TESTING RENAL FUNCTION IN RATS AND RABBITS AFTER ENVENOMATION WITH VENOMS OF RUSSELL'S VIPER (*Vipera russelli*), COBRA (*Naja naja*) AND HUMP NOSED VIPER (*Hypnale hypnale*)

by

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ABSTRACT

Acute renal failure is a serious systemic manifestation encountered in envenomation following snake bite. Renal ischaemia and direct toxic effect of venom are considered to be the major factors contributing towards the development of acute renal failure. However, the mechanism of nephrotoxicity is not elucidated in the Sri Lankan context. The hypotheses regarding the possible mechanisms of nephrotoxicity are based on the clinical and pathological observations in human snake bite victims. Polyvalent antivenom serum imported from India is the only available antidote for the treatment of envenomed patients. Lack of efficacy of this antivenom is a major problem faced by the clinician in treating snake bite victims. Experiments described in this thesis were conducted to investigate the mechanisms of nephrotoxicity following envenomation. The efficacy of the different treatment modalities were also tested on animal models.

Initially, the pathophysiology of the kidney following intraperitoneal injection of Russell's viper venom and cobra venom was studied using an *in vivo* rat model. The pathophysiology of rabbits after subcutaneous injection of Russell's viper venom and hump nosed viper venom was tested *in vitro* in Protocol Two using the isolated perfused kidney model. This included the effect of venom on renal function, renal pathology and renal tubular cell integrity. The contribution of the direct toxic effect of Russell's viper venom, cobra venom and hump nosed viper venom towards the development of nephrotoxicity was studied in Protocol Three using a kidney slice model. The efficacy of polyvalent antivenom serum, monovalent antivenom serum (*Pulchella Tab*) and a combination of polyvalent antivenom serum and intravenous immunoglobulins in neutralizing the effects of Russell's viper venom and cobra venom was studied using *in*

vitro models in Protocols Four and Five. In the last protocol, the LD_{50} values of the venoms were obtained.

The *in vivo* experiments (Protocol One) showed a gradual reduction in the renal functional measurements of rats after injection of Russell's viper venom. This was associated with marked degenerative and early necrotic changes in the cells of the proximal convoluted tubules of the renal specimens. Intravascular haemolysis was evident at 4 hours after injection of venom. A significant reduction in the renal function was observed in rabbits 4 hours after injection of Russell's viper venom (Protocol Two). This was associated with intravascular haemolysis and significantly disturbed renal tubular cell integrity (increased excretion of N-acetyl-β-D glucosaminidase enzyme in urine). In the renal specimens severe degenerative and early necrotic changes were seen in the cells of the proximal convoluted tubules. Ultrastructurally, mitochondrial cristae pattern in the cells of the proximal convoluted tubules in some renal specimens has completely disappeared. There were marked ultrastructural changes in the glomerular capillaries also. Severe necrotic changes in the cells of the glomeruli, proximal and distal convoluted tubules with complete destruction of the cell ultrastructure were observed in the rabbit kidney slices following incubation with 10 mg/ml of Russell's viper venom (Protocol Three).

The disturbance to the renal function observed following injection of Russell's viper venom was consistent with the renal pathological changes observed. When the findings from the first four protocols are considered together, they all suggest that the direct toxic effect of Russell's viper venom is the major contributory factor which is mediating the structural and functional changes seen in the kidneys.

The pathological changes observed in the renal specimens of rats after injection of cobra venom were mild. There was no significant derangement in renal function and there was no evidence of intravascular haemolysis. However, direct application of cobra venom on kidney slices exerted a direct toxic damage on renal tubules.

After injection of hump nosed viper venom to rabbits, there was no significant reduction in the renal function (Protocol Two). The damage to the renal tubular integrity was mild and there was no intravascular haemolysis. The histological and ultrastructural changes observed in the renal specimens of rabbits also were mild. The evidence from the experiments using cobra venom and hump nosed viper venom also favour the hypothesis that nephrotoxicity is mediated by direct effects of venom.

Injections of polyvalent antivenom serum alone or together with intravenous immunoglobulins did not neutralize the effects of Russell's viper venom significantly (Protocol Four). Similarly, incubation of kidney slices with a mixture of Russell's viper venom or cobra venom and polyvalent antivenom serum did not reduce the direct toxic effects of the venoms. The polyvalent antivenom serum was not effective in neutralizing the functional disturbances caused by the direct toxic action of Russell's viper venom. The monovalent antivenom serum (*Pulchella Tab*) significantly reduced the functional and pathological changes caused by Russell's viper venom. However, even with monovalent antivenom serum a complete resolution of the pathological changes was not observed.

The LD_{50} values obtained for the intravenous route of rats were 10.16 μg/100g for Russell's viper venom, 212.1 μg/100g for cobra venom and 7.62 mg/100g for hump nosed

viper venom. Comparison of LD_{50} values of the present study with that of the Medical Research Institute indicated that rats are more susceptible to toxicity of venom than mice.