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Efficacy and Safety of Five Injectable Anesthetic Regimens for Chronic Blood Collection from the Anterior Vena Cava of Guinea Pigs

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Despite several published methods of inducing surgical anesthesia in guinea pigs, viable methods of anesthesia for blood collection from the vena cava are inadequate. We compared 5 anesthesia regimens and their efficacy in inducing anesthesia for blood sampling in guinea pigs: ketamine–xylazine (30 and 2.5 mg/kg) administered subcutaneously, intramuscularly, or intraperitoneally; pentobarbital (37 mg/kg) administered intraperitoneally; and medetomidine (0.5 mg/kg) administered intramuscularly. Parameters measured included time to onset of anesthesia, time to recovery from anesthesia, and complete blood count (CBC) and serum chemistry values. CBC values did not differ among the 5 regimens, but serum glucose, BUN, phosphorous, and creatine phosphokinase levels varied among groups. Based on our data, intraperitoneal ketamine–xylazine appears to emerge as a preferable injectable anesthetic regimen in guinea pigs for blood collection from the anterior vena cava.

Abbreviations: CBC, complete blood count; SOP, standard operating procedure

The guinea pig (*Cavia porcellus*) is a widely used animal model for immunologic studies. One of the most common procedures in immunogenicity studies is blood collection for the analysis of neutralizing antibodies.[27] In guinea pigs, blood typically is sampled from the orbital sinus or anterior vena cava.[27] When performed correctly, vena cava blood sampling results in higher yields and lower incidence of injury.[27] Deep anesthesia (to surgical plane) is ideal for blood sampling retroorbitally or from the anterior vena cava because of trauma to the posterior orbit due to lack of a true retroorbital sinus in this species and the need for complete immobility for vena caval puncture. However, induction and maintenance of deep anesthesia is difficult and variable in guinea pigs.[2,4,6,9,14,17,19,22,27] In addition, short recovery periods are preferable for guinea pigs because longer recoveries are associated with thermoregulatory and metabolic disturbances.[10]

One common measure of determining depth of anesthesia is assessing the time to loss of reflexes, including the time to loss of the righting, pedal withdrawal, and palpebral reflexes.[10,24,27] The righting reflex is the ability of the animal to regain normal posture (sternal recumbency) after placement in dorsal recumbency. The pedal withdrawal reflex is observed by pinching a hindlimb footpad. The palpebral reflex is assessed by monitoring an animal's response to the touching of an eyelid. Recovery from anesthesia is determined to be the time at which the animal has regained all 3 reflexes.[2, 17]

Although typically considered to be a safe method, inhalant anesthesia is difficult to administer in guinea pigs due to their propensity to hold their breath after administration of a noxious inhalant agent.[3] This behavior complicates controlling the dose of inhalant anesthetic and has potential for injury.[3,9,20,27] For this and other reasons, the current study focused solely on injectable regimens. Various injectable regimens have been used to induce deep planes of anesthesia in guinea pigs. A combination of tiletamine–zolazepam (40 mg/kg) and medetomidine (0.5 mg/kg) provided sufficient anesthesia for major surgeries.[2] In another study, a cocktail comprising ketamine (87 mg/kg) and xylazine (13 mg/kg) was adequate for mildly painful procedures.[17] Animal safety must also be considered when selecting an anesthesia regimen. In a study comparing the effects of 4 anesthetic regimens on cardiovascular and ventilatory variables in guinea pigs, ketamine–xylazine combinations resulted in the least depressive effects: the cocktail depressed breathing by only 17% and ventilatory responsiveness to hypoxia and hypercapnia was the least attenuated.[22] However, few methodologies regarding anesthesia in guinea pigs for the purpose of blood sampling have been published. In addition, the effects of chronic administration of anesthesia to the animal's physiology have not been documented.

In this study, we attempt to determine the 'best choice' anesthetic method for vena caval bleeds in guinea pigs. The most efficient anesthetic regimen would provide sufficient duration of anesthesia for blood withdrawal, but with rapid onset and recovery from anesthesia. Although the blood sample procedure itself may be quick (2 to 3 min), typically in a toxicologic research setting, several guinea pigs are anesthetized simultaneously to be bled successively, so a duration of 15 to 25 min of anesthesia is preferred. These goals must be accomplished without compromising the health of the animal and the quality of the sample collected. Blood sampling, especially in immunogenicity studies, is usually repeated frequently over a prolonged observation period. Therefore, an ideal regimen could be given frequently in succession over an extended period of time without affecting animal physiology, thereby eliminating or reducing an

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unwanted experimental variable. In vaccine studies, for example, blood collection may be necessary every week for several weeks. Subsequently, a secondary objective of this study was to evaluate the efficiency of successive use of different anesthesia regimens and their effects on body weight, serum chemistry, and complete blood counts (CBC).

Materials and Methods

This study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*[13]and approved by the Vaccine Research Center's Animal Care and Use Committee. The animal facility in which the guinea pigs were housed is AAALAC accredited.

Animals. Twenty female Hartley guinea pigs (*Cavia porcellus*; Crl:HA; strain code, 051) approximately 4 to 6 mo of age were obtained from a commercial vendor (Charles River Laboratories, Kingston, NC). The guinea pigs were specific pathogen-free; our program screens animals for the following pathogens: *Bordetella bronchiseptica*, Sendai virus, reovirus 3, and *Salmonella*. Animals were provided with irradiated, pelleted guinea pig chow ad libitum and autoclaved water ad libitum and vitamin-C-fortified treats (PRIMA Treats, BioServ, Frenchtown, NJ) and vegetables twice weekly. Autoclaved toys were placed in the cage for environmental enrichment. The animal room was maintained on a 12:12-h light:dark cycle, temperature at 22.2 ± 1.0 °C, and relative humidity at 50% ± 2%. Animals were housed in sterile solid-bottom shoebox style cages with commercial bedding (Carefresh, Absorption Corporation, Ferndale, WA) and placed on ventilated racks (Lab Products, Seaford, DE). Cages were changed twice weekly.

Study design. The study was designed to mimic a frequent (weekly), long-term (3-mo) blood collection protocol in guinea pigs, similar to those commonly performed at our institute. Five different anesthetic regimens were evaluated to determine their utility for anterior vena caval bleeds. The times from administration of anesthesia to loss of the righting, pedal withdrawal, and palpebral reflexes and to recovery from anesthesia were recorded. Prior to the study and at each blood collection time point, each animal received a physical examination and was weighed. Blood was collected for CBC and serum chemistry analysis at weeks 0, 2, 4, 6, 8, and 10. The animals were allocated into 5 groups (A through E). Animals in groups A through C were given a ketamine (30 mg/kg) and xylazine (2.5 mg/kg) cocktail, which was mixed in a sterile glass vial just before injection.[8,17,22] Anesthesia was administered via the subcutaneous (group A), intramuscular (group B), or intraperitoneal (group C) route. Animals in group D received sodium pentobarbital (37 mg/kg IP),[26] and those in group E were given medetomidine (0.5 mg/kg IM).[2,15] Later in the study, the dose of the medetomidine was increased to 1.0 mg/kg because 0.5 mg/kg provided insufficient anesthesia. At the end of the study, animals were euthanized by overdose of pentobarbital–phenytoin sodium (0.5 mg/kg; Beuthanasia, Schering–Plough, Kenilworth, NJ). Death was confirmed by verifying cessation of heartbeats. This study was not randomized; animals were assigned to study groups in a nonsystematic way according to cage location. The number of animals in each group initially varied slightly due to animal availability. In addition, 1 animal each in 3 of the groups (ketamine–xylazine IM, pentobarbital, and medetomidine) died of complications associated with the blood collection, as confirmed by necropsy. Anesthesia-associated death was ruled out based on final diagnostic remarks at necropsy. Causes of deaths were attributed as follows: the animal that received pentobarbital died of hepatic necrosis and sepsis; the animal that received ketamine–xylazine IM died of an iatrogenic perithoracic cavity and jugular hematoma; and the animal that received medetomidine died of cardiac insufficiency. Data from animals that died during the course of the study were excluded from analyses, thereby leaving 3 to 5 animals in each group for analysis.

Blood collection. After administration of anesthetic, each animal was placed in an incubator (Thermocare, Incline Village, NV) and ophthalmic ointment applied to its eyes. Once the animal reached complete anesthesia (loss of all 3 reflexes), 1 ml blood was collected from the anterior vena cava by using a 3-ml syringe fitted with a 1-in., 26-gauge needle and dispensed into lithium–heparin and EDTA microtainers. Each animal then was placed on a circulating warm-water pad until full recovery from anesthesia. Each animal then was placed in a cage and observed for 24 h for any clinical abnormalities. Blood samples were sent to a commercial laboratory (Antech, Rockville, MD) for CBC and serum chemistry analyses.

Histopathology. Samples of liver and kidney tissues were obtained at necropsy of euthanized animals and analyzed histopathologically to determine any anesthetic-induced alterations or injuries.

Statistical analysis. Because of iatrogenic and anesthesia-unrelated deaths in some groups, the number of animals available per group varied for analysis of CBC and clinical chemistry data. Experiments were conducted roughly every 2 wk for each of the animals, resulting in 6 measurements for each parameter per animal. These values were averaged for each animal, resulting in 3 to 5 independent mean values per group. Groups were compared using ANOVA followed by *t* tests, using the average of the multiple measurements for each animal. Comparisons of time points were based on paired *t* tests of the first and last measurements. Differences associated with *P* values less than or equal to 0.05 were considered statistically significant. In addition, Wilcoxon signed-rank tests were used to analyze differences in CBC values.

Results

Anesthesia. Four of the 5 anesthetic regimens induced anesthesia; the medetomidine regimen only achieved surgical anesthesia in one animal. Guinea pigs were considered to have recovered from anesthesia once they had regained sternal recumbency. Mean times to return of the righting, pedal withdrawal, and palpebral reflexes and to recovery from anesthesia are shown in Table 1.

CBC and serum chemistry results. CBC values were analyzed for statistical differences between groups by using Wilcoxon signed-rank tests. Mean cellular hemoglobin concentration decreased over time ($P = 0.004$), whereas the mean cell volume ($P = 0.004$) and red blood cell count ($P = 0.023$) increased over time. However, hemoglobin concentration, mean cellular hemoglobin, and white blood cell count did not change significantly over time. Serum chemistry results (Figure 1) were analyzed by using ANOVA and paired *t* tests, which indicated significant differences in blood glucose levels between groups C and D ($P = 0.0216$), in BUN levels between groups B and D ($P = 0.0199$), in phosphorous levels between groups A and D ($P = 0.0302$), and in creatine phosphokinase levels between groups B and D ($P = 0.0112$). For all experimental groups, glucose concentrations were below the normal range in guinea pigs (125 ± 13 mg/d),[27] whereas phosphorus was present at levels above the normal range (3.0 to 7.6 mEq/dl).[27] BUN values were within normal limits.[27]

Histopathology. Samples of tissues obtained at necropsy of euthanized animals were analyzed histopathologically. No

Table 1. The time of recovery from anesthesia, body weight

	Ketamine–xylazine SC (n = 3)		Ketamine–xylazine IM (n = 3)		Ketamine–xylazine IP (n = 5)		Pentobarbital (n = 3)		Medetomidine (n = 4)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Time (min) to loss of righting reflex	6.6	1.0	2.1	0.4	8.8	0.8	12.7	1.2	[a]	[a]
Time (min) to loss of pedal withdrawal reflex	8.6	1.3	2.6	0.3	10.3	0.5	17.8	3.01	[a]	[a]
Time (min) to loss of palpebral reflex	8.0	1.3	2.8	0.4	10.3	0.6	18.9	2.96	[a]	[a]
Time (min) to recovery	81.8	3.9	83.0	4.7	49.0	7.6	294.3	10.8	57.83	27.2
Body weight (g)	831.4	74.7	791.1	28.9	814.5	38.7	750.4	69.1	820.2	33.4

[a]Only 1 animal lost all 3 reflexes during the study, and 3 animals never lost any reflex after a second dose of medetomidine.

anesthetic-induced histopathologic lesions were found; occasional slides showed rare artifactual alterations.

Discussion

The guinea pig is a commonly used animal model for studies of the respiratory, nervous, and immune system. Immunologic studies often require repeated blood collections over a long period of time, and animals should recover from these blood collections as fast as possible. To successfully sample blood without risking injury to the animal, anesthesia must be induced quickly, with complete immobilization of the animal. Previous studies have focused primarily on anesthetizing guinea pigs for the purpose of major surgical procedures. For studies performed at our institute, the best-choice anesthetic would be one that rapidly induces stage III (surgical) anesthesia and from which the animal recovers quickly, without any changes in physiology over a long period of use.

This study led us to believe that intraperitoneal injection of a ketamine–xylazine cocktail (30 and 5 mg/kg) is the best-choice anesthetic of those tested for blood collection from the vena cava of guinea pigs. However, statistically significant differences were not observed across groups. Induction times were similar across routes and agents. Four of the 5 anesthetic regimens induced anesthesia, with the exception of medetomidine. As an α_2-adrenoreceptor agonist, medetomidine administration typically induces anesthesia and some analgesia.[15] We hypothesized that medetomidine would quickly induce sedation, with rapid recovery after sufficient time for blood collection. However, only 1 animal developed anesthesia sufficient for blood sampling. Subsequently, midway through the study, the dose of the medetomidine was increased from 0.5 mg/kg to 1.0 mg/kg. However, this higher dose also failed to provide adequate anesthesia and caused only sedation.

The significantly higher values for BUN after pentobarbital versus ketamine–xylazine administration may be a direct result of the decreased cardiac output, renal blood flow, and glomerular filtration rate that are well known effects of pentobarbital anesthesia[7,10,22] compared with the increased cardiac output associated with ketamine.[1,7] Although elevated BUN values typically are associated with renal dysfunction, we did not note an associated increase in creatine levels or an effect on the overall clinical wellbeing of animals. The significantly lower glucose values under pentobarbital versus ketamine anesthesia may be related to ketamine-induced increases of epinephrine and norepinephrine.[1] Similar increases in blood glucose levels occur in humans, who develop a slight rise in blood sugar level after ketamine induction and a return to a baseline level after 48 h.[23] An increase in blood sugar also occurs in rats under ketamine–xylazine anesthesia, in which (as in many species) the significant hyperglycemic effect of xylazine is believed to be due to its action on α2-adrenoreceptors on the beta cells of the pancreas, thus inhibiting insulin release.[21] Further, when food was withdrawn 18 h prior to study initiation, the hyperglycemic effect was largely eliminated.[21] This effect would be important to remember when choosing anesthetic agents and using fed versus fasted rodents in a study of diabetes or in other situations where glucoregulatory hormone levels may influence the interpretation of data.

Phosphorus values were slightly higher in groups B through D compared with group A, with a statistically significant difference between group A and D. Higher doses of ketamine likely would progressively inhibit insulin release,[21] and insulin enhances intracellular uptake of phosphorus.[18] These effects might account for the decreased serum phosphorus levels associated with ketamine compared with pentobarbital, which does not have a similar effect.

Anesthetics agents in general increase creatine phosphokinase values,[11,12,15,25] the main source of which is necrosis of skeletal muscle. Acute muscle necrosis or sudden changes in the permeability of the sarcolemma may lead to increases in serum creatine phosphokinase.[5,12] We noted a significant difference in the creatine phosphokinase values of animals anesthetized with intramuscular ketamine–xylazine compared with pentobarbital. Pentobarbital has a longer duration of effect than does intramuscular ketamine–xylazine,[7] and prolonged anesthesia has been shown to be a factor in increased creatine phosphokinase values in humans.[16]

The anesthetic regimens used in this study were limited to injectable anesthetics. Ketamine–xylazine and pentobarbital are 2 of the more common anesthetic regimens used for minor surgical procedures in guinea pigs. The dosages of anesthetics were chosen according to institute-wide practices and standard operating procedures and published methodologies. A more comprehensive study would entail a wider variety of injectable anesthetics at various doses with a larger sample population per group to detect significant differences between groups.

In summary, injection of a ketamine–xylazine cocktail is an effective method of anesthesia in guinea pigs that is associated with minimal alteration to normal blood chemistry values, relatively short induction time, and ease of use. Ketamine–xylazine is suitable for routine procedures that require anesthesia for as long as 45 min, which accommodates bleeding several guinea pigs sequentially and brief surgical procedures.

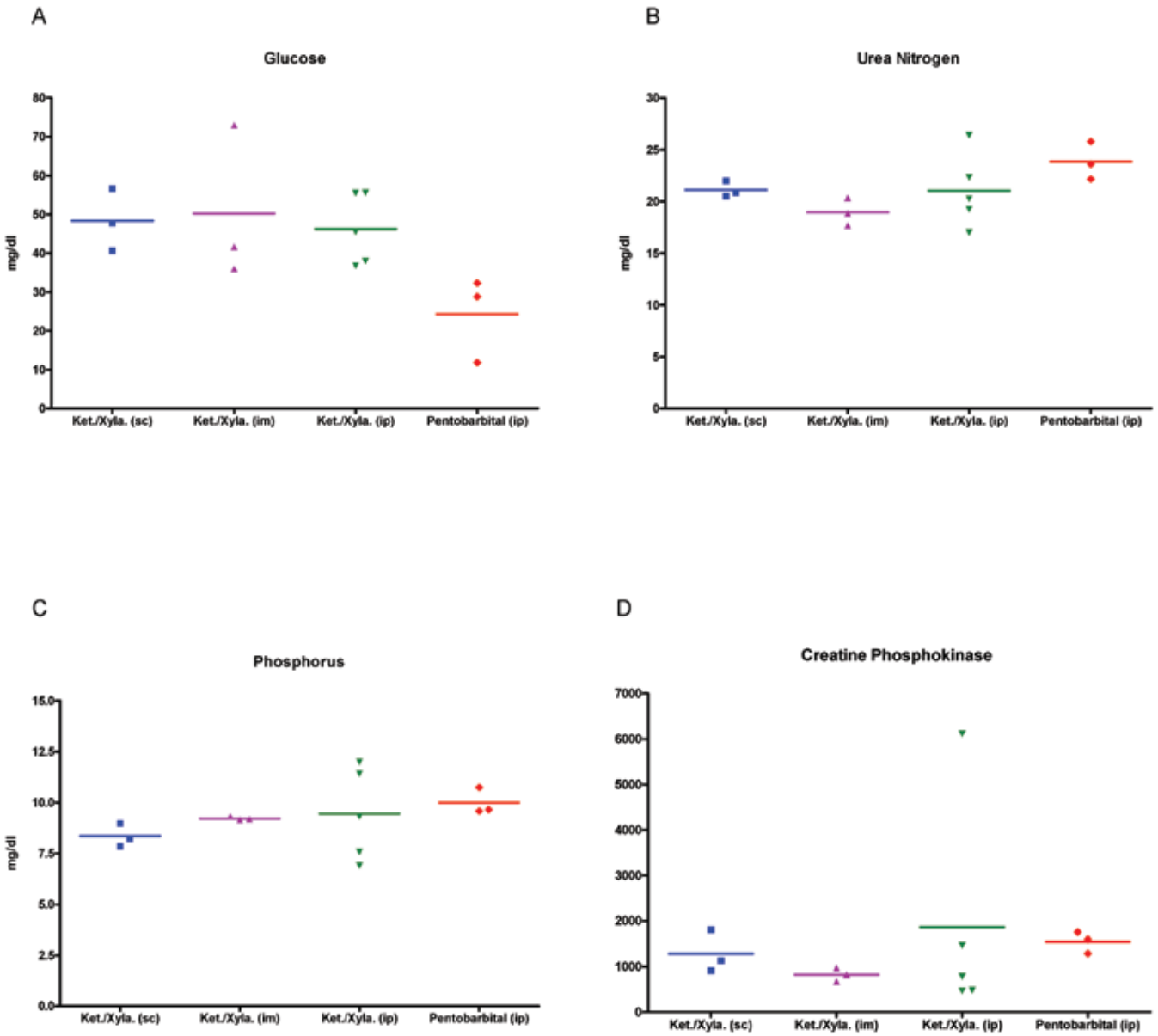

Figure 1. Results of CBC and serum chemistries. CBC values did not differ among experimental groups. Serum chemistries revealed significant differences in blood glucose levels (Fig. 1A) between groups C and D ($P = 0.0216$), BUN levels (Fig. 1B) between groups B and D ($P = 0.0199$), phosphorous levels (Fig. 1C) between groups A and D ($P = 0.0302$), and creatine phosphokinase levels (Fig. 1D) between groups B and D ($P = 0.0112$).

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