Toxicological and immunological characterization of the Sri Lanka hump-nosed pit viper (*Hypnale hypnale*) venom

Choo-Hock Tan.[1], *Christeine Ariaranee Gnanathasan*[3], *Si-Mui Sim*[1], *Shin-Yee Fung*[2], *Nget-Hong Tan*[2]
[1]*Department of Pharmacology, Faculty of Medicine, University of Malaya;* [2]*Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia;* [3]*Department of Clinical Medicine, Faculty of Medicine, University of Colombo, Sri Lanka.*

The humped-nose pit viper, *Hypnale hypnale*, is a medically important venomous snake in Sri Lanka and Southwestern coast of India, with envenomation that results in local tissue destruction, hemostatic dysfunction, acute kidney injury, and even death. The characterization and neutralizing studies of its venom are therefore essential to understand the envenoming pathophysiology and to improve the management. The venom has an intravenous LD_{50} of 0.9 μg/g mouse, and exhibits procoagulant, hemorrhagic, necrotic, phospholipase A_2, L-amino acid oxidase and hyaluronidase activities. Some of the toxin components were partially purified by Resource Q ion-exchange chromatography, noteworthy is the thrombin-like enzymes which likely present in multiple isoforms. The venom clotting activity on fibrinogens showed distinct species differences, and its clot formation on human fibrinogen is followed by a slow dissolution, a phenomenon suggestive of consumptive coagulopathy observed clinically. At an intramuscular sublethal dose, the venom did not cause acute kidney injury in a rodent model. Hence, nephrotoxicity might occur at a higher venom dose in the face of coagulopathy as a complication of the venom hematoxicity. We also showed that the monovalent Malayan pit viper antivenom and Hemato polyvalent antivenom (both produced by Thai Red Cross Society) could effectively neutralize the lethality of *H. hypnale* venom (0.89 and 1.52 mg venom/ml antivenom respectively) as well as its hemorrhagic, procoagulant and necrotic effects. Furthermore, the polyvalent antivenom could also effectively neutralize the lethal effect of *Daboia russelii* venom (2.50 mg venom/ml antivenom), another common biting snake in Sri Lanka and South India. These findings suggested that the Hemato polyvalent antivenom may be beneficial in the antivenom treatment of *H. hypnale* envenoming where antidote is still unavailable. Meanwhile, we continue the work on producing *Hypnale hypnale*-specific antibodies, as conceptually the homologous antivenom should neutralize the venom components more optimally. The venom elicited satisfactory titers of anti-*H. hypnale* (anti-Hh) IgG in rabbits following 3 immunizations. The antisera and IgG were effective in neutralizing the venom lethality and other toxic activities, indicating the feasibility to produce an effective specific antivenom using the common immunization regime. Cross-reactivity studies using indirect ELISA showed that anti-Hh IgG cross-

reacted with several Asiatic pit vipers' venoms particularly that of *Calloselasma rhodostoma* (73.6%), presumably due to the presence of many common venom antigens in view of the close phylogenetic relatedness between these two crotalids. Levels of immunological cross-reactivity were vastly reduced with double-sandwich ELISA, suggesting that the assay can be developed into a specific and sensitive diagnostic tool in *H. hypnale* envenomation. In pharmacokinetic study, the venom injected intravenously in rabbits showed a rapid distribution phase ($T_{1/2\ \alpha}$ = 0.5 h) and a slow elimination phase ($T_{1/2\ \beta}$ = 20 h), with a systemic clearance of 15 ml/h. When given intramuscularly, multiple peak concentrations of the venom were observed, however the elimination half-life was similar ($T_{1/2\ \beta}$ = 19 h) to that of intravenous route. The i.m. bioavailability was low and this might explain for the highly varied LD_{50} between i.v. and i.m. envenomings in animal models, as the venom is principally hematoxic. Intravenous infusion of 4 ml Hemato polyvalent antivenom 1 h after i.m. venom injection markedly reduced the serum venom levels, but subsequently produced a redistribution of venom antigens from the extravascular to vascular space. These free redistributed venom antigens were effectively neutralized by a second dose of 4 ml antivenom, and the mean values of venom antigens concentrations at various time intervals as well as the total antigens amount were significantly reduced in the treated group. This provides an experimental basis guiding towards the optimization for the use of the antivenom at clinical setting.