Special Article

Comparative Studies of Structural and Functional Properties of Snake Venom Metalloproteinases

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Snake venom metalloproteinases (SVMPs) induces local and systemic effects on patients suffering from snakebite, degrading extracellular matrix (ECM) proteins such as collagen, gelatin, elastin, laminin, fibronectin, nidogen (entactin), and thrombospondin that cause local hemorrhage and tissue damage. They cleave or activate coagulation factors such as fibrinogen, fibrin, prothrombin, factor V, factor IX, factor X and protein C that bring about systemic coagulopathy. SVMPs and their truncated forms cleave or interfere with platelet adhesive proteins such as vWF, fibrinogen and collagen, and cleave or interfere with platelet receptors such as GPVI, alpha2beta1, GPIb, GPIX, and GPIIbIIIa that result in platelet aggregation defect. SVMPs induce cancer cell line to form morphological changes and apoptosis in vitro concordant with skin necrosis after snakebite in some cases. These local effects caused by SVMPs have no certain treatments, even with commercial anti-venom. SVMPs researches are focusing on their inhibitors, measurement and replacement of blood coagulation factor defects, or anti-cancer drug.

Keywords: *Snake venom metalloproteinase (SVMP), Extracellular matrix (ECM), Coagulation factor, Platelet aggregation, Anti-cancer drug*

J Med Assoc Thai 2016; 99 (Suppl. 1): S76-S88
Full text. e-Journal: *http://www.jmatonline.com*

Snake venom metalloproteinases (SVMPs)

Snake venoms contain many toxic enzymes. Snake venom metalloproteinases (SVMPs), known as extracellular matrix (ECM) degradation enzyme, constitutes a large proportion of snake venom and cause local tissue damage followed by skin necrosis in some envenomed cases. In addition, they interfere with blood coagulation system and hemostatic plug formation. SVMPs are classified into 3 groups by their domain structures. A group P-I SVMP, Ia, is composed of a single metalloproteinase domain. A group P-II SVMP; IIa, IIb, IIc, IId and IIe, consists of metalloproteinase domain and disintegrin domain. A group P-III SVMP; IIIa, IIIb, IIIc and IIId, consists of metalloproteinase domain and disintegrin-like cysteine-rich domain. VLFXA, RVV X and VAFXA are P-III SVMPs that contain two additional disulfide-linked C-type lectin-like domains. The active form and post-translational modification of different types of SVMPs was well described[1].

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The P-I SVMPs activity

The biological function of P-I SVMPs, as shown in Table 1, have degradation of ECM proteins such as collagen, gelatin, elastin, laminin, fibronectin, nidogen (entactin), and thrombospondin that cause local hemorrhages and tissue damage. The proteolytic activity to ECM proteins of some P-I SVMPs such as fibrolase, atroxase and ACLF, do not show the hemorrhagic activity but they have proteolytic activities against fibrinogen, fibrin, fibronectin, laminin and thrombospondin, implying that in vitro activities may not refer to in vivo effects, or P-I SVMPs may be responsible for systemic effects but not local effects. They also have fibrinogenolytic and fibrinolytic activity especially to alpha-chain. Some P-I SVMPs degrade beta-chain, but almost all cannot degrade gamma-chain of human fibrinogen. In generally, the fibrinogenolytic and fibrinolytic activity of P-I SVMPs does not cause pro-coagulant activity. The researcher tried to apply the fibrolase as a commercial thrombolytic drug, but alfimeprase®, recombinant fibrolase from yeast, did not successful in phase III clinical trials[2]. Interestingly, a P-I SVMP rACLF induces Hela cells to form shape changes, detachment and reduction on cell viability, which the mechanism is unclear. It could be possible that rACLF cleave cancer cell adhesion proteins[3]. It is

Table 1. The example of structural and functional properties of P-I SVMPs

No.	Snake species	SVMPs	Class	Mass* (kDa)	ECM proteins degradation	Hemorrhagic activity	Fibrinogen degradation	Other approaches	Reference
1	*Agkistrodon c. contortrix*	Fibrolase	Ia	23	-	No	Alpha	Alfimeprase®, Fibrinogenolysis agent	(13-15)
2	*Agkistrodon acutus*	Acutolysin A	Ia	22	-	Yes	-	-	(16)
3	*Crotalus adamanteus*	Adamalysin II	Ia	24	-	Weak	-	-	(17)
4	*Crotalus r. ruber*	HT 2	Ia	23	-	Yes	Alpha	-	(18,19)
5	*Crotalus atrox*	Atrolysin-C	Ia	24	Yes	Yes	-	-	(20-22)
6	*Protobothrops flavoviridis*	Trimerelysin-2	Ia	22	-	No	-	Cleave microbial collagenase	(23)
7	*Bothrops lanceolatus*	BlaH1	Ia	28	Yes	Yes	Alpha Beta	-	(24)
8	*Lachesis m. muta*	LHF-II	Ia	22	Yes	Yes	Alpha	Not induce death of cancer cell line	(25,26)
9	*Crotalus atrox*	Atroxase	Ia	23	-	No	Alpha Beta	Not activate plasminogen Not inhibit platelet aggregation	(27)
10	*Agkistrodon c. laticinctus*	ACLF	Ia	23	Yes	No	Alpha Beta	Induce innate immune response Induce death of cancer cell line	(28)
11	*Bothrops asper*	BaP1	Ia	22	Yes	Weak	Alpha Beta	Induce innate immune response Specific antibody developing Not inhibit platelet aggregation Not induce death of cancer cell line	(29-33)

* The protein mass of SVMPs was determined from calculation, SDS-PAGE or mass-spectrometry which is not referred to the mass of active form in some cases.

noteworthy that P-I SVMPs cannot inhibit platelet aggregation. This may due to P-I SVMPs lack other additional domains to bind platelet receptors and related proteins.

Platelet aggregation contributes to hemostasis using complex mechanisms. Binding of subendothelial collagen with platelet receptor glycoprotein (GP) VI (non-integrin) stimulates the signaling pathways and up-regulates platelet integrin expression (inside-out signaling), such as alphaIIbbeta3and alpha2beta1. In addition, stimulated platelets secrete the granule contents, particularly ADP which promotes platelet activations. Like GPVI, the alpha2beta1 intergrin also binds collagen fibers activating platelet adhesion and spreading, as well as thrombus formation. The integrin alphaIIbbeta3 plays an exclusive role in linking platelets to one another through the adhesive action of fibrinogen. Engagements of this receptor further activate platelet spreading and enhance platelet aggregation[4].

The P-II SVMPs activity

As shown in Table 2, a group P-II SVMP; IIa, IIb, IIc, IId and IIe, consists of metalloproteinase domain and disintegrin domain, not only has proteolytic activity to ECM proteins, fibronogen and fibrin, but also has hemorrhagic activity. In generally, the fibrinogenolytic and fibrinolytic activity of P-II SVMPs does not cause pro-coagulant activity. They can inhibit platelet aggregation using conserve tri-peptide sequences located in disintegrin domain. The conserve tri-peptide sequences are either the Arg-Gly-Asp (RGD) or Lys-Gly-Asp (KGD), both of them are a potent inhibitor of integrins. The intergrin-constituent proteins found in human are fibrinogen, vWF, collagen, vitronectin, thrombospondin and also fibronectin. Thus, the venom disintegrins may appear to inhibit platelet aggregation using competitive binding between platelet GPIIbIIIa and fibrinogen as well as between vWF and GPIbIX, collagen and GPVI, and vitronectin/ fibronectin and GPIIbIIIa. However, the tri-peptide sequences of bilitoxin-1 contain Met-Gly-Asp (MGD) which it cannot inhibit platelet aggregation. In addition, snake venom disintegrins may have a therapeutic potential for the treatment of tumor metastasis, a process requiring cell-ECM interaction via integrins. Many reports showed that snake venom disintegrins can block RGD-dependent integrins such as the vitronectin receptors (alphaVbeta3 and alphaVbeta5) and fibronectin receptor (alphaVbeta3) involved in cell migration and invasion of tumor cells[5]. This confirms that the conserve tri-peptide sequences are vital for platelet aggregation inhibition. There are some P-II SVMPs have no hemorrhagic activity, jerdonitin and insularinase-A. Interestingly, insularinase-A can activate prothrombin similar to group A prothrombin activator. It also activate factor X which cause pro-coagulant activity.

The P-III SVMPs activity

As shown in Table 3, a group P-III SVMP; IIIa, IIIb, IIIc and IIId, consists of metalloproteinase domain and disintegrin-like cysteine-rich domain. The disintegrin-like cysteine-rich domain of P-III SVMPs contains the hyper-variable-region (HVR). The ECM proteins proteolysis of P-III SVMPs contributes to the hemorrhagic activity. Non-hemorrhagic P-III SVMPs were reported such as berythractivase, ecarin and HV1. The fibrinolysis and fibrinogenolysis of P-III SVMPs mostly degrade alpha-chain of human fibrinogen and fibrin, and some of them degrade beta-chain subsequently. Nevertheless, that activity cannot be a pro-coagulant activity in human blood coagulation system. Ecarin, the RDD P-III SVMP is not only a non-hemorrhagic venom, but also non-platelet aggregation inhibitor. It can activate prothrombin that cause blood clot, and was developed to the Ecarin chromogenic assay. The usefulness of this assay is for quantitative determination of direct thrombin inhibitors such as hirudin, argatroban and melagatran[6].

Interestingly, there are some high molecular weight P-III SVMPs that can activate factor IX, X or protein C such as VAFXA-I, VAFXA-II, RVV X and VLFXA. The active form of VLFXA is heterotrimer (disulfide-linked) which consists of one heavy chain (metalloproteinase and disintegrin-like cysteine-rich domain) and two light chains of lectin-like domain (LC1 and LC2). The additional two lectin-like domains found in P-IIId SVMP may responsible for factor V, factor IX, factor X and protein C binding and/or activating. The usefulness of RVV X, P-IIId which can inhibit platelet aggregation, was developed to be Lupus Anticoagulant test. The indications for this test are detection of antiphospholipid antibody or detection of some inhibitors that cause APTT prolong[7]. The example of prothrombin activator from P-IIIc is HV1. The active form of HV1 is homodimer suggesting that high molecular weight P-III SVMP can activate prothrombin. However, VaH3 P-IIIc SVMP cleaves prothrombin and factor X without activating them. P-IIIa SVMP, berythractivase can activate prothrombin, but jerdohagin can only cleave without activating.

Table 2. The example of structural and functional properties of P-II SVMPs

No.	Snake species	SVMPs	Class	Mass (kDa)	Hemorrhagic activity	Fibrinogen degradation	Tri-peptide	Platelet aggregation inhibition	Other approaches	Reference
1	*Bothrops insularis*	Insularinase-A	IIa	53	No	Alpha Beta	RGD	Yes	Activate prothrombin and factor X Not induce death of cancer cell line	(34-36)
2	*Gloydius ussuriensis*	Ussurin	IIa	53	-	-	RGD	Yes	-	(37)
3	*Crotalus atrox*	Atrolysin-E	IIa	53	Yes	Alpha	MVD	Yes	ECM proteins degradation Autoactivation	(22,38,39)
4	*Protobothrops flavoviridis*	Flavoridin	IIa	54	-	-	RGD	Yes	-	(40)
5	*Protobothrops flavoviridis*	HR2a	IIa	53	Yes	-	RGD	Yes	-	(41)
6	*Protobothrops gramineus*	Trigramin	IIa	53	-	-	RGD	Yes	-	(42)
7	*Protobothrops albolabris*	Albolamin	IIa	36	-	No	RGD	Yes	ECM proteins degradation	(43)
8	*Protobothrops jerdonii*	Jerdonitin	IIb	54	No	Alpha Beta	RGD	Yes	Induce death of cancer cell line	(44)
9	*Protobothrops jerdonii*	TJM-1	IIb	53	-	-	RGD	Yes	-	(45)
10	*Agkistrodon halys*	Agkistin	IIb	60	-	-	RGD	Yes	Induce death of cancer cell line	(46)
11	*Agkistrodon bilineatus*	Bilitoxin-1	IIc	48	Yes	Alpha	MGD	No	Autoactivation ECM proteins degradation	(47,48)
12	*Agkistrodon c. contortrix*	Contortrostatin	IId	53	-	-	RGD	Yes	Induce death of cancer cell line Block HSV entry and cell fusion	(49,50)
13	*Agkistrodon c. contortrix*	Acostatin B	IIe	54	-	-	RGD	-	-	(51)
14	*Macrovipera lebetina*	Lebetase	IIe	53	Yes	Alpha	VGD	Yes	-	(52-54)

* The protein mass of SVMPs was determined from calculation, SDS-PAGE or mass-spectrometry which is not referred to the mass of active form in some cases.

Table 3. The example of structural and functional properties of P-III SVMPs

No.	Snake species	SVMPs	Class	Mass (kDa)	ECM proteins degradation	Hemor-rhagic activity	Fibrinogen degradation	Tri-peptide	Platelet Aggregation inhibition	Other approaches	Reference
1	*Bothrops jararaca*	HF3	IIIa	52	-	Yes	-	ECD	Yes	Induce innate immune response	(55)
2	*Vipera a. ammodites*	VaH1, VaH2	IIIa	70	-	Yes	Alpha	-	-	-	(56)
3	*Gloydius halys*	Halysase	IIIa	66	Yes	-	Alpha	ECD	Yes	Induce death of cancer cell line	(57,58)
4	*Crotalus atrox*	Atrolysin-A	IIIa	46	Yes	Yes	-	ECD	Yes	Induce death of cancer cell line	(22,59-61)
5	*Deinagkistrodon acutus*	Acurhagin	IIIa	51	Yes	Yes	Alpha Beta	ECD	Yes	Cleave vWF Induce death of cancer cell line	(8,62-64)
6	*Naja kaouthia*	Kaouthiagin	IIIa	51	-	-	No	DCD	Yes	Cleave vWF	(65,66)
7	*Bothrops erythromelas*	Beryth-ractivase	IIIa	78	-	No	Alpha	DCD	Yes	Activate prothrombin Induce innate immune response Not induce death of cancer cell line	(67)
8	*Bothrops jararacussu*	BjussuMP I	IIIa	60	-	Yes	Alpha	RGD	Yes	Bactericidal activity	(68)
9	*Echis carinatus*	Ecarin	IIIa	69	-	No	-	RDD	No	Activate prothrombin Ecarin chromogenic assay	(6,69,70)
10	*Protobothrops jerdonii*	Jerdohagin	IIIa	96	-	Yes	Alpha	ECD	-	Cleve prothrombin	(71)
11	*Protobothrops albolabris*	Alborhagin	IIIb	60	-	-	Alpha	-	No	-	(72,73)
12	*Protobothrops albolabris*	Albocollagenase	IIIb	62	Yes	-	No	DCD	Yes	-	(74)
13	*Bothrops jararaca*	Bothropasin	IIIb	68	-	Yes	-	ECD	Yes	Autoactivation	(75)
14	*Crotalus atrox*	Catrocollastatin	IIIb	55	Yes	Yes	-	ECD	Yes	Induce death of cancer cell line	(76-79)

* The protein mass of SVMPs was determined from calculation, SDS-PAGE or mass-spectrometry which is not referred to the mass of active form in some cases.

Table 3. Cont.

No.	Snake species	SVMPs	Class	Mass (kDa)	ECM proteins degradation	Hemorrhagic activity	Fibrinogen degradation	Tripeptide	Platelet Aggregation inhibition	Other approaches	Reference
15	*Bothrops jararaca*	Jararhagin	IIIb	52	Yes	Yes	Alpha	ECD	Yes	Cleave vWF Induce innate immune response Induce death of cancer cell line Jaracetin induce platelet aggregation No effect on platelet agglutination	(60,76, 80-84)
16	*Protobothrops flavoviridis*	HR1A	IIIb	60	-	Yes	-	ECD	Yes	Autoactivation Induce death of cancer cell line	(85)
17	*Trimeresurus gramineus*	Graminelysin	IIIb	48	-	-	Alpha Beta	ECD	Yes	Induce death of cancer cell line Not bind vWF	
18	*Vipera a. ammodites*	VaH3	IIIc	53	Yes	Yes	Alpha	ECD	Yes	Cleave prothrombin and factor X	(86)
19	*Crotalus atrox*	VAP1	IIIc	67	No	Yes	Alpha	ECD	-	Induce death of cancer cell line	(87)
20	*Protobothrops flavoviridis*	HV1	IIIc	68	-	No	Alpha	ECD	Yes	Activate prothrombin Induce death of cancer cell line	(88)
21	*Vipera a. ammodites*	VaH4	III	65	Yes	Yes	Alpha	ECD	Yes	Induce death of cancer cell line	(89)
22	*Protobothrops pupureomaculatus*	Hemorrhagin	III	72	Yes	Yes	-	-	-	-	(90)
23	*Deinagkistrodon acutus*	AAV1	III	50	No		Alpha Beta	-	Yes	Not cleave prothrombin	(91)
24	*Ophiophagus hannah*	Ohagin	III	50	-	-	Alpha	ECD	Yes	-	(92)
25	*Vipera a. ammodites*	VAFXA-I, VAFXA-II	IIId	58, 70	Weak	-	Weak	-	Yes	Not activate prothrombin and plasminogen Activate factor X	(93)
26	*Daboia russelii*	RVV X	IIId	98	-	-	-	ECD	Yes	Activate factor IX, X and protein C Lupus anticoaggulant	(7,94,95)
27	*Macrovipera lebetina*	VLFXA	IIId	102	-	-	No	ECD	-	Not activate prothrombin Activate factor IX, X and protein C Cleave factor V	(96,97)

* The protein mass of SVMPs was determined from calculation, SDS-PAGE or mass-spectrometry which is not referred to the mass of active form in some cases

Notably, the P-III SVMPs, which can cleave vWF such as acurhagin, kaouthiagin and jararhagin, was developing to anti-cancer drug, but it is not always the case. Acurhagin, P-IIIa SVMP 51 kDa, can induce cell lines to form morphological changes, caspase 8/9, and finally to apoptosis similar to the reduction of cells' viability, proliferation, adhesion, and migration in vitro. The well-known P-IIIb SVMP, jararhagin 52 kDa, can induce cell lines to increase caspase-3 pathway, to reduce G0/G1 period, to form necrosis, and finally to reduce nodules tumor in vivo. The mechanisms of these are not clear and are believed to come from metalloproteinase, disintegrins, or disintegrin-like cysteine rich domain. They may cleave ECM proteins that are vital for cell adhesion similar to previous results that showed that vascular endothelial damages can induce endothelial cell anoikis, a specialized form of apoptosis[8] or they may directly induce cell lines to apoptosis such asgraminelysin, a SVMP from *Trimeresurus (Protobothrops) gramineus*, that causes endothelial apoptosis prior to cell detachment[9].

It was shown that P-III SVMPs were more active in inducing hemorrhage than enzymes comprising only the metalloproteinase domain. In addition to the protease domain, the strong proteolytic activity of the P-III SVMPs may result from a specific interaction between disintegrin-like cysteine-rich domain and basement membrane components. Several studies suggested that the cysteine-rich domain bind collagen receptor on platelet, alpha2beta1, and cleave von willebrand factor (vWF) contributing to the hemorrhagic activity. The recent crystal structure of catrocollastatin revealed the hyper-variable-region (HVR) located at the C terminal part of the cysteine-rich domain, which may be a substrate recognition site for binding of disintegrin-like cysteine-rich domain with ECM proteins. Jararhagin binds collagen using disintegrin-like cysteine rich domain. This data may imply that the mechanism of P-III SVMPs, to induce the local effects of snakebite patients uses disintegrin-like cysteine rich domain attached to ECM proteins at the wound site. The attachment causes the P-III SVMPs degrade ECM proteins and induce inflammation, apoptosis and necrosis using metalloproteinase and disintegrin-like cysteine rich domain in envenomed patients. Therefore, cysteine-rich domain may function as substrate targeting to enhance metalloproteinase domain activities. Furthermore, HVR may also play a role in triggering pro-inflammatory effects by promoting leukocyte rolling[10].

The disintegrin-like cysteine-rich domain of SVMPs is the main part interacting with platelets. The disintegrin-like cysteine-rich domain of jararhagin, jaracetin, was compared with jararhagin for platelet aggregation inhibition test. Both of them inhibit collagen-induced platelet aggregation with IC_{50} of 140 and 40 nM, respectively. As well as halydin was compared with halysase, both of them inhibited platelet aggregation with IC_{50} of 178 and 87 nM, respectively. Furthermore, the disintegrin-like cysteine-rich domain of P-III HF3, DC-HF3, inhibits collagen-induced platelet aggregation with IC_{50} of 768 nM. Therefore, the disintegrin-like cysteine-rich domain of SVMPs was hypothesized to inhibit collagen-induced platelet aggregation. The studies in P-III SVMPs revealed the specific sequences that seem likely to react with platelets. The disintegrin-like cysteine-rich domain was found to block alpha2beta1 integrin binding to collagen and apparently enhanced the hemorrhagic activity of SVMPs[1]. The sequence SECDPA is involved in the inhibition of alpha2beta1 integrin binding to collagen[11].

P-III SVMPs can inhibit platelet aggregation through several proposed mechanisms. First, they can degrade or interact with different platelet receptors. For example, jararhagin degraded the beta subunit of integrin alpha2beta1. Atrolysin A bound to and blocked alpha2beta1. Acurhagin interacted with GPVI. Second, they can degrade or interact with adhesive proteins involved in hemostasis. For example, AAV1 and halysase degraded fibrinogen. Kaouthiagin and jararhagin destroyed vWF. Jararhagin, atrolysin A and catrocollastatin interacted with vWF domain. Jararhagin, acurhagin and catrocollastatin bound collagen fibers. Albocollagenase, albolamin, and catrocollastatin inhibited only collagen (not ADP)-induced platelet aggregation suggesting that the venom protein specifically prevented collagen and collagen receptor (GPVI and/or alpha2beta1 integrin) interactions. Whether this is mediated by enzymatic degradation or non-enzymatic binding mechanisms remains to be determined. It was reported that jararhagin was bound to collagen and alpha2beta1 integrin by two independent motifs located on disintegrin-like and cysteine-rich domain respectively. The collagen binding with jararahgin only appeared to inhibit collagen-induced platelet aggregation[12].

Conclusion

SVMPs are ECM proteins degradation that contributes to hemorrhage in envenomed patients using metalloproteinase domain. The disintegrin domain of SVMPs consists of conserve RGD sequences

responsible to inhibit platelet aggregation and to inhibit cancer progression. The disintegrin-like cysteine rich domain of SVMPs consists of conserve ECD sequences and HVR responsible to inhibit platelet aggregation, to bind specifically with local substrates at snakebite site and to inhibit cancer progression. The additional two lectin-like domains found in P-IIId SVMP may be responsible for factor V, factor IX, factor X and protein C binding and/or activating.

Ethics consideration

The author has no financial or other relationship with people or organizations that may inappropriately influence the work.

What is already known on this topic?

We have examined the activity of recombinant albocollagenase, P-III SVMP of Thai green pit viper, and found that albocollagenase digests collagen type IV and inhibits collagen-induced platelet aggregation in vitro. These results imply that P-III SVMP of Thai green pit viper can induce local hemorrhage and systemic bleeding in envenomed patients.

What this study adds?

This review paper clearly described the activities of P-I, P-II and P-III SVMPs using table presentation. In addition, this review contains the tri-peptide conserve sequences of many SVMPs related to their activities. Thus, we can use these data to characterize the usefulness of recombinant albocollagenase to be the treatment target or drug discovery.

Acknowledgement

The author would like to thank the staff of the Office of International Relations at Ubon Ratchathani University and also appreciation is expressed for the help in English provided.

Potential conflicts of interest

None.

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การศึกษาเพื่อเปรียบเทียบโครงสร้างที่สัมพันธ์กับหน้าที่ของเอนไซม์กลุ่มเมทัลโลโปรตีเนสของพิษงู

อนุวัตร ภิญญะชาติ

เอนไซม์กลุ่มเมทัลโลโปรตีเนสของพิษงูทำให้เกิดการทำลายเนื้อเยื่อเฉพาะที่รอบบาดแผลและพยาธิสภาพทั่วร่างกายในผู้ป่วยที่ถูกงูกัด เอนไซม์กลุ่มนี้ย่อยโปรตีนเนื้อเยื่อเกี่ยวพัน เช่น คอลลาเจน, เจลาติน, อีลาสติน, ลามินิน, ไฟโบรเนคติน, นิโดเจนและธรอมโบสปอนดิน ซึ่งทำให้เกิดการทำลายเนื้อเยื่อเฉพาะที่เป็นเหตุให้เกิดเลือดออกใต้ผิวหนัง เอนไซม์กลุ่มนี้ย่อยหรือกระตุ้นปัจจัยการแข็งตัวของเลือด เช่น ไฟบริโนเจน, ไฟบริน, โปรธรอมบิน, factor V และ factor X ซึ่งทำให้เกิดพยาธิสภาพทั่วร่างกาย เอนไซม์กลุ่มนี้หรือบางส่วนของโมเลกุลสามารถย่อยหรือขัดขวางการทำงานของโปรตีนตัวเชื่อมกับเกล็ดเลือด เช่น von Willebrand factor (vWF), ไฟบริโนเจนและคอลลาเจน อีกทั้งย่อยหรือขัดขวางไกลโปรตีนตัวรับต่างๆ บนผิวเกล็ดเลือดเช่น GPVI, alpha2beta1, GPIb, GPIX และ GPIIbIIIa ทำให้สามารถยับยั้งการเกาะกลุ่มของเกล็ดเลือด เอนไซม์กลุ่มเมทัลโลโปรตีเนสของพิษงูเหนี่ยวนำให้เซลล์มะเร็งเปลี่ยนลักษณะทางสัณฐานวิทยาเกิด apoptosis และ necrosis ในหลอดทดลองสอดคล้องกับการเกิดแผลเน่าตายหลังถูกงูกัดในผู้ป่วยบางราย การทำลายเนื้อเยื่อเฉพาะที่รอบบาดแผลที่ถูกงูกัดยังไม่มีวิธีรักษาที่ดีถึงแม้จะให้เซรุ่มแก้พิษงูแล้วก็ตาม งานวิจัยที่เกี่ยวข้องกับเอนไซม์กลุ่มเมทัลโลโปรตีเนสในพิษงูกำลังมุ่งเน้นไปที่การหาตัวยับยั้ง การวัดและการทดแทนปัจจัยการแข็งตัวของเลือดที่ผิดปกติ หรือยาต้านมะเร็ง