CHAPTER VII

CONCLUSION

The epidemilogical data by prospective and retrospective studies evaluated factors impacting on the severity of bite-site necrosis and systemic symptoms and signs from envenomation in patients bitten by *C. rhodostoma* and *N. kaouthia*.

1. Epidemiology study

1.1. *C. rhodostoma* bites

- Most patients of snakebite came from the southern region , especially Trang and Nakorn Si Thammarat. Surprisingly, they were also found in Lampang, a northern Thailand province. The peak snakebite season was in May and early in the monsoon.
- Snakebite occurred throughout the day (8.00 a.m. to 4 p.m.) ; representing the time that victims work in the field or rubber plantation. Most snakes were of larger size and were brought for identification.
- More male patients with age range of 21 – 60 years experienced snake bites. Most bites occurred in rural areas, outdoors and at dusk. Bites of lower limbs, especially leg, represented 40-60 %.

- The time between bite and arrival at a hospital was 0.01- 60 min. The number of patients who did not applied tourniquet were higher. They required only wound care to prevent or control infection and admitted to the hospital for 1-5 days. The antivenom administered was 1-5 vials.
- The incidence of tissue necrosis at bite site from *C. rhodostoma* bites was higher (95 %) and was with minimal clinical manifestations, such as local pain and mildly inflammed wound.
- Systemic manifestations involved the cenral nervous , cardiovascular, pulmonary, gastrointestinal and hematologic systems. An overall analysis revealed, 0.80-1.00 involving the CNS, 0.40-0.90 the pulmonary, 0.03-0.17 of gastrointestinal, 0.45-0.83 cardiovascular, 1.69-1.84 the hematologic systems and 1.01-1.17 of bite site reactions.. The highest score levels appeared 12 hours after hospitalization.
- Most victims had severe abnormal coagulopathy (VCT > 30 min) in the first day of hospitalization. The VCT gradually decreased to normal by day 5 after treatment with antivenom. The abnormal CPK activity, ranged from 181-256 units/litre, was shown in the first day.
- The degree of clinical snake envenomation was presented with moderate severity in the first 12 hours of hospitalization and decreased to no sign/symptom by day 3.
- Various factors including gender , age, education , location of snakebite and duration of hospitalization, significantly influenced the extent of local tissue necrosis of the victims.

1.2. *N. kaouthia* bites

- Many patients of *N. kaouthia* bites came from southern Nakorn Si Thammarat and Nakorn Sawan (northern). The peak prevalence was in May which is the early part of the rainy season.
- Snakebites were distributed throughout the day between 11.00 a.m. to 4.00 p.m. Species identification was made in 60% of the cases with dead culprit snakes.
- The victims age ranged from 21 to 40 years old, with low education (primary school) and low monthy salary (1,000 – 3,000 baht). Most bites occurred at the lower limbs, especially feet , finger and toe. Most snakebite occurred in rural areas, outdoors and at dusk.
- Time between bite and seeking medical advice ranged between 0.01 - 60 min. The number of patients who applied tourniquet were higher (82.5%) and need only wound care to prevent or control infection. Only one case required amputation of the thumb. The range of hospitalization was 1-5 days. The dose of antivenin administed ranged from 1-29 vials.
- The degree of clinical manifestations was mostly mild , especially at 12 hours after hospitalization.
- The CPK activity was normal (10-180 units/litre) in 75.60 % of these cases.
- Various factors including factors related to treatment, age and the distance between fang marks significantly affected the degree of local tissue necrosis of the victims.

2. Experiments Study

- Venoms from many species of snakes are able to cause variable degree of local tissue damage in the victim. The local toxic effects of snake venoms have been defined as hemorrhage, myonecrosis and edema. The magnitude of these effects depends on the venom route, dose injected, the host and venom type. In severe cases, the tissue damage may lead to serious sequelae such as permanent tissue loss, disability or even amputation. The local toxicities of various snake venoms are not prevented or reversed by antivenom administration. The main enzymes causing hemorrhage, edema and tissue necrosis are hemorrhagic metalloproteinases and myotoxic phospholipase A_2. In addition, cardiotoxins or 'Membrane Active Peptides', which devoid of enzymatic activity, are found in many elapid venoms.

- Metalloproteinases are one of the several classes of proteolytic enzymes and consist almost entirely of zinc containing. Snake venom metalloprotinases play a key role in causing hemorrhage, a prominent local tissue damage characteristic of viperinae and crotalinae snakes.

- Phospholipase A_2 myotoxins have been found in the venoms of many snakes of the families Elapidae, Crotalidae and Viperidae. Non-neurotoxic phospholipase A_2 myotoxins may utilize a different mechanism for myotoxicity. First, some myotoxins of this group have been shown to be directly cytotoxic to immature muscle cells.

Second, a number of natural toxin variants (isoforms) having phospholipase A_2 structure, are unable to hydrolyze phospholipids due to changes in amino acid residues that are essential for the catalytic mechanisms.

- The present study has been carried out to find potent inhibitors of metalloproteinase and/or phospholipase A_2 of *Naja kaouthia* and *Calloselasma rhodostoma* venoms and test whether these inhibitors can reduce the extent of local tissue necrosis caused by these venoms.

- Drugs and chemicals known to inhibit metalloproteinase and/or phospholipase A_2 have been studied *in vitro* on the rate and extent of inhibition of the enzymes from *C. rhodostoma* and *N. kaouthia* venoms. These drugs/chemicals were divided into 2 groups, the water soluble compounds and the water insoluble compounds which were solubilized in dimethyl sulfoxide solution.

  Known inhibitors of metalloprotease studied were:

  L_1 (Desferiprone)

  DFO (Desferrioxamine)

  TEPA (Tetraethylenepentamine)

  N-Phenylglycine

  EDTA (Ethylenediamine tetraacetic acid)

Known inhibitors of phopholipase A_2 studied were:

Quinine

p-BPB (para-bromophenacyl bromide)

Mefloquine

EDTA (Ethylenediamine tetraacetic acid)

- Metalloproteinase inhibitors L1 (10 mM), DFO (20 mM), TEPA (20 mM) and N-phenylglycine (20 mM) have been shown to completely inhibit metalloproteinase and proteolytic activities of both venoms. TEPA (20 mM) and N-phenylglycine (20 mM) also completely inhibited phospholipase A_2 activity *C. rhodostoma* and *N. kaouthia* venoms.

- Phospholipase inhibitors quinine (10 mM), p-BPB (0.5 mM) and EDTA (2 mM) have been shown to completely inhibit phospholipase A_2 activities of *C. rhodostoma* and *N. kaouthia* venoms. These phospholipase A_2 inhibitors were slightly effective and inhibited only 40% of the proteolytic and metalloproteinase activities of both venoms.

- The effects of inhibitors of metalloproteinase and/or phospholipase A_2 on the local tissue necrosis included by *N. kaouthia* or *C. rhodostoma* venoms have been studied in mice.

- Local tissue necrosis has been quantitated using the following parameters: edema by measuring the increase in weight of mouse footpad; myonecrosis by measuring the rise in serum creatine phosphokinase activity. For *C. rhodostoma* venom, an additional parameter of hemorrhage was studied by determining the diameters of hemorrhagic spots induced by the venom.

- Two types of experimental designs have been used in the study of metalloproteinase and/or phospholipase A_2 inhibitors on local tissue necrosis induced by the venoms.
  a) A preincubation type experiment in which the metalloproteinase and/or phospholipase A_2 inhibitors and the venom were preincubated before the mixture was injected into mice
  b) An independent inoculation type experiment in which the venom was first injected, to be followed after certain time intervals by the injection of metalloproteinase and/or phospholipase A_2 inhibitors.

- In the preincubation type experiment:
  - The metalloproteinase inhibitors N-phenylglycine (37.80-151.20 μg per mouse) and TEPA (92.90-371.60 μg per mouse) significantly reduced local tissue necrosis (edema, myonecrosis and hemorrhage) induced by the two venoms.
  - The phospholipase A_2 inhibitors p-BPB (6.96 μg per mouse) and EDTA (93.05 - 372.20 μg per mouse) have been shown to significantly decreased local toxicity (edema and myonecrosis) of *C. rhodostoma* or *N. kaouthia* venom.

Mefloquine has been shown to reduce local tissue damage (edema, myonecrosis and hemorrhage) induced by CR venom.

- In the independent type experiment:

  N-phenylglycine and EDTA, shown to be highly effective in the pre-incubation experiments, were chosen for the independent injection studies. N-phenylglycine (37.80 μg) and EDTA (93.05 μg) have been shown to be 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 3 min or more after the venom injection.

- An 'Inhibitor mixture' containing an inhibitor of metalloprotease N-phenylglycine (37.80 μg), an inhibitor of phospholipase A_2 EDTA (93.05 μg per mouse) and an inhibitor of hyaluronidase sodium aurothiomalate (195 μg per mouse) has been shown to significantly reduce 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.

- N-phenylglycine (75.60 – 151.20 μg), L1 (139 – 278 μg) TEPA (376.10 – 752.20) and DFO (657 – 1,314 μg) prolonged the survival time of mice receiving lethal doses of *C. rhodostoma* or *N. kaouthia* venom.

- Quinine (360.09 μg), mefloquine (21.20 μg) p-BPB (3.47 μg) and EDTA (372.20 μg) prolonged the survival time of mice injected with lethal doses of *N. kaouthia* or *C. rhodostoma* venom.

- These results showed that by inhibiting metalloproteinase and phospholipase A_2, the local and systemic toxicities of *N. kaouthia* and *C. rhodostoma* venoms in mice could be significantly reduced.

- It is concluded that the 'Inhibitor mixture' containing inhibitors of hyaluronidase, metalloprotease and phospholipase A_2 which are the main enzymes implicated in causing local tissue necrosis, is effective in reducing local tissue necrosis caused by *N. kaouthia* or *C. rhodostoma* venom if the 'mixture' is injected at the same site within 3 min after venom injection.