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Safety and Efficacy of Various Combinations of Injectable Anesthetics in BALB/c Mice

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Four combinations of drugs—ketamine–xylazine, ketamine–xylazine–acepromazine (KXA), ketamine–xylazine–buprenorphine, and ketamine–xylazine–carprofen—were compared for their ability to produce anesthesia in BALB/c mice. Induction time, anesthetic duration, blood pressure, pulse rate, and time to recovery were recorded. The anesthesia induced by each anesthetic combination was assessed by using reflex responses to standardized stimuli. The KXA combination produced stable physiologic parameters and was associated with the longest duration of anesthesia (40 ± 8 min); immobility was produced in all other groups (38 ± 5 min), but a surgical plane of anesthesia could not be confirmed. All anesthetic protocols produced significant hypotension. No deaths occurred. We recommend KXA as a safe and reliable anesthetic for mice requiring a surgical plane of anesthesia.

Abbreviations: A, acepromazine; B, buprenorphine; C, carprofen; K, ketamine; MAP, mean arterial pressure, PRF, forelimb pedal withdrawal reflex; PRH, hindlimb pedal withdrawal reflex; TS, reflexive response to a tactile stimulus; X, xylazine

The selection of an anesthetic regimen for use in research depends on several factors, including species and strain of animal to be anesthetized, health status of the animal, safety considerations, type and duration of the procedure to be performed, recovery time, and research goals.[1,8,11,12] Methods of induction and maintenance of anesthesia include gaseous (induction chamber and delivery of anesthetic by mask or intubation) and injectable anesthesia.[8] Gaseous anesthesia has many advantages, including: 1) increased operator control over depth of anesthesia; 2) agents (for example, isoflurane) that require minimal metabolism, biotransformation, or excretion, thus minimizing research variability; and 3) decreased cardiopulmonary depression, leading to improved safety during induction and decreased recovery time.[20,31,39]

Despite the advantages of gaseous anesthesia, injectable anesthesia has been preferred in mice, possibly because minimal equipment and training is required and initial costs are lower. In addition, the small size of mice relative to the anesthetic equipment makes some procedures difficult to perform in gas-anesthetized mice, especially when working in a biological safety cabinet in barrier facilities.[26] Recommended doses of injectable anesthetics for mice vary substantially,[1] and this broad dose range may be due in part to differences between operators, mouse strains (phenotypes and genotypes), or both. One of the anesthetic combinations used most frequently in rodents is ketamine and xylazine (KX).[1,4,22] Published doses range from 80 to 200 mg/kg ketamine and 0.5 to 10 mg/kg xylazine.[1,4,6,9,11,14,20,22,26,31,38,39] Few of the published dosing regimens for KX are supported by objective assessment of the depth of the ensuing anesthesia, and several reports indicate that KX at recommended doses does not provide a surgical plane of anesthesia in mice.[1,3,5,7] A comparison of the doses of KX used in mice (Table 1) confirms that a wide range of doses appears to have produced satisfactory anesthesia, although a standardized, objective method was not used to assess anesthetic depth.

A technique of general anesthesia, 'balanced anesthesia,' has been recommended in veterinary medicine.[17] Balanced anesthesia is the administration of a mixture of sedatives, analgesics, and anesthetics to produce anesthesia by using lower doses than if each component were to be used by itself . This practice has the advantage of synergy and avoids the unwanted effects seen when increased dosages of individual components are used to produce anesthesia. Balanced anesthesia has the potential for smooth induction, an increased safety margin, and, when analgesics are used, minimization of pain during the immediate postoperative period.[9,17] Acepromazine (A) is a phenothiazine tranquilizer that is used frequently as a preanesthetic in dogs[6] but is rarely used in rodents. However, A is reported to be a useful adjunct to KX anesthesia in mice and rats.[1,36]

Preemptive analgesia has been recommended for painful procedures.[8] There is no consensus on the most suitable analgesic agent for mice or on the optimal time of administration.[21] However, buprenorphine (B) and carprofen (C) are frequently used analgesics in rodent research,[8,21,26,27] and preemptive administration of these drugs has been recommended.[8]

The aim of the present study was to identify a balanced anesthetic regimen that would reliably produce safe surgical anesthesia in mice. A, B, or C was added to KX and administered at induction to provide balanced anesthesia with preemptive analgesia. These anesthetic regimens were compared with KX to assess their ability to produce anesthesia.

Materials and Methods

Animals and housing. We obtained 6-mo-old female BALB/cAnNCrlCr mice from the National Cancer Institute (Bethesda, MD). This particular strain was selected because it is one of the most widely used inbred strains in biomedical research.[19] The Institutional Animal Care and Use Committee of the Roswell Park Cancer Institute approved all procedures.

Upon arrival, mice were free of viral, bacterial, and parasitic diseases, and their health status was monitored under the

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Table 1. Published K and X dosages used in mice

Dose (mg/kg)		Comments in publication regarding undesirable effects of KX	Additional comments regarding anesthesia	Reference(s)
K	X			
100	5	Cardiac depression, bradychardia, and hypotension	• Authors measured duration from loss to regaining of righting reflex without assessing depth of anesthesia. • Authors added morphine to KX and then taped mice to operating table.	15,31
60	6	Significant reduction in heart rate with substantial influence in cardiac and hemodynamic parameters	Goal of anesthesia was loss of righting reflex or immobilization for transthoracic echocardiography	22
150	15	Decreased systolic arterial pressure	Only measured with respect to immobility of mice	10
50	10	Produced more pronounced decreases in blood pressure and cardiac index than did isoflurane or pentobarbital	Additional KX was needed before mice showed loss of pedal withdrawal reflex	20
65	4.1	Profound bradychardia	Supplemented mice with half of the initial dose to maintain adequate levels of anesthesia	14
80	10	• Significant reduction in heart rate. • Inhalant anesthetic was considered to be superior to KX for echocardiography analysis	Anesthesia was achieved with isoflurane but not KX	4
100	20	All reflexes were positive to stimulus	Anesthesia was not achieved—only immobilization	1
150	20	Increased death rate by 40%	30% of the animals tested achieved anesthesia.	1
150	15	Significant negative inotropic and chronotropic effects on the mouse heart	Mice only immobilized (not anesthetized) for echocardiography	38
90 or 150[a]	75 or 12.5	Induced changes in heart rate and blood pressure	Anesthesia adequate for superficial surgical procedures	11
80	5	Bradycardia and hypotension	• No description of assessment of depth of anesthesia • Most mice were immobilized for measurement of blood pressure	18

[a]Low dose of 90 mg/kg K and 75 mg/kg X repeated after 20 min. Single, high dose of 150 mg/kg K and 12.5 mg/kg X.

institutional animal health surveillance program. Mice were housed in an AAALAC-accredited facility and the cages were maintained on a ventilated rack system in which each cage was individually ventilated with HEPA filters at a rate of 55 to 60 air changes hourly. Each cage was provided with reverse-osmosis water delivered by an automatic watering system and supplied with sterilized food (mouse diet 7012 with 5% fat, Harlan–Teklad, Madison, WI) and bedding (1/4-in. grit, Bed o'cobs, The Andersons, Maumee, OH). All rodent manipulations and cage changes were performed in biosafety cabinets or laminar airflow workstations. Room temperatures were controlled by reheating units inside rooms and were maintained at 21.7 ± 1.7 °C. The humidity was maintained at 40% to 70%.[29]

Experimental procedures. ***Anesthetic groups.*** Mice were randomly allocated into 4 groups and identified by ear punch. Each group received 1 of 4 anesthetic combinations. All agents were diluted in sterile saline to provide a final dose of 0.1 ml per 10 g of body weight and, except for carprofen, were administered by IP injection. Carprofen was administered at 4 mg/kg SC.

The KX group (n = 7), which served as the control group, received ketamine HCl (100 mg/ml, Fort Dodge Animal Health, Overland Park, KS) at a dose of 100 mg/kg and xylazine (20 mg/ml, The Butler Company, Dublin, OH) at a dose of 10 mg/kg. The KXA group (n = 7) received ketamine HCl (100 mg/kg), xylazine (10 mg/kg), and acepromazine maleate (10 mg/ml, Boehringer Ingelheim, Ridgefield, CT) at a dose of 3 mg/kg. The KXB group (n = 5) was given ketamine HCl (100 mg/kg), xylazine (10 mg/kg), and buprenorphine (0.3 mg/ml, Abbott Labs, Abbott Park, IL) at a dose of 0.05 mg/kg. The KXC group (n = 7) received ketamine HCl (100 mg/kg) and xylazine (10 mg/kg) IP and carprofen (50 mg/ml, Pfizer, New York, NY) at a dose of 4 mg/kg by SC injection.

Hemodynamic parameters. A noninvasive blood-pressure analysis system (BP-2000, Visitech Systems, Apex, NC) was used to measure indirect blood pressure and pulse rate.[24] The system consists of a mouse platform, control unit, and computer. The heated mouse platform has a mounted tail cuff, with an embedded sensor, which is connected to the computer. All animal handling and the platform were maintained inside a biosafety cabinet during the experiment. The blood-pressure analysis system was calibrated prior to each experiment and was programmed to measure average (from 10 consecutive readings) systolic and diastolic blood pressure and pulse rate every 5 min.

Anesthetic induction and monitoring. Animals were weighed and injected with one of the anesthetic combinations. Once the righting reflex was lost, the animals were placed on a heated platform, their eyes lubricated with ointment (ParalubeVet, PharmaDerm, Duluth, GA), and then monitored with the aid of the blood-pressure analysis system. The tail was passed through a cuff to initiate monitoring of physiologic parameters (Figure 1). The variables recorded were: induction time, time to loss of righting reflex, indirect systolic and diastolic arterial blood pressure, pulse rate, time to start of anesthesia (the point

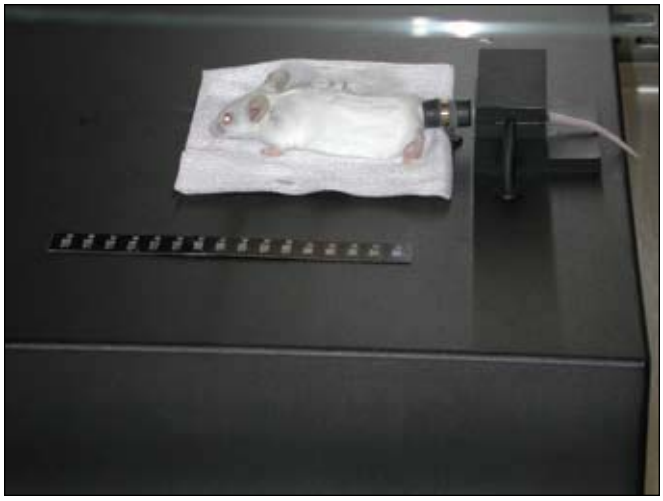

Figure 1. Animal anesthetized and placed in blood-pressure analysis system for monitoring of blood pressure and pulse rate. The platform is maintained at a constant temperature of 98 °F.

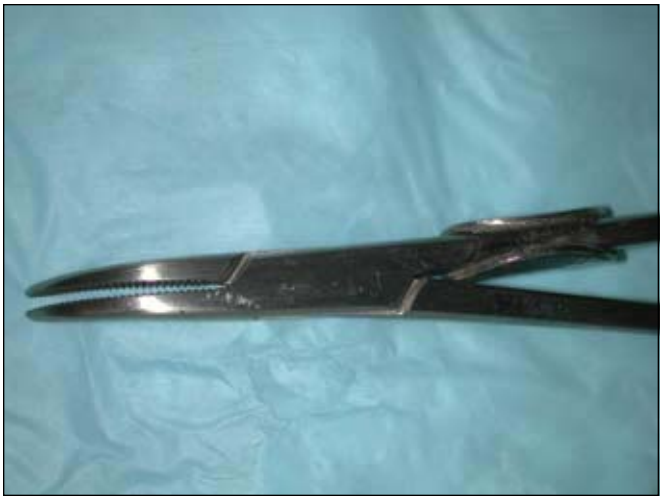

Figure 2. A metal clip was added to forceps to ensure a standardized stimulus for inducing pedal reflexes.

at which a tactile stimulus failed to induce a response and at which the forelimb or hindlimb pedal withdrawal reflex response was absent or delayed), time to loss of anesthesia (the point at which a moderate or rapid pedal withdrawal reflex was regained), and time to recovery of the righting reflex. To standardize the amount of pressure applied to the paw during pedal withdrawal reflex testing, a curved 5-in. Halstead mosquito forceps was modified by inserting a metal clip on 1 arm (Figure 2), thus precluding complete closure of the jaws, standardizing the maximal pressure applied to the interdigital area, and minimizing tissue trauma. Reflexes were recorded as fast (score, 1), moderate (score, 2), slow (score, 3), or absent (score, 4); the lower the score, the stronger the attempt to withdraw the limb after the stimulus. The same scoring system was used for tactile stimulus response.

Assessment of anesthetic depth. To assess the reflexive response to a tactile stimulus (TS), the vibrissae were touched with a sterile forceps. The response to movement was scored from 1 to 4 as described, and the reflex was assessed every 5 min.

The pedal withdrawal reflex was assessed in forelimbs and hindlimbs (PRF and PRH, respectively). To this end, the interdigital web was grasped with the modified forceps, and the extent of any movement of the leg away from the noxious stimulus was scored from 1 to 4, as described. The stimulus was repeated every 5 min on alternate legs until the animal displayed spontaneous locomotor activity, which was recorded as the recovery time.

Surgical anesthesia was defined as the time when both PRF and PRH were greater than or equal to 3 and when the TS score was 4. Animals with scores that did not meet these criteria were considered to be in a light plane of anesthesia and thus unsuitable for major surgery.

Postanesthetic care and monitoring. Once a mouse demonstrated spontaneous movement, 1 ml of 0.9% sodium chloride was administered SC and the mouse returned to its cage. Animals were monitored until they were fully ambulatory. All animals were monitored daily for 3 consecutive days to assess anesthesia recovery.

Statistical analysis. Data were summarized by using simple descriptive statistics. All statistical analyses were carried out using SAS (version 9.1, SAS Institute, Cary, NC), with significance being associated with a P value of less than 0.05. In the examination of longitudinal patterns of mean arterial blood pressures and pulse rate, a random coefficient mixed model, also known as a hierarchical linear model,[2] was fit to each of the 3 parameters. In our model, we assumed that the 4 anesthetic groups potentially differed in regards to the mean measure of the parameter over time. Aproximate F-tests for overall group differences were performed. When overall group differences existed, follow-up testing between pairs of groups was done in conjunction with Bonferroni correction. The hypothesis test results reflect equality of the group means from induction to recovery, not at a specific time point. We felt that this approach to the analysis was appropriate because differences in the overall patterns of the means were of interest.[2] Graphs of the mean parameters over time for each group were generated. The distributions of the times until the mice regained consciousness were compared by using the log-rank test.

Results

In general, clinical experience suggests that addition of an analgesic such as an opioid to an anesthetic combination be accompanied by a reduction in the dose of the anesthetic to prevent excessive cardio-respiratory depression.[20] Initially in the KXB group, the doses of K and X were reduced from 100 and 10 mg/kg, respectively, to 70 and 7 mg/kg when buprenorphine (0.05 mg/kg) was used. However the first 2 animals that received this combination did not lose their righting reflexes. Therefore we increased the K and X doses to 100 and 10 mg/kg, respectively, for the remaining animals in this group (n = 5) and discarded data from the first 2 animals, which could no longer be considered naive, from the statistical evaluation.

Hemodynamic parameters. ***Blood pressure.*** Mean arterial blood pressure (MAP) differed significantly ($P < 0.0001$) between groups, as did the patterns of the group means. All groups demonstrated marked hypotension, compared with the normotensive values measured in a conscious animal.[24]

Blood pressure measurements recorded over time (Figure 3) revealed that the MAP of the KXA and KXB groups at 5 min from loss of the righting reflex were lower than those of the KX and the KXC groups. This trend was maintained at 10 min after induction, when MAP in the KXB group started to increase slowly. By 20 min after induction, the MAP in the KXA group was greater than 40 mm Hg, and that in the KXB group continued to increase. By 30 min, MAP in the KXB group had increased significantly ($P < 0.05$), and that in the KXA group remained above 40 mm Hg. At 40 min after induction, the ani-

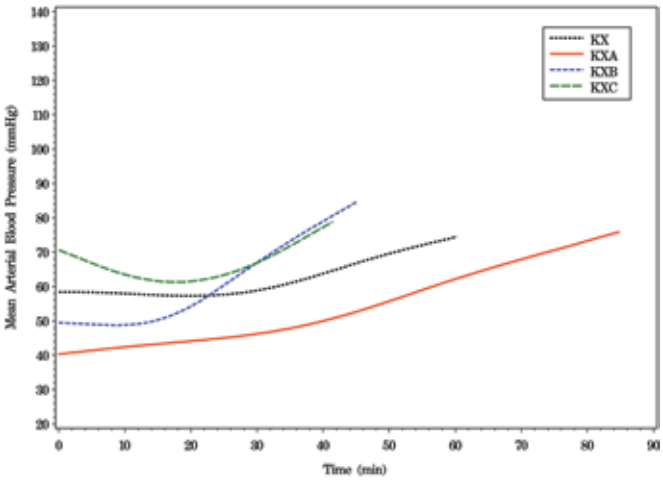

Figure 3. Mean arterial pressure (MAP) in mice over time after administration of 4 anesthetic combinations. Time 0 indicates onset of loss of righting reflex. Groups KXA, KXB, and KXC showed significant ($P < 0.05$) differences from the control group (KX). The normotensive value in a conscious animal that was used for comparison was 119.7 ± 3.3 mm Hg.[24]

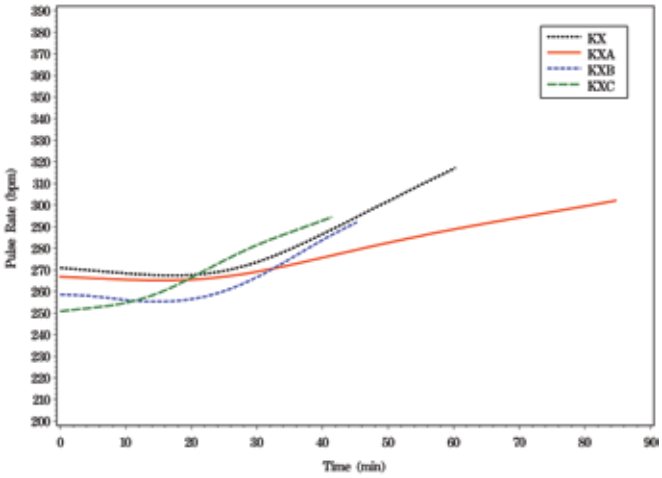

Figure 4. Pulse rates in mice over time after administration of 4 anesthetic combinations. Time 0 indicates onset of loss of righting reflex. Bradycardia was seen after all of the anesthetic combinations. The normal pulse rate of a conscious animal as measured with the blood pressure analysis system is 550 ± 7 beats per minute (bpm).[24]

mals in the KXC group had regained consciousness; mice in the KXB and KX groups also were awake shortly thereafter. At the 40-min point, MAP in the KXB group was at approximately 80 mm Hg. The KXA group had a gradual increase in MAP until they regained consciousness at 74.4 ± 12.0 min.

Pulse. Pulse rate (Figure 4) differed significantly ($P < 0.0001$) between groups, and group mean patterns differed from each other. Marked bradycardia was present in all groups, compared with the pulse rate in a conscious mouse as measured by using the same system.[24] During the first 10 min after induction, KXB mice showed a decrease in pulse rate when compared with all groups. Animals that received KXC had a gradual increase in pulse rate from induction time to recovery time (at 37.0 ± 9.5 min). The KX and KXA groups showed less variation in pulse rate, maintaining ranges of 270 ± 5 beats per minute for the first 20 min after induction and then gradually increasing until regaining spontaneous movement at 52 ± 1 and 74 ± 12 min, respectively, after induction.

Assessment of anesthetic depth. The scores for TS, PRF, and PRH for mice in each group were analyzed by averaging the responses of all animals in the group at the given time point. Interanimal variability in each group was minimal. All mice within a particular group responded fairly consistently to the reflex stimulus, and scores were very consistent throughout the immobilization time.

TS scores in all groups were of 4 (absent) throughout the immobilization time, and lower scores of 2 and 1 (indicating moderate to fast response to the stimulus) were apparent just before the animals regained spontaneous movement.

PRF scores indicated that animals in the KX group lacked responses at a single time point throughout the immobilization time and then displayed either a slow or moderate response to the stimulus at the remaining time points. Groups KXB and KXC had either moderate or fast responses to the stimulus at all time points evaluated. Responses were markedly different for the KXA group, in which all responses were slow to absent throughout 30 min of immobilization .

PRH scores for animals in groups KX, KXB, and KXC were 1 (fast) at all time points measured, indicating a light plane of anesthesia. A marked difference was noticed in the mice in group KXA, in which the reflex was absent (score, 4) for at least 30 min of the immobilization time. Only the KXA group attained a surgical plane of anesthesia, as defined by a TS score of 4 and PRF and PRH scores of 3 or greater. The other groups reached only a light plane of anesthesia, as indicated by immobility with rapid response to the standardized stimulus.

Time-course comparisons. A time-course comparison of the effects of each of the 4 anesthetic protocols is presented in Table 2. Mice in the KX, KXB, and KXC groups lost the righting reflex but failed to attain a surgical plane of anesthesia. The KXA animals achieved a surgical plane of anesthesia. The total immobilization time for KXA mice was significantly longer than for animals in the other 3 anesthetic groups. No mortalities occurred in any group during the 3-d observation period after anesthesia.

Discussion

The combination of KXA was a safe and reliable anesthetic that provided surgical anesthesia for approximately 45 min in BALB/c mice. Mice were ambulatory within 2 h, and no deaths occurred. These results are similar to those from previous work in different strains of mice[1,20,26] and in rats[7,36] in which KXA provided surgical anesthesia.

KXA had substantial cardiovascular effects,[33] as manifested by low pulse rates and hypotension. As a dissociative anesthetic, K causes immobilization, analgesia, and hypotension, and muscle relaxation and analgesia have been recorded in animals after administration of X.[38] Anesthetics are known to affect the circulatory system, and the addition of acepromazine, a phenothiazine tranquilizer, potentates their action. The marked hypotension associated with KXA was most likely due to the combined effects of the anesthetics and the peripheral vasodilatory properties of acepromazine.[9]

Cardiovascular responses differ greatly between anesthetized and conscious animals.[35,38,39] The MAP in anesthetized mice is 80 ± 10 mm Hg, with average systolic pressures of 112 ± 10 and mean diastolic pressures of 42 ± 12 mm Hg.[30] The MAP associated with the KXA combination indicated hypotension, and the pulse rate, although stable, was decreased markedly. Several factors, including strain,[16] sex, and metabolic state,[3,34] can affect hemodynamic parameters during anesthesia. An evaluation of the effects of anesthesia on the hemodynamic parameters of 4 commonly used mouse strains (Swiss, CD1,

Table 2. Time course of immobilization and anesthesia after administration of 4 anesthetic combinations to BALB/c mice

	KX (control)	KXA	KXB	KXC
Time from induction to LRR	1.91 ± 0.013	1.43 ± 0.53	1.85 ± 0.38	2.00 ± 0.58
Time from LRR to surgical plane of anesthesia	surgical anesthesia not achieved	8.17 ± 1.80	surgical anesthesia not achieved	surgical anesthesia not achieved
Duration of surgical plane of anesthesia	surgical anesthesia not achieved	40.0 ± 8.6	surgical anesthesia not achieved	surgical anesthesia not achieved
Duration from loss of surgical plane of anesthesia to recovery	surgical anesthesia not achieved	26.15 ± 9.05	surgical anesthesia not achieved	surgical anesthesia not achieved
Total immobilization time	52.30 ± 0.11	74.43 ± 12.08	37.70 ± 9.50	38.15 ± 5.40

LRR, loss of righting reflex.
Time data are given in minutes and presented as mean ± SEM.

BALB/c, and C57Bl6) revealed that MAP and heart rate were similar among the 4 strains of mice.[39] A comparison of the effects of pentobarbital (80 mg/kg) with those of 2 dosages of KX (90 mg/kg K with 7.5 mg/kg X repeated after 20 min and 150 mg/kg K with 12.5 mg/kg X) in pregnant and nonpregnant ICR mice revealed that mean blood pressure was lower and heart rate was higher in pregnant than in nonpregnant mice for each anesthetic protocol. [11]

Responses to pedal withdrawal reflex stimuli were absent (indicating deep anesthesia) in mice that received KXA. Even though deaths did not occur, the dose for the KXA combination can still be refined by future research to optimize anesthesia while minimizing cardiovascular depression.

Although KX is commonly used and was chosen as the anesthetic standard for this study, it failed to induce anesthesia either alone or in combination with 2 recognized analgesics. At best KX decreased sensory perception and motor responses but did not abolish pain perception. Similar results were reported for the transgenic strain HanIbm:NMRI when the same dose was used to induce anesthesia.[1] When the doses of K and X were increased in an attempt to attain surgical anesthesia, a mortality rate of 40% occurred.[1] Given these similar results in different strains of mice, a reasonable conclusion is that KX has a narrow therapeutic range and provides insufficient analgesia for surgery.

The addition of an opioid analgesic such as buprenorphine[23] and a nonsteroidal antiinflammatory drug, carprofen, to KX at the time of induction neither induced nor prolonged anesthesia compared with that after KX. KX and KXC produced immobilization, but neither combination induced a surgical plane of anesthesia. The addition of carprofen to an anesthetic mixture could provide preemptive analgesia. However, our data suggest that carprofen, and perhaps other nonsteroidal antiinflammatory drugs, will not increase the depth of KX-based anesthesia in mice.

The addition of B to KX did not deepen or prolong the anesthesia induced but did increase cardiovascular depression.[27] The lower blood pressure associated with the KXB combination agrees with previous results, in which the addition of the opioid caused a drop in blood pressure. Coadministration of B with light isoflurane anesthesia in mice depressed blood pressure and increased mortality.[20] Although there were no deaths in the present study, these previous data suggest that addition of B to any injectable or gaseous anesthetic regimen requires prior evaluation of the health of the cardiovascular system and careful monitoring of blood pressure throughout the procedure.

Continuous measurement of physiologic parameters such as heart rate and blood pressure are used routinely in human and veterinary anesthesia (large animals) to assess the adequacy of anesthesia or, conversely, the likelihood that pain is being experienced.[17] Similar monitoring during anesthesia of mice is not always practical, because of their small size and the need for specialized equipment. Close observation and reflex response tests are simple to perform and do not require sophisticated equipment. Although the reliability of reflexes varies across species, reflex assessment is considered an acceptable method of determining anesthetic depth and surgical tolerance.[1,8,30,32,38,39]

In the current study, the tactile stimulus reflex was a poor indicator of levels of anesthesia, because TS was absent before other reflexes were lost, and it reappeared close to recovery. We found that pedal withdrawal reflexes were the most useful reflexes for assessing anesthetic depth in mice. One unanticipated finding was the difference between the responses of the forelimb and hindlimb; this difference was present in all groups. The reflex response of the forelimb was lost before that of the hindlimb and thus well before surgical anesthesia was attained. Our data suggest that the hindlimb reflex is a reliable indicator of anesthetic depth. In contrast, the reflex in the forelimb should not be used as the sole criterion to assess anesthetic depth because its response was reduced markedly compared with that of the hindlimb when pedal withdrawal reflexes were assessed at the same time points by using standardized stimuli.

One of the goals of this study was to evaluate whether the various anesthetic combinations significantly affected cardiovascular parameters such as blood pressure and pulse rate.[33] The current study also provided an opportunity to use blood pressure analysis equipment that had not previously been evaluated in anesthetized mice. Techniques for measuring blood pressure in experimental animals can be divided into direct and indirect methods.[25] In animals, the most common indirect method for monitoring blood pressure is the cuff technique,[9] in which the blood pressure is measured by determining the cuff pressure at which changes in blood flow in the tail or a limb occur during occlusion or release of the cuff. Indirect methods are considered to have 4 main advantages: (1) they are noninvasive and do not require surgery; (2) they can be used to obtain repeated measurements of blood pressure in conscious animals during studies of short or long duration; (3) they require less expensive equipment than some direct methods (for example, telemetry);[5,12,28] and (4) they can be used to screen for systolic hypertension or substantial differences in systolic blood pressure among large number of animals. Although indirect methods clearly are suitable for measuring blood pressure in some circumstances, they have 3 main disadvantages: (1) indirect methods measure blood pressure for only a few cardiac cycles; (2) despite their noninvasiveness and well-intended efforts to acclimate animals to the procedures, indirect methods impose considerable stress that disturbs multiple aspects of the cardiovascular system; and (3) the accuracy of indirect measurement methods in animals

is open to question.[8] Other studies[4,8,9,10] suggest that hemodynamic measures can vary markedly between indirect and direct (invasive) methods of assessing blood pressure in rodents. It generally is advised that the physical accuracy of the indirect methods should be established through calibration against a mercury column and by comparing indirect pressure measurements with simultaneously obtained direct measurements of arterial pressure.[13,28,37] However, most of the recommendations address the measurement of BP in unanesthetized conscious animals.

Many of the current data were recorded by using a noninvasive blood pressure analysis system, which we found to be a useful adjunct for monitoring cardiovascular parameters in anesthetized mice. We also used a series of reflexes to assess anesthetic depth. The hindlimb pedal withdrawal reflex was the most useful reflex for assessing anesthetic depth in our study, whereas the equivalent reflex in the forelimb was less useful and should not be used as the sole parameter for assessment of surgical anesthesia. The drug combinations tested in the present study induced profound changes in MAP and pulse rate when compared with values obtained from conscious mice in other studies.[14] Our findings further suggest that KX, KXB, and KXC at the doses tested are not suitable anesthetics for major surgery in BALB/c mice. In contrast, although further work is needed to identify the optimal mix of components to reduce the significant hypotension induced, we found that KXA (at the doses used in the present study) appeared to be efficacious in providing a long, safe, and satisfactory anesthesia for performing surgical procedures in BALB/c mice.

Acknowledgments

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