Chapter II

Review of Literatures

Venomous snakes of Southeast Asia include three families : The Elapidae, Viperidae and Colubridae. The elapidae snakes are divided into three subfamilies : Elapinae, including cobras, kraits and coral snakes, the other two are seasnakes, Hydrophinae and Laticaudinae. At least 13 species of elapinae are shown. (2) Very little informations about the habits and distribution of sea snakes in this region are known. The viperidae consists of two subfamilies : Viperinae and Crotalinae. The viperinae snakes, true or common vipers, are presented by two species inhabiting this region, the Russell's viper (Vipera russeli) and the Fea's viper (Azemiops feae). The crotaline snakes or pit vipers, having the thermosensitive organ "pit" situated between the eye and the nostril, consists of two genera, Trimeresurus and Ancistrodon. Poisoning from Colubrid snake bite was occasionally reported.

Snakebite is an important medical problem in many parts of this region. Neurotoxic envenoming is a prominent feature for the elapid snake, hemostatic dysfunction for envenoming by viperid and crotalid snakes (3-11), rhabdomyolysis is common effect of sea-snake venom. Renal failure is the main causes of V. russeli mortality. (12)

Local necrosis is a major morbidity and an occasional cause of mortality in patients envenomed by Ancistrodon rhodostoma, newly named Calloselasma rhodostoma, and cobras.(3 , 13-15)

There are several venomous snakes in Thailand. Hemostatic failure can be major features of systemic envenomization by viperid and crotalid snakes. (5-7,16-19) One of the two families, crotalid or true pit vipers, is the common poisonous snakes encountered in this country. They consists of two genera as previously mentioned. The genus Trimeresurus comprised of the great majority of the victims. Green pit viper, Trimeresurus albolabris and Trimeresurus popeorum, are more commonly found mainly inhabiting in the central part, especially in Bangkok. The other, C. rhodostoma, is represented by a single species, which is commonly found in cultivated areas such as ricefields and rubber estates, frequently causing hemorrhagic problems in southern and eastern Thailand. The clinical manifestations of local effects as well as systemic envenomation of these crotalids are well established. (3-6 , 15-19)

Crotalid Venoms and Hemostasis

Like other venomous snakes, it is generally agreed that crotalid venoms contain various enzymes in addition to toxic elements. (20-22) They act in the following ways:

(a) effect local capillary damage and tissue necrosis by proteolytic components, proteinase, phospholipases, arginine ester hydrolase and hyaluronidase ; (b) cause diverse coagulant and anticoagulant actions by various proteinases and phospholipase A_2 ; and (c) induce acute hypotension and pain due to release of vasoactive peptides.

The present review will concentrate on common crotalid snakes in Thailand, Green pit viper and Malayan pit viper, as mentioned. Green pit viper misidentification, which have been appeared in the medical and herpetological literatures from Thailand, have been corrected. (23) T. albolabris and T. popeorum will be focused instead of the previous nomenclature.

Green Pit viper venom

The nature of the hemostatic disorders caused by Green pit viper venom, found in Thailand, is not widely investigated. Mitrakul (1973) and Talalak (1977) reported effects of green pit viper venoms on blood coagulation, platelets, and the fibrinolytic system. (5, 6, 17) The clotting activity of the venoms were thrombin-like action, named thromboserpentin activity (24), direct on fibrinogen to form non-crosslinked fibrin without activation of factor XIII. (25) This action was concentration-dependent, and not interfered by heparin. (5,17) A low concentration of 10 mg/ml changed fibrinogen to gel clot, and only a

minute amount of 0.001 ug converted fibrinogen to fibrin monomer.

The fibrinolytic activity, measured by euglobulin lysis time, is definitely present in those venoms, actually by the fibrinolytic effect of the venom itself and/or activating the activator system involved in the conversion of plasminogen to plasmin. The lytic action is also enhanced by increasing concentration. This activity was shown to be inhibited by epsilon-aminocaproic acid and Trasylol. (6) The fibrinolysis obtained with venoms of the same strength, is rather weak compared with clotting activity. It appeared that thrombin-like action is the main factor in the decrease of plasma fibrinogen observed clinically.

In vitro studies, venom of T. erythrurus has stronger clotting effect but weaker fibrinolytic activity than T. popeorum venom. The venom has fibrinogenolytic action and has minimal fibrinolytic activity. (17)

A direct aggregating effect on platelet was also established (6), but certain concentrations of the venom were necessary, probably as low as 2-10 µg/ml. (17) Platelet aggregating and fibrinogen clotting actions of the venom seem to take place in the different systems.

Striking local effects, subcutaneous swelling, blister formation, hemorrhage and necrosis, were also recognized in patients bitten by crotalid snake. (15, 18, 19, 26) Tu et al demonstrated hemorrhagic and proteolytic activity in the Green pit viper (T. popeorum) venom. (27) This hemorrhagic principle or hemorrhagin causes bleeding by a direct action on the blood vessel wall, as distinct from venom agents that affect hemostasis through an action on blood coagulation, but these effects seemed to be aggravated by alteration in hemostatic mechanisms. (28)

The overall results were studied with crude venoms. The isolation of hematotoxic principles and their specific mode of action from Green pit viper, T.albolabris and T. popeorum, venom have been not yet reported in the literatures.

Malayan Pit Viper Venom

For Malayan pit viper, C. rhodostoma, many evidences of hemostatic abnormalities have been widely reported. (3, 4, 8, 9, 11, 14-16, 29)

The defibrination syndrome following envenoming by the Malayan pit viper was described. (3, 4, 9, 16, 28) Its venom is well known for containing two enzymes which have a direct action on fibrinogen, one with the coagulant

action, thrombin-like component, and the other with a powerful fibrinogenolytic effect. (6, 9, 29-31)

The coagulant properties of venom was to convert fibrinogen to fibrin directly in the absence of the other clotting factors. (31) In vivo, crude venom has a powerful coagulant action, thrombin-like, with venom concentration of between 1-10 μg/ml, complete conversion of fibrinogen to fibrin appears to take place since the addition of a strong thrombin solution produced no further clotting. It depletes plasma fibrinogen by converting it to an unstable form of fibrin, non-crosslinked form, which is rapidly removed from the circulation without producing clinical evidence of vascular occlusion. (32) The venom coagulant either does not release the acidic peptide B from the β(B) chain or releases from it peptides with opposite charge. Approximately 10 μg of whole venom has an equivalent coagulant activity of 1 N.I.H. unit of thrombin. (33)

Esnouf and Tunnah, 1967, isolated and identified the thrombin-like fraction (ancrod) from crude venom by TEAE-cellulose chromatography followed by gel filtration on Sephadex G-100. (34) The fraction is protein with a monomer molecular weight of about 30,000 and contains at least 20% carbohydrate. It has been shown to be a more active enzyme than thrombin against synthetic substrate. It has no effect on blood coagulation factors other than fibrinogen. Ancrod has been used as a defibrinogenating

agent in thrombosis. (35, 36) Contamination of proteolytic enzymes present in this fraction was postulated (37), and a method for purification of the coagulant enzyme from ancrod was described. (38)

As Green pit viper venom, thrombin activity of ancrod is unaffected by heparin unless concentration of heparin above 100 units per ml was used. (6, 7, 25, 31) Furthermore, it is unliked the thrombin in many ways : (i) it has not been shown in vitro to affect substrates other than fibrinogen ; (ii) thrombin act on human fibrinogen by removing four small soluble fibrinopeptides A, AP, AY and B, and polymerization of modified fibrinogen molecules then leads to the formation of a fibrin gel, whereas ancrod cleaved only fibrinopeptide A , AP and AY from the α(A) chain of fibrinogen (25, 39); (iii) it does not activated factor XIII. (25, 40) This thrombo-serpentin component has neither effect on platelet aggregation (31), nor direct fibrinolytic activity. (42)

Concerning fibrinolysis, increase in fibrinolytic activity can be demonstrated in Malayan pit viper bite victims. (30,43) The venom has a very strong lytic effect, but differ from green pit viper venom. This action was not inhibited either by plasminogen activator inhibitors, EACA, or Trasylol. (7, 30, 43) Although it has been shown to contain a powerful fibrinogenolytic and fibrinolytic

enzymes, its contribution to the defibrination produced by whole venom in vivo is minimal. (39)

Ouyang et al, 1983, described the purified fibrino(geno)lytic principle from this snake venom, α-fibrinogenase. (44) It had a single peptide chain with a molecular weight of 25,613, contained less than 1% carbohydrate, and a specific fibrinogenolytic activity of 51 mg fibrinogen per min per mg protein. This activity was inhibited by EDTA and cysteine, but not by EACA or Trasylol. This indicates that the disulfide bond must be important for the fibrinogenolytic activity of this enzyme and the action is not due to the activation of plasminogen.

Effect on platelets, thrombocytopenia is also clinically seen in man with systemic envenomization of malayan pit viper bite (4, 8, 9, 15, 16, 29) but its venom has little or no effect on human platelet aggregation in vitro. (7, 9, 29, 45) According to Davey and Luscher's observations, it indicates that the splitting of fibrinopeptide A from platelet fibrinogen can occur without consequent various metamorphosis. (45)

Ouyang et al, 1986, in a preliminary experiment, they found a potent activating activity of crude C. rhodestoma venom on washed rabbit platelet suspension. They purified and characterized this platelet activating component, named aggregoserpentin. (46) It was a

glycoprotein with molecular weight of 28,160 and may exist as a dimer. It is devoided of phospholipase A_2, TAME esterase, fibrino(geno)lytic and thrombin-like activities, which were found in crude venom. Additionally, they postulated that venom activates platelets through the activation of endogenous phospholipase A_2 or C, leading to intracellular calcium mobilization, but is independent of the ADP release reaction or thromboxane A_2 formation.

Nevertheless, bites by this snake not only cause systemic manifestation, but local effects are also the prominent symptoms, local necrosis, blister and occasional extravasation of the red cells. (3, 15, 16) Hemorrhagic activity can be demonstrated in Malayan pit viper venom. (27, 47, 48) It appeared that there is a correlation between this hemorrhagic activity, hemorrhagin, and venom proteolytic activities, providing the ability to destroy blood vessel walls. It is noteworthy that the hemorrhagic condition may not be a consequence of defibrination of blood by the injected venom, because patient with prolonged defibrination caused by C. rhodostoma were found not to have a hemorrhagic condition. (28, 49) It plays a minor role in the lethality, but occasionally morbidity.