

## INTRAMUSCULAR ADMINISTRATION OF KETAMINE-MEDETOMIDINE ASSURES STABLE ANAESTHESIA NEEDED FOR LONG-TERM SURGERY IN THE ARGENTINE TEGU *SALVATOR MERIANAE*

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**Abstract:** To define a protocol of anesthesia for long-duration invasive surgery in a lizard, eight young adult Argentine tegus (*Salvator merianae*) of mean body weight 3.0 kg (interquartile range [IQR] 3.40–2.65) were anesthetized with a mixture of ketamine (K) and medetomidine (M) at 19°C, injected intramuscularly and equally distributed in the four limbs. As the experimental surgery procedure required a prolonged deep anesthesia with a good myorelaxation (between 16 and 21 hr), reinjections were required and reflexes were checked during surgery. Times for anesthetic induction, anesthetic reinjection, and recovery periods were recorded for five different combinations of ketamine-medetomidine: 1) 66 mg/kg K + 100 µg/kg M; 2) 80 mg/kg K + 100 µg/kg M; 3) 100 mg/kg K + 130 µg/kg M; 4) 125 mg/kg K + 200 µg/kg M; and 5) 150 mg/kg K + 200 µg/kg M. The effect on the recovery speed of the postoperative atipamezole injection was also evaluated. The median induction time was 30 (IQR 35–27.5) min with no statistical difference between all the concentrations tested. The first reinjection of half a dose was administered after a mean of 5 hr (5.64 hr, IQR 5.95–4.84) as were the subsequent reinjections of a quarter dose (3.99 hr, IQR 5.98–3.23). Intramuscular administration of the ketamine-medetomidine combination is a simple, rapid, and efficient anesthesia for long-term surgery (>12 hr). A mix of 100 mg/kg ketamine and 200 µg/kg medetomidine, with reinjections every 4 hr of half a dose of the previous injection can maintain a good quality of anesthesia for at least 16 hr. The injection of atipamezole after the surgery reverses the effects of medetomidine and permits a reduction of the recovery period.

**Key words:** Anesthesia, ketamine, medetomidine, reptile, *Salvator merianae*.

### INTRODUCTION

The number of exotic pets encountered in veterinary clinics is constantly growing.<sup>4</sup> This increase raises the problem of the care for these species, especially when a major surgical intervention is needed. In those cases, inducing a prolonged and stable anesthesia of the animal is necessary. Unfortunately, no published protocol on how to induce and maintain prolonged deep anesthesia in lizards appears to exist. The use of

inhalant anesthetic agents, such as isoflurane or halothane, is common in lizards for small interventions but it requires expensive and dedicated equipment to ensure the best anesthesia.<sup>15</sup> Moreover, when prolonged anesthesia is required these methods tend to desiccate the airways if the anesthetic is not hydrated. Furthermore, intubation renders surgery to the head or buccal cavity difficult and inhalant anesthesia cannot be easily used in the wild. Injectable anesthetics are a good and straightforward alternative but their use and dosage for prolonged anesthesia has not been described in the literature.<sup>3</sup> The focus of the present study was on establishing an injection protocol using a mix of ketamine and medetomidine for the anesthesia of a species of large squamate, *Salvator (Tupinambis) merianae*.<sup>10</sup>

Although many injection protocols have been proposed, dissociative anesthetics appear to be the most appropriate because these agents induce a state of catalepsy, a sort of paralysis in which the animal is dissociated from its environment.<sup>2</sup> Historically, ketamine has been one of the most commonly used injectable dissociative agents by veterinarians for “reptile” anesthesia due to its effective induction time and some degree of

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immobilization.<sup>18</sup> Ketamine is a noncompetitive antagonist of the NMDA receptor, which plays an important role in the modulation of neurotransmission and neuron excitability.<sup>22</sup> This characteristic may explain its dissociative effect because it affects the activity of the limbic system. Nonetheless, this product requires a high dose (170–230 mg/kg), involves a long recovery time,<sup>1</sup> and can induce many side effects when used as a sole agent (increase in muscle tone, hypersalivation, acceleration of apoptosis when administered in high doses).<sup>6,13</sup> Consequently, an  $\alpha_2$  adrenergic agonist, such as medetomidine, is often used in combination with ketamine. Indeed, medetomidine has an analgesic effect and myorelaxant properties, and increases the total anesthesia period.<sup>9</sup> The sedative effect of medetomidine is due to the particular action of this  $\alpha_2$ -agonist on the norepinephrine release in the central nervous system. The norepinephrine liberation is blocked by medetomidine and therefore induces a profound sedation. Moreover,  $\alpha_2$ -agonists also act on the spinal cord by inhibiting the interneurons causing a significant myorelaxation. Additionally, their effects on the brain and spinal cord play a key role in inhibiting the pain pathways and inducing analgesia.<sup>20</sup> Furthermore, medetomidine can be reversed with atipamezole, an adrenoceptor antagonist.<sup>17</sup> Another advantage of these products is that they can be mixed together and injected intramuscularly (im).<sup>7</sup> The intramuscular route is the most commonly used injection route for “reptiles” because it is also the most practical one. Moreover, the im injection avoids the high peak concentration common in iv injection and prevents the harmful side effects this peak could induce.<sup>8</sup> The use of medetomidine furthermore permits a decrease in the dose of ketamine and an optimization of the recovery time.<sup>18</sup> Thus, it is possible to immobilize “reptiles” for a long duration, as when needed in the context of a major surgery.

The hypothesis is that a mix of ketamine and medetomidine could be used to induce a prolonged and stable anesthesia in lizards for a long-term intervention. Therefore different concentrations were tested to determine the optimal protocol.

## MATERIALS AND METHODS

All experiments were conducted applying the 3R principles in animal experiments and in accordance with the European Community Council Directive for the use of research animals (86/609/EEC and 2016/63/EU; <http://ec.europa.eu/environment/>

[chemicals/lab\\_animals/pdf/endorsed\\_awb-nc.pdf](#)). Protocols and procedures used were approved by the local Comité d’Ethique en Experimentation Animale of the University Lyon 1.

### Animals and surgery

Eight adult (1.75 yr, interquartile range [IQR] 2–1.5; two females) Argentine tegus, *Salvator merianae*, (3.0 kg, IQR 3.40–2.65) were used for the experiments. In order to record different physiological parameters simultaneously, a prolonged deep anesthesia with a good myorelaxation was needed. The surgery involved clearing off the top of the skull and multiple trepanations, small skin and muscles incisions, as well as the placement of deep brain electrodes.

### Monitoring

To assess the depth of the anesthesia, several reflexes were checked regularly. The absence of the spinal reflexes (eg failure to breathe when a finger is run down the back and failure to move the tail when pinched) and the palpebral reflex (ie closing of the eyelid when touched) were considered as being signs of a good anesthesia. The conservation of the corneal reflex was also used to ensure the right depth of the anesthesia, as well as the breathing rate, which was evaluated regularly and visually during the surgery. During the recovery, all animals were kept under observation using infrared cameras 24 hr a day.

### Surgery conditions and anesthetics

All surgeries started in the morning, between 6:00 and 8:00 AM, in a temperature-controlled room ( $19 \pm 2^\circ\text{C}$ ). The first injection and recovery took place in the home enclosure of the animal in order to reduce stress (room temperature  $25^\circ\text{C}$ ). The hot spot at  $45^\circ\text{C}$  was turned off 1 day before the surgery and turned on again 3 days after.

The anesthetics used for the experiments were ketamine (Kétamine 1000 Virbac; Virbac, 06517 Carros, France) at five different dosages (66, 80, 100, 125, and 150 mg/kg), and medetomidine (Domitor, Vétoquinol; Orion, 02200 Espoo, Finland) at three different dosages (100, 130, and 200  $\mu\text{g}/\text{kg}$ ). Ketamine concentrations were based on prior experiences with ketamine as a sole induction agent during surgery in different lizards and experience with short-duration surgery using both ketamine and medetomidine.<sup>5,11,12,14</sup> The extemporaneous mixture was injected im in the triceps of the anterior limbs and the iliotibialis muscle of the posterior limbs.

**Table 1.** Anesthesia protocol per animal. Summary of the anesthesia, the weight of the animal, and anesthetic products and concentrations used for the surgery. The induction time of the anesthesia is also provided for each animal.

Animal	Weight (kg)	Ketamine (mg/kg)	Medetomidine ( $\mu\text{g}/\text{kg}$ )	Induction time (min)
Animals 1 to 4	$2.8 \pm 0.8$	66	100	$24.8 \pm 11$
Animal 5	3	80	100	30
Animal 6	3	100	130	58
Animal 7	3.75	125	200	35
Animal 8	2.8	150	200	30

The median injected volume during the surgery was 2.75 ml (IQR 3.79–2.31) and was equally distributed in the four limbs of the animal to prevent pain. Two groups of dosages were defined: a high-dose group with a ketamine dosage between 100 and 150 mg/kg and a low-dose group with a ketamine dosage ranging from 66 to 80 mg/kg. Reinjection of anesthetics were decided when any signs of awakening were observed (eg increase in respiratory rate). Each reinjection was half the concentration of the previous one. In some cases ( $n = 4$ ), atipamezole (Antisedan, Vétoquinol; Orion, 02200 Espoo, Finland) was injected im in the iliotibialis muscle of the posterior limbs, to reverse the effect of medetomidine, the concentration being once or twice the concentration of medetomidine (ie 100–400  $\mu\text{g}/\text{kg}$ ). It was injected 12 hr after the surgery.

#### Data analysis

All statistical analyses were performed using MATLAB® (MathWorks®, Natick, Massachusetts 01760, USA). The difference in the induction time and the reinjection time between the two dosage groups was analyzed using Mann-Whitney *U*-tests. Differences were considered significant at  $P < 0.05$ . All data are expressed as median (IQR third quartile to first quartile).

## RESULTS

#### Induction of anesthesia

Anesthetic concentrations and induction times are presented in Table 1. No statistical difference in induction time between low and high concentrations was observed ( $P = 0.14$ ). The median induction time was about 30.0 (IQR 35.0–27.5) min.

#### Reinjection

For the eight lizards, there were about 2.00 (IQR 2.25–1.75) reinjections needed during the

surgery. The first reinjection of anesthetic was around 5.64 (IQR 5.95–4.84) hr, and in the majority of cases, half a dose was reinjected. The median time between the following reinjections was 3.99 (IQR 5.98–3.23) hr with a concentration of half of the previous injection. There was no statistical difference for the time of reinjection between low and high doses of anesthetic ( $P = 0.88$ ). The results are summarized in Figure 1. The surgeries lasted 15.8 hr (IQR 17.0–14.7) without any signs of awakening for all animals.

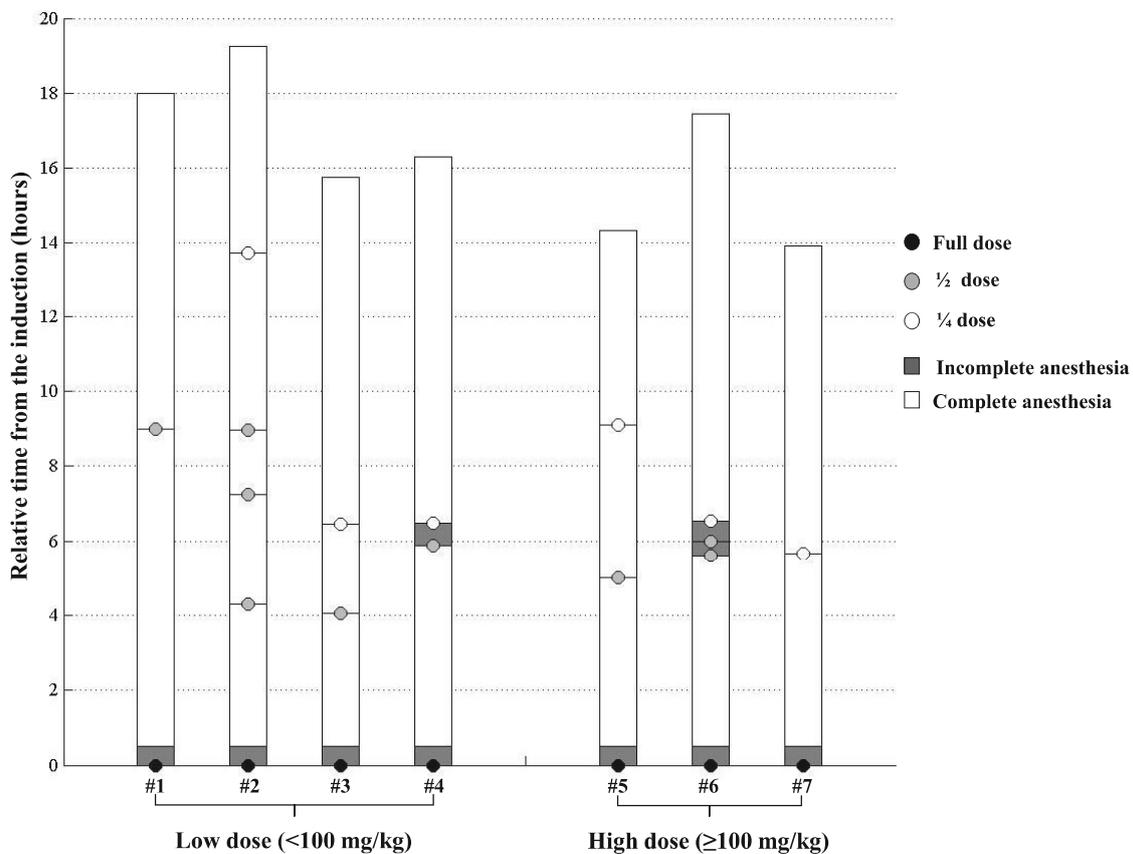
#### Recovery time

Spinal and palpebral reflexes started to appear a few hours after the last injection of anesthetic. Twelve hours after the end of the intervention, signs of consciousness including eye opening, limb movements, and tongue flicks were present. Animals that received atipamezole at this time became more reactive to stimulation. However, all animals preferred to rest with a full voluntary locomotion appearing 3 days (IQR 3.50–3.00) after the last anesthetic dose. After surgery all animals displayed normal behavior including, feeding, locomotion, and sleeping, and were monitored for up to 3 mo consecutively. No signs of pain (eg prostration, increase in aggressiveness, stereotypic movements), stress, or ill health were observed after surgery.<sup>16</sup>

## DISCUSSION

The aim of the study was to determine an anesthesia protocol allowing maintenance of a prolonged anesthesia needed to perform an invasive surgery on a large lizard, *Salvator merianae*. To do so, different concentrations of a mix of ketamine and medetomidine were tested, as those anesthetics allow a deep and stable anesthesia.<sup>18</sup>

The results showed that the protocols induce a rapid and deep anesthesia (median induction time 30 min, IQR 35.0–27.5) and that the induction time was not significantly modified by the dosage



**Figure 1.** Timing of the anesthesia. Relative time (hr) from the first injection for each surgery ( $n = 7$ ). The black, grey, and white markers indicate the concentration and time of an injection or reinjection. White rectangles represent a state of good and deep anesthesia whereas the grey ones represent a state of incomplete anesthesia (induction or animal not completely unconscious or myorelaxed).

of the anesthetic (Table 1). The depth of the anesthesia was regularly controlled by measuring the breathing rate and the assessment of different reflexes (palpebral, spinal, corneal). The protocol induced a deep anesthesia that was maintained for 5 hr (5.64 hr, IQR 5.95–4.84; Fig. 1).<sup>2</sup> If the half-dose reinjection is given too late during the anesthesia plateau, the time necessary to reinduce a proper anesthesia can be considerably increased, however. Therefore, it is crucial to not exceed 5 hr before the first reinjection. If the lizard presents any signs of arousal, a half dose is required; otherwise, a quarter dose suffices. Complex surgeries require that the animal stays unconscious for a long period of time and the protocol described here grants this. It is worth mentioning that, unlike what could be found in the literature, the protocol uses higher ketamine doses (i.e., >60 mg/kg) as these are needed to induce a deep anesthesia, essential for a stereo-

tactic surgery, for example.<sup>2</sup> The robust dissociative effect of those concentrations allows for an invasive procedure without the risk of a subject awaking midsurgery.

Secondly, the im injection of atipamezole postoperatively tended to reduce the time to the reappearance of consciousness. The injection 12 hr after the intervention permitted maintenance of relative immobility of the animal, by keeping the myorelaxant effect of the medetomidine. However, with or without atipamezole, the animal recovered all its spinal and palpebral reflexes within 12 hr after the last injection. Interestingly, animals preferred resting for several days (3 days, IQR 3.5–3). Personal experience with single injections of ketamine and medetomidine without surgery (eg during magnetic resonance imaging), followed by an injection of atipamezole a few hours after, was associated with a recovery time (exploration behavior, voluntary locomotion, feeding,

and tongue flicking) that was much shorter, with a complete recovery within the next 12 hr. In this protocol the atipamezol was injected in the middle of the night. Consequently, the rest induced by the anesthesia cannot be differentiated from the rest associated with sleep. However, as preliminary experiences with smaller species suggest a recovery time within the hour after an injection of atipamezol, a species-specific reaction of the tegu cannot be excluded. The long recovery period could be a limitation when needing to use anesthesia in the wild and further investigation is needed to properly assess this issue. Furthermore, the reversal potential of atipamezole is dose-dependent and it could be interesting to test the effect of a higher dosage of this product on the recovery time.<sup>19</sup>

This anesthesia protocol allowed performance of complex and major surgery on a lizard (*Salvator merianae*) under stable anesthesia. All animals ( $n = 8$ ) recovered well after the surgery. As the metabolism of tegus and other poikilotherms is temperature dependent, and as a consequence also the pharmacokinetic effect, particular attention should be paid to the room temperature.<sup>21</sup> For a long surgery, the anesthesia should be done at a relatively low temperature (around 20°C) without any heating device, in order to reduce the degradation of the anesthetic, thus extending the anesthesia. Moreover, an increase of the recovery room temperature could decrease the recovery time. Therefore, when used in the wild, the anesthesia and recovery period will likely be affected by the ambient temperature.

### CONCLUSIONS

This study suggests that the use of a mixture of 100 mg/kg of ketamine combined with 200 µg/kg of medetomidine injected im is adapted to inducing a deep anesthesia in the tegu. At 20°C this protocol provides immobility and a stable anesthesia for 5 hr. After that, reinjections of half the previous dose every 5 hr are recommended if required. The injection of atipamezole (400 µg/kg) after surgery contributes a reduction of the recovery period. The good response to the anesthetics and the satisfactory recovery of the animal suggests that this protocol induces a sufficiently prolonged and deep anesthesia in the Argentine tegu and could be transposed to other species of lizards.

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