นิพนธ์ต้นฉบับ

Urinary enzymes and renal function changes following VIPERA RUSSELLI SIAMENSIS snake venom injection in rats.*

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The effects of Russell's viper venom (RVV) on urinary excretion of 4 enzymes and renal function changes were studied in 2 groups of 24 Wistar strain rats. Twelve control and 12 experimental rats were injected intraperitoneally with normal saline 1 ml/kg and RVV 1 mg/ml-kg body weight (bw) respectively. Urine collection was done in the period of 12 hours following injection. At the end of 12 hours, cardiac blood samples were taken after the rats were anesthetized with inactin at 120 mg/kg bw.

In the period of 12 hours following injection, the glomerular filtration rate (GFR) of experimental rats decreased significantly, whereas the urinary excretion of total protein increased ($p < .001$) when compared with control rats. The fractional excretion of sodium and potassium also increased significantly ($p < .001$). The urinary excretion of proximal tubular cytosolic enzyme fructose-1,6-biphosphatase (FBP), lysosomal N-acetyl-β-glucosaminidase (NAG) and glutathione-S-transferase (GST) rose drastically, while the pyruvate kinase (PK) fell significantly ($p < .001$).

From this study, both glomerular and tubular functions were reduced following venom injection. The enhanced urinary excretion of total protein and the reduction of GFR may have been due to the toxic damage of glomerular membrane leading to protein leakage into the urine. The increased proximal tubular enzyme excretion along with the fractional excretion of sodium and potassium might have reflected some destruction of proximal tubular cells. We concluded that RVV does exert a primary nephrotoxic action on both glomerular and tubular parts of the nephron. However, the secondary effect from its general circulation may also play a role.

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บังอร ชมเดช, Pfaller W. ผลของพิษงูแมวเซาไทยต่อเอ็นซัยม์ในปัสสาวะและหน้าที่ของไตในหนูแรท. จุฬาลงกรณ์เวชสาร 2531 มิถุนายน ; 32(6) : 573-578

ศึกษาผลของพิษงูแมวเซาไทยในหนูแรทพันธุ์วิสตาร์ 24 ตัว โดยแบ่งหนูออกเป็น 2 กลุ่มเท่า ๆ กัน กลุ่มควบคุมฉีดน้ำเกลือปกติ กลุ่มทดลองฉีดพิษงูขนาด 1 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม โดยละลายในน้ำเกลือปกติเข้าทางช่องท้อง เก็บปัสสาวะในหนูทั้งสองกลุ่มตลอดระยะเวลา 12 ชั่วโมงภายหลังฉีด เมื่อครบ 12 ชั่วโมง นำหนูมาทำให้สลบโดยฉีดอินแอคติน 120 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม เจาะเลือดจากหัวใจนำไปปั่นแยกเอาพลาสมาออกทันทีทันใด

ผลการทดลองพบว่าการทำงานของไตในหนูกลุ่มที่ฉีดพิษงูลดลงไปอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่มควบคุม ($p < .001$) อัตราการกรองของไตลดลงพร้อมกับมีโปรตีนในปัสสาวะเพิ่มขึ้นแสดงถึงมีการทำลายผนังโกลเมอรูไล อัตราการขับถ่ายต่ออัตราการกรองของโซเดียมและโปแตสเซียมเพิ่มขึ้น พร้อมกับมีการขับถ่ายเอ็นซัยม์ของหลอดไตส่วนต้นในปัสสาวะมากขึ้นด้วย จึงพอจะสรุปได้ว่าพิษงูแมวเซามีผลทั้งโกลเมอรูไลและหลอดไตส่วนต้นโดยตรง ผลเสริมจากการเปลี่ยนแปลงของระบบไหลเวียนเลือดก็น่าจะมีส่วนเกี่ยวข้องด้วย

Clinical observations have shown that renal involvement is common following Russell's viper bites.[1,2] The pathological changes of the kidney including acute tubular necrosis and cortical necrosis,[3,4] severe glomerulonephritis[4] and acute interstitial nephritis[5] have been reported.

Many investigators believed that acute renal failure is secondary to intravascular hemolysis and intravascular coagulation.[3,5] The hypotension following envenomation can cause a sudden decrease in renal blood flow leading to acute ischemic renal failure.[7,9] However, acute renal failures have developed in some patients without hypotension[6] and no correlation has been found between the severity of renal failure and coagulopathy.[3]

Enzymes along the nephron have been recorded in some conditions.[9-11] In patients with tubulointerstitial disease, enzymuria was frequent even in the absence of proteinuria.[12,13] Fructose-1,6,-biphosphatase (FBP), glutathione-S-transferase (GST), N-acetyl-β-glucosaminidase (NAG) are enzymes associated with the proximal tubular cells, while the pyruvate kinase (PK) is associated with the distal tubular cell functions.[11,14]

The increased urinary excretion of renal enzymes in response to nephrotoxins may be indicative of various subcellular effects.[15] NAG enzymuria is a sensitive indicator of the activity of renal disease and may prove to be a suitable screening test for significant renal diseases or injury,[13,15,16] since it has been shown to be present in the renal tubules and to have a sufficiently high molecular weight not to ordinarily be filtered by the glomerulus.[13] The high urinary excretion could indicate the conditions that produce primary glomerular as well as tubular damage.[13]

Renal function changes following injection of RVV in an animals have been reported.[7,17,18] The direct or indirect toxicity or both have been attempted to be elucidated. This study investigated the role of venom on urinary excretion of four enzymes which may point out the mechanism of nephrotoxicity of the venom.

Materials and Methods

Four urinary enzymes and the renal functions were performed in the 2 groups of 24 Wistar strain rats, weighing 180-240 gm. Rats were fed with normal pellets in metabolic cage 2 days before the experiment. On the day of experiment, 12 control rats were injected with normal saline solution 1 ml/kg bw and 12 experimental rats were injected with lyophilized crude RVV, dissolved in normal saline 1 mg/ml-kg bw intraperitoneally. Urine samples were collected over a 12 hour period following injection. At the end of 12 hours, rats were anesthetized intraperitoneally with inactin 120 mg/kg bw. The whole cardiac blood was collected, centrifuged and separated for plasma sample at once.

Routine measurements of creatinine, sodium and potassium concentrations in plasma and urine were done. Total protein concentration in urine was also examined. Urinary enzyme activities were detected as described by Kotanko et al.[19] One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol reaction product per minute. Enzyme activities were related to urinary creatinine concentration in order to correct the differences in urinary volume output.[13, 19]

The glomerular injury was indicated by the urinary excretion of protein, and the glomerular filtration rate was expressed as the clearance of creatinine. The tubular damage was exhibited by the fractional excretion of sodium, potassium and also 4 urinary enzyme activities.

The results were demonstrated as mean ± S.D. The differences were analyzed by Student's unpaired t - test.

Results

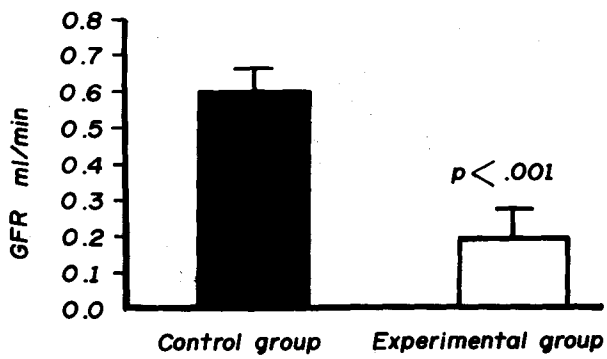

Figure 1 The Effect of Russell's Viper Venom on Glomerular Filtration Rate (GFR)

As illustrated in figure 1, in the 12 hour period following the injection of normal saline and Russell's viper venom in control and experimental groups of rats, the glomerular filtration rate of the experimental rats fell significantly ($p < .001$) when compared with the control rats.

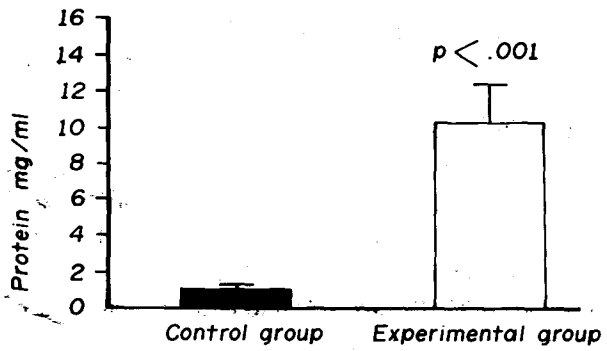

Figure 2 The Effect of Russell's Viper Venom on Urinary Total Protein.

Figure 2. showed the significant enhancement of urinary total protein during the 12 hours in the experimental rats compared to the control rats during the same period.

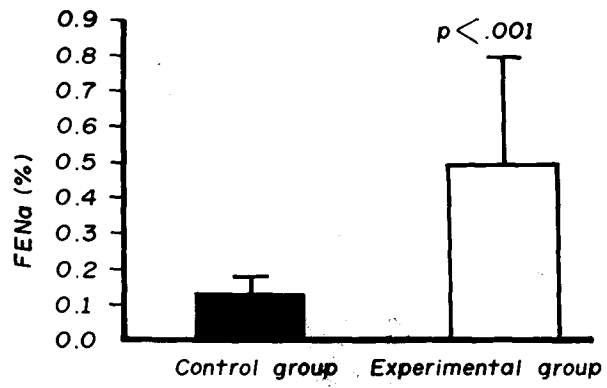

Figure 3 The Effect of Russell's Viper Venom on Fractional Excretion of Sodium (FENa)

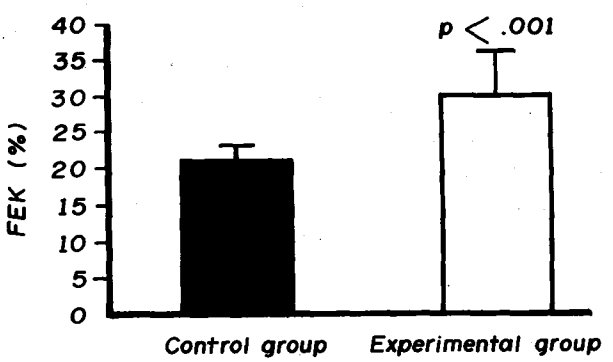

Figure 4 The Effect of Russell's Viper Venom on Fractional Excretion of Potassium (FEK)

Figures 3 and 4 demonstrated the fractional excretion of sodium and potassium in response to Russell's viper venom. The fractional excretion of both electrolytes of Russell's viper venom injected rats increased significantly when compared with the saline injected rats. The percent changes of fractional excretion of sodium were more than those of potassium.

As shown in the table,the proximal tubular enzymes : fructose-1, 6-biphosphatase, glutathione-S-transferase and N-acetyl-β-glucosaminidase increased drastically in the urine, whereas pyruvate kinase, the distal tubular enzyme, diminished in response to Russell's viper venom injection.

Discussion

The RVV consists of a mixture of toxic proteins and enzymes which have hematotoxic and necrotizing properties.[20,21] Acute renal failure is one of the clinical manifestrations that may result from the toxicity.[22] The renal effects of the venom could be direct, or indirect as a consequence of shock, hemorrhage and vasculopathy.[3, 23, 34]

Table The Effect of Russell's Viper Venom on Urinary Enzymes

Urinary Enzymes	mU/mg. Creatinine		
	Control group	Experimental group	p-value
F-1,6-Biphosphatase (FBP)	2.9 ±0.3	17.4 ± 1.4	< .001
G-S-Transferase (GST)	2.9 ±2.5	14,200 ±6,900	< .001
Pyruvate Kinase (PK)	10.1 ±2.6	1.7 ±0.9	< .001
N-acetyl-β-Glucosaminidase (NAG)	34.1 ±2.8	175.0 ±13.1	< .001

In the present study, as shown in figures 1 and 2, RVV reduced GFR significantly along with producing proteinuria. It was possible that RVV interfered the filtering function by changing the glomerular permeability or surface area.[8] The injurious effect of the venom could be mediated through complement activation,[1, 20] causing glomerular membrane damage, hence resulting in an increase in urinary excretion of proteins.

Metabolism of renal cortex is characterized by fatty acid oxidation and gluconeogenesis. Proximal tubule is the main site of renal gluconeogenesis[9, 11] and is the region in which gluconeogenesis is metabolically adapted.[9] The specific enzymes from different parts of the nephron could be used as a marker of the damage.[10, 11]

NAG possibly indicates the direct leakage with the damaged tubular lysosomes. It is also known that such lysosomes occur in renal tubular epithelial cells.[25, 26] High levels appearing in the urine are always associated with active renal parenchymal diseases, particularly with those resulting from tubular injury.[27]

The kidney contains the highest activities of enzymes involved in glutathione synthesis and degradation, with the highest level in the proximal straight tubule as an important site of renal drug metabolism.[14] The pyruvate kinase, an enzyme catalyzing another irreversible step in the glycolytic pathway has the highest activity along the distal part of the nephron.[11, 14]

As demonstrated in figures 3, 4 and the table, there were significant increases in FENa, FEK and urinary enzymes of proximal tubular cells. The enhancement of FENa and FEK could be due to the inhibition of reabsorption function of proximal tubule by RVV.[17] The probable damage was supported by the fact that urinary excretion of the proximal tubular cytosolic enzyme FBP and the lysosomal NAG were enhanced drastically. However, the decrement of PK in this experiment may help in the postulation that the activity of specific enzymes of glycolytic pathway is inhibited by some mechanisms.[9,14]

The correlation between proteinuria and increased enzymuria was also observed in patients with glomerulonephritis secondary to systemic vasculitis syndromes.[13] RVV has also been reported to produce arteritis and severe glomerulonephritis.[4] The increased urinary excretion of these enzymes in response to nephrotoxins may be indicative of various subcellular toxic effects.[15] Nevertheless, it might be pointed out from this study that RVV plays an important role in damaging the kidney directly both at the glomerulus and proximal tubule, with some degrees of indirect toxicity.

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