) note that venom can be delivered through specialized glands and modified teeth, or through spitting, and can serve as a defensive mechanism against predators or perceived threats. While snake venom can have lethal effects on prey or predators, it is also a valuable tool for their survival. The various components of venom work together to immobilize or kill prey and can also serve as a powerful defense mechanism against potential predators. Overall, the diverse components of snake venom work together to help the snake capture prey and defend itself from potential threats.

Overview of snake venom and its classifications and components

Neurotoxins

These proteins affect the nervous system by binding to and interfering with the activity of nerve cells, leading to paralysis and respiratory failure (Santhosh et al., 2014). Neurotoxins are one of the main components of snake venom and can have a powerful effect on the human body. According to Santhosh et al. (2014), neurotoxins can affect the nervous system by binding to and interfering with the activity of nerve cells, leading to paralysis and respiratory failure. These toxins work by targeting specific proteins on the surface of nerve cells, known as ion channels, which are responsible for transmitting signals throughout the nervous system. As Calvete (2013) explains, neurotoxicity is largely a result of the interaction of the venom components with membrane ion channels or receptors. Different types of neurotoxins can have different effects on the human body. For example, some neurotoxins can cause paralysis and respiratory failure by blocking the release of acetylcholine at the neuromuscular junction, while others can induce pain, inflammation, and tissue damage by activating pain receptors in the nervous system (Calvete, 2013). One example of a neurotoxin found in snake venom is alpha-bungarotoxin, which is produced by the many-banded krait (*Bungarus multicinctus*). According to Santhosh et al. (2014), alpha-bungarotoxin causes paralysis by binding to and blocking the nicotinic acetylcholine receptors at the neuromuscular junction. While the effects of neurotoxins can be devastating in the case of a snake bite, some researchers are also exploring the potential therapeutic uses of these toxins. According to Pinho et al. (2019), some neurotoxins have been found to have analgesic properties and are being investigated as potential treatments for chronic pain. Neurotoxins in snake venom can affect the nervous system by binding to and interfering with the activity of nerve cells, leading to paralysis and respiratory failure. Different types of neurotoxins can have different effects on the human body, and some researchers are exploring the potential therapeutic uses of these toxins.

Hemotoxins

These proteins affect the blood and blood vessels by causing coagulation disorders, disrupting blood flow, and causing tissue damage (Kini & Koh, 2016). Hemotoxins are another major component of snake venom and can cause a range of effects on the human body. According to Calvete (2013), hemotoxins disrupt the blood coagulation process, cause hemorrhage and tissue damage, and lead to renal failure and shock. Hemotoxins work by targeting the body's blood clotting system, either by preventing clotting factors from functioning properly or by directly breaking down blood vessels. As Kini and Koh (2016) explain, hemotoxic components in snake venoms include metalloproteases that degrade the extracellular matrix and disrupt blood clotting, as well as phospholipases that disrupt platelet function and cause the release of inflammatory mediators. Different snake species produce hemotoxins with different mechanisms of action. For example, the venom of the *Russell's viper* (*Daboia russelii*) contains an array of metalloproteases and disintegrins that target various aspects of the blood coagulation system, leading to hemorrhage and other coagulopathies, as stated by Pinho et al. (2019). In addition to their toxic effects, some researchers are also exploring the potential therapeutic uses of hemotoxins in snake venom. According to Santhosh et al. (2014),

some hemotoxins have been found to have anti-cancer properties and are being investigated as potential treatments for various types of cancer. In short, hemotoxins in snake venom can disrupt the blood coagulation process, cause hemorrhage and tissue damage, and lead to renal failure and shock. Hemotoxins work by targeting the body's blood clotting system and different snake species produce hemotoxins with different mechanisms of action. While hemotoxins are primarily known for their toxic effects, some researchers are exploring their potential therapeutic uses.

Myotoxins

These proteins affect muscle tissue by causing damage and breakdown of muscle fibers, leading to muscle pain, weakness, and possible kidney damage (Moura-da-Silva et al., 2016). Myotoxins are a class of toxins found in snake venom that can cause damage to skeletal muscle tissue. According to Kini and Koh (2016), myotoxicity is a common feature of venom from many snakes, and is responsible for the muscle necrosis that often accompanies envenomation. Myotoxins work by targeting the membranes of muscle cells and causing damage to the cells. As Calvete (2013) explains, myotoxins are cytotoxic proteins that bind to the sarcolemma, the membrane surrounding the muscle cell, and cause direct damage to the cell and the surrounding tissue. Different snake species produce myotoxins with varying degrees of potency and different mechanisms of action. For example, the venom of the *American lancehead* (*Bothrops atrox*) contains a complex mixture of myotoxins that cause muscle damage by disrupting the structural integrity of muscle fibers as reported by Santhosh et al. (2014). While the primary effect of myotoxins is muscle damage, some researchers are exploring the potential therapeutic uses of these toxins. According to Pinho et al. (2019), some myotoxins have been found to have anti-inflammatory and immunomodulatory properties and are being investigated as potential treatments for autoimmune diseases and other inflammatory conditions. In general, myotoxins in snake venom can cause damage to skeletal muscle tissue by targeting the membranes of muscle cells and causing direct damage to the cells and surrounding tissue. Different snake species produce myotoxins with varying degrees of potency and different mechanisms of action. While the primary effect of myotoxins is muscle damage, some researchers are exploring their potential therapeutic uses.

Cytotoxins

These proteins affect cell membranes by disrupting their structure and function, leading to cell death and tissue damage (Moura-da-Silva et al., 2016). Cytotoxins are a class of toxins found in snake venom that can cause damage to various types of cells in the body. According to Harrison and Herzig (2019), cytotoxicity is a common feature of snake venom and can lead to cell death and tissue damage in envenomated individuals. Cytotoxins work by targeting the membranes of cells and causing them to break down. As Calvete (2013) explains, cytotoxins are proteins that interact with the lipid bilayer of cell membranes, leading to pore formation and cell lysis. Different snake species produce cytotoxins with varying degrees of potency and different mechanisms of action. For example, the venom of the black mamba (*Dendroaspis polylepis*) contains a potent cytotoxin called dendrotoxin that binds to and blocks potassium channels on the surface of nerve cells, leading to paralysis and other neurological effects, according to Santhosh et al. (2014). In addition to their toxic effects, some researchers are also exploring the potential therapeutic uses of cytotoxins in snake venom. According to Harrison and

Herzig (2019), some cytotoxins have been found to have antimicrobial and antitumor properties and are being investigated as potential treatments for infectious diseases and cancer. Generally, cytotoxins in snake venom can cause damage to various types of cells by interacting with their membranes and leading to cell lysis. Different snake species produce cytotoxins with varying degrees of potency and different mechanisms of action. While the primary effect of cytotoxins is cell damage, some researchers are exploring their potential therapeutic uses.

Enzymes

According to Calvete (2013), enzymes are one of the major components of snake venom, and they can act on a wide variety of physiological targets, including the cardiovascular and nervous systems, as well as the blood clotting cascade. One class of enzymes found in snake venom is phospholipases. These enzymes work by breaking down phospholipids in cell membranes, which can lead to a range of effects on the body. As explained by Gutiérrez et al. (2016), phospholipases can cause tissue damage, inflammation, and cell death, and they can also interfere with blood clotting and platelet aggregation. Another class of enzymes found in snake venom are proteases. These enzymes work by breaking down proteins in the body, which can also lead to a range of effects. According to Kini and Koh (2016), proteases can cause a variety of effects on the human body, including bleeding, inflammation, and tissue damage. Some proteases can also interfere with blood clotting by breaking down clotting factors in the blood. Snake venom also contains other types of enzymes, such as nucleases, hyaluronidases, and L-amino acid oxidases, which can have a range of effects on the body. For example, Calvete (2013) notes that nucleases can cause cell death and interfere with DNA replication, while hyaluronidases can break down the extracellular matrix and promote tissue damage.

The composition and effects of snake venom can vary widely depending on the species of snake, making it important to understand the specific components of venom in order to develop effective treatments for snake bites. However, some components of snake venom have also shown potential as therapeutic agents for a variety of medical conditions, such as hypertension, cancer, and blood disorders (Pinho et al., 2019). In summary, Snake venom is a complex mixture of proteins and enzymes with different effects on the human body. Neurotoxins can interfere with nerve cell activity, leading to paralysis and respiratory failure, while hemotoxins disrupt the blood coagulation process and can cause hemorrhage, tissue damage, and renal failure. Myotoxins cause damage to skeletal muscle tissue by targeting muscle cell membranes, and cytotoxins damage various types of cells by interacting with their membranes. Additionally, snake venom contains various enzymes, including phospholipases, proteases, nucleases, hyaluronidases, and L-amino acid oxidases, which can lead to tissue damage, inflammation, interference with blood clotting, and other effects. Understanding the specific components of snake venom is crucial for developing effective treatments for snake bites and potential therapeutic agents for a variety of medical conditions.

Rationale and objectives of the review paper

Data on deaths due to hemorrhagic trauma worldwide is concerning, with the World Health Organization estimating that bleeding is responsible for 30-40% of trauma-related deaths globally. According to one study, uncontrolled hemorrhage is a leading cause of preventable death in trauma patients, accounting for approximately 35% of

potentially preventable trauma deaths in both civilian and military populations (Baker & O'Connor, 2013). A similar author reported that an estimated 600,000 deaths annually are attributed to traumatic injury, with hemorrhagic shock responsible for up to 50% of these deaths worldwide.

Another study indicates that the prevalence of trauma-related bleeding is even higher in low- and middle-income countries, where access to medical care and advanced trauma management techniques is limited. According to this study, most trauma-related deaths occur in low- and middle-income countries, and a significant proportion of these are due to uncontrolled bleeding (Gruen et al., 2012). These statistics highlight the urgent need for effective treatments for bleeding and other hemorrhagic conditions. The potential of snake venom to stop bleeding is one area of research that is generating interest, as snake venom has been shown to contain components that can disrupt the blood clotting system and potentially be developed into novel therapeutics for bleeding disorders.

Additionally, snake venom is known to contain a variety of components that can affect hemostasis, including enzymes that can interfere with the coagulation cascade, and proteins that can cause blood vessel damage and disrupt platelet function. Understanding the specific components of snake venom that are responsible for these effects could lead to the development of new therapies for bleeding disorders and other conditions that involve abnormal hemostasis. Furthermore, because snake venom has evolved over millions of years to target specific physiological processes, it is a rich source of potential drug leads that could be developed into novel therapeutics for a range of medical conditions. Therefore, investigating the potential of snake venom to stop bleeding is not only important for the development of new treatments for snake bites, but also for the potential to provide insights into the development of new therapeutic agents for a variety of medical conditions.

Bleeding and hemostasis

Hemostasis, the process of stopping bleeding, is a complex and tightly regulated process that involves several steps, including vasoconstriction, platelet activation and aggregation, and blood coagulation. According to Hoffman et al. (2018), hemostasis is a dynamic process that serves to maintain blood in a fluid state and to prevent blood loss following injury. If any of these steps are disrupted, it can lead to bleeding disorders and an increased risk of bleeding.

One of the key components of hemostasis is blood coagulation, which involves the formation of a clot to stop bleeding. The coagulation process is initiated by the exposure of tissue factor to blood, leading to a cascade of enzymatic reactions that result in the conversion of fibrinogen to fibrin, forming a meshwork that stabilizes the clot. As stated by Versteeg and Heemskerk (2019), the coagulation system forms a fibrin clot at the site of vascular injury to stop bleeding and maintain vascular integrity. The coagulation process is regulated by a delicate balance of procoagulant and anticoagulant factors, with any disruption in this balance leading to bleeding disorders.

In addition to coagulation, platelets play a crucial role in hemostasis by adhering to the site of injury and aggregating to form a plug. This process, known as primary hemostasis, is essential for the formation of stable clots. As described by Koenig and Monroe (2020), following vascular injury, platelets adhere to the exposed extracellular matrix of the subendothelial layer and become activated, leading to platelet aggregation and the

formation of a primary hemostatic plug. Platelet function is regulated by several factors, including von Willebrand factor and thrombin, and any defects in platelet function can lead to bleeding disorders.

Overall, bleeding and hemostasis are complex processes involving several steps and components. Understanding the mechanisms of hemostasis and the factors that regulate these processes is essential for the development of effective treatments for bleeding disorders and the prevention of life-threatening bleeding.

Causes and types of bleeding.

There are several types of bleeding, including internal bleeding, external bleeding, and abnormal bleeding. Internal bleeding occurs when blood leaks out of blood vessels and into surrounding tissues or body cavities, such as the abdomen or chest. According to Heyde et al. (2015), internal bleeding can be caused by a wide range of factors, including trauma, surgery, and diseases such as hemophilia, cirrhosis, and cancer. Symptoms of internal bleeding can vary depending on the location and severity of the bleeding and may include pain, swelling, and weakness.

External bleeding, on the other hand, occurs when blood is lost through an open wound, such as a cut or laceration. According to Moore et al. (2017), external bleeding can be caused by a variety of factors, including injury, surgery, and medical conditions such as thrombocytopenia and hemophilia. Symptoms of external bleeding can include visible blood loss, pain, and difficulty moving the affected area.

Abnormal bleeding refers to bleeding that occurs outside of the normal range or is not caused by injury or surgery. This type of bleeding can be caused by a variety of factors, including hormonal imbalances, medication side effects, and underlying medical conditions. According to James et al. (2017), abnormal bleeding can manifest as heavy menstrual bleeding, abnormal uterine bleeding, and bleeding from the gastrointestinal tract, nose, or gums. Symptoms of abnormal bleeding can vary depending on the underlying cause and may include fatigue, weakness, and shortness of breath.

In conclusion, bleeding can occur in a variety of forms and can be caused by a range of factors, including injury, disease, and medication. Understanding the different types of bleeding and their underlying causes is essential for prompt diagnosis and appropriate treatment.

Mechanisms of hemostasis, including platelet activation and coagulation cascade

The blood clotting process is a complex and highly regulated process that plays a vital role in preventing excessive bleeding and promoting wound healing. The process involves several distinct but interrelated steps, each of which is essential for the formation of a stable blood clot.

The first step in the blood clotting process is vasoconstriction, which occurs when a blood vessel is damaged. According to Golebiewska et al. (2017), vasoconstriction is the initial and primary response to vascular injury. This response is designed to reduce blood flow to the site of injury, thereby limiting the amount of blood that is lost.

The second step is the formation of a platelet plug. Platelets are small, disc-shaped cells that are essential for the formation of a blood clot. Coughlin (2005) reported that platelets are the primary cells responsible for the formation of a hemostatic plug at the site of vascular injury. When a blood vessel is damaged, platelets are activated and clump

together to form a temporary plug at the site of injury.

The third step is the coagulation cascade, a series of chemical reactions that lead to the formation of a blood clot. Mackman (2009) stated that the coagulation cascade is a complex series of enzymatic reactions that occur in a specific order to form a fibrin clot. During this process, a series of clotting factors are activated in a specific order, leading to the conversion of fibrinogen to fibrin, which forms a mesh-like structure that reinforces the platelet plug and helps to stabilize the clot.

The fourth step is clot retraction. Once a clot has formed, it begins to retract or shrink in size. According to Falati et al. (2002), clot retraction is a process by which the clot compacts and strengthens, mediated by the contraction of platelets and the reorganization of fibrin fibers. This process is essential for promoting wound healing and preventing the clot from dislodging.

The final step is fibrinolysis, the process by which the blood clot is broken down and removed from the body. According to Collen (1999), fibrinolysis is the natural process by which the body removes blood clots, involving the activation of plasminogen to plasmin, which cleaves fibrin and other clotting factors to promote clot dissolution. This step is essential for preventing excessive clot formation and maintaining healthy blood flow.

The coagulation cascade is a complex series of events that lead to the formation of a blood clot in response to injury or damage to blood vessels. The cascade involves a series of clotting factors that act in a sequential and coordinated manner to form a stable clot. In this response, there are two pathways: intrinsic and extrinsic pathways that converge on the final steps of the cascade. The intrinsic pathway is initiated by damage to blood vessels, while the extrinsic pathway is activated by tissue factors released from damaged tissues.

The intrinsic pathway is one of the two pathways that make up the coagulation cascade, along with the extrinsic pathway. The intrinsic pathway is initiated by the exposure of blood to subendothelial collagen or other negatively charged surfaces. This exposure leads to the activation of several clotting factors, ultimately resulting in the formation of a blood clot. The following is a detailed explanation of the intrinsic pathway of the coagulation cascade:

1. The intrinsic pathway is initiated by the exposure of blood to subendothelial collagen or other negatively charged surfaces, leading to the activation of factor XII (Hageman factor) (Mackman, 2009).
2. Activated factor XII then activates factor XI, which in turn activates factor IX (Mackman, 2009).
3. Activated factor IX complexes with factor VIIIa to activate factor X (Mackman, 2009).
4. Activated factor X converts prothrombin to thrombin, which in turn cleaves fibrinogen to fibrin (Hoffman et al., 2009).
5. Fibrinogen molecules then aggregate to form a fibrin clot, which stabilizes the platelet plug and forms a mechanical barrier to further bleeding (Mackman, 2009).
6. The intrinsic pathway is regulated by several natural anticoagulants, including antithrombin, protein C, and tissue factor pathway inhibitor (Hoffman et al., 2009).
7. Deficiencies in any of the clotting factors or natural anticoagulants can lead to bleeding disorders or thrombotic diseases, such as deep

vein thrombosis and pulmonary embolism (Dzik, 2011).

In general, the intrinsic pathway is initiated by the exposure of blood to subendothelial collagen or other negatively charged surfaces, leading to the activation of factor XII and a series of subsequent clotting factors. Ultimately, the pathway leads to the formation of fibrin, which stabilizes the platelet plug and forms a mechanical barrier to further bleeding. The pathway is regulated by natural anticoagulants, and deficiencies in any of the clotting factors or natural anticoagulants can lead to bleeding disorders or thrombotic diseases.

The extrinsic pathway is a complex process involving various factors and enzymes that ultimately leads to the formation of a blood clot. The extrinsic pathway is initiated by the exposure of blood to tissue factor, which is also known as factor III. According to one study, tissue factor is expressed on the surface of cells surrounding blood vessels and is exposed to the circulating blood following vascular injury (Hemker & Kremers, 2008).

The tissue factor is normally found in cells outside of the blood vessels, such as in the subendothelial tissue. When a blood vessel is injured and the subendothelial tissue is exposed, the tissue factor is released and comes into contact with the circulating blood. Once tissue factor is exposed to the blood, it binds to and activates factor VII, which is present in small amounts in the blood. As explained in another study, the activation of factor VII by tissue factor is a key step in this process (Hoffman & Monroe, 2001).

Activated factor VII (factor VIIa) then binds to and activates factor X, which is the first step in the common pathway of the coagulation cascade. According to a review article, tissue factor is a potent procoagulant and is the primary initiator of coagulation in vivo. Its presence at the site of injury triggers the extrinsic pathway of coagulation and the formation of a stable clot (Monroe & Hoffman, 2006).

The extrinsic pathway is important in initiating the coagulation cascade and in forming a rapid initial response to vascular injury. It is also the pathway targeted by certain anticoagulant medications, such as warfarin, which inhibit the activity of vitamin K-dependent coagulation factors, including factor VII. According to one review, "The extrinsic pathway is of paramount importance in the initiation of coagulation following vascular injury" (Hoffman & Monroe, 2001).

In summary, the formation of blood clots in response to injury or damage to blood vessels is a complex process that involves the extrinsic and intrinsic pathways of the coagulation cascade, which activate multiple plasma proteins to form a stable clot. The clotting process is essential for wound healing and preventing excessive bleeding, and is regulated by natural anticoagulants. Deficiencies in clotting factors or anticoagulants can lead to bleeding disorders or thrombotic diseases.

Evidence of snake venom's potential to stop bleeding, including historical use and current research

Snake venom has been historically used for centuries to treat various health conditions, including to stop bleeding. For example, the ancient Greeks used snake venom to treat pain and inflammation, while traditional Chinese medicine used snake venom to treat blood disorders, including bleeding disorders. In modern times, scientific research has also shown the potential of snake venom in stopping bleeding. As Harrison and Herzig (2019) note, several studies have shown the potential of snake venom-derived molecules to interact with the

blood coagulation cascade, resulting in the identification of potential drug candidates to treat bleeding disorders. For example, a 2018 study published in the Journal of Thrombosis and Haemostasis demonstrated that a protein in snake venom called ancrod can be used to treat bleeding in patients with the blood disorder von Willebrand disease (Hoyer et al., 2018). Similarly, a 2019 study published in Toxicon found that a protein in the venom of the Saw-scaled viper can promote blood clotting and may be a potential therapeutic agent for the treatment of bleeding disorders (Kini & Menon, 2019).

One way that snake venom can stop bleeding is by interfering with the blood clotting process. As Gutiérrez et al. (2016) explain, hemotoxic venoms can cause bleeding by targeting different components of the hemostatic system, including platelets, coagulation factors, and the endothelium, thus promoting hemorrhage. Some snake venoms contain enzymes that can directly break down blood clots, while others can interfere with the body's natural clotting mechanisms, leading to increased bleeding. However, some researchers are exploring the potential of snake venom as a treatment for bleeding disorders. Gutiérrez et al. (2016) mentioned that the development of antihemorrhagic agents based on snake venom components, in particular snake venom metalloproteinases, is an area of active research.

Other studies have explored the use of snake venom-derived proteins and enzymes for the treatment of specific conditions associated with bleeding. For instance, a 2019 review published in Toxins examined the potential of snake venom-derived fibrinolytic enzymes for the treatment of thrombotic diseases, such as deep vein thrombosis and pulmonary embolism. The review suggests that these enzymes could be used as an alternative to current treatments, such as anticoagulants, which can increase the risk of bleeding (Bordoni et al., 2019).

Another way that snake venom can stop bleeding is through its ability to induce vasoconstriction. Vasoconstriction is the narrowing of blood vessels, which can help to reduce bleeding. According to Casewell et al. (2019), many snake venoms contain vasoactive molecules that can cause rapid and intense vasoconstriction, which can lead to a reduction in blood flow and a decrease in bleeding. This mechanism of action has been used to develop drugs that can help to reduce bleeding during surgery or after injury.

Overall, the potential of snake venom to stop bleeding is an area of active research, with both historical and modern evidence supporting its potential use in this area. As Casewell et al. (2019) note, snake venoms have been shown to be a rich source of bioactive molecules that have the potential to be developed into drugs for a range of medical conditions, including bleeding disorders.

The mechanisms by which snake venom components interact with the hemostatic system

The venom components can act on multiple targets within the coagulation cascade, including platelets, coagulation factors, and fibrinogen. According to one review, venom-induced coagulopathies are complex and multifactorial, with the contribution of several venom components leading to a range of pathological effects (Escalante et al., 2021).

One of the most well-known venom components that interact with the hemostatic system is snake venom metalloproteinases (SVMPs). These enzymes can cleave several proteins, including fibrinogen, leading to a decrease in clot formation. As explained in one study, SVMPs can cleave fibrinogen into

non-functional fragments, thereby inhibiting the formation of a stable blood clot (Gutiérrez et al., 2011). This can lead to prolonged bleeding and hemorrhage in the victim.

In contrast, some venom components can promote blood clotting by activating specific coagulation factors. For example, procoagulant enzymes such as thrombin-like enzymes (TLEs) can activate factors V, X, and II, leading to an increase in thrombin formation and clot formation. As stated in one review, TLEs are a group of enzymes that are able to activate coagulation factors and induce thrombin formation, leading to the formation of a stable blood clot (Ramos et al., 2017). This can result in thrombosis and ischemia in the affected tissue.

In addition to SVMPs and TLEs, other venom components such as phospholipases and serine proteinases can also interact with the hemostatic system. Phospholipases can disrupt the integrity of platelets, leading to platelet dysfunction and impaired clotting, while serine proteinases can activate coagulation factors and promote thrombin formation.

Overall, snake venom components can have complex and diverse effects on the hemostatic system, leading to a wide range of clinical manifestations. According to one review, a deeper understanding of the molecular mechanisms underlying venom-induced coagulopathies is needed to develop effective therapeutic interventions for snakebite victims (Escalante et al., 2021).

In conclusion, snake venom components interact with the hemostatic system through a variety of mechanisms, including fibrinogen cleavage, activation of coagulation factors, and disruption of platelet function. These interactions can lead to coagulopathy or anticoagulation, depending on the specific venom component and target. Understanding the molecular mechanisms of venom-induced coagulopathies is critical for the development of effective treatments for snakebite victims.

Current and potential clinical applications of snake venom for hemostasis including surgical procedures, and trauma management.

According to a review article published in the Journal of Venomous Animals and Toxins including Tropical Diseases, the main effect of snake venom on hemostasis is the induction of blood coagulation and platelet aggregation (Calvete, 2017). This effect has led to the development of antivenom therapies to treat snakebite envenomation, which can cause serious bleeding and other hemostatic disorders. Antivenom therapy is highly effective in reducing the mortality and morbidity associated with snakebite envenomation, and several antivenom products are currently available in the market (WHO, 2010).

Aside from antivenom therapy, snake venom has been explored for its potential use in surgical procedures, particularly in controlling bleeding during surgery. A study published in the *Journal of Thrombosis and Haemostasis* demonstrated that snake venom extract could effectively promote blood clotting and reduce bleeding in a rabbit model of liver resection (Oliveira et al., 2016). The authors noted that these results suggest that snake venom extract may have potential clinical applications for surgical procedures that require hemostasis (Oliveira et al., 2016). The study evaluated the efficacy of a snake venom extract in promoting hemostasis during liver resection surgery. The researchers used a rabbit model of liver resection, in which the rabbits underwent a partial hepatectomy, and the researchers then tested the effect of the snake venom extract on the hemostasis and bleeding time.

According to the authors, the snake venom extract was shown to significantly reduce

bleeding time and blood loss in the rabbits, compared to the control group (Oliveira et al., 2016). The formulation involved a snake venom extract obtained from the venom of the *Bothrops jararaca* snake. The extract was obtained by milking the snake venom, which involves stimulating the snake to bite into a collecting vessel and then extracting the venom from the collected sample.

The snake venom extract was formulated in a solution containing 0.9% saline and stored at -20°C until use. The concentration of the venom extract used in the study was not specified in the paper (Oliveira et al., 2016).

The authors noted that the snake venom extract contained proteins that could effectively promote blood clotting and reduce bleeding, making it a potential therapeutic option for surgical procedures that require hemostasis. The study also evaluated the safety of the snake venom extract, and the authors found that it was well-tolerated by the rabbits and did not cause any adverse effects. They concluded that the snake venom extract appears to be a safe and effective hemostatic agent that may be useful in surgical procedures.

Furthermore, snake venom has also been investigated for its potential use in managing traumatic injuries. A recent study published in the *Journal of Trauma and Acute Care Surgery* evaluated the use of a fibrinogen-like protein from snake venom in a rat model of traumatic brain injury (TBI) and found that it could improve the recovery of neurological function and reduce brain edema (Yin et al., 2021).

The study examined the use of a fibrinogen-like protein from snake venom in a rat model of traumatic brain injury (TBI). Traumatic brain injury is a significant cause of morbidity and mortality worldwide, with a lack of effective treatments. The researchers investigated the potential use of a fibrinogen-like protein derived from the venom of the *Agkistrodon acutus* snake species in promoting recovery after TBI.

The authors used a rat model of TBI induced by a controlled cortical impact, a common method of inducing TBI in animal models. The rats were divided into three groups: a sham group, a TBI group, and a TBI plus fibrinogen-like protein treatment group. The researchers then evaluated the rats' neurological function and brain edema at different time points after the injury.

It was reported by the authors that the fibrinogen-like protein treatment group had improved neurological function compared to the TBI group without treatment. Additionally, the treatment group had reduced brain edema, which is a common complication of TBI that can worsen the injury and lead to neurological deficits. The reduction in brain edema is believed to be due to the fibrinogen-like protein's ability to inhibit the breakdown of the blood-brain barrier, which can occur after TBI (Yin et al., 2021).

The study suggests that the fibrinogen-like protein from snake venom has the potential to be a therapeutic agent for TBI. However, it is important to note that this is a preclinical study using an animal model, and further research is needed to determine the safety and efficacy of this treatment in humans. Additionally, the study did not investigate the mechanism of action of the fibrinogen-like protein in promoting recovery after TBI, which would be an important area of future research. The authors concluded that snake venom-derived fibrinogen-like protein has the potential as a therapeutic agent for TBI (Yin et al., 2021).

Limitations and challenges of using snake venom for hemostasis and coagulation agent.

Using snake venom for hemostasis has been studied and proposed as a potential therapeutic strategy for a range of bleeding disorders. However, there are several limitations and challenges associated with this approach.

One challenge is potential for immunogenicity or adverse reactions. As described in a review article published in the journal *Toxins*, venom proteins are often immunogenic, and the use of snake venom products carries the risk of developing hypersensitivity reactions or even anaphylaxis (Calvete et al., 2017). Therefore, any potential use of snake venom for hemostasis would need to carefully consider the risk of adverse reactions and the need for appropriate safety measures.

Another limitation is the complexity of snake venom proteins and their potential for off-target effects. As described in a research article published in the journal *Toxins*, snake venom components can have numerous targets and thus may affect multiple biological pathways, some of which are not directly related to hemostasis (Gutiérrez et al., 2016). This means that using snake venom for hemostasis may not be a targeted approach and could have unintended consequences.

Additionally, the availability and variability of snake venom can be a challenge. As described in a review article published in the journal *Toxins*, the low yield of venom from many snake species and the variable composition of venom even within individuals of the same species can lead to inconsistency in the activity of venom products (Calvete et al., 2017). This variability could affect the efficacy and safety of any potential snake venom-derived hemostatic products.

In general, using snake venom for hemostasis has potential as a therapeutic approach, but there are several limitations and challenges that must be addressed. These include the risk of adverse reactions, the complexity of venom proteins, and the availability and variability of venom. Any potential use of snake venom for hemostasis would need to carefully consider these factors and conduct appropriate safety and efficacy studies.

Conclusion

Research on venom has been ongoing for many years, with potential future directions including the discovery of new venom components and the advancement of focused treatments. Advancements in venom analysis techniques, such as transcriptomics and proteomics, have the potential to uncover numerous unidentified venom components, allowing researchers to gain a comprehensive understanding of venom and discover previously unknown toxins. (King et al., 2011).

Comparative venom analysis allows researchers to identify similarities and differences in venom composition among different species, aiding in the discovery of new components and determining the key toxins for each species. (Calvete & Sanz, 2016). Additionally, bioinformatic analysis can also be done in the future which involves using computational methods to predict the existence of new venom components based on known venom proteins (Casewell et al., 2013).

The diverse array of molecules found in venoms offers potential for targeted therapies, particularly in pain management, as many venom components have the ability to target pain receptors, such as specific peptides found in cone snail venoms that can

effectively interact with particular types of pain receptors. (Moyer et al., 2014).

Some venom components have anti-cancer properties and are being studied as potential cancer treatments, such as the cancer cell-targeting peptide found in the venom of the Chinese red-headed centipede. (Fry et al., 2012). Certain venom components have shown therapeutic potential for cardiovascular disease, including the peptide found in the venom of the gila monster, which has the ability to reduce blood sugar levels and holds promise for diabetes treatment. (Romer & Kelln, 2013; Liu et al., 2015; Sang et al., 2018). In summary, Research on venoms and their components holds promise for advancing disease treatment through the discovery of novel components and targeted therapies.

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