Received: October 03, 2009
Accepted: February 25, 2010
Abstract published online: March 3, 2010
Full paper published online: May 30, 2010

J Venom Anim Toxins incl Trop Dis.
V.16, n.2, p.342-354, 2010.
Original paper.
ISSN 1678-9199.

Clinical and electrocardiographic evaluation during experimental toad poisoning in dogs

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ABSTRACT: Accidents involving toad poisoning are frequent and dogs are the most common victims; they become poisoned by biting or ingesting a toad. When released in the organism, the venom is absorbed by both the oral mucosa and the digestive tract, initiating its toxic action. The aim of this work was to evaluate the clinical and electrocardiographic aspects of dogs subjected to experimental toad poisoning, as well as their response to treatment with propranolol. Twenty dogs were divided into two groups, a control group (n = 5) and a poisoned group (n = 15). After general anesthesia, the control group received a placebo, while the poisoned group received a venom aliquot through an orogastric tube. Results were tested through multivariate analysis ($p < 0.05$). The animals in the poisoned group had gastrointestinal symptoms including emesis, intense salivation, hyperemic or congested oral mucosa and pasty diarrhea. Non-responsive mydriasis, nystagmus, depression, stupor, tachypnea, opisthotonus and ataxia were also manifested by 100% of the poisoned animals. Affected dogs had an increase in blood pressure, statistically significant throughout study. Five poisoned animals developed ventricular tachycardia and were treated with propranolol (0.5 mg/kg IV). All propranolol-treated animals returned to normal sinus rhythm, which evidences the efficacy of this drug to treat ventricular arrhythmias caused by toad venom.

KEY WORDS: dogs, toad venom, poisoning, bufotoxin, arrhythmias.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Since toads lack inoculation tools, they are not considered venomous but poisonous animals. There are glands over their skin producing highly toxic venom, and among them are the paratoid glands, located bilaterally in the post-orbital region. They produce and store milky venom (1-3).

Toads (order Anura, family Bufonidae, and genus *Bufo*) have a worldwide distribution, preferably in tropical and humid temperate climate (4, 5). Accidental toad poisoning may occur in dogs, especially in the surroundings of lakes, reservoirs, creeks and rivers, which are the natural habitats of toads (6). Dogs are the most common victims due to their inquisitive nature, being attracted by the toad's slow movements, especially at night (7). They become poisoned by biting or ingesting the toad, which makes the venom come into contact with the oral and digestive tract mucosa, where it is absorbed, expressing its toxic action (4, 8, 9).

Bufo sp. venom has two major groups of active substances, biogenic amines and steroid derivatives. Adrenalin, noradrenalin, serotonin, bufotenines, dihydro-butenines and bufotyonines can be emphasized among biogenic amines due to their toxic relevance. Among the steroid derivatives, bufodienolids and bufotoxins act similarly to digitalis, inhibiting the Na^+-K^+ pump in cardiac muscle cells (10-13).

Clinical signs of toad poisoning can be separated into mild, moderate and severe. Mild cases are usually oral mucosa irritation and sialorrhea. Moderate cases includes, in addition to oral mucosa irritation and sialorrhea, emesis, depression, weakness, ataxia and neurological signs like walking in circles, cardiac rhythm abnormalities, spontaneous evacuation and urination. Severe cases can also comprise diarrhea, abdominal pain, sternal decubitus, pupils not responsive to light, seizures, pulmonary edema, and cyanosis, possibly evolving to death (8, 9, 14-16).

Electrocardiographic alterations include gradual deterioration of the cardiac rhythm normal patterns and arrhythmia, which may develop to multiform ventricular tachycardia (5, 16, 17). According to Eubig (12), the most common electrocardiographic findings in dogs exposed to bufotoxin are sinus tachycardia and normal sinus rhythm.

The success in treating poisoning by bufotoxin can increase by washing the animal's oral cavity in order to remove non-absorbed toxic secretions. Since bufotoxin has cardio-active properties, the use of adrenergic blockers as antiarrhythmics is recommended. Phenoxybenzamine is indicated for α-adrenergic block, and

propranolol for β-adrenergic block. When propranolol is administered after ventricular fibrillation, the electrocardiographic profile rapidly returns to the normal sinus rhythm (16).

Thus, in the present study, a clinical and electrocardiographic evaluation of dogs subjected to toad poisoning was carried out; moreover, the effectiveness of propranolol therapy was assessed.

MATERIALS AND METHODS

Twenty adult dogs of different breeds, males (n = 10) and females (n = 10), weighed approximately 10 kg, were used in this study. They were from the Central Animal Facility of São Paulo State University (UNESP), Botucatu campus, Brazil. The animals were divided into control group (n = 5) and poisoned group (n = 15).

Venom was collected from 20 toads of the species *Bufo schineideri*, from the Herpetology Laboratory, Department of Zoology, Botucatu Biosciences Institute (IBB), UNESP, Botucatu campus, Brazil. The venom was extracted by manually compressing the paratoid glands, located in the post-orbital region. A venom pool was stored in a glass container in order to eliminate individual variations of the venom toxicity. The venom sample was lyophilized in a conventional lyophilizer (Edwards do Brasil, Brazil), in the Laboratory of Immunology, IBB, UNESP, Botucatu, Brazil.

All dogs were subjected to a thorough physical examination of general condition, body temperature, mucosa color, hydration condition, abdominal palpation and cardiopulmonary auscultation. Laboratorial exams such as hemogram, urinalysis and hemogasometry were performed one day before the animals were used in the experiment. Dogs with abnormal results in the physical or laboratorial exams were excluded from the experiment.

For anesthetic induction, sodium thiopental 25 mg/kg IV was used and for anesthesia maintenance, isofluorane at 3%. Body temperature was kept from 37 to 38^{o}C throughout the anesthetic period by using a heated mattress. Ventilation was controlled by the inhaling anesthesia equipment, maintaining the respiratory frequency between 10 and 15 mpm. The electrocardiographic monitoring was maintained throughout the assessment period and the tracing was recorded at a bipolar II deviation, at the speed of 25 mm/s and amplitude n.

Venom aliquots (70% of the mean quantity of venom in a toad, approximately 0.1003 g) were resuspended in 10 mL water and administered through an orogastric tube to each animal. The control group received 10 mL saline solution (0.9% NaCl).

The animals that had multiform ventricular tachycardia were treated with propranolol 0.5 mg/kg IV. Multiform ventricular tachycardia was considered when more than 3 consecutive premature ventricular complexes (PVC) were recorded.

Each animal was kept anesthetized for two and a half hours, with cardiac frequency and rhythms verified at every ten minutes. Blood pressure (diastolic, systolic and mean) was measured by an invasive method at every 20 minutes throughout the period the animal kept anesthetized and after recovery, until values returned to normal. Every clinical sign was monitored and recorded, including sialorrhea, oral mucosa irritation, urination, evacuation, respiratory alterations, mucosa color and body temperature. These parameters were evaluated during 48 hours after treatment.

Profile multivariate analysis was used to compare the effects of periods on each group for every variable of the experiment, as well as to compare, on average, the effect between groups at each time.

The project was approved by the Ethical committee.

RESULTS

Control dogs had no changes in clinical signs during the anesthesia period, waking up right after the inhaling anesthetic equipment was turned off.

All animals (100%) of the poisoned group had gastrointestinal clinical signs from 3 to 6 hours after poisoning, with recurrent emetic episodes of varying frequency (3 to 6 episodes) and foamy yellowish liquid content. Six dogs (40%) kept presenting emesis until 24 hours after the venom administration. Brown pasty diarrhea occurred for five poisoned dogs (33%) until 48 hours after the venom administration. These signs disappeared without any specific treatment.

Salivation was noticed for 100% of the poisoned animals in the immediate post-anesthesia period. Six dogs (40%) had congestion and hyperemic oral mucosa. Eight animals (55%) had spontaneous urination and defecation during anesthesia.

Neurological changes were detected for all animals of the poisoned group until 96 hours after the toad venom administration. These signs varied, as shown in Table 1.

Clinical signs such as mydriasis, horizontal nystagmus, depression, tachypnea and stupor were detected for all animals of this group.

Table 1. Frequency of neurological changes in dogs subjected to toad poisoning (n = 15)

Neurological clinical signs	Frequency %
Non-responsive mydriasis	100
Nystagmus	100
Depression	100
Tachypnea	100
Stupor	100
Opisthotonus	73
Higher limbs spastic contractions	66
Ataxia	53
Disorientation	53
Unconsciousness	46
Gaze	46
Indifference to its surrounding	46
Proprioception deficit	26
Vocalization	20
Hypermetria	13
Compulsive walking	13
Circular walking	06

For control dogs, the heart rate varied from 73 to 118 bpm, values below the normal range for dogs (70 to 140 bpm). Animals of the poisoned group had a heart rate ranging from 42 to 181 bpm (18). These values had a wide variation; four animals had bradycardia (26%), seven had tachycardia (46%) and four had values within the normal range. The mean heart rate values were statistically analyzed by comparing the results within and between groups at 0 (basal), 20, 60 and 120 minutes after the administration of placebo or toad venom. There was no significant difference ($p > 0.05$) among times and between groups, as shown in Table 2.

Table 2. Mean and standard deviation of heart rate for control dogs (n = 5) and dogs subjected to toad poisoning (n = 15)

	Time (minutes)			p-value
	20	60	120	
Control	87.6 ± 9.63	92.2 ± 7.20	90.4 ± 7.30	(p = 0.283)
Poisoned	104.1 ± 27.40	110.8 ± 33.6	105.6 ± 36.40	(p = 0.709)
p-value	(p = 0.211)	(p = 0.242)	(p = 0.374)	

The systolic arterial pressure (SAP) of control dogs ranged from 75 to 168 mmHg. Only one animal in this group had values slightly above normality (120 mmHg). For the poisoned group, SAP ranged from 85 to 269 mmHg. Thirteen dogs (86%) in this group had increased SAP, and two dogs (13%) had normal SAP values throughout the evaluation period (18).

The diastolic arterial pressure (DAP) for control dogs ranged from 44 to 96 mmHg, and only one animal had values above normality. Poisoned dogs had DAP values ranging from 44 to 178 mmHg, which were above normal values for 13 animals (86%) and below normal values for two (13%) animals (70 mmHg).

Mean arterial pressure (MAP) for control animals ranged from 50 to 122 mmHg. Only one animal of this group had values above normality (18). MAP values for the poisoned group ranged from 59 to 183 mmHg, and all animals of this group reached values above normality.

Mean values of SAP, DAP and MAP were analyzed by comparing the two groups at 0 (basal), 20, 60 and 120 minutes after placebo or venom administration. There was no statistically significant difference ($p > 0.05$) in SAP values at time 0 between groups. At 20, 60, and 120 minutes, there was a statistically significant difference between groups ($p < 0.05$). There was also a statistically significant difference among times for the poisoned group ($p < 0.05$). At 20 minutes, SAP was significantly lower than at 60 and 120 minutes (Table 3). There was a significant difference between groups for DAP values at all times ($p < 0.05$). Poisoned dogs had DAP significantly higher than that of control animals ($p < 0.05$), as shown in Table 4. There was no statistical difference among times for each group ($p > 0.05$). Considering MAP values, there was a significant difference between groups at 60 and 120 minutes ($p < 0.05$). MAP values for the poisoned group were significantly higher than those for the control group at these times (Table 5).

Table 3. Mean and standard deviation of systolic arterial pressure for control dogs (n = 5) and dogs subjected to toad poisoning (n = 15)

	Time (minutes)			p-value
	20	60	120	
Control	97.6 ± 25.12^{a}	113.4 ± 22.72^{a}	109.60 ± 14.81^{a}	(p = 0.134)
Poisoned	142.1 ± 29.12^{Ab}	177.7 ± 30.72^{Bb}	164.9 ± 25.44^{Bb}	(p = 0.001)
p-value	(p = 0.007)	(p = 0.001)	(p = 0.001)	

Capital letters: comparison between lines. Small letters: comparison among columns. Values with different superscripts are significantly different ($p < 0.05$).

Table 4. Mean and standard deviation of diastolic arterial pressure for control dogs (n = 5) and dogs subjected to toad poisoning (n = 15)

	Time (minutes)			p-value
	20	60	120	
Control	53.00 ± 15.21^{a}	65.00 ± 11.94^{a}	64.41 ± 9.02^{a}	(p = 0.181)
Poisoned	86.3 ± 22.30^{b}	93.60 ± 20.94^{b}	82.10 ± 18.45^{b}	(p = 0.214)
p-value	(p = 0.006)	(p = 0.010)	(p = 0.050)	

Small letters: comparison among columns. Values with different superscripts are significantly different ($p < 0.05$).

Table 5. Mean and standard deviation of arterial pressure for control dogs (n = 5) and dogs subjected to toad poisoning (n = 15)

	Time (minutes)			p-value
	20	60	120	
Control	69.0 ± 17.82^{a}	81.6 ± 15.98^{a}	78.0 ± 8.25^{a}	(p = 0.170)
Poisoned	102.9 ± 26.11^{Aa}	120.7 ± 24.43^{Bb}	107.1 ± 18.56^{ABb}	(p = 0.035)
p-value	(p = 0.015)	(p = 0.004)	(p = 0.004)	

Capital letters: comparison between lines. Small letters: comparison among columns. Values with different superscripts are significantly different ($p < 0.05$).

As regards the electrocardiographic analysis at time 0 (before toad venom administration), there was no significant difference ($p > 0.05$) for the variables: P wave duration (s), P wave amplitude (mV), PR segment duration (s), QRS complex duration (s), R wave amplitude (mV), and ST segment duration (s).

Throughout the experiment, control animals did not have any type of arrhythmia, whereas in the poisoned group, 7 (46%) dogs had arrhythmia, all (100%) dogs had ventricular premature complex (VPC) (Figure 1A), and 5 (71%) of these animals had

ventricular tachycardia (VT). The observed VT was positive and negative, called multiform ventricular tachycardia (Figure 1 – B and C). When intercalated with sinus rhythm, this cardiac rhythm alteration was called paroxysmal ventricular tachycardia (Figure 1D).

All five animals showing VT had to receive the antiarrhythmic propranolol: one dose for one animal, two doses for two animals and three doses for one animal. After propranolol administration, all animals had their sinus rhythm returned to normality.

No animal used in this experiment died due to the treatments applied.

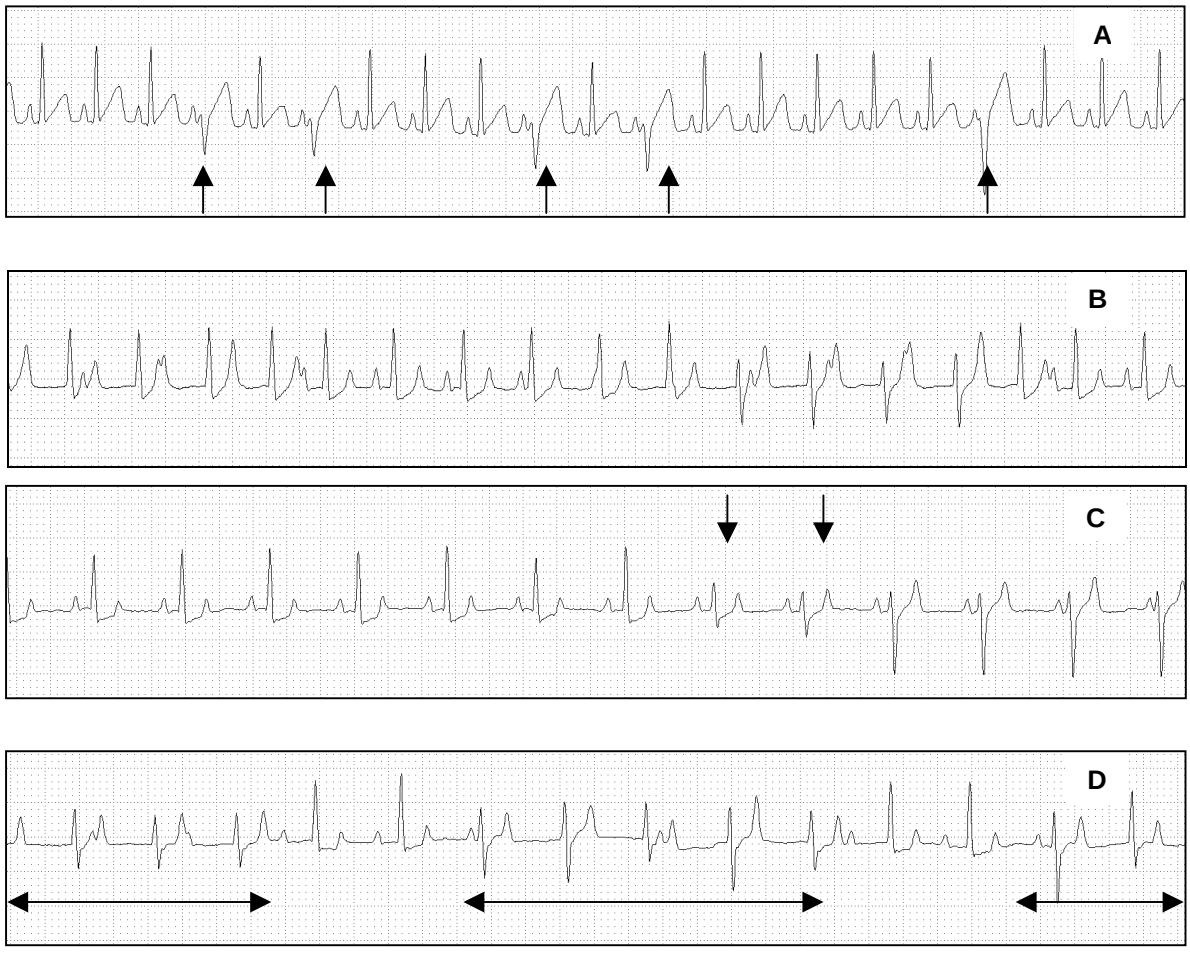

Figure 1. Electrocardiographic profile for poisoned dogs presenting: (**A**) sinusal tachycardia with isolated VPC (arrows); (**B**) multiform ventricular tachycardia; (**C**) ventricular tachycardia after sinusal rhythm with two fusion beats (arrows); and (**D**) Paroxysmal ventricular tachycardia (above arrows). Speed 25 mm/s, derivation II, n.

DISCUSSION

The gastrointestinal signs manifested by the dogs subjected to toad poisoning included frequent yellow emetic content, intense salivation, hyperemia or congestion of the oral mucosa, and pasty diarrhea. These signs are related to the action of bufotoxin on the gastrointestinal mucosa (12, 17). All gastrointestinal signs were self-limited and disappeared with no specific treatment 48 hours after the administration of *Bufo schineideri* venom. These results agree with data in the literature (8, 9, 14-16).

Cardiac alterations were not present in all animals subjected to toad poisoning. Heart rate (HR) and rhythm changes were noticed, as well as ventricular arrhythmias (VPC and VT) and blood pressure changes. These results disagree in part with the statement that systemic signs of toad poisoning are especially cardiotoxic since neurological signs were also evident, with variable signs, and present in 100% of the poisoned dogs in this experiment (3, 8, 10, 11).

The most common neurological disorders were non-responsive mydriasis, nystagmus, depression, stupor, tachypnea, opisthotonus and ataxia, present in all poisoned animals. The neurological signs observed during this experiment agreed with those described in the literature (7-9, 14-16) and developed due to bufotenines, dihydro-butenines and bufotyonines. These substances have hallucinogenic effects on the central nervous system (17).

The HR of the poisoned group had a wide variation over time, progressively increasing; this increase usually preceded ventricular tachycardia events. For this group, sinus bradycardia was present in 26% animals, while sinus tachycardia was present in 46%. These findings agree with those of Chi *et al*. (19), who reported sinus bradycardia and tachycardia, as well as ventricular arrhythmias, which are commonly observed.

Bufotoxins increase the intracellular calcium concentration by blocking the Na^+-K^+ pump, leading to an increase in the cardiac contraction strength and a reduction in the heartbeat due to an action of vagal reflex. However, the catecholamines present in the venom may overcome this effect, which explains the possible occurrence of both sinus bradycardia and tachycardia (17). Although there was no significant difference ($p > 0.05$) at all times, HR mean values for poisoned dogs kept higher than

those of the control group throughout the experimental period but still within the normal HR values for dogs (18).

Arterial pressure (AP) (systolic, diastolic and mean) monitoring indicated that the animals subjected to toad poisoning had a significant AP increase, suggesting hypertension. This elevation was statistically significant at all evaluated times (20, 60 and 120 minutes), compared to the control group. When times were compared, the AP increase was also significant at 20 minutes for systolic and mean values, which is due to the venom causing a gradual AP increase and values keeping above normality at 30 minutes after the venom administration (3, 9). After this time, AP (systolic, diastolic and mean) values were above the normal values for dogs (18). This result can be considered important since, after 30 minutes, almost 90% of the poisoned animals had AP values above normality (hypertension).

The hypertension caused by toad poisoning can be justified by the presence of biogenic amines such as adrenalin, noradrenalin and serotonin in the venom composition, besides hydroxy-methyltryptamine, also known as bufotenine. These substances are powerful vasoconstrictors, increasing the resistance of peripheral blood vessels and leading to an increase in the arterial blood pressure (3, 9). The findings of the present work disagree with those of some authors, who describe that dogs subjected to experimental toad poisoning by the oral route do not have arterial pressure elevation (12, 16, 20). They explain that substances such as adrenalin, noradrenalin and serotonin are degraded and inactivated by the digestive tract and the liver but, when present in the blood stream, they potentiate the poisoning. According to some authors, when intravenously administered, bufotoxin causes a rapid arterial pressure increase and dyspnea (16, 20). In the present experiment, the toad venom was administered by using an orogastric tube and the AP increase was evident.

Hypotension did not occur in any animal. In humans, bradycardia, hypotension and bradyarrhythmias such as atrial ventricular blocking are described as part of toad poisoning symptoms (19, 21).

The emergence of arrhythmias is due to the substances bufodienolides and bufotoxins present in the toad venom, which have an action similar to that of digitalis (12, 22, 23), blocking the Na^+-K^+ pump in the cardiac muscle cells by linking to Na-K-ATPase receptors, producing clinical signs that resemble a cardiac glycoside overdose (3, 9). Bufotenolides and bufotoxins can also reduce the speed of the

conduction of the cardiac electric impulse from the sinus node to the atrioventricular node, developing focal ventricular ectopy and causing premature ventricular contraction, which can lead to ventricular fibrillation (11, 12, 22). Supraventricular arrhythmias are not usually reported but may also occur, responding very well to β-blockers or vagal stimulus treatments (19). Roberts *et al*. (24) studied 94 bufotoxin intoxication cases in dogs between 1997 and 1998 and observed that 68% of the animals had electrocardiographic changes.

The use of β-blockers to treat ventricular arrhythmias caused by toad venom is indicated by several authors (3, 9, 16). For Eubig (12), propranolol is the most efficient treatment for bufotoxin intoxication. According to this author, this β-blocker has shown an outstanding capacity to reverse cardiac changes and prevent ventricular fibrillation in dogs subjected to experimental toad poisoning. Some researchers have recommended the use of propranolol as bolus and/or continuous infusion (12, 5). In the present study, the propranolol efficacy at the dose of 0.5 mg/kg IV was evidenced in the treatment of ventricular arrhythmias caused by toad venom. The animals that had ventricular tachycardia were treated with this medication, recovering the sinus rhythm. However, Sakate and Lucas de Oliveira (5) reported that verapamil is an alternative in the treatment of arrhythmias but does not prevent the development of ventricular arrhythmia.

CONCLUSION

Considering the conditions of the present experiment, the main arrhythmias observed during electrocardiographic recording were ventricular premature complex and ventricular tachycardia. Hypertension is a common alteration in dogs subjected to toad poisoning, present in 90% of the poisoned dogs. Neurological signs were evident and varied; the most common alterations were mydriasis, nystagmus, depression, tachypnea and stupor. Gastrointestinal signs were emetic episodes of yellowish content, pasty diarrhea, salivation, congestion or hyperemia of the oral mucosa. Propranolol, at the dose of 0.5 mg/kg IV, is an efficient treatment for ventricular tachycardia, not causing severe side effects.

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