10.2478/abm-2010-0051

Asian Biomedicine Vol. 4 No. 3 June 2010; 421-428

Original article

Effects of *Bungarus candidus* (Malayan krait) venom on general circulation and renal hemodynamics in experimental animals

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Background: Many studies have reported the occurrence of lethal acute renal failure after snakebites. *Bungarus candidus* (Malayan krait) is a medically important venomous snake distributed widely throughout Southeast Asia. The best known features of systemic envenoming by *B. candidus* are neurotoxic.
Objective: Obtain more information on effects of *B. candidus* venom on changes in systemic and renal hemodynamics in experimental animals.
***Methods*:** Twelve adult male New Zealand white rabbits were used to study the effect of *B. candidus* venom on general circulation and renal hemodynamics. An anesthetized animal was intravenously injected with *B. candidus* venom at a dosage of 50μg/kg bodyweight. All changes of parameters were observed after initial post venom injection and recorded at 30 min intervals until 150 minutes after envenomation.
Results: After envenomation, cardiovascular responses showed a marked decrease in mean arterial pressure within two minutes, afterwards gradually returning closely to baseline values. There were stepwise decreases in heart rate and cardiac output, while total peripheral resistance was slightly increased. The renal hemodynamics significantly decreased by glomerular filtration rate, effective renal plasma flow and effective renal blood flow, while the filtration fraction significantly increased. Envenomed animals showed a reduction in renal fraction, while renal vascular resistance stepwise increased. The plasma potassium level tended to increase. Animals showed stepwise decreases in urinary excretion of Na^+, K^+ and Cl^-. A marked decrease in plasma calcium level was apparent at 120 minutes, while plasma creatine phosphokinase and lactate dehydrogenase levels increased at 30-120 minutes.
Conclusion: A significant drop in blood pressure was attributed to a sustained fall in cardiac output, which would be associated with a reduction in heart rate. Sustained hypotension would contribute to reduction of renal blood flow, which results in decreased GFR.

Keywords: Animal experiment, *Bungarus candidus*, general circulation, renal hemodynamics, snake venom

Snakebite is a public health problem in rural tropical and subtropical regions with more than 85,000 deaths and 150,000 permanent sequelae worldwide each year [1]. *Bungarus candidus* Linnaeus, 1758 (Malayan krait) is an important venomous snake living in large area of Southeast Asia. In Thailand, the mortality rate from *B. candidus* bite is as high as 33% before the availability of antivenom [2]. Slow recovery has been observed in neurotoxic snakebite following respiratory and other supportive treatment without antivenom administration [3, 4]. Laothong and Sitprija [5] described decrease in parasympathetic activity manifested by mydriasis, prolonged hypertension, and tachycardia has been described. The hypotensive effect of *B. candidus* venom with secondary renal function changes has been described in a victim envenomed by *B. candidus* in northeastern Thailand despite monospecific *B. candidus* antivenom administration (Wirat Leeprasert, personal

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communication). Hemodynamic changes are thus important in *B. candidus* envenomation, but have received little attention. In this study, we evaluated the effect of *B. candidus* venom on systemic and renal hemodynamics in experimental animals.

Methods

Animal preparation

Twelve adult male New Zealand white rabbits weighing 2.5-3.5 kg were used. The animals were deprived of food, but not of water, for 12 hours prior to the study. On the day of experiments, the animal was anesthetized with pentobarbital sodium (25mg/kg) by intravenous injection. The animal was tracheotomized, and an endotracheal tube was inserted. A carotid artery was catheterized with a polyethylene tube for recording cardiac output, arterial blood pressure and heart rate (Grass Polygraph Model 79 E, Grass Instrument, Quincy, USA), and for blood sample collections. The jugular vein was catheterized with a polyethylene tube for fluid infusion. A left flank incision was made via a retroperitoneal approach, and the left ureter was cannulated with a polyvinyl catheter for urine collection and for renal clearance studies.

All the experimental procedures were approved by the Animal Ethics Committee of the Queen Saovabha Memorial Institute, and were in compliance with the National Research Council of Thailand guidelines for care and use of animals in research. Animals were supplied by the Queen Saovabha Memorial Institute and housed in conventional animal facilities.

Experimental protocol

Before the experiment, pilot studies for determination of the minimal lethal doses of *B. candidus* venom were performed in 12 anesthetized rabbits. The *B. candidus* venom in lyophilized form from the Queen Saovabha Memorial Institute was used for the study. One hundred percent of rabbits died within 167 minutes when they were envenomed with *B. candidus* venom solution at a dosage of 150 μg/kg bodyweight by intravenous injection (lyophilized crude venom dissolved in 0.9 % saline solution). Eighty-three percent died within 5-153 minutes among animals given 75 μg/kg bodyweight of venom. A dose of 50 μg/kg of venom was then selected to use in rabbit for this study. The effects of *B. candidus* venom on cardiovascular function and renal function were studied.

Measurements of cardiovascular and renal functions

Twelve rabbits were divided into three groups of four animals each. Animals in group 1 received normal saline and served as control group. Group 2 received *B. candidus* venom from southern Thailand (BC-S), and group 3 received *B. candidus* venom from northeastern Thailand (BC-NE) (**Fig.1**). Measurements of renal and cardiovascular functions in each group were divided into pretreatment and treatment periods. For treatment period, envenomation was performed by intravenous injection of *B. candidus* venom (1 mg of lyophilized venom dissolved in 1 mL of 0.9 % saline solution) at a dosage of 50 μg/kg bodyweight. All changes of parameters were observed after initial post venom injection and recorded at 30 minutes intervals after envenomation.

Systemic and renal hemodynamics was determined in terms of mean arterial blood pressure (MAP), cardiac output (CO), glomerular filtration rate (GFR), renal blood flow (RBF), packed cell volume (PCV), plasma and urinary electrolytes. The cardiac output (CO) was measured by the dye dilution technique. Evans blue (T-1824) was used instead of indocyanine green dye (ICG) as previously described [6]. In brief, one mL of Evans blue (0.03 % in normal saline) was injected into the jugular vein, and serial samples of arterial blood were collected (0.8 mL/sec) by a peristaltic pump (Eyela, MP-3, Tokyo Rikakikai, Tokyo, Japan) for a period of eight seconds. The concentration of dye in plasma was determined at every second from the beginning of the curve by spectrophotometry. Measurements of effective renal plasma flow (RPF) and glomerular filtration rate (GFR) were performed by *p*-aminohippurate and inulin clearances using standard techniques [7] with modification. The renal blood flow was calculated from the measured PCV.

Total peripheral vascular resistance (TPR) and renal vascular resistance (RVR) were calculated from CO, MAP and RBF using the standard formula. Filtration fraction was obtained by dividing GFR by ERPF. Plasma and urinary sodium (Na^+) and potassium (K^+) were measured by a flame photometer (Clinical Flame Photometer 410C; Corning Co, England), chloride (Cl^-) with a Chloride Analyzer 925. Fractional excretion of electrolytes (FE), representing urinary electrolyte excretion (E) expressed in percent of urinary electrolytes filtered per minute, was calculated. Plasma lactate dehydrogenase (LDH) and

Fig. 1 *Bungarus candidus* snake from southern (upper) and northeastern (lower) Thailand.

creatine phosphokinase (CPK) were determined by immunoturbidimetric assay using Cobas Integra 800 (Roche Diagnostics GmbH, Manheim, Germany). Plasma calcium concentrations were determined using the Cresolphthalein Complexone method [8].

Statistical analysis

All data are expressed as mean ± standard deviation. The results were analyzed by analysis of variance (ANOVA), post hoc tests for comparisons of each value of post-treatment periods against the value of pre-treatment period using Bonferroni t-test. Comparisons of mean values among groups of studies were performed using the Duncan's test. The significant differences among treatments were determined at $p < 0.05$.

Results

Cardiovascular function and renal hemodynamics

The cardiovascular responses occurred within two minutes after venom infusion in the animal receiving a venom dose of 50 μg/mL bodyweight (**Table 1**).

A marked decrease in MAP occurred within 2-5 minutes after envenomation, afterwards gradually returning closely to baseline values (**Fig. 2**).

Table 1. Effect of *Bungarus candidus* venom on cardiovascular function in rabbits.

Parameters	Group	Pre-treatment	Post-treatment (minutes)				
			30	60	90	120	150
HR	Control	235 ± 14.6 [a]	235 ± 17.3	235 ± 13.3	234 ± 12.0	234 ± 12.9	233 ± 11.4
(beats / min)	BC-NE venom	208 ± 9.0 [b]	180 ± 14.7*	174 ± 17.7*	162 ± 12.9*	148 ± 5.7*	122 ± 17.2*
	BC-S venom	219 ± 7.7 [ab]	206 ± 9.0	200 ± 11.4	186 ± 15.5	164 ± 35.1*	132 ± 32.1*
RR	Control	45 ± 10.4 [a]	50 ± 7.5 [a]	51 ± 6.0 [a]	50 ± 7.5 [a]	50 ± 7.5 [a]	49 ± 8.6 [a]
(breaths/min)	BC-NE venom	25 ± 2.9 [b]	28 ± 6.6 [b]	30 ± 10.6 [b]	21 ± 11.9 [b]	14 ± 5.9 [b]	9 ± 1.3 [b]*
	BC-S venom	33 ± 6.0 [b]	42 ± 7.1 [ab]	44 ± 13.3 [ab]	31 ± 21.0 [ab]	13 ± 4.1 [b]	10 ± 5.4 [b]*
PP	Control	25.0 ± 8.2	29.0 ± 9.0	28.3 ± 17.3	28.8 ± 14.4	28.0 ± 17.8	25.5 ± 17.2
(mmHg)	BC-NE venom	28.1 ± 2.4	26.3 ± 3.2	29.4 ± 3.2	29.4 ± 8.3	23.7 ± 9.7	23.1 ± 6.6
	BC-S venom	37.5 ± 10.4	39.4 ± 18.3	38.1 ± 16.1	36.3 ± 17.9	25.0 ± 4.6	29.4 ± 9.7
MAP	Control	92.0 ± 6.9	90.7 ± 13.4	89.5 ± 14.6	86.5 ± 14.9	85.2 ± 15.5	86.7 ± 15.4
(mmHg)	BC-NE venom	105.1 ± 10.1	86.8 ± 19.0	83.5 ± 27.0	87.9 ± 22.4	96.0 ± 23.0	100.2 ± 28.1
	BC-S venom	90.7 ± 10.9	78.7 ± 10.3	77.2 ± 10.3	78.4 ± 7.1	75.2 ± 22.1	75.5 ± 31.6
PCV	Control	36.7 ± 1.5	36.5 ± 1.3	35.5 ± 1.3 [a]	35.3 ± 1.7 [a]	34.5 ± 1.3 [a]	34.3 ± 1.5 [a]
(%)	BC-NE venom	35.5 ± 0.6	35.2 ± 1.0	34.5 ± 0.6 [ab]	34.0 ± 0.8 [ab]*	33.8 ± 0.5 [ab]*	33.0 ± 0.8 [ab]*
	BC-S venom	35.8 ± 0.5	35.3 ± 1.0	33.8 ± 0.9 [b]*	33.3 ± 0.5 [b]*	32.8 ± 0.5 [b]*	32.5 ± 0.6 [b]*
CO	Control	121.7 ± 10.3	121.3 ± 20.3 [a]	122.3 ± 21.9 [a]	nd	124.7 ± 13.3 [a]	nd
(ml / min / kg)	BC-NE venom	118.2 ± 15.3	100.1 ± 25.0 [ab]	76.1 ± 24.8 [b]	nd	74.0 ± 27.0 [b]	nd
	BC-S venom	103.2 ± 31.9	82.8 ± 32.4 [b]	72.7 ± 23.2 [b]	nd	55.4 ± 4.1 [b]	nd
TPR	Control	0.76 ± 0.11	0.73 ± 0.23	0.76 ± 0.24	nd	0.70 ± 0.21 [a]	nd
(mmHg/ml/min/kg)	BC-NE venom	0.90 ± 0.15	0.88 ± 0.24	1.13 ± 0.38	nd	1.34 ± 0.23 [b]	nd
	BC-S venom	0.97 ± 0.33	1.11 ± 0.37	1.21 ± 0.37	nd	1.64 ± 0.16 [cc]	nd
SV	Control	0.52 ± 0.05	0.52 ± 0.15	0.52 ± 0.09	nd	0.54 ± 0.07	nd
(ml/beat/kg)	BC-NE venom	0.57 ± 0.09	0.54 ± 0.15	0.43 ± 0.11	nd	0.51 ± 0.18	nd
	BC-S venom	0.48 ± 0.25	0.40 ± 0.18	0.36 ± 0.13	nd	0.41 ± 0.15	nd

All values are shown as mean ± SD; n = 4 rabbits in each group. Control group and pre-treatment period = no venom injection. Mean values within the row indicated with superscripts are significantly different from pre-treatment by Bonferroni *t*-test (*p <0.05). Comparison between groups using Duncan's test indicated with different superscripts are significantly different ([a, b, c]p <0.05). nd = not determined. HR = heart rate; RR = respiratory rate; PP = pulse pressure; MAP = mean arterial pressure; PCV = pack cell volume or hematocrit; CO = cardiac output; TPR = total peripheral resistance; SV = stroke volume.

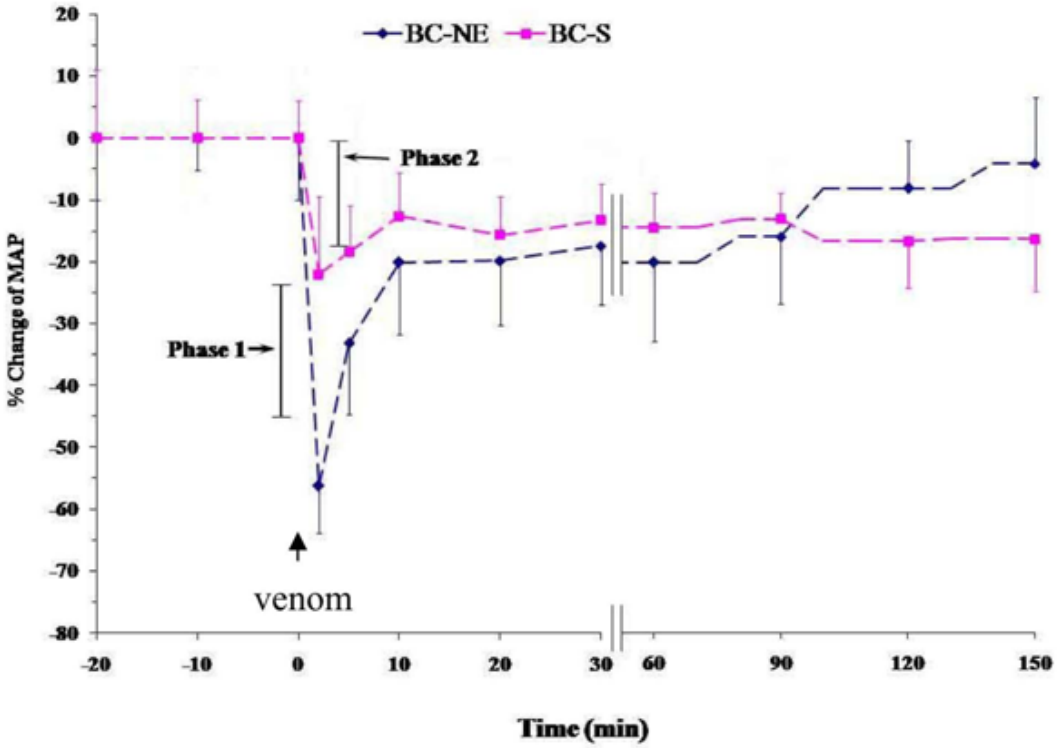

Fig. 2 Effect of *Bungarus. candidus* venom from northeastern (BC-NE) and southern (BC-S) Thailand on the mean arterial pressure (MAP) of rabbits. The points show the mean value of % change ± SD.

There were stepwise decreases in heart rate (HR) and cardiac output (CO). In comparison, the heart rate (HR) and mean arterial pressure (MAP) of BC-NE group immediately dropped compared to the BC-S group. Significant decreases in respiratory rate (RR) appeared after 150 minutes following envenomation in both groups. The slight increase in total peripheral resistance (TPR) was apparent. Packed cell volume (PCV) significantly decreased after 60 minutes in both groups. The alterations in pulse pressure and stroke volume after envenomations were not significant in both groups.

The renal hemodynamics after envenomation is shown in **Table 2**. Glomerular filtration rate (GFR) significantly decreased to 150 min in both groups. In the BC-NE treated group, effective renal plasma flow (ERPF) and effective renal blood flow (ERBF) significantly decreased at 60-150 minutes, while filtration fraction (FF) significantly increased at 150 minutes. Renal fraction (RF) revealed a reduction, and renal vascular resistance (RVR) increased stepwise throughout the experimental period of 150 minutes in both groups. The rate of urine flow markedly decreased at 150 minutes in both groups.

Plasma concentration and urinary excretion of electrolytes, plasma creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

In both envenomed groups, there were no differences in the plasma concentrations of electrolytes in comparison to pretreatment values *vs.* post-treatment values at 150 min for plasma Na^+ (BC-NE group, P_{Na+} 140.3±2.9 *vs.* 141.0±6.0 mEq/L; BC-S group, P_{Na+} 145.3±2.1 *vs.* 144.3±4.3 mEq/L) and for plasma Cl^- (BC-NE, P_{Cl}^-: 103.7±2.6 *vs.* 105.3±3.8 mEq/L; BC-S group, P_{Cl}^- 101.3±5.7 *vs.* 106.7±6.0 mEq/L). The plasma concentration of potassium (P_{K+}) trended to increase after envenomation (BC-NE group, P_{K+} 2.4±0.4 *vs.* 3.3±0.3 mEq/L; BC-S group, 2.4±0.1 *vs.* 2.9±0.6 mEq/L). Urinary excretion (E) of electrolytes showed stepwise decreases, while there were no alterations in fractional excretion (FE) of electrolytes in comparison of pretreatment values vs post-treatment values at 150 min, for Na^+ (BC-NE group, E_{Na+} 51.7±14.0 *vs.* 23.1±17.7 µEq/min; BC-S group, E_{Na+} 60.5±33.3 *vs.* 19.8±15.9 µEq/min); for K^+ (BC-NE group, E_{K+} 2.7±0.7 *vs.* 1.5±1.0 µEq/min; BC-S group, E_{K+} 3.2±1.4 *vs.* 1.9±1.4 µEq/min); for Cl^- (BC-NE group, E_{Cl-}, 41.1±13.2 *vs.* 21.4±7.0 µEq/min;

Table 2. Effect of *Bungarus candidus* venom on renal hemodynamics in rabbits.

Parameters	Group	Pre-treatment	Post-treatment (minutes)				
			30	60	90	120	150
Urine flow	Control	0.36 ± 0.21	0.33 ± 0.21	0.29 ± 0.20	0.30 ± 0.20	0.28 ± 0.15	0.29 ± 0.21
(mL/min)	BC-NE venom	0.31 ± 0.12	0.22 ± 0.15	0.27 ± 0.19	0.29 ± 0.18	0.27 ± 0.15	0.15 ± 0.12
	BC-S venom	0.41 ± 0.26	0.24 ± 0.18	0.23 ± 0.15	0.24 ± 0.19	0.19 ± 0.15	0.14 ± 0.12
GFR	Control	2.87 ± 0.83	2.81 ± 0.85	2.50 ± 0.97	2.70 ± 0.86	2.65 ± 0.81	2.71 ± 0.61[a]
(mL/min/kg)	BC-NE venom	3.26 ± 0.82	2.39 ± 0.64	2.17 ± 0.40	2.15 ± 0.17	2.09 ± 0.37*	1.23 ± 0.70[b]*
	BC-S venom	2.78 ± 0.66	1.75 ± 0.61	2.02 ± 0.18	1.88 ± 0.57	1.60 ± 0.74	1.34 ± 0.70[b]*
ERPF	Control	12.17 ± 3.42	10.50 ± 1.11	9.79 ± 0.50	11.25 ± 3.32	10.47 ± 4.47	11.13 ± 3.33[a]
(mL/min/kg)	BC-NE venom	13.98 ± 4.11	11.40 ± 2.99	7.94 ± 3.44*	6.84 ± 1.38*	5.86 ± 0.99*	3.11 ± 2.20[b]*
	BC-S venom	11.91 ± 4.95	6.60 ± 3.12	7.83 ± 2.78	6.58 ± 3.60*	4.98 ± 3.65	4.78 ± 4.09[b]
ERBF	Control	19.26 ± 5.38	16.59 ± 1.95	15.19 ± 0.92	17.47 ± 5.52	16.02 ± 6.96	17.02 ± 5.47[a]
(mL/min/kg)	BC-NE venom	21.69 ± 6.45	17.69 ± 4.71	12.10 ± 5.18*	10.39 ± 2.14*	8.85 ± 1.54*	4.65 ± 3.29[b]*
	BC-S venom	18.52 ± 7.69	10.20 ± 4.84	11.79 ± 4.05	9.84 ± 5.38	7.42 ± 5.46	7.10 ± 6.11[b]
FF	Control	25.3 ± 5.8	26.8 ± 8.2	25.3 ± 8.9	23.9 ± 1.0	26.4 ± 6.6	24.9 ± 5.4
(%)	BC-NE venom	23.8 ± 3.1	21.2 ± 4.1	29.8 ± 8.7	32.1 ± 5.7	35.7 ± 2.4	45.6 ± 11.9*
	BC-S venom	26.1 ± 11.2	28.9 ± 10.8	28.3 ± 9.6	32.4 ± 11.2	38.4 ± 16.2	36.7 ± 18.1
RF	Control	16.1 ± 5.6	13.9 ± 2.9	12.8 ± 3.0	nd	13.9 ± 6.2	nd
(%)	BC-NE venom	18.7 ± 6.1	15.7 ± 1.7	15.8 ± 3.2	nd	9.7 ± 6.1	nd
	BC-S venom	21.4 ± 16.7	16.3 ± 10.3	15.3 ± 7.2	nd	14.1 ± 9.7[b]	nd
RVR	Control	5.01 ± 1.16	5.20 ± 0.82	5.87 ± 0.66	5.20 ± 1.13	5.89 ± 1.96[a]	5.25 ± 0.66[a]
(mmHg/mL/min/kg)	BC-NE venom	5.39 ± 2.51	5.13 ± 1.59	7.48 ± 3.29	8.49 ± 1.86	10.80 ± 1.58[ab]	32.40 ± 20.54[b]*
	BC-S venom	5.50 ± 2.12	9.11 ± 4.38	7.33 ± 3.08	9.73 ± 4.22*	13.53 ± 6.87[b]	14.26 ± 6.37[ab]

All values are shown as mean±SD; n = 4 rabbits in each group. Control group and pre-treatment period = no venom injection. Mean values within the row indicated with superscripts are significantly different from pre-treatment by Bonferroni *t*-test (*$p < 0.05$). Comparison between groups using Duncan's test indicated with different superscripts are significantly different ($^{a,b,c}p < 0.05$). GFR = glomerular filtration rate; ERPF = effective renal plasma flow; ERBF = effective renal blood flow; FF = filtration fraction; RF = renal fraction; RVR = renal vascular resistance.

BC-S group, E_{Cl} 69.2±10.3 *vs.* 19.8±10.6 μEq/min). The plasma levels of CPK increased from 272.3 ± 56.6 U/L at the pre-treatment values to 464.5±198.1, 445.2±168.9, 382.7±160.0 and 344.0±195.7 U/L at 30, 60, 90 and 120 min after envenomation, respectively. The plasma LDH level increased from 124.7±83.6 U/L at the pre-treatment values to 159.3 ± 94.5, 172.2 ± 108.2, 135.8±68.0 and 149.2±73.9 U/L at 30, 60, 90 and 120 minutes after envenomation, respectively. Measurements of plasma calcium levels showed significant decreases from 8.7±1.1 mg/dL at the pre-treatment period to 6.5±1.4 mg/dL at 120 minutes post-treatment ($p < 0.05$, $n = 8$).

Discussion

The aim of the present study was to determine whether *B. candidus* venoms (BCV) play any effects on changes in the general circulation and renal function. The present results show that systemic hypotension was observed during 150 minutes of monitoring *B. candidus* venom (50 μg/kg *iv*). The effect of venom produced concentration-dependent biphasic responses in mean arterial blood pressure in anaesthetized rabbits. This response consisted of an initial short-lasting depressor phase (2-5 minutes; phase 1) and a long-lasting hypotension phase (30-150 minutes; phase 2). Neither phase 1 nor phase 2 of the response to venom was affected by the muscarinic receptor antagonist, atropine sulphate (0.20 mg/kg *iv.*). Thus, the scavenge acetylcholine inducing hypotension by stimulating M1, M2, and M4 muscarinic receptors [9] was not apparent in *B. candidus* envenomation. The hypotensive effect of BCV was accompanied by an increase in total peripheral vascular resistance. This is contrast to the hemodynamics effects of Russell's viper venom in which hypotension is due to decreased systemic vascular resistance [10].

In this experiment, decreased cardiac output was a striking finding and importantly accounted for hypotension. Decreased heart rate and hypotension persisted despite the compensatory mechanism through activation of the sympathetic nervous system. Persistent decrease in cardiac output and heart rate, despite hypotension, suggest direct cardiac effect of the venom. It has been reported that Bucain, isolated from the venom of *B. candidus*, is similar to cardiotoxin [11, 12]. β-cardiotoxin purified from the venom of *Ophiophagus hannah* acted directly on the cardiac tissue as a dose-dependent decrease in heart rate without affecting contractility [13]. It is possible that *B. candidus* venom has calcium channel blocking effects. Calcium channel blockers are a well-known antihypertensive agent. Thromboxane A_2 released as an inflammatory mediator can decrease cardiac output through pulmonary artery constriction and could play a contractility role [14].

Our findings reveal that the *B. candidus* venom could affect both general circulation and renal hemodynamics, resulting in a reduction of the renal fraction (ERBF/CO). The magnitude of an increase in renal vascular resistance appeared to be more than an increase in total peripheral resistance throughout the experimental periods, thereby leading to reductions in GFR and ERPF after envenomation. A disproportionate decrease in ERPF and GFR resulted in significant increase in filtration fraction after envenomation. As expected in hypotension, the urinary electrolyte excretion decreased. The increments of plasma creatine phosphokinase and the plasma potassium levels after envenomation suggest rhabdomyolysis [15]. The CPK and LDH levels were slightly increased. A marked increase might occur with a higher dose of venom. It may be possible that severe envenomation may cause acute renal failure.

Conclusion

The present experiment is the first demonstration of the action of *B. candidus* venom on hemodynamics. Hemodynamic changes were characterized by decreased hypotension, decreased cardiac output, increased total peripheral resistance and increased renal vascular resistance. Renal blood flow and glomerular filtration rate were reduced. Hypotension was attributed to decreased cardiac output due to the effect of the venom on cardiac muscle. A similar effect to calcium blocking agents was postulated.

Acknowledgements

This study has been supported by a Thesis Grant from the Graduate School of Chulalongkorn University and a grant from the Chongkolneenithi foundation. L. Chanhome is recipient of a grant from QSMI. The authors thank the staffs of the Snake farm, Queen Saovabha Memorial Institute, and Nakhon Ratchasima Zoo, for their assistance.

The authors have no conflict of interest to declare.

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