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The acute effect of Russell's viper venom on renal functions in rats.

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The renal functions following intraperitoneal injection of 1 mg. per kg. B.W. of Russell's viper venom have been investigated in Wistar rats. Rats were anesthetized with inactin 120 mg. per kg. B.W. intraperitoneally. Arterial blood and urine samples were collected every 30 minutes for three hours following venom injection. After venom injection, the mean arterial pressure (MAP), glomerular filtration rate (GFR) and urine flow rate (UF) declined proportionally, but were followed by a small return. After the second hour, MAP maintained a slight decrease, while GFR and UF gradually decreased until the end of the experiment. The increment of the fractional excretion of sodium (FE.Na) was significant during the second hour, whereas that of potassium (FE.K) rose slightly. These data may indicate that the venom has some important effects in reducing renal functions, both by direct and indirect means, although more detailed mechanisms need to be further investigated.

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ฉีดพิษงูแมวเซาด้วยขนาด 1 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม เข้าทางช่องท้องของหนูขาว 12 ตัว ที่ทำให้สลบด้วยอินแอคตินขนาด 120 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม และศึกษาการทำงานของไตตลอดระยะเวลา 3 ชั่วโมง ภายหลังฉีดพิษงู เก็บตัวอย่างเลือดและวัดความดันโลหิตทางหลอดเลือดแดงฟีมอรัลซ้าย เก็บปัสสาวะทางกระเพาะปัสสาวะผ่านหน้าท้อง วัดอัตราการกรองของไตโดยอาศัยอินนูลิน ทันทีทันใดหลังจากฉีดพิษงูพบว่า ความดันโลหิต อัตราการกรองของไต และการขับถ่ายปัสสาวะลดลงเป็นสัดส่วนกัน หลังจากนั้นจะค่อย ๆ เพิ่มขึ้นแต่ไม่ถึงระดับปกติ ภายหลัง 2 ชั่วโมงพบว่า ความดันโลหิตลดต่ำลงและค่อนข้างคงที่ในขณะที่อัตราการกรองของไต และการขับถ่ายปัสสาวะลดลงเรื่อย ๆ จนสิ้นสุดการทดลอง ในขณะเดียวกัน อัตราการขับถ่ายต่ออัตราการกรองของโซเดียมและโปแตสเซียมสูงขึ้นในชั่วโมงที่สอง หลังจากนั้นจะกลับลดลงอีกเล็กน้อย แต่ยังคงสูงอยู่ จากการทดลองนี้ชี้ให้เห็นได้ว่า พิษงูแมวเซาน่าจะมีผลโดยตรงต่อไต ทำให้การดูดซึมโซเดียมและน้ำกลับได้ลดลงเมื่อเทียบกับอัตราการกรองของไต อย่างไรก็ตามผลทางอ้อมของพิษต่อระบบไหลเวียนเลือดก่อนที่จะทำให้การทำงานของไตลดลงก็อาจจะมีร่วมไปด้วย เพื่อจะอธิบายให้ได้ชัดเจนจำเป็นต้องทำการศึกษาต่อไปอีก

Acute renal failure is an important complication in patients who survive the early effects of severe viper envenomation.[1-4] Clinical observations have demonstrated hematuria, oliguria, anuria, hematemesis, decreased platelets and increased fibrinolytic activity.[2-4] No correlation between severity of renal failure and hemostatic abnormality was shown.[3] Tubular ischemia or intravascular hemolysis might result in tubular necrosis and subsequent renal failure.[4]

Many investigators believed that the snake venom might have some direct cytotoxic effect on renal tubular cells,[5-7] presumably by the very high concentration in the kidneys.[8] Recent work was conducted by Huei-chen Huang in 1984,[9] using phospholipase A_2 isolated from Russell's viper venom.[10] He postulated that the hypotensive effect in rats was probably due to the increase in plasma prostacyclin and thomboxane A_2 levels. He also found the reduction in plasma renin activity.[11] The obstruction of glomerular capillaries by coagulated material could also reduce the blood supply to the tubules. Intravascular clotting can enhance intravascular hemolysis and vice versa,[12-13] creating a vicious cycle which accentuates the severity of renal failure.[4] Acute renal failure could be caused by direct effect of venom on vasculature. Arteritis has been observed. The narrowing of vascular lumen could accelerate intravascular coagulation leading to acute renal failure.[14]

The effect of Russell's viper venom on renal function has been demonstrated extensively in patients some days following envenomation. Very little has been reported from animal models, especially during the earlier period. This study addressed the role of venom on glomerular filtration rate, urine flow and arterial pressure as well as the fractional excretion of sodium and potassium during the first three hours after venom injection.

Materials and Methods

Experiments were performed on 12 Wistar rats, anesthetized with inactin intraperitoneally, 120 milligram per kilogram body weight. Tracheostomy was performed and the animals were allowed to breathe room air spontaneously. A jugular vein catheter was introduced for administration of 1 percent inulin in Tris-Ringer solution at the rate of approximately 20 milliliters per kilogram per hour. Left femoral artery was isolated and catheterized to record the systemic pressure and collect blood samples. Urine flow rate was measured via bladder catheter.

A period of 45 minutes was allowed to elapse for the stabilization of plasma inulin concentration and general conditions, after which two or three timed collections of urine were taken along with arterial blood samples at the midpoint of each urine collection. The venom was injected at the dosage of 1 milligram per kilogram body weight intraperitoneally. Blood and urine samples were taken every thirty minutes for three hours after venom injection.

At the end of the experiment, both kidneys were excised, stripped of surrounding fat and tissue, blotted dry and weighed. The glomerular filtration rate and urine flow rate were expressed as microliters per minute per gram kidney weight.

Routine measurements of sodium and potassium concentrations in plasma and urine were determined by flame photometry. Plasma and urine inulin concentrations were detected by an anthrone colorimetric techique. The results were shown as the value of mean ± S.D. The differences were analyzed by the Student's paired t-test.

The Effect of Russell's Viper Venom on Mean Arterial Pressure (MAP), Glomerular Filtration Rate (GFR) and Urine Flow Rate (UF) on Rats (Mean ± S.D.)

Parameter \ Time (hour)	0	0.5	1.0	1.5	2.0	2.5	3.0
MAP (mm.Hg)	104.50 ± 6.08	82.00 ± 7.28 P<.001	88.50 ± 8.80 P<.001	93.00 ± 9.98 P<.010	97.50 ± 9.87 P<.025	87.50 ± 11.29 P<.005	87.00 ± 13.21 P<.005
GFR (µl / min - gm. kidney weight)	104.83 ±14.71	76.17 ±13.05 P<.001	80.17 ±15.21 P<.001	87.92 ±19.26 NS	79.92 ±13.79 P<.025	72.50 ±10.28 P<.001	65.33 ± 11.53 P<.001
UF (µl / min - gm. kidney weight)	23.37 ± 7.84	7.68 ± 7.43 P<.010	6.82 ± 6.49 P<.005	8.20 ± 5.23 P<.010	8.25 ± 3.83 P<.005	6.81 ± 3.49 P<.001	5.52 ± 3.33 P<.001

NS = Nonsignificance

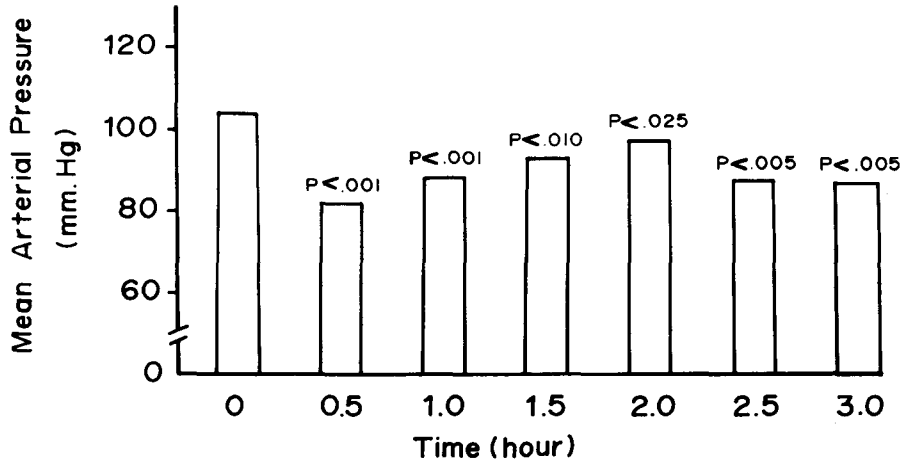

Figure 1 The Effect of Russell's Viper Venom on Mean Arterial Pressure

Results

As illustrated in table and figure 1, following the injection of Russell's viper venom 1 mg per kg body weight intraperitoneally, the mean arterial pressure (MAP) decreased gradually. The MAP decreased from 104.50 ± 6.08 to 82.00 ± 7.28 mm.Hg ($p < .001$) in 30 minutes, and then returned to 97.50 ± 9.87 mm.Hg by the end of the second hour. The decrement in MAP was about 20-30 percent of control value. After 2 hours, it fell again and was maintained at about 87.00 ± 13.21 mm.Hg. The decrement of MAP was statistically significant when compared with that of the control period.

The decrement of glomerular filtration rate (GFR) was seen at 30 minutes following venom injection. GFR dropped from the control value of 104.83 ± 14.71 to 76.17 ± 13.05 microliter per minute per gram kidney weight ($p < .001$). As demonstrated in the table and figure 2, after 30 minutes, GFR increased to 87.92 ± 19.26 microliter per minute per gram kidney weight. The increase was not statistically significant when compared with the control. After one and a half hour, GFR decreased gradually until the end of the experimental period.

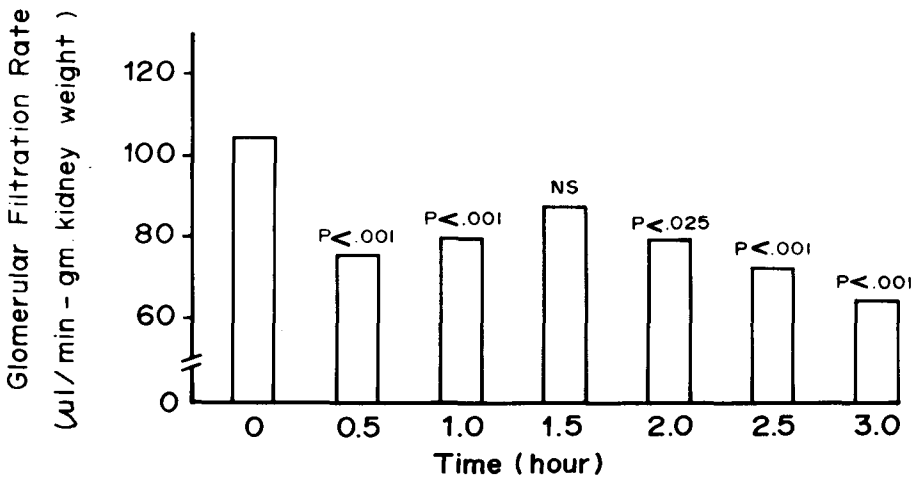

Figure 2 The Effect of Russell's Viper Venom on Glomerular Filtration Rate

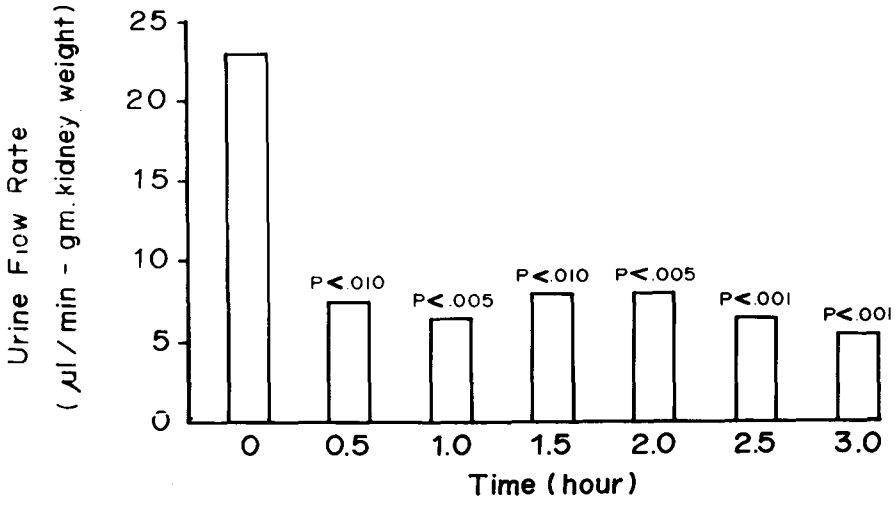

Figure 3 The Effect of Russell's Viper Venom on Urine Flow Rate

The urine flow rate (UF) decreased immediately after venom injection from 23.37 ± 7.84 to 7.68 ± 7.43 and lasted until the end of the first hour (figure 3). During the second hour, UF increased from 6.82 ± 6.49 to 8.25 ± 3.83 microliter per minute per gram kidney weight. However, after 2 hours, UF fell continuously throughout the experimental period as shown in table and figure 3.

Figure 4 and 5 demonstrate the changes of fractional excretion of sodium (FE.Na) and potassium (FE.K) following venom injection. The FE. Na rose significanctly during the second hour ($p < .025$), whereas the FE.K increased slightly. After 2 hours, the fractional excretion of both electrolytes showed a small decline.

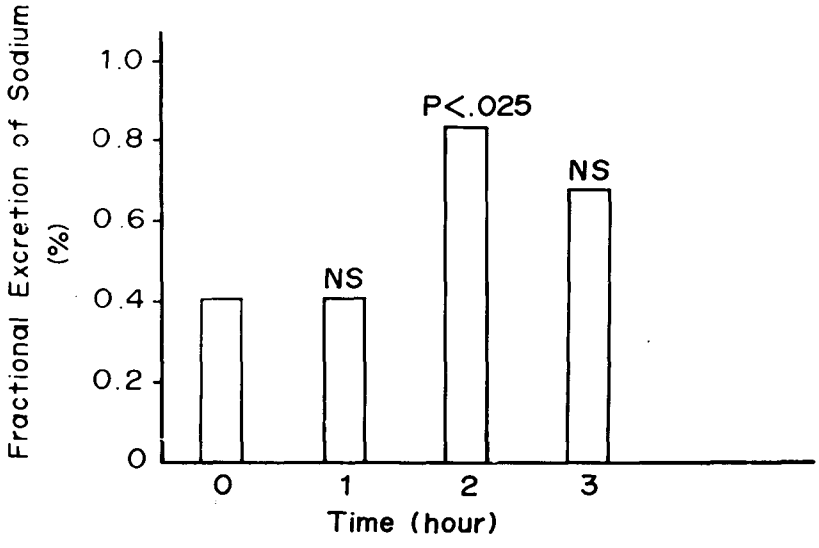

Figure 4 The Effect of Russell's Viper Venom on Fractional Excretion of Sodium

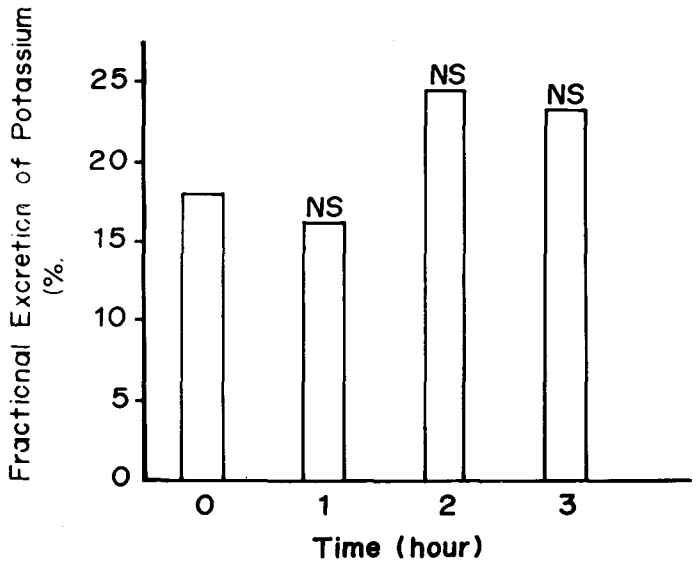

Figure 5 The Effect of Russell's Viper Venom on Fractional Excretion of Potassium

Discussion

Snake venom is well known to cause toxic damage to the kidney. Acute renal failure is one of the clinical manifestrations that may result from the toxicity.[5,15] The venom has been found to consist of toxic proteins and enzymes which have hematotoxic and necrotizing properties. It has been believed that hemolysis produced by these snakes was attributed to the action of phospholipase A_2 of the venom.[16] The pathogenesis of hypotensive effect of the venom has been suggested as due to the vasodilatative action of histamine, PGI_2 and leukotriene which are released from the tissue by the toxic effect of the venom.[9,17] Huei-Chen Huang[9] also reported that some fractions of the venom could increase lung perfusion pressure, which might restrict blood return to the heart and lead to a decrease in cardiac output, inducing hypotension indirectly.

Renal changes have been studied extensively by kidney biopsies in snake bite patients. The renal effects of the toxin could be direct or indirect as a consequence of shock, hemorrhage and vasculopathy.[18] In the present study, as shown in figures 1,2,3 and the table, Russell's viper venom produced hypotension and reduced filtering function of the kidney as well as urine flow rate. The responses were seen immediately after venom injection. After 1 hour of experiment, changes of GFR and UF seemed to have been independent of arterial pressure. A thirty percent decrease in MAP shorter than 1 hour was probably not the sole cause of GFR changes. It was possible that glomerular changes could also be due to direct injurious effect of the venom.[4,19]

Previous studies have suggested that Russell's viper venom might damage renal tubular cell directly.[4,5,10,20] The indirect effects resulting from anaphylactic shock which causes renal hypoxia by collapsing of blood vessel and vasoconstriction have also been reported.[5,14] As illustrated in figures 4 and 5, there were some increments of the FE.Na and FE.K following venom injection. The increase in FE.Na was demonstrated significantly in the second hour period (p .025). Russell's viper venom might have possibly damaged tubular cell and interfered with renal function during this period. The rise of UF at this point was probably due to the decrease of reabsorptive function of the renal tubules. The enhancement of FE.K could have been potentiated by the diminution in the renal tabular function or by some hemolytic effects of the venom.[12] Recently, the direct nephrotoxicity of Russell's viper venom on isolated perfused kidney has been reported[21,22] Thus, from this study, it might be postulated that Russell's viper venom may have both direct and indirect toxic effects on the kidney. The specific pathophysiologic changes need further investigation.

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