# CHAPTER VIII

# DISCUSSION

The majority of venomous snakebites in Thailand are caused by *Naja kaouthia* and *Calloselasma rhodostoma*. The venoms of these snakes induced systemic and local effects. While neurotoxicity is the most pronounced effect of the envenomation by *Naja kaouthia*, envenomation by *Calloselasma rhodostoma* snake is usually characterized by local and, in severe cases, systemic hemorrhage. In addition to systemic life-threatening effects, the venoms from many species of snake cause striking tissue damage at the site of injection, classically defined as consisting of hemorrhage, myonecrosis, and edema (Ohsaka, 1979). In severe cases of envenoming, these local effects may lead to permanent tissue loss, disability or amputation. In 1988, Pongprasit et al. showed that 51% of cobra bite victims presented with severe tissue necrosis and 27.6 % required full-thickness skin grafts. More recently, Chulalongkorn University Hospital (CHU) found that 60.3% of patients bitten by cobras presented with tissue necrosis (Pochanugool, Limthongkul and Wilde, 1997).

Prospective and retrospective studies of *C. rhodostoma* envenomation presented here revealed some degree of bite-site tissue injury in almost all victims. None of the patients required amputation and tissue necroses usually resolved within 2-3 weeks. Rarely, this can take 2-3 months, in some victims, the limb may remain permanently swollen due to vascular and/or lymphatic damage (Reid and Theakston, 1983). Severe

tissue necrosis was found in 2 of 145 cases (1.4 %) and both required surgical debridement. Similarly, the *N. kaouthia* studies presented most patients bitten suffered from minimal tissue necrosis and no cases had severe tissue necrosis. However, one victim required amputation of the thumb and required minor surgical debridement. The hand structures lack soft tissue cover, making them more vulnerable to snake toxin injury (Huang et al., 1978).

Traditionally, patients bitten by venomous snakes in Thailand applied tourniquets to the bite site in an attempt to delay absorption of venom into the circulation. This is no longer recommemded as it was found to be dangerous, especially in victims bitten by *C. rhodostoma* (Ho et al., 1986). In prospective study four patients who had first been treated by traditional healers, required surgical wound debridement. A previous study of patients bitten by *C. rhodostoma* in Thailand showed that tourniquets applied by patients failed to inhibit the spread of venom into the general circulation (Ho et al., 1986; Tun et al., 1987; Khin et al., 1984). In another report, gangrene was observed following tourniquet application (Warrell et al., 1986). In cases envenomed by *N. kaouthia* 27 of 45 victims (60 %) used tourniquets in prospective study and 7 of 27 patients (25.92 %) required intubation and assisted respiration. Among patients who did not apply tourniquets, 38.89 % (7 of 18) developed respiratory failure. There was no relationship between the use of restriction bandages and the kinetics of the serum venom level in patients after bites by *N. kaouthia* (Hung, Liau and Lin-Shiau, 2003). Neurotoxic symptoms and respiratory failure developed in victims

who required intubation and assisted respiration and one case died due to delay in treatment and irreversible CNS damage.

In the SSS scoring used in clinical grading of CNS, cardiovascular, pulmonary, gastrointestinal and/or hemoatological systems, it appeared that the "score" was "severe" usually only on the first day, and that there was rapid subsequent improvement. In the *N. kaouthia* group, on the first day of hospitalization, 14 of 45 victims (31.11 %) in prospective study and 5 of 40 victims (12.50 %) in the retrospective group, had dysphagia, flaccid paralysis and respiratory failure. They were intubated. Only one patient died from respiratory failure in the prospective study. He was 66 years old and came to the hospital deeply comatous in advanced respiratory failure. He was intubated, given 50 mL of antivenin for the first dose and received the same antivenin dosage 2 hours later. He never regained consciousness over 4 days of hospitalization and was taken home to die at the request of his family. Similarly, *C. rhodostoma* bites, coagulopathies usually appeared within the first 24 hours. Abnormal VCT (Venous Clotting Time) was significantly more common in patients who also had severe bites site tissue necrosis. Recurrences of coagulopathy after an initial response to antivenin were not uncommon and occurred more frequently in subjects with initial severe clotting abnormalities. Previous studies suggested that such recurrence of coagulopathy as related to a fall in circulating antivenin level and/or reversible binding of antivenin to venom protein. There may also be a depot of unneutralized venom at the bite site which is released slowly (Ho et al., 1986; Smith et al., 1979; Meyer et al., 1997). However, none had developed septicemia but two subjects had the disseminated intravascular

coagulation syndrome and died from intracranial hemorrhages. The first case, a 60 year old man, came to hospital comatous, with severe coagulopathy (VCT > 30 min and hematuria). He had been bitten by a *C. rhodostoma* 3 days previously and was first treated by a traditional healer He was moribund and had moderately severe tissue necrosis (score 2) at the bite site. He was intubated, given 30 ml of antivenin and his VCT returned to normal after 6 hours. He was found to have an intracranial hemorrhage and never regained conciousness. The second fatality was a 72 years old man. He was admitted to the hospital one hour after having been bitten by a MPV. On the first day of admission, he had pain at the bite site, was very apprehensive but no abnormal systemic signs/symtoms and no coagulopathy (VCT 10 min). On the morning of his second hospital day, he developed bleeding from gums and had a VCT > 30 min. Unfortunately, there was no antivenin available. In the afternoon, he went into shock, lost conciousness and developed, hematuria, hematemesia, and thrombocythemis. He remained deeply unconscious and required vasopressors. At the afternoon of the second day, when it was decided that his case was hopeless, he was taken home by his family to die.

The rational for snakebite envenomation is the i.v. administration of equine specific antivenom. The treatment is effective for the neutralization of systemic toxicities and has reduced the mortality rate. However, experimental evaluations and clinical observations of the neutralizing of antivenin against the local tissue-damaging actions of venom have shown only partial inhibition (Reid et al., 1963; Laing et al., 1992), especially hemorrhage, even when the antivenom was administered immediately after envenomation (Gutierrez et al., 1981).

In the present study, all patients presenting with *C. rhodostoma* bites who had a venous clotting time of more than 30 min on admission received the corresponding specific antivenin. Even after the patients had received 15 vials (150 ml) of specific antivenin, there were still progression of local necrosis. Cases with severe local necrosis (score 2-3) consisted of 23 among 145 patients or 15.9 %. Six of 23 cases (26 %) had moderate to severe local tissue necrosis (score 2-3) that required surgical debridement. There was no need for amputation in both groups.

Most patients presenting with *N. kaouthia* bites showed minimal tissue necrosis, there was no case of severe tissue necrosis in both groups. In the prospective study 29 of 45 victims (64.5 %) and 77.5 % in the retrospective group required some surgical wound care to prevent or control infection but they were of moderate severity (33.6 % and 20 % in prospective and retrospective groups respectively). Only one case required amputation of the thumb in the retrospective group. The duration of hospitalization was prolonged significantly in patients with tissue necrosis.

The main reason for the failure of potent specific antivenom to prevent local tissue necrosis is the rapidity by which the venom toxins/enzymes act to damage the microvasculature. Furthermore, thrombosis, formed as a result of venom enzymes, may hinder the antibody from reaching the venom at the site of the bite. The local pathogenesis is mainly due to the concomitant actions of myotoxic, $PLA_2$ and metalloproteinase (Ohasaka, 1979; Mebs and Ownby, 1990). Myotoxic phospholipase

$A_2$ and cardiotoxins are present in cobra venoms while hemorrhagic metalloproteinase, phospholipase $A_2$ and proteases are abundant in viper venoms.

Snake venom hemorrhagic metalloproteinases are zinc dependent enzymes possessing a high degree of similarity to matrix metalloproteinases (Bode et al., 1993; Paine et al., 1994). These enzymes play essential roles in developing local tissue damage frequently seen in snakebite victims. Venom hemorrhagic metalloproteinases provoked rapid spreading of venom components from the injected area into systemic circulation, as well as causing local tissue damage. Although the precise mechanisms remain unclear, it is likely that the degradation of extracellular matrix and vascular basement membrane by metalloproteases increase the rate of diffusion of venom components into the tissue and absorbed into vessels. $PLA_2$ activity is not required for membrane damage, several observations indicated that $PLA_2$ activity may have an enhancing role for some of these toxins.

Hemorrhagic metalloproteinase, inconcomitance with hyaluronidase ('spreading factor'), may enchance toxins/enzymes diffusion from the bite site. Thus, inhibition of metalloproteinase should limit the distribution of the toxins and as a result confine, at least to some extent, the toxins to the site of bite. The time at which the venom concentration reaches systemic toxic level should be longer and the survival time of animal should increase. Furthermore, if the re-distribution of toxins is reduced to the extent that toxin level does not reach the systemic toxic level, the animals might survival altogether.

Recently, it was reported that local injection of a synthetic matrix metalloproteinase inhibitor (Bastimastat) effectively neutralized local tissue damage induced by a purified venom metalloproteinase, when the inhibitor was administered rapidly after the toxin injection (Escalante et al., 2000). The chelating agent, by chelating the zinc ion, affected the structure of metalloproteinase, as zinc ion is required for the hemorrhagic action (Bjarnson and Fox, 1994). Moreover, metal chelators have been reported in previous studies to completely block the cleaving action of fibrinectin and fibrinogen. These findings suggest that the neutralization or inhibition of venom hemorrhagic metalloproteinases may be effective in not only reducing local lesions, but also the systemic coagulopathy (Anai et al., 2002).

It is for the reasons discussed above that alternative approaches are needed to complement antivenom administration in the prevention and/or reduction of local tissue necrosis induced by snake venoms. There are a few drugs/chemicals which have been shown to inhibit metalloproteinases and phospholipase $A_2$. Some of these inhibitors have been used clinically in man; their therapeutic benefits may result from the inhibition of these enzymes. It is interesting to see whether these drugs/chemicals also inhibit the metalloproteinases and phospholipase $A_2$ of *C. rhodostoma* and *N. kaouthia* venoms, and if so, whether the inhibition could reduce local tissue necrosis caused by the venoms.

The metalloproteinase and phospholipase $A_2$ inhibitors studies here can be devided into 2 types: the water soluble (Desferrioxamine, Desferiprone, Teterethylene pentamineand EDTA) and the poorly soluble compounds (N-phenylglycine, para-bromophenacyl bromide, mefloquine and quinine). The water soluble compounds have the advantage that they can be easily prepared and tested on the enzyme. However unless being injected into the venom depot, the polar and charged agents may not readily penetrate biological membrane and thus may limit its accessibility to the venom metalloproteinases and phospholipase $A_2$. Those metalloproteinase and phospholipase $A_2$ inhibitors which are poorly soluble in water require the presence of DMSO which may itself interfere in some way with the diffusion of venom and/or enzyme inhibitors in the tissue. The presence of DMSO therefore may add another difficult to predict parameter to the study and might make the interpretation of experimental results difficult. The experiments using these water insoluble compounds were carried out by limiting the final concentration of DMSO in both *in vivo* and *in vitro* studies to only 5% (v/v). The final concentrations of the metalloproteinase and phospholipase $A_2$ inhibitors were, as a consequence, limited by their solubility in this solvent i.e., 5% DMSO.

This study has shown by in vitro experiments that metalloproteinase inhibitors: L1 at 10 mM; DFO at 20 mM; TEPA at 20 mM and N-phenylglycine at 20 mM completely inhibited metalloproteinases of *C. rhodostoma* and *N. kaouthia* venoms. TEPA (20 mM) and N-phenylglycine (20 mM) also completely inhibited phospholipase $A_2$ activity of both venoms. The phospholipase $A_2$ inhibitors quinine (10 mM), p-BPB (0.5 mm) or EDTA (2 mM) completely inhibited the enzymes of *C. rhodostoma* and

*N. kaouthia* venoms. These inhibitors inhibited only about 40% of the proteolytic and metalloproteinase activities of both venoms.

In *in vivo* experiments, the inhibitors were shown to significantly reduced hemorrhage, edema and myonecrosis induced by venom injection. In the preincubation type experiment, metalloproteinase inhibitors N-phenylglycine (37.80 – 151.20 μg/mouse) and TEPA (92.90 – 371.60 μg/mouse) significantly reduced local tissue necrosis (edema and myonecrosis) induced by the two venoms while hemorrhage caused by *C. rhodostoma* venom was also inhibited. The phospholipase $A_2$ inhibitors p-BPB (6.96 μg/mouse) and EDTA (93.05 – 372.20 μg/mouse) significantly decreased local toxicity (edema and myonecrosis) of *C. rhodostoma* or *N. kaouthia* venom. Mefloquine (10.60 μg/mouse) reduced local tissue damage induced by CR venom. In the independent type experiments, N-phenylglycine (37.8 μg/mouse) and EDTA (93.5 μg/mouse) were effective if injected 1 to 3 min after the injection of *C. rhodostoma* venom. EDTA was ineffective in reducing myonecrosis induced by *N. kaouthia* venom if injected more than 3 min after venom injection.

In order to effectively reduce local tissue damage caused by venoms, an 'inhibitor mixture' composing of inhibitors of myotoxic phospholipase $A_2$, hemorrhagic metalloproteinase and 'the spreading factor' hyaluronidase was formulated. N-phenylglycine a matrix metalloproteinase inhibitor effectively neutralized local tissue damage induced by both venoms. EDTA inhibits phospholipase $A_2$ (a $Ca^{2+}$ dependent enzyme) (Shina et al., 1992). Sodium aurothiomalate, a hyaluronidase inhibitor has been

shown to limit the distribution of the toxins and as a result confine, at least to some extent, the toxins to the site of bite (Yingprasertchai et al., 2003). The 'inhibitor mixture' containing N-phenylglycine (37.80 µg/mouse), EDTA (93.05 µg/mouse) and sodium aurothiomalate (195 µg/mouse) significantly reduced local necrosis when injected 1,3 or 10 min after *C. rhodostoma* venom injection. The mixture was effective if injected immediately but less so if it was injected 10 min after the injection of *N. kaouthia* venom. Moreover, the 'inhibitor mixture' was shown to significantly prolong the survival time in mice receiving lethal doses of the two venoms.

The results from the present study strongly indicated that it is possible to drastically reduce the venom-induced local tissue damage by rapid injection of inhibitors of metalloprotease, phospholipase $A_2$ and hyaluronidase. In order to neutralize non-enzymatic tissue damaging toxins eg., cardiotoxins of cobras, $F(ab')_2$ antibody can also be included in the 'Inhibitor mixture'. Such 'cocktail of inhibitors', in a prefilled syringe, can be used by immediate injection into the fang marks as first-aid treatment after snake envenomation. Although local injection of 'cocktail of inhibitor' into a tight anatomical compartment such as the finger can be very painful and the solution itself may cause some local tissue injury, these problems are minor when compared to the severe local damage caused by snake venoms.

**Public health implication :**

The epidemiological data showed the incidence of tissue necrosis at bite sites from *C. rhodostoma* and *N. kaouthia to be* more than 90 %. There were only minimal clinical manifestations, such as pain and mildly inflammed wounds. Local necrosis from viper bites appears to be mostly ischaemic, developing slowly, but also affecting deep tissues including muscle. Local effects with cobra bites are different. Tissue necrosis develops rapidly within a few days (Reid and Theakston, 1983). In addition, the superficial maninfestations in cobra bite wound as measured by SSS do not reflect the tissue extent of tissue damage which occur much deeper into the subcutaneous and muscular layers. Therefore, the Snake Severity Score (SSS) may be more appropriate to determine local effects in viper bites but is not a good parameters for cobra bites.

The degree of snake envenoming can be unpredictable in the early stages. Fear can be an important factor in rural areas. Systemic signs of viper and cobra envenoming are sometimes evident within 20 minutes, but may not appear until several hours after the bite. All patients who may have been bitten by a venomous snake should be taken to a health care facility as soon as possible. They should be observed closely, especially cobra bites, although they may have no immediate signs and symptoms of neurotoxic envenomations. The capture of the responsible snake should not be encouraged because of the risk of further envenomations. Knowledge of the local snake ecology and clinical features are usually enough to differentiate viper from cobra bites and plan appropriate treatment.

In systemic snakebite poisoning, specific antivenom is the most effective therapeutic agent available. It should not be given routinely in all cases of snake bite

because it is expensive and can cause reactions. Its misuse (e.g. if given by the wrong route or in an inadequate dose) must also be discouraged. Moreover, antivenom therapy doe not prevent or lessen the local effects of viper and cobra bites such as skin and tissue necrosis. The 'Inhibitor mixture' is effective in decreasing local effects in an experimental model if given within 10 minutes or less after vemom injection. Whether the manufacture and use of such a substance is practical in the field for human use, would have to be evaluated by further studies and also be the subject of a cost-benefit analysis.

The initial care of a patient bitten by a snake should consist of resuscitation (mechanical ventilator), rapid clinical assessment and simple tests such as the 20 min whole blood clotting time (indicating coagulable blood from consumption coagulopathy), a complete blood count , prothrombin time (PT) and partial throboplastin time (PTT). All blood studies should be repeated at four to six hour intervals until abnormal parameters stabilize.

Snakebite is mainly a rural and occupational hazard, most bites occur during the day when more people are exposed to risk. Many bites could be prevented by wearing boots while working in the field or in the jungle.

Community hospitals can manage victims bitten by venomous snakes. They have the facilities (initial laboratory tests and ventilators) and anitvenom. This should be enough to take care of virtually all victims. They should not be transfered to provincial hospitals in almost all cases since this entails delay and added costs. Standards guidelines, facilities, laboratory test and knowledge of health care personnel at the community hospitals need to be strengthened to deliver good care to snakebite victims as

soon as possible to minimize systemic symptoms and local tissue necrosis. The role of Snake Severity Scroe (SSS) in contributing to good care must be highlighted in such endeavour.