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REVIEW ARTICLE

Bothrops Moojeni Snake Venom: A Source of Potential Therapeutic Agents Against Hemostatic Disorders

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Central Illustration: ***Bothrops moojeni*** **proteins acting on the different components of hemostasis.**

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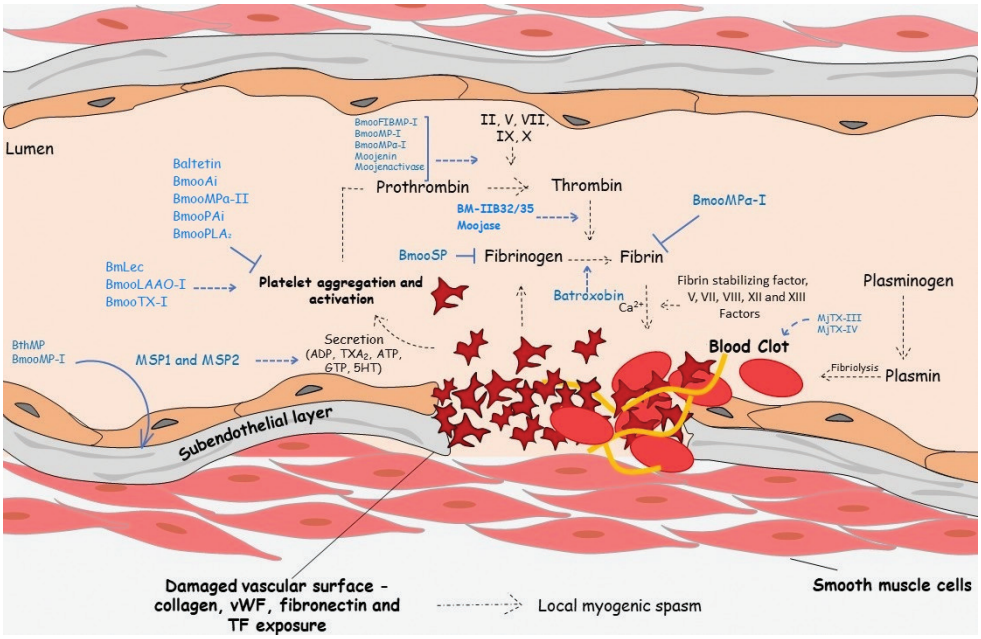

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Elements of hemostasis: a blood coagulation cascade is initiated by release of chemical messengers such as TF and vWF injured tissue, activating the extrinsic coagulation pathway. Platelets meet tissue and release active compounds such as ADP, TXA2, ATP (Adenosine Triphosphate), GTP (Guanosine Triphosphate), and 5HT (Serotonin) that aggregate platelet amplification. Then, there is the conversion of prothrombin to thrombin by intrinsic and extrinsic factors of the coagulation cascade. Thrombin, together with fibrinogen and Ca2+ (calcium ions), form fibrin and activate factors V, VII, VIII, XII, and XIII for fibrin clot formation and stabilization. It occurs the clot fibrinolysis and plasmin-mediated destruction of coagulation factors, local from plasminogen and its activators. The dashed and solid arrows indicate, respectively, the activation and inhibition of various stages of the blood clotting process. TF: tissue factor; vWF: von Willebrand factor.

Abstract

Hemostasis is a complex set of biological processes responsible for blood fluidity within normal vessels and for the physiological interruption of bleeding in cases of vascular injury. *Bothrops moojeni* snake venom is rich in bioactive compounds of pharmacological and clinical interest since its protein components are capable of interfering with many points of the hemostatic process. Here, we present the *B. moojeni*

Keywords

Hemostasis; Snake venoms; Therapeutics.

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venom proteins that affect hemostasis and discuss their pharmacological and clinical potential. This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. Data were obtained from the CAPES Journal Portal database, using the terms "*Bothrops*" AND "hemostasis", in a search for scientific articles made available in the last 20 years. Many components isolated from *B. moojeni* snake venom are characterized for their effect on hemostasis and possible application in the diagnosis and treatment of hemostatic disorders.

Hemostasis

Hemostasis is a complex set of biological processes responsible for blood fluidity within normal vessels and for the physiological interruption of hemorrhage in the case of vascular injury. Preventing coagulation in the normal vascular system depends on endothelial surface factors such as cell uniformity and the presence of glycocalyx-thrombomodulin, and intravascular anticoagulants such as protein C, antithrombin III and heparin. However, to contain bleeding, when a vessel is sectioned or ruptured, hemostasis develops through four main events: vascular constriction, platelet plug formation, blood coagulation, and fibrous organization/clot dissolution[1] (Central Illustration).

The first hemostatic event, vascular constriction, is the result of local myogenic spasms and nerve reflexes in response to local factors released by traumatized tissues and by platelets (such as thromboxane A_2 — TXA_2). Simultaneously, the second event is initiated when platelets come into contact with an injured area, triggering the platelet plug formation mechanism. They begin to dilate and emit pseudopods; then, contractile proteins (actin, myosin, and thrombostenin) cause the release of active compounds such as adenosine diphosphate (ADP), serotonin, TXA_2, epinephrine, fibrinogen and von Willebrand factor (vWF), which maintain and amplify the initial platelet response and also stimulate the formation of platelet aggregates in the injured vessel wall.[1–3]

The blood clotting mechanism, the third event, can be subdivided into three main stages: formation of the prothrombin activating complex; conversion of prothrombin to thrombin; and conversion of fibrinogen to fibrin. Under experimental conditions, the first stage can be activated by two distinct pathways: the extrinsic pathway, triggered by tissue trauma, with consequent release of tissue factor (TF); and the intrinsic pathway, which occurs when there is blood trauma or blood contact with injured endothelial cells or collagen. Both pathways trigger the activation of coagulation cascade factors (plasma proteins), specific and/or common to both pathways, which culminates in the second stage of the coagulation process with the formation of the prothrombin activating complex and consequent conversion of prothrombin (factor II) in thrombin (factor IIa). It is important to emphasize the role of vitamin K in the normal activation of prothrombin and in the formation of most coagulation factors, in addition to the role of calcium ions (Ca^{2+}) in the promotion or acceleration of all blood coagulation reactions. In the last coagulation step, the formation of a fibrin clot occurs. Thrombin removes four low molecular weight peptides from fibrinogen, forming a fibrin monomer, with the ability to polymerize and produce fibrin fibers, establishing a "weak" clot. Under biological conditions (*in vivo*), thrombin also activates the fibrin stabilizing factor (factor XIII), present in normal plasma globulins and released by platelets retained in the clot, promoting the establishment of covalent bonds and cross-links that increase the strength of the fibrin mesh and produce a dense and stable blood clot.[1,4]

The last event in the hemostatic process can occur in two ways: the clot can be invaded by fibroblasts or it can be dissolved. Generally, when the clot develops in a small orifice of the blood vessel, it can be invaded by fibroblasts to form fibrous tissue at the site. However, in a major vascular trauma where tissue clots occur, enzymes are activated to promote clot dissolution. Plasmin is the main component involved in this process; it digests fibrin fibers and destroys many of the clotting factors. When a clot is formed, large amounts of plasminogen and other plasma proteins are retained, and, with the slow and gradual release of plasminogen activators by injured tissues and vascular endothelium, for example, the conversion of plasminogen to plasmin and consequent fibrinolysis of the clot occurs.[1,4]

Disturbances in hemostatic balance may arise due to genetic, nutritional, or traumatic events. In this sense, the clinical manifestation of hemostatic disorders can be observed as hemorrhages or thrombogenesis. In both cases, they represent a medical concern of major importance, as they can lead to death, as in

episodes of venous thromboembolism, stroke, acute myocardial infarction, pulmonary thromboembolism, and hemophilia.[5] The prevalence of these phenomena in the world population is significant. In Brazil, for example, up to 200 cases of venous thromboembolism are recorded for every 100,000 inhabitants, with episodes of pulmonary thromboembolism in 5 to 15% of untreated cases, and more than 100 deaths from acute myocardial infarction.[6,7] According to the Pan American Health Organization, ischemic heart disease and stroke are the biggest killers worldwide, accounting for a total of 15.2 million deaths in 2016.[8]

Bothrops moojeni proteins acting on hemostasis

Bothrops snakes are responsible for about 85% of snakebites registered in Brazil, with more than 20,000 accidents being notified each year.[9] *Bothrops moojeni* snake (Hoge, 1966) (Figure 1A), commonly known as "caiçaca", is a representative venomous snake widespread in South America, including Brazil.[10,11] This species is considered of great clinical importance in the country.[12–14] The *B. moojeni* snake venom is rich in bioactive compounds of pharmacological and clinical interest. This systematic review discourses and discusses proteins isolated from *B. moojeni* snake venom that have been characterized for their action on hemostasis using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method.[15] The bibliographic survey was carried out through the CAPES Journal Portal (from the Coordination for the Improvement of Higher Education Personnel), using the descriptors "*Bothrops*" AND "hemostasis" and searching for scientific articles made available from 2001 until 2023. The search was conducted in December 2021 and updated in September 2023, resulting in a total of 125 studies. According to the inclusion and exclusion criteria, the authors selected and analyzed the titles and abstracts of 93 articles with open access and published in English, Spanish, or Portuguese. Records not related to the scope of this review were excluded and other articles were searched to complement the study. Figure 2 summarizes the criteria and results of this process. Of the 84 articles included in this review, 23 were analyzed in detail to summarize the results presented in Table 1. This review showed 23 proteins isolated from *B. moojeni* venom already characterized regarding their effect on hemostasis and possible application in the diagnosis and treatment of hemostatic disorders (Central Illustration).

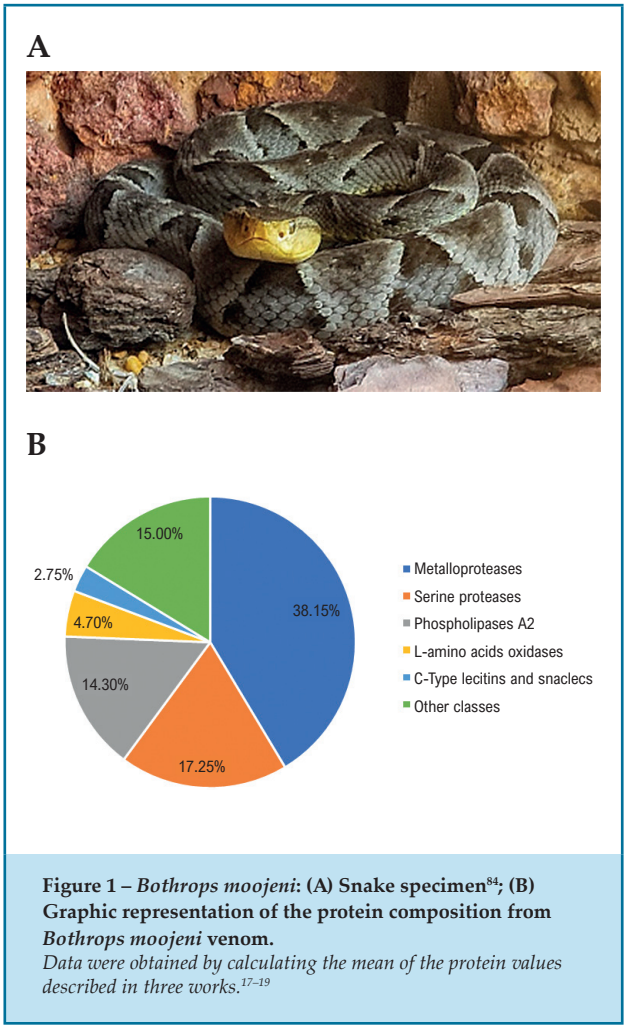

Figure 1 – *Bothrops moojeni*: (A) Snake specimen[84]; (B) Graphic representation of the protein composition from *Bothrops moojeni* venom.
Data were obtained by calculating the mean of the protein values described in three works.[17–19]

Snake venoms are composed of numerous substances, such as ions and biomolecules, especially proteins (about 90%). In general, the most representative protein components in snake venoms are metalloproteases (SVMPs), serine proteases (SVSPs), and phospholipases A_2 (PLA_2s).[16] *B. moojeni* venom is mainly composed of SVMPs (36.5–39.8%), SVSPs (14.7–19.8%), PLA_2 (11.5–17.1%), L-amino acid oxidases (LAAO) (4.2–5.2%), and lectins (2.4–3.1%) (Figure 1B). Among the less abundant toxins are vascular endothelial growth factors (VEGF), bradykinin-enhancing peptides (BPP), cysteine-rich secreting protein (CRISP), 5'-nucleotidases and hyaluronidases, which together, represent less than 15% of total protein in the venom.[17–19]

SVMPs comprise a broad group of proteins that are catalytically dependent on metal ions, especially zinc and calcium. They are subdivided into three classes according to their domains: P-I, P-II, and P-III. The P-I class is compounded of low molecular weight proteins

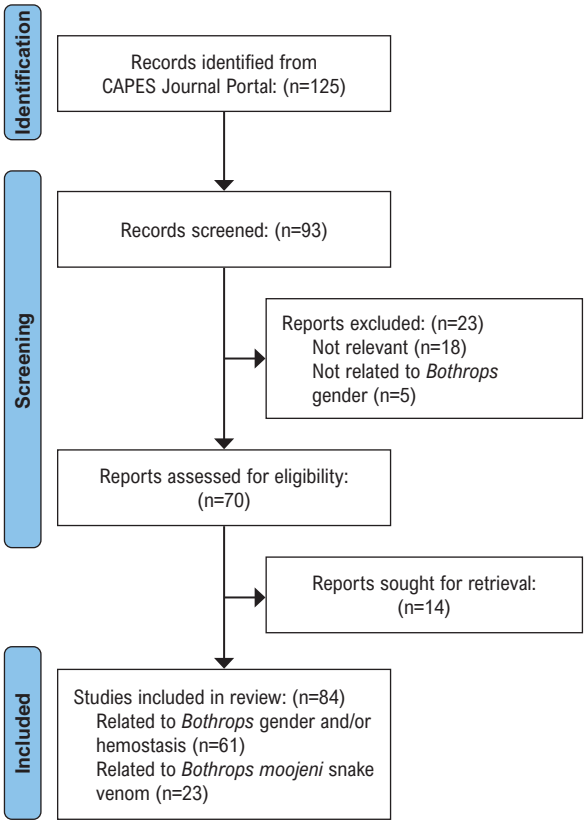

Figure 2 – PRISMA flow diagram for systematic reviews..

(20–30 kDa) that contain only the proteolytic domain. Class P-II contains medium molecular weight (30–60 kDa) proteases, with the addition of the disintegrin domain. The P-III class comprises proteins with a molecular mass of 60–100 kDa, which contain additional domains to the proteolytic region, such as disintegrin-like, rich in cysteine and lectin-like domains. The presence of additional domains in metalloproteases favors the enhancement and specificity of the biological effects of these snake venom components, especially hemorrhage.[20–23]

SVSPs are generally single-chain glycoproteins with a large diversity of substrates and have a highly reactive serine residue, which is stabilized by the presence of histidine and aspartic acid residues in the active site. The main classifications of serine proteases consist of thrombin-like enzymes, fibrinogenolytic enzymes and prothrombin, factors (F) V, VII and X, protein C, platelet aggregation, and plasminogen activators.[24,25]

PLA_2s are low molecular weight enzymes (14–18 kDa) that catalyze the specific hydrolysis of the 2-acyl-ester bond in the sn-2 position of phospholipids in the presence of calcium, promoting the release of fatty acids and lysophospholipids, such as arachidonic acid and lysophosphatidic acid, respectively. These enzymes are classified according to their origin, amino acid sequence, catalytic mechanisms, and biochemical, functional, and structural characteristics. *Bothrops* venom PLA_2s belong to group II. The best-studied group II PLA_2s are classified according to the amino acid located at position 49. Asp49 PLA_2s show catalytic activity on artificial substrates, while Lys49 PLA_2s show little or no activity. Fatty acids released during cleavage are precursors of bioactive lipids and potential inflammatory mediators, pain and platelet activation, such as eicosanoids (prostaglandins, thromboxanes, prostacyclins, and leukotrienes). Therefore, phospholipid hydrolysis is related to important PLA_2s pharmacological activities, such as the effect on platelets, anticoagulant and hypotensive activity, edema induction, neurotoxicity, cardiotoxicity, and myotoxicity.[26–29]

LAAOs are flavoproteins, which are proteins that contain riboflavin nucleotide derivatives as a prosthetic group. Generally, they are homodimeric glycoproteins with a molecular mass of about 110–150 kDa, under native conditions. These enzymes catalyze the stereospecific oxidative deamination of L-amino acids, producing an α-keto acid, ammonia, and hydrogen peroxide (H_2O_2). The mechanism of action of the LAAOs is still unclear; however, several biological effects attributed to the enzymes are related to the H_2O_2 produced during enzymatic catalysis and the resulting oxidative stress, such as bactericidal, cytotoxic, antiparasitic, antitumor and antiplatelet actions.[30–33]

Two groups of lectins are found in snake venoms: C-type lectins and C-type lectin-like or snaclecs (snake C-type lectins). By definition, lectins are non-enzymatic proteins that bind to sugars due to the presence of a characteristic recognition region (carbohydrate recognition domain). C-type lectins are homodimeric proteins, with a molecular mass of approximately 15 kDa that recognize and bind to carbohydrates in a Ca^{2+}-dependent manner and are able to induce hemagglutination via the recognition of glycoconjugates of erythrocyte surface. On the other hand, snaclecs, most often found in snake venoms, are not able to specifically interact with sugars. Snacles have homologous sequences to C-type lectin, but are heterodimeric and their basic

Table 1 – Bothrops moojeni venom proteins acting on hemostasis.

Proteins*	Characteristics	Action on hemostasis		
		Platelets	Coagulation	Fibrinolysis
Metalloproteases				
BmooFIBMP-I [37]	P-I; 22.8 kDa; non-hemorrhagic; α-fibrinogenase	--	Procoagulant in vitro	--
BmooMP-I [41]	P-I; 25 kDa; hemorrhagic; α-fibrinogenase	--	Coagulation alteration	--
BmooMPα-I [38]	P-I; 24.5 kDa; non-hemorrhagic; α-fibrinogenase	--	Anticoagulant in vivo	Fibrinolytic
BmooMPα-II [39]	PI; 22.5 kDa; non-hemorrhagic; α-fibrinogenase	Inhibition of aggregation	Fibrinogenolytic Not pro/anticoagulant	--
BthMP [40]	P-I; 23.5 kDa; hemorrhagic; α-fibrinogenase	--	Anticoagulant in vivo	Fibrinolytic
BmooPAi [42]	PIII, 32 kDa, non-proteolytic, non-hemorrhagic	Activation of aggregation	--	--
Moojenactivase [43,44]	PIII; 85.7 kDa, non-hemorrhagic; α-fibrinogenase	Activation of aggregation	Procoagulant in vitro Activation of factors II and X;	Non-fibrinolytic
Moojenin [45]	PIII; 45 kDa; non-hemorrhagic; α-fibrinogenase	--	Procoagulant in vitro Anticoagulant in vivo	--
Serine proteases				
Batroxobin (Defibrase®) [24,61]	25.5 kDa; thrombin-like	--	Fibrinogenolytic Procoagulant in vitro Anticoagulant in vivo	Fibrinolytic
BmooSP [55]	~30 kDa; thrombin-like	Activation of aggregation	Fibrinogenolytic Procoagulant in vitro Anticoagulant in vivo	Fibrinolytic
Moojase [56]	30.3 kDa; thrombin-like	Activation of aggregation	Fibrinogenolytic Procoagulant in vitro Anticoagulant in vivo	Fibrinolytic
MSP 1 [50,51]	2 chains: 34 and 32.5 kDA	Activation of aggregation	αβγ-Fibrinogenolytic	Fibrinolytic
MSP 2 [50,51]	38 kDa	Activation of aggregation	αβγ-Fibrinogenolytic	Fibrinolytic
BM-IIB32 [85]	~32 kDa; thrombin-like	--	αβ-Fibrinogenolytic Procoagulant in vitro	--
BM-IIB35 [85]	~35 kDa; thrombin-like	--	α-Fibrinogenolytic Procoagulant in vitro	--
PLA_2s				
BmooPLA$_2$ [68]	13.6 kDa; Asp49	Inhibition of aggregation	--	--
BmooTX-I [67]	15 kDa; Asp49	Activation of aggregation	--	--

MjTX-III [69]	13.8 kDa; Lys49	--	Extends blood clotting time	Neutralizes heparin
MjTX-IV [69]	13.8 kDa; Lys49	--	Extends blood clotting time	Neutralizes heparin
Other classes				
Baltetin [82]	Snaclec, 25 kDa	Inhibition of aggregation	--	--
BmLec [81]	Snaclec, 16.3 kDa	Activation of aggregation	--	--
BmooAi [83]	New toxin, 15.2 kDa	Inhibition of aggregation	--	--
BmooLAAO-I [32]	LAAO, 64.9 kDa	Activation of aggregation	--	--

** Proteins isolated from Bothrops moojeni snake venom that have been characterized for their action on hemostasis. The bibliographic search was carried out through the CAPES Journal Portal, using the terms Bothrops and hemostasis and searching for scientific articles made available in the last 20 years. PLA_2s: Phospholipases A_2*

structure includes two homologous subunits: the α subunit (14 and 15 kDa) and the β subunit (13 and 14 kDa). They interact with a multitude of molecules related to hemostasis present in endothelial cells, clotting factors, and platelet receptors, playing a role in the formation of thrombi and inflammation, including anticoagulant effects and activation/inhibition of platelet function.[34–36]

SVMPs

Several SVMPs isolated from the *B. moojeni* venom have already been characterized for their action on hemostasis, such as: BmooFIBMP-I,[37] BmooMPα-I,[38] BmooMPα- II,[39] BthMP,[40] and BmooMP-I[41] belonging to the P-I class; and BmooPAi,[42] Moojenactivase (MooA),[43,44] and Moojenin[45] from P-III class (Table 1).

The proteases BmooMP-I, BmooMPα-II, BmooPAi, and MooA are capable of affecting platelet function and altering the balance of the hemostatic system. SVMP P-I BmooMPα-II, for example, causes the inhibition of platelet aggregation, probably due to the hydrolysis of the ⍟IIb⍟3 integrin since, because of the absence of additional domains, the interaction with membrane receptors is unlikely.[39] Tests performed with the MooA protein, on the other hand, showed that pre-incubation with prothrombin promoted an 8.3-fold increase in the ability of this protein to induce platelet aggregation, suggesting that this SVMP acts on platelets directly and indirectly through thrombin, due to the activation of prothrombin.[44] BmooPAi is a platelet aggregation inhibiting factor derived from SVMP class P-III proteolysis; it has only disintegrin-like and cysteine-rich domains and has no proteolytic activity. This toxin is a platelet aggregation inhibitor that interferes with the interaction of the vWF with platelets and could potentially be used as a tool for the development of novel antithrombotic agents.[42]

All of the analyzed metalloproteases are fibrinogenolytic enzymes, which preferentially hydrolyze the Aα chain of fibrinogen, being called α-fibrinogenases. Although these enzymes primarily cleave the Aα chain of fibrinogen, they can also hydrolyze the Bβ chain and rarely affect the γ chain of fibrinogen. The α-fibrinogenases produce truncated fibrinogen, which is unable to form a stable fibrin clot and thus, under biological conditions, inhibit blood clotting.[20,21] Many SVMPs also have fibrinolytic action, being able to cause dissolution of the fibrin clot, which also makes the blood incoagulable. However, unlike fibrinogen, fibrin has a cross-linked structure and is much less susceptible to proteolysis. The SVMP BmooMPα-I, for example, hydrolyzes only the β chain of fibrin, without affecting the structure of the α chain and of the γ dimer.[38] Due to their fibrinogenolytic properties, these SVMPs may have clinical applications in the treatment of occlusive thrombus.[23,46]

BmooFIBMP-I is an α-fibrinogenase that has coagulant action *in vitro*, but it does not prevent coagulation *in vivo*, as it is inhibited by endogenous factors such as α_2 macroglobulin; thus, it is not capable of causing the depletion of endogenous fibrinogen stores. On the other hand, BmooMPα-I, in addition to acting on fibrinogen, has fibrinolytic activity and an

anticoagulant effect *in vivo*. Therefore, this protease should have greater potential as a therapeutic agent in the treatment and prevention of arterial thrombosis compared to BmooFIBMP-I.[37,38] BthMP and Moojenin also showed fibrinogenolytic activity *in vitro* and, under biological conditions, caused coagulation disorders, which indicates that these proteases are capable of causing the depletion of plasma fibrinogen stores *in vivo*, favoring fluidity and reducing viscosity and coagulability of blood.[40,45]

MooA is also an α-fibrinogenase; however, unlike those already mentioned, it cleaves the γ chain after a longer incubation period. In addition, it is capable of inducing platelet aggregation and plasma coagulation *in vitro*, but has no fibrinolytic action. MooA also induces TF expression on the membrane surface of peripheral blood mononuclear cells (PBMC), which leads these cells to adopt procoagulant characteristics. This SVMP is also capable of causing important systemic disorders such as intravascular hemolysis and disseminated intravascular coagulation, and elevated plasma levels of creatine, aspartate transaminase, and urea/creatinine; it can also cause morphological changes in the erythrocytes, heart, kidney, and lungs associated with thrombosis and hemorrhage.[43,44]

It is interesting to note that some SVMPs have hemorrhagic activity; in other words, they are capable of producing hemorrhage at the envenomation site. It is known that the presence or absence of certain domains does not necessarily denote the hemorrhagic capacity; however, in terms of potency, class P-III SVMPs are the most potent of the three classes in general. BthMP and BmooMP-I, for example, are described as weakly hemorrhagic.[40,41] As they are P-I class enzymes, hemorrhage must be due to their proteolytic action on the basement membrane components.[47]

Accordingly, it is noted that SVMPs are possible therapeutic agents for thrombotic disorders in a very expressive way. Compared to all current antiplatelet therapies to treat cardiovascular events, SVMPs have remarkable biochemical attributes: they are resistant to plasma serine protease inhibitors, have the potential to avoid the risk of bleeding, are inactivated by α_2-macroglobulin, which limits their range of action in the circulation, and others impair platelet aggregation, which represents an important target for therapeutic intervention.[48]

SVSPs

SVSPs are a group of toxins widely studied for their action on the hemostatic system. They affect several physiological functions, including blood clotting, fibrinolysis, blood pressure, and platelet aggregation. These enzymes act in the platelet function, in the components of the coagulation cascade, in the fibrinolytic and kallikrein-quinine systems, and can also cause nonspecific proteolytic degradation, triggering an imbalance in the hemostatic system. SVSPs with an action similar to kallikrein, for example, act by releasing bradykinin or its kininogen precursor and are capable of degrading angiotensin, thus having a hypotensive activity.[2,24,49]

The action of SVSPs on platelet function can occur in different ways. Some cause nonspecific proteolysis, and others activate or deactivate specific factors. Among the mechanisms of action of this protein class is the induction of calcium mobilization in platelets, which favors the action of thrombin, and platelet activation through interactions with the GPIba receptor, a thrombin receptor.[2] The serine proteases MSP 1 and MSP 2 induce platelet aggregation, mainly through the ADP modulation mechanism.[50,51]

Serine proteases classified as thrombin-like are a pominent group for their action on hemostasis, as they have coagulant activity that is similar to plasma thrombin, but are not inhibited by heparin and do not activate coagulation factor XIII. They are capable of cleaving fibrinogen Aα and/or Bβ chains, which triggers the formation of a "loose" clot that is rapidly degraded *in vivo* by fibrinolysis. This action promotes the depletion of endogenous fibrinogen stores and allows the use of SVSPs as antithrombotic and anticoagulant agents.[52] Some SVSPs also activate platelet aggregation through a mechanism similar to thrombin; that is, they can hydrolyze protease-activated receptors (PARs), especially PAR1 and PAR4, which are found in human platelets. Two other thrombin activities that are mimicked by some SVSPs are the activation of factor V and protein C, which trigger an anticoagulant effect.[2,24,53,54]

BmooSP, Batroxobin and Moojase are thrombin-like serine proteases that have fibrinogenolytic activity and, characteristically, have coagulant effects *in vitro* and anticoagulant effects *in vivo*. These three enzymes have high potential for use, or are already commercially used in the treatment and prevention of cardiovascular disease. In experimental studies on BmooSP activities, it was

observed that it decreases blood viscosity and improves blood circulation. In addition, it induces the formation of a stable fibrin clot for more than 10 days, which can be used as an alternative for the production of natural surgical sealants (biodegradable fibrin glue).[55] Moojase, on the other hand, is characterized as a potent acidic coagulating enzyme. Its sequencing indicates that it is an isoform of Batroxobin. Moojase has shown to be able to clot platelet-poor plasma and fibrinogen solutions in a dose-dependent manner, indicating its thrombin-like properties. This SVSP also induces fibrinolysis of fibrin clots formed in vitro and induces platelet aggregation. This enzyme also has significant amidolytic activity on substrates for thrombin, plasma kallikrein, factor XIa and factor XIIa.[56] The SVSPs MSP1 and MSP2 also have fibrinogenolytic activity; however, they have only traces of blood coagulation. It is noteworthy that these enzymes are able to cleave all fibrinogen chains.[50,51]

Batroxobin is a thrombin-like SVSP and, like BmooSP, is associated with reduced plasma fibrinogen levels and increased anticoagulation and fibrinolysis. This protein strongly converts fibrinogen to fibrin by specific cleavage of the Arg16-Gly17 bond in the α chain of fibrinogen releasing fibrinopeptide A. Importantly, it can be isolated from *Bothrops atrox* snake venom and from *B. moojeni*, since its cDNA was considered found in both species.[57] Defibrase® (Batroxobin) is already used to treat a number of diseases, including stroke, pulmonary embolism, deep vein thrombosis, myocardial infarction, and perioperative bleeding.[54]

Batroxobin is used as a defibrinogenating agent as it forms fibrin I from fibrinogen and releases tissue plasminogen activator (t-PA) from the endothelium, leading to a dose-dependent decrease in plasma fibrinogen concentration and a reduction in blood viscosity and coagulability. The absolute highlight of Batroxobin, however, was the development of Vivostat®, a medical device that allows the pre- or intraoperative manufacture of an autologous fibrin sealant from the patient's blood. The Vivostat® system depends entirely on fibrinogen and thrombin from the patient's own blood, and Batroxobin is only needed to start the clotting process, causing the activation of the patient's fibrinogen. It is very useful in maintaining hemostasis in various surgeries, such as liver, neurological, cardiac, plastic, head and neck, skin grafts, etc. Batroxobin is also used for diagnostic purposes as an alternative to the thrombin time test (because it is insensitive to heparin) to determine plasma fibrinogen levels, to investigate dysfibrinogenemias, and to test the platelet contractile system (clot retraction - Reptilase® Test).[58,59] There are clinical studies that demonstrate these properties of this protease. Chowta et al. demonstrated the safety and efficacy of Bothropase, an enzyme preparation with Batroxobin, in effectively reducing clotting and bleeding time in healthy human volunteers.[60] In the study by Zhang et al., 15 patients with deep vein thrombosis succeeded in limb salvage after administration of Batroxobin [61] Hu et al. also demonstrated that the prophylactic use of Batroxobin provided an effective and cheap method to reduce blood loss during or after operations, as patients undergoing spinal surgery had the total amount of perioperative blood loss reduced.[62]

PLA_2

PLA_2s have a wide variety of pharmacological activities, including hemolytic and anticoagulant activities, as well as effects on platelet aggregation and hypotension.[26,63,64] Those that affect platelet function can be divided into three groups at least: the ones capable of inducing platelet aggregation, the enzymes that inhibit platelet aggregation, and those with a biphasic response, which may initiate platelet aggregation at low concentrations or after a short incubation period, but may also inhibit aggregation at high concentrations or after prolonged incubation. This interference on platelets occurs through different mechanisms: the activation of platelet aggregation by the cleavage of phospholipids present in the membrane, thus releasing arachidonic acid and its metabolites such as TXA_2; the inhibition of platelet aggregation by the separation of arachidonic acid by-products; or via mechanisms that are partially independent of catalysis, for example, inducing relevant morphological changes in the platelet cytoskeleton.[2,65,66]

BmooTX-I and $BmooPLA_2$ are phospholipases classified as Asp49 PLA_2, due to the presence of the aspartate residue at position 49 of the polypeptide chain; characteristically, they are catalytically active, that is, they have phospholipase activity. These two enzymes are capable of causing the inhibition of platelet aggregation induced by collagen or ADP in a concentration-dependent manner. $BmooPLA_2$ can also decrease blood pressure in a dose- and time-dependent manner, with a pronounced effect in the first 15 minutes.[67,68]

Phospholipases MjTX-III and MjTX-IV, on the other hand, are classified as class II Lys49 PLA_2 and, therefore, have no catalytic action. They can interact with heparin

and neutralize its anticoagulant effects; in tests, they were able to neutralize the anti-FXa activity of heparin to the same extent as protamine C hydrochloride, the current antidote used. These PLA_2 also prolong blood clotting time in normal plasma by binding to a prothrombinase complex component; and tests have suggested that they may compete with FVa for binding to FXa. Thus, they have great therapeutic potential because it is possible to use the PLA_2 binding domain to prevent excessive bleeding during treatment with heparin.[69]

Other classes

After snake envenomation, LAAOs (SV-LAAOs) are responsible for important physiological changes, including the induction or inhibition of platelet aggregation, hemorrhage and anticoagulant activities.[30] LAAOs induce vascular endothelial apoptosis and inhibit angiogenesis; these activities can be inhibited by the addition of catalase and other H_2O_2 eliminators. LAAOs bind directly to the cell surface and the released H_2O_2 is then accumulated in the localized area at a relatively higher concentration, and apoptosis is triggered by membrane oxidative stress.[70] LAAOs also show significant effects on platelet aggregation, as some inhibit platelet aggregation, while others induce this aggregation. H_2O_2 is also considered an important factor responsible for this function because its platelet activities can be equally reduced or abolished by catalase.[71] The inhibitory activity is due to interference of H_2O_2 in the interaction between the integrin GPIIb/IIIa expressed in activated platelets and fibrinogen or, in the reduction of the binding of ADP to platelets.[72] On the other hand, the H_2O_2 production can promote the greater synthesis of TXA_2 to induce platelet aggregation and is also related to the hemolytic action of some LAAOs.[33] BmooLAAO-I is capable of inducing platelet aggregation in a dose-dependent manner. Therefore, its biological activities are of therapeutic interest in diseases that cause hemorrhage and it can also be a therapeutic agent for diseases in which the induction of H_2O_2 production can be beneficial, such as chronic inflammatory diseases (asthma, atherosclerosis).[32]

Snaclecs also have several activities in hemostasis, including anticoagulant effects and the activation/inhibition of platelet aggregation.[73,74] GPIb and GPVI receptors, $\alpha 2\beta 1$ integrin, as well as the C-type lectin-like receptor (CLEC-2) are the main receptors present on the platelet membrane to which the snaclecs bind.[75] Enzymes with anticoagulant properties, in the presence of calcium, recognize the Gla domain of factor X and IX, inhibiting their actions. On the other hand, procoagulant snaclecs activate factor X and prothrombin, also via Gla recognition. These venom proteins are even capable of binding directly to GPVI receptors, GPIb and vWF, inducing platelet aggregation; most of them act by inducing vWF to bind to GPIb in platelets, causing agglutination. Snaclecs capable of inhibiting platelet aggregation act mainly through $\alpha 2\beta 1$ and GPIb receptors; most of them interact directly with GPIb and inhibit the binding of vWF to GPIb.[76–79] It should be noted that CLEC-2 is strongly expressed in platelets/megakaryocytes; this receptor triggers strong platelet activation through tyrosine kinase-dependent pathways and, thus, snaclecs can bind to this receptor, inhibiting or activating it.[80] Baltetin and BmLec are snaclecs capable of inhibiting and inducing platelet aggregation, respectively.[81,82]

BmooAi is a protein that inhibits platelet aggregation, but its class has not yet been determined. It showed a very specific inhibitory effect on platelet aggregation induced by collagen, ADP, or epinephrine in human platelet-rich plasma in a dose-dependent manner, whereas it had little or no effect on platelet aggregation induced by ristocetin. Therefore, it seems that this toxin acts on the αIIbβ3 integrin, and the lack of inhibition in the activity induced by ristocetin suggests that this enzyme does not interfere with vWF. Thus, BmooAi could be a new tool of medical interest for the development of new therapeutic agents for the prevention and treatment of thrombotic.[83]

Final considerations

Hemostasis is a complex process that involves interactions between different components such as platelets, blood proteins, and endothelial cells. The action of snake venom proteins on this process is very relevant in the medical field. Despite the number of proteins from *Bothrops moojeni*, already known to interfere in the various events of hemostasis, little is known about their mechanisms of action and there are still several less-known protein classes in which few proteins have been characterized. In this context, a more detailed investigation of the pharmacologically active compounds present in this venom can contribute as a valuable research tool for future clinical use in the diagnosis and treatment of various hemostatic disorders, but mainly thrombotic disorders.

Author Contributions

Conception and design of the research, acquisition of data and analysis and interpretation of the data: Silva NB, Mamede CCN; writing of the manuscript: Silva NB, Dias EHV, Costa JO, Mamede CCN; critical revision of the manuscript for intellectual content: Dias EHV, Costa JO, Mamede CCN.

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