CLINICAL REPORT

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Anesthesia of toad (*Rhinella icterica*) premedicated with dextroketamine and morphine for femur ostheosyntesis

*Anestesia de sapo cururu (*Rhinella icterica*) pré-medicado com dextrocetamina e morfina para osteossíntese de fêmur*

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ABSTRACT: In the present work, the objective is to relate the anesthetic procedures performed in a specimen of *Rhinella icterica* that underwent femoral osteosynthesis, as well as the results obtained regarding anesthetic efficacy and safety, and vital signs measured during the perioperative period. Pre-anesthetic medication was performed with dextroketamine and morphine, from which light anesthesia was obtained. Anesthetic induction was performed in gas chamber with isoflurane to reach surgical anesthetic plane. The animal was monitored by evaluation of anesthetic plane, electrocardiogram, pulse oximetry, counting of gular movements and hart rate by vascular Doppler positioned in the sternum. During the entire perioperative period, care was taken to maintain adequate room temperature and skin moistening, in order to preserve organic functions at physiologic standards for the species. Complete anesthetic recovery occurred quickly compared to previously existing literature. The protocol proved to be effective in promoting general anesthesia and safe in terms of stability of evaluated vital signs, becoming an alternative to the use of tricaine methanesulfonate (MS-222) and eugenol. More study is necessary regarding particular anesthetic effects on each species, and, given the lack of information, it is important to consider physiologic characteristics of amphibians in general to minimize the risks.

KEYWORDS: Amphibian; Isoflurane; Recovery.

RESUMO: No presente trabalho, objetiva-se relatar os procedimentos anestésicos realizados em um exemplar de *Rhinella icterica* submetido a osteossíntese de fêmur, bem como os resultados obtidos em relação à eficácia e segurança anestésicas, e os sinais vitais aferidos durante o período perioperatório. A medicação pré-anestésica foi realizada com dextrocetamina e morfina, com a qual se obteve anestesia leve. A indução anestésica foi realizada em câmara de isoflurano com a finalidade de obter plano anestésico cirúrgico. O animal foi monitorado por avaliação do plano anestésico, eletrocardiograma, oximetria de pulso, contagem dos movimentos gulares e frequência cardíaca por Doppler vascular posicionado sobre o esterno. Durante todo o período perioperatório, cuidados foram tomados para manter a temperatura ambiente adequada e o umedecimento da pele, a fim de preservar as funções orgânicas nos padrões fisiológicos para a espécie. A recuperação anestésica completa ocorreu rapidamente em comparação à literatura existente. O protocolo mostrou-se eficaz na promoção da anestesia geral e seguro em termos de estabilidade dos sinais vitais avaliados, tornando-se uma alternativa ao uso de metanossulfonato de tricaina (MS-222) e eugenol. Mais estudos são necessários em relação a efeitos anestésicos particulares para cada espécie, e, dada a falta de informações, é importante considerar as características fisiológicas de anfíbios em geral para minimizar os riscos.

PALAVRAS-CHAVE: Anfíbios; Isoflurano; Recuperação.

INTRODUCTION

The use of certain species of amphibian in scientific research, especially *Xenopus laevis*, provided a lot of information about the anesthesia of these animals. However, the fact that this class is composed of a wide variety of species means that effects of anesthetic protocols for some specimen are unknown. Results obtained using certain techniques should not be extended to all members of the class, and care must be taken carrying out protocols when there are no references for the species worked on (Mitchell, 2009). Over the past years, the reports of amphibian anesthesia are more related to clinical practice, and less related to research (Chai, 2014). We believe

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that studies regarding amphibian anesthesia for clinical purpose tend to become more important as veterinary care for exotic pets' increase.

In this present work, we describe the anesthetic procedures performed in a specimen of *Rhinella icterica* that underwent femoral osteosynthesis. The objective is to describe not only the anesthetic protocol and its efficacy, but care taken from preoperative period to full recovery, monitoring techniques and parameters obtained during every moment of perioperative period.

CASE REPORT

An adult wild toad, weighing 58 g, was referred to the Veterinary Hospital of Federal University of Parana with a history of attack by domestic cat. On physical examination, it presented right pelvic limb instability and body scratches. Radiographic images showed a complete simple fracture in the right femur diaphysis. Surgical correction by intramedullary pin implantation was indicated to promote stabilization of limb and consolidation of the fracture.

As pre-surgical care, the animal remained in a closed enclosure on wet compresses and under controlled room temperature, regulated by air conditioning at 23°C. On pre-anesthetic evaluation, the animal was presented alert, with corneal, postural and pain reflexes preserved. Deep and superficial pain reflexes were checked respectively by finger and interphalangeal membrane pinching. Heart rate (HR) was measured by vascular Doppler (Doppler Flow Detector 811-B, Parks Medical Electronics Inc., Aloha, OR, USA) positioned on chest, and was at 98 beats per minute (bpm). Gular movements were present at 122 movements per minute (mpm).

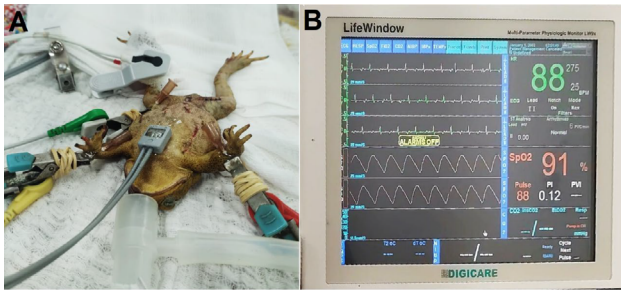
Intramuscular premedication was performed with 50 mg/kg dextroketamine (Ketamine NP, 50 mg/mL, Cristália Ltda., Itapira, SP, Brazil) and 38 mg/kg morphine (Dimorph®, 10 mg/mL, Cristália Ltda., Itapira, SP, Brazil). Five minutes after premedication, only postural and superficial pain reflexes were absent. The animal was positioned on wet compresses inside a gas chamber under pre-oxygenation (0.5 L/min) for 10 minutes. During this period, gular movements remained between 36 and 60 mpm, and HR between 80 and 84 bpm. Anesthesia was induced in gas chamber (Fig. 1) with 1.3% isoflurane (Isoforine®, 100%, Cristália Ltda., Itapira, SP, Brazil) delivered in 100% oxygen (0.5 L/min). Deep pain was checked every minute during induction time and was considered the main criteria for induction interruption at 11 minutes, when this reflex became absent. Gular movements gradually ceased, and were completely absent by the time of surgical positioning.

The patient was positioned in dorsal recumbence on wet compresses for the procedure, which lasted for 60 minutes. It was monitored by counting of gular movements, HR counting by Doppler, electrocardiogram (EKG) and pulse oximetry by multipara meter monitor (LifeWindow™ 9X, Digicare Animal Health, Boynton Beach, FL, USA). The contact of EKG electrodes was provided by superficial skin transfixation of 26 G needles and conductive gel (Fig. 2). The skin was moistened with clean, chlorine-free water every 10 minutes and the room temperature was controlled at 23°C.

Fresh gas flow was maintained close the toad's face, providing only oxygen (0.5 L/min) during most of the procedure. Light anesthetic plane returned 30 minutes after induction, noticed by presence of gular movements and withdrawal reflex. The surgery was paused and 2% isoflurane was provided for the first 3 minutes, then it was reduced to 1% and provided for another 2 minutes, until surgical plane was achieved. The patient returned to light

Source: author's collection

Figure 1. *Rhinella icterica* in gas chamber for anesthetic induction.

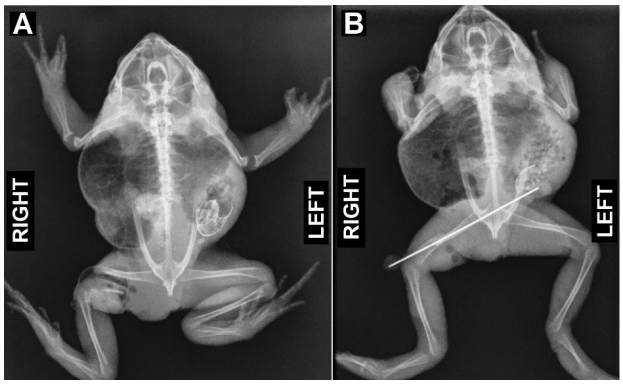


Source: author's collection.

Figure 2. Positioning of EKG electrodes, pulse oximeter and vascular Doppler for *Rhinella icterica* monitoring (A) and monitored parameters displayed on monitor screen (B).

anesthesia again by the end of procedure, 32 minutes after last isoflurane administration.

Short-term anesthetic recovery was provided under periodic skin moistening. Parameters and reflexes were evaluated every 5 minutes from pre-anesthetic period until complete recovery (Tab. 1). Long-term recovery took place in the University's Veterinary Hospital, where the toad received morphine (38 mg/kg, subcutaneous [SC], q8h, 5 days), meloxicam (Elo-xicam®, 0,2%, Chemitec Agroveterinária Ltda., São Paulo, SP, Brazil) (1 mg/kg, SC, q24h, 10 days) and enrofloxacin (Chemitril®, 2,5% Chemitec Agroveterinária Ltda., São Paulo, SP, Brazil) (5 mg/kg, SC, q24h, 10 days). Post-surgical radiographic image showed satisfactory alignment of the fragments (Fig. 3). The intramedullary pin was removed after 45 days, and the toad regained limb function and full condition to return to nature.



Source: author's collection

Figure 3. Pre-surgical radiography on dorsoventral projection showing right femur fracture (A) and post-surgical radiography on dorsoventral projection showing alignment of bone fragments with an intramedullary pin (B).

Table 1. Parameters, isoflurane administration and reflex evaluation of *Rhinella icterica* undergoing femoral osteosyntesis on different moments (M) of procedure, distinguished by numbers that represent time of anesthesia in minutes.

Moments	Isoflurane (%)	Heart Rate (bpm)	Gular movement (mpm)	Oxygen saturation (%)	Postural reflex	Superficial pain reflex	Deep pain reflex	Corneal reflex
$M0_{(a)}$	0.0	98	122	-	+++	+++	+++	+++
$M5_{(b)}$	0.0	80	36	-	-	-	++	++
M10	0.0	84	48	-	-	-	++	++
$M15_{(c)}$	1.3	80	60	-	-	-	++	++
M20	1.3	72	40	-	-	-	+	+
$M25_{(d)}$	1.3	88	16	-	-	-	-	-
M30	0.0	91	0	88	-	-	-	-
$M35_{(e)}$	0.0	78	40	89	-	-	-	-
M40	0.0	88	16	90	-	-	-	-
M45	0.0	91	0	91	-	-	-	-
M50	0.0	102	0	-	-	-	-	-
M55	0.0	103	0	-	-	-	-	-
M60	2 to 1	102	36	-	-	-	+	+
M65	0.0	85	0	-	-	-	-	-
M70	0.0	96	0	-	-	-	-	-
M75	0.0	93	0	-	-	-	-	-
M80	0.0	94	0	-	-	-	-	-
M85	0.0	90	0	-	-	-	-	-
M90	0.0	94	0	-	-	-	-	-
$M95_{(f)}$	0.0	91	20	-	-	-	-	-
M100	0.0	108	16	-	-	-	+	+
M105	0.0	88	10	91	-	+	+	++
M110	0.0	114	24	90	-	+	++	+++
M115	0.0	101	40	90	-	++	++	+++
M120	0.0	91	52	91	+	++	+++	+++
M125	0.0	108	88	-	++	++	+++	+++

Moments: (a) pre-anesthetic evaluation; (b) 5 minutes after premedication; (c) beginning of induction; (d) end of induction; (e) beginning of surgery; (f) end of surgery. Reflexes: (-) nonexistent; (+) mild; (++) moderate; (+++) normal.

DISCUSSIONS

Anatomical and physiological particularities of amphibian have an important impact on anesthetic procedures. Their skin has several functions, such as sensitive, secretory, thermoregulatory and respiratory. It actively participates on fluid and electrolyte balance (Chai, 2014). Care was taken to preserve skin functions during the procedure, especially by periodic moistening, wearing wet and powder free gloves, controlling room temperature, and using wet compresses to position the patient. EKG original electrodes can be harmful to their skin and alcohol can be absorbed, so the contact with EKG electrodes was provided by conductive gel and superficial skin transfixation of needles.

Amphibians are ectothermic, so the control of room temperature to maintain optimal condition during perioperative period is recommended to prevent changes in parameters and to guarantee that anesthetic induction, maintenance and recovery follow a more consistent pattern (Mitchell, 2009). Optimal temperature varies according to species, but values between 15 and 23°C are often considered adequate (Baitchman; Stetter, 2014). It was demonstrated in another species of *Rhinella* that cardiac baroreflex response is related to temperature (Zena; Gargaglioni; Bicego, 2015). Although amphibian metabolism increases at higher temperature, optimal zone should not be exceeded to hasten recovery, as oxygen demand may increase to levels than cannot be supplied by cutaneous respiration (Stetter, 2001). The use of a cloacal or esophageal thermometer was avoided due to the size of the patient, so we cannot affirm that the temperature of the toad was consistent with the environment.

Adult toad breath mostly through lungs and oropharynx, but the skin has also an important role (Chai, 2014). Lungs are inflated by gular movements (Baitchman; Stetter, 2014), which ceased after the induction. Amphibians may become apneic during anesthesia (Baitchman; Stetter, 2014; Chai, 2014), and in this case gas exchange was probably provided mostly by skin. Intubation is optional (Stetter, 2001) and was not performed due the possibility of cutaneous respiration, the small size of the patient and the fact that these animals keep their airway tightly closed during anesthesia, which also makes fasting optional (Chai, 2014). If intubated, significant caution would be needed concerning the pressure applied during assisted ventilation in order to prevent injuries due to the size of the toad. In addition, the minimum fresh gas flow provided by our equipment is 0.3 L/min, which would deliver to the toad a flow of 5000 mL/kg/min. Even in a system with scape of excessive flow, we do not know how harmful it could be to the airway.

Fresh gas flow was kept close to the animal's face for better use in case of return of gular movements. The presence of a plastic surgical drape over the animal may have created an environment similar to a chamber, keeping administered gas at higher fractions under the drape. This may have contributed to better absorption of isoflurane when it was provided, since satisfactory change in anesthetic plane was observed. However, the fact that a sealed system was not used to provide gas certainly resulted in gas waste and affected the actual fraction of isoflurane and oxygen delivered.

Amphibian's anesthetic plane is monitored mostly by evaluation of reflexes. Light anesthesia is achieved by loss of postural reflex and abdominal breathing, and surgical anesthesia by loss of withdrawal reflexes and gular movements (Stetter, 2001). Light anesthesia was obtained after premedication, and induction promoted surgical plane.

Premedication with morphine and dextroketamine was chosen to promote analgesia and sedation. Analgesia is recommended for painful procedure and is able to potentiate anesthetic effects and reduce time of recovery (Chai, 2014). Amphibians have nociceptors and nerves, but the paths of information processing at supraspinal level is not completely well defined (Stevens, 2011). However, dose-dependent response to opioid has been proven with great analgesic potency demonstrated by agonists of mu (μ) receptors.

At the dose of 38 mg/kg, peak of action of morphine is achieved 1 hour after subcutaneous administration in amphibians, and its maximum percentage of effect lasts for at least 240 minutes (Stevens; Klopp; Facello, 1994). We believe that, just like in mammals, intramuscular premedication may promote faster absorption in amphibians. In the present case, surgery started 35 minutes after intramuscular administration, and no signs that could be related to pain were noticed until 60 minutes after premedication, when a single moment of return of spontaneous movements occurred. Considering that at this point morphine had probably reached its peak of effect (Stevens; Klopp; Facello, 1994), it is more reasonable to think that the movements were correlated to light anesthetic plane. The use of morphine in the post-operative period did not induce sedation. Therefore, the effect of light anesthesia obtained after premedication was related to dextroketamine.

Ketamine hydrochloride is effective in promoting anesthesia and somatic analgesia in amphibians (Mitchell, 2009), but there is concern among authors regarding prolonged anesthetic recovery time (Baitchman; Stetter, 2014; Mitchell, 2009), reaching up to 12-18 hours (Gentz, 2007). Prolonged recovery was not observed in the present report. However, by the time of this publication no other report included the use of dextroketamine in amphibians. Further study regarding the use of dextrorotatory isomer of ketamine is necessary, as its effects may be different from the racemic ketamine. In dogs, for example, recovery is faster with dextroketamine and its potency is greater than racemic ketamine at the ratio of 1:1.29 (Duque *et al.*, 2008). Since there is no previous report of dextroketamine in amphibians and considering the possibility of a higher potency, we decided to use half the dose of 100 mg/kg of ketamine (Gentz, 2007).

Anesthetic induction of amphibians is frequently reported with tricaine methanesulfonate (MS-222) or eugenol (Baitchman; Stetter, 2014; Chai, 2014; Gentz, 2007; Mitchell, 2009; Mylniczenko, 2009; Stetter, 2001), not available in our service. Induction with isoflurane can be achieved in gas chamber or by bubbling in bath. Some authors consider that these methods promote longer induction times and excessively fast recovery, while immersion baths and topic application are referred to as more effective and longer-lasting methods (Baitchman; Stetter, 2014; Mitchell, 2009; Stetter, 2001). Excitement during induction with isoflurane is also reported (Stetter, 2001). The sedative effects of premedication are the possible reasons why none of these undesirable effects occurred in the present case. Even though the induction was fast, the fact that the gas chamber was opened to check reflexes certainly prolonged its duration.

Isoflurane was not provided continuously to maintain anesthesia. Surgical plane obtained with induction lasted for 30 minutes, and isoflurane was then administered only one more time in order to provide deepening of anesthetic plane. At this moment, the vaporizer was initially adjusted to a higher setting (2%) compared to induction time (1.3%) because the system was not sealed. Then, 3 minutes later, gular movements were absent and it was reduced to 1% for another 2 minutes to avoid over-deepening and reach surgical plane more safely. After another 32 minutes the patient returned to light anesthetic plane, but no further interventions were required as the surgery reached its end.

Although the vaporizer was calibrated, the fact that a gas analyzer was not used during induction and maintenance, combined with the absence of a sealed gas supply system, makes it impossible to determine the exact amount of isoflurane being delivered, and is considered a limitation in our interpretation.

Monitoring can be challenging due to the size of some specimen and absence of validated measurement techniques. The scarcity of reference values for the species was also a limiting factor for the interpretation of parameters obtained during monitoring.

Pulse oximetry is not validated in amphibians, but it can show trends and allows the detection of peripheral pulse (Baitchman; Stetter, 2014). Oxygen saturation was not measured throughout the procedure due to interferences of surgical manipulation. The values obtained showed low amplitude of variation, which suggests that saturation had a tendency to stability when measurement was feasible. However, it is not possible to establish conclusions about the accuracy of the values obtained, neither correlate them with the actual partial pressure of blood oxygen.

Heart rate should be measured before anesthesia to obtain baseline values and better interpret changes in the anesthetic period (Mitchell, 2009). No significant changes on HR were noted during most of the procedure, except for the 10 minutes preceding the beginning of return to light anesthesia plane. During lightening of anesthetic plane, HR reached maximum value of 114 bpm. This indicates that variation in HR occur according to the anesthetic plane. Interpretation of HR should not be used as single monitoring parameter, as heartbeats are still present in amphibians for some time after death (Mitchell, 2009).

Amphibians are considered recovered from anesthesia when the pre-anesthetic reflexes and parameters return (Mylniczenko, 2009), which was reached shortly after 125 minutes' post premedication and approximately 60 minutes after last isoflurane offer. In another report, complete recovery of a *Rhinella marina* occurred nearly 6 hours after premedication, and 4 hours after the end of procedure. However, isoflurane was provided via facemask during induction and the first 25 minutes of anesthetic maintenance, and was dripped topically during most of the surgical period, that lasted 105 minutes. The exact time extent of topical administration and its frequency were not reported, but it totalized 6 ml of isoflurane. In addition, racemic ketamine was administered in premedication instead of dextroketamine (Gorczak *et al.*, 2021). Gorczak *et al.* (2021) do not report which criteria were used to determine when it would be necessary to provide isoflurane during the procedure.

In our study, the use of anesthetic plane evaluation as criteria to provide isoflurane probably prevented excessive administration of maintenance agents and prevented prolonged recovery. We believe that premedication and care regarding maintenance of temperature and skin moistening actively contributed the short induction time, low anesthetic requirement and satisfactory recovery observed in our case.

The anesthetic protocol was effective in promoting anesthesia of the specimen reported and provided stability of parameters during perioperative period. It was proposed an alternative way to promote amphibian anesthesia, often related to the use of MS-222 or eugenol. These are not always available in veterinary care services and may not provide adequate analgesia. Further studies are necessary to determine effects and safety of anesthetic protocols for each species. Given the lack of information, it is important to know physiological particularities to minimize risks of procedures.

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