Sveriges lantbruksuniversitet
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Jämförelse mellan anestesi med sufentanil-midazolam och sevofluran för ovariohysterektomi av kaniner premedicinerade med medetomidin

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SUMMARY

Rabbits carry a high risk of anesthesia related death. This study was part of a project to develop a form of total intravenous anesthesia (TIVA) with minimal effects on cardiac, respiratory and metabolic parameters. In the study, TIVA with sufentanil and midazolam (group TIVA, n=9) was compared with inhalation anesthesia with sevoflurane (group SEVO, n=9) in female rabbits undergoing surgery (ovariehysterectomi). All rabbits were pre-medicated with medetomidin and carprofen before anesthesia induction. Anesthesia was induced with TIVA (2.3 µg/ ml sufentanil, 0.45 mg/ ml midazolam) at a rate of 4 ml/ kg/ h and the mean induction dose was 0.4 µg/ kg of sufentanil and 0.1mg/ kg of midazolam. The induction time was 4.7 ± 1.7 min (mean ± SD) and time to intubation 7.4 ± 6.7 min. After induction the TIVA infusion was maintained at rate of 0.6 ml/ kg/ h in group TIVA during preparations. In group SEVO, the TIVA infusion was stopped after anesthesia induction, and sevoflurane was administered at a concentration of 1 %. During surgery, the TIVA infusion rate and the sevoflurane concentration were adjusted to maintain a surgical plane of anesthesia in the respective groups. The mean infusion rate during anesthesia was 2.9 ± 1.5 ml /kg/ h in group TIVA and in group SEVO the mean sevoflurane concentration was 1.7 ± 1.0 % during maintenance of anesthesia. Mechanical ventilation was necessary in all rabbits in group TIVA and in four of the rabbits in group SEVO to maintain a $PaCO_2$ between 5 and 6 kPa. Time to extubation was 19 ± 14 min in group TIVA, and time to recovery of the righting reflex was 56 ± 33 min. In group SEVO time to extubation was 5 ± 4 min and time to recovery of the righting reflex 21 ± 11 min. No differences between groups could be detected in terms of circulatory parameters.

SAMMANFATTNING

Anestesi av kaniner är förenat med hög dödlighet. Den här studien var en del av ett projekt att utveckla en form av total intravenös anestesi (TIVA) med minimala effekter på kardiologiska, respiratoriska och metabola parametrar. I studien jämfördes TIVA med sufentanil + midazolam (grupp TIVA, n=9) med inhalationsanestesi med sevofluran (grupp SEVO, n=9) hos honkaniner som genomgick ett kirurgiskt ingrepp (ovariehysterektomi). Alla kaniner premedicinerades med medetomidin och carprofen innan induktion av anestesin. Anestesin inducerades med TIVA (2.3 µg/ ml sufentanil, 0.45 mg/ ml midazolam) med en flödeshastighet av 4 ml/ kg/ h och medelvolym för induktion var 0.4 µg/ kg sufentanil och 0.1mg/ kg midazolam). Tiden för induktion var 4.7 ± 1.7 min (medel ± SD) och tiden för intubering var 7.4 ± 6.7 min. Efter induktionen hade TIVA infusionen en flödeshastighet av 0.6 ml/ kg/ h i grupp TIVA under förberedelserna. I grupp SEVO stoppades TIVA infusionen efter induktionen och sevofluran administrerades med en koncentration av 1 %. Under kirurgin justerades koncentrationen sevofluran och flödeshastigheten för TIVA för att bibehålla kirurgisk anestesi i respektive grupp. Flödeshastigheten under anestesi var 2.9 ± 1.5 ml/kg/h i grupp TIVA och i grupp SEVO var koncentrationen sevofluran för underhåll av anestesi 1.7 ± 1.0 %. Mekanisk ventilation var nödvändigt för alla kaninerna i grupp TIVA och för fyra av kaninerna i grupp SEVO för att bibehålla $PaCO_2$ mellan 5-6 kPa. Tid till extubering var 19 ± 14 min i grupp TIVA och till återhämtning av rättningsreflexen 56 ± 33 min. I grupp SEVO var tiden till extubering 5 ± 4 min och till återhämtning av reflexer 21 ±

11 min. Inga skillnader kunde påvisas mellan grupperna gällande de cirkulatoriska parametrarna.

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INTRODUCTION

The rabbit is the third most common animal species undergoing anesthesia in veterinary small animal practice today, and carries a high risk of anesthesia-related death. A study by Brodbelt et al. (2008) showed that the risk of death in healthy rabbits is approximately 14 times higher than in dogs and six times higher than in cats. In sick rabbits it is five times higher than in both dogs and cats. The overall risk of anesthesia-related death is approximately eight times higher than in dogs and six times higher than in cats.

General risk factors

All anesthetics have a negative effect on the body's vital functions such as the cardiovascular, respiratory and thermo-regulatory systems and their use must therefore be monitored closely. According to the Association of Veterinary Anaesthetists (AVA) there are five requirements for performing safe anesthesia. Every veterinary surgeon that performs general anesthesia must be able to:

1. Ensure free airways
2. Administer oxygen
3. Perform manual ventilation (intermittent positive pressure ventilation, IPPV) by the use of e.g. an Ambu bag or an anesthetic breathing system
4. Administer IV drugs and fluids, i.e. venous access should be secured, ideally with an IV catheter
5. Perform cardio-pulmonary resuscitation (CPR)

Cardiovascular and respiratory risks

Cardio-respiratory complications are a major cause of death in small animal anesthesia, representing over 70 % of deaths in cats and dogs and almost 40 % in rabbits (Brodbelt et al., 2008).

Cardiac failure can be caused by pre-existing heart disease, cardiac arrhythmias due to an increased amount of circulating catecholamines, myocardial hypoxia, cardiac depression caused by relative anesthetic overdose or hypovolemia. Cardiac arrest and circulatory collapse result in failure of delivering blood to the vital tissues which leads to hypothermia, hypoxia and shock.

Respiratory complications include failure of maintaining open airways and inadequate ventilation. Other documented problems are failed intubation, trauma to the upper airways and delivery of hypoxic inspired gas mixtures (See Brodbelt, 2012).

Other medical risks

Less reported medical complications are e.g. neurological causes such as postoperative seizures or failure to regain consciousness, sepsis, multiple organ failure, postoperative renal failure and anaphylactic reactions against drugs or fluids administered (Brodbelt et al., 2008).

Patient and procedure related risks

Health status, age and weight are important patient-related risk factors. Amongst procedure-related factors it is found that duration, complexity and urgency are crucial, as well as the continuity of monitoring and the anesthetists being familiar with the anesthetic-agents used (Brodbelt, 2006).

Risk factors in rabbits

In a study by Brodbelt (2006) the total risk of anesthesia-related death in rabbits (n=8209) was 1.4 %. It concludes that of rabbits dying during anesthesia, 5 % died at induction, 31 % during maintenance and 64 % postoperatively. Of the postoperative deaths more than 60 % occurred within three hours after the procedure. The major number (63 %) of anesthesia-related rabbit deaths reported in the study was of unknown cause. The available information in many cases only allowed for a broad classification, hence aggravating the determination of the exact death cause, which in addition often can be multi-factorial. Necropsies were performed in less than 10 % of the cases, making the exact cause even more difficult to establish. The large number in addition to the timing of most deaths could suggest that patients were insufficiently monitored postoperatively and it was shown that intra-operative monitoring generally held a lower standard in rabbits than in dogs or cats (Brodbelt et al., 2008).

Size

Rabbits have a large surface area to volume ratio compared to larger animals, which increases the risk of hypothermia during anesthesia and thereby respiratory and circulatory collapse. Hypothermia also increases the risk of relative drug overdose and can lead to long recovery periods due to a slower metabolism of the drugs administered. Rapid recovery is important, especially in small animals which have a high rate of metabolism. Small rabbits can develop hypoglycemia and hepatic lipidosis in 12 hours if they do not eat (Bonath el al., 1982). Rabbits are sensitive to overgrowth of pathogenic bacteria in the intestines and postoperative ileus (POI) due to their sensitive gastro-intestinal tract (Krempels et al., 2000). POI is not uncommon after surgery, in particular abdominal surgery. Opioids (administered as part of anesthesia and postoperative pain management) are known to reduce intestinal motility (Bauer and Boeckxstaens, 2004). Overweight can lead to gastrointestinal stasis and ileus, and in addition, rabbits are sensitive to stress which can cause imbalance of the cecal flora and overgrowth of bacteria (*Clostridium spp*.) which in turn can lead to toxinemia, lipidosis and death if left untreated (Krempels et al., 2000).

In addition, their small size and the narrow therapeutic index for many of the anesthetics used make it very important to calculate accurate doses and even diluting the drugs to reduce the risk of overdosing. The same precautions must be taken regarding the amount of fluids given. During rabbit surgery the recommended volume for fluid administration is 10 ml/ kg/ h (The Royal School of Veterinary Studies, 2012).

Health status

Poor health status has been identified as a major anesthesia risk in rabbits (Brodbelt, 2006). In pet rabbits, respiratory, digestive and fluid-balance disorders (Brodbelt et al., 2008) are common, as well as dental problems. Body weight is also an important health factor (Brodbelt, 2006), even though a study by Courcier et al. (2012) shows that obesity in rabbits does not appear to be as common as in e.g. dogs or cats. Inadequate diets affect bodyweight and promote gastrointestinal as well as dental problems (Meredith, 2012).
For different reasons the clinical history of rabbits is often limited (e.g. outdoors housing with little human contact, or the primary care taker being a child), which emphasizes the importance of a thorough clinical evaluation, preferably including a blood sample for hematology, before anesthesia (Self, 2007).

Stress

Being prey animals, rabbits are easily stressed. At the clinic they should be kept separated from other animal species such as dogs and cats (Self, 2007). Given their fragile bone structure combined with the flight instinct, they require careful handling due to the risk of damaging the spinal column. Stress has a number of unwanted effects which in the worst case scenario can be life threatening. Stress leads to release of catecholamines, which increase the risk of cardiac arrhythmias, especially if the animal is hypoxic. Stress is immunosuppressive, can cause gastric ulcers and oliguria, and it causes anorexia, which in combination with reduced intestinal motility and disrupted carbohydrate metabolism can cause hepatic lipidosis, liver failure and death (Harcourt-Brown, 2005).
Rabbits also exhibit stress on induction of anesthesia, especially with the use of volatile agents, which is why they are not recommended for this purpose (Hedenqvist et al., 2001, Flecknell et al., 1999).

Poor venous access

Small rabbits have few easily accessible veins which can make venous catheterization more demanding (see Brodbelt, 2008). The ear veins are the most frequently used.

Lack of familiarity with the species

Many veterinarians have limited experience with rabbits as a species. This could lead to deficiencies in the clinical evaluation and difficulties with i.e. intubation and resuscitation (Self, 2007).

Anatomy

Rabbits have a specific anatomy which makes them prone to developing hypoxia. The lungs are small relative to the abdominal viscera and they have a narrow nasopharynx (Harcourt-Brown, 2005). Rabbits can only breathe through their nose due to their soft palate being permanently locked around the epiglottis (Fraser and Girling, 2009). These anatomical conditions make intubation a challenge that requires some experience (Self, 2007). Furthermore, breathing is primarily controlled by the diaphragm and not by the intercostal

musculature (Harcourt-Brown, 2002) which can affect the rabbit's ability to breathe if placed in a position where the large abdominal viscera press on the diaphragm.

Recovery

To reduce the risk of hypothermia a heat source should be provided both during and after anesthesia, and body temperature should be measured to assure that the rabbit it neither too cold nor too warm. If the rabbit has not started to eat within 12 hours supplemental feeding by syringe should be initiated and if dehydrated fluid therapy.

Anesthesia

There are two methods of administration of drugs for induction and maintenance of anesthesia; inhalation or injection. In rabbits, anesthesia is best induced by injection, whereas maintenance can be achieved by injection or inhalation. There are advantages and disadvantages with both methods and a short overview is given here.

Inhalation anesthesia

The most prominent advantage with inhalation anesthesia is that anesthetic depth is easy to control and that it allows for rapid recovery. Most volatile agents undergo little biotransformation and therefore affect liver function and metabolism of other drugs to a minor extent. They are mostly exhaled in an unchanged form (Tranquilli et al., 2007). Oxygen is commonly used as carrier gas for delivery of volatile agents, which improves tissue oxygenation.

Inhalation anesthetics cause dose-related cardiovascular and respiratory depression (Steffey, 1996) and they are irritating to the airway mucosa. Upon induction with volatile agents, rabbits react with distress, violent struggling and extended periods of breath holding (reflex apnea) which in turn causes hypoxia and severe bradycardia (Flecknell et al., 1996). Other induction-related reflex responses that have been reported in dogs are coughing, bronchoconstriction, laryngospasm and excessive mucus secretion (Mutoh et al., 2001). In addition, inhalation anesthesia requires special equipment for both delivery and evacuation of gas (Hedenqvist, 2008) and contributes to environmental pollution (Marx et al., 2000).

Injection anesthesia

Injection anesthetics can be administered intravenously (IV), intramuscularly, subcutaneously or intraperitoneally, with the intravenous route providing the best control of anesthetic depth. By slow injection or infusion the drug can be administered until the desired depth of anesthesia is reached. With the use of rapidly metabolized agents (e.g. propofol) the depth and duration of anesthesia can be tightly regulated. This is not possible with injection by other routes, in which case depth and duration are more unpredictable.

With the use of slowly metabolized or accumulating agents prolonged recovery is a potential problem, not least in small animals that need to resume eating and drinking as soon as possible. As mentioned earlier, the risk of hypothermia is prominent in small animals. Hypothermia leads to reduction of rate of drug metabolism which lengthens the recovery even

further. This problem can be avoided by using rapidly metabolized drugs or an agent which effects can be reversed by administration of an antidote.

Supplemental oxygen administration and/or mechanical ventilation is required and in addition, injectable anesthetics often induce enzyme activity in the liver which can affect metabolism of other drugs (Hedenqvist, 2008).

AIM

The study was part of a project with the aim to develop and evaluate a form of total intravenous anesthesia (TIVA) with minimal effects on cardiac, respiratory and metabolic parameters in rabbits. This is motivated by high lethality from anesthesia in rabbits and the disadvantages of currently used protocols. In the current study, circulatory as well as respiratory and metabolic parameters were recorded in 18 Himalayan rabbits undergoing ovariehysterectomi (OHE) during anesthesia with sufentanil-midazolam TIVA (n=9) or sevoflurane (n=9). This report focuses on the circulatory parameters.

TIVA WITH SUFENTANIL-MIDAZOLAM

The synthetic opioid sufentanil is more potent than morphine (Niemegeers et al., 1976) and fentanyl (see Sebel and Bovill, 1982). Further, it provides good cardiovascular stability during anesthesia (Reddy et al., 1980) and has a high safety margin (Niemegeers et al., 1982). It allows for a rapid anesthesia induction as well as recovery, provides good analgesia and is suitable for continuous administration (Sanford et al., 1986). However, due to the high doses required to achieve adequate hypnosis if used as a mono-anesthetic (Hellebrekers and Sap, 1991) it is best combined with a potent sedative to provide a sufficient level of anesthesia. Total intravenous anesthesia (TIVA) with a combination of sufentanil and midazolam has previously been studied in dogs undergoing emergency surgery for gastric dilatation/volvulus. In light of the results, showing that it was safe and reliable in these dogs with circulatory disturbances and thus classified as high-risk patients (Hellebrekers and Sap, 1991), we chose to evaluate the combination in rabbits undergoing surgery. An appropriate dose combination was developed in a previous study (Hedenqvist et al., 2013). The evaluation included recording and analyses of various physiological parameters (see material and methods).

MATERIALS AND METHODS

Animals and housing

18 female Himalayan rabbits from Charles Rivers Laboratories (Sulzfeld, Germany) were included in the study. At arrival they were approximately 13 weeks old. At the study start the average body weight (BW) ± SD was 2.1 ± 0.1 kg. According to a health monitoring report (Charles River Laboratories, France) the colony was free from the following agents: Rabbit haemorrhagic disease virus, Rabbit rotavirus, Lymphocytic choriomeningitis virus, *Carbacillus, E. cuniculi, Treponema culiculi, C. piliformis , B. bronchiseptica, P. multocida, P. pneumotropica, Salmonella spp.,* arthropods, helminths, *Eimeria sp., Giardia sp*. and *Spironucleus sp*. The rabbits arrived two weeks prior to the study for acclimatization to the

environment and to handling. They were kept in pairs in cages (L1646 x W 660 x H 650 mm) with autoclaved straw for bedding, a shelf for hiding and sitting on, and with unlimited access to autoclaved hay and fresh water. They were fed restricted rations of a commercial pelleted diet (1.5 dl per rabbit per day of Lactamin K1, Lactamin AB, Kimstad, Sweden) and small amounts of carrots for environmental enrichment. The cages were cleaned once a week and the room temperature was 17 ± 1 °C.

Study design

The rabbits were randomly divided into two groups. In both groups anesthesia was induced by infusion with the TIVA solution. In rabbits in group TIVA (n=9), anesthesia was maintained by continuous infusion with the TIVA solution and in rabbits in group SEVO (n=9) by inhalation of sevoflurane.

Preparation

The rabbits were weighed daily starting five days prior to surgery. On the day before surgery a clinical examination was performed and the fur on the ears was clipped in preparation for vessel catheterization.

Premedication and preparation for anesthesia and surgery

On the day of surgery, a local anesthetic cream (lidocaine 25 mg/ g and prilocaine 25 mg/ g, EMLA, AstraZeneca, Södertälje, Sweden) was applied to the rabbit's ears and the rabbit was injected with 0.1 mg/ kg medetomidin (Domitor vet 1 mg/ ml, Orion Pharma, Sollentuna, Sweden) and 5 mg/ kg carprofen (Rimadyl vet 50 mg/ ml, Orion Pharma Animal Health, Sollentuna, Sweden) subcutaneously (SC). After 30 min, the rabbit was moved to the operating theatre. An arterial catheter (BD Neoflon™, 24 GA, BD Medical Surgical Systems, Stockholm, Sweden) was placed in the central ear artery for registration of blood pressure and collection of arterial blood samples, and a venous catheter (BD Neoflon™, 24 GA, BD Medical Surgical Systems, Stockholm, Sweden) was placed in the marginal ear vein in each ear for separate infusions of TIVA and Ringer-Acetate solution (10 ml/ kg/ h, Fresenius, Sweden). Blood was collected before induction and at predetermined time points during (every 30 min) and after anesthesia (every 45 min for 3h) for examination of blood gases, lactate, glucose and total protein.

The arterial catheter was connected to a pressure transducer (Gabarith™, BD Medical Surgical Systems, Stockholm, Sweden,) and surveillance monitor (AS/3, Datex-Engstrom, DOTmed.com Inc, New York, USA) for continuous registration of arterial blood pressure. The pressure transducer was placed at the level of the rabbit's heart. The arterial catheter was connected to a hemodynamic monitor (LiDCO™ plus Hemodynamic Monitor, LiDCO Cardiac Sensor Systems, London, UK) for registration of cardiac output (CO). The system uses lithium dilution for determination of CO. A bolus dose of 5.6 µg/ kg lithium chloride (0.15 mmol/ ml solution) was injected into the venous catheter. By withdrawing blood past a lithium sensor attached to the arterial catheter a lithium concentration-time curve was obtained. The curve was used to calibrate the pulse contour analysis software, which then provides continuous CO data by analyzing the arterial pressure waveform. ECG-electrodes were placed laterally on the left elbow and both knees and connected to the AS/3 monitor for

continuous recording. The AS/3 monitor had an upper limit in measuring HR which was 250 beats per minute (bpm).

After the preparations were finished the following parameters were recorded: respiratory rate (RR), heart rate (HR), blood pressure (MAP), and CO.

Induction

Anesthesia was induced with a TIVA solution consisting of 2.3 µg/ ml sufentanil and 0.45 mg/ ml midazolam. The solution was attained by mixing 1 ml of sufentanil (Sufenta 50 µg/ ml, Janssen-Cilag, Sollentuna, Sweden) with 10 ml of midazolam (Midazolam Actavis 1 mg/ ml, Actavis, Stockholm, Sweden) and 11 ml of physiological NaCl. An infusionpump (Perfusor Compact S, B. Braun, Kronberg, Germany) with a 20 ml luer-lock syringe (B. Braun, Kronberg, Germany) was used and the flow rate during induction was 4 ml/ kg/ h. Time of induction (i.e. loss of the righting reflex) was noted. The infusion flow rate was then reduced to 0.6 ml/ kg/ h and the laryngopharynx was sprayed with 1 mg/ kg lidocain (Xylocain 20 mg/ ml, AstraZeneca AB, Södertälje, Sweden). After one minute, during which oxygen was provided via face mask, the trachea was intubated blindly or with help of a small animal laryngoscope (Flecknell™, Alstoe Ltd. Animal Health, York, UK). The number of intubation attempts and the time to intubation were noted. The uncuffed endotracheal tube (PVC, 2.5 mm ID-3.3 mm OD, Kruuse, Solna, Sweden) was connected to a ventilator (SV 300, Siemens, Sweden). In rabbits in group TIVA, the infusion rate was maintained at 0.6 ml/ kg until start of surgery. In group SEVO the TIVA infusion was stopped at this time and sevoflurane was administered at a concentration of 1 % until time of surgery. In both groups, the airways were ventilated as needed to maintain a $PaCO_2$ between 5-6 kPa. The air:oxygen ratio was 2:1 and the inspiration:expiration ratio 1:3.

Fig. 1. Demonstrating the technique for blind endotracheal intubation of a Himalayan rabbit.

Preparations for surgery and maintenance of anesthesia

The fur on the rabbit's tail was clipped and a pulsoximeter finger clip sensor (Datex-Ohmeda, Madison, USA), applied and connected to the AS/3 monitor. The rabbit was placed on a heating blanket in dorsal recumbency and a rectal thermometer was inserted for measuring of body temperature (BT). The surgical area was clipped, disinfected and draped.

In group TIVA the anesthesia infusion rate was increased to 2 ml/ kg/ h before start of surgery. In the sevoflurane group the concentration was increased to 0.7-3.4 % at the start of surgery depending on the level of anesthesia. In the respective groups, the TIVA infusion rate and the sevoflurane concentration were adjusted to maintain a surgical plane of anesthesia throughout surgery.

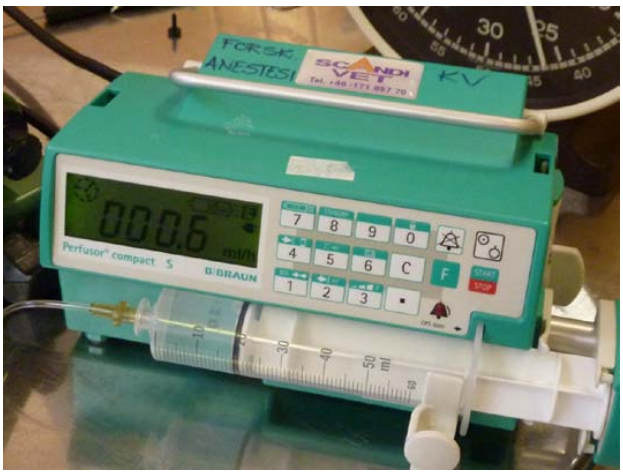

Fig. 2. TIVA infusion pump (Perfusor Compact B. Braun) with a 20 ml luer-lock syringe.

Monitoring

ECG, RR, tidal volume, positive end expiratory pressure (PEEP) , HR, MAP, O_2-sat and BT were continuously measured and recorded on the AS/3 monitor. CO was continuously measured by the LidCO. For statistical evaluation, values recorded every 10 min during anesthesia were used. Arterial blood samples were withdrawn every 30 min for measurement of blood gas, lactate, glucose and total protein.

Surgery

OHE was performed by a veterinary surgeon and a veterinary student. After a 3-5 cm ventral midline skin incision, starting 1-2 cm caudal of the umbilicus and ending at the cranial rim of the pubis, the peritoneal cavity was opened by a stab incision in the linea alba. The incision was extended with the scalpel almost to the full length of the skin incision. The reproductive organs were localized. The ovarian pedicle (including *a.* and *v. ovarica* and the suspensory ligament, *ligamentum suspensorium*) was clamped, using two Mosquito clamps, and a single circumferential ligature was placed in the groove after removing the distal clamp before transecting the pedicle. One or two mass ligatures were placed over the broad ligament and the tissue was transected. *Arteria* and *v. uterina* were ligated with single circumferential ligatures lateral to the vagina on the side of the cervix, and the process was repeated

bilaterally. A single circumferential ligature was placed over the vagina, caudal to the cervices, the tissue was transected and the uterus and ovaries were removed. For all ligatures except in the skin 3-0 Polyglactin 910 (Vicryl, Ethicon, USA) was used. After each transection and again before closing the wound a thorough control was made to assure hemostasis. The linea alba was closed in a simple continuous pattern Due to the thin tissue no sutures were placed SC. The skin was closed with an intradermal continuous suture, using 3-0 Poliglecaprone 25 (Monocryl, Ethicon, USA). Time of start and end of surgery was noted.

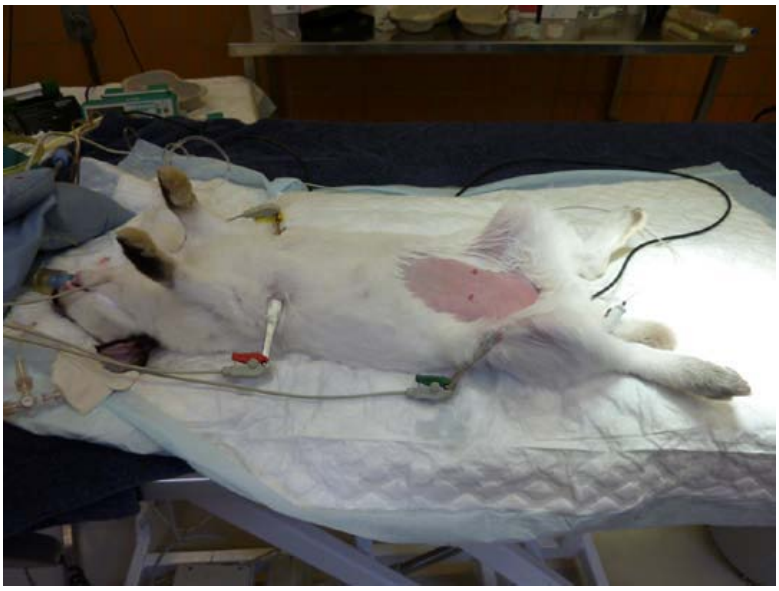

Fig. 3. Rabbit prepared for surgery. The airways are intubated and the ECG-electrodes, pulse oximeter clip and rectal thermometer are attached.

Recovery

After completed surgery, the anesthesia administration was turned off and the times to extubation and to recovery of the righting reflex were recorded. The rabbit was thereafter placed in a cage under a heating lamp. During 180 minutes after ending anesthesia administration, RR, HR and BT were recorded every 15 minutes. An arterial blood sample was collected every 45 min for measuring of blood gases.

The study included postoperative evaluation of pain by scoring of facial expression (Keating et al 2012). For that reason no opioid drug was administered until the rabbits were fully awake (approx. 180 min) and had been filmed for scoring. For filming, the rabbit was placed in a transparent cage for 20 minutes. Thereafter 0.05 mg/ kg buprenorfin (Temgesic 0.3 mg/ ml, RB Pharmaceuticals, Slough Berkshire, England) was administered SC and the rabbit was placed in the home cage. After one hour it was filmed again for comparison of pain scores. The results of the pain scoring will be reported elsewhere. The following day a clinical examination was performed. If a rabbit had not started eating within 12 hours, it was hand fed by syringe with the regular pellet feed crushed and mixed with water. All rabbits were weighed daily for seven consecutive days after surgery.

Analyses

Arterial blood gases, blood glucose and lactate levels were analyzed with a portable analyzer (i-STAT, Abbott, Solna, Sweden) with CG4+ and CG8+ cassettes. Serum was frozen for later analysis of sufentanil and midazolam levels.

Statistic evaluation

For comparison of non-repeated measures between groups, Student's t-test (Shapiro-Wilk) or Mann-Whitney Rank Sum Test were used (time to induction, intubation, extubation, recovery of righting reflex, duration of anaesthesia and surgery, pre-values of HR and BW). For comparison of repeated measures within groups, repeated measures ANOVA was used (BW, HR, MAP, BT). For comparison between groups, mean values were calculated for each animal during and/or after anaesthesia and compared with t-test (BW, HR, MAP, BT). A P-value less than 0.05 was considered significant.

All data are presented as mean ± SD and range. The statistical evaluations were made with Sigma Plot 12.0 (Systat Software, Inc, USA), which automatically tests for normal distribution of data.

RESULTS

There were no differences between groups in induction time or time to intubation. Induction time (n=18) was 4.7 ± 1.7 min (range 2-10). Time to intubation (n=18) was 7.4 ± 6.7 min (range 1-28). The number of intubation attempts was 1-12.

There was no difference between groups for the TIVA induction volume. The mean volume was 0.3 ± 0.1 ml/ kg (n=18), corresponding to a mean dose of sufentanil and midazolam of 0.4 µg/ kg and 0.1 mg/ kg, respectively. For group TIVA the mean infusion rate during anesthesia was 2.9 ± 1.5 ml/ kg/ h (n=9). In group SEVO the mean sevoflurane concentration for maintenance was 1.7 ± 1.0 % (n=9).

All rabbits in group TIVA had to be ventilated throughout anesthesia. In group SEVO four rabbits had to be ventilated occasionally.

Surgery and duration of anesthesia

The surgery lasted 59 ± 14 min (range 34-89, n=18) and the total time of anesthesia was 103 ± 14 minutes in group TIVA (n=9) and 114 ± 13 minutes in group SEVO (n=9). There were no differences between groups for either parameter.

Heart rate

There were no differences in heart rate between groups before, during or after anesthesia (Fig. 1). In group TIVA (n=9), the HR was higher post-operatively compared to pre-values. In group SEVO (n=9) the HR was higher during and post-operatively compared to pre-values. Observe that pre-values were recorded after administration of medetomidine.

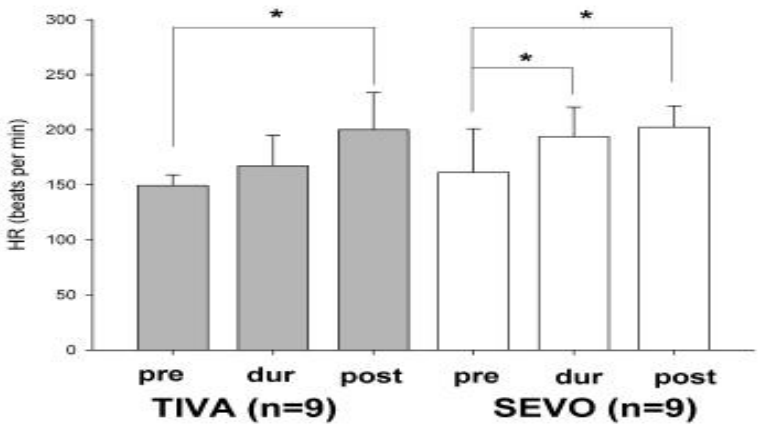

Fig. 1 Heart rate (mean ± SD) before, during and after anesthesia in 18 female Himalayan rabbits undergoing ovariehysterectomi. TIVA = anesthesia maintenance with sufentanil-midazolam, SEVO = anesthesia maintenance with sevoflurane.*p< 0.05 (One Way Repeated Measures Anova with Holm-Sidak posthoc test). Note that pre-values were recorded after medetomidine administration.

Blood pressure

There was no difference in mean arterial blood pressure before (pre) or during anesthesia between or within groups (Fig.2). Note that pre-values were recorded in medetomidine sedated animals.

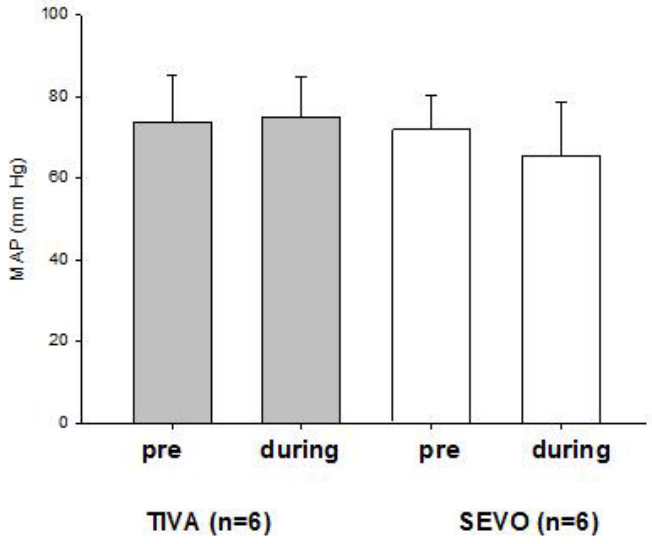

Fig.2 Mean arterial blood pressure (± SD) before (pre) and during anesthesia in female Himalayan rabbits undergoing ovariohysterectomy, TIVA = anesthesia maintenance with sufentanil-midazolam, SEVO = anesthesia maintenance with sevoflurane. Note that pre-values were recorded after administration of medetomidine.

Cardiac output

Due to the small size of the rabbits it was not always possible to get the LiDCO system to withdraw enough blood for calibration past the lithium sensor. Therefore, cardiac output was only successfully recorded in five rabbits, three from group TIVA and two from group SEVO (Fig. 3). The mean cardiac output in all five during anesthesia was 0.4 ± 0.4 L/ kg/ min.

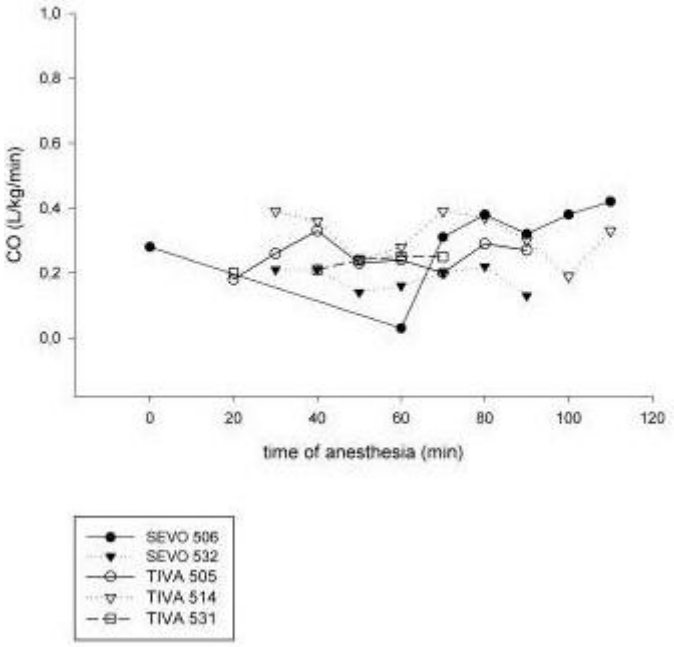

Fig. 3 Cardiac output during anesthesia in five female Himalayan rabbits undergoing ovariohysterectomy, TIVA = anesthesia maintenance with sufentanil-midazolam, SEVO = anesthesia maintenance with sevoflurane.

Body temperature

In group TIVA (n=9), the BT was 37.7 ± 0.9°C (range 35.4-39.6) during anesthesia and 37.5 ± 1.1°C (range 34.4-39.4) after anesthesia. In group SEVO (n=9), the BT was 38.1 ± 0.9°C (range 36.4-39.8) during anesthesia and 37.6 ± 0.9°C (range 36.0-39.3) after anesthesia. There was no difference between groups neither during nor after anesthesia or within groups. The body temperatures during surgery are displayed in Fig. 4.

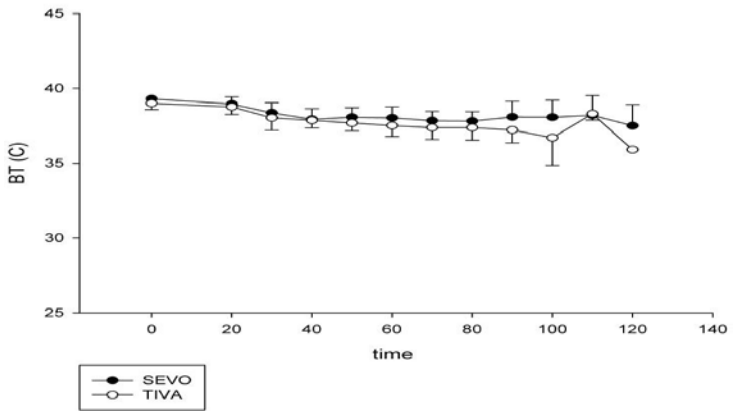

Fig. 4 Body temperature (mean ± SD) during and after surgery in female Himalayan rabbits undergoing ovariohysterectomy, TIVA = anesthesia maintenance with sufentanil-midazolam, n = 9, SEVO = anesthesia maintenance with sevoflurane, n = 9.

Recovery

Time to extubation was longer in group TIVA [19 ± 14 minutes (range 7-45), n=9] than in group SEVO [5 ± 4 minutes (range 0-117), n=9]. Time to recovery of the righting reflex was longer in group TIVA [56 ± 33 minutes (range 15-110), n=9] than in group SEVO [21 ± 11 minutes (range 8-36), n=9].

Two rabbits in group TIVA were still heavily sedated 45-60 minutes after ending anesthesia, and two suffered from bradypnoea (one at extubation and one at 90 minutes after ending anesthesia) and all four were administered naloxone intravenously (0.15 ml/ kg Naloxon B. Braun, 0.4 mg/ ml, Melsungen, Germany). One rabbit in group SEVO was still somnolent 90 minutes after ending anesthesia and was administered atipamezole intramuscularly (0.05 ml/ kg Antisedan® vet., 5 mg/ ml, Orion Pharma Animal Health, Sollentuna, Sweden)

One rabbit from group TIVA showed transient neurologic effects in form of head tilt, nystagmus and anisocoria and had to be syringe fed for three days postoperatively, and another rabbit from the same group showed head tilt after surgery but did not need hand feeding. One rabbit from group SEVO suffered from seizures postoperatively, lasting for approx 60 s. All animals eventually recovered. Three rabbits developed necrosis on part of the ear (2x5 cm) in the area of the arterial catheter placement.

Body Weight

The body weight before surgery was 2.1 ± 0.1 kg in both groups (range 1.8-2.3, n=18). The rabbits lost weight during the first days following surgery, but within a week all animals were gaining body weight. The mean weight loss was 68 ± 32 g in group TIVA (n=9) and 89 ± 19 g in group SEVO (n=9), with no difference between groups. The body weight difference is displayed in Fig 5.

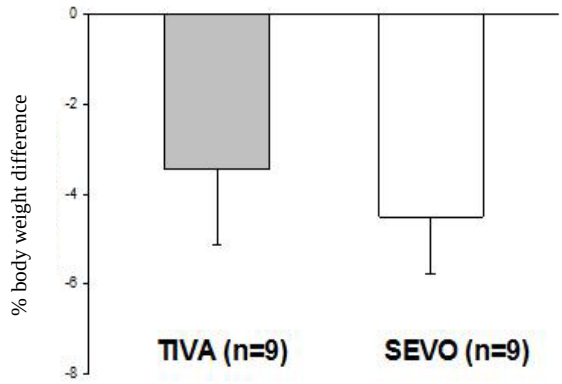

Fig. 5 Percent body weight difference (mean ± SD) in the week following ovariohysterectomy in female Himalayan rabbits, TIVA = anesthesia maintenance with sufentanil-midazolam, SEVO = anesthesia maintenance with sevoflurane.

DISCUSSION

The purpose of this study was to compare cardiac, respiratory and metabolic parameters in medetomidine premedicated rabbits undergoing ovariohysterectomy during anesthesia with either intravenous infusion of sufentanil-midazolam or inhalation with sevoflurane. Only the cardiovascular parameters are discussed here.

Main findings

There were no detectable differences between groups for HR or MAP. CO could not be evaluated due to the difficulties in obtaining data. The increase in HR during anesthesia in group SEVO could be a caused by a direct positive chronotropic effect of sevoflurane, which has been shown to increase heart rate when compared to awake, calm dogs (Steffey, 1996). Other inhalation agents (i.e. isoflurane, desflurane, enflurane) can have similar effect (Steffey, 1996). Inhalant anesthetics are also known to produce respiratory acidosis and hypercapnia by means of respiratory center depression (Johnson, 2008). Elevated levels of arterial pCO_2 cause sympathetic activation, which increases HR, CO and blood pressure (Johnson, 2008) and acidosis can lead to cardiac arrhythmias and heart failure if left untreated (see Launila, 2011). However, in this study pCO_2 reached hypercapnic levels (> 8 kPa, see Launila, 2011) only on a few occasions. The increased HR in this case could also be caused by stress related to surgery. The fact that a similar elevation in HR could not be seen in group TIVA may be due to the depressant effect most opioids have on HR (Schmeling et al., 1989). Several studies have shown a decreased heart rate with the use of sufentanil alone in unsedated dogs (Schmeling et al., 1989) as well as in combination with midazolam during surgery in critically ill dogs with circulatory disturbances (Hellebrekers and Sap, 1991). Benzodiazepines (midazolam) can also cause a decrease in heart rate (FASS VET, 2012).

Surgery is created injury that triggers a variety of physiological changes (so called stress response). The magnitude of the response depends on the severity, intensity and duration of the stimulus and results in secretion of many anabolic and catabolic hormones, which, among many other reactions, causes cardiovascular changes, i.e. rise in cardiac output, heart rate and blood pressure (Singh, 2003). Furthermore, the release of catecholamines increases the risk of cardiac arrhythmias (Harcourt-Brown, 2007). To prevent the negative effects it is important to keep the procedure as short as possible, minimize tissue handling, and keep the animal well hydrated and warm. To ascertain that the animal is kept in a surgical plane of anesthesia at all times, as well as free from pain after surgery is crucial (Singh, 2003). In this study the procedure was longer than usual because a veterinary student performed the surgery, guided by a veterinary surgeon. The tissue handling was careful and kept to a minimum. The rabbits were given fluids and the anesthesia infusion/concentration was continuously adjusted to keep a surgical level of anesthesia.

The rabbits were sedated before baseline parameters were recorded, which could explain that the HR was lower when compared to levels after anesthesia. The elevated HR could also be due to postsurgical pain/stress given the fact that no opioid was administered directly after surgery.

Blood pressure was well maintained during anesthesia with both TIVA and sevoflurane, with a mean of 75 and 65 mm Hg respectively. For comparison, MAP during anesthesia with ketamine/xylazine in rabbits not undergoing surgery, was approximately 60 mm Hg in a study by Lipman et al. (1990). Normal blood pressure in the awake rabbit is 80-91 mm Hg (Fraser and Girling, 2009) and hypotension is common in rabbit anesthesia (Harvey et al., 2012). It may be a result of cardiovascular depression caused by anesthetic and sedative agents and it can be exacerbated by surgical hemorrhage (Harvey et al., 2012). According to Haskins (2007), a MAP below 60 mmHg should be avoided, due to risk of ischemic damage to the kidneys. In previously mentioned studies (Hellebrekers and Sap, 1992; Schmeling et al. 1989), MAP was maintained within physiological limits in both critically ill dogs undergoing surgery and healthy dogs, when anesthetized with sufentanil-midazolam or sufentanil respectively. In this present study, fluid was administered throughout the anesthesia and the surgical hemorrhage was minimal.

There was no difference in body temperature between groups, nor was there any difference in duration of anesthesia. According to Haskins (2007) core body temperature below 40° C and down to 36° C is not detrimental to animals. The body temperature in this study was mainly kept within physiological limits, except on a few occasions in rabbits in group TIVA, on which the temperature was low. Almost all of them could be explained by practical circumstances, such as the rectal probe not being properly inserted (false low value) or the rabbit not being positioned correctly on the heating blanket (true low value). In each case the temperature quickly returned to normal values. Hypothermia increases the risk of circulatory and respiratory collapse, as well as the risk of relative drug overdose and long recovery due to slower drug metabolism.

Regrettably, because of the difficulties to get enough blood for calibration past the lithium sensor due to the small size of the rabbits, the number of successfully recorded measurements of cardiac output was too low to draw any conclusion. CO provides a real-time and continuous assessment of the patient's hemodynamic status and can be an aid to prognosis and diagnosis, and to monitor the adequacy of fluid and inotropic therapy. The technique (LIDCO™ plus system) is minimally invasive, requiring only peripheral arterial and venous access, and allows measurement of CO as long as necessary. In addition to CO and arterial blood pressure parameters it also provides a number of calculated parameters, including: body surface area, pulse pressure variation, systolic pressure variation, cardiac index, stroke volume, stroke volume index, stroke volume variation, systemic vascular resistance and systemic vascular resistance index (Pearse et al., 2004).

The prolonged time to extubation and recovery of reflexes in group TIVA was most likely an effect of sufentanil and could probably have been shorter if buprenorphine had been administered immediately after surgery. Being a partial opioid agonist, buprenorphine would have reversed the effects of sufentanil while still providing analgesia.

The weight loss in both groups was significant but it did not differ between groups. The weight loss was similar to that described in a study by Cooper et al. (2009) where it was approximately 4 % on day two after OHE, but less than in a study by Weaver et al. (2010)

where the average weight loss two days after OHE and telemeter placement was almost 8 % (in total of three treatment groups receiving buprenorphine, ketoprofen, fentanyl respectively, and one control group). The weight loss could be due to decreased appetite, which was shown in both studies, and/or to postoperative catabolism, and can reflect inadequate postoperative pain control (Weaver et al., 2010). The decrease in appetite can also be related to the type of postoperative pain management. Known side effects of buprenorphine are anorexia and gut stasis, and in the study by Cooper et al. (2009) rabbits receiving buprenorphine on a daily basis after OHE had a slower return of appetite than rabbits receiving meloxicam. In the current study all rabbits were gaining weight within a week, similarly to the rabbits in the other studies (Cooper et al., 2009; Weaver et al, 2010).

Seizures could be caused by hypoxia, hypoglycemia, and hypersensitivity to administered drugs (i.e. lidocain) or electrolyte disturbances [i.e. hypocalcemia, hyper- and hyponatremia, and magnesium deficiency (Tranquilli et al., 2007). Intense pain can also trigger seizure-like behavior [the rabbit throwing itself on its side, eyes rolling in their socket (MediRabbit)]. Head tilt is a relatively common clinical presentation in the rabbit and is usually acute in onset and can be presented with the rabbit falling/rolling over and with nystagmus. It has a number of potential causes (i.e. toxoplasmosis, listeriosis, bacterial otitis media/interna). Another possible cause is cerebrovascular damage/trauma (Keeble, 2006) which is most likely to have caused the neurological symptoms in this study. Necropsy was performed in one of the rabbits with neurological symptoms, and it showed extensive coagulative necrosis in the cerebral cortex, consistent with a massive infarct. No thrombus was found but it is likely that coagulated blood in the arterial catheter was flushed back into the carotid artery and ended up in the brain. To prevent this, the catheter should have been filled with a heparin solution after anesthesia. This solution should then be withdrawn before the blood samples were collected.

The necrosis that developed in some of the rabbits' ears was most likely caused by damage of the ear artery by catheter placement.

The possibility to rapidly reverse the effects of TIVA with a partial opioid agonist/ antagonist and a benzodiazepine antagonist must be considered beneficial in comparison to anesthesia with medetomidine-ketamine, which is a relatively common combination in rabbit anesthesia, after which reversal of medetomidin shortly after drug administration can leave unwanted effects of the ketamine (catalepsy). Another advantage of TIVA is that the length of anesthesia is more easily varied according to needs.

Conclusion

In this study there were no detectable differences in HR and MAP between anesthesia with sufentanil-midazolam and sevoflurane. TIVA with sufentanil-midazolam in medetomidine premedicated rabbit could be of use in the small clinic without access to equipment needed for inhalational anesthesia. However, the airways needs to be intubated and ventilated due to marked respiratory depression.

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