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The renal effects of *Bothrops jararacussu* venom and the role of PLA_2 and PAF blockers

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Abstract

The most common complication in the lethal cases of ophidian bites in Brazil is acute renal failure, but its pathogenesis is obscure. The effects of *Bothrops jararacussu* venom (3, 10 and 30 μg/ml) were examined using the isolated perfused kidney from Wistar rats. Dexamethasone, and WEB 2086, a triazolobenzodiazepine substance, which is a platelet activating factor receptor antagonist, were tested for a possible blockade of the renal effects in the presence of 10 μg/ml of venom. The most intense effects of the venom were noticed at 120 min after using 30 μg/ml. We observed a decrease in the perfusion pressure and in the renal vascular resistance. However, the glomerular filtration rate (GFR) and the urinary flow (UF) increased significantly. The percent of sodium ($\%Na^{+}_{tot}$) and potassium ($\%K^{+}_{tot}$) tubular transport were also decreased. Dexamethasone was unable to block the effects of *B. jararacussu* in the kidney, while WEB 2086 blocked its effect in glomerular filtration rate, urinary flow and in the percentage of total tubular potassium reabsorption. We suggest that this venom promotes diuresis independently of perfusion pressure drop. The alterations in GFR, UF and $\%K^{+}_{tot}$ are probably mediated by platelet activating factor. Dexamethasone did not block the renal effects maybe because of the concentration used in this work or maybe the renal effects are promoted by the myotoxin, which does not have PLA_2 activity. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Bothrops jararacussu*; Kidney; Dexamethasone; WEB 2086

1. Introduction

Bothrops species in Brazil causes almost 90% of all snake envenomations, representing a serious health problem in our country. Amongst snakes of this genus, *Bothrops jararacussu*, when fully grown, can reach large sizes and can inject up to 4 ml of crude venom, corresponding to 1 g of dry crude venom (Belluomini, 1984).

The patients bitten by *Bothrops* sp. normally show signs of local tissue damage such as abscesses, edema and necrosis, promoted by the presence of proteolytic enzymes. Those signs can be aggravated by the presence of clotting factors in the venom (Sanchez et al., 1992). Besides systemic bleeding, this venom can cause hemodynamic shock and the process of disseminated intravascular coagulation. All the above signs are common features in *Bothrops* envenomations. However, *B. jararacussu* has an additional myotoxic effect that can intensify the symptoms and also result in myoglobinuria that can cause further harm to the kidney (Milani et al., 1997).

The most common complication amongst lethal cases after ophidian bites in Brazil is the process of acute renal failure (ARF) (Ribeiro et al., 1998). This process can happen even after specific antivenom treatment, but its pathogenesis is not well understood. Some evidence suggests disseminated intravascular coagulation as a possible cause for this renal lesion, but this one cannot exclude that the presence of proteolytic enzymes and vasoactive substances could promote, or even potentiate, the coagulant process in renal sites (Amaral et al., 1986). Other investigators also reported the possible existence of a direct nephrotoxic agent, which was not well described up to now (Sitprija and Chaiyabutr, 1999; Boer-Lima et al., 1999).

Ophidian envenomations promote intense inflammatory reactions with the release of many inflammatory mediators

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(Barraviera et al. 1995). Monteiro and Fonteles (1999), described that the platelet activating factor (PAF) was involved as a mediator of the *B. jararaca* effects in the isolated perfused rat kidney. They showed that WEB 2086, a PAF receptor antagonist, could block the reductions promoted by this venom in the parameters of glomerular filtration rate and urinary flow. Martins et al. (1998), showed that dexamethasone could block some of the renal effects caused by *Crotalus durissus cascavella*. Considering those data, we decided to study the direct renal effects promoted by the venom of *B. jararacussu*, using WEB 2086 and dexamethasone as possible blockers. To test this possibility, we used the isolated perfused rat kidney, which can evaluate renal physiology changes and metabolism without the influence of extrarenal factors.

2. Materials and methods

Adult Wistar rats of either sex, weighing between 240 and 280 g, were fasted for 24 h with free access to water. They were anesthetized with sodium pentobarbital (50 mg/kg), and after careful dissection of the right kidney, the right renal artery was cannulated through the mesenteric artery, without blood flow interruption (Bowman, 1970; Niishiitsutsuji-Uwo et al., 1967). The perfusion method initially described (Hamilton et al., 1974) was modified in our laboratory by the addition of an artificial lung for better oxygenation (Fonteles et al., 1998, and the introduction of a 1.2 μm millipore filter (Pegg, 1971), placed between the peristaltic pump, which maintain the flow rate, and the artificial lung. The perfusion fluid was a modified Krebs–Henseleit with the following composition (in M): 2.36 NaCl, 0.09 KCl, 0.023 KH_2PO_4, 0.023 $MgSO_47H_2O$, 0.025 $CaCl_22H_2O$, and 0.025 $NaHCO_3$. Six grams of bovine serum albumin (BSA fraction V), purchased from Sigma Chemical Co. (St Louis, MO, USA), were added to 100 ml of modified Krebs–Henseleit solution. The solution was dialyzed for 48 h at 4°C to withdraw possible commercial serum albumin contaminators such as citrate, pyruvate and lactate (Hanson and Ballard, 1968; Fonteles et al., 1983; Lima et al., 1992). After dialysis, the final perfusate solution was adjusted by the addition of urea 0.075 g, inulin 0.075 g and glucose 0.15 g to 100 ml of perfusate, and pH adjusted to 7.4. The rate of perfusion was maintained between 25 and 35 ml/min per kidney. The perfusion pressure was measured at the tip of the stainless steel cannula and was allowed to fluctuate under experimental conditions, but was carefully kept at 120–140 mmHg during the 30-min internal control period. In each experiment, the recirculating perfusion system employed 100 ml of the solution and lasted 120 min (Monteiro et al., 1999). After mounting the kidney in the perfusion system, at least 20 min were allowed for complete equilibration. Perfusion pressure was measured at 5 min intervals. Every 10 min samples of urine, obtained by the cannulated right ureter, and perfusate were collected for further analysis of sodium, potassium, inulin, and osmolality, which were done for all experimental groups. Clearance measurements were made according to Pitts (1971), and Martinez-Maldonado and Opava-Stitzer (1978). Sodium and potassium were measured by flame photometry (Instrumentation Laboratory model 445). Inulin was determined according to Walser et al. (1955) and modified by Fonteles et al. (1983). Osmolality was measured in a vapor pressure osmometer (Wescor 5100C, USA). The glomerular filtration rate (GFR), the fractional tubular reabsorptions of sodium and potassium ($\%Na^+_{tot}$, $\%K^+_{tot}$), the renal vascular resistance (RVR) and urinary flow (UF) were determined by conventional formulas described by Martinez-Maldonato and Opava-Stitzer (1978) and Fonteles and cooworkers (1983). All chemicals were reagent grade and purchased from Sigma Chemical Co. The venom of *B. jararacussu* was a gift of Dr Carlos Ribeiro Diniz from Ezequiel Dias Institute (Minas Gerais, Brazil). The first 30 min of each experiment were used as internal control. The venom was always added to the recirculating solution at the time of 30 min. The triazolobenzodiazepine substance (WEB 2086), a receptor antagonist of platelet activating factor (100 μg/ml), which was a gift of Boehringer Manheim (Germany), and Dexamethasone (20 μg/ml) were added to the recirculating system into the solution always at the beginning of each experiment. Data were averaged of four periods of 30 min. There were six perfused-kidney experiments for each data point. Statistical differences were determined by an analysis of variance (ANOVA) with two levels of significance set at $*P < 0.05$ and $**P < 0.001$. Bonferoni *t*-test was used to compare the control group against the three treated groups in the first part of the experiments, and then, to compare the control group with the three groups treated with venom (10 μg/ml), dexamethasone and WEB 2086.

3. Results

3.1. Effects of the B. jararacussu venom in the rat kidney perfusion

Initial experiments investigated the physiology and the metabolism of the kidney after perfusion, using only the modified solution (Krebs–Henseleit + albumin + urea + glucose + inulin), and, thereafter, three different concentrations (3, 10 and 30 μg/ml) of the venom of *B. jararacussu*. Following an analysis of variance no significant differences were found for all parameters evaluated in the control group (Table 1).

All three concentrations of *B. jararacussu* venom had their maximum effect at the period of 120 min, which represents the average of the values for the last 30 min of each experiment. The most intense effects were seen in the concentration of 30 μg/ml. After the addition of 30 μg/ml there was a significant decrease in the perfusion pressure ($control_{120} = 130.50 \pm 0.50$ mmHg; $venom_{120} =$

Table 1
Effects of *B. jararacussu* venom on kidney function and the role of dexamethasone and WEB 2086[a]

Variables	30 min	60 min	90 min	120 min
PP (mmHg)				
Control	125.8 ± 1.07	129.9 ± 0.85	130.80 ± 0.58	130.5 ± 0.50
Venom	122.8 ± 1.12	103.7 ± 4.87	88.40 ± 2.69**	70.20 ± 3.10**
Venom ± Dexa	127.1 ± 0.68	95.6 ± 10.66	74.30 ± 0.35**	71.60 ± 2.55**
Venom ± WEB2086	125.3 ± 0.71	109.0 ± 9.43	76.00 ± 3.03**	63.30 ± 2.45**
RVR (mmHg/ml/g/min)				
Control	5.79 ± 0.04	5.94 ± 0.04	6.36 ± 0.25	6.17 ± 0.17
Venom	5.73 ± 0.04	4.89 ± 0.25	4.09 ± 0.14**	3.22 ± 0.14**
Venom ± Dexa	5.68 ± 0.05	4.20 ± 0.48	3.21 ± 0.02**	3.10 ± 0.10**
Venom ± WEB2086	5.41 ± 0.02	4.74 ± 0.38	3.34 ± 0.14**	2.78 ± 0.10**
GFR (ml/g/min)				
Control	0.78 ± 0.04	0.75 ± 0.01	0.78 ± 0.05	0.74 ± 0.02
Venom	0.95 ± 0.12	0.25 ± 0.18	0.39 ± 0.10	1.39 ± 0.02**
Venom ± Dexa	0.75 ± 0.01	0.35 ± 0.19	0.48 ± 0.15	0.97 ± 0.07**
Venom ± WEB2086	0.76 ± 0.01	0.30 ± 0.12	0.36 ± 0.10	0.76 ± 0.05
UF (ml/g/min)				
Control	0.14 ± 0.01	0.14 ± 0.001	0.15 ± 0.001	0.15 ± 0.001
Venom	0.15 ± 0.01	0.08 ± 0.05	0.15 ± 0.04	0.37 ± 0.04**
Venom ± Dexa	0.17 ± 0.09	0.10 ± 0.04	0.14 ± 0.04	0.41 ± 0.05**
Venom ± WEB2086	0.15 ± 0.03	0.09 ± 0.03	0.09 ± 0.02	0.21 ± 0.02
$\%Na^{+}_{tot}$				
Control	81.20 ± 0.10	80.50 ± 0.18	79.40 ± 0.37	80.10 ± 0.10
Venom	81.90 ± 1.35	64.20 ± 2.82**	61.70 ± 2.50**	54.50 ± 0.95**
Venom ± Dexa	79.20 ± 0.90	69.50 ± 2.11**	67.80 ± 1.05**	67.90 ± 1.91**
Venom ± WEB2086	80.20 ± 0.30	69.70 ± 1.20**	69.50 ± 0.80**	71.20 ± 0.25**
$\%K^{+}_{tot}$				
Control	74.50 ± 0.35	74.20 ± 0.84	76.40 ± 1.15	75.40 ± 0.14
Venom	71.40 ± 2.33	53.40 ± 1.80**	62.80 ± 2.65*	55.40 ± 0.87**
Venom ± Dexa	74.20 ± 1.46	65.50 ± 0.30**	64.60 ± 1.90*	67.30 ± 1.09*
Venom ± WEB2086	76.90 ± 0.40	65.80 ± 0.83**	69.30 ± 0.92	71.20 ± 0.26

[a] Results are expressed as means ± S.E.M. from six different animals for each group. Statistical analysis were done by ANOVA, comparing the three treated groups with the control group with $*P < 0.05$ and $**P < 0.001$. All data were averaged between 30 min intervals (30, 60, 90 and 120 min) in triplicate. The venom was added always after the first 30 min of each experiment. Dexamethasone and WEB 2086 were always added 30 min before the venom. PP, perfusion pressure; RVR, renal vascular resistance; GFR, glomerular filtration rate; UF, urinary flow; $\%Na^{+}_{tot}$, percent of sodium tubular transport; $\%K^{+}_{tot}$, percent of potassium tubular transport; Dexa, Dexamethasone (20 μg/ml) and WEB 2086 (100 μg/ml).

53.70 ± 2.70 mmHg, $P < 0.001$) and in the renal vascular resistance ($control_{120} = 6.17 \pm 0.17$ mmHg/ml/g/min; $venom_{120} = 2.66 \pm 0.14$ mmHg/ml/g/min, $P < 0.001$). The percentage of total tubular sodium transport ($\%Na^{+}_{tot}$) decreased statistically after venom addition. The values for control period were 80.10 ± 0.09% and after the venom administration (30 μg/ml), at the period of 120 min, we noticed a decrease to 53.41 ± 1.62% ($P < 0.001$). The percentage of total tubular potassium transport ($\%K^{+}_{tot}$) also diminished after venom administration. The control averaged 75.40 ± 0.15%, while the venom dropped to 52.14 ± 1.89% ($P < 0.001$).

At 120 min, there was a marked increase in glomerular filtration rate (GFR) and urinary flow (UF). Control GFR was 0.74 ± 0.02 ml/g/min, after venom we found 1.98 ± 0.06 ml/g/min ($P < 0.001$). Control UF was 0.15 ± 0.001 ml/g/min, and after venom 0.81 ± 0.04 ml/g/min ($P < 0.001$).

In the period of 120 min, using the same level of significance ($P < 0.001$), we observed in the concentration of 3 μg/ml that the parameters of PP, RVR, $\%Na^{+}_{tot}$, $\%K^{+}_{tot}$ were statistically different from the control group. Those alterations were mild when compared to the effects observed in the concentration of 30 μg/ml. However, in the parameters of GFR and UF the concentration of 3 μg/ml was not statistically different from the control values, even after reducing the level of significance to $P < 0.05$.

3.2. The renal effects of *B. jararacussu* after dexamethasone (dexa) and WEB 2086

Inflammatory mediators could cause alterations in renal

function following venom administration. Therefore, we tested the effects of dexamethasone and WEB 2086 to inhibit the changes in renal function seen in the previous section. Two previous articles from our group (Martins et al., 1998; Monteiro et al. 1999) showed no significant effect of either dexamethasone (20 μg/ml) or WEB 2086 (100 μg/ml), when administered alone.

When attempting to abolish the effects of the venom of *B. jararacussu*, we had chosen the intermediate dose of 10 μg/ml. Drugs were always administered 30 min before the venom, at the zero time, after the equilibration period. None of the effects of the venom were blocked by dexamethasone when compared to the control group. WEB 2086 did not block the effects of the venom on PP, RVR and $\%Na^+_{tot}$. However, this drug inhibited the effects of the *B. jararacussu* venom on GFR, UF and $\%K^+_{tot}$ when compared to the control group (Table 1).

4. Discussion

In a recent review, Stiprija and Chaiyabutr (1999) described the main renal alterations caused by snakebite. They reported that there are three possible mechanisms responsible for its pathogenesis: hemodynamic alterations, immunologic reactions and direct nephrotoxicity. Swe et al. (1997) affirmed that the renal damage after snake accidents can be promoted by the disseminated intravascular coagulation (DIC), associated with a direct tubulotoxic effect. Some researchers assure the importance of the systemic lesions caused by the coagulative process after *Bothrops* accidents, relating them to the lethal cases (Ribeiro et al., 1998; Milani et al., 1997). However, Win-Aung et al. (1998) described a direct renal effect after snakebites of *Daboia russelii*, whose venom is closely related to *Bothrops* venoms. However, disseminated intravascular coagulation possibly has no relevance to the current experiments since our perfusate is devoid of fibrinogen and clotting factors.

After renal perfusion with the venom of *B. jararacussu*, we noticed a statistically decrease in the perfusion pressure (PP) and renal vascular resistance (RVR) in the two intervals of 90 and 120 min. Despite of the vascular parameters that decreased after kidneys treatment with 10 and 30 μg/ml in the intervals of 90 and 120 min, we found an intense increase of the GFR and UF in the same groups and intervals. The intense diuresis promoted by *B. jararacussu* venom, which was independent of the vascular pressure drop, led us to postulate that perhaps those effects were promoted by different substances. One of these substances could be the bradykinin-potentiating peptides (BPP). Ferreira et al. (1992) reported at least six bradykinin-potentiating peptides presented in the venom of *B. jararacussu*, but the one called Peptide P was more active than captopril in its inhibition of the conversion of angiotensin I to angiotensin II.

Barraviera et al. (1995) reported that kidney cells could release prostaglandin, cytokines, bradykinin, complement fractions and platelet activating factor. Koeppen and Stanton (1997) stated that bradykinin, by stimulating the liberation of nitric oxide from renal endothelium, could promote the increase of the GFR.

Many bradykinin-potentiating peptides were isolated from the venom of *B. jararaca* (Ferreira et al., 1970). Monteiro and Fonteles (1999), using the same method described herein, when evaluating *B. jararaca* venom, found no increase in the GFR or in the UF. On the contrary, they observed an intense decrease in GFR and in UF after using 10 μg/ml of the venom. However, the same decrease in the perfusion pressure was found in both works. These results show that even though both venoms come from serpents of the same genus, their renal effects are different. Perhaps other substances present in the *B. jararacussu* venom lead to a specific diuresis, which was not seen with the use of *B. jararaca* venom.

Murayama et al. (1997) identified a gene that code seven BPP peptides, and also a natriuretic peptide C type in the venom of *B. jararaca*. Recently, many natriuretic peptides presented in ophidian venom have been reported in the literature (Higuchi et al., 1999; Schweitz et al., 1992; Ho et al., 1997). Lisy et al. (1999) described the diuretic and natriuretic effects promoted by the natriuretic peptide, called DNP, isolated from the serpent *Dendroaspis angusticeps* (Green Mamba). DNP showed structure similarities with atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). If the diuresis found in our experiments were caused by a natriuretic peptide it was probably different from the CNP found in *B. jararaca*, since this serpent did not promoted this effect.

Dexamethasone is a glucocorticoid that inhibits phospholipase A_2, suppressing the release of lipid mediators and vasoactive amine (Barnes, 1995). Martins et al. (1998) showed, in the isolated perfused rat kidney method, that dexamethasone (20 μg/ml) did not affect renal function when compared with the control group. However, this drug abolished all renal alterations induced by *Crotalus durissus cascavella* venom.

Bon (1997) affirmed that almost all snake species have phospholipase A_2 in their venoms. After fractionation of *B. jararacussu* venom, Homsi-Brandeburgo et al. (1988) reported the existence of a fraction that had all the phospholipase A_2 activity (PLA_2) presented in this venom. From this fraction they identified five subfractions. One of them had no PLA_2 activity, though it showed itself to be highly myotoxic. The other subfractions, where PLA_2 were detected, were not myotoxic. Using the same amount of dexamethasone as Martins et al. (1998), we observed that this drug was unable to block the renal effects caused by *B. jararacussu* venom. Therefore, we showed that the renal effects caused by this venom could not be blocked with the same amount used for Martins et al. (1998) or the effects are promoted by the myotoxin, which does not have phospholipase A_2 activity.

In the same method of isolated rat kidney, Monteiro et al. (1999), reported that after administration of WEB 2086 (100 μg/ml), kidneys remained stable throughout the perfusion time, but did inhibit the glomerular and renal tubular effects induced by cholera toxin. This drug, in the same concentration described above, was used to inhibit renal alterations induced by *B. jararaca*. WEB 2086 abolished the decrease in GRF and UF, but did not attenuate sodium tubular transport nor the perfusion pressure (Monteiro and Fonteles, 1999). Our results showed many similarities with the latter. WEB 2086 inhibited the alterations of GFR and UF, but did not attenuate the decrease of $\%Na^{+}_{tot}$, PP and RVR. In addition, the diminution of potassium tubular transport was blocked, starting at the period of 90 min and lasting until the end of the experiments.

We conclude that the *B. jararacussu* venom altered all the renal functional parameters evaluated in this work. We also suggest that the increases in GFR and UF were independent from the vasoactive parameters PP and RVR, being promoted by PAF. The tubular sodium transport is probably not influenced by PAF, but it affects the potassium transport. Maybe the amount of dexamethasone we used was not able to block the renal effects. However, the myotoxin found in the *B. jararacussu* could be involved in this effect since it does not have PLA_2 activity.

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