

Direct nephrotoxic effects produced by venoms of Sri Lankan cobra, Russell's viper and hump nosed viper

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Abstract

Nephrotoxicity is the principal cause of death following Russell's viper envenomation. Envenomation following the bite of several other snakes is also known to cause nephrotoxicity. The nephrotoxicity can be due to direct effects of venom or secondary to circulatory disturbances (eg. ischaemia), which these patients often manifest. As separating out the contributions of direct toxic effects and ischaemic effects are difficult in the *in vivo* situation, experiments were carried out using the kidney slice model to study and compare the direct toxic effects of venom of cobra, Russell's viper and hump nosed viper.

The effect of cobra venom (CV) on rat kidney slices and the effects of Russell's viper venom (RVV) and hump nosed viper venom (HNVV) on rabbit kidney slices were examined. Healthy male animals were anaesthetized and kidneys were harvested. Kidneys were decapsulated, bisected and sliced. Rat kidney slices were incubated with CV and rabbit kidney slices were incubated with RVV and HNVV for different time periods. Rat and rabbit kidney slices were incubated with 0.9% sodium chloride as the control. At the end of each observation period kidney slices were preserved for light and electron microscopy (LM and EM).

When CV was used, complete necrosis was seen in proximal and distal convoluted tubular cells (PCT and DCT). When rabbit kidney slices were incubated with RVV for 4 hours there was complete necrosis of glomeruli and PCT with the preservation of the basement membrane. LM and EM changes were mostly confined to PCT when HNVV was used.

The results of this experiment provide evidence that the venoms studied produce direct damage on renal tissue. Different areas of the nephron are differentially susceptible to the effects of the three venoms.

Key words : direct nephrotoxicity, Russell's viper venom, cobra venom, hump nosed viper venom, kidney slice model

Introduction

The death rate due to snake bite envenomation in Sri Lanka is one of the highest in the world (1). These fatalities are often caused by cobra, Russell's viper and the common krait. Though cobra and krait venom are more potent than Russell's viper venom, Russell's viper is the most dreaded snake in the island (2) and it is responsible for 40% of deaths due to envenomation (1).

Although death following Russell's viper envenomation could be due to different reasons (shock, haemorrhage, disseminated intravascular coagulation, neurotoxicity, respiratory failure or acute renal failure), nephrotoxicity leading to acute renal failure has been identified as the principal cause (1). In one study it was found that envenomation by Russell's viper contributes for 49% of cases of acute renal failure reported at the National Hospital of Sri Lanka (3). Envenomation following the bite of several other snakes (e.g. cobra, pit vipers and sea snake) is also known to cause nephrotoxicity (4, 5).

The development of nephrotoxicity in envenomation can be due to direct effects of venom or secondary to circulatory disturbances (ischaemia), which these patients often show. Separating out the contribution of direct

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nephrotoxicity and ischaemic effects towards the development of acute renal failure is difficult in the *in vivo* situation. Hence, experiments were carried out using the kidney slice model in order to study the direct nephrotoxic effects of venom of Russell's viper (RVV), hump nosed viper (HNVV) and cobra (CV). This *in vitro* model had been used by other workers to demonstrate direct nephrotoxic effect of *Vipera russelli russelli* (6).

Material and Methods

Animals

The protocol reported here is part of a detailed investigation on effects of venom on renal structure and function (7, 8, 9). In this protocol studying the direct toxic effects of venom, CV was tested in rats and RVV and HNVV were tested in rabbits.

For the experiments using rats, healthy Sprague-Dawley male animals (approximately 8 months of age) weighing 200-300 g were obtained from an inbred colony maintained at the animal house of the Faculty of Medicine, Colombo. These rats were housed under tropical laboratory conditions and given a pelleted diet with sweet potato.

For the experiments using rabbits, local cross-bred white male animals (approximately 9 months of age), weighing 1 to 2 kg were used. Rabbits were kept in the animal house for 2 weeks prior to the experiments. They were given a diet of carrot and green leaves. Ethical clearance for the experiments was obtained from the institutional review body.

Source of venom

CV, RVV and HNVV were obtained from the reptilium of the National Zoological Garden, Dehiwela, Sri Lanka. The snakes were identified by the curator and the venom was milked by him. Venom was milked into pre-weighed, sterilized, universal bottles and care was taken to prevent the venom mixing with saliva. The venom containing universal bottles were kept in ice and brought to the laboratory. In the laboratory the venom was weighed and divided into 2 or 3 aliquots and stored at -70°C .

Anaesthesia of animals and harvesting of kidneys

Rats were anaesthetized by ether inhalation for harvesting of kidneys. Kidneys were harvested through a mid ventral laparotomy. During the dissection anaesthesia was maintained by injecting diazepam intramuscularly at a dose of 0.3 mg/kg. After harvesting the kidneys animals were killed by a cardiac puncture.

Rabbits were anaesthetized using intravenous thiopentone sodium (20 mg/kg body weight). During dissection anaesthesia was maintained by ether inhalation using a mask. Once the animal was anaesthetized the kidneys were harvested through a mid ventral laparotomy and subsequently the animals were killed by a cardiac puncture.

The kidneys were decapsulated and bisected using sterile instruments while keeping in 0.9% sodium chloride. Uniform slices of 0.3 mm thickness were obtained from the bisected kidneys using a tissue slicer. Rat kidney slices were incubated with 10 mg/ml of CV and rabbit kidney slices were incubated with 10 mg/ml of RVV and HNVV separately for 1, 2, 3 or 4 hours in a water bath at 37°C ($n=5$ for each stage). This dose of venom (10 mg/ml) was decided based on the experiments by Soe-Soe *et al.*, (6). In their experiments varying doses of RVV (*Vipera russelli russelli*) were used (300ng/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml). They found effects only at doses of 5 mg/ml and 10 mg/ml. Hence, for our experiments only the 10 mg/ml dose was used. Kidney slices incubated with 0.9% sodium chloride were used as controls. After incubating for different time periods (1 hour, 2 hours, 3 hours or 4 hours), kidney slices were fixed in modified Boins solution or with 2% glutaraldehyde for light microscopy (LM) or electron microscopy (EM) respectively. Pathological changes in kidney slices were expressed as percentages. The percentage of change (degeneration or necrosis) were categorized as described below after examining ten fields under low and high dry power (10 X 20, 10 X 40) in two slides.

changes in 5 - 10% of structures - very mild
 changes in 11 - 33% of structures - mild
 changes in 34 - 66% of structures - moderate
 changes in 67 - 85% of structures - marked
 changes in 86 - 100 % of structures - severe

Results

Effects of CV

In the rat kidney slices incubated with CV the glomeruli were histologically normal in appearance. The proximal convoluted tubular cells (PCT) showed complete necrosis 2, 3 and 4 hours after incubation. The nuclei of the distal convoluted tubular cells (DCT) were pyknotic at 4 hours after incubation. Marked degeneration was there in the medulla at 3 and 4 hours after incubation. At electron microscopy both normal and collapsed capillary loops in the glomerular tufts were seen. The ultrastructure of the PCT cells was completely destroyed. However, the basement membrane was preserved (Figure 1). Changes were more marked with the increase in incubation time.

Effects of RVV

In the rabbit kidney slices incubated with RVV, glomerular tuft of the surface glomeruli showed necrosis and rest of the glomeruli had pyknotic nuclei at 3 and 4 hours after incubation. Histologically, the PCT showed complete necrosis at 2, 3 and 4 hours after incubation. All the cortical structures (PCT, DCT and glomeruli) showed complete necrosis at 4 hours after incubation. The renal medulla in the specimens was histologically normal in appearance. Electron microscopy showed complete destruction of glomerular tufts and the cells of the PCT (Figures 2 and 3). However, the basement membrane of the cells was preserved.

Effects of HNJV

In the rabbit kidney slices incubated with HNJV, the glomeruli showed occasional pyknotic nuclei at 3 and 4 hours after incubation. There was severe necrosis of the PCT epithelium with occasional viable cells at 3 and 4 hours after incubation. Appearance of small endocytic vacuoles was visible in the cytoplasm of the podocyte and endothelial cells of the glomeruli in some areas

when kidney slices were incubated with HNJV for 4 hours. Most of the mitochondria and their membranes of the PCT cells were completely destroyed. Appearance of vacuoles and myeloid bodies in the PCT cell cytoplasm was also observed (Figure 4).

Appearance of control specimens

Glomeruli in the control specimens were normal in appearance. Necrotic changes were not observed in the PCT or DCT cells. Electron microscopically swollen mitochondria and disappearance of cristae were noticed in a few PCT cells.

Discussion

These experiments confirm production of direct damage on the kidney tissue by the venoms of cobra, Russell's viper and hump nosed viper found in Sri Lanka. The damage was most severe with RVV and least with HNJV. Similar findings using venom of *Vipera russelli siamensis* on rat kidney slices have been reported by Tin-Nu-Swe *et al.*, (10). They describe extensive renal damage including damage to the glomeruli when rat kidney slices were incubated with 5 mg/ml of Russell's viper venom. Soe-Soe *et al.*, (1990) also have observed necrosis of PCT when rabbit kidney slices were incubated with 5 and 10 mg/ml of Russell's viper venom. However, they describe only about damage to the PCT cells (6). Our findings contrast with those of Ratcliffe *et al.*, who did not demonstrate any renal damage with venom of Thai cobra (*Naja kauothio*) on isolated perfused rat kidney model (11). The direct toxic effect of HNJV has not been subjected to experiments earlier. However, the observation is that venom of hump nosed viper is less nephrotoxic than the venoms of Russell's viper and the cobra.

The variation in the direct nephrotoxic effects of venoms may be due to the different amounts of phospholipase A₂ present in them or due to differences in their iso-enzymes (12).

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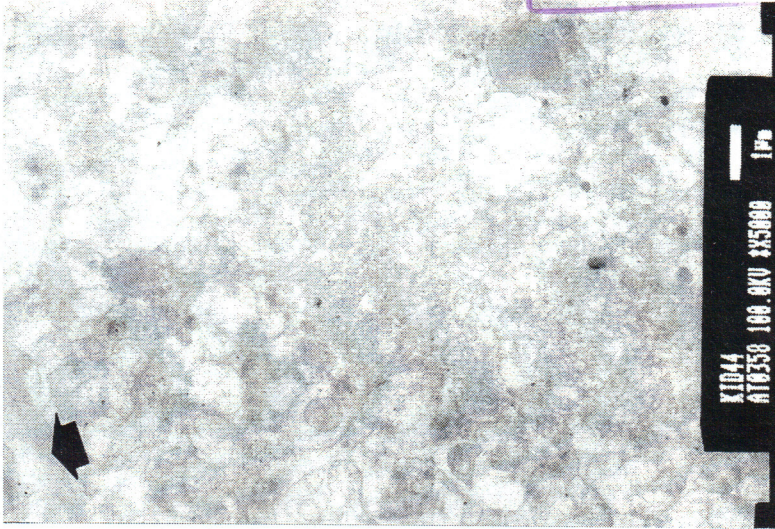


Figure 1. Electron micrograph appearance of rat kidney slices when incubated with cobra venom for 4 hours. Complete destruction of the ultrastructure of the PCT cells with the preservation of the basement membrane is seen (arrowhead). Magnification X 10000, bar = 1 mm.

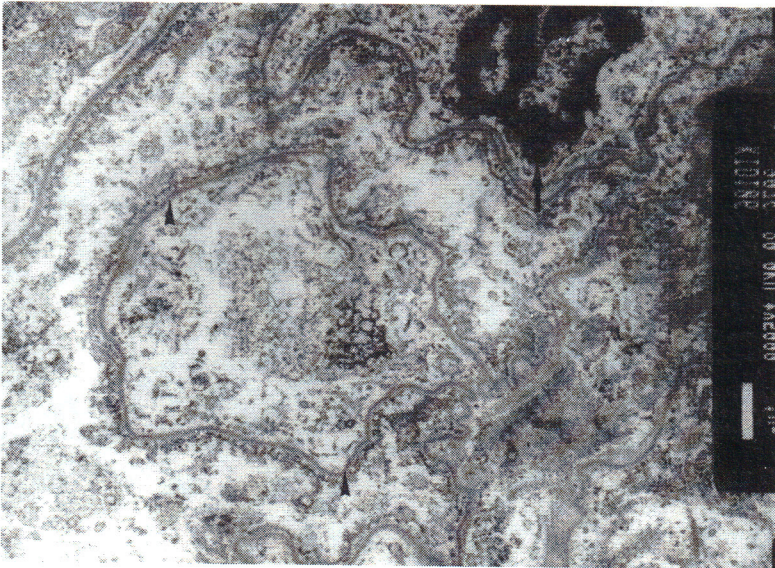


Figure 2. Electron micrograph appearance of the completely destroyed ultrastructure of a glomerulus of a rabbit kidney slice incubated with Russell's viper venom for 4 hours. Basement membrane is preserved (arrowhead). Clumping of chromatin in the nucleus of a white blood cell is seen (arrow). Magnification X 10000, bar = 1 mm.

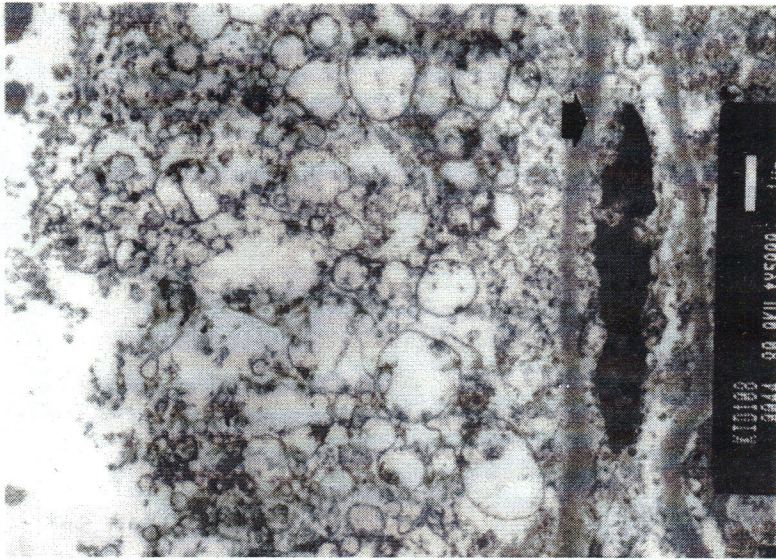


Figure 3. Electron micrograph of the completely destroyed ultrastructure of a PCT cell when rabbit kidney slices were incubated with Russell's viper venom for 4 hours. Basement membrane of the cell is preserved (arrowhead).

Magnification X 10000, bar = 1 mm.

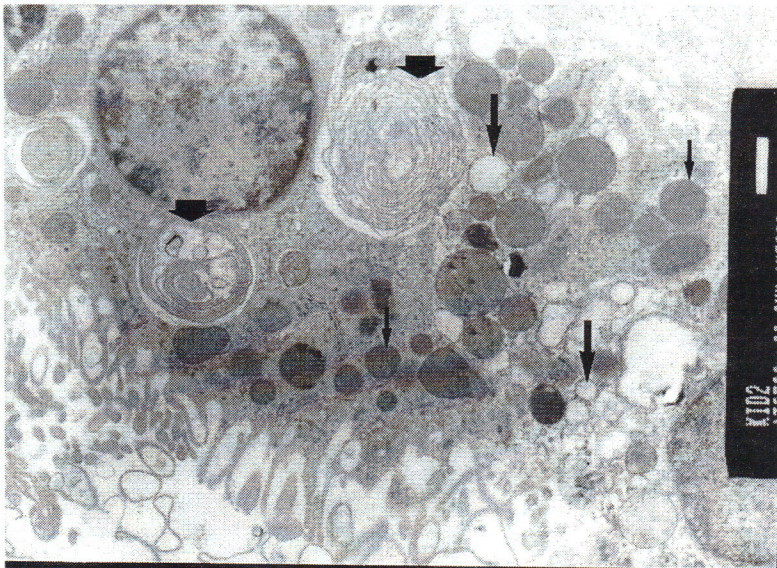


Figure 4. Electron micrograph appearance of a PCT cell of a rabbit kidney slice when incubated with hump nosed viper venom for 4 hours. Appearance of myelin bodies; concentrically laminated electron-dense bodies which represents altered lysosomes (arrow heads), dense bodies (small arrows) and vacuoles (large arrows) are seen.

Magnification X 10000, bar = 1 mm.