Chapter V

Discussion and Conclusion

It is well-known that bleeding can be a major symptom of envenomization by crotaline snakes, commonly green pit viper (Trimeresurus spp.) and Malayan pit viper (Calloselasma rhodostoma) in Thailand. (3, 5, 8, 15, 16, 18, 19) Bleeding following snakebites may be local hemorrhage, at the site of the bite, and systemic bleeding in various organs. The pathogenesis of snake bite hemorrhage involves coagulation disturbances, coagulant and fibrinolytic activities, thrombocytopenia and vessel wall damage caused by venom hemorrhagins.

In this study, both T. popeorum and C. rhodostoma venoms possess coagulant activity, thrombin-like action (Table 1, 2), fibrinolytic (Table 4, 5), and hemorrhagic activities (Table 6). They were all dose-dependent. Neither direct platelet aggregating nor aggregation inhibition activities were demonstrated (Fig. 7, 8, 9).

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Thrombin-like activities, thromboserpentin, of T. popeorum venom is much less potent than C. rhodostoma venom about 120 times either on fibrinogen solution or normal pool plasma (Fig. 1, 2). Interestingly, Malayan pit viper venom could produced reprolongation of clotting time on normal pool plasma with the higher concentration than

100 ug/ml (Table 2, Fig. 4). This may be due to in vitro effect of more prominent fibrinogenolytic component of large concentration venom on limited amount of fibrinogen in pool plasma. This effect seem to be disappeared when fibrinogen solution was used as substrate.

Following the DEAE-cellulose chromatography, the coagulant enzymes distributed in fractions II, V and VI of T. popeorum venom (Table 7). Peak V appeared to be the most potent one, about 20 times stronger than crude venom (Fig. 14). Of C. rhodostoma venom, they were recovered in various different fractions: I, II, III, IV, V and VI (Table 8.1, 8.2), wheseas the fraction IV was the most active peak (Fig. 15). It was approximately 3.7 times more active than crude venom. The reasons for coagulant activity appearing in many venom fractions could be either contamination of truely coagulant component in those fractions or properly presence of more than one form of the coagulant enzymes or subunits. The electrophoretic patterns of those were shown in Fig. 20 and 21. Ancrod was the purified coagulant fraction from C. rhodostoma venom (34, 38), of which 2 µg is equivalent of to 1 unit thrombin. (33)

Like thrombin-like activities, the fibrinolytic principles of T. popeorum venom is about 17.5 times less active than of C. rhodostoma venom. They have both potent fibrinogenolytic and fibrinolytic actions. Inspite of

powerful fibrinogenolytic effect, defibrination produced by venoms have mainly contributed to coagulant activities. (39) Their fibrino(geno)lytic components were shown to have much weaker action than clotting activity with the same concentration of venoms.

The fibrinolytic activities were found in fraction I and V of T. popeorum venom. The fraction I was slightly stronger than the crude venom. Of C. rhodostoma venom, this action was shown in fraction I, but it appeared to be less potent than the crude venom. This may be due to unsuitable condition used for isolation of this fraction or instability of the component. Ouyang et al showed that the purified fraction of fibrinogenolytic activity was completely destroyed at 60°C at pH 5.6, 7.4 or 8.8. (44) More appropriate condition should be further studied.

The fibrinolytic activity of T. popeorum venom was quite different from that of C. rhodostoma venom. (6, 7, 44) The former was inhibited by epsilon-aminocaproic acid and Trasylol, but the latter was not. These suggested that the mechanisms for fibrinolysis of Green pit viper venom might be not only due to direct lytic action on fibrinogen or fibrin, but also probably from activation of plasminogen activator system. For Malayan pit viper venom, the strong lytic activity should be due to direct action of venom, but not involved in plasminogen activation.

Platelet aggregating action of T. popeorum venom, which was previously demonstrated by some studies in literature (6, 17), was not found in this study although the optimum contentrations of venom were used, as well inhibition of ADP- or adrenaline-induced platelet aggregation (Fig. 7, 8). In Mitrakul's (1973) and Talalak's works (1977), it seemed that critically low concentrations of venom, about 10-78 ug/ml, caused platelet aggregation. In contrast to in vivo findings, thrombocytopenia was clinically observed in more severe cases that was expected to be poisoned by large amount of venom. (18, 19, 26) Thus, the mechanism of thrombocytopenia in patients with Green pit viper bite remained unclear. They may be a direct damaging action on the circulating platelets, direct aggregating effect, or even injury to megakaryocytic pool, or combination of these.

C. rhodostoma venom did not have either direct human platelet aggregating or platelet aggregation inhibition effects (Fig. 9), as reported by some investigatiors. (7, 28, 45) Except for the study of Ouyang et al, they demonstrated potent activating activity, name aggregoserpentin, on washed rabbit platelet suspension.(46) The different action of venom on platelets of different species should be considered in the term of different platelet structures or specific receptors for venom. This aspect remains for further clearification. Because of the very powerful coagulant activity of venom,

using platelet-rich plasma for aggregation study was limited to interference by fibrin clot.

Unlike thrombin-like and fibrinolytic activities, T. popeorum crude venom possess about 25 time stronger hemorrhagic activity than C. rhodostoma venom (Fig. 10, 11). By chromatography, the hemorrhagic component appeared in many different fractions of T. popeorum venom (Fig. 18), more potent in peak I, and also in fraction I of C. rhodostoma venom (Fig. 19).

Concerning the fibrinolytic and hemorrhagic principles, it may be similar to the works on crotalid venoms by many investigators. The fibrinolytic and hemorrhagic activities were distributed in more than one fractions of purified components of other Trimeresurus spp. (56, 57) Purified 2 fibrino(geno)lytic enzymes with different modes of action, α-fibrinogenase digesting specifically the α(A) chain of fibrinogen and β-fibrinogenase cleaving preferentially the β(B) chain, were demonstrated in Trimeresurus gramineus (56), and in Trimeresurus mucrosquamatus venoms (57, 58). Two hemorrhagic principles were separated from Trimeresurus flavoviridis (Habu snake), HR1 and HR2 (HR2a & HR2b), by Ohsaka and colleagues. (59-61) In contrast to Agkistrodon spp., fibrino(geno)lytic activities from either Agkistrodon (Calloselasma) rhodostoma or Agkistrodon acutus (Hundred-pace snake) venoms were mainly in one of

the fractions, belonging to the class of α-fibrinogenase. (44, 62, 63) In addition to the fibrinolytic, fibrinogenolytic and caseinolytic activities, the purified fibrinolytic component of Agkistrodon acutus venom possessed hemorrhagic activity. (63)

From electrophoretic studies, they seemed that fibrinolytic and hemorrhagic principles of both venoms moved faster, with higher R_f value, than the thrombin-like activity (Fig. 20, 21). Thus, it was implied that the fibrinolytic and hemorrhagic principles had lower molecular weight than the coagulant enzymes. The result was similar to the study of Hatton on C. rhodostoma venom, as shown in Fig. 22. (38)

This study showed that effects of crotalid venoms on hemostatic process : thrombin-like, fibrino(geno)lytic, hemorrhagic activities, and even on platelets, took place in the different seperated fractions. The distributions of those hematotoxic principles of T. popeorum and C. rhodostoma were summarized in Table 11 and 12, respectively. This was only preliminary study in this field, the venom fractions obtained were partial purified. Further steps in seperation and identification of purified components should be proceeded.

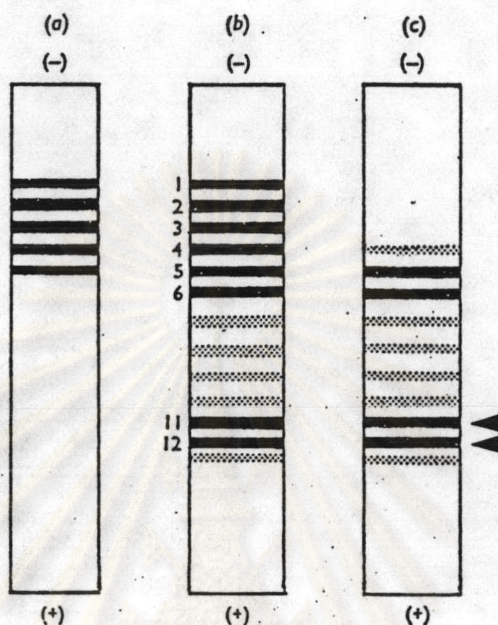

Figure 22. Polyacrylamide-gel electrophoresis at pH 8.6 of the coagulant enzyme (a), venom peak 6 (b), and the hemorrhagic and caseinolytic fractions (c) of C. rhodostoma venom, by M.W.C. Hatton. (38)

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Table 11. Distribution of hematotoxic principles in T. popeorum venom fractions

Activity	Fraction number								
	I	II	III	IV	V	VI	VII	VIII	IX
Thrombin-like activity	-	++	-	-	+++	++	-	-	-
Fibrinolytic activity	+	-	-	-	++	-	-	-	-
Direct platelet aggregating activity	-	-	-	-	-	-	-	-	-
Platelet aggregation inhibition	-	-	-	-	-	-	-	-	-
Hemorrhagic activity	+++	w	+	-	-	w	+	++	-

+ to ++++ represented degree of potency

w = weak

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Table 12. Distribution of hematotoxic principles C. <u>rhodostoma</u> venom fractions.

Activity	Fraction number						
	I	II	III	IV	V	VI	VII
Thrombin-like activity	+	++	++	++++	+++	++	-
Fibrinolytic activity	+++	-	-	-	-	-	-
Direct platelet aggregating activity	-	-	-	-	-	-	-
Platelet aggregation inhibition	-	-	-	-	-	-	-
Hemorrhagic activity	+	-	-	-	-	-	-

+ to ++++ represented degree of potency

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