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Distribution of ^{131}I-labeled *Bothrops erythromelas* venom in mice

C.M.L. Vasconcelos[1,3], R.C. Valença[1], E.A. Araújo[1], J.C.A. Modesto[2], M.M. Pontes[2], T.K. Brazil[4] and M.C. Guarnieri[2]

[1]Departamento de Biofísica e Radiobiologia and [2]Departamento de Zoologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, PE, Brasil
[3]Departamento de Ciências Biológicas, Universidade Tiradentes, Aracajú, SE, Brasil
[4]Departamento de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, Salvador, BA, Brasil

Abstract

Bothrops erythromelas is responsible for many snake bites in northeastern Brazil. In the present study we determined the *in vivo* distribution of the venom following its subcutaneous injection into mice. *B. erythromelas* venom and albumin were labeled individually with ^{131}I by the chloramine T method, and separated in a Sephacryl® S-200 column. The efficiency of labeling was 68%. Male Swiss mice (40-45 g), which had been provided with drinking water containing 0.05% KI over a period of 10 days prior to the experiment, were inoculated dorsally (*sc*) with 0.3 ml (2.35 x 10^5 cpm/mouse) of ^{131}I-venom (N = 42), ^{131}I-albumin or ^{131}I (controls, N = 28 each). Thirty minutes and 1, 3, 6, 12, 18 and 24 h after inoculation, the animals were perfused with 0.85% NaCl and skin and various organs were collected in order to determine radioactivity content. There was a high rate of venom absorption in the skin (51%) within the first 30 min compared to albumin (20.1%) and free iodine (8.2%). Up to the third hour after injection there was a tendency for venom and albumin to concentrate in the stomach (3rd h), small intestine (3rd h) and large intestine (6th h). Both control groups had more radioactivity in the digestive tract, especially in the stomach, but these levels decreased essentially to baseline by 12-18 h postinjection. In the kidneys, the distribution profiles of venom, albumin and iodine were similar. Counts at 30 min postinjection were low in all three groups (1.37, 1.86 and 0.77, respectively), and diminished to essentially 0% by 12-18 h. Albumin tended to concentrate in muscle until the 3rd h postinjection (1.98%). There was a low binding of labeled venom in the liver (<0.54%), thyroid (<0.11%) and lungs (<0.08%), and no iodinated venom was detected in brain, heart, diaphragm, spleen or bladder. The low venom binding observed in most internal organs, comparable to that of albumin, suggests that *B. erythromelas* venom does not specifically target most internal organs. That is, the systemic effects of envenomation are mainly due to an indirect action.

Correspondence
M.C. Guarnieri
Laboratório de Animais Peçonhentos e Toxinas
Departamento de Zoologia
CCB, UFPE
Av. Prof. Moraes Rego, s/n
50670-901, Recife, PE
Brasil
Fax: 55 (081) 271-8359
E-mail: mcg@npd.ufpe.br

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Key words
- *Bothrops erythromelas*
- Snake venom
- Protein iodination

Introduction

Bothrops erythromelas, commonly known as "jararaca-da-seca" or "jararaca-malha-de-cascavel", is responsible for many snake bites in northeastern Brazil (1,2). The venom of this snake lacks thrombin-like activity (3-5), but exhibits powerful procoagulant action on factors X and II (3-6). Patients with systemic envenomation may develop disseminated intravascular coagulation followed by blood incoagulability due to the consumption of coagulant factors (5). *Bothrops erythromelas* venom also displays considerable hemorrhagic (5,6), fibrinolytic, proteolytic (4,7) and phospholipase A_2 (8) activities, induces edema and necrosis (6,7) and inhibits platelet aggregation (9). The direct myotoxic activity of the venom is low (10), suggesting that the phospholipases it contains have other biological targets. Recent studies on dogs performed in our laboratory have shown that *B. erythromelas* venom induces hemostatic changes involving hypercoagulable blood followed by incoagulable blood (6) and intense hemorrhage in the lungs, kidneys and liver (11). Despite the medical importance of this species in this region, there are no studies on the distribution of this venom in simulated envenomations. In the present study we examined the distribution of ^{131}I-labeled *B. erythromelas* venom in mice following subcutaneous injection.

Material and Methods

Radioiodination

Bothrops erythromelas venom was obtained from specimens collected in Itaparica (Bahia, Brazil) and maintained in the Departamento de Zoologia, UFBA. Venom and bovine serum albumin (25 mg each) were labeled with 3.1 x 10^8 cpm of Na^{131}I using the chloramine T method (12). Excess free iodine was removed by gel filtration on a Sephacryl® S-200 column (1.3 x 97.3 cm) (Pharmacia Biotech, Uppsala, Sweden) equilibrated with 50 mM sodium phosphate buffer, pH 7.4, containing 0.1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO).

Biodistribution

Male Swiss mice (40-45 g) were provided with drinking water containing 0.05% KI for 10 days prior to the experiments in order to saturate the thyroid gland with cold iodine. Mice were divided into two groups: controls (N = 56) were injected dorsally (*sc*) with 0.3 ml of ^{131}I or ^{131}I-labeled albumin while experimental animals (N = 42) received ^{131}I-labeled venom. The injected amount of ^{131}I, ^{131}I-albumin and ^{131}I-venom was 235,000 cpm/mouse. Mice were then perfused with 0.85% NaCl through the right atrium after incision of the left ventricle at 0.5, 1, 3, 6, 12, 18, or 24 h postinjection. The brain, thyroid, heart, lungs, diaphragm, liver, stomach, small intestine, large intestine, spleen, kidneys, bladder, muscle and skin were removed for counting using a gamma scintillation counter (GAMBYT CR 10/20, DPC, Los Angeles, CA).

After labeling, labeled venom was applied to a Sephacryl® S-200 column to separate labeled crude venom from free iodine. Labeling efficiency was 68.8%, which is relatively high.

Results and Discussion

At 30 min postinjection, skin accounted for more that 50% of the ^{131}I-labeled venom injected. All tissues together accounted for 56.2% of injected counts 30 min postinjection. Of this total, skin contained 51.04% of ^{131}I-labeled venom, mainly at the site of injection (Table 1, Figure 1A). This level dropped continuously until it reached 1.8% at 18 h. Control animals receiving ^{131}I-labeled albumin displayed the same pattern

with a phase shift. After 30 min, 20.1% of injected counts remained in the skin; however, this value rose to a maximum of 41.1% at 1 h and declined steadily to 2% at 18 h in precisely the same manner as observed for labeled venom (Table 1, Figure 1A).

In control animals receiving ^{131}I, only 8.2% free iodine was found in the skin after 30 min. This level diminished steadily thereafter in the same manner as observed for ^{131}I-labeled venom. Thus, the pattern of radioactivity in the skin was the same for iodine and for labeled proteins, but ^{131}I alone was cleared from the site of injection much more rapidly. This observation suggests that the remaining venom at the site of injection may be due to the high relative molecular size related to iodine, making its absorption and elimination difficult. The high concentration of the venom in the skin is compatible with clinical effects caused by this venom, including edema, pain, blisters, ecchymosis and hemorrhage (1).

The distribution of a hemorrhagic protease (jararafibrase-I) purified from *B. jararaca* venom suggests that this venom may

Table 1 - Distribution of ^{131}I-labeled *Bothrops erythromelas* venom (V), ^{131}I-labeled albumin (A) and ^{131}I (I) in mice.

Radioactivity is reported as percentage of the total dose administered (235,000 cpm). Data are reported as the mean ± SD for 3 experiments. No radioactivity was detected in the brain, heart, diaphragm or bladder.

		Time (h)					
Organ		0.5	1	3	6	12	18
Skin	V	51.04 ±13.86	35.84 ±13.48	17.55 ± 6.61	6.38 ± 3.27	2.10 ± 0.58	1.84 ± 1.86
	A	20.08 ± 3.33	41.04 ±12.30	28.85 ±10.79	19.24 ± 6.21	4.31 ± 0.94	2.01 ± 0.80
	I	8.24 ± 0.82	4.27 ± 0.55	2.46 ± 0.31	2.07 ± 0.62	0.15 ± 0.03	0.32 ± 0.25
Muscle	V	1.80 ± 1.01	0.61 ± 0.15	0.66 ± 0.65	0.24 ± 0.07	0.07 ± 0.04	0.05 ± 0.02
	A	1.14 ± 0.35	1.31 ± 0.28	1.98 ± 1.68	0.85 ± 0.52	0.41 ± 0.19	0.12 ± 0.04
	I	1.49 ± 0.16	0.82 ± 0.33	0.64 ± 0.20	0.54 ± 0.29	0.01 ± 0.01	0
Kidney	V	1.38 ± 0.31	1.37 ± 0.24	0.86 ± 0.17	0.59 ± 0.29	0.21 ± 0.12	0.09 ± 0.03
	A	1.86 ± 0.79	1.71 ± 0.23	1.60 ± 0.94	0.68 ± 0.07	0.36 ± 0.09	0.42 ± 0.18
	I	0.77 ± 0.13	0.56 ± 0.19	0.29 ± 0.08	0.28 ± 0.08	0	0
Stomach	V	0.62 ± 0.22	1.51 ± 0.29	1.57 ± 1.03	1.39 ± 0.60	0.13 ± 0.04	0.10 ± 0.07
	A	1.71 ± 0.97	2.93 ± 0.93	3.07 ± 1.11	1.86 ± 0.86	0.69 ± 0.22	0.25 ± 0.08
	I	10.26 ± 0.72	5.04 ± 1.75	3.60 ± 1.75	1.92 ± 0.62	0.05 ± 0.01	0.04 ± 0.01
Small intestine	V	0.59 ± 0.13	0.78 ± 0.19	0.98 ± 0.33	0.82 ± 0.45	0.04 ± 0.04	0.02 ± 0.04
	A	1.06 ± 0.49	1.58 ± 0.27	1.75 ± 0.62	1.05 ± 0.29	0.58 ± 0.13	0.16 ± 0.20
	I	3.38 ± 1.04	2.14 ± 1.26	1.39 ± 0.66	0.99 ± 0.65	0.20 ± 0.04	0
Large intestine	V	0.17 ± 0.04	0.23 ± 0.08	0.33 ± 0.04	0.36 ± 0.19	0.04 ± 0.02	0.03 ± 0.02
	A	0.38 ± 0.33	0.30 ± 0.04	0.70 ± 0.10	0.89 ± 0.15	0.34 ± 0.15	0.41 ± 0.20
	I	1.04 ± 0.28	0.72 ± 0.22	0.35 ± 0.04	0.32 ± 0.06	0.02 ± 0.01	0
Liver	V	0.48 ± 0.08	0.54 ± 0.15	0.38 ± 0.09	0.50 ± 0.08	0.06 ± 0.09	0
	A	0.81 ± 0.49	0.80 ± 0.10	0.81 ± 0.41	0.47 ± 0.08	0.28 ± 0.02	0.12 ± 0.06
	I	1.58 ± 0.20	0.79 ± 0.20	0.55 ± 0.11	0.42 ± 0.07	0	0
Spleen	V	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	0.04 ± 0.01	0	0
	A	0.10 ± 0.08	0.10 ± 0.04	0.14 ± 0.08	0.04 ± 0.01	0.02 ± 0.01	0.12 ± 0.12
	I	0.27 ± 0.04	0.17 ± 0.04	0.05 ± 0.01	0.05 ± 0.01	0	0
Lung	V	0.04 ± 0.02	0.06 ± 0.01	0.08 ± 0.04	0.06 ± 0.03	0	0
	A	0.04 ± 0.02	0.06 ± 0.04	0.06 ± 0.04	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
	I	0.14 ± 0.04	0.16 ± 0.01	0.08 ± 0.01	0.06 ± 0.02	0	0
Thyroid	V	0.03 ± 0.01	0.06 ± 0.05	0.08 ± 0.05	0.11 ± 0.09	0.03 ± 0.02	0.04 ± 0.02
	A	0	0.08 ± 0.02	0.05 ± 0.05	0.06 ± 0.02	0.02 ± 0.02	0.03 ± 0.01
	I	0.12 ± 0.04	0.11 ± 0.04	0.13 ± 0.02	0.12 ± 0.03	0.06 ± 0.04	0

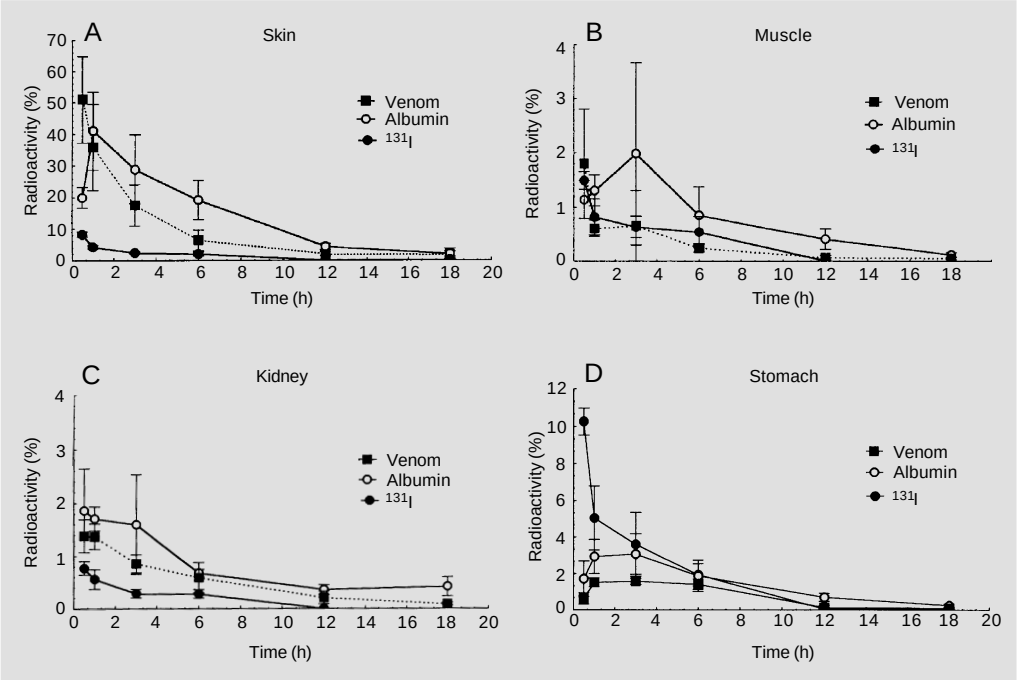

Figure 1 - Distribution of *Bothrops erythromelas* venom, albumin and iodine in the skin (A), muscle (B), kidneys (C) and stomach (D) of animals submitted to perfusion. Radioactivity is reported as percentage of the total injected dose. Data are reported as the means ± SD for 3 experiments.

also remain at the bite site for a long period of time, due to the diminished local circulation of blood caused by the hemorrhagic toxins (13). Maruyama et al. (14) also reported that the hemorrhagic factors may influence the venom absorption rate by changing vascular permeability.

With regard to the distribution of radioactivity in muscle and kidney, experimental and control animals exhibited the same accumulation and clearance pattern as observed for venom in skin. The highest values were recorded at 30 min for experimental and ^{131}I control. In contrast albumin control peaked at the 3rd h. All three groups declined steadily thereafter (Table 1, Figure 1B and 1C). These results suggest that *B. erythromelas* venom has a low capacity to bind to muscle tissue, which is consistent with the previously reported low myotoxicity of this venom (10).

Kinetics of labeled venom and albumin exhibited a completely different pattern in the stomach and small and large intestines (Table 1, Figures 1D, 2A and 2B). In these tissues radioactivity increased steadily after injection reaching maximum levels of 1.6%, 1% and 0.4%, after 3 to 6 h, for the stomach and small and large intestines, respectively. In contrast, control animals receiving ^{131}I showed highest radioactivity in the gastrointestinal tract 30 min after injection. As was the case for the other tissues, radioactive NaI counts decreased continuously thereafter. The presence of the venom in the stomach and intestines is compatible with the digestive system bleeding characteristic of envenomation by this snake (1).

Tanigawa et al. (13) showed that the metabolization of hemorrhagin (*B. jararaca*) occurs mainly in the liver, following intravenous administration to experimental animals which were not perfused. On the other hand, the levels of radioactivity detected in liver of mice injected with *B. erythromelas* venom were lower than in control animals (Table 1). These results can be explained by perfusion of the animals and the subcutaneous route of administration.

Negligible levels of radioactivity (<0.05%) were observed in the spleen and lung of experimental mice (Table 1) and those receiving labeled albumin. As is evident from the overlapping errors, these values appeared to be essentially static across all time inter-

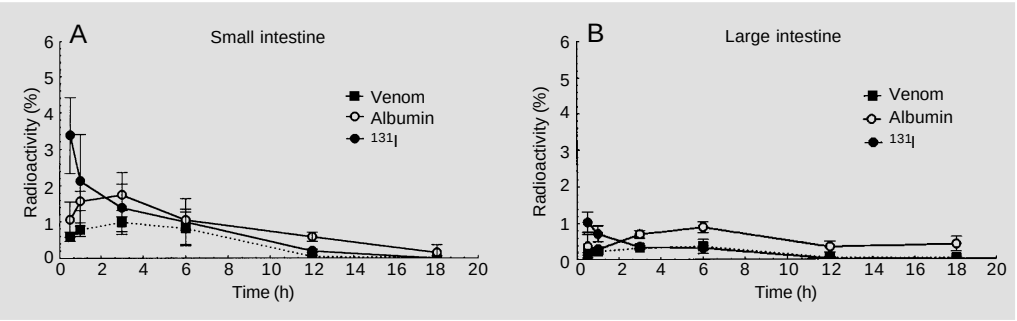

Figure 2 - Distribution of *Bothrops erythromelas* venom, albumin and iodine in the small intestine (A) and large intestine (B) of animals submitted to perfusion. Radioactivity is reported as percentage of the total injected dose. Data are reported as the means ± SD for 3 experiments.

vals. ^{131}I controls displayed marginally higher values at the outset, but these were also minimal (<0.15%). In thyroid tissue, radioactivity levels (Table 1) were maximal at 6 h postinjection in animals receiving ^{131}I and decreased thereafter.

Radioactivity was not detected in the brain, heart, bladder or diaphragm, indicating that *B. erythromelas* venom does not act directly on these organs. The extremely low concentration of the venom in the brain suggests that *B. erythromelas* venom could not penetrate the blood-brain barrier. Twenty-four hours after the administration of ^{131}I-labeled venom, no radioactivity was detected in all organ samples.

Although *B. erythromelas* venom may induce acute renal failure (1) and intense pulmonary hemorrhage (11), the present results show that it has a low capacity to bind to these organs and suggest that the effects observed occur mainly through indirect mechanisms, perhaps through the formation of microemboli in the circulation.

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