Electrocardiographic, Hematologic, Histopathologic, and Recovery Characteristics From Repeated Morphine-Chloralose Anesthesia in Dogs

Daise Nunes Queiroz da Cunha[1]
Matthew Buccellato[1]
Bruce W. Keene[2]
Paivi Rajala-Schultz[3]
Yoshinori Nishijima[1]
Yunus Ozkanlar[4]
Robert Louis Hamlin[1]

[1]*Department of Veterinary Biosciences*
The Ohio State University
Columbus, Ohio, USA
[2]*Department of Clinical Sciences*
North Carolina State University
North Carolina, USA
[3]*Department of Veterinary Preventive Medicine*
The Ohio State University
Columbus, Ohio, USA
[4]*Department of Veterinary Internal Medicine*
Ataturk University
Turkey

KEY WORDS: Alpha-chloralose; pre-anesthetic medication; baroreceptor depression; morphine-chloralose

ABSTRACT

Alpha-chloralose is an intravenous anesthetic that produces minimal cardiovascular effects. The safety of repeated administration of alpha-chloralose is uncertain. This study evaluated the electrocardiographic, hematologic, and histologic effects of repeated alpha-chloralose anesthesia in dogs. Six dogs were given 1.5 mg/kg of IV morphine sulfate as pre-anesthetic medication and then anesthetized with 100 mg/kg of IV alpha-chloralose as slow bolus injection, followed by maintenance infusion of 70 mg/kg/hour for 3 hours. This anesthetic sequence was performed 3 times, with 48 hours in between each anesthetic period. The RR interval, PQ, QRS, QT, and QTc-$_{\text{Frederichia}}$ (QT/RR⅓) durations were determined. Hematologic measurements were obtained before the first and last episodes of anesthesia. Following the last anesthetic episode, dogs were euthanatized and major organs removed and examined for gross and histopathologic lesions. The QT interval lengthened significantly by 35 (±8) ms between baseline measurements and 3-hour post-anesthetic recordings when data from all 3 anesthetic episodes were combined ($P < 0.0001$); however, differences in the QT interval on individual days between baseline and 3-hour measurements were not statistically significant. The RR interval also lengthened between baseline and 3-hour recordings across days ($P < 0.0001$). This finding was also true for the anesthetic episode of Day 1 ($P = 0.03$) alone, but differences in the RR interval were not statistically significant on Days 2 or 3 examined individually. No histologic, hematologic, or blood biochemical changes were found between baseline and terminal measurements of these parameters.

Mean duration of each anesthetic episode was 180 (±20) minutes, and mean duration from cessation of anesthesia to coordinated walking was 119 (±36) minutes. Recovery from each anesthetic episode was uneventful. Morphine-chloralose appears to produce safe and effective anesthesia when used repeatedly.

INTRODUCTION

Alpha-chloralose is recommended for use in non-survival experiments requiring prolonged anesthesia with minimal surgical intervention.[3,5] In particular, it has been popularized for its lack of baroreceptor depression, that is, alpha-chloralose produces a stable, "physiologically awake animal" that is immobilized and unconscious. One study suggested that alpha-chloralose anesthesia may be inadequate for use during major and/or minor painful surgical procedures, precluding its use as the sole anesthetic agent under those circumstances.[10]

Controversially, other investigators performed thoracotomies to chronically instrument dogs using alpha-chloralose as the sole anesthetic agent, without the use of any preanesthetic.[2] In this study, alpha-chloralose did not affect heart rate, PQ interval, or the response to isoproterenol or atropine.[2] In contrast, pentobarbital was found to increase heart rate, prevent second-degree AV block at all pacing rates, not affect the response to isoproterenol, and blunt effects of atropine[2] The study, however, did not evaluate the safety of repeated alpha-chloralose usage.[2]

There is disagreement in the literature about whether alpha-chloralose is a true anesthetic agent or a hypnotic with little analgesic action.[1] Morphine-chloralose is used primarily for physiologic studies to preserve the vagal and central baroreceptor reflexes, or in acute cardiovascular studies to preserve myocardial function for greater at least 3 hours after induction.[6] While alpha-chloralose is generally considered to have no application in survival studies or in clinical veterinary medicine,[8] alpha-chloralose, after thiopental induction, has been used to anesthetize dogs 10 times between 80 and 300 days of age.[4] The investigators in that study observed no seizures, and although no physiologic parameters were measured and no histopathologic examinations were conducted, the dogs in that study grew normally (within the 95% confidence interval for normal dogs).[4]

Other investigators have conducted studies to measure hemodynamics and to identify an anesthetic regimen that does not significantly influence cardiovascular physiology.[6] The hemodynamics of dogs anesthetized with morphine-chloralose most closely resembled those of conscious animals when compared to dogs that received fentanyl citrate and/or no anesthesia (ie, trained conscious preparation).[6]

The uniform transmural distribution of phosphogens is also apparently sustained with alpha-chloralose anesthesia, but not with pentobarbital,[9] and anesthesia with alpha-chloralose changed ventricular performance in dogs less than anesthesia with sodium pentobarbital.[11] The scientific literature supports the contention that anesthesia with alpha-chloralose is superior to other anesthetic agents with regard to maintaining normal autonomic, physiologic, and biochemical responses, but alpha-chloralose is rarely cited in the literature as being used repeatedly in mature dogs.[2,4]

The purpose of this study was to examine the effects of 3 episodes of anesthesia with morphine-chloralose on the electrocardiographic (ECG) and hematologic parameters, and gross and histopathologic organ appearance in normal adult dogs. Additionally, we observed the nature and time of anesthetic recovery from repeated anesthetic episodes using this regimen.

MATERIALS AND METHODS

This study used 6 hound-type, unconditioned male dogs (Columbus, Ohio, USA) between 3 and 5 years of age and averaging 20 kg in weight. This study was conducted under the protocol number 2003A0153, which was approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

Dogs were pre-anesthetized with morphine sulfate, 1.5 mg/kg given IV. Fifteen minutes later, they were anesthetized with alpha-chloralose, 100 mg/kg, and anesthesia was maintained with a constant infusion of 70 mg/kg/hour of alpha-chloralose for 3 hours. Dogs were intubated and given intermittent positive pressure ventilation (Harvard Apparatus Co., Inc. Millis, Massachusetts, USA) with room air at 12 breaths/

minute with a tidal volume of 15 mL/kg, such that end-tidal PCO2 (Airway Monitor, Datex, Ohmeda, INC., Madison, Wisconsin, USA) was kept between 35 and 45 mmHg. This procedure was performed in each dog on 3 different days with 48 hours in between anesthetic episodes. Mean times between cessation of anesthesia and the time of the first spontaneous breath, as well as the time between the first spontaneous breathing and the first voluntary motion, the time from first voluntary motion until the first staggering gait, and from staggering gait to coordinated walking were all recorded to the nearest minute. The total time from cessation of anesthesia to coordinated walking was the sum of those durations. Recovery time from anesthesia was obtained only twice for each dog, since the dogs were euthanatized before they awakened from the third anesthetic episode. After each 3-hour period of anesthesia, dogs were kept on a heating pad, the ventilator was stopped, and if the dog did not breath spontaneously within 1 minute, positive pressure ventilation was reinstituted.

Lead I and II ECGs were obtained 15 minutes after onset of the maintenance infusion of alpha-chloralose. This is termed the baseline value and each day (Days 1, 2, and 3) of anesthesia was called an anesthetic episode. The RR intervals (the interbeat interval between consecutive R waves on the ECG) and durations of P waves, QRS complexes, QT interval, and corrected QT intervals ($QTc\text{-}_{Frederichia}$) were measured as the average of 6 consecutive cardiac cycles chosen randomly during 60 seconds of recording. The QT intervals were corrected using the Fredericia formula, which is expressed by the QT interval divided by the cubed root of the RR interval ($QT/RR^{1/3}$). Venous blood samples (Table 1) were obtained before the first anesthetic episode, and before the last episode of anesthesia. After the last anesthetic episode, dogs were euthanatized while still fully anesthetized, and examined at necropsy to identify any grossly apparent lesions. Samples were taken from the following organs for histologic examination (Table 2): kidney (right and left), adrenal gland (right and left), lung, heart (interventricular septum [IVS], left ventricular free wall [LVFW], left and right atrium [LA and RA, respectively]), liver, spleen, stomach wall, duodenum, pancreas, and brain. All organ samples were removed and fixed in 10% neutral buffered formalin. Histologic sections were cut at 5 µm thicknesses, stained with hematoxylin and eosin, and analyzed by light microscopy.

Statistical Analysis

The RR interval (the reciprocal of heart rate), PQ interval, QRS duration, QT, and $QTc\text{-}_{Frederichia}$ ($QT/RR^{1/3}$) were measured for 6 consecutive cardiac cycles taken during baseline (ie, approximately 15 minutes after induction when dogs achieved an apparent steady state) and 3 hours after the baseline on each of 3 days. Each parameter (RR, PQ, QRS, QT) was measured in 6 different cardiac cycles. The mean of the 6 measurements for all parameters at these 2 time points on Days 1, 2, and 3 were compared by analyzing the data with a repeated measures model, using PROC MIXED in SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA).[7] A separate model was built for each parameter. Time (baseline and 3 hours) and day (Days 1, 2, and 3) were included as covariates and dog was treated as a random effect to account for the correlated nature of the data. Interaction terms between time and day were also included in the models if either of the covariates was significant ($P < 0.05$) as a main effect. In such a case, pairwise comparisons of time-day combinations were also evaluated, using the Tukey-Kramer adjustment for the multiple comparisons.

Values of hematologic parameters (Table 1) obtained before the first and before the last anesthetic episodes were compared by paired Student's t-test requiring a P-value < 0.05 for significance.

RESULTS

ECG: Figure 1 shows a lead II ECG from 6 dogs at the baseline and 3-hour recordings for each anesthetic episode (Days 1, 2, and 3). Although T-waves from dogs named HO,

Table 1: Hematology values for first and last day of anesthesia with morphine-chloralose. Descriptive statistics (mean ± SD) on blood chemistry and hematology are from 6 dogs before the first and before the third anesthetic episodes. The parameters did not differ significantly between the 2 time points.

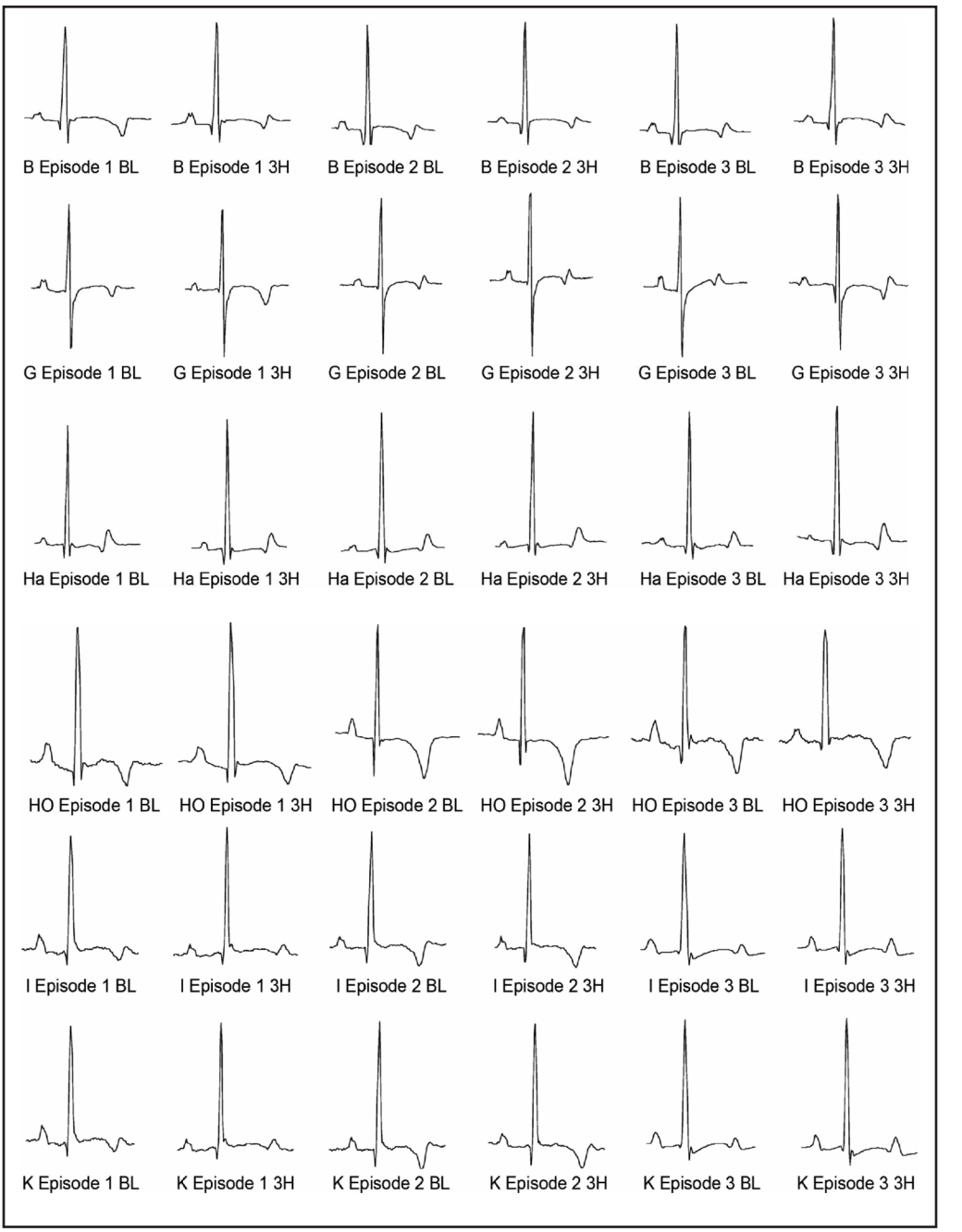
Chemistry	Baseline	SD	Last Day	SD
Urea/nitrogen	16.6	2.4	16.3	4.5
Creatinine	1.1	0.1	1.0	0.2
Phosphorus	5.2	0.5	4.9	0.4
Calcium	11.0	0.2	34.1	1.7
Na	148.8	2.3	125.5	59.1
K	4.4	0.4	22.7	44.2
Cl	112.4	2.2	98.0	37.5
Anion gap	20.4	2.6	77.3	3.4
Serum osmolarity	296.8	4.4	242.0	124.8
Bicarbonate	20.4	2.4	42.6	3.8
ALT	58.0	60.7	35.2	5.6
AST	43.4	46.5	29.2	26.4
ALK phosphatase	44.8	15.5	41.0	11.4
ALP/CAP	2.2	1.0	2.7	1.2
CK	115.2	48.4	144.0	75.4
Cholesterol	173.6	20.6	179.0	17.0
Bilirubin total	4.1	8.0	0.1	0.0
Total protein	7.0	0.8	6.8	0.7
Albumin	3.5	0.3	3.5	0.5
Globulins	3.5	1.1	3.3	0.9
Albumin/globulin ratio	1.1	0.4	1.1	0.4
Glucose	91.4	9.2	92.5	16.4
Lipemic index	9.2	4.0	6.2	9.5
Hemolytic index	26.2	39.5	12.0	20.7
Icteric index	0.0	0.0	0.0	0.0
Blood count				
Plasma protein	7.8	0.6	7.5	0.4
Packed cell volume	43.6	1.9	46.9	6.6
Hemoglobin	15.1	1.0	16.0	2.3
Red blood cells	6.7	0.1	7.1	0.8
MCV	65.4	3.4	66.4	3.9
MCHC	34.5	1.0	34.0	0.7
RDW	16.3	0.8	15.4	1.4
Nucleated cells	9.4	2.4	8.2	2.3
Seg neutrophils	7.8	1.3	7.5	1.1
Lymphocytes	43.6	0.7	46.9	1.3
Monocytes	15.1	2.2	16.0	0.2
Eosinophils	6.7	0.7	7.1	0.7
Leukocyte morphology (reactive lymphocytes)				
Platelet count	251000.0	77116.5	197000.0	38661.4
Platelet evaluation	adequate		adequate	

Table 2: Histopathology.

Organs	Dog ID					
	B	G	HA	HO	I	K
Kidneys (right and left)	Multifocal, minimal, subacute lymphoplasmacytic interstitial nephritis[1]	NSF	Multifocal, mild to moderate, subacute lymphoplasmacytic interstitial nephritis[1]	NSF	NSF	NSF
Adrenal gland. (right and left)	NSF	NSF	NSF	NSF	NSF	NSF
Lungs	Multifocal, mild, chronic lymphohystiocytic peribronchitis[1]	Multifocal, mild lymphoplasmacytic interstitial aggregates[1]	NSF	NSF	NSF	NSF
Heart (IVS, LVFW, LA, RA)	NSF	NSF	IVS: Focal eosinophilic and histiocytic myocarditis with eosinophilic abscess formation LVFW: Multifocal, minimal lymphoplasmacytic, histiocytic, and eosinophilic interstitial myocarditis[4]	NSF	NSF	NSF
Liver	Moderate widespread congestion[3]	Focal chronic abscess, minimal to mild lymphoplasmacytic portal hepatitis[2]	Multifocal, minimal plasmacytic and eosinophilic portal hepatitis[2]	NSF	Widespread congestion[3]	NSF
Spleen	NSF	Mild lymphoid depletion	NSF	NSF	NSF	NSF
Stomach wall	Widespread, mild to moderate eosinophilic, lymphocytic gastritis[5]	NSF	NSF	NSF	Widespread, mild eosinophilic and lymphocytic gastritis[5]	NSF
Duodenum	NSF	NSF	NSF	NSF	NSF	NSF
Pancreas	NSF	Multifocal, mild to moderate eosinophilic and lymphoplasmacytic pancreatitis[6]	NSF	NSF	NSF	NSF
Brain	NSF	NSF	NSF	NSF	NSF	NSF

NSF = no significant findings; IVS = interventricular septum; LVFW = left ventricular free wall; LA = left atrium; RA = right atrium; 1 = Non-specific inflammation related to local antigen expression, cause not apparent; 2 = Related to past parasite migration, specific cause not apparent. 3 = Postmortem change, no significance; 4 = May be related to local bacterial or parasite infection, cause not apparent; 5 = Non-specific inflammation, may be related to local allergens, cause not apparent; 6 = May be local bacterial or parasite infection, cause not apparent.

Figure 1: Electrocardiographic morphology. Lead II ECGs from all dogs taken before and during each of 3 episodes of anesthesia with morphine-chloralose.



I, and K became large and negative after episode 2, they returned to normal by episode 3, and no similar changes were observed in the other 3 dogs. As seen on Table 3, there were no significant differences in PQ intervals identified across days (Days 1, 2, and 3; $P > 0.05$) or between baseline and the 3-hour recording after the onset of anesthesia ($P > 0.05$) for all days taken together. There were no significant differences in the duration of QRS complex across days (Days 1, 2, and 3; $P > 0.05$) or between baseline

and after 3 hours of anesthesia ($P > 0.05$) for all days taken together. The QT duration did not differ between days ($P > 0.05$); however, the QT interval lengthened significantly (by 35 ms) between baseline and the 3-hour recording when all days were taken together ($P < 0.0001$). When considering the pairwise comparisons between individual days, the QT difference (40 ms) was significant only on Day 2 ($P = 0.041$); however, the RR interval lengthened significantly from baseline to 3-hour recording on Day 1 ($P = 0.01$) and on Day 3 ($P = 0.005$), but not on Day 2 ($P = 0.313$). The $QTc\text{-}_{Frederichia}$ did not differ between days ($P > 0.05$); however, when data from all days were considered, it lengthened 13 (±4) ms between baseline and 3 hours ($P = 0.017$).

Hematology: Table 3 showing biochemical and cellular constituents of blood obtained immediately prior to the first episode of anesthesia and 48 hours after the second or just before the third episode. There are no differences of statistical and clinical significance in any parameter.

Gross and histologic pathology: In all tissues examined (Table 2) there was no evidence of peracute or acute gross and/or histologic pathology associated with the morphine-chloralose anesthetic. Four of 6 dogs used in the study did have evidence of minimal to mild subacute to chronic inflammatory disease in various organs. Subject B had minimal to mild interstitial infiltrates of lymphocytes and plasma cells within the kidney and lung sections, with an additional eosinophilic component in the stomach. Infiltrates of a similar nature and severity were seen in dog G, this time in the lung, liver, and pancreas (the latter also containing eosinophils), and there was also a focal chronic microabscess in the liver. Dog named HA had histiocytic, lymphoplasmacytic and eosinophilic interstitial infiltrates in the myocardium (IVS and LVFW), with focal eosinophilic microabscess formation in the IVS, as well as a minimal plasmacytic and eosinophilic portal hepatitis. Finally, dog named I had a widespread, mild eosinophilic and lymphocytic gastritis. In all of these subjects, there was no evidence of active bacterial or parasitic infectious disease, nor of biochemical alterations or clinical illness associated with the specific tissue lesions described above.

Recovery: All dogs recovered from the initial 2 anesthetic episodes. Mean duration from cessation of anesthesia to coordinated walking was 119 (with standard deviation of 36) minutes. The durations from cessation of anesthesia to spontaneous ventilation, from spontaneous ventilation to first voluntary motion, from first voluntary motion to staggering gait, and from staggering gait to coordinated walking were 17 (±11), 19 (±12), 26 (±11), and 57 (±13) minutes, respectively. All dogs defecated sometime between the first voluntary motions to the staggering gait. Recoveries were without seizures, and no dog manifested violent behavior.

DISCUSSION AND CONCLUSIONS

We report that 3 episodes of anesthesia with morphine-chloralose did not produce detectable changes in ECGs, blood biochemistry and cellularity, and histopathology, and that dogs recovered from anesthesia without violence or injury. Morphine (1.5 mg/kg) and chloralose (100 mg/kg induction and 70 mg/kg/hour maintenance) can be used to produce desired immobilization and unconsciousness required for studying the autonomic and cardiovascular physiology at least 3 times without causing significant changes in these parameters, and all dogs maintained sinus rhythm during all episodes. The venous blood was analyzed 2 times, once before the first and once before the third anesthetic episode. The rationale for this timing is that the alpha-chloralose is only soluble in a large amount of warm saline, which produces temporary hemodilution, thus distorting the blood biochemistry and cellularity. This distortion (hemodilution) was avoided by sampling before the first and last anesthetic episodes.

There were a number of apparent histologic changes, some of which may be explained by the fact these dogs were uncon-

Table 3: Descriptive statistics (mean ± SD) for different ECG parameters measured at baseline and 3 hours of recording for 3 episodes (Days 1, 2, and 3).

	RR Interval (ms)		Difference From 3H to BL	PQ Interval (ms)		Difference From 3H to BL	QRS Duration (ms)		Difference From 3H to BL	QT Interval (ms)		Difference From 3H to BL	QTc-Frederichia (ms)		Difference From 3H to BL
	BL	3 HR	3H - BL	BL	3H	3H - BL	BL	3H	3H - BL	BL	3H	3H - BL	BL	3H	3H - BL
Episode 1	1144* (± 244)*	1347* (± 267)	203	147 (± 17)	168 (± 29)	21	78 (± 10)	80 (± 7)	80 (± 7)	326 (± 29)	352 (± 14)	26	313 (± 24)	322 (± 21)	9
Episode 2	1213 (± 165)	1478 (± 150)	265	152 (± 16)	154 (± 18)	2	83 (± 9)	76 (± 7)	-7	320† (± 8)	360† (± 22)	40	301 (± 19)	318 (± 26)	17
Episode 3	1109† (± 121)	1403† (± 306)	294	140 (± 21)	153 (± 30)	13	79 (± 8)	81 (± 9)	2	313 (± 18)	351 (± 39)	38	304 (± 21)	317 (± 30)	13
All episodes	1155 (± 53)	1,409 (± 66)	254 (± 46)	146 (± 6)	158 (± 8)	12 (± 10)	80 (± 3)	79 (± 3)	-1 (± 5)	320‡ (± 7)	354‡ (± 5)	35 (± 8)	306† (± 6)	319† (± 3)	13 (± 4)

BL= baseline; 3 HR= 3 hours of recording; ms= milliseconds.
The measurements did not differ between different days for any of the parameters.
*$P < 0.01$; †$P < 0.05$; ‡$P < 0.001$ between baseline and 3-hours recordings is based on the repeated measures models.

ditioned. They were underweight, and may have been parasitized prior to entering the University's research colony for this study. The histologic changes observed may have been attributable to parasitism. While the use of unconditioned dogs reduces the uniformity of the results, it may more closely approximate the variability seen in a clinical setting and may also optimize the chance of observing toxicity in response to an anesthetic regimen. Unfortunately, similar unconditioned dogs that did not undergo morphine-chloralose anesthetic episodes were not available to serve as controls for the pathologic evaluations.

From the ECG, the RR interval (the reciprocal of heart rate) lengthened slightly (approximately 7 beats/minute) between baseline and 3 hours during Days 1 and 3, but not during Day 2; overall there was no physiologically significant alteration in chronotropy attributable to the anesthetic regimen. When comparing the QT interval during baseline with that at 3 hours, there was a slight lengthening on each of the 3 days. When comparing $QTc_{Frederichia}$ between baseline and 3 hours for each of the 3 days, there was no change; however, when taking all 3 days together, $QTc_{Frederichia}$ lengthened trivially 13 (±4) ms. This lengthening achieved statistical significance, likely because of a larger sample size, with data from all 3

days combined, thus it is unlikely to be physiologically significant. The lengthening of the QT interval may be attributed in part to a reduction in heart rate (lengthening of RR interval), or to a direct action on ion channels specific for ventricular repolarization (eg, IKR, IKS, IKTO, ICa). The lengthenings of QT and $QTc_{Frederichia}$ were small and heart rate slowed slightly, therefore it is unlikely that morphine-chloralose anesthesia would be torsadogenic or would be permissive for other test articles being torsadogenic unless it amplified a QT-lengthening effect of another drug.

It appears from this study that at least 3 episodes of anesthesia should be safe and should present no issues of torsadogenicity, suggesting that dogs anesthetized with alpha-chloralose may be used for studies to evaluate torsadogenicity of test articles.[12,13] However, the combination of various test articles and chloralose has not been studied yet.

We did not explore possible adverse effects that might have evolved over subsequent weeks (eg, genetic alterations), or that ultramicroscopic (eg, mitochondrial swelling) or physicochemical lesions (eg, alterations in ion channels) might have occurred. Furthermore, we do not know if the anesthetic, singly or repeatedly, might alter the physiologic responses to drugs or to disease. That was not the purpose of this study. It must be verified that results of any investigation of a pharmacologic or pathologic process that utilizes this anesthetic regimen would not be altered by the anesthetic. A previous study in which puppies were anesthetized repeatedly with morphine chloralose demonstrated normal growth, but there were no reports of reproductive properties. Thus morphine-chloralose anesthesia appears to be a safe and effective anesthetic regimen for repeated anesthesia required for less than major surgical interventions.

ACKNOWLEDGEMENTS

Thanks to Dr. William W. Muir, III for assistance with interpretation of these findings. Work was conducted at the Cardiovascular and Physiology Laboratory in the Veterinary Hospital building. DNQC, BK, and RLH conceived and designed the experiments. MB performed histopathology. DNQC, YN, and YO performed the experiments. DNQC, RLH, and PRS analyzed the data. DNQC, BK, PRS, and RLH wrote the paper.

REFERENCES

1. Croft P. Actions and dosage of chloralose. *Nature*. 1964;203:1086.
2. Duchene-Marullaz P, Fabry-Delaigue R, Gueorguiev G, Kantelip JP. Influence of chloralose and pentobarbitone sodium on atrioventricular conduction in dogs. *Br J Pharmacol*. 1982;77:309-317.
3. Fleckenell P. *Laboratory Animal Anesthesia*. London, England: Harcourt Brace Jovanovick; 1987:135-137.
4. Grad R. Witten M, Quan S. Intravenous chloralose is a safe anesthetic for longitudinal use in beagle puppies. *Lab Anim Sci*. 1988;38:422-425.
5. Holzgrefe H, Everitt J, Wright E. Alpha-chloralose as a canine anesthetic. *Lab Anim Sci*. 1987;37:587-595.
6. Lang RM, Marcus R H, Neuman A, et al. A time-course study of the effects of pentobarbital, fentanyl, and morphine chloralose on myocardial mechanics. *J Appl Physiol*. 1992;73:143-150.
7. VandeBerg JL. Genetics of nonhuman primates. In: Bennett BT, Abee CR, Henrickson R, eds. *Nonhuman Primates in Biomedical Research, Biology and Management*. San Diego: Academic Press, Inc.; 1995:129-146.
8. Littell RC, Milliken GA, Stroup WW, Wolfinger RD. *SAS System for Mixed Models*. Cary, NC: SAS Institute. Inc.; 1996:633.
9. Lumb W, Jones E. *Veterinary Anesthesia*. Philadelphia, PA: Lea & Febiger; 1984:315-325.
10. Rath DP, Little CH, Zhang H, et al. Sodium pentobarbital versus alpha-chloralose anesthesia – experimental production of substantially different slopes in the transmural CP/ATP ratios within the left ventricle of canine myocardium. *Circulation*. 1995;91:471-475.
11. Silverman J, Muir WW III. Special topic Overview. A review of laboratory animal anesthesia with chloral hydrate and chloralose. *Lab Anim Sci*. 1993;43:210-216.
12. Van Citters RL, Franklin DL, Rusmer RF. Left ventricular dynamics in dogs during anesthesia with alpha-chloralose and sodium pentobarbital. *Am J Cardiol*. 1964;13:349-354.
13. Hamlin RL, Cruze CA, Mittelstadt SW, et al. Sensitivity and specificity of isolated perfused guinea pig heart to test for drug-induced lengthening of QTc. *J Pharmacol Toxicol Methods*. 2004;49(1):15-23.
14. Hamlin RL, Kijtawornrat A, Keene BW, Hamlin DM. QT and RR intervals in conscious and anesthetized guinea pigs with highly varying RR intervals and given QTc-lengthening test articles. *Toxicol Sci*. 2003;76(2):437-442.