

Thesis Title : Studies on the Improvement of
 Immunogenicity of Elapid Snake
 Postsynaptic Neurotoxins.

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ABSTRACT

Nine polymeric forms and conjugates of purified principal postsynaptic neurotoxins of Naja naja siamensis (NNS), Ophiophagus hannah (OH) and Bungarus fasciatus (BF) have been synthesized by controlled polymerization in which only 25-60 % of the toxins were allowed to react. A carbodiimide (E CDI) and glutaraldehyde (GA) were used as coupling agents while BSA, diphtheria toxoid (DT) and tetanus toxoid (TT) were used as carriers. The antigenic mosaic of these immunogens were: I, NNS-E CDI; II, NNS-OH-BF-E CDI; III, NNS-BSA-E CDI; IV, NNS-TT-E CDI; V, NNS-OH-BF-TT-E CDI; VI, NNS-GA; VII, NNS-OH-BF-GA; VIII, NNS-DT-E CDI and IX, NNS-OH-BF-DT-E CDI. By using SDS-PAGE and radioactive toxin, each immunogen preparation was characterized in terms of molecular size and abundance of protein components, percent toxin reacted and toxin density. The relative

immunogenicities of seven immunogens (I to VII) along with those of crude NNS venom and pure NNS postsynaptic neurotoxin were evaluated in groups of 8 rats using incomplete Freund's adjuvant (IFA). The levels of specific antibody against each of the neurotoxins were determined by ELISA's. Multiple comparisons between antibody responses to these immunogens were made. All the chemically modified immunogens were found to be at least as immunogenic as NNS venom. NNS-TT-ECDI gave the highest antibody response (2.7-6.2 fold higher than that induced by NNS venom) while pure NNS postsynaptic neurotoxin was among the least immunogenic. The 3 multispecific immunogens (II, V and VII) induced comparable specific antibodies to BF,OH and NNS neurotoxins. The results showed that the presence of protein carrier and the relative degree of toxin density affected the immunogenicities.

In another set of experiment, the effect of 3 adjuvants: IFA, bentonite and squalene-Arlacel A were studied in groups of 8 rats using one monospecific (VIII) and two multispecific (VII and IX) immunogens while crude NNS venom was used as control. It was found that IFA gave the highest antibody responses with each of the 4 immunogens. The 3 chemically modified immunogens were also shown to be more immunogenic than the crude NNS venom in most adjuvants. The two multispecific immunogens (VII and IX) also stimulated high antibody titers against OH and BF neurotoxins in all 3 adjuvants.

The results of this study should pave the way to the production of potent, polyvalent antivenoms against these and other deadly elapid snakes.