Effective and Safe Anesthesia for Yorkshire and Yucatan Swine with and without Cardiovascular Injury and Intervention

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The goal of this study was to identify an injectable anesthetic protocol that provides sedation sufficient for peripheral vascular catheterization, intubation, and transport while minimizing cardiovascular changes in Yorkshire and Yucatan pigs with and without cardiovascular injury and intervention (CI). Phase 1 examined the safety and efficacy of acepromazine–ketamine, diazepam–ketamine, midazolam–ketamine, and medetomidine–ketamine in 5 healthy Yorkshire pigs. For each drug combination, we obtained multiple measurements of heart rate, blood pressure, respiratory rate, temperature, sedation score, ability to catheterize and intubate, and recovery score. Phase 2 evaluated and refined the dose of the most effective Phase 1 anesthetic combination (midazolam–ketamine) in healthy and CI Yorkshire pigs (n = 53 trials). Phase 3 mirrored Phase 2 but tested midazolam–ketamine in healthy and CI Yucatan pigs (n = 34 trials). Midazolam (0.5 mg/kg)–ketamine (25 to 27 mg/kg) was the most effective anesthetic combination in healthy Yorkshire pigs, but this dose was less effective in healthy Yucatan pigs and CI Yorkshire and Yucatan pigs. Midazolam–ketamine resulted in tachycardia and apnea more frequently in CI pigs than healthy pigs. This combination also caused vomiting in one CI Yucatan pig. Overall, midazolam–ketamine provided safe and effective sedation for catheterization and intubation of both healthy and CI pigs. This study suggests Yucatan pigs may require a higher dose midazolam–ketamine to achieve the same level of sedation as that in Yorkshire pigs. Although anesthetic complication rates were higher in CI pigs, our results indicate that midazolam–ketamine can be safely used for sedation of both pig breeds with and without CI.

Abbreviation: CI, cardiovascular injury and intervention.

Pigs (*Sus scrofa*) are common models of cardiovascular injury and intervention (CI) that largely have replaced traditional canine cardiology models. Advantages of swine compared with dogs include anatomic and physiologic characteristics similar to humans, such as similar coronary artery distribution and effective collateralized blood flow to the myocardium after coronary artery blockage.[23] However, pigs are difficult to restrain and anesthetize due to their size and resistance to sedative drug combinations, including those with morphine.[24] Injectable sedative drugs may result in cardiovascular and respiratory effects such as increased cardiac work and oxygen consumption, tachycardia, bradycardia, apnea, hypertension, and hypotension.[5,6,8-12,14,19,20,25-29] These side effects can pose considerable problems for CI pigs, and anesthesia protocols with minimal effects on cardiovascular function are needed. A literature review revealed no published studies of anesthetic protocols in swine with existing cardiovascular injury; published research is limited to investigating anesthesia protocols for experimental induction of CI or determining in vitro and in vivo drug effects on healthy cardiovascular systems.[4-6,8-12,14,19-21,25-29] Other published studies have limited investigations to studying sedative and physiologic effects in healthy Yorkshire, Yucatan, mixed-breed, Landrace, and Gottingen miniature swine.[2,3,10,13,17,18,20-22]

We conducted the current study to address the need for a systematic investigation of anesthetic protocols in CI Yorkshire and Yucatan pigs. The goals of this study were to determine an injectable-only anesthetic protocol for both Yorkshire and Yucatan pigs that yielded sufficient sedation for peripheral vascular catheterization, sufficient duration for transport, and minimal cardiovascular effects while being safe and effective for CI pigs.

Materials and Methods

Animals. All procedures were performed as part of animal research protocols approved by the Office of Research Services and National Heart, Lung, and Blood Institute animal care and use committees. This research was conducted in compliance with the Animal Welfare Act,[1] other federal regulations, and the *Guide for the Care and Use of Laboratory Animals* at an AAALAC-accredited facility.[10]

All animals were purchased as SPF for pseudorabies and brucellosis from commercial swine vendor closed herds (S and S Farms, Vicksburg, MI; Sinclair Bio Resources, Windham, ME; and Archer Farms, Darlington, MD). SPF status for additional diseases and vaccination programs varied among vendors. All animals were processed through an off-site quarantine facility before being transferred to the final housing facility, where they were provided a 5-d adaptation period before any procedures were conducted. All pigs were housed in indoor–outdoor runs, and environmental conditions were maintained as recommended by the *Guide for the Care and Use of Laboratory Animals*.[10] Animals were fed twice daily with a commercial chow (NIH 2004 Swine Diet, Zeigler Bros, Gardners, PA). Fresh water was provided ad libitum by an automated system. Pigs were housed

in pairs when possible and were provided opportunities to root and chew for enrichment.

Phase 1 involved 5 healthy 2.5-mo-old male Yorkshire pigs. The study population for phase 2 included 43 healthy naïve male and female Yorkshire pigs (age, 2 to 8 mo) and 10 male and female Yorkshire pigs (age, 2 to 8 mo) with experimentally induced myocardial infarctions, mitral regurgitation, or aortic valve replacements. Phase 3 involved 21 healthy naïve male and female Yucatan pigs (age, 2 to 8 mo) and 13 male and female Yucatan pigs (age, 2 to 8 mo) with experimentally induced myocardial infarctions or aortic valve replacements.

CI pigs were maintained on lisinopril (10 mg PO once daily; West Ward Pharmaceutical, Eatontown, NJ), atenolol (0.7 mg/kg PO once daily; Sandoz, Princeton, NJ), aspirin (80 mg PO once daily; Gold Line Laboratories, Miami, FL), amiodarone (200 mg PO twice daily; Sandoz), clopridogrel (75 mg PO once daily; Bristol-Myers Squibb, Bridgewater, NJ), and furosemide (0.5 to 3 mg/kg PO once or twice daily; Qualitest Pharmaceuticals, Hunstville, AL), depending on the experimental procedure and clinical signs indicating decompensation of congestive heart failure. Clinical signs included coughing, increased respiratory effort, serous nasal discharge, lethargy, and inappetance. Pulmonary edema was confirmed by using thoracic radiography and MRI.

Study design. Phase 1. A pilot study was conducted with 5 healthy male Yorkshire pigs (age, 2.5 mo) to evaluate anesthetic drug combinations and doses. These included acepromazine (1 to 2 mg/kg SC; Vedco, St Joseph, MO) with ketamine (20 to 27 mg/kg SC; Fort Dodge Animal Health, Fort Dodge, IA), diazepam (2 to 5 mg/kg SC; Hospira, Wake Forest, IL) plus ketamine (20 to 27 mg/kg SC), midazolam (0.2 to 1 mg/kg SC; Bedford Laboratories, Bedford, OH) with ketamine (20 to 27 mg/kg SC), and medetomidine (0.1 to 0.2 mg/kg SC; Orion Pharma, Espoo, Finland) plus ketamine (5 mg/kg SC).

For each trial, designated personnel randomly assigned and prepared one of the drug combinations and doses, starting at the lowest dose. The chosen drug combination was administered as a subcutaneous injection in the lateral cervical muscle region. Personnel blind to the selected drug and dose scored the effects and attempted catheterization with a 22-g catheter (BD Teflon, Helsinborg, Sweden) in the auricular vein. We used the following if catheterization and 30 min of sufficient sedation (score of 4 or 5, as described later) was not achieved. When needed, doses were increased successively by 0.5 mg/kg for acepromazine, 1.5 mg/kg for diazepam, 0.4 mg/kg for midazolam, 0.1 mg/kg for medetomidine, and 7 mg/kg for ketamine. When we achieved catheterization and 30 min of sufficient sedation, we tested the combination and dose in additional trials and excluded higher doses from subsequent testing. This pattern was repeated until optimum sedation and catheterization with minimal change in cardiovascular parameters was achieved. Each pig was sedated no more than once weekly.

For each trial, personnel blinded to the drug and dose recorded heart rate, blood pressure (5098-70 Tycos TR2 Hand Aneroid, Welch-Allyn, Skaneateles Falls, NY), temperature, and respiratory rate every 5 min beginning just prior to drug administration until recovery from sedation was complete (pig was ambulatory). If the drug for a given trial did not provide sedation, these parameters were measured every 5 min for a total of 30 min. In addition, sedation was scored every 5 min using the scale in Figure 1, beginning 10 min after drug administration until recovery. Stimuli included needle pricks, body shaking, hand clapping, and nose pinching. The time from injection to catheterization was measured also. If the drug for

a given trial did not provide sedation, these parameters were measured every 5 min for a total of 30 min. Furthermore, all pigs were allowed to recover after drug administration and were monitored until walking. Time between drug administration and full recovery was measured. Recovery was scored by using the scale in Figure 2. If sedation sufficient for catheterization was achieved, blood was drawn from the auricular artery catheter and analyzed using a handheld clinical analyzer (model JAMS058A, iStat, Heska, Waukesha, WI). Measured parameters included pH, pCO_2, pO_2, HCO_3, base excess, anion gap, sodium, potassium, chloride, and glucose.

Phase 2. The goal of Phase 2 was to evaluate the best Phase 1 sedative combination in a larger population of healthy and CI Yorkshire pigs. Based on the data obtained in Phase 1, the chosen drug combination and dose was studied in healthy and CI Yorkshire pigs (n = 53 trials). Yorkshire pigs used in Phase 1 were not used in Phase 2.

During each trial, a pig received the anesthetic drug chosen from Phase 1 and glycopyrrolate (0.01 mg/kg SC; American Regent, Shirley, NY) or atropine (0.01 mg/kg SC; American Pharmaceutical Partners, Schaumberg, IL) in the lateral cervical muscle region. After sedation, the animal was catheterized and intubated. If after 20 min the pig was not sufficiently sedated for intravenous catheter placement and intubation, isoflurane was administered by facemask until catheterization and intubation could be achieved; the pig was maintained on isoflurane until transportation began. If a pig's plane of anesthesia lightened during transportation to the imaging and surgery facility, the animal was redosed with ketamine (50 to 100 mg IV) as needed to maintain light anesthesia. Transport consisted of 5 to 10 min in a climate controlled vehicle without the availability of isoflurane. For each trial, the anesthetizing technician completed an anesthesia questionnaire (Figure 3).

Phase 3. Procedures mirrored those of phase 2 with the exception that phase 3 involved healthy and CI Yucatan pigs (n = 34 trials) instead of Yorkshire pigs.

Statistical analyses. Statistical analyses performed included 2-way ANOVA, Mann–Whitney U tests, and Fisher exact tests (SAS Version 9.1, SAS Institute, Cary, NC). Statistical significance was defined as a P value of less than 0.05.

Results

Phase 1. Various numbers of trials were conducted in healthy Yorkshire using the 4 anesthetic drug combinations at various doses (Tables 1 and 2). All clinical parameters and body temperatures were within normal limits from for all phase 1 pigs (data not shown).

Acepromazine–ketamine. This combination resulted in an increased heart rate from baseline more frequently than other combinations. During 1 trial, the pig experienced multiple seizures and had profuse vomiting prior to recovery (Tables 1 and 2). Due to these complications, we did not test acepromazine doses higher than 1 mg/kg.

Diazepam–ketamine. Sedation with the diazepam–ketamine combination was so poor that we did not test doses lower than 3.5 mg/kg diazepam and 27 mg/kg ketamine. This drug combination had the lowest average sedation score of all combinations tested in Phase 1. We did not attempt more than 1 trial for which the diazepam dose was 3.5 mg/kg or higher because the injection volume (50 mL) required for large (50 kg) pigs was excessive (Tables 1 and 2).

Medetomidine–ketamine. This combination resulted in seizure activity and a rough recovery in 1 pig given the lower dose of 0.1 mg/kg medetomidine and 5 mg/kg ketamine. One pig

Sedation score	Criteria
1	No sedation, able to stand and ambulate, responsive to stimuli, catheterization not possible
2	Able to stand but ataxic, responsive to stimuli, catheterization not possible
3	Sternal recumbency, responsive to stimuli, catheterization not possible
4	Lateral recumbency, responsive to stimuli but catheterization possible, makes voluntary movements
5	Lateral recumbency, unresponsive to stimuli, catheterization possible, makes few or no voluntary movements

Figure 1. Phase 1 sedation scoring system.

Recovery score	Criteria
1	No or little sedation achieved, therefore no true recovery phase
2	Smooth: Sternal recumbency regained with no struggling, able to stand and walk with no struggling
3	Acceptable: Sternal recumbency regained with some struggling, able to stand and walk with some struggling
4	Rough: Prolonged struggling, numerous failed attempts to rise or place self in sternal recumbency, vocalization, paddling

Figure 2. Phase 1 recovery scoring system.

given the higher dose of 0.2 mg/kg medetomidine and 5 mg/kg ketamine experienced profound bradycardia (less than 65 bpm). In addition, this combination yielded the largest observed percentage change in respiratory rate, but all respiratory rate changes were increases from baseline ($P < 0.05$ compared with average percentage change in respiratory rate due to midazolam and ketamine).

Midazolam–ketamine. The greatest success occurred with 0.6 mg/kg midazolam and 27 mg/kg ketamine. Among all drug combinations tested during phase 1, midazolam–ketamine had the most negligible effect on respiratory rate. Although this combination resulted in the greatest percentage change in heart rate, the rates themselves remained within normal physiologic limits for pigs. Although one pig demonstrated seizure activity and a very long recovery to this regime, these undesirable outcomes did not occur when the same pig was retested with the same combination and dose.

Phase 1 was conducted as a pilot study to provide us direction about which drug dose and combination to further study in phases 2 and 3. Only the percentage changes in respiratory rate for the midazolam and medetomidine combinations achieved statistical significance. These differences in addition to various adverse reactions and general trends led us to choose midazolam and ketamine for further study in phases 2 and 3.

Phase 2. While conducting Phase 1, we were successfully using a lower midazolam–ketamine dose (0.5 and 25 mg/kg) in other Yorkshire pigs not on this study. We therefore evaluated this lower dose in 10 healthy Yorkshire pigs (data not shown). This lower dose was effective and caused no complications. We therefore used this dose in the remaining phase 2 trials. Reactions to midazolam–ketamine injections were mild but more pronounced in CI Yorkshire pigs compared with healthy York-

shire pigs (Table 3). CI pigs required significantly ($P < 0.02$) more injection attempts to administer drugs and generally had more pronounced injection reactions than did their healthy counterparts. Compared with healthy Yorkshire pigs, CI Yorkshire pigs were more difficult to sedate with midazolam–ketamine at 0.5 and 25 mg/kg, respectively. In addition, CI pigs had a significantly ($P < 0.05$) greater rate of complications than did their healthy counterparts.

Phase 3. While conducting Phases 1 and 2, we were unsuccessfully in using 0.5 mg/kg midazolam and 25 mg/kg ketamine in other Yucatan pigs not on this study. We therefore evaluated a higher midazolam–ketamine dose (0.6 and 27 mg/kg) in 10 healthy Yucatan pigs (data not shown). This higher dose was effective and caused only mild apnea (approximately 5 min) in 2 pigs and marked apnea (approximately 30 min) in a single pig. We therefore continued Phase 3 trials at this dose level.

Reactions to midazolam–ketamine injections were mild but more pronounced in CI Yucatan pigs compared with healthy Yucatan pigs (Table 3). CI pigs required significantly ($P < 0.02$) more injection attempts for drug administration and generally had greater injection reactions than did their healthy counterparts. Compared with healthy Yucatan pigs, CI Yucatan pigs were more difficult to sedate by using this higher dose of midazolam–ketamine ($P = 0.02$ when comparing isoflurane needs for catheterization). We observed tachycardia (200 bpm) and vomiting (one case each; different animals) in CI Yucatan pigs.

Discussion

In our facility, a small number of pigs with experimentally induced myocardial infarction and congestive heart failure died after receiving xylazine, ketamine, atropine, and butorphanol. In addition, a systematic investigation of anesthetic protocols in CI Yorkshire and CI Yucatan pigs has been needed. These factors prompted us to attempt to identify an injectable-only anesthetic protocol for both healthy and CI Yorkshire and Yucatan pigs. This cardiovascular-safe anesthetic protocol needed to provide, by injectable drugs alone, effective sedation for catheterization, intubation, and transport. Injectable-only protocols were necessary because our pigs are transported on trucks without gas anesthetic machines from our housing facility to an imaging and surgery facility. In addition, pigs at our institution must remain sedated while in the imaging and surgery facility until they can be placed on isoflurane. This delay may be several minutes long, so it is vital that our injectable anesthetic protocol provide long-lasting sedation. Due to this requirement and the difficulty of redosing during truck transport, we focused on studying long-lasting injectable drugs, such as ketamine, rather than short-acting agents, such as propofol, fentanyl, or etomidate. Although ketamine can be hazardous in animals with heart failure, the need for a long-lasting injectable sedative drug outweighed this potential contraindication. We sought to evaluate ketamine in combination with other injectable drugs, in light of ketamine's reported muscle rigidity and lack of surgical anesthesia when used as a sole agent.[3,24] Finally, pigs in our facility are large because they are maintained on long-term cardiovascular studies, and isoflurane mask induction of anesthesia is not an option due to safety concerns for staff and pigs.

In addition to the need for injectable-only anesthesia, we had to consider an additional requirement: investigators in our facility use both Yorkshire and Yucatan pigs for CI procedures, currently necessitating anesthetic regimens that are breed-specific. Our technicians historically have reported that Yucatan pigs are not effectively sedated by using doses prescribed for

Pig Anesthesia Questionnaire

Date_____   Pig Number_____

Was any drug lost during administration? Y   N

How many attempts did it take to administer the drug? _____

Was there a dramatic reaction to the injection? Y   N   If yes, how dramatic was this on a scale of 1 to 4? _____
                                                                1 = No reaction
                                                                2 = Scratching at injection site
                                                                3 = Scratching, kicking, minor vocalizations
                                                                4 = Scratching, kicking, biting, loud squealing,
                                                                      frantic escape attempts

Were the initial drugs given enough to sedate for catheterization? Y   N

Was isoflurane needed for catheterization? Y   N

Was isoflurane needed for intubation? Y   N

Estimated time between drug administration and catheterization (min)_____

Was additional ketamine needed for transport? Y   N

          If yes, how much additional ketamine was used? _____

Please indicate if any of the adverse events took place:
          Apnea
          Tachycardia
          Bradycardia
          Vomiting
          Other:_____

Figure 3. Phase 2 and 3 anesthesia questionnaire.

Yorkshire pigs. We therefore were prompted to systematically study differences in dosing requirements for both Yorkshire and Yucatan pigs.

In phase 1, we studied incremental dose increases of acepromazine–ketamine, diazepam–ketamine, midazolam–ketamine, and medetomidine–ketamine in healthy Yorkshire pigs. The goal of this phase was to determine a drug combination that provided sufficient sedation for catheterization with minimal cardiovascular side effects. Compared with other combinations, acepromazine–ketamine had the lowest success rate for catheterization and most frequently led to increased heart rates. In addition, acepromazine–ketamine was associated with the longest recovery time and caused vomiting and seizures. Because seizures also were observed with other ketamine-containing regimens, these seizures likely were ketamine-induced. The higher heart rate observed with acepromazine is consistent with reflex tachycardia secondary to decreased blood pressure.[17] It is surprising that acepromazine resulted in the longest recovery, because this drug has a short half-life. One author[24] recommends using 1.1 mg/kg acepromazine and 33 mg/kg ketamine for pig anesthesia. In light of complications in pigs that received 1 mg/kg acepromazine and 27 mg/kg ketamine, we conducted limited trials with this combination.

In the present study, diazepam–ketamine resulted in very little sedation of pigs. When catheterization was possible, the catheterization window was the shortest of all drug combinations. In addition, diazepam–ketamine required a large volume for injection, given the low concentration of diazepam; one suggested dosing regimen for pigs is 2 mg/kg diazepam and 15 mg/kg ketamine.[24] In the present study, however, pigs required as much as 3.5 mg/kg diazepam and 27 mg/kg ketamine for sedation adequate for catheterization.

At the higher dose levels we tested, medetomidine–ketamine provided sedation adequate for catheterization, but delay between injection and catheterization was the longest of all combinations. This finding is in contrast to a previous study,[17] in which medetomidine created profound sedation compared with that of midazolam and acepromazine. Medetomidine–ketamine in our pigs also caused seizure activity, rough and prolonged recovery, dramatic injection reactions, and bradycardia, a well-known and described side effect of this drug.[20,26,28] Medetomidine has been reported to result in prolonged recoveries, and our recovery times were consistent with those of other studies.[17-19] One recommended dosing regimen[24] is 0.2 mg/kg medetomidine and 10 mg/kg ketamine, yet we found that catheterization could be achieved by using 0.2 mg/kg medetomidine and 5 mg/kg ketamine.

Midazolam–ketamine had the greatest success rate for catheterization, and the time window during which catheterization could be accomplished was the longest among all combinations. Although midazolam–ketamine caused the greatest percentage change in heart rate, they were within normal physiologic limits for pigs. This midazolam-induced change in heart rate has been described in the literature.[4,6,21] In addition, midazolam has minimal effects on several other cardiopulmonary parameters, indicating that compared with other drug combinations, midazolam–ketamine may be less detrimental to CI pigs.[4,6,21] In addition, the reaction to injection and recovery were very favorable in the current studies. The use of 0.5 mg/kg midazolam and 33 mg/kg ketamine in pigs has been recommended,[24] and we had the greatest success with 0.5 to 0.6 mg/kg midazolam and 25 to 27 mg/kg ketamine. Although our phase 1 study involved a limited number of trials, we selected midazolam–ketamine for

Table 1. Phase 1: drug combinations, doses, and effects in healthy Yorkshire pigs

	Acepromazine–Ketamine		Diazepam–Ketamine		Midazolam–Ketamine			Medetomidine–Ketamine	
	1 mg/kg + 20 mg/kg	1 mg/kg + 27 mg/kg	2 mg/kg + 20 mg/kg	3.5 mg/kg + 27 mg/kg	0.2 mg/kg + 20 mg/kg	0.6 mg/kg + 20 mg/kg	0.6 mg/kg + 27 mg/kg	0.1 mg/kg + 5 mg/kg	0.2 mg/kg + 5 mg/kg
Average % change in heart rate	29.8	26.2	24.5	19.4	28.4	34.4	32.5	22.6	24.3
No. of trials with increased heart rate	1 of 2	1 of 3	0 of 2	0 of 1	1 of 2	0 of 3	1 of 3	0 of 2	0 of 2
Average% change in respiratory rate	15.6	46.0	45.0	32.0	36.5	32.8	17.8	141.5	64.6
No. of trials with decreased respiratory rate	1 of 2	1 of 3	2 of 2	0 of 1	2 of 2	1 of 3	0 of 3	0 of 2	0 of 2
No. of trials with sedation score ≥3	2 of 2	3 of 3	0 of 2	1 of 1	2 of 2	3 of 3	3 of 3	2 of 2	2 of 2
Average sedation score	3.0	3.3	1.0	4.0	3.5	3.3	4.3	3.5	4.0
No. of trials in which pigs could be catheterized	0 of 2	1 of 3	0 of 2	1 of 1	1 of 2	2 of 3	3 of 3	1 of 2	2 of 2
No. of trials with dramatic reactions[a]	1 of 2	0 of 3	0 of 2	0 of 1	0 of 2	0 of 3	1 of 3	1 of 2	0 of 2
No. of trials with adverse reactions[b]	1 of 2	0 of 3	1 of 2	0 of 1	0 of 2	0 of 3	1 of 3	1 of 2	0 of 2
Average time to catheterization (min)	NP	10.0	NP	10.0	25.0	12.5	15.0	10.0	15.0
Average time during which pigs could be catheterized (min)	NP	10.0	NP	5.0	10.0	15.0	16.7	15.0	10.0
Average time to recovery (min)	190.0	345.0	90.0	70.0	172.5	330.0	165.0	210.0	225.0
Average recovery score	1.0	1.3	1.0	2.0	1.5	2.0	2.6	1.5	2.0
No. of trials with recovery scores of 2 or 3	0 of 2	1 of 3	0 of 2	2 of 2	1 of 2	3 of 3	1 of 3	1 of 2	2 of 2

NP, catheterization was not possible.
[a]Dramatic reactions included scratching at injection site, kicking, biting, loud vocalizations, and frantic escape attempts.
[b]Adverse reactions included seizure activity, vomiting, and rough recovery.

Table 2. Data calculated by combining trials for each Phase 1 drug combination independent of dose in healthy Yorkshire pigs

	Acepromazine–Ketamine	Diazepam–Ketamine	Midazolam–Ketamine	Medetomidine–Ketamine
Average% change in heart rate	28	22	31.8	23.5
No. of trials with increased heart rate	2 of 5	0 of 3	2 of 8	0 of 4
Average % change in respiratory rate	33.9	40.7	28.1[c]	103.0[c]
No. of trials with decreased respiratory rate	2 of 5	2 of 3	3 of 8	0 of 4
No. of trials with sedation score ≥3	5 of 5	1 of 3	8 of 8	4 of 4
Avgrage sedation score	3.2	2	3.75	3.75
No. of trials in which pigs could be catheterized	1 of 5	1 of 3	6 of 8	3 of 4
No. of trials with dramatic reactions[a]	1 of 5	0 of 3	1 of 8	1 of 4
No. of trials with adverse reactions[b]	1 of 5	1 of 3	1 of 8	1 of 4
Average time to catheterization (min)	10.0	10.0	11.9	13.3
Average time during which pigs could be catheterized (min)	10.0	5.0	15.0	11.7
Average time to recovery (min)	352.0	83.3	202.5	217.5
Average recovery score	1.2	1.3	2.1	1.4
No. of trials with recovery scores of 2 or 3	1 of 5	1 of 3	6 of 8	3 of 4

[a]Dramatic reactions included scratching at injection site, kicking, biting, loud vocalizations, and frantic escape attempts
[b]Adverse reactions included seizure activity, vomiting, and rough recovery
[c]These 2 values differed significantly ($P < 0.05$).

use in the remainder of the study, given the number of positive attributes.

We had 3 goals in conducting phases 2 and 3 of the current study. The first goal was to validate in both Yorkshire and Yucatan pigs the drug combination and dose we identified through phase 1. Although the success rates of catheterization with midazolam–ketamine were not as high as in phases 2 and 3 as in phase 1, injection reactions and time to catheterization were similar to those in phase 1. Complications were more frequent during phases 2 and 3 compared with phase 1, but this difference may reflect the larger sample sizes in phases 2 and 3. Despite these differences, midazolam–ketamine still provided a safe and relatively effective method of anesthesia for all pigs, with mild apnea (marked in one pig), one case of tachycardia, and one case of vomiting as the only complications. Seizures did not occur during any of the 87 trials conducted during phases 2 and 3. Overall, midazolam–ketamine performed satisfactorily in phases 2 and 3 compared with phase 1.

The second goal of phases 2 and 3 was to determine whether Yorkshire and Yucatan pigs demonstrate breed-specific differences in their response to anesthetic drugs. Both breeds of pigs had similar average numbers of injection attempts, similar average injection reactions, and similar times to catheterization. In addition, Yorkshire and Yucatan pigs exhibited similar needs for isoflurane for intubation. However, some important differences were observed. Complications occurred less frequently in Yucatan pigs than in Yorkshire pigs, but the events were more serious in Yucatan pigs. A key observation was that Yucatan pigs required more midazolam–ketamine for both sedation and transport than did Yorkshire pigs. Although we attempted to adjust doses for this need, Yucatan pigs still required isoflurane

more frequently for catheterization than did Yorkshire pigs. This increased dose requirement for Yucatan pigs may be due to a number of factors. Body fat composition differs between Yorkshire and Yucatan pigs, with Yucatan pigs having a greater amount of body fat per kilogram of body weight. Some authors[7] have demonstrated increased volume of midazolam distribution in obese people, and midazolam may be abnormally sequestered in adipose tissue of Yucatan pigs. In addition, genetic influences may result in drug receptor or blood–brain barrier differences. Other factors that may contribute to dose requirement differences were ruled out because our Yucatan and Yorkshire pigs were similar in age and number of sedations, and doses were prescribed on a milligram per kilogram basis. To our knowledge, the only study to observe interspecies differences in the drug responses of Yorkshire and Yucatan pig demonstrated that cocaine induces cardiodepression in Yorkshire pigs and a hyperadrenergic state in Yucatan pigs.[16]

The third, and most important, goal of phases 2 and 3 was to determine a safe and effective anesthetic regimen for CI pigs. We noted several important differences between healthy and CI pigs. Both Yorkshire and Yucatan CI pigs required isoflurane for catheterization more frequently than did their healthy counterparts. CI Yorkshire pigs also required more ketamine to maintain anesthesia during transport than did healthy Yorkshire pigs, and the same was the case for Yucatan pigs. These results indicate that CI pigs of both breeds require higher doses of sedative drugs for catheterization and maintenance than do healthy pigs of both breeds. CI pigs were maintained on various combinations of lisinopril, atenolol, furosemide, clopridogrel, and amiodorone, and none of these medications are expected to affect the action of midazolam–ketamine. The increased dose

Table 3. Summary of results of Phase 2 trials using 0.5 mg/kg midazolam and 25 mg/kg ketamine in healthy and CI Yorkshire pigs and Phase 3 trials using 0.6 mg/kg midazolam and 27 mg/kg ketamine in healthy and CI Yucatan pigs

	No. of injection attempts		Degree of injection reactions		Pigs needing isoflurane		Average time (min) to catheter placement (range)	Pigs needing additional ketamine		Complications	
	Mean	Range (mode)	Mean	Range (mode)	For catheterization (no. [%])	For intubation (no. [%])		For transport (no. [%])	Mean dose (mg/kg [range])	No. (%)	Type (no. [%])
Healthy Yorkshire pigs	1.8	1–5 (1)	2	1–4 (1)	17 of 33 (51.5)	28 of 33 (84.8)	14.1 (5–35)	11 of 33 (33.3)	2.3 (1.5–7)	14 of 33 (42.4)	Apnea 14 of 14 (100)
CI Yorkshire pigs	3.3	2–7 (3)	2.4	1–3 (3)	7 of 10 (70.0)	9 of 10 (90.0)	15.7 (5–25)	3 of 10 (30.0)	2.9 (1.5–5.7)	8 of 10 (80.0)	Apnea 8 of 8 (100)
Healthy Yucatan pigs	1.5	1–2 (3)	1.5	1–2 (2)	5 of 11 (45.4)	11/11 (100)	13.0 (4–20)	4 of 11 (36.3)	2.0 (1.4–2.3)	3 of 11 (27.2)	Apnea 3 of 3 (100)
CI Yucatan pigs	2.5	1–4 (2)	1.8	1–3 (1)	12 of 13 (92.3)	12/13 (92.3)	15.5 (5–30)	3 of 13 (23)	2.7 (1.5–3.4)	6 of 13 (46.0)	Apnea, 4 of 6 (66.6); Vomiting, 1 of 6 (16.6); Tachycardia, 1 of 6 (16.6)

requirement for CI pigs may reflect slower drug distribution due to a CI-related decrease in cardiac output. A 2002 review described cardiac output as a significant source of variability in drug response, supporting the notion that decreased cardiac output in CI pigs may influence the timing and magnitude of anesthetic drug effects.[15] Both CI Yucatan and Yorkshire pigs experienced tachycardia, apnea, and vomiting more frequently than did healthy pigs. Although complications were infrequent and manageable in our phase 3 Yucatan pigs, other investigators reported profound midazolam–ketamine-associated tachycardia (exceeding 200 bpm) and profuse vomiting after these Yucatan pigs were delivered for procedures. Tachycardia in CI pigs may be in response to hypotension caused by a combination of lisinopril, furosemide, and atenolol or a previously undescribed reaction of CI Yucatan pigs to midazolam–ketamine.

In summary, midazolam–ketamine provides a relatively safe and effective means of sedation sufficient for catheterization, intubation, and transport of healthy Yorkshire and Yucatan pigs. This drug combination can provide a prolonged time for catheterization, minimal cardiovascular effects, mild reaction to injection, and a favorable recovery. Although seizures occurred in 2 phase 1 pigs, we did not observe seizures in any of the 87 trials conducted in phases 2 and 3. The dose of midazolam–ketamine will depend on the pig breed, need for transport, and cardiovascular status. In general, Yucatan pigs require more midazolam–ketamine than Yorkshire pigs for catheterization. If sedation and anesthesia are conducted in the same facility, doses of 0.5 mg/kg midazolam and 25 mg/kg ketamine likely will be sufficient for Yorkshire pigs, and doses of 0.6 mg/kg midazolam and 27 mg/kg ketamine likely will suffice for Yucatan pigs. However, if transport is required, an additional 1.5 to 7 mg/kg ketamine may be required to maintain anesthesia in Yorkshire pigs (1.4 to 3.4 mg/kg in Yucatan pigs) during a 5- to 10-min transport period. CI pigs may require even higher doses for catheterization and transport. However, tachycardia may occur with higher doses of ketamine, especially in CI Yucatan pigs. Complications including tachycardia and apnea can be expected to occur more frequently in CI pigs. We adopted these guidelines, and with continued use of midazolam–ketamine at the doses described, we have found that the observations of this study remain valid.

Pigs are common models for cardiovascular research, necessitating safe and effective protocols for sedation, induction, and maintenance of general anesthesia. This study is the first to compare the anesthetic responses of Yorkshire and Yucatan pigs and to attempt to determine a safe and effective anesthesia protocol in CI pigs. We hope our work will prompt additional investigations into finding safe and effective anesthetic methods for CI pigs.

Acknowledgments

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