Chapter 5

DISCUSSION

Our study demonstrated that Green pit viper bite patients had fibrinolytic system activation ie. hypofibrinogenemia, elevated FDPs, shortened ELT, hypoplasminogenemia and hypoantiplasminemia. Higher plasminogen activator activity in case group suggests a hyperfibrinolytic state. Elevated tissue-type plasminogen activator levels in patient group depicted that t-PA was likely a contributing factor for plasminogen activator activity elevation.

Subjects in this study were only in a subset of Green pit viper bites because some patients who were bitten by snakes might not come to doctors. Some patients might perceived that green pit viper causes only mild symptoms and requires no treatment. It is unknown if there is any difference in symptoms and/or severity between a patients who came and did not come to the hospital. The patients who came to doctors probably had more severe symptoms. Moreover, there is a geographical difference between snake species in Bangkok. Suebsan Mahasandana and Paiboon Jintakune (1990), demonstated that *T. albolabris* is more prevalent on Bangkok area but *T. macrops* is more prevalent on Thonburi area. Green pit viper other than these two species are very rare in Thailand. Chulalongkorn University hospital is located in Bangkok area, so *T. albolabris* is the most common species we found. Clinically, *T. albolabris* causes more severe systemic symptoms (Suebsan Mahasandana et al, 1990 and Hutton et al , 1990). In this study, *T. macrops* seemed to cause the same hematological effects but in less intensity than *T. albolabris*. Therefore, this study may not represent hematological changes of average Green pit viper of Thailand quantitatively, but at least qualitative changes probably the same. The patients who brought the biting snakes to the hospitals were less than 10% of total patients. There is no known scientific reason causing dissimilarity between cases who did and did not bring the snakes with them. Therefore, hematological changes in the

group of patients in this study probably represents the directions of changes in Green pit viper bites in general, but differences in severity are unknown.

Various physiologic stimuli could affect fibrinolytic activity and caused some impact on our study. The control group in this study had higher proportion of male subjects than the case group. Koh et al (1991) found that normal female had higher plasminogen activator activity than male and this possibly contributes to the result of this study. However, the differences of fibrinolytic parameters between sexes were not observed in both control and case group (table 6). In subgroup analysis, female patients had significantly higher t-PA levels than female control. t-PA levels and plasminogen activator activity were elevated in both sexes, but not statistically significant. This may due to the small sample size (n) in subgroup analysis. Fibrinolytic activity is decreasing with age (Krishnamurti et al, 1988). patients were averagely older than case group, so the differences in plasminogen activator antigen and activity in this study were not caused by the difference of age. Time of specimen collections also results in variation in fibrinolytic parameters. Morning specimen had lower fibrinolytic activity (Bachman, 1994). In this study, plasminogen activator of the patients were consistently elevated irrespectable to the time of specimen collection (table 12). In conclusion, the differences in plasminogen activator antigen and activity in this study were unlikely to be caused by physiologic variation.

APTT, PT and TT prolongation in Green pit viper bites was mainly due to hypofibrinogenemia. The term defibrination syndrome has been used because fibrinogen (factor I) is the only one factor consumed (Chulee Mitrakul et al, 1973 and Suebsan Mahasandana et al, 1980). This is dissimilar to other models of disseminated intravascular coagulation in which other coagulation factors are also prominently utilized (Spero et al, 1980). TT which measures only fibrinogen levels was the most sensitive test of the three. TT prolongation was the first abnormality detected by coagulogram followed by PT and APTT respectively. Adding PT and/or APTT to TT test did not increase the positive rate. Therefore, TT is one of the most sensitive tests of coagulopathy in Green pit viper

envenomation. TT prolongation can result from hypofibrinogenemia and/or FDP elevation because FDPs interfere with fibrin polymerization. FDPs elevations were found in almost all cases of Green pit viper bites except only one who was bitten by *T. macrops*. In contrast, FDPs were undetectable in all control subjects. Therefore, FDP assay was the most sensitive screening test. In this study, FDPs measured by semiquantitative particle agglutination immunoassay can be done easily and quite rapidly. The tests took less than 5 minutes. However, the test is rather expensive (about 300 baht per test) and can not differentiate between cases with and without coagulopathy. Fibrinogen level assay was more sensitive than TT and ELT assay was more sensitive than fibrinogen levels. Both tests are inexpensive but more time-consuming than TT. They also cannot differentiate cases with and without coagulopathy. Our institute routinely uses VCT that is the least sensitive test. The reasons of its use are it is simple and its result is readily available. The aim is as a therapeutic test or to make a decision in patient treatment and monitor responses to treatment.

Fibrinolytic activation which was reflected by shortened ELT, low plasminogen and low antiplasmin activity was observed in all hypofibrinogenemic cases. Plasminogen and antiplasmin activities were strongly correlated with fibrinogen and FDPs levels. t-PA antigen and activity were 2 times higher than controls and were elevated more in cases with than without fibrinolytic activation (table 10). t-PA antigen levels were significantly correlated with fibrinogen, plasminogen and antiplasmin levels. These imply that t-PA may contribute significantly to hyperfibrinolytic states. However, the correlation coefficients between t-PA antigen and activity with other parameters were less strong than plasminogen and antiplasmin with other parameters. Moreover, t-PA antigen did correlated with its activity. These discrepancies may result from the unknown factor(s) that contribute(s) to fibrinolytic activation. Plasminogen activator inhibitors may be released and inactivate t-PA activity. Direct fibrinolysis from fibrin deposition without t-PA release may play an essential role.

Elevated PA activity confirmed the hyperfibrinolytic states that was probably contributed by tissue-type plasminogen activator release. However, fibrinopeptide A levels were markedly elevated in comparison with t-PA levels which were elevated only about 2 times of normal. In contrast to DIC of liver diseases which is another example of hyperfibrinolysis, t-PA elevation is about 10 times of normal (Francis and Seyfert, 1987). Moreover, after antivenin administration plasminogen activator antigen and activity did not respond in all cases, in contrast to fibrinopeptide A levels, plasminogen, antiplasmin and coagulogram as well as clinical bleeding. This suggests that coagulant effect of the venom is the most essential effect that causes coagulopathy and bleeding and can be inferred that fibrinolysis from fibrin deposition may play an important role in fibrinolysis. Synthetic plasminogen activator inhibitors such as tranexamic acid to inhibit plasminogen activator activity are unlikely to be beneficial in systemic bleeding at least initially. Because antifibrinolysis in conditions with intense hypercoagulation may be harmful. However, after antivenin, coagulant effect was matkedly subside but hyperfibrinolysis may be persisted or even more pronounced. The paradoxical increase in fibrinolytic activity after antivenion was also found in Russell's viper bites (Woodham et al, 1989). The mechanism of this phenomenon is unclear and its clinical significance is unknown. Further study is required. If it really has clinical importance, antifibrinolytic agents after antivenin is to be considered. The effects of local fibrinolysis have not been studied. Oral cavities had high plasminogen activator levels (Stump et al, 1992). This may explain a high rate of gingival bleeding in Green pit viper bite patients (Ponlapat Rojnuckarin, 1996). Plasminogen activator inhibitors in oral bleeding may have some theoretical benefit.

Disseminated intravascular coagulation with hyperfibrinolysis was confirmed in green pit viper bite models in contrary to Russell's viper (Woodhams et al, 1989). ELT was shortened in more than half of our cases. The t-PA was definitely elevated. Plasminogen activator activity also was elevated depicting that plasminogen activator inhibitor did not exert a significant role in this model. The direct fibrinolytic effect of the venom has not been excluded. Plasminogen activator inhibitor assay will be performed in a further study. If it is normal, t-PA antigen must contribute to plasminogen activator

activity elevation. However, if it is markedly elevated, the activity of other types of plasminogen activator such as direct fibrinolytic action of the venom may have an important role. In addition, the mechanism of t-PA release has to be studied in further experiments. Effects of the venom to cultured endothelial cells are worthwhile studying. The component(s) of the venom causing t-PA release has to be clarified in the future and possibly has clinical usefulness.

In this study, changes in *T. macrops* seemed to be less severe than *T. albolabris*. The statistic significance could not be demonstrated due to small subgroups of the patients. Nevertheless, the direction of changes seemed to be the same. The biological differences of these two species have to be clarified.

Thrombocytopenia was also a common finding in Green pit viper bites. Degrees of thrombocytopenia were correlated with coagulopathy. However, profound thrombocytopenia with modest changes in coagulation can be observed clinically (Ponlapat Rojnuckarin et al, 1996).