

# Direct nephrotoxic effect of Sri Lankan Russell's viper venom – an experimental study using *in vitro* models

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## Abstract

Nephrotoxicity manifesting as acute renal failure is the principal cause of death in Russell's viper envenomation. The mechanisms responsible for nephrotoxicity are not clear and lack of experimental data in the Sri Lankan context prompted us to do this study. In the present experiments, the time related physiopathological changes in rabbits after injection of Russell's viper venom were studied using two *in vitro* models, viz. the isolated perfused kidney model and the kidney slice model. The isolated perfused kidney model experiments showed a significant time related reduction in renal function 4 hours after envenomation. This was associated with disturbances to the renal tubular cell integrity and renal pathology. Experiments using venom on kidney slices showed complete necrosis of the glomeruli and proximal convoluted tubular cells with the preservation of the basement membranes of tubular cells suggestive of direct damage caused by the venom. Data from these experiments are highly suggestive that Russell's viper venom causes renal damage mainly by a direct action on the kidney.

**Key words:** Russell's viper venom, renal failure, direct nephrotoxicity, isolated perfused kidney model, kidney slice model

## Introduction

Russell's viper (*Vipera russelli pulchella*) contributes to the highest number of fatalities (40%) due to snake bite in Sri Lanka (1). Acute renal failure is the commonest cause of death in these patients and it can occur due to several mechanisms. These include direct nephrotoxicity, renal

haemodynamic alterations, non specific effects of venom (such as hypotension, intravascular haemolysis, myoglobinuria, haemoglobinuria and disseminated intravascular coagulation) and immune mechanisms or due to the combined effect of several of these mechanisms (2, 3). Although death following the local viper bite is common hardly any experimental studies have been conducted here using the venom of the Sri Lankan Russell's viper. In the *in vivo* situation it is difficult to obtain evidence of a direct effect of venom on the kidney because of the difficulty in separating renal damage that can occur due to haemodynamic changes (4, 5). Therefore, the *in vitro* experiments described in this paper were carried out using the isolated perfused rabbit kidney (IPK) model and kidney slice model to elucidate the possible mechanisms for renal damage following envenomation by the local Russell's viper venom.

## Material and Methods

### Animals

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

### Source of venom

Russell's viper venom was obtained from the reptilium of the National Zoological Garden, Dehiwela, Sri Lanka. The snakes were identified

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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  $-70^{\circ}\text{C}$ . A stock solution of the venom was prepared by adding 0.9% sodium chloride solution to an aliquot and stored at  $0^{\circ}\text{C}$  for one week for use in experiments. Measured amounts of the stock solution were used according to the body weight of the animal and the dose of venom.

#### Experiments using the isolated perfused kidney model (Protocol 1)

In this protocol  $2\text{LD}_{50}$  doses ( $\text{LD}_{50} = 1.5 \text{ mg/kg}$  body weight for the intravenous route) of Russell's viper venom (RVV) was injected subcutaneously into healthy rabbits (6). After injection of venom animals were observed for 1 h ( $n=5$ ), 2 h ( $n=5$ ), 3 h ( $n=7$ ) and 4 h ( $n=8$ ). At the end of each observation period animals were anaesthetised for removal of the kidneys. Anaesthesia was induced using intravenous thiopentone sodium (20 mg/kg body weight) and during the dissection anaesthesia was maintained by ether inhalation using a mask (7). A separate set of control animals ( $n=10$ ) were injected with 0.9% sodium chloride solution and subjected to the same experimental procedure.

Once the animal was anaesthetised, a mid ventral laparotomy was performed. The renal vessels were clamped and the left kidney was removed along with the ureter and renal vessels. The ureter was cut leaving about 5-7 cm from the kidney. The right kidney was also removed at the same time to get the wet weight of the kidney and for pathological examination.

Immediately after removal the left kidney was perfused with the perfusate solution using a 20 gauge scalp vein set in order to remove blood from the kidney. Then it was connected to the isolated perfused kidney apparatus where the perfusate was maintained at  $37^{\circ}\text{C}$ .

The isolated perfused kidney (IPK) model used in

these experiments was set up according to the method described for the determination of reperfusion damage and renal preservation (8) and modified in our laboratory (5). The perfusate solution used in this apparatus had the following composition ( $\text{Na}^+$  155 mmol/l,  $\text{K}^+$  6.8 mmol/l,  $\text{Ca}^{2+}$  2.0 mmol/l,  $\text{Cl}^-$  139 mmol/l,  $\text{HCO}_3^-$  25.0 mmol/l,  $\text{H}_2\text{PO}_4^-$  1.5 mmol/l, glucose 5.0 mmol/l, creatinine 0.2 mmol/l, mannitol 100.0 mmol/l and bovine serum albumin 1g/l). The osmolality of the perfusate solution was 400 mOsm/kg. After preparation of the perfusate solution, until the experiment was over, this solution was continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide.

After mounting the kidney the IPK apparatus was operated for about 15 minutes until a steady flow of urine was established. After this stabilization period the machine was operated for 1 hour, during which urine, perfusate and effluent samples were collected at 15 minute intervals (15, 30, 45 and 60 minutes). During the initial 15 minute period the perfusion pressure was maintained between 100 and 120 mm Hg by adjustment of the flow rate of the perfusate delivered to the kidney. After that arterial pressure was kept constant at 120 mm Hg.

Urine and perfusate samples collected were used to assess the renal function. Renal function was assessed using urine flow rate (UFR), perfusate flow rate (PFR), creatinine clearance ( $\text{Ccr}$ ), percentage tubular reabsorption of glucose (PTRG), proteinuria and the percent fractional excretion of  $\text{Na}^+$  and  $\text{K}^+$  ( $\text{PFNa}^+\text{E}$ , and  $\text{PFK}^+\text{E}$ ). Creatinine, glucose and protein in the samples were determined spectrophotometrically using reagent kits and  $\text{Ccr}$ , PTRG and proteinuria were calculated (4, 8).  $\text{Na}^+$  and  $\text{K}^+$  were determined by flame photometry and  $\text{PFNa}^+\text{E}$  and  $\text{PFK}^+\text{E}$  were calculated (8). N-acetyl- $\beta$ -D glucosaminidase enzyme (NAG) level in urine was determined spectrophotometrically to assess the renal tubular cell integrity following injection of venom (9).

Pieces from the right kidney of rabbits were fixed in modified Boins solution and with 2% glutaraldehyde for light microscopy (LM) and electron microscopy (EM) respectively. Pathological changes in kidneys are expressed as percentages. The percentage of changes (degeneration or

necrosis) were categorized as described below after examining ten fields under low and high dry power ( $10 \times 20$ ,  $10 \times 40$ ) 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

### Experiments using the kidney slice model (Protocol 2)

Kidneys from healthy rabbits were removed as described in the previous experiments. 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. Kidney slices were incubated with 10 mg/ml of RVV for 1, 2, 3 and 4 hours ( $n=5$  for each) and LM and EM changes were studied. Kidney slices incubated with 0.9% sodium chloride solution were used as the control.

### Statistical Analysis

The data are expressed as means  $\pm$  standard deviation (SD) and a  $p$  value less than 0.05 was considered to be statistically significant. Results (except pathological findings) were initially analysed by Kruskal-Wallis test (ANOVA). When the differences of means between groups were statistically significant (when the calculated  $H$  value was significant) Wilcoxon's rank sum test was applied in order to identify the significant groups.

### Results

#### Experiments using the IPK model (Protocol 1)

In this protocol experiments were carried out to determine the time related effect of venom on renal function, renal tubular cell integrity and renal pathology.

### Effect of venom on renal function

The glomerular and tubular functions were determined using UFR, Ccr, proteinuria, PTRG, PFNa<sup>+</sup>E and PFK<sup>+</sup>E. The mean values for these measurements in the control and experimental groups are given in Tables 1, 2 and 3.

The control group showed a gradual increase in the UFR during 1 hour of perfusion (Table 1). The experimental animals showed a progressive decrease in the UFR. This decrease was highest in the animals who had the longest exposure to the venom (*i.e.* those killed 4 hours after envenomation). Similar to the UFR, the Ccr showed a progressive decrease with time and the fall of Ccr was highest in animals which had the longest exposure to the venom (Table 1).

The PTRG showed a progressive decrease in the control and experimental groups. However the decrease was much greater in the experimental group compared to the controls. The fall in PTRG was highest in the animals who had the longest exposure to venom (Table 2). Proteinuria, which was expressed as a percentage of the perfusate protein concentration, was significantly higher during first and last 15 minutes of perfusion in animals who were exposed to the venom for 4 hours (Table 2).

Both PFNa<sup>+</sup>E and PFK<sup>+</sup>E showed a gradual increase during 1 hour of perfusion in both the control and experimental groups. However, allowing for changes in control animals with time, the experimental animals who were exposed to the venom for the longest period showed a significant increase in both PFNa<sup>+</sup>E and PFK<sup>+</sup>E (Table 3).

There was no significant change in the PFR (ml/min/g wet weight of the kidney) in the control and experimental groups during perfusion. In the control group ( $n=10$ ) PFR varied between  $1.42 \pm 0.42$ ,  $1.44 \pm 0.39$ ,  $1.42 \pm 0.42$ , and  $1.43 \pm 0.42$  during 1 hour of perfusion. The PFR values for the experimental groups were  $1.30 \pm 0.53$ ,  $1.28 \pm 0.48$ ,  $1.33 \pm 0.50$  and  $1.38 \pm 0.49$  at 1 hour,  $1.40 \pm 0.19$ ,  $1.39 \pm 0.20$ ,  $1.41 \pm 0.19$  and  $1.43 \pm 0.16$  at 2 hours,  $1.47 \pm 0.28$ ,  $1.48 \pm 0.32$ ,  $1.50 \pm 0.34$  and  $1.50 \pm 0.37$  at 3 hours and  $1.41 \pm 0.21$ ,  $1.55 \pm 0.29$ ,  $1.46 \pm 0.25$  and  $1.46 \pm 0.27$  at 4 hours after injection of venom respectively.

**Table 1. Urine flow rate (UFR) and creatinine clearance (Ccr) in rabbits after injection of Russell's viper venom during 15, 30, 45 and 60 minutes of perfusion in the isolated perfused kidney model**

		No. of animals	UFR and Ccr (ml/min/g wet weight of the kidney)			
			15 min	30 min	45 min	60 min
Control	10	UFR	0.10±0.03	0.15±0.07 <sup>1</sup>	0.19±0.07 <sup>1</sup>	0.21±0.08 <sup>1</sup>
		Ccr	0.18±0.07	0.20±0.07	0.24±0.09	0.23±0.08
Experimental						
1 h	5	UFR	0.21±0.12*	0.23±0.10*	0.21±0.11	0.18±0.12
		Ccr	0.39±0.19*	0.29±0.11*	0.26±0.11	0.17±0.11
2 h	5	UFR	0.12±0.02	0.15±0.02	0.16±0.03	0.14±0.03
		Ccr	0.29±0.13*	0.23±0.10	0.18±0.07	0.13±0.07*
3 h	5 <sup>a</sup>	UFR	0.16±0.08	0.16±0.06	0.14±0.03	0.12±0.04*
		Ccr	0.21±0.03	0.21±0.05	0.17±0.06	0.14±0.04
4 h	5 <sup>b</sup>	UFR	0.04±0.01*	0.05±0.01*	0.05±0.01*	0.05±0.01*
		Ccr	0.06±0.02*	0.06±0.02*	0.05±0.02*	0.04±0.01*
<hr/>						
1. <i>H</i> value (ANOVA) -		UFR	16.43	15.68	14.74	15.18
		Ccr	19.98	15.78	14.99	15.17

2. \* Significantly different from the control ( $p < 0.05$ , Wilcoxon's rank sum test on unpaired data).

3. <sup>1</sup> The UFR of the control animals at 30, 45 and 60 minutes are significantly different from the value at 15 minutes ( $p < 0.05$ ).

4. <sup>a</sup> In the experiments done 3 hours after venom injection, one animal died and another did not produce urine. This data was not included in the analysis.

5. <sup>b</sup> In the experiments done at 4 hours after venom injection, one animal died and another two experiments did not produce urine. This data was not included in the analysis.

**Table 2. Percentage tubular reabsorption of glucose (PTRG) and proteinuria in rabbits after injection of Russell's viper venom during 15, 30, 45 and 60 minutes of perfusion in the isolated perfused kidney model**

		No. of animals	PTRG and proteinuria (expressed as a percentage of the perfusate protein concentration)			
			15 min	30 min	45 min	60 min
Control	10	PTRG	59.8±8.44	47.5±8.51 <sup>1</sup>	40.4±11.03 <sup>1</sup>	30.1±7.67 <sup>1</sup>
		Protein	99.2±5.05	103.1±4.54	103.9±13.4	94.1±6.68
Experimental						
1 h	5	PTRG	68.2±7.64	51.4±7.22 <sup>1</sup>	39.3±10.50 <sup>1</sup>	20.8±3.22 <sup>1*</sup>
		Protein	97.2±2.45	99.4±4.44	100.6±2.89	96.2±3.98
2 h	5	PTRG	63.9±5.79	45.7±9.85 <sup>1</sup>	37.0±11.43 <sup>1</sup>	25.6±9.56 <sup>1</sup>
		Protein	91.9±7.79	96.2±6.1*	99.5±2.92	100.7±4.75
3 h	5 <sup>a</sup>	PTRG	61.4±7.02	38.7±13.13	37.9±11.00 <sup>1</sup>	9.6±6.53 <sup>1*</sup>
		Protein	95.3±4.52	98.2±2.09*	100.6±6.68	98.0±8.69
4 h	5 <sup>b</sup>	PTRG	25.6±0.7*	18.8±2.01 <sup>1*</sup>	14.7±1.66 <sup>1*</sup>	4.2±3.43 <sup>1*</sup>
		Protein	116.7±3.98*	107.3±4.79 <sup>1</sup>	102.4±5.02 <sup>1</sup>	111.8±9.97*

1. *H* value (ANOVA) – PTRG 14.13 14.56 12.22 22.20  
Protein 15.03 12.03 0.613 11.377

2. \* Significantly different from the control ( $p < 0.05$ , Wilcoxon's rank sum test on unpaired data).

3. <sup>1</sup> The PTRG of the control and experimental animals and proteinuria at 4 hours after venom injection at 30, 45 and 60 minutes are significantly different from the value at 15 minutes ( $p < 0.05$ ).

4. <sup>a</sup> In the experiments done 3 hours after venom injection, one animal died and another did not produce urine. This data was not included in the analysis.

5. <sup>b</sup> In the experiments done at 4 hours after venom injection, one animal died and another two experiments did not produce urine. This data was not included in the analysis.

**Table 3. Percent fractional excretion of Na<sup>+</sup>/K<sup>+</sup> (PFNa<sup>+</sup>E/ PFK<sup>+</sup>E) in rabbits after injection of Russell's viper venom during 15, 30, 45 and 60 minutes of perfusion in the isolated perfused kidney model**

	No. of animals		PFNa <sup>+</sup> E and PFK <sup>+</sup> E			
			15 min	30 min	45 min	60min
Control	10	PFNa <sup>+</sup> E	56.7±16.82	70.7±15.24 <sup>1</sup>	76.8±14.21 <sup>1</sup>	85.3±7.95 <sup>1</sup>
		PFK <sup>+</sup> E	70.1±18.28	87.2±26.22	95.2±22.54 <sup>1</sup>	107.2±18.61 <sup>1</sup>
Experimental						
1 h	5	PFNa <sup>+</sup> E	56.4±11.04	80.0±11.52 <sup>1</sup>	82.1±12.44 <sup>1</sup>	104.1±8.12 <sup>1*</sup>
		PFK <sup>+</sup> E	85.6±18.24	100.4±11.42	103.0±5.55	115.1±4.96 <sup>1</sup>
2 h	5	PFNa <sup>+</sup> E	49.8±13.04	64.5±4.97 <sup>1</sup>	82.7±14.38 <sup>1</sup>	92.7±14.29 <sup>1</sup>
		PFK <sup>+</sup> E	73±21.2	85.5±8.4	105.8±11.26 <sup>1</sup>	106.3±13.28 <sup>1</sup>
3 h	5 <sup>a</sup>	PFNa <sup>+</sup> E	69.8±7.07	81.6±7.11 <sup>1</sup>	90.8±6.11 <sup>1</sup>	97.4±3.57 <sup>1*</sup>
		PFK <sup>+</sup> E	82.4±10.28	90.4±10.08	104.1±7.75 <sup>1</sup>	116.4±10.91 <sup>1</sup>
4 h	5 <sup>b</sup>	PFNa <sup>+</sup> E	82.8±3.18*	95.9±2.03 <sup>1*</sup>	101.8±2.32 <sup>1*</sup>	123.5±7.29 <sup>1*</sup>
		PFK <sup>+</sup> E	104.9±6.18*	117.1±1.08 <sup>1*</sup>	123.5±6.33 <sup>1*</sup>	148.8±8.17 <sup>1*</sup>
1. H value (ANOVA) -		PFNa <sup>+</sup> E	13.89	16.3	13.84	18.66
		PFK <sup>+</sup> E	11.75	12.53	11.41	13.46

2. \* Significantly different from the control ( $p < 0.05$ , Wilcoxon's rank sum test on unpaired data).

3. <sup>1</sup> The PFNa<sup>+</sup>E and PFK<sup>+</sup>E of the control and experimental animals at 30, 45 and 60 minutes are significantly different from the value at 15 minutes ( $p < 0.05$ ).

4. <sup>a</sup> In the experiments done 3 hours after venom injection, one animal died and another did not produce urine. This data was not included in the analysis.

5. <sup>b</sup> In the experiments done at 4 hours after venom injection, one animal died and another two experiments did not produce urine. This data was not included in the analysis.

**Table 4. N-acetyl- $\beta$ -D-glucosaminidase enzyme (NAG) in urine of rabbits after injection of Russell's viper venom during 15, 30, 45 and 60 minutes of perfusion in the isolated perfused kidney model**

	No. of animals	NAG(U/mg of urinary creatinine)			
		15 min	30 min	45 min	60min
Control	10	0.56 $\pm$ 0.16	0.70 $\pm$ 0.43	0.53 $\pm$ 0.28	0.93 $\pm$ 0.57
Experimental					
1 h	5	1.40 $\pm$ 1.18	2.69 $\pm$ 2.09*	2.43 $\pm$ 0.88*	3.70 $\pm$ 1.77*
2 h	5	1.86 $\pm$ 1.17*	1.97 $\pm$ 1.04*	3.57 $\pm$ 2.17*	4.99 $\pm$ 1.59 <sup>1</sup> *
3 h	5 <sup>a</sup>	2.22 $\pm$ 0.98*	4.19 $\pm$ 1.82*	4.77 $\pm$ 0.89 <sup>1</sup> *	5.84 $\pm$ 2.03 <sup>1</sup> *
4 h	5 <sup>b</sup>	3.12 $\pm$ 0.54*	5.26 $\pm$ 1.88 <sup>1</sup> *	9.03 $\pm$ 1.55 <sup>1</sup> *	16.73 $\pm$ 3.99 <sup>1</sup> *

1. *H* value (ANOVA) – 18.31 18.93 25.16 24.51

2. \* Significantly different from the control ( $p < 0.05$ , Wilcoxon's rank sum test on unpaired data).

3. <sup>1</sup> The NAG of the control and experimental animals at 30, 45 and 60 minutes are significantly different from the value at 15 minutes ( $p < 0.05$ ).

4. <sup>a</sup> In the experiments done 3 hours after venom injection, one animal died and another did not produce urine. This data was not included in the analysis.

5. <sup>b</sup> In the experiments done at 4 hours after venom injection, one animal died and another two experiments did not produce urine. This data was not included in the analysis.

### Effect of venom on renal tubular cell integrity and renal pathology

Urinary NAG levels were used to determine the effect of venom on renal tubular cell integrity. The concentration of this enzyme was very low in the control group during perfusion. A progressive increase of concentration of NAG in urine with time was observed. The excretion of the enzyme was highest in animals who were exposed to the venom for the longest period, *i.e.* 4 hours (Table 4).

### Light microscopy

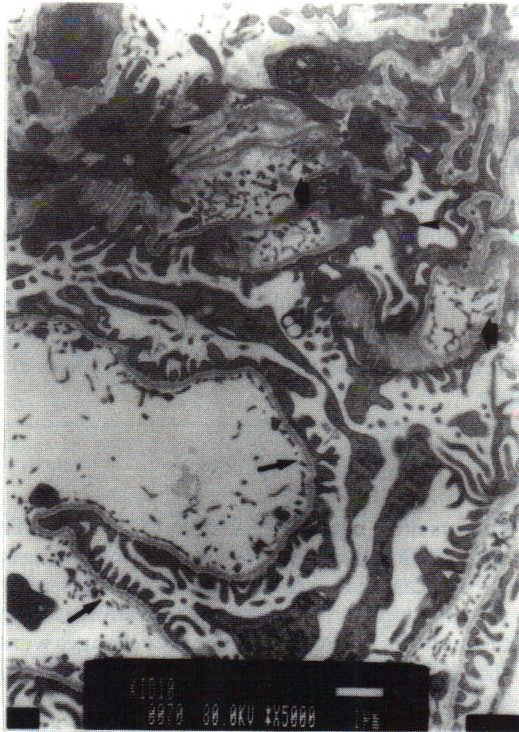
Congestion of the glomerular tuft and occasional pyknotic nuclei were noticed in the glomeruli

4 hours after injection of venom ( $n=8$ ). The cells of the PCT showed severe degenerative changes with occasional foci of necrosis 4 hours after venom injection. Necrotic tissue was observed in the lumen of the PCT. Degenerative changes were seen in the cells of the DCT and collecting ducts. The renal medulla was congested and in a few areas there was necrosis of the collecting ducts. Clots or fibrin threads were not observed in the capillaries. Pigment casts were not observed in the tubular lumen.

Normal cellularity was noticed in the glomeruli of the control specimens. No degenerative or necrotic changes were observed in the cells of the proximal convoluted tubule (PCT) and distal convoluted tubule (DCT). Medulla appeared normal in the control specimens.

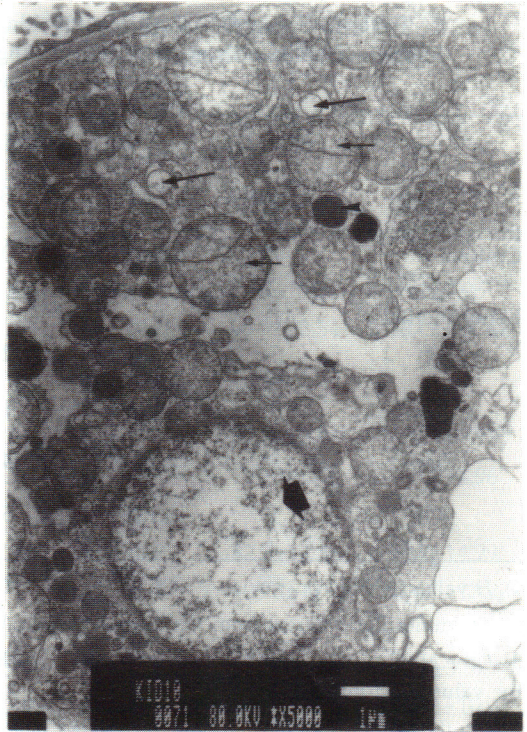
### Electron microscopy

At 3 (n=7) and 4 hours (n=8) after injection of venom few capillary loops of the glomerular tuft and some podocytes were constricted and there was appearance of vacuoles in the podocytes. In some glomerular capillary loops endothelial cell lining was sloughing (Figure 1). There was swelling of mitochondria with disappearance of mitochondrial cristae in the cells of the PCT. In some specimens the pattern of mitochondrial cristae has completely disappeared (Figure 2).



**Figure 1.** Electron micrograph of kidney specimen obtained from rabbit 4 hours after injection of Russell's viper venom. Endothelial cells of the capillaries of the glomerular tuft show sloughing (arrows); constricted appearance of some capillary loops (small arrowheads) and podocytes (large arrowheads) are seen.

Magnification  $\times 10000$ ; bar =  $1\mu\text{m}$



**Figure 2.** Electron micrograph of kidney specimen obtained from rabbit 4 hours after injection of Russell's viper venom. A proximal convoluted tubule cell with prominent intracellular oedema, disappearance of mitochondrial cristae (small arrows) swelling of mitochondria, intracytoplasmic vacuolation (large arrows), appearance of dense bodies (small arrowhead) and dissolution of nuclear material (large arrowhead) is seen.

Magnification  $\times 10000$ ; bar =  $1\mu\text{m}$

Appearance of glomeruli was normal in the control specimens. In the cells of the PCT, the generally elongated shape of mitochondria and the regular arrangement of the microvilli of the brush border was clearly seen. Chromatin material of the nucleus was not clumped.

### Experiments using the kidney slice model (Protocol 2)

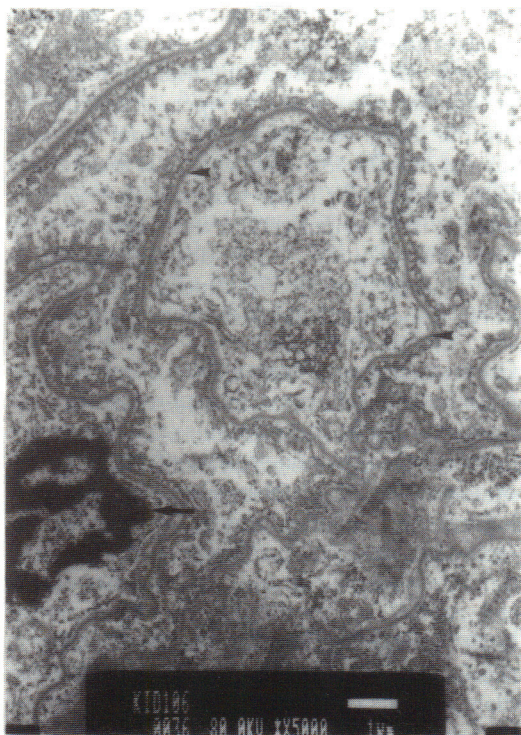
#### Light microscopy

At 3 and 4 hours after incubation of kidney slices with venom, there was necrosis of the glomerular



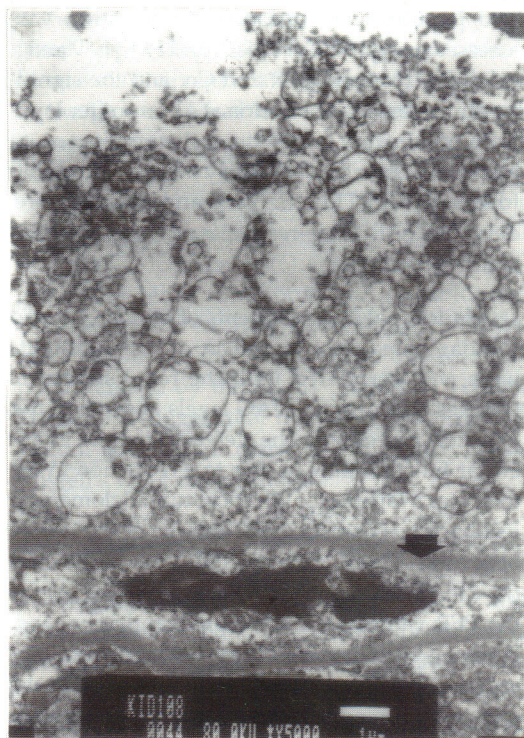
tuft of the surface glomeruli and presence of pyknotic nuclei in the rest of the glomeruli. At 2, 3 and 4 hours after incubation, there was complete necrosis (100%) of the PCT. Four hours after incubation of the rabbit kidney slices with venom, there was complete necrosis of all cortical structures (PCT, DCT and glomeruli). The renal medulla appeared normal in the specimens.

Glomeruli appeared normal in the control specimens. There were mild degenerative changes in the PCT cells. There were no necrotic changes.



**Figure 3.** Electron micrograph of a rabbit kidney specimen 4 hours after incubation with Russell's viper venom. The ultrastructure of a glomerulus is completely destroyed. The intact basement membrane (arrowhead) is seen. The arrow points to clumped chromatin in the nucleus of a white blood cell.

Magnification  $\times 10000$ ; bar =  $1\mu\text{m}$



**Figure 4.** Electron micrograph of a rabbit kidney specimen 4 hours after incubation with Russell's viper venom. The ultrastructure of the proximal convoluted tubular cell is completely destroyed. The basement membrane is intact (arrowhead).

Magnification  $\times 10000$ ; bar =  $1\mu\text{m}$

### Electron microscopy

Complete destruction of the structure of the glomerular tufts and the cells of the PCT was seen after incubation of kidney slices with venom (Figures 3 and 4). Glomeruli were normal in appearance in the control specimens. Swollen mitochondria and disappearance of cristae were noticed in a few PCT cells.

### Discussion

In Protocol 1, the time related effects of Russell's viper venom on glomerular and tubular function and renal tubular cell integrity were assessed. Glomerular function was assessed using UFR, Ccr

and proteinuria, and tubular function was assessed using PTRG, PFNa<sup>+</sup>E and PFK<sup>+</sup>E. The significantly reduced UFR and Ccr and the significantly increased proteinuria at 4 hours after injection of venom suggests a derangement of glomerular function. Similarly the tests of tubular function (i.e. PTRG, PFNa<sup>+</sup>E and PFK<sup>+</sup>E) were, deranged. All these results indicate glomerular and tubular damage.

The functional measurements of glomerular and tubular damage observed in our experiments were well correlated with the renal structural changes and urinary NAG levels observed after injection of venom. The deterioration of glomerular function may be due to a reduction in the total area available for filtration, tubuloglomerular feedback mechanism, back leakage of the filtrate or several of these reasons. Reduction in the fluid flow to the kidney cannot be a reason for this as the PFR values were not significantly changed. Interestingly, when the ultrastructure of the glomerulus was studied after injection of venom, the endothelial lining in some areas of the glomerulus was sloughing. On histological examination there were pyknotic nuclei in the glomeruli. These are good indications for the destruction of the glomerular structure following envenomation. This reduces available surface area for filtration. There were severe degenerative and necrotic changes in the cells of the PCT. Obstruction to the tubular flow by the presence of degenerated and necrotic material in the tubular lumen exerts a back pressure towards the glomeruli. This tubuloglomerular feedback mechanism is also a possible reason for the reduction in the glomerular filtration. In addition to these, a variable degree of change was observed for UFR in the control and experimental groups at 1, 2 and 3 hours after injection of venom. This may be due to the structural damage that took place in the thick ascending limb of the loop of Henle in the form of mitochondrial swelling during perfusion with an acellular medium leading to failure in urine concentrating ability of the kidney (10, 11, 12).

On the other hand the significant time related changes observed in the tubular function (PTRG,

PFNa<sup>+</sup>E, PFK<sup>+</sup>E) indicate an injury to the PCT reducing the reabsorptive capacity. The cells of the PCT are active sites of renal metabolism and are important for Na<sup>+</sup>, K<sup>+</sup> and glucose reabsorption from the filtrate. When the kidney is perfused with an acellular medium the cells of the PCT are subjected to the effects of hypoxic damage reducing their functional capacity (4, 11). The measurement of renal tubular cell integrity (urinary NAG levels) is good evidence for continuous structural damage taking place at the PCT during perfusion. This enzyme is mainly located in the lysosomal fraction of the PCT cells (13). When cells are damaged this enzyme is released and finally it is excreted in the urine. Increased levels of urinary NAG were found in patients without any renal functional abnormality following envenomation by Russell's viper venom indicating the direct toxic effect of venom on renal tubular cells (14, 15). Findings similar to ours have been reported by other workers. For example when Russell's viper venom was included in the perfusate medium a rapid, dose related fall in inulin clearance and a dose dependent increase in PFNa<sup>+</sup>E and PFK<sup>+</sup>E has been observed (4).

Other than direct damage the other reasons considered as aetiogenic for nephrotoxicity were immunological mechanisms, renal haemodynamic changes, non-specific effects of venom or a combination of these. However, immunological mechanism cannot be a contributory factor for the renal functional and structural damage observed in our experiments as the changes have occurred within a few hours. When renal haemodynamic changes are considered as causing the nephrotoxicity one of the main mechanisms would be by renal ischaemia. In these experiments we did not make measurements of renal blood flow. However in the IPK apparatus the perfusate flow rate values in the control and experimental groups were not significantly different and the perfusion pressure was held constant during 1 hour of perfusion. These observations indicate that ischaemia is unlikely. However these observations are insufficient to exclude ischaemia definitely. The absence of fibrin threads and micro clots in the renal vessels

and lack of pigment casts in the renal tubules of rabbits tends to exclude non specific effects on renal structure and function. Hence, direct toxicity of venom is the likely explanation for the observed structural changes and functional changes.

The light microscopic changes seen in kidneys were also not suggestive of ischaemic damage. During the 1, 2, 3 and 4 hour observation periods following injection of venom the majority of animals did not show any changes that suggested that they were in hypotension or shock. They had to be anaesthetised prior to removal of the kidneys.

The severe degenerative and necrotic changes observed after injection of venom were mostly confined to the cells of the PCT although there were some changes in the glomeruli, DCT and collecting ducts also. The proximal tubules were affected equally and evenly and the underlying basement membrane of the cells was well preserved. These pathological findings do not suggest ischaemia. If ischaemia was responsible we would have seen collapsed architecture of the tubules due to the damaged basement membrane and an increased interstitial space between tubules (16). The data from our experiments point that renal haemodynamic changes to be unlikely as an aetiological factor for the kidney damage. However 2 animals each died 3 and 4 hours after injection of venom and no reason was found for their deaths.

The pathological findings of Protocol 1 were well supported by the findings of the Protocol 2. When rabbit kidney slices were incubated in Russell's viper venom (10 mg/ml) complete necrosis of all the cortical structures (PCT, DCT and glomeruli) was observed at 4 hours. However the basement membranes of the cells were intact. The renal medulla appeared normal. Histological observations were well correlated with the ultrastructural changes in the PCT and the glomeruli (complete destruction of the ultrastructure with the preservation of the basement membrane). Renal ischaemia due to non-specific effects or alterations

in the renal blood flow cannot be a reason for the observed pathological changes in this experiment. More extensive renal damage including damage to the glomeruli has been observed when rat kidney slices were incubated with 5 mg/ml of Russell's viper venom (17). Another research group has observed necrosis of PCT in the rabbit kidney slices when incubated in 5 and 10 mg/ml of Russell's viper venom (6). All this evidence suggests that renal functional and pathological changes observed after injection of Russell's viper venom are more likely to be due to direct renal damage by the toxins present in venom. These data suggest that the direct toxic effect of venom is the main cause of nephrotoxicity in Russell's viper envenomation.

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