PROPOFOL ANESTHESIA IN LOGGERHEAD (CARETTA CARETTA) SEA TURTLES

Robert A. MacLean,1,2,5 Craig A. Harms,1,3,6 and Joanne Braun-McNeill4

1 Environmental Medicine Consortium and the Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, North Carolina 27606, USA
2 North Carolina Zoo, 4401 Zoo Parkway, Asheboro, North Carolina 27205, USA
3 Center for Marine Sciences and Technology, 303 College Circle, Morehead City, North Carolina 28557, USA
4 National Marine Fisheries Service, NOAA Beaufort Laboratory, 101 Pivers Island Road, Beaufort, North Carolina 28516, USA
5 Current address: Audubon Nature Institute, 6500 Magazine Street, New Orleans, Louisiana 70118, USA
6 Corresponding author (email: craig_harms@ncsu.edu)

ABSTRACT: Rapid, safe, and effective methods of anesthetic induction and recovery are needed for sea turtles, especially in cases eligible for immediate release. This study demonstrates that intravenous propofol provides a rapid induction of anesthesia in loggerhead (Caretta caretta) sea turtles and results in rapid recovery, allowing safe return to water shortly after the procedure. Forty-nine loggerhead sea turtles were recovered as local fishery by-catch in pound nets and transported to a surgical suite for laparoscopic sex determination. Treatment animals (n = 32) received 5 mg/kg propofol intravenously (i.v.) as a rapid bolus, whereas control animals (n = 17) received no propofol. For analgesia, all animals received a 4 ml infusion of 1% lidocaine, locally, as well as 2 mg/kg ketoprofen intramuscularly (i.m.). Physiologic data included heart and respiratory rate, temperature, and a single blood gas sample collected upon termination of the laparoscopy. Subjective data included jaw tone and ocular reflex: 3 (vigorous) to 0 (none detected). Anesthetic depth was scored from 1, no anesthesia, to 3, surgical anesthesia. Turtles receiving propofol became apneic for a minimum of 5 min with a mean time of 13.7 ± 8.3 min to the first respiration. Limb movement returned at a mean time of 21.1 ± 16.8 min. The treatment animals were judged to be sedated for ~30 min (mean anesthetic depth score ≥ 1.5) when compared to controls. Median respiratory rates for treatment animals were slower compared to controls for the first 15 min, then after 35 min, they became significantly faster than the controls. Median heart rates of control animals became significantly slower than treatment animals between 40 and 45 min. Physiologic differences between groups persisted a minimum of 55 min. Possible explanations for heart rate and respiratory rate differences later in the monitoring period include a compensatory recovery of treatment animals from anesthesia-induced hypoxia and hypercapnia or, alternatively, an induced response of the nonsedated control animals. The animals induced with propofol were easier to secure to the restraint device and moved less during laparoscopy. In conclusion, propofol is a safe and effective injectable anesthetic for use in free-ranging loggerhead sea turtles that provides rapid induction and recovery.

Key words: Anesthesia, Caretta caretta, free ranging, propofol, reptile, sea turtle.

INTRODUCTION

Loggerhead (Caretta caretta) sea turtles are listed globally as an endangered species (International Union for Conservation of Nature and Natural Resources, 2007), and as a result, rehabilitation efforts for these animals have increased in their intensity and sophistication in recent years. Propeller and collision injuries are not uncommon in areas where recreational boating and ship traffic are intense, and encounters with fishing gear and other debris are also problematic. Thus, many animals require medical and occasional surgical intervention, and rapid, safe, and effective methods of anesthetic induction and recovery are needed (for a review of sea turtle anesthesia, see Chittick et al., 2002). Invasive procedures on these animals are common in research efforts as well, consisting largely of biopsy samples for genetic and toxicologic work. Due to the late occurrence of sexual dimorphism in sea turtles (estimated to be 15–30 yr in loggerheads), these invasive procedures also include sex determination by laparoscopy (Eckert et al., 1999).

Anesthesia in chelonians can be challenging largely due to the prolonged
induction and recovery times often associated with both inhalational and injectable protocols. Adequate monitoring of anesthetic quality is also difficult (Read, 2004). In sea turtles, induction by gas inhalation is a lengthy process and can require well over an hour, due in part to the long periods of breath-holding or apneusis commonly observed in chelonians (Bennet, 1996; Naganobu et al., 2000; Chittick et al., 2002). Moreover, recovery from gas anesthesia is often protracted. Forced intubation and ventilation quickens induction considerably; however, it is extremely difficult for personnel and the animal (Moon and Stabenau, 1996). Few controlled studies are available concerning injectable anesthesia in sea turtles, and induction and recovery periods have, until recently, been similarly inadequate or prolonged (Chittick et al., 2002). Advances in our understanding of reptile physiology have improved our ability to manage these animals under anesthesia (Bennett, 1998).

The use of intravenous propofol in reptile anesthesia has gained acceptance in recent years, and several reports and controlled studies have demonstrated its effectiveness and safety at dosages between 2 and 10 mg/kg (Stahl and Donoghue, 1997; Bennett et al., 1998; Pye and Carpenter, 1998; Anderson et al., 1999; Heard, 2001; Stahl, 2002; Johnson, 2004; Wellehan and Gunkel, 2004). Propofol (2,6-diisopropylphenol) is a hindered phenol that produces a smooth and rapid loss of consciousness when administered intravenously (i.v.) and a rapid redistribution and subsequent recovery that is noted to be “clear-headed” in humans (Digger and Viira, 2003). Propofol is quickly eliminated through hepatic metabolism to glucuronide metabolites and renal excretion (Digger and Viira, 2003).

Free-ranging sea turtles undergoing laparoscopy for population biology studies have traditionally not received general anesthetics or sedative analgesics due to concerns over potential mortality following a quick return to water (Owens, 1999). We hypothesized that intravenous propofol would provide rapid induction of anesthesia in loggerhead sea turtles with rapid recovery, allowing safe return to water shortly after the procedure. This study investigates the effects of propofol on anesthetic depth and physiology as part of a larger study involving the validation by laparoscopy of a hormonal sex determination assay in juvenile loggerheads.

**MATERIALS AND METHODS**

**Animals**

Immature loggerhead sea turtles incidentally captured in commercial pound nets set in Core and Pamlico Sounds, North Carolina, were brought to the laboratory as part of an ongoing study to verify sex determination by serum testosterone assay (Braun-McNeill et al., 2007). Forty-nine turtles \((n=18\) in June [season one] and \(n=31\) in October [season 2] of 2004) were examined.

**Presurgical procedures**

Within 15 min of recovery, a blood sample was collected from the dorsal cervical sinus as part of the serum testosterone study as well as for health assessment. Carapace measurements and epibiotic burden were noted, and scute scrapings were collected for later heavy metal analysis. After scrubbing the carapace with a stiff nylon brush and using fresh water to remove loose debris, turtles were kept in dry, individual plastic tubs in the shade with a wet towel keeping the carapace moist.

Animals were assigned to a treatment group by either a coin toss or the surgeon’s preference (where sedation was determined desirable for expediency with regards to the primary objective of sex determination). Treatment animals \((n=32)\) received propofol (Rapinovet, Schering-Plough Animal Health, Union, New Jersey, USA; 5 mg/kg) intravenously into the dorsal cervical sinus using a 20 ml syringe and a 22 gauge 37 mm needle, whereas control animals \((n=17)\) received no propofol. Animals were then moved into dorsal recumbency, noting the directional change in orientation, and strapped, using nylon ropes and cleats, onto a padded wooden restraint device lying flat on the floor. Turtles were placed in head down (season one) or right lateral (season two) recumbency to facilitate laparoscopic access to the reproductive organs.
Surgical orientation was altered in season two in an attempt to reduce the dependent erythema, ocular secretions, and increased intraocular pressure observed previously in the traditional head down position (Chittick and Harms, 2001). The site of laparoscopic incision in the prefemoral fossa (right for season one, left for season two) was surgically scrubbed using betadine scrub and 70% isopropyl alcohol. All animals then received an infusion of local anesthetic (1% lidocaine; Lidotject Rx, Vetus Animal Health, St. Paul, Minnesota, USA; 4 ml subcutaneously (s.q.), intramuscularly (i.m.)) into the site of incision, and ketoprofen (Ketofen, Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA; 2 mg/kg, i.m.) was administered for short-term presumptive postoperative analgesia without sedation at a dose studied in green iguanas (Iguana iguana; Tuttle et al., 2006). All turtles also received oxytetracycline (Liquamycin LA-200, Pfizer Animal Health, New York, New York, USA; 25 mg/kg, i.m.) as a biologic marker of bone and as prophylaxis against surgical infection (Frazier, 1985; Harms et al., 2004). The turtle and restraint apparatus were then moved to the surgical area and leaned against a table at approximately 10 degrees from vertical.

Physiologic data collected

The observers were not blinded to the treatment status of the subjects. Prior to surgical procedures, an average resting respiratory interval in seconds was determined using a handheld stopwatch to time the duration between at least three breaths. One observer was responsible for an individual turtle’s respirations throughout its procedure. Turtles from season one were then moved to a scale for weighing. Four leads from a portable electrocardiograph (ECG) monitor (Silogic EC-60, Silogic Design Ltd., Stewartstown, Pennsylvania, USA) were then placed with alligator clips moistened with 70% isopropyl alcohol, one on each limb, and a preanesthetic heart rate was determined. Due to periodic movement during manual restraint on the scale, resting heart rates for season two were determined in the holding tub without additional restraint, and the turtle was weighed afterward.

Jaw tone, ocular reflex, and pedal withdrawal were scored once prior to manual restraint and then frequently during the procedures using subjective grades: 3 (vigorous) to 0 (none detected). Jaw tone and ocular reflexes were determined by hand, while pedal withdrawal was determined using Carnalt forceps closed with increasing force onto a distal phalanx of a forelimb. Anesthetic depth was scored from 1 (no apparent sedation), to 3 (surgical anesthesia). Upon termination of the laparoscopy, a cloacal temperature was recorded (Digi-Sense Thermocouple Thermometer, Model 91100-20, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA), and a single blood gas sample was collected from the dorsal cervical sinus and analyzed immediately on a handheld blood gas analyzer (i-STAT, Heska Corporation, Fort Collins, Colorado, USA) using the CG+ cartridge.

Surgery

A stab incision was made in the prefemoral fossa using a number 15 scalpel blade. A 14.5 French (Fr.) operating sheath with a 5 Fr. instrument channel and two stopcocks (Model 67065C, Karl Storz Veterinary Endoscopy, Goleta, California, USA) was fitted with a blunt obturator (Model 67065CO, Karl Storz) and advanced cranioomedially through fascia and musculature into the coelom. The obturator was removed, allowing room air to enter the coelom, and then was replaced with a 2.7 mm wide-angle 30° oblique laparoscope (Model R7218BSA, Karl Storz) attached to a 175 W xenon Nova light source (Model 20131520, Karl Storz). The coelom was explored, and the reproductive organs were visualized and identified using video imaging (Telecam 20212130U and SL ncsc 20212120, Karl Storz). In order to displace the coelomic air for proper buoyancy, the turtle was placed into dorsal recumbency on a table and padded so that the incision was the highest point. One liter of sterile 0.9% saline (Baxter Healthcare, One Baxter Parkway, Deerfield, Illinois, USA) was infused into the coelom through the cannula. Manual pressure was applied to the plastron to force out coelomic air while manipulating the cannula. The cannula was removed, and the incision was closed, under plastron pressure, using 3-0 PDS (PDS II, polydioxanone, Ethicon, Novartis Animal Health US, Inc., Greensboro, North Carolina, USA) in single muscle and skin sutures. The turtle was then surgically prepared in dorsal recumbency in the opposite prefemoral fossa. Lidocaine was infused as before, and a 2 cm incision was made through the skin to expose fat. Fat was sharply excised in a manner appropriate for later organochlorine analysis, and the incision was closed in two layers using 3-0 PDS in a simple interrupted pattern. The turtle was returned to a dry holding tub for observation until it appeared alert. Recovered and responsive animals were then moved into
an individual tank with flow-through seawater for overnight observation. Animals that had normal buoyancy and that were feeding on frozen-thawed squid were released into Core Sound, usually the following day.

Data Management and Statistics

Because data were collected at variable time points during the procedure, individual turtle data were averaged into 5 min bins for analysis. For respiratory intervals, the maximum interval per 5 min period was entered for that bin. Respiratory rates were calculated from this maximum respiratory interval and entered as the rate for that bin as well as for previous bins where no respirations were observed (i.e., breath holding or apneusis for longer than 10 min). Much of the data was not normally distributed (Shapiro-Wilk W test), so all data were analyzed for significance using the Wilcoxon rank sums test or contingency analysis through JMP 5.1.2 software (SAS Institute, Inc., Cary, North Carolina, USA). Significance is reported when \( P < 0.05 \). Temperature manipulations were not performed to adjust the i-STAT calculations from 37°C to the turtles’ cloacal temperature. To facilitate analysis by contingency table, all reflex score data indicating some reduction in response (0, 1, 2) were merged. Similarly, for anesthetic depth score data, all values indicating some sedation (2, 3) were merged.

RESULTS

Weights (median \( [10^{th} \) and \( 90^{th} \) percentiles] of 40.5 [21.4, 67.9] for propofol-treated turtles and 37.8 [27.7, 57.0] kg for control turtles) and sex ratios (3.6F:1M for propofol-treated turtles and 4.7F:1M for control turtles) did not differ overall between treatment groups. The median time to incision closure, signaling the end of the laparoscopy, was not different between propofol-treated \((30 \ [27.0, 57.3])\) and control groups \((33 \ [27.1, 49.4])\) min).

All propofol-treated animals became apneic for a minimum of 5.3 min post-induction; the median \( (10^{th} \) and \( 90^{th} \) percentiles) time was 10 (6.3, 26.1) min, ranging from 5.3 to 37.7 min, to the first respiration. Limb movement ceased within \(~2\) min of induction in treatment animals and returned at 13.5 (8, 50.9) min. These animals did not struggle during the initial postinduction restraint, and they moved their limbs less often during the surgery. In addition, unlike controls, animals treated with propofol were judged to be sedated, with a median anesthetic depth score \(~1.5\), for 30 min when compared to controls. Pedal withdrawal scores were collected during season one and were not significantly different. Both the ocular reflex and jaw tone were significantly reduced in propofol-treated turtles between 5 and 45 min. Median respiratory rates for propofol-treated turtles were slower compared to controls for the first 10 min, and then became significantly faster than the controls at 15 min and between 35 and 55 min (Fig. 1). Median heart rates did not differ between treatments or from the pretreatment values for the first 25 min of procedures. Median heart rates of propofol-treated turtles then became significantly faster than controls at 30 min \((38 \ [29.2, 43.4]\) and 30 \([8, 41.6]\) beats per minute \((bpm)\), respectively) and 45 min \((36 \ [32, 40]\) and 9 \([9, 20]\) bpm, re-
spectively) (Fig. 2). The overall preprocedural heart rates, however, did differ by season; the heart rate from season one was significantly faster than that of season two (36 [26.4, 40.2] and 28 [12, 36] bpm, respectively).

The median temperature for all turtles was different in season one and season two (29.4 [27.4, 31.0] and 24.2 [22.2, 27.1] C, respectively) but did not differ between treatments. Blood gases were measured for 17 turtles in each group. Median blood gas collection times for propofol-treated and control turtles were 19 (14, 36.4) and 21 (12, 36.6) min, respectively. Blood gas values did not differ significantly between treatments except for sO$_2$%, a calculated value, which was significantly lower for propofol-treated turtles (54% [37.8, 95.2] vs. 93% [78, 96]); pO$_2$, however, was not different. When blood gas data from propofol-treated turtles with collection times above 21 min are excluded from analysis, significant differences between propofol-treated and control groups, respectively, include TCO$_2$ (39.5 mmol/L [20.3, 47.6] vs. 41 mmol/L [31.4, 48]), pH (7.30 [7.16, 7.33] vs. 7.34 [7.25, 7.44]), pO$_2$ (30.5 mmHg [28, 62] vs. 65 mmHg [48, 96]), and sO$_2$% (46% [36, 88] vs. 93% [78, 96]). When data are analyzed within each season, other differences are elucidated. For season one, differences are noted between groups in pH (7.31 [7.15, 7.36] vs. 7.35 [7.29, 7.49]), pCO$_2$ (73.2 mmHg [50.4, 82.2] vs. 60 mmHg [49.3, 70]), pO$_2$ (31 mmHg [28, 67] vs. 64 mmHg [48, 74]), and sO$_2$% (52% [36, 91] vs. 90% [78, 94]), but no differences in blood gases are noted for season two alone.

Propofol-treated turtles were returned to water in individual holding tanks 51 – 170 min after propofol injection, and they swam vigorously prior to settling down. Most turtles in both groups were transiently positively buoyant; however, all but four animals achieved neutral buoyancy within 12 hr. Two animals achieved proper buoyancy after undergoing a percutaneous coelomic aspiration using a spinal needle under local anesthesia to remove excess air. The remaining two animals suffered a small laceration of the visceral pleura upon trocar insertion and were released after 4 mo of rehabilitation at the Karen Beasley Sea Turtle Rescue and Rehabilitation Center in Topsail Island, North Carolina, USA. In the course of continued monitoring of pound nets in the study area through 2006, there was no significant difference in live recapture rates between propofol-treated (12 of 32, 37%) and control (2 of 17, 12%) turtles ($P=0.0959$). Three propofol-treated and no controls have been recovered dead through the North Carolina sea turtle stranding network. One became stranded in January, 3 mo following the procedures, and was a presumed victim of hypothermic stunning. One was a victim of net entanglement 7 mo following the procedures. The third was stranded 6 mo later, ~190 km southeast of the study area, emaciated but having grown 2 cm in length.

**DISCUSSION**

Intravenous propofol at 5 mg/kg provided for a period of ~10 min of apparent
surgical anesthesia followed by a longer period of mild to moderate sedation of ~30 min. Physiologic differences remained between treatments and controls for at least 45 min in heart rate and 55 min in respiratory rate. Once past the initial period of apnea, the treatment animals maintained a respiratory rate similar to the resting, preprocedural level. The controls, however, gradually reduced their respiratory rate by between two- and sixfold after ~30 min. With regard to heart rate, the treatment animals maintained a preprocedural pace, whereas the control animals dropped their heart rate by ~1.5-fold to threefold starting at about 40 min. Possible explanations for these differences include a compensatory circulatory and respiratory recovery of treatment animals from an anesthesia or apnea-induced hypoxia and hypercapnia, which is supported by the trends in the blood gas data. Alternatively, the nonsedated control animals may have been responding to procedural stimulation by lowering their heart rate and respiratory rate. It should be noted that the overall preprocedural heart rates were lower in season two than season one, but this was likely due to the decreased handling during ECG monitoring. The relative decreases in control heart rate, however, are still significant.

All treatment animals entered a period of apnea for a minimum of 5.3 min up to a maximum of 37.7 min. No attempt was made to intubate this latter animal due to the fact that its heart rate was steady, it had some limb movement starting at 11 min, and it maintained a mild to moderate ocular reflex and jaw tone throughout the apneic period. This turtle began to breathe regularly 5 min after the start of the fat biopsy. Although this period of apnea appears prolonged, it was still well within the maximum dive durations of up to 2 hr (Bentivegna et al., 2003) and 7 hr (Hochscheid et al., 2005) reported for loggerheads in winter, and it is comparable to routine dive durations of subadult loggerheads of 19–30 min (Lutcavage and Lutz, 1997). The fact that all treatment turtles experienced apnea may be attributable to the method of i.v. delivery as a bolus. In dogs and other species, rapid injections of propofol can lead to profound respiratory depression and apnea (Muir and Gadawski, 1998). A slower induction may prevent the observed apnea in sea turtles. Additionally, as our respiratory data were collected by visual observation only, a part or all of the periods of apnea may have been periods of apneusis or sustained inspiration, which can be difficult to distinguish in sea turtles. It should also be noted that although injection pain has been reported in humans with propofol injection, no reaction attributable to pain on injection was noted in these sea turtles (Digger and Viira, 2003). Other observations include a lack of any excitatory effects upon induction with propofol, as has been reported to occur in some dogs and humans (Smedile, 1996; Aun, 1999; Digger and Viira, 2003). It is possible, however, that some limb and head movements noted commonly during surgery may have been myoclonal in nature and an effect of the propofol sedation.

The animals induced with propofol were easier to secure to the restraint device and moved much less vigorously and less frequently during laparoscopy. The controls moved their limbs frequently, but the median time to first limb movement for propofol-treated turtles was 13.5 min, which usually allowed sufficient time to complete the laparoscopic sexing, but not the evacuation and closure. Either periodic readministration of the propofol or use of an i.v. catheter with a constant rate infusion (CRI) would likely resolve this problem of insufficient surgical anesthesia time. Propofol has a very low toxicity in mammals, even when administered as a CRI (Short and Young, 2004). No toxic effects from the propofol or other procedures were noted in sea turtles during this investigation. Recoveries were rapid and appeared complete after ~55...
min. Propofol would likely be appropriate for use in wild loggerheads destined for release after at least 1 hr postinduction, as long as proper buoyancy was maintained. In conclusion, propofol is a safe and effective injectable intravenous anesthetic for use in wild loggerhead sea turtles, at 5 mg/kg, that provides for a rapid induction and recovery with a short period of surgical anesthesia and sedation sufficient for brief procedures.

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