Chapter 2

RATIONALE AND OBJECTIVES

The three possible mechanisms of fibrinolytic system activation in viper bite patients are the followings.

1. Tissue-type plasminogen activator (t-PA) is released from endothelium by the effect of venom.

or 2. Fibrin deposition (generated by the coagulant effect of Green pit viper venom) promotes plasminogen activator (PA) activity.

or 3. Plasminogen activator (PA) activity is exerted by the Green pit viper venom itself.

The studies in various types of venom yielded contradictory results. In Russell's viper, fibrinolytic system activation was demonstrated and t-PA level was not elevated in bitten patients (Than-Than et al, 1988 and Woodhams et al, 1989). The venom had no direct fibrinolytic effect (Chulee Mitrakul, 1979). The Russell's viper venom has thromboplastin-like effect causing thrombin effect in contrast to Green pit viper venom that causes thrombin-like effect. Therefore, there is fibrin deposition as shown by microangiopathic hemolytic blood picture (Suebsan Mahasandana et al, 1980) and the fibrinolytic mechanism is believed to be secondary to fibrin deposition (mechanism 2). In Malayan pit viper, the more closely related species to Green pit viper, t-PA release from endothelial cells were detected in experimental animals (Krishnamurti et al, 1987). Therefore, the first mechanism is likely. Similar to Green pit viper venom, Malayan pit viper had only thrombin-like effect and no fibrin deposition *in vivo* was found. Therefore,

the mechanism 2 is unlikely. Both Green pit viper venom and Malayan pit viper venom have merely weak direct fibrinolytic effect *in vitro*. Therefore, the mechanism 3 is also improbable. In conclusion, Green pit viper venom fibrinolytic action *in vivo* is more likely to be the same as that of Malayan pit viper (mechanism 1) because of the very similar *in vitro* effects.

Although the main *in vitro* effect of Green pit viper venom is coagulation (Chulee Mitrakul, 1973, Phumara Talalak, 1977, Gaffney et al, 1979, Chulee Mitrakul, 1979), the main *in vivo* effect is fibrinolysis (Visudhiphan et al, 1989, Hutton et al, 1990 and Chan et al, 1993). Because there is not only plasma component of coagulation system but also cellular component, experiments in test tubes cannot explain all phenomena in human. The most important cellular component of fibrinolytic system *in vivo* is endothelial cells which are lack in *in vitro* study. Absence of endothelial cell *in vitro* is the main reason why *in vitro* and *in vivo* effects are different. Therefore, we propose that fibrinolytic system activation *in vivo* is caused by **tissue-type plasminogen activator (t-PA) released from endothelium by the effect of venom.**

In the current study, quantitative measurement of t-PA antigen in plasma of Green pit viper bite patients are performed and compared with that of the controls who are healthy subjects. If t-PA antigen is increased in the patient group, t-PA antigen levels between the patients with and without fibrinolytic activation will be compared. If t-PA antigen is higher in the group with fibrinolytic activation, this will be a convincing evidence that t-PA is the cause of fibrinolysis because it indicates that t-PA elevation is not a nonspecific response to snake bite itself. Fibrinopeptide A (FpA) will be measured to confirm the hypothesis that *T. albolabris* venom cuts fibrinopeptide A from fibrinogen. This hypothesis has not been adequately explored. Follow-up studies of FpA and t-PA after antivenin therapy will be done to assess the relative responses of coagulant effect (FpA level) and fibrinolytic effect (t- PA) to antivenin.

Research Questions

1 Primary question

Do plasma tissue-type plasminogen activator (t-PA) levels elevate in Green pit viper bite patients ?

2 Secondary questions

2.1 Do plasma fibrinopeptide A (FpA) levels elevate in *Trimeresurus albolabris* bite patients ?

2.2 Do plasma t-PA levels respond to antivenin at the same rates as FpA ?

Objectives

1. To study the *in vivo* fibrinolytic effect of green pit viper venom by measuring fibrinolytic parameters especially plasma t-PA (tissue-type plasminogen activator) level in the patients who are bitten and comparing with normal persons.

2. To study the *in vivo* coagulant effect of green pit viper venom by measuring plasma FpA (fibrinopeptide A) level in patients who are bitten and comparing with normal persons.

Hypothesis

H_O (Null Hypothesis) = The mean plasma level of tissue-type plasminogen activator in patients with Green pit viper bite is equal to that of normal individuals. (mean t-PA_1 =mean t-PA_2)

H_A (Alternative Hypothesis) = The mean plasma level of tissue-type plasminogen activator in patients with Green pit viper bite is higher than that of normal individuals. (mean t- PA_1 > mean t-PA_2)

Clinical Implications of the Study

1. In Green pit viper bite patients : After antivenin therapy, the coagulant effect is ameliorated (Chulee Mitrakul, 1982 and Suebsan Mahasandana, 1980) but hypofibrinogenemia and FDPs elevation are persisted for several days. This indicated continuing fibrinolytic action. The antifibrinolytic agents can inhibit the action of t-PA (mechanism 1) and direct effect of the venom (mechanism 3). If the main mechanism is one of these mechanism, the antifibrinolytic agents may be helpful to inhibit fibrinolytic process after coagulant effect is stopped by antivenin. A trial of antifibrinolytic agents in conjunction to antivenin in animal experiment may be justified. On the other hand, If fibrin deposition (mechanism 2) is the main mechanism, antifibrinolytic agents will be contraindicated because fibrinolytic inhibition in this case will be resulted in vascular thrombosis.

2. In disseminated intravascular coagulation (DIC) : DIC in different models of diseases has different balance between coagulant and fibrinolytic action (Takahashi et al, 1990). In some models such as sepsis, coagulation system activation is predominant but in the others such as acute promyelocytic leukemia, fibrinolysis is predominant. This is because the mechanisms of fibrinolytic activation are different among various models of DIC. The model of green pit viper has not been studied before. The knowledge from this

project will result in another step of understanding the mechanism to control fibrinolysis in a pathological state. The next step is to explore in detail about the molecular basis of the mechanism which is found. For example, it is interesting to know how the venom causes endothelial cells to release t-PA. Is it receptor-mediated? What is the signal transduction? There are many questions that required further studies in the future.

3. Many useful substances are derived from viper venom such as the application of Russell's viper venom for lupus anticoagulant assay, Malayan pit viper venom for anticoagulant (ancrod) and albolabrin, an interesting disintegrin protein from green pit viper venom, has been shown to inhibit the binding of cancer cells (B6-F10 mouse melanoma cell) to fibronectin or laminin, which is believed to be the mechanism of cancer cell metastasis (Soska et al, 1991). Most importantly, the understanding of the fibrinolytic properties of Green pit viper venom clarified by the present study may lead further to the identification of a novel thrombolytic agents. Therefore, the particular chemical substance causing fibrinolysis (either by direct or indirect effect) in Green pit viper venom and its molecular mechanism are to be identified in the future.