Chapter 1

LITERATURE REVIEW

Introduction

Hematotoxic snake bite is a common problem in Thailand. In a report of 1631 venomous snakes bites from 67 provinces of Thailand, Chaisin Viravan et al (1992) found that hematotoxic snakes comprised 80% of all snakes. Of these, Green pit viper was the most widely distributed species which attributed 26.5% of all venomous snakes. In this series, patients had to bring the snakes to the hospital for species identification. In Bangkok, Kaysorn Meemano, Charn Pochanugool and Sakchai Limthongul (1987) found that green pit viper was account for 1,118 cases or up to 94% of all venomous snake bites coming to Chulalongkorn hospital in one year (in 1985). Green pit vipers in this series were identified by patients or doctors as the green snakes with red tails. The two most common species of green pit viper in Bangkok are *Trimeresurus albolabris* and *Trimeresurus macrops* (Suebsan Mahasandana and Piboon Jintakune, 1990).

Although most victims of Green pit viper bites experienced mild toxicity, Suebsan Mahasandana , Yupa Rungruxsirivorn and Veena Chantarangkul (1980) and Chulee Mitrakul (1982) reported a fatal case in each series of patients with Green pit viper bites. In addition, acute renal failure from Green pit viper bites was also reported (Boonyuan Dumavibhat, Sukon Visudhiphan and Prida Malasit, 1987). In order to ensure safety from systemic toxicity, the patients must have blood checked every day for more than 3 days (Ponlapat Rojnuckarin et al, 1996). As a result, they have to be absent from work. Suffering from pain and swelling can be as long as four weeks. In some cases the limbs are necrosed. The antivenin has severe side effects such as anaphylaxis (Suebsan Mahasandana, S. Ratananda and S. Khunprayoon, 1984) that may be fatal. In conclusion, because of the high incidence, the significant morbidity and the possible mortality, Green pit viper bite is an important health problem in Thailand.

Effects of Green Pit Viper Venom on Hemostatic System

1. *In Vitro* Studies

1.1 Coagulant Effect is the strongest effect. The venom makes blood clotted through its "thrombin-like effect". It cuts fibrinopeptide A (FpA) from fibrinogen and causes FpA-less fibrinogen to polymerize like the effect of thrombin (Gaffney, March and Phumara Talalak, 1979). However, the venom can not cut fibrinopeptide B (FpB) and can only weakly stimulate factor XIII that opposes to thrombin effects (Gaffney et al, 1979 and Chulee Mitrakul, 1979). Therefore, clots are friable (Chulee Mitrakul, 1973 and Phumara Talalak, 1977). Species of snakes studied were reported to be *T. erythrurus* and *T. popeorum* (Chulee Mitrakul, 1973, Phumara Talalak, 1977 and Chulee Mitrakul, 1979). *T. erythrurus* might be a misnomer of *T. albolabris* and *T. popeorum* was a misnomer of *T. Macrops* (Hutton et al, 1990). There is currently no published evidence that the venom of *T. albolabris* - the most important green pit viper species in Thailand - cuts FpA from human fibrinogen. There is an evidence that different species of the same genus have different effects on fibrinogen. *Agkistrodon contortrix contortrix* venom cuts FpB (not FpA) but *Agkistrodon rhodostoma* (Malayan pit viper) cuts FpA (not FpB) from fibrinogen (Gaffney et al, 1979). Therefore, *T. albolabris* may have the effects that differ from other *Trimeresurus species* which were already published.

1.2 Fibrinolytic Activity The Green pit viper venom increased fibrinolytic activity *in vitro* but this effect was much weaker than the coagulant effect (Chulee Mitrakul, 1973). A synthetic plasminogen activator inhibitor, EACA, inhibits this effect (Chulee Mitrakul, 1973). This plasminogen activator inhibitor binds specifically to the sites on plasminogen or plasminogen activator molecules in order to inhibit them. This indicates that fibrinolytic enhancement *in vitro* is mediated either by endogenous plasminogen activator system in human plasma or a fibrinolytic agent in the venom which has the binding sites analogous to human plasminogen activator, so it can be

inhibited by EACA. Studies in related genera revealed different results. Russell's viper venom did not have direct fibrinolytic effect (Mitrakul, 1979). Malayan pit viper venom has direct fibrinolytic effect but, in contrast to Green pit viper venom, can not be inhibited by plasminogen activator inhibitor (Chulee Mitrakul, 1979). Arnuparp Lekhakula (1988). revealed that thrombin-like effect and fibrinolytic effect of green pit viper venom were each in the different fractions of the venom .

1.3 Platelet Aggregation : Green pit viper venom has platelet aggregating activity in a certain range of concentrations (Chulee Mitrakul, 1973 and Phumara Talalak, 1977). Peng, Lu and Kirby (1991) and Soszka et al (1991) extracted Alboaggregin A, B and C from *T. albolabris* venom. These proteins bound to platelet glycoprotein Ib and led to platelet aggregation (Peng et al, 1991 and Peng, Lu and Kirby, 1992). These substances may be useful in studying function of platelet glycoprotein Ib in certain diseases.

2. *In Vivo* Studies

2.1 Defibrination syndrome : Hypofibrinogenemia with normal levels of other clotting factors is characteristic of this syndrome (Chulee Mitrakul and Chaweewan Impun, 1973, Suebsan Mahasandana et al, 1980, Chulee Mitrakul, 1982, Sukon Visudhiphan et al, 1981, Cockram, Chan and Chow, 1990, Hutton et al, 1990, Chan et al, 1993). The fibrinolytic system is hyperactivated i.e. shortened euglobulin lysis time (Chulee Mitrakul and Chaweewan Impun, 1983, Sukon Visudhiphan et al, 1981 and Chan et al, 1993), low plasminogen and antiplasmin (Hutton et al, 1990). The mechanism of *in vivo* fibrinolytic activation is still not well defined. In human, fibrinogen - fibrin degradation products (FDPs) had been found to be markedly elevated (Sukon Visudhiphan et al, 1989, Cockram et al, 1990, Hutton et al, 1990 and Chan et al, 1993). However, cross-linked FDPs or D-dimer is modestly elevated (Hutton et al, 1990) due to weak activation of factor XIII. In addition, the effects of intravascular coagulation such

as microangiopathic hemolytic anemia and organ ischemia due to thrombotic vascular occlusion are not found.

Hypofibrinogenemia and FDP elevation take more than a week to return to normal (Suebsan Mahasandana et al, 1980) and the fibrinolytic activity in patient plasma was reported to be persistent as long as 6 days after bites (Sukon Visudhiphan, Boonyuen Dumavibhat and Mukda Trishnananda, 1981).

2.2 Thrombocytopenia : It was proposed that the platelet aggregating activity of the green pit viper venom may consume circulating platelets *in vivo* (Chulee Mitrakul, 1979). Malayan pit viper venom also has the thrombocytopenic effect *in vivo* like that of Green pit viper. Nevertheless, Malayan pit viper venom action was platelet aggregation inhibition *in vitro* (Chulee Mitrakul, 1979). The exact mechanism of thrombocytopenia in each species is presently not known.

Physiology of Fibrinolytic System

Fibrinolysis is a normal mechanism *in vivo* to lyse fibrin clot and maintain patency of blood vessels. In human body it is caused by a broad spectrum proteolytic enzyme, plasmin (Hajjar and Nachman, 1994). In addition to fibrin, plasmin digests fibrinogen, clotting factor V (Lee and Mann, 1989), clotting factor VIII, complement and various extravascular protein. Therefore, at baseline, plasmin must be in the inactive form called plasminogen. Fibrinolysis results from plasminogen activator converting inactive plasminogen into its active form or plasmin. Various kinds of plasminogen activators are found, such as

1. Tissue-Type Plasminogen Activator (t-PA) It is named so because it can be extracted from many types of tissue (Collen, Lijnen and Verstraete, 1995). t-PA is the most important plasminogen activator in plasma (Wun and Capuno, 1985, Wun and Capuno, 1987 and Collen et al, 1995) and contributes most of plasminogen activator activity in normal plasma. The main source of plasma t-PA is endothelial cells (Kooistra et

al, 1994). t-PA in blood is rapidly metabolized in the liver (Emeis, van den Hoogen and Jense, 1985) with a half-life of only 5 minutes. Various physiologic and pathologic changes stimulate the release of endothelial storage of t-PA and thus cause highly fluctuating plasma t-PA levels.

2. Urokinase-Type Plasminogen Activator (u-PA) u-PA is produced by various tissue as well as stimulated endothelium (Grondahl-Hansen et al, 1989 and Niedbala and Picarella, 1992). In physiologic state, u-PA activity in plasma is not important. It is now believed that u-PA functions are mainly extravascular (Vassalli, Sappino and Belin, 1991), for instance, tissue remodelling.

3. Exogenous Plasminogen Activator Many plasminogen activators from many biological sources are used or have potential to be used therapeutically as thrombolytic agents. The examples are Streptokinase (ISIS-3 Collaborative group, 1992), Staphylokinase (Collen and Lijnen, 1994) and Vampire bat plasminogen activator. Green pit viper venom has weak fibrinolytic effect (Chulee Mitrakul, 1973). It remains to be clarified whether there is any plasminogen activator in the venom.

In addition to plasmin, some other snake venom enzymes have direct fibrinogenolysis (Ouyang, Teng and Huang, 1992 and Marsh, 1994). However, this effect has not yet been demonstrated in green pit viper venom.

The crucial mechanism of normal fibrinolysis is that t-PA activates plasminogen into plasmin (Francis and Marder, 1994). The effect of t-PA is enhanced markedly during thrombolysis by two mechanisms.

1. Fibrin Deposition When there is fibrin deposition, t-PA and plasminogen will bind avidly to lysine residues on fibrin molecules. Fibrin binding enhanced plasminogen activation reaction up to 1500 times of baseline (Hoylaerts et al, 1982). This mechanism localizes fibrinolysis to only on thrombus surface and prevents systemic fibrinogenolysis.

2. t-PA release t-PA is released from endothelial cells after many stimuli. Thrombin effect and venous occlusion occuring during clotting process cause t-PA release (Kooistra et al, 1994). This mechanism is predominant in the microcirculation which occupies relatively high endothelial surface area (Francis et al, 1994).

Fibrinolytic inhibitors also play vital roles in controlling this system. α_2 antiplasmin is a specific inhibitor of active plasmin. It rapidly forms complex with plasmin and inactivates plasmin instantly (Wiman and Collen, 1978). In pathologic fibrinolysis, excessive plasmin can overcome antiplasmin and results in fibrinogenolysis. Low antiplasmin activity reflects fibrinolytic activation causing antiplasmin depletion (Collen et al, 1995). Plasminogen activator inhibitor 1 (PAI-1), the main inhibitor of plasma plasminogen activator activity, instantaneously forms complex with t-PA released from endothelium. The sources of PAI-1 include liver, endothelium, megakaryocyte and other tissue (Colucci, Paramo and Collen, 1985). It is an acute phase protein (Brommer et al, 1993) that is elevated by endotoxin and cytokines (Colucci et al, 1985, Bevilacqua et al, 1986 and Luskutoff, 1993). PAI-1 levels in green pit viper bites have not been studied.

Antifibrinolytic drugs such as Tranexamic acid and EACA are lysine analogues. They block plasminogen binding to fibrin and thus inhibit plasminogen activating effect of plasminogen activators (Hoylaerts, Lijnen and Collen, 1981). Direct fibrinolytic action of green pit viper venom is inhibited by EACA (Chulee Mitrakul, 1973) suggesting the roles of plasminogen, plasminogen activators and fibrin interactions.

Fibrinolysis in Various Models of DIC

Fibrinolysis is considered to be a normal reaction to DIC and so-called secondary fibrinolysis. The t-PA elevation is a typical finding in various models of DIC (Francis and Seyfert, 1987 and Fukao et al, 1992). However, plasminogen activator activity is uncommonly elevated (Francis et al, 1987). In a large study of DIC, shortened euglobulin

lysis time (ELT) was found in only 10% of cases (Spero, Lewis and Hasiba, 1980) suggesting that most released t-PA in DIC was complexed with plasminogen activator inhibitor (Asakura et al, 1991 and Fukao et al, 1992). In some models of DIC with shortened ELT and elevated plasminogen activator (PA) activity, fibrinolysis is considered inappropriate to coagulant effect. These conditionsshould indeed be called "DIC with hyperfibrinolysis" rather than "pure DIC" that has undetectable PA activity.

Fibrinolytic responses to DIC are determined by the underlying diseases of patients (Takahashi et al, 1990). In common causes of DIC such as sepsis and malignancy, fibrinolysis is not prominent. Particularly in sepsis, an acute phase protein, PAI-1, is markedly increased in plasma (Fukao et al, 1992). The proportion of t-PA to PAI-1 is less than normal in sepsis (Fukao et al, 1992), suggesting hypofibrinolytic states. DIC in Russell's viper bites seems to be the same. The t-PA levels are not elevated (Than-Than et al, 1988 and Woodhams et al, 1989) in this condition. Fibrinolysis in these models is most likely resulted from fibrin deposition enhancing plasminogen activator activity locally.

DIC with hyperfibrinolysis can be demonstrated in liver cirrhosis (Takahashi et al, 1990) and acute promyelocytic leukemia (Takahashi et al, 1990). Liver is the most important organ that metabolizes t-PA (Emeis et al, 1985). This explains t-PA elevation in liver diseases (Leebeek et al, 1991, Paramo et al, 1991 and Violi et al, 1993). However, results regarding the PAI-1 levels in this condition are contradictory. High (Boks et al, 1986, Tran-Thang et al, 1989 and Leebeek et al, 1991), normal (van Wersch, Russel and Lustermans, 1992), or low (Huber et al, 1991 and Violi et al, 1993) levels have been reported. In acute promyelocytic leukemia, fibrinolysis was mediated by releasing u-PA from leukemic cells (Stump et al, 1990). However, the mechanism of hyperfibrinolysis in green pit viper bites has never been explored. Green pit viper envenomation is probably another example of DIC with hyperfibrinolysis because shortened ELT is consistently demonstrated. Nevertheless, ELT is an inaccurate test (Booth, 1991). It depends on fibrinogen level. In hypofibrinogenemis states, euglobulin clots are very small or even

imperceptable and result in false positive ELT. Moreover, ELT depends on endogenous plasminogen activity in plasma and plasminogen depletion in hyperfibrinolytic states may result in false negative ELT. Therefore, determinations of fibrinolytic parameters are important for elucidating the exact mechanisms of the hyperfibrinolytic conditions.