RELATIONSHIP OF TIME AND SERUM CONCENTRATIONS OF RUSSELL'S VIPER VENOM WITH BLOOD COAGULATING FACTORS AND RENAL HEMODYNAMICS IN ANESTHETIZED DOGS INJECTED WITH RUSSELL' S VIPER VENOM AND THE EFFECT OF DOPAMINE PLUS FUROSEMIDE

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ABSTRACT

The relationship of time and serum concentrations of Russell's viper venom (RVV), as well as blood coagulating factors, hemodynamics, and renal hemodynamics were studied, in comparison with the co-administration of dopamine plus furosemide, iu anesthetized male mongrel dogs (N=18), weighing 10-15 kg. Intravenous infusion of normal saline solution (NSS) containing inulin and para-aminohippuric acid (PAH) was given for renal hemodynamic studies. Twelve dogs were intramuscularly injected with RVV (0.1 mg/kg) . Six of these dogs were given with RVV (0.1 mg/kg) followed by infusion of dopamine (D, 3 μg/kg/min) plus furosemide (F, a bolus dose of 1 mg/kg and infusion of 1 mg/kg/h). The other 6 dogs were injected with NSS and served as a control group. Blood and urine samples collected at time 0,2,6,12 and 24 hour were analysed for inulin, PAH, electrolytes, complete blood count (CBC) and blood coagulating factors including fibrinogen, factor V, factor X and fibrin degradation product (FDP). Calculation were made to obtain renal blood flow (RBF), glomerular filtration rate (GFR), renal vascular resistance (RVR), filtration fraction (FF), fractional excretion (FE) of electrolytes. Serum concentrations of RVV, in twelve dogs given with RVV, were measured by ELISA at time 0, 15 min, 30 min, 1,2,4,6,9,12 and 24 hour. The serum concentrations of RVV were plotted against time on semilog paper. Kinetic parameters were calculated from the graphs. When comparing the group given with RVV alone and the group given with RVV plus D and F, the results showed gradual absorption of RVV from the injection site into systemic circulation with time of maximum absorption (T_{max}) of 9 hours. In both groups, maximum concentration (C_{max}) of 13.0 ± 13.34 ng/ml and 13.0 ± 2.83 ng/ml, elimination half-life ($T_{½}$) of 26.0 ± 18.53 hours and 16.4 ± 10.96 hours, the elimination rate constant (K_e) of 0.0267 ± 0.0194 h^{-1} and 0.0423 ± 0.0211 h^{-1} and volume of distribution (V_d) of 5.56 ± 3.23 l/kg and 6.06 ± 3.85 l/kg, were observed, respectively. Mean arterial blood pressure (MABP) was significantly decreased at time 12 and 24 hours after RVV injection, but slightly decreased in the group given with RVV+D+F. Heart rate was increased owing to sympathetic stimulation as a compensatory mechanism. In the group given with RVV, RBF, GFR, urine flow rate (V) were markedly decreased throughout the period of 24 hours of experiment whereas RVR was significantly increased. In contrary, D plus F could restore the renal function that being altered by RVV. The increase of FF and FE_K and the decrease of FE_{Na} and FE_{Cl} were not significant. Among blood components, there was no significant difference among the 3 groups. Blood cell components were slightly increased. Blood coagulating factors, including fibrinogen, factor V, and factor X, were significantly decreased where as FDP was increased throughout the experiment. Dopamine plus furosemide seemed to attenuate the effect of RVV on blood coagulation. These results indicated the failure of blood coagulation induced by RVV. The effects of RVV were closely related to serum RVV concentrations. Binding of RVV to some tissues may be responsible for high apparent V_d and long T½, thus duration of RVV action is prolonged. Dopamine plus furosemide synergistically improved the renal functions, thus increased renal clearance of RVV. This may result in lesser effects on blood coagulation. This should be taken into an account when human victims of Russell's viper bites are treated. The conventional treatment using antivenom and maintenance of adequate volume should be monitored and observed over a longer period of time than 24 hours. Dopamine plus furosemide may considerably be useful in treatment of the viper bites.

Key words : serum concentration of Russell's viper venom, blood coagulating factors, dopamine, furosemide

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ความสัมพันธ์ของเวลาและความเข้มข้นของพิษงูแมวเซาในซีรัมกับแฟคเตอร์เกี่ยวกับการแข็งตัวของเลือด และการทำหน้าที่ของไตในสุนัขที่ได้รับการฉีดพิษงูแมวเซาและฤทธิ์ของโดปามีนร่วมกับฟูโรซีไมด์

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บทคัดย่อ

ศึกษาความสัมพันธ์ของเวลาและความเข้มข้นของพิษงูแมวเซาในซีรัมตลอดจนแฟคเตอร์เกี่ยวกับการแข็งตัวของเลือด การทำหน้าที่ของไต เปรียบเทียบกับการให้ยาโดปามีนและฟูโรซีไมด์ร่วมด้วยในสุนัขพันธุ์พื้นเมืองหนัก 10-15 กิโลกรัม ที่ทำให้สลบ ทำการหยดน้ำเกลือปกติที่มีอินนูลิน และกรดพาราอะมิโนฮิปพิวริก เพื่อศึกษาการทำงานของไต กลุ่มที่ 1 สุนัข 6 ตัวได้รับการฉีดพิษงูแมวเซา (0.1 มก./กก.) ทางกล้ามเนื้อ กลุ่มที่ 2 สุนัข 6 ตัวได้รับการฉีดพิษงูแมวเซา (0.1 มก./กก.) แล้วตามด้วยยาโดปามีน (3 มคก./กก./นาที) ร่วมกับยาฟูโรซีไมด์ (ขนาดเดียว 1 มก./กก. ตามด้วยการหยด 1 มก./กก./ชม.) สุนัขอีก 6 ตัวได้รับการฉีดน้ำเกลือปกติเป็นกลุ่มควบคุมเก็บตัวอย่างเลือดและปัสสาวะที่เวลา 0, 2, 6, 12 และ 24 ชั่วโมง เพื่อวิเคราะห์ระดับอินนูลิน กรดพาราอะมิโนฮิปพิวริก อิเล็คโตรไลท์ นับเม็ดเลือด วิเคราะห์ระดับแฟคเตอร์ที่เกี่ยวข้องกับการแข็งตัวของเลือด ได้แก่ไฟบริโนเจน แฟคเตอร์ห้า แฟคเตอร์สิบ และผลิตผลจากการสลายตัวของไฟบริน คำนวณค่าการไหลเวียนของเลือดไปยังไต อัตราการกรองที่โกลเมอรูลัส ความด้านทานในหลอดเลือดที่ไต สัดส่วนการกรอง สัดส่วนการขับถ่ายอิเล็คโตรไลท์ วิเคราะห์หาความเข้มข้นของพิษงูแมวเซาในซีรัมของสุนัข 12 ตัวที่ได้รับการฉีดพิษงูแมวเซาที่เวลา 0, 15 นาที, 30 นาที, 1, 2, 4, 6, 9, 12 และ 24 ชั่วโมง โดยวิธีอิไลซา สร้างกราฟระหว่างเวลากับล็อกความเข้มข้นของพิษงูแมวเซาในซีรัม คำนวณค่าพารามิเตอร์ทางจลนศาสตร์จากกราฟ เมื่อเปรียบเทียบสุนัขกลุ่มที่ได้รับพิษงูแมวเซาอย่างเดียวกับสุนัขกลุ่มที่ได้รับพิษงูแมวเซาร่วมกับยาโดปามีนและฟูโรซีไมด์ ผลการทดลองแสดงว่าพิษงูแมวเซาค่อย ๆถูกดูดซึมจากตำแหน่งที่ฉีดเข้าสู่กระแสโลหิต เวลาที่ระดับพิษงูแมวเซาสูงสุดในซีรัมคือ 9 ชั่วโมงในทั้งสองกลุ่ม ความเข้มข้นสูงสุดพอ ๆกัน คือ 13.0 $\pm$ 13.34 นนก./มล. และ 13.0 $\pm$ 2.83 นนก./มล. ค่าครึ่งชีวิตของการกำจัดเท่ากับ 26.0 $\pm$ 18.53 ชั่วโมงและ 16.4 $\pm$ 10.96 ชั่วโมง ค่าคงที่ของการกำจัดเท่ากับ 0.0267 $\pm$ 0.0194 ชั่วโมง$^{-1}$ และ 0.0423 $\pm$ 0.0211 ชั่วโมง$^{-1}$ และปริมาตรการกระจายเท่ากับ 5.56 $\pm$ 3.23 ล./กก. และ 6.06 $\pm$ 3.85 ล./กก. ตามลำดับ ค่าเฉลี่ยของความดันเลือดแดงลดลงอย่างมีนัยสำคัญที่เวลา 12 และ 24 ชั่วโมงหลังจากฉีดพิษงูแมวเซาแต่ลดลงเล็กน้อยในกลุ่มที่ได้รับพิษงูแมวเซาร่วมกับโดปามีนและฟูโรซีไมด์ อัตราการเต้นของหัวใจเพิ่มขึ้นเป็นผลมาจากการกระตุ้นประสาทซิมพาเธติค ซึ่งเป็นกลไกเพื่อชดเชยการลดความดันเลือด ในกลุ่มที่ได้รับพิษงูแมวเซาอย่างเดียวมีการไหลเวียนของเลือด อัตราการกรองที่โกลเมอรูลัสและอัตราการไหลของปัสสาวะลดลงอย่างเด่นชัดตลอดระยะเวลาการศึกษา 24 ชั่วโมง ขณะที่ความด้านทานในหลอดเลือดที่ไตเพิ่มขึ้นอย่างมีนัยสำคัญ ในทางตรงกันข้ามยาโดปามีนและฟูโรซีไมด์สามารถทำให้การทำงานของไตที่เปลี่ยนแปลงเนื่องจากผลของพิษงูแมวเซากลับคืนมา การเพิ่มสัดส่วนการกรองและสัดส่วนการขับถ่ายโปแตสเซียมและการลดลงของสัดส่วนการกรองและสัดส่วนการขับถ่ายโซเดียมและคลอไรด์ไม่มีนัยสำคัญ ในบรรดาส่วนประกอบของเลือดพบว่ามีความแตกต่างระหว่างสุนัข 3 กลุ่มอย่างไม่มีนัยสำคัญ เม็ดเลือดชนิดต่าง ๆเพิ่มขึ้นเล็กน้อย แฟคเตอร์ที่เกี่ยวกับการแข็งตัวของเลือดได้แก่ ไฟบริโนเจน แฟคเตอร์ห้า และแฟคเตอร์สิบลดลงอย่างมีนัยสำคัญ ขณะที่ผลิตผลจากการสลายตัวของไฟบรินเพิ่มขึ้นตลอดการทดลอง ยาโดปามีนร่วมกับฟูโรซีไมด์ทำให้ฤทธิ์ของพิษงูแมวเซาต่อการแข็งตัวของเลือดลดน้อยลง ผลการทดลองแสดงว่าพิษงูแมวเซาทำให้การแข็งตัวของเลือดบกพร่อง ฤทธิ์ของพิษงูแมวเซามีความสัมพันธ์กับระดับพิษงูแมวเซาในซีรัม พิษงูแมวเซาอาจจับกับเนื้อเยื่อทำให้มีปริมาตรการกระจายมาก และค่าครึ่งชีวิตยาวจึงทำให้ฤทธิ์ของพิษงูแมวเซายาวนาน ยาโดปามีนร่วมกับฟูโรซีไมด์เสริมฤทธิ์กันช่วยให้การทำงานของไตดีขึ้นจึงเพิ่มการขับถ่ายพิษงูแมวเซา ผลนี้อาจทำให้ฤทธิ์ของพิษงูแมวเซาต่อปัจจัยเกี่ยวกับการแข็งตัวของเลือดมีน้อยลง ผลการศึกษานี้ควรนำมาพิจารณาเมื่อทำการรักษาคนที่ถูกงูแมวเซากัด วิธีการรักษาดั้งเดิมที่ใช้สารต้านพิษงู (แอนตีวีนอม) และควบคุมให้มีปริมาตรเลือดเพียงพอ นอกจากนี้ควรเฝ้าระวังและสังเกตอาการในช่วงเวลาที่นานกว่า 24 ชั่วโมง อาจนำยาโดปามีนร่วมกับฟูโรซีไมด์มาใช้ประโยชน์ในการรักษาการถูกงูแมวเซากัดได้

คำสำคัญ : ระดับพิษงูแมวเซาในซีรัม โดปามีน ฟูโรซีไมด์ แฟคเตอร์เกี่ยวกับการแข็งตัวของเลือด

INTRODUCTION

The venom of snake is a complex mixture of enzymes, peptides and proteins of low relative molecular weight with specific chemical and biological activities which may cause multifarious clinical manifestation. Russell's viper venom (RVV) contains isoenzymes of phospholipase A_2[1], coagulation factor-activating proteases[2], hyaluronidase[3], nuclease, hemorrhagins, and several other constituents[4], which can instantly induce hematological abnormalities and circulatory failure[5]. The clinical manifestations associated with most viper bite are local swelling, local necrosis, systemic symptoms, spontaneous hemorrhage, hematological effects, shock and death[5-7]. The kidney is one of the organ frequently involved with snake bite. Acute renal failure (ARF) is an important cause of death in Russell's viper envenomation[8]. The nonspecific effects of RVV leading to renal hemodynamic alterations, together with direct nephrotoxicity, favor the development of ARF. Early treatment with specific antivenom and adequate fluid administration are preferred. However, the effective dose and frequency of antivenom administration should be based on the amount of venom envenomation and the kinetics of venom distribution in the body. As the severity of poisoning are directly related to the serum venom levels. The relationship of time and venom concentrations with the blood coagulating factors and renal hemodynamics may be useful in determining the proper antivenom therapy and the period of time the patients should be closely monitored. The results from our previous report showing the synergistic effect of dopamine and furosemide in the model of RVV-induced nephrotoxicity[9]. Dopamine combined with furosemide may accelerate the urinary excretion of RVV as a consequence of renal function improvement. It is, therefore, the purpose of this study to describe the changes of serum RVV in relation to its effects on blood coagulating factors and renal hemodynamics, in comparison with the co-administration of dopamine plus furosemide, in dogs injected intramuscularly with RVV.

MATERIALS AND METHODS

Eighteen male mongrel dogs, weighing 10-15 kg, were studied. They were anesthetized with intravenous pentobarbital sodium (25 mg/kg). If necessary, supplementary doses of 25-50 mg were given to maintain the anesthetized state throughout the study. In all dogs, femoral artery and vein cannulations were made for measuring the mean arterial pressure (MAP), heart rate (HR), blood collection, intravenous infusion of normal saline solution (NSS) and drug solution, and renal clearance study. The urine flow rate (V) was made by mean of ureter cannulation. Russell's viper venom in the lyophilized form was produced by the Queen Saovabha Memorial Institute of the Thai Red Cross Society.

Experimental Design

Dogs were divided into three groups of six dogs each. In all dogs, 1.2% of para-aminohippurate (PAH) and 7.5% of inulin dissolved in NSS were intravenously injected at the dose of 0.5 ml/kg and then 0.12% of PAH and 0.75% of inulin dissolved in NSS were continuously infused with the rate of 1.8 ml/min throughout the study. After a control period of 60 min, RVV (0.1 mg/kg body weight) dissolved in 2 ml of the NSS was intramusculary injected into twelve dogs. In six dogs given with RVV, a bolus dose of furosemide (1 mg/kg) was intravenously injected followed by continuous infusion of furosemide (1 mg/kg/h) and dopamine (3 μg/kg/min), dissolved in NSS, for 5 hours. Blood samples were taken at time 0,15,30 min, 1,2,4,6,9,12, and 24 hour. Serum was separated and quantitative analysis of RVV in the serum were performed by the method of Enzyme Linked-Immuno-Sorbent Assay (ELISA) developed in-house. At time 0,2,6,12 and 24 hour, determination were made of MAP, HR, renal blood flow (RBF), glomerular filtration rate (GFR), urine flow rate (V), renal vascular resistance (RVR), filtration fraction (FF), fractional excretion of sodium (FE_{Na}); potassium (FF_K); and chloride (FE_{Cl}). The renal plasma flow (RPF) and GFR were measured by PAH and inulin clearances, using the standard techniques[10,11]. Plasma and urine concentrations of inulin and PAH were measured by method described by Davidson and Sackner[12], and Smith[11]. RBF was calculated from RPF and packed cell volume (PCV). RVR was calculated from MAP and RBF. Sodium and potassium were measured by flame photometry (Klina Flame, Beckman). Chloride was measured by a chloride analyzer (Instrumentation Labs, model 279). Blood coagulation study including complete blood count (CBC), fibrin degradation product (FDP), fibrinogen, factor V, and factor X were

performed. CBC was measured (Sysmex Meditop, Japan), FDP was measured by Rapid Latex Test[13,14], fibrinogen and factor V were measured by Clotting Assay[15,16], and factor X was measured by Chromigenic Assay[17].

STATISTICAL ANALYSIS

All values were expressed as mean ± SD. The data were analyzed by Student's paired t-test. A P-value of <0.05 was considered significant.

RESULTS

Serum concentrations of RVV in relation to time

Figure 1 and Table 1 show the changes of serum concentrations of RVV during 24 hours in dogs injected with RVV (0.1 mg/kg body weight) intramuscularly, compared to the dogs injected with RVV at the same dose and same route combined with dopamine and furosemide. The levels of RVV gradually increased in both groups with the maximum concentration (C_{max}) of 13 ng/ml at 9 hours (T_{max}) after RVV injection. In RVV group, serum RVV was higher than that observed in the group given RVV with D+F. Moreover, C_{max} in the RVV group was sustaimed for at least 3 hours of observation,

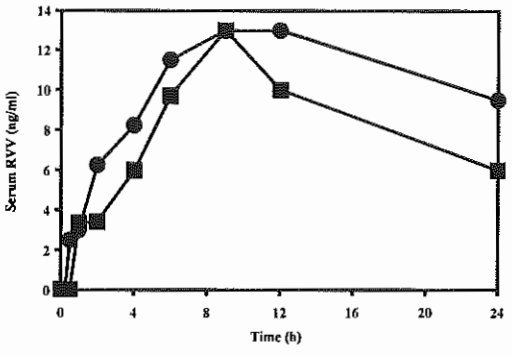

Figure 1. Time-serum concentration curves of RVV in dogs injected with 0.1 mg RVV/kg body weight (●) and dogs given sustained RVV and dopamine combined with furose-mide (■)

whereas C_{max} in the RVV+D+F group declined to 10 ng/ml within 3 hours. At the end of 24 hours of observation, serum RVV in the group given RVV+D+F was lower than that observed in the RVV group. However, the concentrations of RVV were still high showing that RVV was slowly eliminated. Assume that the kinetics of RVV follows the one-compartment model, calculated values of kinetic parameters are shown in table 2. As elimination of RVV in the group given RVV+D+F was accelerated as considered from elimination rate constant (K_e) and clearance (CL). Half-life ($T_{½}$) was shortened and area under the curve (AUC) was lower than that observed in the group given RVV alone.

Effects of RVV on blood components and coagulating factors

Table 3 summarizes the effects of RVV on blood components, and table 4 shows the effects of RVV on blood coagulating factors in dogs injected with RVV (0.1 mg/kg) intramuscularly, compared to the dogs injected with RVV at the same dose and same route combined with dopamine and furosemide. White blood cells (WBC) were increased in all groups, however in the groups given RVV showed higher amount of the increased WBC during the first 12 hours. Red blood cells were slightly decreased in the groups given RVV during the first 12 hours, whereas markedly decreased in the group given RVV alone as observed at the end of 24 hours. Platelets (PLT) were slightly increased at 2 hour time and then decreased toward the end of 24 hours, while those in the control group remained unchanged. Hemoglobin was substantially decreased in the group given RVV alone as observed at the end of 24 hours. Hematocrit was slightly increased at the first 2 hours and then decreased toward the end of 24 hours, particularly in the groups given RVV. Blood clotting factors, including fibrinogen; factor V, and factor X, were significantly decreased in the group given RVV alone and markedly decreased in the group given RVV+D+F. Whereas the fibrin degradation product (FDP) was significantly increased in the groups given RVV.

Table 1. Serum concentrations of RVV in dogs injected with 0.1 mg RVV/kg body weight compared with the group given RVV with dopamine (D) and furosemide (F), expressed as mean ±S.D. (N =6)

Group	Serum RVV at Time (h)									
	0	15 min	30 min	1	2	4	6	9	12	24
RVV	0	0	2.50 ± 2.00	3.00 ± 2.83	6.25 ± 3.20	8.25 ± 7.18	11.50 ± 10.30	13.00 ± 13.34	13.00 ±12.03	9.50 ± 8.23
RVV +(D+F)	0	0	0	3.33 ± 3.77	3.40 ± 3.58	6.00 ± 4.24	9.70 ± 2.86	13.00 ± 2.83	10.00 ± 2.35	6.00 ± 5.57

Table 2. Estimated values of RVV kinetic parameters in dogs injected with RVV (0.1 mg/kg) compared with the dogs given RVV with D and with F. (N =6)

Group	$T_{½}$ (h)	V_2 (l/kg)	K_e (h^{-1})	CL (l/h/kg)	AUC (mg-h/l)
RVV	26.00 ± 18.53	5.56 ± 3.23	0.0267 ± 0.0194	0.1485 ± 0.1073	0.6734 ± 0.4528
RVV +(D+F)	16.40 ±10.96	6.06 ± 3.85	0.0423 ± 0.0211	0.2563 ± 0.1215	0.3902 ± 0.1083

Table 3. Effects of RVV on blood components: white blood cell (WBC), red blood cell (RBC), platelet (PLT), hemoglobin (HGB) and hematocrit (HCT), in dogs injected with 0.1 mg RVV/kg body weight compared with the group given RVV with dopamine (D) combined and furosemide (F) and the control group. (N = 6)

Blood Components	Group	Time (h)				
		0	2	6	12	24
WBC (x 10^3 cells/μl)	Control	4.07 ± 2.39	8.02 ± 4.71	15.37 ± 10.83	16.90 ± 10.83	15.00 ± 10.84
	RVV	4.40 ± 3.20	12.98 ± 10.79	18.54 ± 16.79	26.90 ± 23.61	7.7 ± 6.93
	RVV +(D+F)	4.64 ± 2.33	10.45 ± 6.08	16.25 ± 10.16	23.78 ± 16.40	11.46 ± 6.13
RBC (x 10^6 cells/μl)	Control	4.95 ± 1.54	5.13 ± 1.62	5.25 ± 1.28	5.21 ± 1.55	4.51 ± 1.55
	RVV	4.88 ± 1.23	4.76 ± 0.86	4.66 ± 0.91	4.37 ± 1.43	2.06 ± 0.79
	RVV + (D+F)	4.65 ± 1.31	4.81 ± 1.04	4.87 ± 1.06	4.45 ± 1.35	3.87 ± 0.94
PLT (x10^3 cells//μl)	Control	69.00 ± 37.67	81.33 ± 41.64	74.50 ± 58.74	68.50 ± 38.96	77.00 ± 43.66
	RVV	69.50 ± 31.45	84.50 ± 40.63	36.60 ± 14.51	41.50 ± 21.67	36.00 ± 7.07
	RVV + (D+F)	67.30 ± 41.15	78.50 ± 38.79	46.67 ± 23.79	45.53 ± 21.69	40.35 ± 18.49
HGB (g/dl)	Control	11.45 ± 3.33	12.10 ± 3.94	12.20 ± 2.95	12.08 ± 3.17	11.10 ± 4.16
	RVV	10.80 ± 2.48	10.55 ± 1.79	10.43 ± 2.04	10.0 ± 3.28	4.9 ± 1.84
	RVV + (D+F)	10.79 ± 3.16	11.08 ± 2.67	11.27 ± 3.41	10.26 ± 3.28	10.00 ± 3.01
HCT (%)	Control	33.27 ± 9.97	34.65 ± 11.11	32.60 ± 9.73	35.42 ± 9.32	32.07 ± 11.40
	RVV	32.38 ± 7.76	33.98 ± 5.25	26.54 ± 6.68	26.90 ± 10.05	17.70 ± 5.52
	RVV + (D+F)	29.25 ± 5.85	33.88 ± 2.39	27.75 ± 4.35	24.88 ± 7.47	23.25 ±7.5

Table 4. Effects of RVV on blood coagulating factors in dogs injected with 0.1 mg RVV/kg body weight, compared with the group given RVV with dopamine and furosemide. (N = 6)

Blood Coagulating Factor	Group	Time (h)				
		0	2	6	12	24
Fibrinogen (mg/dl)	Control	2.25 ± 0.27	2.07 ± 0.34	2.14 ± 0.61	2.53 ± 0.95	2.57 ± 1.45
	RVV	2.28 ± 0.68	1.83 ± 0.60	0.95 ± 0.23	0.58* ± 0.26	0.24* ± 0.12
	RVV +(D+F)	2.15 ± 0.39	1.94 ± 0.52	1.38 ± 0.43	1.07 ± 0.35	0.94 ± 0.48
Factor V (mg/dl)	Control	149.2 ± 16.36	125.23 ± 26.90	118.02 ± 39.52	108.6 ± 27.69	119.67 ± 36.12
	RVV	126.67 ± 36.80	44.50 ± 32.08	16.77* ± 13.72	12.60* ± 11.50	17.77* ± 13.90
	RVV + (D+F)	120.25 ± 24.04	65.50 ± 25.60	30.95 ± 15.62	27.53* ± 10.32	31.25* ± 11.22
Factor X (mg/dl)	Control	125.80 ± 36.80	110.40 ± 42.04	101.20 ± 43.05	106.4 ± 43.33	112.33 ± 35.92
	RVV	110.50 ± 61.65	102.50 ± 38.26	56.17 ± 42.12	39.20* ± 19.26	39.67* ± 30.67
	RVV + (D+F)	110.16 ± 53.68	100.20 ± 45.50	74.90 ± 31.11	48.75 ± 20.05	50.60 ± 21.47
Fibrin Degradation Product (FDP) (mg/dl)	Control	0	0.40 ± 0.29	1.28 ± 0.89	1.21 ± 0.32	1.11 ± 0.42
	RVV	0	0.40 ± 0.29	2.80 ± 1.10	2.97 ± 1.11	54.00* ± 25.0
	RVV + (D+F)	0	0.38 ± 0.19	2.12 ± 1.05	2.26 ± 0.97	27.35* ± 11.41

*P< 0.05, compared with the time zero within the same group

Effects of RVV on systemic and renal hemodynamics

Table 5 summarizes the effects of RVV on MAP, BP, RBF, GFR, V, RVR, FF, FE_{Na} , FE_K, FE_{Cl}. There was a significant reduction in MAP in the group given RVV alone. The HR was increased in the group given RVV+D+F. RBF, GFR and V were significantly decreased in the group given RVV alone. In contrary, RBF, GFR and V were increased in the group given RVV+D+F. RVR was significantly increased in the RVV group, whereas in the RVV+D+F group, RVR was decreased. FF was slightly increased during the first 12 hours in the RVV group. FE_{Na}, FE_K and FE_{Cl} were increased in the group given RVV+D+F.

DISCUSSION

The serum levels of RVV in dogs given a single dose of RVV intramuscularly peaked at 9 hours after venom injection. This result is somewhat different from that observed in the envenomated rabbit[18] whose peak venom levels were detected between 2 -4 hours after venom injection. Whereas in RVV bite victims, C_{max} was unidentifiable since the patients must be treated immediately on arrival to the doctors. However the initial serum levels of RVV in RVV bite victims ranged from less than 10 ng/ml to 290 ng/ml[18]. Since RVV and/or its metabolites are excreted by the kidney[18]. A combination of dopamine and furosemide seemed to enhance the excretion of RVV. Although at the end of 24 hours, elimination of RVV was not completed, however the calculation of kinetic parameters were made to obtain the relative values between the two groups. As expected, clearance of RVV in dogs receiving RVV+D+F was relatively faster when compared with the dogs given RVV alone. This effect may be due to the combination of dopamine and furosemide. Furosemide is a very potent diuretic acting on the thick ascending limb of Henle's loop and inhibits Na^+-K^+-$2Cl^-$ symporter[19]. Dopamine itself acts on the DA_1-dopaminergic receptors thereby giving rise to renal vasodilatation[20] and facilitates furosemide to the site of its action in the nephron, thus synergises the action of furosemide. The effects of a combination of dopamine and furosemide on renal hemodynamics in dogs injected with RVV has confirmed our previous report[9]. Dopamine plus furosemide was reported to be effective in the

Table 5. Effects of RVV on mean arterial pressure (MAP), heart rate (HR), renal hemodynamics: renal blood flow (RBF), glomerular filtration rate (GFR), urine flow rate (V), renal vascular resistance (RVR), filtration fraction (FF), fractional excretion of sodium, potassium, and chloride (FF_{Na}, FE_K, FE_{Cl}) in dogs injected with 0.1 mg RVV/kg alone or in combination with dopamine and furosemide. (N of each group = 6)

Parameter	Group	Time (h)				
		0	2	6	12	24
MAP (mmHg)	Control	96.01 ± 36.04	106.0 ± 33.34	93.74 ± 22.47	94.4 ± 15.08	93.01 ± 20.16
	RVV	107.13 ± 23.57	108.6 ± 18.55	104.67 ± 12.5	56.73*† ± 36.9	28.24*† ± 10.96
	RVV +(D+F)	100.75 ± 27.18	90.42 ± 29.1	95.75 ± 29.02	91.88 ± 12.16	85.28 ± 15.68
HR (beats/min)	Control	135 ± 34	135 ± 38	139 ± 40	147 ± 39	133 ± 26
	RVV	175 ± 44	161 ± 22	149 ± 74	147 ± 70	140 ± 42
	RVV + (D+F)	158 ± 40	141 ± 37	162 ± 53	180 ± 40	179 ± 32
RBF (ml/min)	Control	198.97 ± 33.64	134.84 ± 38.48	176.37 ± 39.61	175.05 ± 39.18	221.56 ± 57.59
	RVV	162.39 ± 63.52	93.85* ± 36.16	29.08*† ± 16.37	4.73*† ± 5.07	9.35*† ± 1.57
	RVV + (D+F)	183.34 ± 82.54	154.96 ± 28.9	264.96 ± 235.63	343.33 ± 484.85	218.8 ± 170.45
GFR (ml/min)	Control	63.11 ± 36.11	39.31 ± 34.16	57.41 ± 55.93	76.66 ± 80.31	80.08 ± 60.06
	RVV	22.57 ± 3.96	18.64 ± 5.98	4.36*† ± 2.57	1.66*† ± 1.92	1.49*† ± 0.51
	RVV + (D+F)	22.29 ± 8.37	24.93 ± 12.18	33.03 ± 14.15	29.37 ± 10.06	32.09 ± 13.55
V (ml/min)	Control	0.82 ± 0.53	0.84 ± 0.86	1.29 ± 0.96	1.12 ± 0.77	0.88 ± 0.44
	RVV	1.77 ± 0.67	0.52 ± 0.46	0.25*† ± 0.19	0.06*† ± 0.04	0.12*† ± 0.06
	RVV + (D+F)	1.22 ± 0.82	2.54 ± 2.67	1.94 ± 1.68	1.11 ± 0.98	1.0 ± 0.35
RVR (x1,000 dyne-sec/cm^5)	Control	45.61 ± 23.19	71.64 ± 44.85	62.82 ± 47.87	58.37 ± 36.73	36.3 ±15.07
	RVV	65.94 ± 27.14	106.56 ± 45.68	409.15† ± 211.79	2328.22*† ± 30882	252.98 ± 136.41
	RVV + (D+F)	71.38 ± 75.08	49.01 ± 14.07	60.22 ± 47.24	34.95 ± 20.98	41.11 ± 21.22
FF (%)	Control	48.33 ± 29.05	43.66 ± 27.91	46.92 ± 28.72	59.16 ± 53.03	51.91 ± 28.96
	RVV	23.09 ± 5.86	31.45 ± 11.47	26.76 ± 21.89	48.79 ± 40.88	19.44 ± 1.71
	RVV + (D+F)	18.17 ± 6.91	24.64 ± 14.69	18.74 ± 10.09	12.14 ± 15.66	20.99 ± 4.36
FE_{Na} (%)	Control	1.43 ± 1.65	1.35 ± 2.1	2.66 ± 1.64	3.28 ± 4.31	1.67 ± 1.07
	RVV	3.9 ± 1.95	1.18 ± 0.84	4.72 ± 5.26	2.84 ± 2.59	7.69 ± 4
	RVV + (D+F)	3.54 ± 0.41	8.81 ± 6.1	7.08 ± 4.73	3.31 ± 2.03	4.56 ± 3.33
FE_K (%)	Control	20.69 ± 11.08	31.65 ± 24.19	26.65 ± 18.71	40.14 ± 37.29	21.31 ± 20.19
	RVV	104.64 ± 71.05	45.42 ± 14.65	79.98 ± 64.23	151.57 ± 130.2	54.98 ± 47.08
	RVV + (D+F)	28.68 ± 25.36	80.49 ± 36	84.7 ± 56.05	49.63 ± 21.14	56.57 ± 54.95
FE_{Cl} (%)	Control	1.67 ±1.99	3.87 ± 5.5	1.91 ± 1.47	3.85 ± 3.88	1.71 ± 1.68
	RVV	4.08 ± 2.31	1.18 ± 1.05	4.73 ± 6.35	2.41 ± 2.14	2.83 ± 2.12
	RVV + (D+F)	3.61 ± 1.41	9.17 ± 7.72	9.5 ± 6.31	3.06 ± 3.19	6.94 ± 3.36

* $P < 0.05$, compared with the time zero within the same group.

† $P < 0.05$, compared with the other groups

treatment of oliguric acute renal failure induced by several causes[21-23]. RVV clearly alters renal hemodynamics. The reduction of MAP, RBF and GFR observed in this study confirms the results from our previous report[24]. Hemodynamic alterations in snake envenomation are believed to be the result of interactions of various vasoactive mediators[25,26], some of which are vasoconstrictors, for examples: norepinephrine, angiotensin II, endothelin, thromboxane A_2, which contribute to renal vasoconstriction and cause a reduction of RBF and GFR. Some are vasodilators, for examples: prostacyclin (PGI_2), PGE_2, bradykinin and also NO, which contribute to vasodilatation and hypotension. The effects of RVV on blood coagulating factors as observed in this study show the decrease of fibrinogen as well as the factor V and factor X. This coagulopathy is the frequent consequence of viper bites[6]. Viperine venoms act indirectly by activating prothrombin or factor X, then intravascular coagulation occurs and the factor X is decreased. Viper venom also acts continuously on fibrinogen, producing a fibrin more susceptible to lysis and resulting in non-clotting or poorly clotting blood because of the absent or very low levels of fibrinogen whereas levels of fibrin degradation products are elevated[27], abnormal bleeding may follow[6]. Most viper venoms, in man, act predominantly on the haemostatic system, particularly on capillary endothelium[6]. Locally, this causes swelling rapidly as observed at the site of RVV injection in this study. Together with hemorrhage, the exudation may consist of plasma or whole blood as observed in this study that RBC was decreased. Hemodynamic changes are very much similar to those observed in sepsis[25,28]. Snake bite is thus involved the inflammatory processes. It is not surprising that the WBCs, the major blood component playing important roles in the inflammatory processes, are found increasing during the first 12 hours in the groups given RVV. However, at the end of 24 hours WBCs which are increased in the control group may reflect some inflammation resulted from the operation procedure performed in the experiments. However the increase of WBCs is prominent in the groups given RVV. The slight increase of hematocrit and blood platelets during the first 2 hours after RVV injection is similar to the previous study in dogs[29]. This is probably related to a reflex mechanism that causes systemic vasoconstriction and perhap splenic contraction in response to the decrease in MAP and plasma volume[30].

CONCLUSION

In conclusion the effects of RVV on blood coagulation and renal hemodynamics were closely related to serum RVV concentrations. Binding of RVV to tissue compartment was responsible for the high apparent volume of distribution and long half-life of the venom, thus duration of RVV action is prolonged. Dopamine combined with furosemide not only improved the renal function, they might also accelerate the urinary elimination of RVV. However the conventional treatment using specific antivenom and maintenance of adequate volume should be monitored and observed over a period of longer than 24 hours.

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