THE EFFECT OF GREEN PIT VIPER (*TRIMERESURUS ALBOLABRIS*) VENOM ON PLATELET MORPHOLOGY BY ELECTRON MICROSCOPY

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Abstract. The incidence of venomous snake bites increases every year in Thailand, especially due to green pit viper. After the bite, there is bleeding due to thrombin-like property of the venom. The mean platelet volume has been reported to be decreased in those who have been bitten by this snake. In this study we investigate the effect of green pit viper venom (*Trimeresurus albolabris*) on platelet volume (MPV), number and morphology of platelets *in vitro*. The test was carried out by washing platelets in phosphate buffer at pH 7.2 to remove fibrinogen, then the washed platelets were mixed with green pit viper venom. Platelet morphology was examined by scanning electron microscope (SEM).The morphology of platelets was smaller than normal which ranges from 1.1- 1.2 µm. Green pit viper venom can directly effect platelet morphology, decreasing platelet volume.

INTRODUCTION

The green pit viper (*Trimeresurus albolabris* and *Trimeresurus macrops*) is a common venomous snake in Thailand. This venomous snake has increased its importance in society because biting rates have increased dramatically, up 73.58 % (Dumavibhat, 1977). The venom is found to have a thrombin-like effect *in vitro* and can cause a defibrination syndrome *in vivo* (Visudhiphan *et al*, 1981). Clinical features of this venomous snake bite vary from asymptomatic to fatal bleeding. The venom of *Trimeresurus albolabris* can increase fibrinolytic activity by shortening euglobulin time (Kamnerdnond and Jitprommeta, 2004). A recent report (Rojnuckarin *et al,* 1999) studied a group of patients bitten by the green pit viper (*Trimeresurus albolabris* and *Trimeresurus macrops*). The study found fibrinolytic system activation was very common as indicated by low plasminogen, low antiplasmin and elevated fibrin-fibrinogen degradation products levels (FDPs). A significant decrease in total platelet count and mean platelet volume (MPV) was demonstrated in envenomated blood. The changes may have been partly due to the effect of green pit viper venom on platelet morphology (Soogarun *et al,* 2003). In this study we evaluated the *in vitro* effect of mixing green pit viper venom and platelet rich solution, then observed the changes in morphology by SEM.

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MATERIALS AND METHODS

Crude venom preparation

Lyophilized crude venom (*Trimeresurus albolabris*) was obtained from the snake farm of the Thai Red Cross. One milligram of crude venom was dissolved in normal saline solution (NSS) as described previously (Soogarun, *et al*, 2005).

Platelet preparation

Platelet concentrate without fibrinogen was prepared using 10 ml of EDTA blood mixed with 150 ml 0.1 M phosphate buffer at pH 7.2 (40.5 ml of 0.2M dibasic sodium phosphate and 9.5 ml of monobasic sodium phosphate, then added to an equal volume of distilled water) (Anonymous, 2005). The solution was then centrifuged in a refrigerated centrifuge at 3,000*g* for 15 minutes. The supernatant was discarded and another 145 ml was added, gentle agitation was carried out so as to disperse any clumped platelets. The solution was re-centrifuged 50*g* for 10 minutes. The platelets in the supernatant were used for the SEM study.

Platelet morphology study

Study the morphology of the platelets was carried out by fixing a mixture of platelets and venom with 2.5% glutaraldehyde for 4-6 hours, then observed the morphology by scanning electron microscope (SEM), the process was repeated twenty times.

RESULTS

Before the study, the platelet count was within normal limits at 216 ± 101 x 10^9 /l and the MPV was 8.9 ± 1.2 fl. Under electron micrograph, the platelets appeared regular in shape with a smooth surface, ranging from 1.4-2.0 µm (Fig 1). Because red cells were in the supernatant, we evaluated the red blood cells and found them to have a smooth surface, round disc-like shape measuring 5-6 µm in diameter. After addition of green pit viper venom to the platelet solution, by one minute the red blood cells were irregularly shaped with multiple cytoplasmic projections. Most red cells had shrinkage to diameters of 3-4 µm. The platelets also had reduced diameters of 1.1-1.2 µm. Their surfaces were irregular and rough. Most platelets adhered firmly to one another (Fig 2).

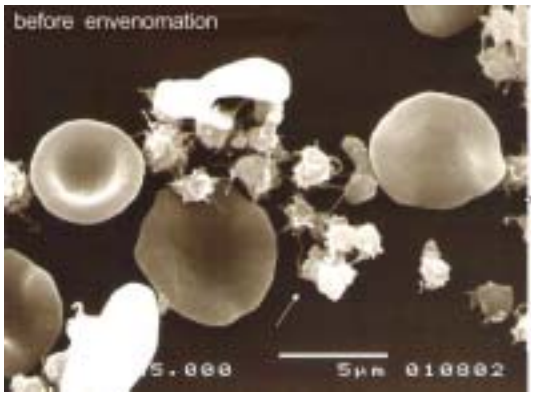

Fig 1–Electron micrograph of platelets and red blood cells before envenomation with green pit viper venom. Platelets appear regular in shape with a smooth surface, ranging in diameters from 1.4-2.0 µm. Red blood cells have a smooth surface, round disc-like shape measuring 5-6 µm.

Fi g 2–Electron micrograph of platelets after envenomation with green pit viper venom. Their surfaces are irregular and rough, diameters range from 1.1-1.2 µm and most of them adhere closely. Red blood cells show spherocytes.

DISCUSSION

This study found the number of platelets were decreased after exposure to green pit viper venom. The fewer platelets *in vivo* may be a result of a reaction to the venom and some may have been consumed by clotting formation. Patients with a large amount of envenomation may have severe bleeding. This study supports a previous report of decreased MPV *in vivo* due to snake venom (Soogarun *et al,* 2003). The SEM showed changes occurred not only to the platelets but the red cells as well, which had spiny protrusions and a spherical shape with diameters of 3-4 µm, compared to 5-6 µm before envenomation. That may result in a decreased MCV, as reported previously (Wiwanitkit and Suwansaksri, 2001). The report did not mention whether thalassemia trait was present. This study found red cell morphology mixed with green pit viper venom had morphologic changes similar to Russell's viper venom (Nopathorn *et al,*1998), however Russel's viper venom caused significant increase in hematocrit.

Altered morphology was observed at 1 minute and reached a maximum at 30 minutes (Nopathorn *et al,* 1998; Soogarun *et al,* 2005). Green pit viper venom may have some properties different from the Russell's viper but both caused sphero-echinocytes. The decreased platelet count at one minute may have been due to cell lysis, but some cells were able to tolerate and persist in a toxic environment. However, further study is warranted.

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