CHAPTER I

INTRODUCTION

Snake envenomation is a serious medical and economic problem which could result in death and disability in victims. The high incidence of snake bites is found in many parts of the world especially in tropical countries of Africa, South America and South-east Asia (W.H.O, 1981). In Thailand, snake envenomation constitutes a relevant public health hazard in most parts of the country. From 1993 to 1999, about 8,000 – 10,000 of venomous snakebite cases with approximate 20 death were reported annually (Division of Epidemiology, Ministry of Public Health, 1993 -1999).

Poisonous snakes can be divided into 4 families which include Elapidae, Viperidae, Crotalidae and Hydrophidae. The majority of snake envenomation in Thailand is caused by snakes of the Elapidae and Viperidae families (Trishnananda, 1979). Among the Elapidae family, the Thai cobra (*Naja kaouthia*) is the most dangerous. It causes the highest mortality from snake envenomation in Thailand. Malayan pit viper (*Calloselama rhodostoma*), of the Viperidae family, is an extremely dangerous and abundant species responsible for nearly 40 % of all snakebites from 1993 to 1997. It is found in most parts of the country, especially in the southern region (Division of Epidemiology, Ministry of Public Health, 1993-1997).

The clinical manifestations of snake envenomation may be either local or systemic or both. Systemic manifestations depend upon the particular species of the culprit snakes involved. Snake venom is classified as neurotoxic (cobra and kraits),

hemorrhagic (vipers) and myotoxic (sea snakes). Snake neurotoxins, found mainly in elapid venom, cause skeletal muscle paralysis by inhibiting neuromuscular transmission resulting in respiratory failure. It is the main cause of death in elapid bites. In viper bites, hemorrhagic effects are the main manifestations consisting in coagulopathy and bleeding distant from the bite site. This could include macrohematuria, epitaxis, uterine bleeding, hemoptysis and shock. These symptoms are caused by venom enzymes such as various proteases (Seegeres and Ouyang, 1979; Ouyang, Teng and Huang, 1992). The myotoxic sea snake venoms produce skeletal muscle necrosis, myoglobinuria, hyperkalemia and myalgia. Almost all the systemic effects can be successfully treated by antivenom which is species specific. Monospecific antivenom is manufactured in Thailand for treatment of bites by the 6 terrestrial snakes originally considered to be the most medically important ones (Ganthavorn, 1969): *Naja kaouthia* (common monocellate cobra), *Ophiophagus hannah* (King cobra), *Bungarus fasciatus* (banded krait), *Calloselasma rhodostoma* (formerly *Ancistrodon* or *Agkistrodon rhodostoma*) (Malayan pit viper), *Daboia russellii siamensis* (formerly *Vipera russellii* (Russell's viper) and *Trimeresurus albolabris* (green pit viper). Antivenom, the principle and effective therapy for systemic symptoms caused by snake envenomation have saved the lives of most victims. However, local toxicity of various snake venoms such as edema, hemorrhage and myonecrosis, are not prevented or reversed by antivenom treatment (Gutierrez et al., 1998).

Local toxicity of viper venoms frequently include pain, swelling and echymoses and appear within minutes of the bite. Such signs are followed by necrosis of the area surrounding the bite site. Tissue necrosis often appears to be mainly ischaemic,

developing slowly within a few weeks and presenting as dry gangrene. Local effects of cobra venom is different, swelling does not usually appear until 2-3 hours after the bite, but necrosis develops rapidly, presenting as wet gangrene within a few days, with a putrid smell (Reid and Theakston., 1983). These effects may prolong the duration of hospitalization and increase morbidity which may result in loss of a digit or limb, or in crippling deformity (Warrell et al., 1986). Although victims may be given antivenom, it is often less effective in preventing the tissue necrosis. Since the local effects are caused by a variety of toxic components with rapid onset of pathological changes, it has been difficult to prevent these effects with antivenom, especially if there is a delay in serotherapy (Gutierrez et al., 1998).

In Thailand, factors contributing to tissue necrosis outcome have been many. Delayed arrival at the hospital was encountered in most parts of rural areas. Thus, many snakebite patients suffer from the progressive severity of systemic and local effects. Some victims, treating themselves by using traditional medicine, made the lesion worse and went to the hospital only when their condition deteriorated. For example, a 30 year old man was brought to the hospital after 17 days of traditional treatment for a *C. rhodostoma* bite. He had a gangrenous leg and was febrile, jaundiced, semi-comatose and obviously septicaemic. (Looareesuwan, Viravan and Warrell, 1988). Misdiagnosis and incorrect treatment may also lead to severe morbidity and death. Delayed treatment with monospecific antivenom in *Calloselasma rhodostoma* bites may increase the degree of local necrosis and chronic disability (Reid et al., 1963). Warrell et al (1986) defined treatment failure in their study of *Calloselama rhodostoma*

bites, as recurrence or persistence of incoagulable blood after a total of 20 ampules (200 ml) of antivenom. In part, an incorrect diagnosis may be caused by derive from a bias toward the snake of high prevalence in a particular region.

Although local tissue necrosis caused by snake envenomation has been well recognized to result in serious morbidity, the extent of the problem has not been studied. This is partly due to the success of antivenom in saving lives of snakebite victims. The problem of local tissue necrosis seems to be minor in comparison. The use of antivenom has been found ineffective against local necrosis. Yet, there is no alternative in the treatment. However, it remains possible that new approaches in preventing or reducing local tissue necrosis can be found. The use of enzyme inhibitors, as a first-aid injection kit, may inhibit the causative hydrolytic enzymes of the venom.

In order to arrive at an effective treatment of local tissue necrosis, more information is needed. Although it has been observed by clinicians that *N. kaouthia* and *C. rhodostoma* cause a high incidence of local tissue necrosis, data on the prevalence and severity of injuries caused by these snakes need to be gathered. In addition, factors contributing to the degree of local tissue necrosis must be identified. If verified, these snake venoms will be the targets for the search of enzyme inhibitors. Inhibitors can then be tested in an experimental animal model for their ability to prevent local tissue damage caused venoms.

Objectives of Research

1. To study the severity of tissue necrosis in individuals bitten by *Naja kaouthia* or *Calloselasma rhodostoma.*
2. To determine the associated factors of tissue necrosis from bites by *Naja kaouthia* and *Calloselasma rhodostoma* .
3. To study the effectiveness of inhibitors of phospholipase A_2 , metalloproteinase and hyaluronidase in preventing or reducing local tissue necrosis caused by these venoms in an experimental model.

Expected results and benefits

1. Epidemiology study

From the epidemiology survey, information on the seriousness and predictors of local tissue necrosis caused by *N. kaouthia* and *C. rhodostoma* will be obtained. This information may give useful guidelines for the prevention or reduction of severity of tissue necrosis after snakebites. Furthermore, snakebite severity score (SSS) may be of benefit for physicians to assess the clinical manifestations before making decision on antivenom administration.

2. Studies on the enzyme inhibitors

From the *in vitro* studies, it is expected to find potent inhibitors of the venom enzymes. The *in vivo* study of myonecrosis, in the absence and presence of various enzyme inhibitors, will give results on the potential benefit of these inhibitors as first-aid

treatment of snakebites. Lastly, the experiment of the effects of enzyme inhibitors on the survival time of mice receiving lethal doses of the venom may give some indication of the possible delay of onset and extent of systemic toxicity produced by these inhibitors.