EXPERIMENTAL ENVENOMATION WITH Crotalus durissus terrificus VENOM IN DOGS TREATED WITH ANTIOPHIDIC SERUM – PART II: LABORATORY ASPECTS, ELECTROCARDIOGRAM AND HISTOPATHOLOGY

NOGUEIRA R. M. B. (1), SAKATE M. (2), SANGIORGIO F. (1), LAPOSY C. B. (3), TOSTES R. A. (4)

(1) Department of Small Animals, Medical Clinic, University of Western São Paulo, UNOESTE, Presidente Prudente, São Paulo State, Brazil; (2) Department of Small Animals, Medical Clinic, School of Veterinary Medicine and Animal Husbandry, São Paulo State University, UNESP, Botucatu, São Paulo State, Brazil; (3) Department of Clinical Pathology, UNOESTE, Presidente Prudente, São Paulo State, Brazil; (4) Department of Pathological Anatomy, UNOESTE, Presidente Prudente, São Paulo State, Brazil.

ABSTRACT: The present work shows laboratory aspects, electrocardiogram and histopathology results during experimental envenomation by Crotalus durissus terrificus in dogs treated with antiophidic serum. Twenty-one dogs were divided into three groups of seven animals each. Group I received 1mg/kg venom (sc); Group II received 1mg/kg venom (sc), 50mg antiophidic serum (iv) and fluid therapy including 0.9% NaCl solution (iv); and Group III received 1mg/kg venom (sc), 50mg antiophidic serum (iv) and fluid therapy including 0.9% NaCl solution containing sodium bicarbonate diluted to the dose of 4mEq/kg. Urinalysis showed brown urine, proteinuria, occult blood and myoglobinuria. Respiratory acidosis and hypotension were also observed. At the venom inoculation site, there was discreet edema, popliteal lymph node response, musculature presenting whitish areas and necrotic myositis with myoregenerative activity. There was not evidence of electrocardiographical and biochemical alterations.

KEY WORDS: Crotalus durissus terrificus, dog, laboratory aspects, histopathology.

CONFLICTS OF INTEREST: There is no conflict.

CORRESPONDENCE TO: ROSA MARIA BARILLI NOGUEIRA, Rua Santo Brugnoli, 249, 19025-160, Presidente Prudente, São Paulo, Brasil. Email: noballi@ig.com.br.
INTRODUCTION

Accidents by snakes of the genus *Crotalus* constitute the second most frequently reported accidents in Brazil, which usually show high severity (7, 8, 15). Crotalic venom is composed of the fractions crotoxin (50%), crotamine, giroxin and convulxin (6, 22, 25). It produces neurotoxic, myotoxic, nephrotoxic and hemolytic effects (2, 3, 9, 26). The neurotoxic activity of *C. durissus terrificus* venom is mainly attributed to crotoxin, the major component of this venom (10). Recently, crotoxin has been proved to cause systemic skeletal muscle injury in mice, being selective for types I and IIa fibers (11, 27). The thrombin-like enzyme is responsible for the venom coagulant activity (23).

Ophidic accidents can be classified as light, moderate and severe, and the only effective treatment to neutralize the action of crotalic venom is intravenous administration of antiophidic serum. Acute renal failure with tubular necrosis can be the main complication of a crotalic accident (18, 26).

MATERIALS AND METHODS

This experiment was approved by the Ethics Committee of University of Western São Paulo, UNOESTE, Presidente Prudente, Brazil.

Twenty-one dogs (1–4 years old, no defined breed, and 4–15kg) from the Central Kennel of UNOESTE were used in the study. Based on complete physical examination and laboratory tests, animals showing normal values were selected (17). Dogs were kept in individual stalls at the kennel of the Veterinary Hospital at UNOESTE. They received water and food *ad libitum*.

Animals were divided into three groups of seven animals each, Group I: animals inoculated with crotalic venom (1mg/kg); Group II: animals inoculated with crotalic venom (1mg/kg) and treated with antiophidic serum (50mg) and fluid therapy including 0.9% NaCl solution 6h after venom inoculation (AV); Group III: animals inoculated with crotalic venom (1mg/kg) and treated with antiophidic serum (50mg) and fluid therapy including 0.9% NaCl solution containing sodium bicarbonate diluted to the dose of 4mEq/kg 6h AV.

Antiophidic serum (bothropic-crotalic) was from Vencofarma Laboratory (serum sample 001/03); each flask of 10ml serum neutralizes 10mg crotalic venom.
Lyophilized crotalic venom supplied by the Center for the Study of Venoms and Venomous Animals, CEVAP, UNESP, Brazil, was reconstituted in sterile saline solution and subcutaneously inoculated, at the dose of 1mg/kg body weight, into the lateral surface of the animals thigh; antiphidic serum was intravenously administered 6h after venom inoculation (AV).

Laboratory studies included biochemical tests for urea and creatinine, (blood samples were collected by jugular vein puncture using a 30X08mm hypodermic needle and a 10ml syringe), blood gas analysis (blood samples were collected by femoral artery puncture using a 20X5.5mm hypodermic needle and a 1ml syringe containing heparin), urinalysis (urine was collected by cystocentesis using a 25X07mm hypodermic needle and a 10ml syringe), systolic blood pressure (Doppler), histopathology and electrocardiogram.

Table 1. Moments (M) of laboratory tests – urinalysis, electrocardiogram, serum biochemistry, blood gas analysis, systolic blood pressure and histopathology.

|                         | M0 | M1  | M2  | M3  | M4  | M5            |
|-------------------------|----|-----|-----|-----|-----|---------------|
| Urinalysis              | Control | 6h AV | 24h AV | 48h AV | 72h AV | 144h AV       |
| Electrocardiogram       | Control | 6h AV | 24h AB | 48h AV | 72h AV | 144h AV       |
| Biochemistry            | Control | 6h AV | 48h AV | 144h AV | -     | -             |
| Blood gas analysis      | Control | 6h AV | 48h AV | 144h AV | -     | -             |
| Systolic blood pressure | Control | 2h AV | 6h AV | 9h AV  | -     | -             |
| Histopathology          | -     | -    | -    | -    | -    | euthanasia after 144h |

AV: after venom inoculation; Antiphidic serum was administered 6h after venom inoculation.

**Statistical analysis**

Each variable was studied by analysis of repeated measures or profiles (19) of differences between each evaluation moment and the control moment in Groups I, II and III throughout the experiment.
For variables whose results were given by scores, groups were compared at every moment using Kruskal-Wallis non-parametric test, and the effect of moments on each group was compared using Friedman non-parametric test (19, 29). A significance level of $p<0.05$ was adopted.

**RESULTS AND DISCUSSION**

**Urinalysis**

Animals of Group III presented increased urine pH due to the alkalization process of the used urine.

Brown color and positive myoglobin were observed at M1 in all three groups, at M2 in Groups I and II and at M3 in Group I (Figure 1A).

Alteration in the urine color is due to the venom systemic myotoxic activity, denominated rhabdomyolysis and characterized by myoglobinuria (1-5, 12, 18). Proteinuria was also observed (500mg/dl) in Groups II and III at M1 and M2, and in Group I at M1, M2, M3 and M4.

Occult blood was observed at every moment starting from M1 in all groups.

According to Rosenfeld *et al.* (26), occult blood in the urine is due to the hemolytic action of the crotalic venom. Nogueira and Sakate (20) and Collicchio *et al.* (12) also reported occult blood in the urine.

**Biochemistry and blood gas analysis**

Urea and creatinine levels were within normal values for the studied species at every moment for all three groups.

Respiratory acidosis was observed, with increased $pCO_2$ and decreased blood pH values at M1 in all groups, which kept up to M2 for animals of Group I (28).

**Systolic blood pressure and electrocardiogram**

Systolic blood pressure significantly decreased ($p<0.05$) in animals of Groups I and III at M2, relative to the control moment, and in animals of all three groups at M3. Other authors have reported hypotension and shock in an accident involving humans (24).
Differences in the electrocardiogram were not observed among groups for each moment and among moments for each group. Sousa and Silva et al. (30) did not observe electrocardiogram alterations in an experimental study with dogs.

**Histopathology**

At the inoculation site, discreet edema, as well as delimited focal and multifocal areas of whitish coloration, was observed due to degeneration and necrosis (Figure 1B). Alterations in biceps femoris and semitendinous muscles were characterized by moderate to severe necrotic myositis. In the adjacent musculature, there was no alteration (Figure 1C) and the complete muscle, including form, color and texture, was different from those reported by other authors such as Azevedo-Marques et al. (2) and Koscinczuk et al. (16). The absence of alterations in other muscles might be attributed to the evaluation moments and the inoculated venom dose.

Inflammatory process, varying from focal to diffuse and multifocal, was noticed in animals of all three groups. With regard to fiber hyalinization, Group I showed diffuse hyalinization accompanied by necrotic areas; Group II presented marked hyalinization and Group III showed one animal with rare fiber hyalinization, another animal with marked hyalinization and a third animal with diffuse hyalinization. In Group I, diffuse fiber necrosis prevailed, whereas in Groups II and III, focal fiber necrosis and multifocal necrosis were observed (Figure 1D). Groups I and III showed edema and marked hemorrhage and Group II presented edema and light hemorrhage (Figures 1E and 1F).

Myoregenerative activity was discreet and noticeable in animals of all three groups. In 50% animals of Groups I and II, popliteal lymph nodes response was observed. The present histological findings agree with those described by Dal Pai & Neto (13) and Salvini et al. (27).

According to Ownby & Colberg (21), muscle lesions within the first hours after the accident are related to the components present in the venom. In a later stage, necrosis is common and, in a final stage, partial or complete regeneration of the muscle cells occur (27).

The venom fraction showing phospholipase $A_2$ activity damages the muscle, promoting rupture of intracellular organelles allowing the opening of calcium channels and release of creatine-kinase (14).
Figure 1. A. Urine color in Group II at control moment, 6h after venom administration (AV), 24h AV, 48h AV, 72h AV, 144h AV. B. Musculature of an animal of a control group showing normal coloration, and musculature of an animal of Group I showing focal whitish areas. C. Transversal section showing integrity of the muscle fiber (HE, 400X). D. Transition between the intact musculature and an extensive area of myonecrosis in the biceps femoris muscle (HE, 50X). E. Longitudinal section of muscle fibers showing necrosis and edema between fibers (HE, 100X). F. Longitudinal section of muscle fiber showing necrosis and debris.

CONCLUSION
Urinalysis showed brown urine, proteinuria, occult blood and myoglobinuria six hours after the venom administration. Alteration in the urine color is due to the venom myotoxic activity (rhabdomyolysis) caused by the components crotoxin and crotamine which induce myonecrosis.
The levels of urea and creatinine were within normality for the studied species at all moments for all three groups. The findings show that there was not renal compromising because of the employed venom dose.

Respiratory acidosis in blood gas analysis and dyspnea were observed six hours after venom inoculation in all three groups, which kept only up to 48h after envenomation in animals of Group I. Systolic blood pressure decreased at M3 (9h AV) in animals of all three groups. These effects are attributed to the neurotoxic action of this venom and to the shock developed.

No alterations were observed in the electrocardiogram for all three groups.

At the inoculation site there was discreet edema besides well-marked focal and multifocal areas of whitish coloration due to degeneration and necrosis, popliteal lymph node response and discreet myoregenerative activity. These effects are due to the venom myotoxic action and rhabdomyolysis.

The employed antiophidic serum dose was effective in neutralizing the venom, all animals survived and recovered after one week.

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