PHARMACOLOGICAL CHARACTERIZATION OF THE MAJOR NEUROTOXIN AND MYOTOXIN FROM SRI LANKAN RUSSELL'S VIPER (*DABOIA RUSSELLI*) VENOM

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Objectives: Envenoming by Russell's viper (*Daboia* spp.) is common in Asiabutparalysis and rhabdomyolysis are onlyreported from Sri Lanka and South India (*D. russelli*). The objectives were to identify the neurotoxins and myotoxins in *D. Russelli* venom and to characterize their pharmacological activities.

Methods: *D. russelli* venom was collected from Sri Lanka. A combination of size-exclusionchromatography (SEC) and reverse-phase high performance liquid chromatography (RP-HPLC) was used to isolate and purify toxins from the venom. Toxin purity was confirmed with SDS-PAGE and mass spectrometry. Pharmacological activity was tested *in vitro* using the isolated chick biventer cervicis nerve-muscle (CBCNM) preparation and the isolated rat phrenic nerve-hemidiaphragm (RPNH) preparation. Toxins were subjected to partial N-terminal sequencing, and interaction between the Indian Polyvalent antivenom and toxins were studied using western blotting.

Results: β-viperotoxin-Dr1 (13.6 kDa), constituted approximately 22-28% of the venom, had an N-terminal sequence of: SLLEFGMMILEETGKLAVPF, similar to the basic phospholipases A_2 previously isolated from Russell's viper venom in the region. This toxin concentration-dependently inhibited nerve mediated twitches in the CBCNM preparation without inhibiting responses to acetylcholine or carbachol. The time to inhibit the amplitude of twitches by 50% (i.e. t_{50}) was 48 minutes and concentrations as high as 0.6μM did not completely abolish twitches after 180min suggesting β-viperotoxin-Dr1is a relatively weak pre-synaptic neurotoxin. The t_{50} for 0.6μM was similar in the CBCNM and RPNH. Removal of β-viperotoxin-Dr1from the venom abolished almost all neurotoxicity. β-viperotoxin-Dr1 also displayed weak myotoxic activity. A second toxin, viperomyotoxin-Dr1(13.5 kDa), constituted approximately 35-48% of the venom and had an N-terminal sequence of: SLLEFGKMILEETGKLAIPS. Viperomyotxin-Dr1 displayed concentration-dependent myotoxic activity in the CBCNM preparation, characterised by an increase in baseline tension and inhibition of responses to exogenous KCl. However, even concentrations as high as1μM were unable to inhibit the amplitude of twitches by 50% suggesting viperomyotxin-Dr1 is a weak myotoxin. Removal of both toxins from crude venom results in a loss of myotoxic activity. Western blotting showed effective binding of Indian polyvalent antivenom with both toxins.

Conclusion: Paralysis and myolysis seen in Sri Lankan Russell's viper envenoming is likely to be due to these two toxins. Although these toxins constitute most of the venom, their low potency is likely to be why neurotoxicity and myotoxicity is mild in humans.