Medetomidine-ketamine-sevoflurane anaesthesia in juvenile Nile crocodiles
(Crocodylus niloticus) undergoing experimental surgery

Abstract

Objective To describe the anaesthetic, physiological, and side effects of intramuscular
(IM) medetomidine and ketamine, followed by inhalational anaesthesia with
sevoflurane, in Nile crocodiles (Crocodylus niloticus).

Study design Observational trial.

Animals Ten juvenile captive bred Nile crocodiles undergoing surgical implantation of
skeletal beads and muscular electrodes.

Methods During pre-anaesthetic examination, the following variables were assessed:
heart (HR) and respiratory ($f_R$) rates, and response to palpebral, corneal and toe- and
tail-pinch withdrawal reflexes. The crocodiles were injected IM with an initial
combination of medetomidine and ketamine and re-evaluated at 5 minute-interval for 20
minutes, or until they appeared unresponsive. If that did not occur, the drugs were re-
dosed according to a decision tree based on the observed effects. The righting, biting
and palatal valve reflexes were assessed in the unresponsive crocodiles, and used to
confirm anaesthetic induction. Anaesthesia was maintained with sevoflurane in oxygen.
At the end of surgery, medetomidine was antagonised with IM atipamezole.

Result The decision tree identified 0.3 mg kg^{-1} medetomidine and 15 mg kg^{-1} ketamine
as a useful drug combination, which resulted in anaesthetic induction and surgical
anaesthesia 15.6 ± 8 and 16 [25-20] minutes after injection, respectively. Compared to
baseline, HR and $f_R$ significantly decreased after anaesthetic induction (P < 0.001), but
then remained stable throughout surgery. Intraoperatively, cloacal temperature ($T$; 27
[26-30 °C) did not change over time (P= 0.48). The total dose of atipamezole was 2 [1-3] mg kg\(^{-1}\) and time to recovery was 36 [20-60] minutes. Peri-operative complications were not observed.

**Conclusion and clinical relevance** Medetomidine and ketamine, injected IM and followed by sevoflurane anaesthesia, may be regarded as a useful anaesthetic technique for juvenile Nile crocodiles undergoing minimally invasive experimental surgery.

**Keywords** anaesthesia, *Crocodylus niloticus*, immobilization, Nile crocodile, reptile,
Introduction

When clinical or experimental procedures involving potentially dangerous animals are to be performed, safe and effective anaesthesia is important for both the personnel and the patients. Nile crocodiles (*Crocodylus niloticus*) are often kept in captivity in zoos and used as animal model for research, and may be anaesthetized for diagnostic or experimental purposes. Nevertheless, there are a few reports describing the anaesthetic management of this animal species (Di-Poi and Milinkovitch 2013; Stegmann et al. 2017), none of which reported details about quality and duration of anaesthesia and complications.

The aim of this work was to describe the anaesthetic and physiological effects, as well as the possible side effects and related complications, of IM ketamine and medetomidine, followed by sevoflurane anaesthesia, in ten Nile crocodiles undergoing experimental surgery.

Material and Methods

Ten female juvenile captive-bred Nile crocodiles (La Ferme aux Crocodiles; Pierrelatte, France) were anaesthetised to undergo surgical implantation of either tantalum skeletal beads or electromyography electrodes, to be used thereafter for a locomotion study. The latter was conducted in accordance to the Animals Scientific Procedures Act (Home Office License number: P0806ABAD).

The animals were deemed healthy based on physical appearance and behavior, as assessed by trained personnel. The crocodiles were housed in groups of 2-6 in a humidity (70-80 %) and temperature-controlled (26-28°C) enclosure, with free access to water ponds and ground areas, and fed daily with chopped dead mice. On the day of
surgery, the animals were captured and head-tail restrained by two operators, who
secured the mouth with tape. Fasting time was 48 hours. Body weight, heart rate (HR,
with the Doppler probe positioned over the ventral aspect of the coelom) and respiratory
rate (fR, by looking at abdominal/gular excursions) were measured and recorded as part
of the preanaesthetic assessment. Moreover, the following were scored, always by the
same investigator: the toe- and tail-pinch withdrawal reflexes (PWR, defined as the
ability to withdraw the limb/tail in response to hard pinch of the front limb-second digit
or of the tail, respectively, with haemostatic forceps applied for 2 seconds), the
palpebral and the corneal reflexes (ability to close the eyelid in response to gentle touch
of the eyelid and of the cornea, respectively). A scoring system ranging from 0 to 2 (0:
absent; 1: delayed, > 1 sec; 2: normal, < 1 sec) was employed for all but the corneal
reflex, which was assessed with a binary system (0: absent reflex; 1: present reflex). For
each parameter, the value recorded during preanaesthetic assessment was defined as
baseline.
The crocodiles were injected in one triceps brachii muscle with 0.2 mg kg⁻¹
medetomidine (Sedastart; Animalcare, UK) and 10 mg kg⁻¹ ketamine (Ketamidor;
Chanelle, UK), and placed in a carrier. Room temperature in the operation theatre was
23 ± 2 °C. The reflexes were assessed every 5 minutes to monitor the progression of
sedation/anaesthesia. The righting reflex (RR; defined as the ability to regain sternal
recumbency after positioning in dorsal recumbency), the biting reflex (mouth opening,
hissing and/or attempts to bite the catch pole) and the palatal valve reflex (closure of the
palatal valve after gentle touch of the gular fold with a syringe plunger) were evaluated
only when the animals appeared unresponsive to tactile stimulation with a stick, the
former one using the 0-2 scoring system as above described, and the other two with a
binary system (0: absent reflex; 1: present reflex).
Sedation was defined as delayed righting, palpebral and corneal reflexes, whereas anaesthesia was considered induced when these reflexes were absent. The possible complications were classified as major (too deep anaesthetic depth if induction was achieved in less than 5 minutes from injection, and severe cardiovascular depression when HR decreased by more than 50% of the baseline values), and minor ($f_R$ less than 50% of the baseline values and apnea for at least one minute, and HR decreased by less than 50% of the baseline values).

A decision tree, developed based on the possible scenarios and associated courses of action, was used as follows:

- Neither sedation nor anaesthetic induction were achieved; complications were not observed. Medetomidine (0.2 mg kg$^{-1}$) and ketamine (10 mg kg$^{-1}$) were repeated IM 20 minutes after the first injection.

- Sedation, but not anaesthetic induction, was achieved, and complications were not observed. Medetomidine (0.1 mg kg$^{-1}$) and ketamine (5 mg kg$^{-1}$) were administered IM 20 minutes after the previous injection.

- Anaesthetic induction was achieved and no complications were observed. The drug combination was tested in two other crocodiles and used in the remaining ones if the findings were consistent.

- Anaesthetic induction was achieved but minor complications were observed. The next animal received drug doses decreased by 25%.

- Anaesthetic induction was achieved, but major complications were observed. The next animal received drug doses decreased by 50%.

- Occurrence of any complication considered unacceptable by the investigator. The trial was aborted, and the study plan revised to establish a new protocol.
The time to anaesthetic induction was defined as the minutes elapsed from the first IM injection to induction of general anaesthesia. The time to surgical anaesthesia was defined as the minutes elapsed from the IM injection to loss of RR, palpebral, corneal and toe-PWR and tail-PWR.

After anaesthetic induction was achieved, the tracheas were intubated with an uncuffed tube, then connected to a circle system to deliver sevoflurane (Sevoflo; Abbott, UK) in oxygen and initiate IPPV with pressure-controlled mode. Active warming (Bair Hugger 505; Augustine, Canada) was provided during anaesthesia. A Doppler probe (Model 811; Parks Medical, NV, USA) was placed over the ventral aspect of the abdomen to monitor the HR. The crocodiles were instrumented with a standard electrocardiogram with blunt clip electrodes placed on the skin of the front left feet and on the dorsal aspect of the neck. A multi-parametric module (Datex Ohmeda S/5; GE Healthcare, TN, USA), equipped with a pediatric Pitot tube to monitor spirometry, and with a temperature (T, °C) probe placed 5 cm into the cloaca, was used intraoperatively. The physiological parameters (HR, \( f_R \) and T) and the palatal valve, corneal, palpebral and withdrawal reflexes were scored and recorded every 10 minutes. Crystalloids (Hartmann’s solution; Baxter, UK) were administered as bolus (5 ml kg\(^{-1}\)) via the caudal vein after the beginning of surgery. Venous blood was collected once 60 minutes after the beginning of mechanical ventilation, either from the cervical sinus or from the caudal vein, and analysed with a portable device (i-STAT; Abbott, UK). Any occurrence of major and minor complications was recorded.

At the end of surgery sevoflurane was discontinued and atipamezole (1 mg kg\(^{-1}\)) (Sedastop; Animalcare, UK) administered in one triceps brachii, and repeated after 30 minutes in case of residual sedation. When regular spontaneous breathing was regained, the tracheas were extubated and the mouth taped. The reflexes were monitored until the
crocodiles could keep the head lifted and responded to tactile stimulation with a stick by turning and attempting to bite. Time to recovery was defined as the minutes elapsed from the first atipamezole administration to returned ability to lift and hold up the head.

Data distribution was assessed with a Kolmogorov-Smirnov test. Continuous variables were analyzed either with one-way repeated measures analysis of variance, followed by the Holm-Šídák method for pairwise multiple comparisons, or with the Friedman test where it applied. Commercially available software (SigmaStat 14, Systat software Inc., CA, USA) was used. P values < 0.05 were considered statistically significant. Data are presented as means and SD, or medians and interquartile 25 and 75% ranges where applicable.

Results

The crocodiles had body masses of 4.2 ± 1.7 kg, chest circumferences of 34 ± 2.6 cm and their length from the nares to the distal tip of the tail was 99.4 ± 17.8 cm. The baseline HR and \( f_R \) were 50 ± 10 beats minute\(^{-1}\) and 10 ± 6 breaths minute\(^{-1}\), respectively. At preanaesthetic examination, the toe-PWR was found delayed in five animals, absent in one and normal in the remaining four. The palpebral, corneal and tail-PWR reflexes were normal in all the crocodiles.

The decision tree was useful and easy to use. The first crocodile was anaesthetised with 0.2 mg kg\(^{-1}\) of medetomidine and 10 mg kg\(^{-1}\) of ketamine, which resulted in neither sedation nor adverse effects. The drug combination was administered again 20 minutes after the first injection, and anaesthetic induction and surgical anaesthesia were achieved. Based on these findings, the second crocodile received 0.4 mg kg\(^{-1}\) of medetomidine and 20 mg kg\(^{-1}\) of ketamine, which resulted in profound anaesthesia within 5 minutes from administration. Based on the decision tree, the third crocodile
was administered with the previous doses decreased by 25% (0.3 mg kg\(^{-1}\) of medetomidine and 15 mg kg\(^{-1}\) of ketamine). This new combination resulted in anaesthetic induction and surgical anaesthesia and was tested in the next two crocodiles.

Owing to consistent findings, it was then used in the remaining five crocodiles, in which it produced anaesthetic induction and surgical anaesthesia 15.6 ± 8 and 16 [15-20] minutes after injection, respectively.

The size of the endotracheal tubes ranged from 2.5 to 4 mm (inner diameter).

Surgery lasted 246 ± 61 min. During surgery all the crocodiles were mechanically ventilated with \(f_R\) ranging from 4 to 5 breaths minute\(^{-1}\), and with peak airway pressures from 5 to 7 cm H\(_2\)O, which resulted in 40 [27-51.5] mL V\(_T\) and 10 [6.1-15] mL kg\(^{-1}\) lung compliance. Blood gas results are shown in Table 1.

Crocodiles 1 and 2 had a basal HR of 64 and 46 breaths minute\(^{-1}\), and intraoperative HR values of 48[32-52] and 28[20-56], respectively. Their intraoperative cloacal T and P\(_{\text{E}^\prime}\)CO\(_2\) were 27 [25-30] °C and 24 [19-42] mmHg; F\(_E\)Sevo was 2 [0.7-2.4] %. Crocodile 1 received a total dose of atipamezole of 0.8 mg kg\(^{-1}\) while crocodile 2 required 6 mg kg\(^{-1}\). Time to recovery was 64 and 178 minutes, and cloacal temperatures at recovery were 29.6 and 31 °C, respectively.

Data obtained from the eight crocodiles that received the same doses of medetomidine and ketamine were analysed together. Compared to baseline, HR and \(f_R\) decreased after anaesthetic induction (\(P < 0.001\)), but then these variables did not change over time and remained stable throughout surgery. The F\(_E\)Sevo was 1 [0.7-1.9] % and the intraoperative values of cloacal T (27 [26-30] °C) and P\(_{\text{E}^\prime}\)CO\(_2\) (25 ± 8 mmHg) did not change over time. The total dose of IM atipamezole was 2 [1-3] mg kg\(^{-1}\); time to recovery was 36 [20-60] minutes, and cloacal T measured at recovery was 29.6 ± 0.5 °C. Vomiting and regurgitation were not observed, and the crocodiles were
returned to their enclosure, isolated from the other animals, as soon as they appeared bright and active, which occurred within 2 hours from recovery. Access to the water pond was restricted until the day after recovery. None of the crocodiles had post-operative complications.

**Discussion**

The main finding of this study was that IM medetomidine-ketamine combination, at the doses identified by using the decision tree, was effective to immobilize the Nile crocodiles. Moreover, medetomidine and ketamine followed by sevoflurane anaesthesia resulted in adequate anaesthetic depth during the experimental surgeries.

Some of the challenges encountered during the trial were identification of parameters useful to evaluate the anaesthetic depth, interpretation of blood gas analysis, and prevention of hypothermia.

The tail-PWR was the first reflex that the crocodiles regained during lightening of anaesthesia, and unlike the toe-PWR, which was found delayed or absent in many crocodiles before anaesthesia, could be evoked consistently during the preanaesthetic assessment. It is challenging to provide a reasonable explanation to these findings. The inability to evoke the toe-PWR in some crocodiles despite the presence of a strong tail-PWR might be the result of physical restraint, which may prevent the limbs, but not the tail, from moving freely. Based on the findings of this study, the tail-PWR may be regarded as a more accurate indicator of inadequate surgical anaesthesia than toe-PWR. Similarly, the identification of reliable indicators of regained consciousness at recovery from anaesthesia was an issue. The RR was initially assessed for this purpose but found unreliable, as the crocodiles could maintain the head elevated, and respond to noxious stimuli, without turning into sternal recumbency. It was therefore concluded that the
ability to lift and keep up the head in the presence of normal palpebral and corneal
reflexes may be a more useful parameter to evaluate recovery from anaesthesia in this
species.

Regarding the blood gas analysis, common findings were high lactate blood
concentrations and pH lower than 7.3. Crocodilians mainly rely on anaerobic
metabolism during strenuous exercise (Seymour et al. 1987), and manual restraint has
been associated with increased lactates for up to 48 hours (Franklin et al. 2003).
However, the collection site might have also played a role, as pH is lower in peripheral
than in central venous blood (Lawrence 1999).

In reptiles, body temperature was shown to have an influence not only on the
anesthetic depth and duration (Kischinovsky et al. 2013), but also on cortisol plasma
levels and on the immune response (Huchzermeyer 2003). Preventing hypothermia is
challenging in crocodiles as, being poikilothermic animals, their body temperature is
directly influenced by the environment. In the study crocodiles, T dropped significantly
during the surgical preparation; however, it then remained stable throughout surgery for
periods up to 300 minutes. This may indicate that the use of active warming is effective
in this species in preventing further decreases in temperature. Ideally, in juvenile Nile
crocodiles the cloacal T should not drop below 29-30°C during anaesthesia in order to
avoid delayed recoveries (Fleming 2014).

The present study has some limitations. Being all females and bred in the same
facility, the study crocodiles may poorly represent the whole population of Crocodylus
niloticus, a species that exhibits considerable genetic divergence across its modern
biogeographic range (Schmitz et al. 2003). The baseline physiological values, obtained
during restraint, might have been affected by the autonomic nervous system response
(Fleming 2001). This hypothesis is supported by previous work, that reported in
juvenile Nile crocodiles lower baseline HR than the ones of the current study, under similar environmental conditions (Klide & Klein 1973). Furthermore, baseline values for T, biting and palatal/gular fold reflexes could not be obtained so as not to jeopardize the personnel safety.

Conclusions

Intramuscular medetomidine and ketamine, followed by inhalation of sevoflurane in oxygen, may be regarded as a useful and effective anaesthetic technique for juvenile Nile crocodiles undergoing minimally invasive experimental surgery.

References


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BE, base excess; nm, not measured; PCV, packed cell volume; Pe′CO<sub>2</sub>: end-tidal carbon dioxide, P CO<sub>2</sub>, partial pressure of venous carbon dioxide; P VO<sub>2</sub>, partial pressure of venous oxygen; SvO<sub>2</sub>, venous saturation of oxygen; TCO<sub>2</sub>, total carbon dioxide.

a. Sample was collected from the cervical venous sinus instead of the caudal vein.

b. Sample was collected 60 minutes into recovery of the Nile crocodile that experienced prolonged recovery (178 minutes).