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THE EFFECTS OF TWO DOSAGES OF MIDAZOLAM ON SHORT-DURATION ANESTHESIA IN THE HARP SEAL (*PHOCA GROENLANDICA*)

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Abstract: The purpose of this study was to provide safe anesthesia for bronchoalveolar lavage and assess the utility of premedication with i.m. midazolam for short-duration anesthesia with isoflurane in harp seals (*Phoca groenlandica*). Fourteen yearling harp seal pups were anesthetized three times each as part of a prospective, cross-over, blinded study. Each animal received i.m. premedication with saline, low-dose, or high-dose midazolam (respectively 0.1 and 0.2 mg/kg). Following premedication, anesthesia was induced with 4% isoflurane in oxygen delivered through a mask and connected to a Bain non-rebreathing system. A significantly longer time was taken from the end of general anesthesia to head movement in the high-dose group compared with the saline group ($P = 0.002$). A significantly longer time was taken from the end of general anesthesia to ambulation in the high-dose group compared with the saline group ($P = 0.006$). There were no significant differences between groups in the subjective assessment of anesthetic quality or ease of intubation. Premedication with i.m. midazolam at the dosages used did prolong recovery from anesthesia, although to a degree unlikely to be significant clinically.

Key words: Anesthesia, bronchoalveolar lavage, harp seal, midazolam, *Phoca groenlandica*, phocids.

INTRODUCTION

General anesthesia in phocids is potentially more complicated than in other mammals because of anatomic and physiologic adaptations to diving.[4,7,8] Furthermore, their response to sedative and anesthetic agents is not fully understood. Short-duration general anesthesia was required for bronchoalveolar lavage as part of a comprehensive study looking at the effect of the lungworm, *Otostrongylus circumlitus*, on the health of harp seals (*Phoca groenlandica*). Bronchoalveolar lavage is a technique commonly used to investigate respiratory disease in domestic species[2] and has been used in pinnipeds.[14] Bronchoalveolar lavage is not a benign procedure and can result in a decrease in oxygen saturation of hemoglobin and hypoxemia.[10] An ideal anesthetic protocol for this type of procedure would employ drugs with minimal cardiovascular and respiratory effects, allow a good degree of control over the depth of anesthesia, undergo rapid metabolism and elimination from the body (without complete dependence on a single organ system), be reversible, and have little or no residual effect during the recovery period. Rapid and total reversibility of anesthesia allowing safe return to the aquatic environment and minimizing the duration of the post-operative surveillance period are also desirable.

The aim of this study was to provide safe anesthesia for bronchoalveolar lavage and assess the utility of premedication with i.m. midazolam for short-duration anesthesia with isoflurane in harp seals.

MATERIALS AND METHODS

This project was carried out according to animal utilization protocols approved by the animal care committees of the institutions involved, both of which operate under the auspices of the Canadian Council on Animal Care.[1] The animals used in this project were collected as weaned pups on the ice pack in the Gulf of St. Lawrence during the month of March. After 1 wk of fasting in a dry tank to complete the molt, seals were kept in two indoor tanks (3.71 m × 3.55 m × 1.36 m) filled with salt water (flow rate: 50–60 L/min, at −1.0°C to 10°C, 24–30‰). Each tank had a haul-out area of 1.82 m × 3.55 m. The ambient air temperature was maintained between 8°C and 10°C during the duration of the study with a complete air exchange every 5 min. The seals were fed once a day ad lib. with shrimp, capelin, and herring, and a vitamin/mineral supplement (SeaTab®, Pacific Research Laboratories, El Cajun, California 92019, USA).

A group of 14 harp seals with a mean ± SD weight of 26.9 ± 3.8 kg were used for the anesthesia study. A prospective, cross-over, blinded protocol was prepared to assess the effects of two dosages of midazolam on induction, intubation, and re-

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covery parameters. Each animal was anesthetized three times, 2 wk apart, and given either i.m. midazolam hydrochloride (Versed® 5 mg/ml, Hoffmann—La Roche Limited, Mississauga, Ontario L5N 6L7, Canada) high dose (HD; 0.2 mg/kg), low dose (LD; 0.1 mg/kg), or saline control (SAL; equal volume to midazolam) as preanesthetic medication. For the first procedure, animals (approximately 6 wk old) were randomized to receive either midazolam LD or saline. For the second procedure, animals (approximately 8 wk old) that received midazolam LD for the first procedure received saline and vice versa. For the third procedure, all animals (approximately 11 wk old) received midazolam HD. Food was withheld for 12 hr prior to anesthesia. Each seal was netted and weighed within the tank (drained the evening before). Following removal from the tank, the test drug and aminophylline (Hospira Healthcare Corporation, Saint-Laurent, Quebec H4N 2X6, Canada) were injected in opposite muscle groups (2 mg/kg i.m.; semitendinosus or femoral biceps) and a general clinical examination, including thoracic auscultation, was performed. Following completion of general clinical examination, mask induction of anesthesia with isoflurane (AErrane®, Baxter Corporation, Mississauga, Ontario L4Z 3Y4, Canada) commenced at a vaporizer setting of 4%. The isoflurane was delivered with oxygen as a carrier gas via a Bain non-rebreathing system and a flow rate of 200 ml/kg/min. Mask induction of anesthesia was accomplished using an opaque mask fashioned from a semirigid rubber traffic cone fitted with an adaptor allowing connection to the Bain non-rebreathing system. Once muscular relaxation was apparent (assessed as loss of muscle tone in the thoracic limb), endotracheal intubation was attempted with a cuffed endotracheal tube of 6–7 mm internal diameter. If the first attempt to intubate failed, isoflurane delivery was continued and intubation was reattempted within 5 min. Following intubation, intermittent positive-pressure ventilation (IPPV) was instituted manually and monitoring equipment was attached. Cardiovascular and respiratory parameters were monitored with a Doppler ultrasound probe (Ultrasonic Doppler Flow Detector, model 811-B, Parks Medical Electronics, Inc., Aloha, Oregon 89119, USA) placed directly on the globe of the eye, a reflectance pulse oximeter (NPB-75®, Nellcor Puritan Bennett, Pleasanton, California 94588, USA) placed on the hard palate to determine oxygen saturation of hemoglobin, and a sidestream end-tidal carbon dioxide analyzer (NPB-75®, Nellcor Puritan Bennett) connected to the endotracheal tube. After placement of monitoring equipment and stabilization, bronchoalveolar lavage was performed. Briefly, a sterile urinary catheter (8 Fr, 56 cm long; Sovereign® Sherwood Medical, St. Louis, Missouri 63146–3367, USA) was inserted in the endotracheal tube and gently advanced until resistance was encountered or up to full insertion of the catheter. A total of 9–17 ml ($\bar{x}$ = 15.3 ml) of warm sterile saline was then slowly injected and immediately aspirated using a 60-ml catheter-tip syringe (Terumo® Corporation, Elkton, Maryland 21921, USA) attached to the urinary catheter. At the end of the lavage procedure, isoflurane was discontinued and the breathing system was flushed with 100% oxygen. Weaning from IPPV involved stopping ventilation for 1-min periods until the animal began spontaneous ventilation or nostril movements occurred in an effort to ventilate. Extubation followed return to spontaneous ventilation or when it was believed that breath holding was occurring following adequate recovery from anesthesia (i.e., breath holding in the presence of return of spontaneous movement). Animals were returned to the tank following complete recovery from anesthesia. The following time periods were recorded: start of induction (delivery of isoflurane) to loss of muscle tone, start of induction to intubation, the total duration of general anesthesia (GA) from delivery of isoflurane until the vaporizer was turned off, end of GA to return of spontaneous respiration, end of GA to head movement, end of GA to ambulation, and time from initiation of spontaneous respiration to extubation. Heart rate, end-tidal partial pressure of carbon dioxide (ET_{CO2}) and oxygen saturation of hemoglobin (SpO_2) were recorded during each procedure. In addition, each animal was scored for quality of the anesthetic procedure, the ease of intubation, laryngeal state at intubation and the quality of recovery (Table 1). Assessors were blinded to the premedication received by each animal.

Parametric data were analyzed with analysis of variance for repeated measures followed by a Bonferroni's post hoc test (SAS version 9.1, SAS Institute, Cary, North Carolina, USA). Confidence intervals (95%) were calculated for significant differences between groups. Ordinal data and data not following a normal distribution were analyzed with a Kruskal–Wallis test and Friedman's test, respectively (SPSS 11.0, SPSS Inc., Chicago, Illinois, USA). A P value of <0.05 was taken as significant.

RESULTS

No fatality occurred during procedures. One seal was euthanized and was removed from statistical analysis. At necropsy this seal had a large s.c. abscess on its shoulder not believed to be associated with the anesthetic procedure. The mean ± SD for

Table 1. Scoring system used for the subjective evaluation of the anesthesia.

Score	0	1	2	3
Ease of intubation	Easily achieved	Slightly difficulty	Moderate difficulty	Marked difficulty
State of larynx	Closed	Partially open	Open	—
Quality of recovery	Long and calm	Rapid and calm	Moderate agitation	Marked agitation
Quality of anesthesia	No problems encountered	Some problems, but no consequence	Some problems with potential consequences	Death

each group of the different time intervals recorded are presented in Table 2. The time from i.m. injection of the test drug to start of induction was 10.62 ± 2.49 min (mean ± SD). There was no significant difference in total GA time, time from start of induction to loss of muscle tone, or time from induction to intubation. A trend for a longer time between the end of GA to spontaneous respiration as the dosage of midazolam increased was observed (Table 2); however, there was no significant difference between groups. The time from spontaneous respiration until extubation showed a tendency to being longer in the midazolam HD compared with the SAL group (Table 2).

For the time period from the end of GA to head movement, a significantly longer time to head movement was present in the HD compared to the SAL group (Table 2). A tendency for a longer time to head movement was present in the LD compared with the SAL group and between the HD and LD groups. However, these tendencies were not statistically significant ($P = 0.080$ and $P = 0.065$, respectively). The time from end of GA to ambulation was significantly longer in the HD group compared to the SAL group (Table 2). Significance was not achieved for the SAL and LD comparisons ($P = 0.147$) or LD and HD comparisons ($P = 0.243$).

The heart rate (bpm) of the HD group (mean ± SD [range]; 103 ± 12.2 [78–131]) was significantly lower ($P = 0.007$) than the saline group (109 ± 11.6 [83–133]) and neither differed significantly from the LD group (115 ± 16.7 [69–138]). The partial pressure of end-tidal CO_2 was maintained between 20 and 60 mm Hg during the procedures by adjusting the ventilatory rate. Use of a pulse oximeter and reflectance probe did not generate consistent readings because of the difficulty in maintaining probe position.

The subjective scoring systems used for laryngeal state ($P = 0.542$), quality of recovery ($P = 0.368$), and overall quality of anesthesia ($P = 0.513$), were not significantly different between groups.

DISCUSSION

Phocid anesthesia is, as is anesthesia of other species of marine mammals, associated with a va-

Table 2. Time periods in anesthesia experiment showing comparison between premedication with high dose of midazolam (0.2 mg/kg; $n = 13$), low dose of midazolam (0.1 mg/kg; $n = 13$), and saline control ($n = 13$).

Time (min)	Saline (mean ± SD)	Midazolam (mean ± SD)		*P* values
		Low dose	High dose	
Total GA[a] time	15.08 ± 2.53	15.85 ± 6.23	15.31 ± 5.56	0.885
Induction to loss of muscle tone	2.85 ± 1.63	2.77 ± 1.54	2.85 ± 0.80	0.976
Induction to intubation	10.23 ± 2.09	11.31 ± 6.09	11.92 ± 5.25	0.642
End of GA to spontaneous respiration	5.69 ± 2.87	6.31 ± 2.81	8.23 ± 3.92	0.126
Spontaneous respiration to extubation	0.38 ± 0.87	0.54 ± 1.39	2.15 ± 2.88	0.050[b]
End of GA to head movement	7.31 ± 2.66	9.54 ± 3.33	12.85 ± 4.54	0.002[c]
End of GA to ambulation	10.00 ± 2.97	12.31 ± 4.31	16.15 ± 5.66	0.006[d]

[a] GA: General anesthesia.

[b] Tendency to a significant difference between saline and HD. (Mean difference [95% confidence interval]: 1.77 [0.35–3.19] min.)

[c] Significant difference between saline and HD. (Mean difference [95% confidence interval]: 5.54 [2.79–8.29] min.)

[d] Significant difference between saline and HD. (Mean difference [95% confidence interval]: 6.15 [2.95–9.35] min.)

riety of potential complications from apnea to severe bradycardia and death.[4,7,8] It is difficult to assign risk factors or determine the cause of many of these complications because of the wide variety of anesthetic protocols, procedure duration, limited knowledge of response to anesthetic and sedative agents in these species, and experience of the anesthesiologist. Of the phocids, harp seal anesthesia is not well documented, further limiting the available pool of information.[8] Bradycardia is a potential complication of pinniped anesthesia[4,7] but was not observed during this study. Together with apnea, these phenomena are part of the dive response. It is unclear if these responses to anesthesia represent elicitation of the true dive response or a particular response to anesthesia in diving species (although their appearance is variable).[4,7,8,11] The inclusion of atropine in the anesthetic protocol is common practice and has been advocated to offset the occurrence of bradycardia.[3,4,7,11,13,17] We elected to have a dose of atropine calculated, though none was given. The lower heart rate recorded in the HD group is unlikely to be clinically significant. Mask induction of anesthesia is a recognized technique in phocids.[4,6,7] It has the advantages of allowing induction where i.v. access is not easily achievable and, because of the limited metabolism of isoflurane, it allows a rapid recovery from anesthesia at the end of the procedure or during the induction period should problems arise. During this experiment, mask induction was extremely well tolerated without the struggling or the breath holding often associated with the use of this technique in phocids.[4] The age of the animals may have played a role in this, although it may be possible that this is a feature of this species.[6,8] This technique presents some disadvantages, including the lack of rapid airway control, increasing the risk of aspiration of stomach contents should regurgitation occur, and exposure of personnel and the environment to anesthetic gases. A mask induction technique was chosen in this case because it allowed rapid recovery from anesthesia and the size of the animals facilitated manual restraint. Of the benzodiazepines, both diazepam and midazolam act centrally to provide sedation and muscle relaxation via GABA (gamma-aminobutyric acid) receptor stimulation.[15] Midazolam was investigated in this study because diazepam (with a propylene glycol vehicle) has been associated with muscle necrosis at the site of injection and its uptake from i.m. injection is variable.[7,12] The pharmacokinetic properties of midazolam may provide an advantage in field use because of a faster onset of action and greater potency than diazepam.[12] Doses were based on those frequently used in domestic species and those reported in other pinniped species.[7,13,17] Midazolam is frequently used in anesthesia of domestic species for the provision of muscle relaxation without significant cardiorespiratory effects at clinical dosages.[9] Furthermore, it is reversible with flumazenil if necessary.[12,13] To the authors' knowledge, midazolam alone as a sedative agent has not been reported in pinnipeds, although it does provide effective sedation in conjunction with other sedative and anesthetic agents.[7,13,17]

The aims of this study were twofold: 1) to provide a safe anesthetic for a short, although not benign procedure and 2) to investigate the effects of i.m. midazolam on both objective and subjective measures of anesthesia quality. The first aim was fulfilled, with no fatality experienced in a total of 39 anesthetics delivered. Reported perioperative mortality rates for phocids are highly variable, ranging from 0–100 %.[4] These ranges do not include harp seals, for which the mortality rate in one study was two out of a total of 12 anesthetics delivered to eight individuals.[8] There was no significant difference between groups for the time from induction (placement of mask) to loss of muscle tone. Assessment of loss of muscle tone was highly subjective because manual restraint was only necessary during the initial period of induction and, in general, the mask was well tolerated. This was expected because covering the head of pinnipeds has been reported to have a calming effect.[11] The induction-to-intubation time did not vary greatly between groups and this was intentional in an effort to standardize the time of first attempt at intubation because of the difficulty in assessing readiness for intubation. However, this time was similar to that reported for sea lion pups also undergoing mask induction.[6]

Intubation of phocids can be difficult because of physical obstruction of the upper respiratory tract by copious pharyngeal soft tissue in adult animals.[7,11] Visibility of the larynx was not a problem in this species. However, the larynx itself appeared highly sensitive and would close tightly, blocking attempts at intubation if the endotracheal tube was not passed through the rima glottis rapidly. Increasing the depth of anesthesia did not appear to improve intubation conditions. Although not documented in this study, laryngospasm has been noted in other phocids.[11]

The use of midazolam in this study did not improve intubation conditions. However, this might be because of the relatively short period of time between the premedication and start of induction. Hypoventilation and apnea are a common occurrence during anesthesia in phocids and institution of IPPV

has been recommended.[4] Monitoring of end-tidal CO_2 allows assessment of adequacy of ventilation. However, normal values have not been reported in this species. Elevated levels of end-tidal CO_2 under general anesthesia have been reported in other pinniped species and this may indicate hypoventilation as a result of the sedative and anesthetic agents used and/or a natural degree of carbon dioxide tolerance.[3,5] During this study, IPPV was provided manually and constantly throughout the anesthetic period although it was still difficult to maintain an end-tidal CO_2 within normal limits for domestic species. The reasons for this are unclear. Re-breathing expired CO_2 was unlikely because flow rates of fresh gas were calculated for each animal and ventilation was controlled. Resistance to ventilation was significant in many animals, suggesting a decrease in pulmonary and or chest wall compliance. This may have been because of muscular bronchioles, a collapsible trachea, and a compressible thorax in phocids.[7,11,16] The high degree of anatomical adaptation of the phocid respiratory tract to diving has been implicated in mortality under general anesthesia.[11] All animals received aminophylline IM at the same time as midazolam, therefore ease of ventilation without aminophylline could not be assessed. Its use was indicated as a result of clinical experience of bronchospasm following bronchoalveolar lavage in domestic species.

Statistically significant differences between groups were found for the time interval between the end of GA to head movement, and from the end of GA to ambulation. Between the end of GA and head movement there was a tendency for an increase in time in the groups receiving midazolam (with a significant difference between the HD and SAL groups). A similar result was seen for the time period from end of GA to ambulation. These findings agree with the expected clinical effect of midazolam. Because of the short duration of the procedure, it is likely that the sedative and or muscle relaxant effects of midazolam remained at recovery from anesthesia. It was not possible to identify if these findings were a result of sedation, muscle relaxation, or a combination of these factors. Sedation and muscle relaxation may be beneficial in domestic species where further confinement is required following an anesthetic. However, these effects may not be advantageous in a field situation or where large numbers of animals need to be anesthetized without the availability of holding facilities.[6] Close monitoring during the recovery period may not be possible in either of these situations and premature return to the aquatic environment may be hazardous where sedation and muscle relaxation persists.

For the time interval between the end of GA to return of spontaneous respiration, a nonsignificant trend was observed for an increase in the time taken for the return of spontaneous respiration in the groups receiving midazolam. Again, this may be a disadvantage where anesthesia is required for a group of animals within a limited time, particularly given the length of time taken for return to spontaneous respiration in the HD group (Table 2). A confounding factor for measurement of this time period was the behavior of the seals. Extubation occurred prior to spontaneous respiration in 24 of 39 anesthetics when it was believed that animals were breath holding although no longer anesthetized. In these cases, nostril and or head movement were taken as a sign of recovery from anesthesia. In every case, breathing recommenced upon extubation. This may have been a response to handling, the environment, or presence of the endotracheal tube.[8] Therefore, it is likely in at least some cases that this time period was overestimated. This breath holding following apparent recovery to consciousness has been previously documented in harp seals and other pinniped species.[7,8,17] There was a tendency to a significant difference between groups for the time period from spontaneous respiration to extubation. It was apparent that animals in the HD group tolerated the endotracheal tube to a greater degree and a longer period of respiration was observed prior to extubation. A greater degree of sedation in this group may have accounted for this.[17]

The effect of i.m. midazolam on subjective measures of anesthetic quality was difficult to assess because no significant difference was found between groups for the scoring systems used (laryngeal state, quality of recovery, and overall anesthetic quality). It is possible that the time allowed for the midazolam to achieve therapeutic plasma levels may not have been sufficient. Pharmacokinetic data for this species are not available, therefore the time allowed for midazolam to take effect was extrapolated from our clinical experience with small domestic species and a range of 6 to 14 min passed before induction began. A 20-min preanesthetic medication time has been recommended in southern elephant seals (*Mirounga leonine*) where midazolam was part of the anesthetic protocol.[17] This could not be achieved in the present study because of time constraints, and it was felt that any advantage achieved by extending the duration of the preanesthetic medication time was outweighed by the degree of stress likely experienced with the associated increase in preinduction handling, or in the event of recapture following release after delivery of the injection. Furthermore, it is possible that

the scoring systems used (Table 1) were not sensitive enough to detect subtle differences and that the response of harp seals to midazolam may not fall within the categories examined in each scoring system.

CONCLUSIONS

Intramuscular midazolam, used at 0.1–0.2 mg/kg, does not confer benefits in terms of improved intubating conditions, induction, or recovery when an inhalational technique is used. Recovery from anesthesia may be prolonged although this prolongation is insignificant clinically. Use of midazolam at higher doses was associated with a longer period of autonomous respiration during the recovery period with the endotracheal tube in place. Because of the inherent difficulty of re-intubating extubated phocids should complications arise in the postoperative period, a longer period of autonomous respiration during the recovery period with the endotracheal tube in place would be beneficial in ensuring return of spontaneous respiration prior to extubation.

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