A randomized clinical trial on effects of alfaxalone combined with medetomidine and midazolam in preventing stress-related neurohormonal and metabolic responses of isoflurane-anesthetized cats undergoing surgery

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OBJECTIVE
To evaluate the effects of IM and IV administration of alfaxalone alone and in combination with medetomidine, midazolam, or both on key stress-related neurohormonal and metabolic changes in isoflurane-anesthetized cats undergoing ovariohysterectomy or castration.

ANIMALS
72 client-owned mixed-breed cats undergoing ovariohysterectomy or castration between October 4, 2018, and January 10, 2020.

PROCEDURES
For each type of surgery, cats were assigned to 1 of 6 premedication protocols groups, with 6 cats/group: physiologic saline (0.9% NaCl) solution (0.5 mL, IM) and alfaxalone (5 mg/kg, IV); physiologic saline solution (0.5 mL, IM) and alfaxalone (5 mg/kg, IM); medetomidine (50 μg/kg, IM) and alfaxalone (5 mg/kg, IV); medetomidine (50 μg/kg, IM) and alfaxalone (5 mg/kg, IM); midazolam (0.5 mg/kg, IM) and alfaxalone (5 mg/kg, IM), medetomidine (50 μg/kg, IM), and alfaxalone (5 mg/kg, IV); or midazolam (0.5 mg/kg, IM), medetomidine (50 μg/kg, IM), and alfaxalone (5 mg/kg, IV). Venous blood was taken before pretreatment, pre- and postoperatively during anesthesia with isoflurane and oxygen, and during early and complete recovery.

RESULTS
Compared with baseline concentrations, plasma adrenaline and noradrenaline concentrations decreased during anesthesia in cats premedicated with alfaxalone alone and in combination with medetomidine. The combination of medetomidine, midazolam, and alfaxalone prevented an excessive increase in catecholamines during anesthesia and surgery in cats. Postoperative plasma cortisol concentration after ovariohysterectomy was lower for cats premedicated with the combination of medetomidine and alfaxalone or the combination of medetomidine, midazolam, and alfaxalone, compared with cats premedicated with alfaxalone alone. Cats treated with combinations that included medetomidine and midazolam had hyperglycemia during anesthesia. Cats treated with medetomidine or medetomidine and midazolam in combination with alfaxalone, compared with alfaxalone alone, had lower concentrations of nonesterified fatty acids during anesthesia. Behavioral recovery scores were lower (better) for cats that received medetomidine in addition to alfaxalone, compared with alfaxalone alone.

CLINICAL RELEVANCE
Results indicated that pretreatments with medetomidine and alfaxalone or with medetomidine, midazolam, and alfaxalone were useful for preventing stress-related hormonal and metabolic responses, other than hyperglycemia, during isoflurane anesthesia and surgery in cats.

Alfaxalone (3α-hydroxy-5α-pregnane-11, 20-dione) is a newly developed anesthetic agent that is useful for the sedation, induction, and maintenance of anesthesia in cats.1,2 However, during the recovery period, this agent produces more adverse events than propofol, including ataxia and muscular tremors.3,4 The quality of recovery from anesthesia with alfaxalone is reported to be improved with the use of...
other sedatives, inhalant anesthetic agents, or both.\(^1\) The \(\alpha_2\)-adrenoceptor agonist medetomidine is widely used in feline veterinary practice as an excellent sedative; however, it induces undesirable effects, such as bradycardia, hyperglycemia, and emesis.\(^5\)-\(^7\) A combination of medetomidine with midazolam and ketamine produces good analgesia and anesthesia in cats, with better analgesia potentiation.\(^8\)-\(^11\) Therefore, there is a need to investigate the effects of using an injectable anesthetic, alfaxalone, in combination with medetomidine and midazolam as a pre-anesthetic medication in feline veterinary practice.

Stressors such as anxiety, excitement, pain, anesthesia, and surgery induce neurohormonal and metabolic changes in animals; these changes are characterized by elevated concentrations of blood cortisol, catecholamines, glucose, and nonesterified fatty acid (NEFA).\(^12\)-\(^14\) Actions mediated by \(\alpha_2\)-adrenoceptors are closely coordinated with these stressors.\(^5\) In cats, anesthesia with medetomidine, midazolam, and ketamine suppresses the release of catecholamine and cortisol, suppresses lipolysis, and induces hyperglycemia.\(^15\) Thus, there is a need to evaluate cats' stress-related hormonal and metabolic responses during anesthesia and postoperatively in clinical practice.

Medetomidine prevents or delays the stress response induced by ovariohysterectomy in isoflurane-anesthetized dogs.\(^16\) In halothane-anesthetized dogs undergoing ovariohysterectomy, medetomidine prevents an increase in plasma cortisol concentrations both during the surgery and during early recovery.\(^17\) Medetomidine appears to offer some advantages over acepromazine in terms of decreasing perioperative concentrations of stress-related hormones, including plasma catecholamine and cortisol.\(^18\) However, to the best of our knowledge, there are no published reports on the effects of pretreatment with alfaxalone alone, and in combination with other sedatives, on stress responses in anesthetized cats undergoing surgery. Therefore, this study aimed to evaluate the effects of IM and IV administrations of alfaxalone alone and in combination with medetomidine, midazolam, or both on key stress-related neurohormonal and metabolic changes in isoflurane-anesthetized cats undergoing ovariohysterectomy or castration. This study was designed to assess stress responses during clinically important perioperative stages in feline veterinary practice.

**Materials and Methods**

**Animals**

Seventy-two client-owned, mixed-breed cats (36 males, 36 females) were prospectively recruited at the Kamohara Animal Hospital for ovariohysterectomy or castration between October 4, 2018, and January 10, 2020. Cats were healthy and ranged from 6 months to 1 year of age. The mean ± SD body weight of males and females were 3.76 ± 0.39 kg and 3.13 ± 0.54 kg, respectively. Informed consent was obtained from the owner of each cat for the procedure undertaken and data collection. Physical and routine hematologic examinations prior to the study revealed that all values were within the reference ranges. All cats were fasted for 12 hours but had ad libitum access to water. Owners brought their cats to our hospital early in the morning on the day of surgery. After preparation for surgery and anesthesia, each cat rested in a darkened cage for 2 to 3 hours before anesthesia. After complete recovery from anesthesia, all cats received water ad libitum and food.

Ethical approval from a committee was not necessarily required, because the drugs and anesthesia procedures used in this study were already accepted in veterinary practice. Informed consent was essential to obtaining from the owner of all animals for the procedure undertaken. Specific attention was paid for preoperative and postoperative analgesia in this study.

**Study protocol**

For each type of surgery (ovariohysterectomy or castration), cats were randomly assigned to 1 of 6 treatment groups (6 cats in each group): physiologic saline (0.9% NaCl) solution (0.5 mL, IM) and alfaxalone (5 mg/kg, IV; alfaxalone IV group); physiologic saline solution (0.5 mL, IM) and alfaxalone (5 mg/kg, IM; alfaxalone IV group); medetomidine (50 μg/kg, IM) and alfaxalone (5 mg/kg, IV; medetomidine + alfaxalone IV group); medetomidine (50 μg/kg, IM) and midazolam (0.5 mg/kg, IM; medetomidine + midazolam IV group); midazolam (0.5 mg/kg, IM), and alfaxalone (5 mg/kg, IV; medetomidine + midazolam + alfaxalone IV group); or midazolam (0.5 mg/kg, IM), medetomidine (50 μg/kg, IM), and alfaxalone (5 mg/kg, IM; medetomidine + midazolam + alfaxalone IM group).

Medetomidine, midazolam, or both were administered 10 minutes prior to administration of alfaxalone. For cats that received medetomidine and midazolam, the 2 drugs were mixed in the same syringe immediately prior to injection. In the alfaxalone IM group and the medetomidine + alfaxalone IM group, anesthesia was induced with 4% isoflurane in oxygen at a total gas flow rate of 1.5 L/min using a facemask attached to the veterinary anesthesia delivery system (ADS 1000; Engler, Hialeah, FL). Then, cats were intubated with a cuffed endotracheal tube. In the alfaxalone IV group and the medetomidine + alfaxalone IV group, anesthetic induction was assisted with a face mask when the effect on tracheal intubation was insufficient. In the medetomidine + midazolam + alfaxalone IV group; or midazolam (0.5 mg/kg, IM), medetomidine (50 μg/kg, IM), and alfaxalone (5 mg/kg, IM; medetomidine + midazolam + alfaxalone IM group).

Medetomidine, midazolam, or both were administered 10 minutes prior to administration of alfaxalone. For cats that received medetomidine and midazolam, the 2 drugs were mixed in the same syringe immediately prior to injection. In the alfaxalone IM group and the medetomidine + alfaxalone IM group, anesthesia was induced with 4% isoflurane in oxygen at a total gas flow rate of 1.5 L/min using a facemask attached to the veterinary anesthesia delivery system (ADS 1000; Engler, Hialeah, FL). Then, cats were intubated with a cuffed endotracheal tube. In the alfaxalone IV group and the medetomidine + alfaxalone IV group, anesthetic induction was assisted with a face mask when the effect on tracheal intubation was insufficient. In the medetomidine + midazolam + alfaxalone IV group; or midazolam (0.5 mg/kg, IM), medetomidine (50 μg/kg, IM), and alfaxalone (5 mg/kg, IM; medetomidine + midazolam + alfaxalone IM group).
solution (10 mL/kg, IV) was administered throughout anesthesia and surgery. The duration of isoflurane anesthesia was 30 and 60 minutes in castrated and ovariohysterectomized cats, respectively, after which time isoflurane inhalation was completely stopped. The endotracheal tube was extubated once a laryngeal reflex was visible. A laryngeal reflex was assessed by the response of the larynx when the tracheal tube was moved slightly or the tongue was pulled.

During the recovery process, the cats remained in separate cages in a room with an air temperature of 25°C. General postoperative management and care were performed for all cats. After complete recovery, butorphanol (0.1 to 0.4 mg/kg, IM) was administered to cats with signs of pain, such as those exhibiting vocalizations, anorexia, and pain-related postures.

**Anesthesia and intraoperative monitoring**

An agent-specific precision vaporizer was used to administer isoflurane. Gas samples were collected from the breathing circuit through a tube attached to an adapter positioned at the oral end of an endotracheal tube. During anesthesia, we assessed the expired end-tidal isoflurane and carbon dioxide concentrations, arterial oxygen saturation using pulse oximetry, heart rate, respiration rate, rectal temperature, and mean blood pressure (mean arterial pressure [MAP]) using the oscillometric method, either continuously or intermittently, with a multiparameter monitor (BSM-5192; Nihon Kohden, Tokyo, Japan). The MAP was calculated by the following formula: MAP = diastolic pressure + (systolic pressure − diastolic pressure)/3. Averaged MAP, heart rate, and expired end-tidal isoflurane concentration were calculated as a mean of multiple measurements throughout anesthesia for each animal. Airway gas was continuously sampled at the rate of 200 mL/min, and the gas was not returned to the circuit. During controlled ventilation, the respiration rate was adjusted to a range of approximately 25 to 35 mm Hg of expired end-tidal carbon dioxide. Maximum inspiration pressure was set at 20 cm H2O.

The adjustment of anesthesia was carried out by a veterinarian skilled in anesthetic methods. The person was aware of the treatment group. The depth of anesthesia was judged by the lack of response to surgical stimuli. A positive response to stimuli was defined as gross purposeful muscular movement of the head or extremities. If an animal showed a positive response, the end-tidal isoflurane concentration was increased by 10% to 20%.

**Behavioral scoring of recovery**

The overall quality of recovery from anesthesia was assessed using previously published scoring methods, as follows: score 1 = excellent; score 2 = good; score 3 = moderate; score 4 = poor; and score 5 = extremely poor. The observer was blinded to treatment. Times to extubation and the head-up motion after discontinuing isoflurane anesthesia were also assessed in all groups.

**Blood sample collection**

Blood sample collection was conducted during clinically important perioperative stages. Blood samples (2 mL) were collected from a 24-gauge catheter inserted into a cephalic vein on 5 occasions: immediately before pretreatment (baseline); 5 minutes after induction of anesthesia and before the surgical procedure during anesthesia (preoperatively); after completing the surgical procedure during anesthesia (postoperatively); at head-up motion after removal of the tracheal tube after discontinuation of anesthesia (early recovery); and 180 minutes after the discontinuation of anesthesia (complete recovery). Postoperative blood samples were collected at 25 and 55 minutes in the castrated and ovariohysterectomized groups, respectively.

**Sample processing and analysis**

Blood was mixed with EDTA to prevent clotting. Samples were immediately centrifuged (2,000 X g, 15 minutes); the plasma was separated and frozen at −76°C until analysis. Glucose, NEFA, cortisol, adrenaline, and noradrenaline concentrations were measured according to previously published methods. In brief, glucose and NEFA concentrations were determined via an enzyme assay (Glucose CII-test and NEFA C-test, respectively; FUJIFILM Wako Pure Chemical Co., Osaka, Japan) and a spectrophotometer (U-2001; Hitachi High-Tech Science Co., Tokyo, Japan). Cortisol concentrations were measured via a solid phase-antibody radioimmunoassay (Cortisol Kit FR; Fujirebio Co., Tokyo, Japan). Catecholamines were extracted on activated alumina and measured using high-performance liquid chromatography and an electrochemical detector (Coulochem II; ESA Biosciences, Inc., MA).

**Statistical analysis**

All data were analyzed using commercially available software (Prism, version 7.0; GraphPad Software Inc., San Diego, CA). All data, except for recovery score data, were reported as mean ± SD. Score data were reported as median and range. All data other than score data were tested for normality using the Shapiro-Wilk test. A repeated-measures 1-way ANOVA was used to examine differences across variables within each group. Post hoc Dunnett multiple-comparisons tests were used to identify differences from baseline within each group. One-way ANOVA and post hoc Tukey multiple-comparisons tests were used to determine differences across groups. In all tests, the significance level was set at P < .05. Score data were analyzed using the Wilcoxon-Mann-Whitney test for treatment comparisons; P < .00833 was considered significant by a Bonferroni correction.

**Results**

The number of cats that received butorphanol was 2 in the alfaxalone IV group and 5 in the alfaxalone IM group in castration and 3 in the alfaxalone IV group, 5 in the alfaxalone IM group, 1 in the...
medetomidine + alfaxalone IV group, and 1 in the medetomidine + alfaxalone IM group in ovariohysterectomy. Although butorphanol was a short-acting analgesic, analgesia seemed to be effective with its administration for these cats. There were no issues with surgery or anesthesia in any of the cats. Since butorphanol was administered after the last blood sampling at 180 minutes after the discontinuation of anesthesia, its administration did not have any influence on the blood values of hormonal and metabolic variables measured in this study.

The mean respiration rate during anesthesia in the castrated and ovariohysterectomized groups ranged between 11.0 and 13.7 breaths/min for the castration groups and between 10.1 and 12.5 breaths/min for the ovariohysterectomy groups. Since gas samples were collected from the oral end of an endotracheal tube, expired carbon dioxide concentrations may have been a little low in this study. The difference between inspired and end-tidal isoflurane concentrations was apparent (approximately 0.2 to 0.5%). Arterial oxygen saturation was > 98% in all cats. Mean rectal temperature in each group ranged between 37.4 °C and 38.2 °C preoperatively and between 36.5 °C and 37.6 °C postoperatively in the castration groups, and between 37.2 °C and 38.4 °C preoperatively and 35.8 °C and 36.8 °C postoperatively in the ovariohysterectomy groups. There were no significant differences in these variables across groups for both types of surgery.

**Expired end-tidal isoflurane concentration, heart rate, and MAP during anesthesia**

For both surgeries, mean expired end-tidal isoflurane concentration was significantly \( (P < .05) \) lower in the medetomidine-combined groups than in the nonmedetomidine groups and was lowest in the medetomidine + midazolam + alfaxalone groups (Figure 1). Similarly, for both surgeries, the mean heart rate was significantly lower in the medetomidine-treated groups than in the nonmedetomidine groups. Also, for both surgeries, the averaged MAP was significantly higher in the medetomidine-treated groups than in the nonmedetomidine groups. In the alfaxalone, medetomidine + alfaxalone, and medetomidine + midazolam + alfaxalone treatment groups, all variables were not significantly different between the IV and IM routes of administration.

**Adrenaline**

In all treatment groups except for the medetomidine + midazolam + alfaxalone IM group, adrenaline concentration decreased preoperatively.
postoperatively, or both compared with baseline concentration; this was true for both types of surgery (Figure 2). In ovariohysterectomized cats, postoperative adrenaline concentration was significantly higher in the medetomidine + midazolam + alfaxalone IM group than in the alfaxalone IM group. Adrenaline concentration did not significantly differ across groups during the early and complete recovery phases.

**Noradrenaline**

For both surgeries, noradrenaline concentration decreased significantly preoperatively, postoperatively, or both, compared with baseline concentration in all groups except for the medetomidine + midazolam + alfaxalone IM group, in which it did not significantly change (Figure 3). In ovariohysterectomized cats, postoperative noradrenaline concentration was significantly higher in the medetomidine + midazolam + alfaxalone IM group than in the alfaxalone IV group. Noradrenaline concentration did not significantly differ across groups during the early and complete recovery phases.

**Cortisol**

In castrated cats in the alfaxalone IV and IM groups, cortisol concentration did not significantly differ from baseline during anesthesia and recovery. Conversely, in the medetomidine + alfaxalone IM and medetomidine + midazolam + alfaxalone IM groups undergoing castration, cortisol decreased significantly postoperatively, compared with baseline concentration (Figure 4). In ovariohysterectomized cats in the alfaxalone IV and IM groups, compared with baseline concentrations, cortisol concentration did not significantly change during anesthesia but increased significantly in the early recovery phase. On the other hand, in the medetomidine + midazolam + alfaxalone group, cortisol concentration decreased significantly postoperatively, compared
Cortisol concentration was significantly lower in the medetomidine + alfaxalone IM and IV groups than in the alfaxalone IV group. Cortisol concentrations did not significantly differ across the medetomidine-combined groups in any phases.

**Glucose**

In castrated cats in the alfaxalone IM group, glucose concentration increased slightly, significantly preoperatively, postoperatively, or both compared with baseline concentrations (Figure 5). On the other hand, glucose concentration in the medetomidine-combined groups undergoing castration increased largely, significantly pre- and postoperatively compared with baseline. Pre- and postoperative glucose concentrations were significantly higher in the medetomidine + alfaxalone IV and IM and medetomidine + midazolam + alfaxalone IV groups than in the alfaxalone IV and IM groups. Glucose concentration during early recovery was also significantly higher in the medetomidine + alfaxalone IM group than in the alfaxalone IM group.

In ovariohysterectomized cats in the alfaxalone IV and IM groups, glucose concentration increased significantly preoperatively, postoperatively, or both, and during early recovery, complete recovery, or both, compared with baseline. Glucose concentration also increased significantly preoperatively, postoperatively, or both compared with baseline in the medetomidine-combined groups. Preoperative glucose concentration was significantly higher in the medetomidine + alfaxalone IV and IM, and medetomidine + midazolam + alfaxalone IV groups than in the alfaxalone IV and IM groups.

**NEFA**

In castrated cats in the alfaxalone IV and IM groups, NEFA concentration decreased significantly postoperatively compared with baseline but
thereafter it during early and complete recovery was not significantly different from baseline concentration (Figure 6). In the medetomidine-combined groups, NEFA concentration decreased significantly preoperatively, postoperatively, or both, compared with baseline. Preoperative NEFA concentration was significantly lower in the medetomidine + midazolam + alfaxalone IV group than in the alfaxalone IV group.

In ovariohysterectomized cats in the alfaxalone IV and IM groups, NEFA concentration did not significantly change preoperatively, postoperatively, or both from baseline, and thereafter increased significantly during the recovery phases in the alfaxalone IV group. In the medetomidine-combined groups, NEFA concentration decreased significantly preoperatively, postoperatively, or both, compared with baseline. Pre- and postoperative NEFA concentrations were significantly lower in the medetomidine + alfaxalone IM and IV groups than in the alfaxalone IV group.

**Recovery time and behavioral recovery score**

Recovery times (mean ± SD) to extubation and head-up in alfaxalone IV; alfaxalone IM; medetomidine + alfaxalone IV; medetomidine + alfaxalone IM; medetomidine + midazolam + alfaxalone IV; and medetomidine + midazolam + alfaxalone IM groups of castrated cats were 8.3 ± 1.6 and 24.2 ± 8.9 min; 9.3 ± 3.2 and 29.2 ± 9.0 min; 18.2 ± 4.6 and 46.2 ± 7.6 min; 20.3 ± 6.2 and 55.8 ± 7.4 min; 22.5 ± 4.5 and 48.8 ± 4.9 min; and 21.0 ± 3.7 and 58.3 ± 8.7 min, respectively. In ovariohysterectomized cats, recovery times to extubation and head-up in alfaxalone IV; alfaxalone IM; medetomidine + alfaxalone IV; medetomidine + alfaxalone IM; medetomidine + midazolam + alfaxalone IV; and medetomidine + midazolam + alfaxalone IM groups were 9.3 ± 2.0 and 42.8 ± 13.2 min; 12.5 ± 2.7 and 40.0 ± 3.4 min; 28.8 ± 7.2 and 56.7 ± 12.5 min; 26.2 ± 4.6 and 60.5 ± 6.8 min; 36.7 ± 4.3 and 57.8 ± 8.5 min; and 38.2 ± 5.1 and 61.5 ± 11.7 min, respectively. In both castrated and ovariohysterectomized cats, recovery times to extubation and head-up were significantly longer in the medetomidine-combined groups than in the alfaxalone IV and IM groups. There were no significant differences in recovery times between the alfaxalone IV and IM groups and between the medetomidine-combined groups, other than in ovariohysterectomized cats, in which time to extubation was significantly longer in the medetomidine + midazolam + alfaxalone IM group than in the medetomidine + alfaxalone IV group and longer in both the medetomidine + midazolam + alfaxalone IV and IM groups than in the medetomidine + alfaxalone IM group.

Median behavioral recovery scores after castration and ovariohysterectomy were 3 (range, 2 to 4) for both surgery types in the alfaxalone IV group; 3 (range, 2 to 4) for both surgery types in the alfaxalone IM group; 2 (range, 2 to 3) and 2 (range, 1 to 3), respectively, for the medetomidine + alfaxalone IV group; 3 (range, 2 to 3) and 2 (range, 1 to 3), respectively, for medetomidine + alfaxalone IM group; 2 (range, 1 to 3) and 2 (range, 1 to 2), respectively, for medetomidine + midazolam + alfaxalone IV group; and 1.5 (range, 1 to 3) and 2 (1 to 3), respectively, for medetomidine + midazolam + alfaxalone IM group. For both types of surgeries, behavioral scores were significantly lower (better) for cats in the medetomidine-combined groups versus the alfaxalone IM group. For all cats, behavioral scores were significantly lower for cats in the medetomidine + alfaxalone IV group or the medetomidine + midazolam + alfaxalone IV or IM group versus the alfaxalone IV group.

**Discussion**

The rationale for using fixed dosages of alfaxalone alone and in combination with medetomidine or midazolam has been outlined in earlier studies.4,12,20
The present study revealed that, in cats undergoing castrated or ovariohysterectomy, the combination of medetomidine or medetomidine + midazolam with alfaxalone reduced isoflurane concentration and maintained higher blood pressure during anesthesia. Since the dose of medetomidine used in this study is known to induce peripheral vasoconstriction and decrease cardiac output, the higher blood pressure in combination with medetomidine may be due to the vasoconstrictive effect of medetomidine. In this regard, changes in cardiac output with this combination may need to be further investigated. The present study further found that, in both castrated and ovariohysterectomized cats, the addition of medetomidine or medetomidine + midazolam to alfaxalone compared with alfaxalone alone improved the quality of recovery from isoflurane anesthesia and surgery. These results support previous findings that the quality of recovery from anesthesia with alfaxalone may be improved with the simultaneous use of other sedatives.

The present study revealed that both adrenaline and noradrenaline concentrations decreased during isoflurane anesthesia in castrated or ovariohysterectomized cats premedicated with alfaxalone with and without medetomidine, except for cats in the medetomidine + midazolam + alfaxalone IM group. Isoflurane inhibits the secretion of catecholamines in adrenal chromaffin cells at concentrations within the range encountered during general anesthesia, suggesting that isoflurane anesthesia itself inhibits the release of catecholamines due to a suppression of sympathetic-adrenomedullary activity in cats. As medetomidine is known to decrease plasma adrenaline and noradrenaline concentrations in both cats and dogs, we postulated that the addition of medetomidine to alfaxalone may reduce plasma catecholamine concentrations during isoflurane anesthesia. However, the present study revealed that, during isoflurane anesthesia, medetomidine or medetomidine + midazolam premedication did not enhance the inhibition of catecholamine release, compared with alfaxalone alone; this may be due to the reduction of isoflurane concentration induced by the medetomidine and medetomidine + midazolam treatments. The present results also revealed that postoperative catecholamine concentrations tended to be higher in the medetomidine + midazolam + alfaxalone IM group than in the alfaxalone IV or IM groups in ovariohysterectomized cats; this may be due to the effect of midazolam on catecholamine release since plasma adrenaline and noradrenaline concentrations are increased by midazolam in cats. Although a reduction in catecholamines as a sign of decreased neurohumoral stress response is a beneficial effect, extremely low concentrations of blood catecholamines (near to zero concentrations) may also be a disadvantage to good maintenance of the sympathetic activity under anesthesia. In this regard, midazolam can prevent an excessive decrease in catecholamines during anesthesia and surgery, without an increase in blood catecholamines. The use of midazolam combined with medetomidine and alfaxalone may be useful for the good maintenance of sympathetic activity during anesthesia and surgery. Therefore, both the reduction of isoflurane concentration with the medetomidine + midazolam + alfaxalone treatment and the effect of midazolam on catecholamine release may be useful in appropriate maintenance without excessive increases in catecholamine concentrations during isoflurane anesthesia and surgery.

Previous studies have shown that medetomidine alone or medetomidine + midazolam does not significantly affect plasma cortisol concentrations in healthy cats without inhalant anesthesia and surgery. In the present study, plasma cortisol concentration increased during the early recovery phase in the alfaxalone IV and IM groups of ovariohysterectomized cats, whereas it was significantly lower postoperatively in the medetomidine + alfaxalone IM and medetomidine + midazolam + alfaxalone IV groups than in the alfaxalone IV groups. These findings in cats are similar to those in previous reports in dogs, in which medetomidine premedication reduced or delayed the increase in cortisol concentrations that is induced by ovariohysterectomy. On the other hand, postoperative cortisol concentrations in the alfaxalone IV and IM groups and during the early recovery phase in all groups tended to be greater for ovariohysterectomy than for castration, supporting the belief that cortisol release depends on the type of surgery, nociceptive stimulation, and the degree of trauma. Overall, the present findings indicate that combinations of medetomidine or medetomidine + midazolam with alfaxalone are useful for suppressing excessive postoperative adrenocortical activity in isoflurane-anesthetized cats.

The present results revealed that blood glucose increased slightly during isoflurane anesthesia and the early recovery phase in the alfaxalone IV and IM groups undergoing either ovariohysterectomy or castration. This increase in blood glucose may be due to several side effects, including surgical injuries, increased cortisol, decreased insulin, and decreased peripheral use of glucose associated with inhalant anesthesia. In the present study, during anesthesia, postoperative glucose concentrations were higher in the medetomidine + alfaxalone and medetomidine + midazolam + alfaxalone groups than in the alfaxalone groups, demonstrating that medetomidine premedication causes hyperglycemia during isoflurane anesthesia in cats. This enhancement of hyperglycemia by medetomidine may be mainly due to the medetomidine-induced inhibition of insulin release via α2-adrenoceptors on pancreatic β-cells. Hyperglycemia may limit the use of medetomidine and medetomidine + midazolam in combination with alfaxalone in cats with metabolic and neurohormonal problems, such as diabetes mellitus, ketosis, and glycosuria.

Changes in NEFA concentrations are important metabolic indicators of the stress response since NEFA is affected by hormones like cortisol and catecholamines. Lipolytic activity is stimulated by cortisol and catecholamines. In the present study, NEFA concentrations in the alfaxalone IV and IM
groups tended to decrease preoperatively, postoperatively, or both, and increase during the recovery phases in both castrated and ovariohysterectomized cats. These results may be attributed to the complicated effects of decreased catecholamine and increased cortisol during anesthesia and post-surgery. In unanesthetized cats, medetomidine is reported to reduce plasma NEFA concentration; however, medazolam does not reduce plasma NEFA.\textsuperscript{12,25} In the present study, pretreatments with medetomidine or medetomidine + midazolam in combination with alfaxalone, especially compared with alfaxalone IV, greatly reduced preoperative, postoperative, or both NEFA concentrations during anesthesia.

Lipolytic activity is suppressed by medetomidine via α₂-adrenoceptors on adipose tissues, which also decreases cortisol and catecholamine.\textsuperscript{3,10} Although NEFA concentration fluctuations may not be directly harmful to anesthetized cats, a decrease in plasma NEFA concentration induced by pretreatments of medetomidine or medetomidine + midazolam combined with alfaxalone may be a clinically significant metabolic index of inhibition of sympathetic-adrenal activation in isoflurane-anesthetized cats undergoing surgery.

In the present study, there were no significant differences between alfaxalone IV and IM administration in hormonal and metabolic effects and recovery scores. Although not significant, the overall quality of recovery from anesthesia appeared to be inferior in cats received alfaxalone IM compared with IV, suggesting that alfaxalone IV may be preferable to IM.

In conclusion, the addition of medetomidine or medetomidine + midazolam to alfaxalone improved the quality of recovery from isoflurane anesthesia in both castrated and ovariohysterectomized cats. Both plasma adrenaline and noradrenaline concentrations decreased during isoflurane anesthesia in cats premedicated with alfaxalone with and without medetomidine, except for medetomidine + midazolam + alfaxalone IM. Treatment with medetomidine + midazolam + alfaxalone prevented an increase in catecholamine concentrations during anesthesia and surgery. Combinations of medetomidine or medetomidine + midazolam with alfaxalone suppressed a postoperative increase in plasma cortisol in ovariohysterectomized cats. The addition of medetomidine or medetomidine + midazolam to alfaxalone facilitated hyperglycemia during isoflurane anesthesia in cats. Pretreatments with medetomidine or medetomidine + midazolam in combination with alfaxalone are useful for the prevention of stress-related hormonal and metabolic responses, other than hyperglycemia, during isoflurane anesthesia and surgery in feline veterinary practice.

Acknowledgments

The authors thank Kumiko Maeda and Erika Chikami for their assistance with data collection.

No external funding was used in this study. The authors declare that there were no conflicts of interest.

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