Comparison of high (5%) and low (1%) concentrations of micellar microemulsion propofol formulations with a standard (1%) lipid emulsion in horses

Pedro Boscan, DVM, PhD; Eugene P. Steffey, VMD, PhD; Thomas B. Farver, PhD; Khursheed R. Mama, DVM; Nick J. Huang, BSc; Steven B. Harris, MD

Objective—To compare anesthesia-related events associated with IV administration of 2 novel micellar microemulsion preparations (1% and 5%) and a commercially available formulation (1%) of propofol in horses.

Animals—9 healthy horses.

Procedures—On 3 occasions, each horse was anesthetized with 1 of the 3 propofol formulations (1% or 5% microemulsion or 1% commercial preparation). All horses received xylazine (1 mg/kg, IV), and anesthesia was induced with propofol (2 mg/kg, IV). Induction and recovery events were quantitatively and qualitatively assessed. Venous blood samples were obtained before and at intervals following anesthesia for quantification of clinicopathologic variables.

Results—Compared with the commercial formulation, the quality of anesthesia induction in horses was slightly better with the micellar microemulsion formulas. In contrast, recovery characteristics were qualitatively and quantitatively indistinguishable among treatment groups (eg, time to stand after anesthesia was 34.3 ± 7.3 minutes, 34.1 ± 8.8 minutes, and 39.0 ± 7.6 minutes in horses treated with the commercial formulation, 1% microemulsion, and 5% microemulsion, respectively). During recovery from anesthesia, all horses stood on the first attempt and walked within 5 minutes of standing. No clinically relevant changes in hematologic and serum biochemical analytes were detected during a 3-day period following anesthesia.

Conclusions and Clinical Relevance—Results suggest that the micellar microemulsion preparation of propofol (1% or 5%) has similar anesthetic effects in horses, compared with the commercially available lipid propofol formulation. Additionally, the micellar microemulsion preparation is anticipated to have comparatively low production costs and can be manufactured in various concentrations. (*Am J Vet Res* 2006;67:1476–1483)

Abbreviations

MMF	Micellar microemulsion formulation
PGH	Polyethylene glycol-15-hydroxystearate

Propofol (2,6-diisopropylphenol) is an anesthetic agent that is administered IV and is widely used for both induction and maintenance of anesthesia in human and small animal patients.[1–3] Despite the facts that propofol has a rapid onset of action and is metabolized rapidly, and that awakening from propofol-associated anesthesia is also rapid, the use of this agent in large animals such as horses is rare. The high purchase cost and low concentration (1%, which necessitates administration of high-volume injections) of commercially available formulations are major factors that limit current use of the drug in horses. A new MMF of propofol is presently under development. The MMF of propofol is optically clear (containing 12-nm-diameter micelles) and is being developed to provide a marketable high-concentration product at what is expected to be reduced production cost, compared with the commercially available product. In our laboratory, it has been determined that the MMF has increased resistance to microbial growth. By use of the new formulation procedure, propofol can be provided in preparations at concentrations that are considerably greater than the concentrations in commercially available preparations. The purpose of the study reported here was to compare anesthesia-related events associated with IV administration of 2 novel MMFs (1% and 5%) and a commercially available formulation (1%) of propofol in horses.

Materials and Methods

Propofol preparations—A commercially available 1% formulation of propofol[a] for injection was obtained from the general stock of the Veterinary Medical Teaching Hospital of the University of California, Davis (originally purchased from the hospital's usual commercial sources).

The propofol MMFs (1% and 5%)[b] for injection were produced by 2 of the authors (NJH and SBH). These transparent liquid preparations are micellar solutions or

Received October 25, 2005.
Accepted March 10, 2006.
From the Department of Surgical and Radiological Sciences and Veterinary Medical Teaching Hospital (Boscan, Steffey) and Department of Population Health and Reproduction (Farver), School of Veterinary Medicine, University of California, Davis, CA 95616; the Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 (Mama); and MEDDS (Micro Emulsion Drug Delivery Systems) Inc, Civic Center Dr, Rancho Cucamonga, CA 91730 (Huang, Harris).
Partial financial support and supplies of propofol microemulsions were provided by MEDDS Incorporated.
Presented as a poster abstract at the Fall Meeting of the Association of Veterinary Anaesthetists, Vienna, Austria, September 2004.
The authors thank Don Hermes, Chris Scarlett, and Doug West for technical assistance.
Address correspondence to Dr. Boscan.

microemulsions of propofol in physiologic saline (0.9% NaCl) solution stabilized with a nonionic parenteral pharmaceutical emulsifier (polyethylene glycol-600 15-hydroxystearate or PGH[c]). The 1% propofol MMF was prepared (according to patent number WO 2005/079758) by mixing 1 part of propofol (2,6 diisopropylphenol[d]) with 10 parts (wt/wt) of molten PGH and then adding 89 parts of physiologic saline solution, resulting in a 1% (wt/vol) propofol-in-saline solution mixture. The mixture was stirred at 50°C for a few minutes. Once the clear mixture was formed, it was cooled to room temperature (approx 20°C), filtered through a conventional 0.2-µm syringe filter into a sterile 250-mL IV solution bag, and refrigerated at 5°C; the preparation was used within 6 weeks. The preparation can be stored either in an anhydrous base form before adding saline solution or following dilution with saline solution. The 5% propofol MMF was prepared by mixing 25 parts of molten PGH, 5 parts (wt/wt) of propofol (2,6 diisopropylphenol[d]), 5 parts (wt/wt) of 95% ethanol (USP), and 2.5 parts (wt/wt) of ethyl oleate[e] to make a liquid emulsion base. The 5% injectable MMF preparation was formed when 62.5 parts (by weight) of 50°C physiologic saline solution was added to this base, resulting in a 5% (wt/wt) propofol-in-saline solution mixture. Once the microemulsion had cooled to room temperature and cleared, it was filtered and stored as mentioned above and used within 6 weeks.

Horses and drug administration protocol—After approval was obtained from the Animal Care and Use Committee of the University of California, Davis, 9 healthy university-owned horses were used in the study. There were 5 geldings and 4 mares; mean ± SD age was 10.6 ± 1.0 years (range, 6 to 14 years), and mean weight was 563 ± 50 kg (range, 512 to 622 kg). The group of horses consisted of 7 Thoroughbreds, 1 warmblood, and 1 Quarter Horse, and none of the horses had been anesthetized within at least the previous 3 years.

In a crossover study, all 9 horses were anesthetized with each of the 3 drug formulations on 3 separate occasions (ie, each horse was anesthetized with 1% commercial propofol,[a] 1% propofol MMF,[b] and 5% propofol MMF[b]); administration of each of the formulations was separated by at least 14 days. Food (but not water) was withheld for approximately 12 hours prior to each anesthetic exposure. Xylazine[f] (1.0 mg/kg) was administered IV to each horse 5 minutes before anesthetic induction with propofol (2.0 mg/kg, IV); the propofol formulation was administered by continuous hand injection over a period of 45 seconds. The drug doses were selected on the basis of results of a previous study[4] performed in our laboratory. The formulation of propofol to be used each time was determined at the beginning of the study by use of a randomized scheme (developed by one of the investigators [TBF]).

At least 1 hour prior to anesthesia, a 14-gauge, 13-cm catheter[g] was placed in an external jugular vein, and the horse was weighed. Following premedication, the horse was walked into a padded recovery stall for induction of anesthesia. For anesthetic induction, the horse's head was supported by an experienced person using a long rope, one end of which was attached to the horse's halter and the other passed through a ring embedded in the wall of the recovery stall above the level of the horse's head. Following propofol injection, the horse was allowed to move from standing to a recumbent position with minimal or no assistance beyond restraint provided by the head rope. Following recumbency, the horse's halter was removed, and the horse was observed but not assisted until it was standing. Timing and quality of induction of and recovery from anesthesia were always evaluated directly by 2 of the investigators (PB and EPS) who for safety reasons were not blinded to the drug formulation administered. For each horse, the induction of and recovery from anesthesia were recorded on videotape. After the recordings were digitized and identifying marks removed, compact disc records of the original tapes were reviewed by 2 investigators (1 unaware [KRM] and 1 aware [EPS] of the drug formulation administered) and 2 other individuals who were experienced in equine behavior and anesthetic recovery assessment and were unaware of the drug formulation administered.

For each horse, the time to first anesthetic effect, time to lateral recumbency, and observations regarding the quality of induction of anesthesia were recorded. During recovery from anesthesia, the time to movements of an ear, head, and a limb; lifting the head; first attempt to move to a sternal posture; attaining and remaining in sternal recumbency; and standing, and number of attempts to gain sternal recumbency and standing were recorded. In addition, rectal temperature, pulse rate, respiratory rate, and results of a CBC and serum biochemical analyses were also evaluated 20 minutes before and 24 hours after induction of anesthesia. A blood sample was also obtained from all horses for serum biochemical analyses 3 days after the second and third anesthetic episodes. Subjective quality assessments for behaviors during induction of and recovery from anesthesia were provided by the same aforementioned 4 observers by use of a previously described numeric scale of 1 (worst) to 5 (best).[4]

Statistical analysis—Results are grouped and expressed as mean ± SD values except the subjectively derived anesthesia induction and recovery scores, which are summarized as median. By use of computer software,[h] inferential analyses were performed involving a repeated-measures comparison with propofol formulation and order of use as factors. A Bonferroni pairwise multiple comparison test was used as indicated post hoc. Nonparametric data were analyzed by use of a Friedman repeated-measures ANOVA on ranks followed, when appropriate, by the Bonferroni nonparametric multiple comparison procedure. Significance level for all statistical tests was set at a value of $P < 0.05$.

Results

All horses developed a similar degree of sedation following administration of xylazine. At 2 to 4 minutes after the IV injection of xylazine, horses stood with head down in a wide-based stance and had reduced sensitivity or unresponsiveness to surroundings. Induction of anesthesia occurred within 1 to 2 minutes after injection of each of the 3 propofol formulations (**Table 1**).

Comparison of behavioral responses following propofol administrations—The time to first anesthetic effect following drug injection was 0.94 ± 0.19 minutes, 1.07 ± 0.15 minutes, and 1.16 ± 0.33 minutes for the commercially available 1% propofol formulation, 1% propofol MMF, and 5% propofol MMF, respectively. Administration of the commercially available propofol formulation was associated with a slightly faster onset of action, compared with the propofol MMFs (Table 1). Time to lateral recumbency was not significantly different among groups. The anesthesia induction–related behavior characteristics varied among the 9 horses. Some horses had a smooth induction of anesthesia and slow, easy transition from standing to lateral recumbency. After becoming recumbent, horses laid quietly on the recovery stall floor until entering the recovery phase. Other horses also made an easy transition from standing to recumbency but then stiffened or began to paddle

with 1 or more limbs. The latter behavior was evident in 5 of the 9 horses; there were 12 instances of this behavior (nearly equally distributed among administrations of the 3 drug formulations; horses affected in this manner by 1 formulation were typically similarly affected by the other formulations). The paddling appeared as coordinated running of 5- to 20-seconds' duration. A difference in anesthesia induction quality was detected between the commercially available propofol and each of the propofol MMFs; the quality appeared to be better following administration of either propofol MMF.

All horses lay quietly in lateral recumbency during anesthesia, except 1 horse during the anesthesia episode involving commercially available propofol; that horse did not complete the transition to lateral recumbency but remained in sternal position throughout the recumbency phase. During the anesthesia period, all horses breathed rhythmically and periodically blinked their eyelids. The first movement was nearly always a gentle ear, head, or limb movement. The first movement occurred at a similar time after administration of each propofol formulation (**Table 2**). Following the first movement, horses remained calm in lateral recumbency, moving ears, eyelids, limbs, and head, and began swallowing until they started to lift their head. Unlike events during spontaneous recovery from anesthesia in horses in clinical settings, there was no head slapping by the horses of this report. During the later phases of lateral recumbency, the horses generally seemed awake despite their lateral posture and often calmly looked around the recovery room. At 25 to 29 minutes after induction of anesthesia, horses attempted to attain sternal recumbency, which was usually not achieved during their first attempt. These attempts were coordinated and calm. With respect to sternal recumbency, there were no differences in the times to the first attempt, the number of attempts, or the time to actually achieve a sternal position among horses following treatment with the 3 propofol formulations. After remaining in a sternally recumbent posture, the horses slowly and in a coordinated manner stood on the first attempt. The times required for horses to achieve a standing position were also not different among propofol treatments. After administration of each propofol formulation, the quality of the standing event was judged as excellent, and the overall recovery of horses from anesthesia was considered very good to excellent.

Table 1—Responses of 9 clinically normal horses after administration of 3 formulations of propofol (2 mg/kg, IV) in a crossover study (14-day intervals between treatments).

Propofol preparation	Time to first anesthetic effect (min)	Time to lateral recumbency (min)	No. of horses paddling during anesthesia	Quality of induction of anesthesia*
Commercially available 1% product	0.94 ± 0.19†	1.49 ± 0.27	4	2.0 ± 1.4 (1.0)†
1% MMF	1.07 ± 0.15	1.55 ± 0.18	5	2.5 ± 1.4 (3.0)
5% MMF	1.16 ± 0.33	1.73 ± 0.50	3	2.8 ± 1.6 (3.0)

Data are expressed as mean ± SD values; the median is also given in parentheses for induction quality. There were no significant differences in any variable among the first, second, and third anesthetic episodes.
*Quality of the induction of anesthesia was evaluated by use of a 5-point scoring scale (from 1 [worst] to 5 [best]). †Within a column, value significantly ($P < 0.05$) less than the values for the 2 MMFs.

Table 2—Recovery responses of 9 clinically normal horses that were anesthetized via administration of 3 formulations of propofol (2 mg/kg, IV) in a crossover study (14-day intervals between treatments).

	Propofol preparation				Anesthetic episode			
Variable	Commercially available 1% product	1% MMF	5% MMF	*P* value	First	Second	Third	*P* value
Time to first movement (min)	15.6 ± 3.8	14.6 ± 4.4	17.3 ± 1.5	0.24	14.2 ± 4.3	17.2 ± 2.2	15.9 ± 3.5	0.14
Time to first ear movement (min)	15.7 ± 3.5	14.7 ± 4.4	17.9 ± 2.4	0.1	14.9 ± 4.8	17.3 ± 2.2	15.9 ± 3.5	0.11
Time to first head movement (min)	16.1 ± 8.2	16.8 ± 4.1	19.4 ± 3.1	0.4	14.6 ± 4.7[a]	18.7 ± 1.9	20.7 ± 3.8[a]	0.003
Time to first head lift (min)	22.1 ± 9.7	24.5 ± 9.7	22.2 ± 5.1	0.8	17.4 ± 5.7[a]	24 ± 9.9	27.4 ± 5.6[a]	0.03
Time to first limb movement (min)	18.3 ± 7.4	19.7 ± 6.3	19.9 ± 3.6	0.83	15.2 ± 5.2[a,b]	20.8 ± 3.9[a]	21.9 ± 6.1[b]	0.03
Time to first attempt to attain sternal recumbency (min)	27.2 ± 8.7	28.3 ± 9.6	25.3 ± 4.5	0.5	22.8 ± 8.9	28.1 ± 8.6	29.4 ± 4.5	0.98
Time to attainment of sternal position (min)	32.4 ± 7.6	33 ± 8.7	34.6 ± 7.9	0.71	34.1 ± 8.9	33.1 ± 7.5	32.9 ± 8	0.91
No. of attempts to attain sternal recumbency	2.7 ± 2.5	3 ± 2.6	3.2 ± 1.9	0.77	4 ± 2.3[a]	2.8 ± 2.5	2.1 ± 1.8[a]	0.03
Time to standing (min)	34.3 ± 7.3	34.1 ± 8.8	39 ± 7.6	0.31	36.6 ± 9.1	35.7 ± 7.3	35 ± 8.2	0.91
Quality of standing (score)*	4.7 ± 0.8 (5)	4.8 ± 0.4 (5)	4.7 ± 0.6 (5)	0.69	4.6 ± 0.6 (5)	4.8 ± 0.4 (5)	4.8 ± 0.4 (5)	0.15
Quality of recovery from anesthesia (score)*	4.0 ± 0.9 (4)	4.1 ± 0.8 (4)	4.2 ± 0.8 (4)	0.67	4.0 ± 0.9 (4)	4.2 ± 0.8 (4)	4.1 ± 0.8 (4)	0.67

Data are expressed as mean ± SD values; the median is also given in parentheses for quality data.
*Qualities of standing and recovery from anesthesia were evaluated by use of a 5-point scoring scale (from 1 [worst] to 5 [best]).
[a,b]Within a row, values for the first, second, and third anesthetic episodes with the same superscript letters are significantly ($P < 0.05$) different.

All horses were able to walk to their corral within 5 minutes after standing. The horses were alert, and their walk was coordinated or with only mild ataxia similar to that detected after administration of xylazine to otherwise unmedicated horses.

Effect of number of anesthetic episodes on behavioral responses—The induction data did not depend on the number of anesthetic episodes; there were no significant differences in time to first anesthetic effect, time to lateral recumbency, number of horses paddling during anesthesia, and quality of induction of anesthesia among the first, second, and third anesthetic episodes. Similarly, during recovery, the onset time for first movement and first ear movement did not differ among anesthetic episodes. However, times for head movement, limb movement, and head lifting progressively increased, whereas the

Table 3—Mean ± SD hematologic variables in 9 clinically normal horses before and 24 hours after anesthesia induced via administration of 3 formulations of propofol (2 mg/kg, IV) in a crossover study (14-day intervals between treatments).

	Propofol preparation					
	Commercially available 1% product		1% MMF		5% MMF	
Variable	Before anesthesia	After anesthesia	Before anesthesia	After anesthesia	Before anesthesia	After anesthesia
RBCs ($\times 10^6$ cells/μL)	9.3 ± 0.9	8.5 ± 0.6	9.2 ± 1.3	8.5 ± 0.5	9.3 ± 0.7	8.5 ± 0.7*
Hemoglobin (g/dL)	16.1 ± 1.8	14.5 ± 0.7	15.6 ± 1.9	14.5 ± 0.7	16 ± 1.1	14.5 ± 0.9*
Hct (%)	41.5 ± 4.4	37.7 ± 1.9	40.5 ± 4.8	37.6 ± 1.6	41.5 ± 2.7	37.7 ± 2.1*
WBCs ($\times 10^3$ cells/μL)	7.2 ± 1.7	7.1 ± 1.2	7.5 ± 2.1	6.7 ± 1.4	7.2 ± 1.6	6.6 ± 1
Neutrophils ($\times 10^3$ cells/μL)	4.8 ± 1.3	4.8 ± 1	4.9 ± 1.5	4.3 ± 1.1	4.8 ± 1.5	4.2 ± 0.8
Lymphocytes ($\times 10^3$ cells/μL)	2 ± 0.6	1.9 ± 0.5	2.1 ± 0.8	1.9 ± 0.7	2.1 ± 0.7	2 ± 0.6
Monocytes ($\times 10^3$ cells/μL)	0.3 ± 0.04	0.3 ± 0.05	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.08
Eosinophils ($\times 10^3$ cells/μL)	0.09 ± 0.06	0.09 ± 0.06	0.16 ± 0.26	0.2 ± 0.25	0.07 ± 0.05	0.08 ± 006
Basophils ($\times 10^3$ cells/μL)	0.02 ± 0.01	0.02± 0.01	0.02 ± 0.01	0.03 ± 0.04	0.02 ± 0.01	0.01 ± 0.01
Platelets ($\times 10^3$ platelets/μL)	127 ±16.5	129 ± 17.5	126 ± 20.6	128 ± 21.9	132 ± 26.3	123 ± 17.7

*Value significantly ($P < 0.05$) different from the value before anesthesia with this preparation.

Table 4—Mean ± SD serum biochemical variables in 9 clinically normal horses* before, 24 hours after, and 3 days after anesthesia was induced via administration of 3 formulations of propofol (2 mg/kg, IV) in a crossover study (14-day intervals between treatments).

	Propofol preparation								
	Commercially available 1% product			1% MMF			5% MMF		
Variable	Before anesthesia	1 day after anesthesia	3 days after anesthesia	Before anesthesia	1 day after anesthesia	3 days after anesthesia	Before anesthesia	1 day after anesthesia	3 days after anesthesia
Sodium (mmol/L)	137 ± 4	136 ± 5	134 ± 1	137 ± 3	136 ± 5	137 ± 3	138 ± 2	139 ± 2	135 ± 4
Potassium (mmol/L)	3.5 ± 0.3	3.5 ± 0.5	4 ± 0.1	3.6 ± 0.3	3.7 ± 0.5	3.8 ± 0.5	3.8 ± 0.2	3.7 ± 0.3	3.7 ± 0.5
Chloride (mmol/L)	97 ± 3	96 ± 3	94 ± 1	96 ± 3	97 ± 3	96 ± 3	98 ± 2	98 ± 2.2	94 ± 3.5
Calcium (mg/dL)	13 ± 1	12 ± 1	13 ± 1	13 ± 1	13 ± 1	13 ± 1	13 ± 1	13 ± 1	12 ± 1
Phosphate (mg/dL)	3.4 ± 0.8	2.8 ± 0.5	3.4 ± 0.6	3.5 ± 0.6	3 ± 0.4	3.4 ± 0.6	3.6 ± 0.8	3.2 ± 0.5	3.3 ± 0.3
Creatinine (mg/dL)	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1 ± 0.1
BUN (mg/dL)	19 ± 1	18 ± 2	20 ± 1	20 ± 1	19 ± 2	19 ± 1	20 ± 2	18 ± 1†	19 ± 2
Glucose (mg/dL)	91 ± 16	87 ± 14	92 ± 9	92 ± 13	97 ± 16	89 ± 12	90 ± 7	97 ± 18	91 ± 6.8
Total protein (g/dL)	7.0 ± 0.5	6.8 ± 0.4	6.5 ± 0.2	6.9 ± 0.2	6.8 ± 0.6	6.8 ± 0.4	6.9 ± 0.4	7.0 ± 0.4	6.5 ± 0.4
Albumin (g/dL)	3.1 ± 0.2	3.0 ± 0.2	2.9 ± 0.1	3.1 ± 0.2	3.0 ± 0.3	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	2.9 ± 0.1
Globulin (g/dL)	3.9 ± 0.3	3.8 ± 0.3	3.6 ± 0.2	3.8 ± 0.2	3.8 ± 0.4	3.8 ± 0.3	3.9 ± 0.4	3.9 ± 0.4	3.6 ± 0.4
Aspartate aminotransferase (U/L)	259 ± 48	268 ± 56	244±56	244 ± 24	254 ± 35	253 ± 24	252 ± 31	272 ± 35	224 ± 28
Creatine kinase (U/L)	233 ± 58	482 ± 589	218 ± 76	201 ± 41	281 ± 160	401 ± 327	202 ± 49	876 ± 1220	179±93
Alkaline phosphatase (U/L)	101 ± 21	102 ± 22	108 ± 15	100 ± 21	100 ± 21	105 ± 25	96 ± 12	99 ± 13	95 ± 20
γ-Glutamyltransferase (U/L)	11 ± 3	11 ± 4	12 ± 4	10 ± 3	11 ± 2	11 ± 2	11 ± 2	12 ± 2	11 ± 3
Total bilirubin (mg/dL)	2.9 ± 0.4	2.8 ± 0.4	2.1 ± 0.4	2.8 ± 0.5	3.0 ± 0.5	1.8 ± 0.6	2.6 ± 0.7	2.8 ± 0.8	1.9 ± 0.4

*At 3 days after anesthesia, data were obtained from 6 horses. †Value significantly ($P < 0.05$) different from the value before anesthesia with this preparation.

Table 5—Mean ± SD weight and values of physical variables in 9 clinically normal horses before and 24 hours after anesthesia was induced via administration of 3 formulations of propofol (2 mg/kg, IV) in a crossover study (14-day intervals between treatments).

	Propofol preparation					
	Commercially available 1% product		1% MMF		5% MMF	
Variable	Before anesthesia	After anesthesia	Before anesthesia	After anesthesia	Before anesthesia	After anesthesia
Heart rate (beats/min)	44 ± 6	40 ± 8	40 ± 3	42 ± 6	39 ± 4	41 ± 6
Respiratory rate (breath/min)	26 ± 5	24 ± 7	29 ± 8	20 ± 2	27 ± 6	20 ± 6
Rectal temperature (°C)	37.6 ± 0.4	37.6 ± 0.2	37.7 ± 0.5	37.7 ± 0.2	37.5 ± 0.4	37.6 ± 0.2
Weight (kg)	561 ± 51	NE	564 ± 50	NE	562 ± 46	NE

NE = Not evaluated.

number of attempts to attain a sternal position decreased with increasing anesthetic experience (Table 2). Thus, in the first and second anesthetic episodes, the horses moved earlier and made more attempts to achieve sternal recumbency during recovery; at the third anesthetic episode, the horses lay for a longer period without movement and made fewer attempts to attain a sternal position during recovery, compared with events after the first anesthetic episode. Nevertheless, multiple anesthetic episodes did not influence the times to attainment of sternal recumbency and standing, and the quality of standing was considered excellent in all horses for each of the 3 anesthetic events.

Results of CBCs—For each horse, a CBC was performed before premedication with xylazine, and another was performed the morning after anesthesia (24 hours after anesthesia; **Table 3**). Compared with the CBC findings before anesthesia, there were significant decreases in RBC count, hemoglobin content, and Hct in association with administration of the 5% propofol MMF only. However, these changes were not considered clinically relevant.

Results of serum biochemical analyses—The original intent was to collect blood samples for serum biochemical analyses only before and 24 hours after anesthesia. However, during the first phase of the study, the investigators elected to also collect blood on the third day after anesthesia to better characterize changes in blood analytes. As a result, complete analyte data for the third day after anesthesia were only available for the second and third anesthetic episodes. The only serum biochemical variable for which a significant change was detected was BUN concentration. In our opinion, this change was not clinically relevant. The concentration of BUN decreased the day after anesthesia, but the change from the value before anesthesia was significant only in horses receiving 5% propofol MMF; by the third day after anesthesia, the BUN value was similar to the value determined prior to anesthesia (**Table 4**).

Serum biochemical data obtained during the first, second, and third anesthetic episodes were also compared. With regard to BUN concentration, there was a significant difference between the values before anesthesia and immediately after anesthesia during the second anesthetic episode only (20 ± 1 mg/dL vs 18 ± 1 mg/dL, respectively). With regard to creatinine concentration, there was a significant difference between the values before anesthesia and after anesthesia during the third anesthetic episode only (1.3 ± 0.1 mg/dL vs 1.1 ± 0.1 mg/dL, respectively). Although the BUN and creatinine concentrations varied according the anesthetic episode, the values remained within the reference ranges for horses.

For all other serum biochemical variables, there were no significant differences between the values obtained 24 hours or 3 days after anesthesia and those obtained before anesthesia. By the third day after anesthesia, all serum biochemical values were similar to the values before anesthesia (ie, not significantly different).

Comparison of weight, rectal temperature, heart rate, and respiratory rate among treatment groups—There were no differences in body weight, temperature, heart rate, and respiratory rate before and after anesthesia among the 3 treatment groups (**Table 5**). Similarly, there were no differences in those variables before and after anesthesia among the 3 anesthetic episodes. All horses completed the study with no apparent adverse effects.

Discussion

The primary focus of the present study was to characterize the effects of novel 1% and 5% MMFs of propofol (as yet commercially unavailable) in horses. The effects of the novel micellar microemulsions were compared with those of a readily available 1% commercial soybean formulation of propofol. Under conditions of our study, no important behavioral, hematologic, or serum biochemical changes resulted from administration of the 1% or 5% MMFs. Except for time to first anesthetic effect and quality of induction of anesthesia, there were no differences in the variables assessed between horses that were administered either of these MMFs and horses that were administered the commercially available form of propofol. Any differences between the MMF treatments and the

commercial treatment were judged to be small, and further studies are necessary to verify whether the statistical differences detected are of biological or clinical importance.

The qualities of induction of and recovery from anesthesia associated with use of the commercially available propofol product and the MMFs compare favorably with results previously reported[4] from our laboratory. As part of both the present study and our previous investigations, horses were premedicated with 1.0 mg of xylazine/kg administered IV 5 minutes prior to receiving an IV injection of a commercially available propofol preparation (2 mg/kg). In both studies, the quality of induction of anesthesia was variable. Excitatory behavior and increased muscle activity (including running movements in lateral recumbency) were commonly detected after propofol administration. Similarly, recovery characteristics of horses in the 2 studies were similar; all standing events following the similar drug exposure in both studies were judged qualitatively as excellent. The induction and recovery values did not differ significantly between studies (nonpaired *t* test analysis).

The undesirable induction behavior of some horses in the present study is also similar to that reported elsewhere.[5–8] The observations made in our study further support the opinion that propofol alone or in combination with xylazine is not a desirable drug protocol for routine induction of anesthesia in horses.[4,9]

It is a commonly held clinical notion that recovery behavior of horses that are anesthetized on multiple occasions changes with increasing number of anesthetic episodes. Some of the findings of the present study seem to support this at least in part. For example, although the time to first movement following induction of anesthesia did not depend on the number of the anesthetic episodes, the time at which the horses moved their heads, lifted their heads, or moved their limbs increased with increasing number of anesthetic episodes; the number of attempts to move from lateral to sternal recumbency decreased with increasing number of anesthetic episodes. We interpreted this to mean that although a degree of wakefulness (ie, first movement and time to attain a sternal posture) occurred at similar times in the process of recovery following administration of the 3 propofol preparations, horses were more likely to remain in lateral recumbency until later in the recovery stage and move into a sternal position with improved efficiency (as indicated by fewer attempts) after repeated anesthetic events (ie, the horses were learning in our study).

Importantly, results of the present study and other investigations in horses[4-6,8,10] indicate that independent of the propofol formula used, recoveries from propofol anesthesia are likely to be smooth and calm, and during that phase, minimal attempts to stand are made.

Previous studies[11–13] involving horses performed in our laboratory and elsewhere have revealed that episodes of inhalation anesthesia and recumbency of various durations may be associated with mild transient alterations in some hematologic and serum biochemical variables. Results of the present study involving a short period of recumbency and anesthesia induced by only xylazine and propofol are in qualitative agreement with those earlier findings but are much less striking quantitatively. On consideration of the data obtained in the study of this report along with results of our previous studies, it is suggested that duration of anesthesia or type of anesthetic agent, or both, impacts the magnitude of change in at least some serum biochemical analytes (eg, aspartate aminotransferase and creatine kinase activities and phosphate concentration). The low magnitude of changes in serum biochemical variables (especially the change in creatine kinase activity) associated with anesthesia in the present study may also, in part, be attributable to the favorable recovery behavior of horses following anesthesia with propofol.

The commercially available 1% propofol product[a] used in the present study carries formal FDA labeling regarding approval for anesthetic use in dogs. The product consists of hydrophobic water-insoluble liquid propofol loaded onto a soybean oil–phospholecithin emulsion in physiologic saline solution. This type of oil-in-water emulsion is similar to the intralipid emulsions used for parenteral lipid supplementation.[14] The oil-droplet size of commercial propofol emulsions is approximately 100 to 200 nm; this relatively large size causes the milk-like visual appearance of the preparations. The micelles in the 1% and 5% MMFs of propofol are transparent because of the much smaller droplet diameters (12 nm for both preparations as measured by a laser Doppler light scattering technique). These microemulsions appear to be (but are not) chemical solutions in water, with variable degrees of yellowish color provided by the propofol they contain.

The hydrophobic property of propofol is similar to xylene, with an octanol-to-water partition coefficient of 6,761:1 at pH 6.0 to 8.5. For this reason, the MMF uses an emulsifier such as PGH. The emulsifier is a soft waxy material at room temperature (approx 20°C), which when melted is freely miscible with propofol liquid. The micellar solubilized mixture of drug in water is thermodynamically stable at the temperatures used (25° to 37°C). A 5% (wt/vol) transparent microemulsion of propofol in physiologic saline solution cannot be produced with only PGH emulsifier; addition of 1 part liquid ethyl oleate to 2 parts propofol (wt/wt) greatly increases the propofol-carrying capacity of PGH. The addition of ethanol to liquid emulsion bases greatly reduces the time, temperature, and stirring that are typically necessary to produce high-concentration propofol-PGH emulsions in saline solution. As with the 1% mixture, the 5% microemulsion finally produced from this combination is the thermodynamically stable form.

The propofol in commercial products is carried in droplets of soybean oil, in which the propofol is present as a relatively minor (10%) component; the 100- to 200-nm oil droplets are homogenized with a phospholecithin surfactant to remain in aqueous emulsion. This emulsion is formed by homogenization with input of energy and is thereafter stable. It can be destabilized by centrifugation or other dehomogenization procedures. By contrast, the MMFs are dependent on the fact

that PGH surfactant molecules (uncoiled length, approx 7 nm) spontaneously form micellar solutions in water or saline solution; the micelles are aggregates of approximately a hundred surfactant molecules that are arranged in a spherical form (approx 2 molecules [12 nm] in diameter) with stearate heads located on the inner aspect and 15-mer polyethylene glycol tails located on the outer aspect. The micelles form spontaneously in water without the addition of additional hydrophobic material to form a droplet. When loaded with a hydrophobic drug such as propofol (approx 1 mole of propofol/mole of surfactant), the size of these micelles is not altered, indicating that the drug is probably interspersed in spaces between stearate moieties in the hydrophobic core of the micelles. Even when ethyl oleate is added (2 parts propofol to 1 part ethyl oleate [wt/wt]) to increase the drug-loading capacity of the microemulsion, the size of the micelles is not altered, indicating that the ethyl oleate (molar ratio approx one fourth that of drug or surfactant) probably is also able to fit into preexisting spaces in the micelle core. Thus, the structure of the microemulsions differs considerably from that of the classical oil-in-water pharmaceutical emulsions.

The structure of the MMFs is similar to the first solubilized propofol preparations for injection. These solutions were prepared in polyethoxylated castor bean oil. However, use of the polyethoxylated castor bean oil–based solution was not successful because of allergic reactions to castor beans among some of the recipients.

The results of the present study in horses involving microsolublized preparations of propofol agree with findings of comparative pharmacokinetic studies in humans involving propofol microemulsion systems made with different surfactant poloxymers or block copolymers of polypropylene glycol solutions. According to data from a phase I trial in humans,[i] administrations of poloxymer propofol microemulsions and commercial propofol preparations result in essentially time-identical blood concentrations and clinical responses. The results of the present study also are in agreement with the kinetics and dynamics of propofol in pigs,[15] as determined by comparison of a commercial propofol emulsion[j] with a preparation of 1% solubilized propofol containing a modified cyclodextrin. The solubilized propofol preparation is a transparent drug formulation, which, like the MMFs, consists of much smaller particles than commercial soybean emulsions.[15]

The data obtained in the present study point to the existence of a drug-reservoir system in the blood, which nearly completely buffers the effects of various formulations of propofol so that although those formulations differ greatly in composition, all produce similar effects over time. In circulation, propofol is thought to be bound approximately equally to RBCs and serum albumin, but not bound to blood lipids.[16] The mechanism and rate of transfer of propofol from emulsion or microemulsion to these final within-blood carriers are unknown. Whatever the rate of transfer, the surface area of the micelles in the 1% MMF of propofol is several hundred times larger than that of the currently available soybean oil preparations of the drug, yet there are no differences in time of onset or duration of effect following administration of the 2 types of drug formulation. This suggests that transfer of drug out of commercial emulsions is already so fast that further reduction of this interval is unimportant to the clinical results. Because it is essentially the circulation time from vein to brain that determines the time scale for onset of the anesthetic action of propofol, a substantial amount of the drug must leave the emulsion system within a time frame that is rapid, compared with the blood circulation time.

Pharmacokinetic data[15] for an emulsion similar to the 1% MMF suggest that the blood reservoir of bound drug (which determines the duration of anesthesia) is not dependent on the emulsion in which the drug was delivered. Rather the duration of anesthesia is probably determined by 1 or more secondary drug-binding systems in blood. The results of the present study strengthen the general finding that the form in which propofol enters the blood does not influence propofol pharmacokinetics, as long as the emulsion size is at least smaller than present commercial products.

Overall, the data obtained in the present study have indicated that the characteristics of the induction of and recovery from anesthesia achieved via administration of a commercial 1% propofol product or 1% and 5% MMFs of propofol are similar in horses. By use of propofol, the quality of induction of anesthesia may present a potential for injury, whereas the quality of recovery from anesthesia appears to be excellent. The propofol MMFs used in our study are examples of a new generation of high-concentration propofol micellar microemulsion products that are likely to offer several advantages, compared with currently available preparations. Lipid emulsions from commercial propofol products contain both nitrogen and phosphorus, which support microbe growth. Propofol MMFs contain only the elements carbon, hydrogen, and oxygen in physiologic saline solution; in our laboratory, these elements and base appear to prevent and retard microorganism growth. The liquid self-microemulsifiable base of the MMFs allows the preparations to be stored as an anhydrous base solution (a saline solution-free form), which can be reconstituted in minutes with physiologic saline solution prior to injection. The anticipated low cost of production of propofol microemulsions encourages further development of these preparations for clinical use in horses and other species. However, further studies are necessary to fully reveal possible advantages and disadvantages, such as adverse effects on cardiopulmonary parameters in horses or other species.

a. Propoflo, Abbott Laboratories Inc, Animal Health Division, North Chicago, Ill.
b. MEDDS Inc, Rancho Cucamonga, Calif.
c. PGH or Solutol HS-15, BASF Corp, Ludwigshafen, Germany.
d. Sigma-Aldrich, Milwaukee, Wis.
e. Crodamol EO brand NF, Croda Inc, Parsippany, NJ.
f. Anased, Akorn Inc, Decatur, Ill.
g. Angiocath, Franklin Lakes, NJ.
h. SPSS, version 11, SPSS Inc, Chicago, Ill.
i. Maelor Pharmaceuticals Ltd, Wrexham, UK: In-house corporate data, obtained May 7, 2006.
j. Diprivan, AstraZeneca, Newark, Del.

References

1. Short CE, Bufalari A. Propofol anesthesia. *Vet Clin North Am Small Anim Pract* 1999;29:747–778.

2. Wagner AE, Hellyer PW. Survey of anesthesia techniques and concerns in private veterinary practice. *J Am Vet Med Assoc* 2000;217:1652–1657.

3. Ilkiw JE, Pascoe PJ, Haskins SC, et al. Cardiovascular and respiratory effects of propofol administration in hypovolemic dogs. *Am J Vet Res* 1992;53:2323–2327.

4. Mama KR, Steffey EP, Pascoe PJ. Evaluation of propofol for general anesthesia in premedicated horses. *Am J Vet Res* 1996;57:512–516.

5. Frias AF, Marsico F, Gomez de Segura I, et al. Evaluation of different doses of propofol in xylazine pre-medicated horses. *Vet Anaesth Analg* 2003;30:193–201.

6. Mama KR, Steffey EP, Pascoe PJ. Evaluation of propofol as a general anesthetic for horses. *Vet Surg* 1995;24:188–194.

7. Matthews NS, Hartsfield SM, Hague B, et al. Detomidine-propofol anesthesia for abdominal surgery in horses. *Vet Surg* 1999;28:196–201.

8. Oku K, Yamanaka T, Ashihara N, et al. Clinical observations during induction and recovery of xylazine-midazolam-propofol anesthesia in horses. *J Vet Med Sci* 2003;65:805–808.

9. Wagner AE, Mama KR, Steffey EP, et al. Behavioral responses following eight anesthetic induction protocols in horses. *Vet Anaesth Analg* 2002;29:207–211.

10. Nolan AM, Hall LW. Total intravenous anaesthesia in the horse with propofol. *Equine Vet J* 1985;17:394–398.

11. Steffey EP, Zinkl J, Howland D Jr. Minimal changes in blood cell counts and biochemical values associated with prolonged isoflurane anesthesia of horses. *Am J Vet Res* 1979;40:1646–1648.

12. Steffey EP, Farver T, Zinkl J, et al. Alterations in horse blood cell count and biochemical values after halothane anesthesia. *Am J Vet Res* 1980;41:934–939.

13. Stover SM, Steffey EP, Dybdal NO, et al. Hematologic and serum biochemical alterations associated with multiple halothane anesthesia exposures and minor surgical trauma in horses. *Am J Vet Res* 1988;49:236–241.

14. Baker MT, Naguib M. Propofol: the challenges of formulation. *Anesthesiology* 2005;103:860–876.

15. Egan TD, Kern SE, Johnson KB, et al. The pharmacokinetics and pharmacodynamics of propofol in a modified cyclodextrin formulation (Captisol) versus propofol in a lipid formulation (Diprivan): an electroencephalographic and hemodynamic study in a porcine model. *Anesth Analg* 2003;97:72–79.

16. Mazoit JX, Samii K. Binding of propofol to blood components: implications for pharmacokinetics and for pharmacodynamics. *Br J Clin Pharmacol* 1999;47:35–42.