RESEARCH PAPER

Intravenous sufentanil-midazolam *versus* sevoflurane anaesthesia in medetomidine pre-medicated Himalayan rabbits undergoing ovariohysterectomy

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Abstract

Objective To compare physiological effects of sufentanil-midazolam with sevoflurane for surgical anaesthesia in medetomidine premedicated rabbits.

Study design Prospective, randomized controlled experimental study.

Animals Eighteen female Himalayan rabbits, weight 2.1 ± 0.1 kg.

Methods Premedication with 0.1 mg kg^{-1} medetomidine and 5 mg kg^{-1} carprofen subcutaneously, was followed by intravenous anaesthetic induction with sufentanil (2.3 µg mL^{-1}) and midazolam (0.45 mg mL^{-1}). After endotracheal intubation, anaesthesia was maintained with sufentanil-midazolam ($n = 9$) or sevoflurane ($n = 9$). Ovariohysterectomy was performed. Intermittent positive pressure ventilation was performed as required. Physiological variables were studied perioperatively. Group means of physiologic data were generated for different anaesthetic periods. Data were compared for changes from sedation, and between groups by ANOVA. Post-operatively, 0.05 mg kg^{-1} buprenorphine was administered once and 5 mg kg^{-1} carprofen once daily for 2–3 days. Rabbits were examined and weighed daily until one week after surgery.

Results Smooth induction of anaesthesia was achieved within 5 minutes. Sufentanil and midazolam doses were 0.5 µg kg^{-1} and 0.1 mg kg^{-1}, during induction and 3.9 µg kg^{-1} $hour^{-1}$ and 0.8 mg kg^{-1} $hour^{-1}$ during surgery, respectively. End-tidal sevoflurane concentration was 2.1% during surgery. Assisted ventilation was required in nine rabbits receiving sufentanil-midazolam and four receiving sevoflurane. There were no differences between groups in physiologic data other than arterial carbon dioxide. In rabbits receiving sevoflurane, mean arterial pressure decreased pre-surgical intervention, heart rate increased 25% during and after surgery and body weight decreased 4% post-operatively. Post-operative problems sometimes resulted from catheterization of the ear artery.

Conclusion Sevoflurane and sufentanil-midazolam provided surgical anaesthesia of similar quality. Arterial blood pressure was sustained during sufentanil-midazolam anaesthesia and rabbits receiving sevoflurane lost body weight following ovariohysterectomy. Mechanical ventilation was required with both anaesthetic regimens.

Clinical relevance Anaesthesia with sufentanil-midazolam in medetomidine premedicated healthy rabbits is useful in the clinical and the research setting, as an alternative to sevoflurane.

Introduction

The rabbit (*Oryctolagus cuniculi*) is the third most common pet animal species undergoing anaesthesia in the United Kingdom (Brodbelt et al. 2008) and the third most commonly used laboratory animal species in Europe (European commission 2010). A surgical intervention routinely performed in female rabbits and which requires general anaesthesia is ovariohysterectomy. For largely unknown reasons, anaesthesia of rabbits carries a high mortality risk in comparison with dogs and cats (Brodbelt 2009). Possible anaesthetic risk factors in rabbits include; a high stress level, high prevalence of clinical and subclinical disease, small body size and relatively small lungs. Moreover, rabbits are not routinely endotracheally intubated and possibly are less carefully monitored during anaesthesia compared with cats and dogs (Brodbelt 2006). In addition, anaesthesia in rabbits has been studied less than in the aforementioned species.

Anaesthesia can be achieved with inhalation or injection of anaesthetic drugs. Some benefits of using inhalation anaesthesia are that most veterinarians are familiar with the technique and that anaesthetic depth can be precisely controlled. Sevoflurane (SEVO) has a lower blood: gas partition coefficient (0.7) than isoflurane (1.4), resulting in more rapid induction and recovery and possibly a greater ability to control anaesthetic depth. Total intravenous anaesthesia (TIVA) has the benefit of separating provision of anaesthesia from ventilation and reducing atmospheric pollution. If rapidly metabolized drugs which action can be antagonized are administered by target control infusion, adjustment of anaesthetic depth and time to recovery is rapid. High dose opioid anaesthesia provides cardiovascular stability in humans as well as in dogs (Bovill et al. 1984; Benson et al. 1987; Flecknell et al. 1989). In human cardiac surgery, sufentanil-based protocols have been shown to be superior to fentanyl in respect of cardiac output and haemodynamic stability during and after anaesthesia (Howie et al. 1991; Sato et al. 1995). In combination with midazolam, sufentanil has been shown to produce safe surgical anaesthesia in cardiovascularly compromised dogs suffering from gastric dilatation/volvulus (Hellebrekers & Sap 1991) and doses have been established in New Zealand White (NZW) rabbits premedicated with medetomidine (Hedenqvist et al. 2013; Larsson et al. 2014). Similarly to opioids, alpha-2-adrenergic agonists have been shown to reduce the surgical stress response in humans and dogs, which may prevent cardiac complications (Wijeysundera et al. 2009). Our aim was to compare sufentanil-midazolam (SUF-MID) anaesthesia with SEVO anaesthesia in medetomidine premedicated Himalayan rabbits regarding cardio-respiratory variables, lactate, glucose and recovery after surgery. Our hypothesis was that SUF-MID would have less effect on cardiovascular and metabolic variables than would SEVO.

Materials and methods

The study was granted permission from the regional ethics committee of animal experiments in Uppsala (C 83/12) and met the requirements of Swedish and EU legislation regarding the use of experimental animals.

Animals

Eighteen female Himalayan rabbits, with an age of 15 weeks and a mean ± SD body weight of 2.1 ± 0.1 kg were acquired from a certified breeder (Crl:CHBB[HM], Kissleg, Germany). According to standard health monitoring (Nicklas et al. 2002), the breeding colony was free from known rabbit pathogenic and opportunistic agents (rabbit haemorrhagic disease virus, rabbit rotavirus/rabbit corona virus, *Bordetella bronchiseptica*, *Clostridium piliforme*, *Pasteurella multocida*, *Salmonella* spp, endo- and ectoparasites, dermatophytes). The rabbits were housed in pairs in double cage systems (EC2, Scanbur Technology A/S, Denmark), with a total floor area of 0.86 m^2 and a combined hiding place/shelf. Autoclaved straw was used as bedding and the cages were cleaned once a week. Autoclaved hay and water *ad lib*, and a limited ration of pelleted feed (SDS Standard Rabbit, Special Diet Services, UK) was provided daily. The rabbits were acclimatised for two weeks, and during five days prior to surgery they were accustomed to handling by daily weighing. The light cycle was 08:00–17:00; the room temperature was 17 ± 1 °C and the humidity 50 ± 20%. The number of air changes was 15 hour^{-1}.

Premedication and preparations for anaesthesia

A clinical examination, including general body condition, mental status, auscultation of heart and lungs, inspection of eyes and nose and mucous membranes, was performed the day before surgery on all rabbits. Approximately 30 minutes before anaesthetic induction, the animals were premedicated with 0.1 mg kg^{-1} medetomidine (Domitor vet, Orion Pharma Animal Health, Sweden) and 5 mg kg^{-1} carprofen (Rimadyl vet, Orion Pharma Animal Health, Sweden) subcutaneously (SC). The fur on the ears was clipped and a local anaesthetic cream (EMLA, AstraZeneca, Sweden) was applied to the skin. Before preparations for anaesthetic induction started, respiratory rate (f_R) and heart rate (HR) were recorded manually. Preparations included placement of catheters (BD Neoflon, 24 gauge, BD Medical Surgical Systems, Sweden) in the central auricular artery and the marginal auricular vein. The fur of the tail was clipped for placement of a pulse oximeter finger clip sensor (Datex-Ohmeda GE Health Care, Sweden).

Induction of anaesthesia

Anaesthesia was induced in the same way in all rabbits, by intravenous (IV) infusion with a mixture of sufentanil (Sufenta, 50 μg mL^{-1}, Jansen-Cilag, Sweden) and midazolam (Midazolam, 1 mg mL^{-1}, Actavis AB, Sweden). Sufentanil and midazolam were mixed with sterile water to a concentration of 2.3 μg mL^{-1} and 0.45 mg mL^{-1}, respectively. The mixture was administered at 0.3 mL kg^{-1} hour^{-1}, equivalent to a dose of 0.7 μg kg^{-1} hour^{-1} sufentanil and 0.14 mg kg^{-1} hour^{-1} midazolam, with a syringe pump (Braun Compact S, B. Braun Melsungen AG, Germany). Additionally, every 20 seconds a bolus (0.05 mL) of the mixture was injected until the righting reflex was lost and the jaw muscles relaxed (end of induction). The larynx was sprayed with 0.25 mL of lidocaine (Xylocaine 10 mg mL^{-1}, AstraZeneca,, Sweden), and oxygen was delivered at 1 L minute^{-1} via face mask for 60 seconds. The airways were intubated blindly with an uncuffed PVC endotracheal tube with a 2.5 mm inner diameter (Rusch, SweVet, Sweden) and the times to end of induction and completed intubation were recorded.

Maintenance of anaesthesia

The rabbits were assigned randomly using a random number generator (Microsoft Office Excel 2007, Microsoft, Sweden) to receive either SEVO by inhalation ($n = 9$), or IV infusion with SUF-MID ($n = 9$) for maintenance of anaesthesia.

In group SEVO, the endotracheal tube was connected to the anaesthesia machine (Anmedic Q-Circle System, Anmedic AB, Sweden) via a paediatric circular breathing system (Intersurgical Ltd, UK). The SEVO vaporizer (Ohmeda Tech 5, Datex Ohmeda, Sweden) was set initially at a delivery concentration of 1%, delivered in 1.5 L oxygen minute^{-1} and 0.5 L medical air minute^{-1}. The rabbits were allowed to breathe spontaneously, unless apnoea occurred (no breath in 30 seconds), in which case manual compression of a 0.5 L breathing bag was performed. Additional blood gas examinations were performed after initiation of manual ventilation to maintain a partial pressure of arterial carbon dioxide ($PaCO_2$) between 5 and 8 kPa (38–60 mmHg).

In group SUF-MID the endotracheal tube was connected to a ventilator (Servo Ventilator 300, Siemens-Elema AB, Solna, Sweden) in case of apnoea. The ventilator was not compatible with the use of volatile anaesthetic agents and therefore not used in group SEVO. A mixture of 1.5 L oxygen and 0.5 L air was provided via the same breathing system as in group SEVO. The ventilator was used in pressure mode with a pressure of 8–19 cm H_2O, a respiratory frequency of 12–30 minute^{-1} and an inspiratory: expiratory duration ratio of 1:3. The aim was to maintain $PaCO_2$ between 5 and 8 kPa (38–60 mmHg).

The rabbits were placed in dorsal recumbency on an electric heating pad and prepared for surgery. The fur was clipped on the ventral abdomen, the surgical site cleansed with soap and water and scrubbed with antiseptic solution. Sterile surgical drapes were applied to prevent contamination of the surgical site. Intravenous Ringer acetate was administered at 20 mL kg^{-1} hour^{-1} during anaesthesia. During surgery, the administration of SUF-MID or SEVO was adjusted to a plane of surgical anaesthesia, by increasing and decreasing the rate of administration by 10% from baseline depending on the degree muscle relaxation, motor reactions and/or changes in HR and mean arterial blood pressure (MAP) during surgery. An experienced veterinary nurse was in control of the anaesthesia delivery.

Ovariohysterectomy

Laparatomy and ovariohysterectomy was performed according to Mehler (2006) by ventral midline

incision and under aseptic conditions by an experienced surgeon and a veterinary student. The surgical procedure lasted approximately one hour.

Monitoring

The arterial catheter in the auricular artery was connected via saline filled tubing to a pressure transducer (Gabartith, BD Medical, Sweden) that was calibrated to atmospheric pressure at the level of the heart. The pressure transducer, the pulse oximeter probe, a side-stream gas analysis line and a temperature probe were connected to a calibrated monitor (CS/3, Datex-Engstrom, Datex Ohmeda, Sweden) for continuous recording. The sampling rate for gas analysis was 200 mL minute^{-1}. Arterial blood samples (0.2 mL) were collected for analyses of blood gases, glucose and lactate with a portable analyzer (CG4+ and CG8+ cartridges, i-STAT1 Portable Clinical Analyzer, Abbott Laboratories, Abbott Scandinavia AB, Sweden).

The following variables were recorded in medetomidine premedicated rabbits: f_R, HR, saturation of peripheral arterial haemoglobin (SpO$_2$), PaCO$_2$, pressure of arterial oxygen (PaO$_2$), MAP, blood glucose and lactate. During anaesthesia and surgery, f_R, HR, SpO$_2$, MAP, rectal temperature (RT), SUF-MID infusion rate (group SUF-MID) and end tidal SEVO concentration (group SEVO) were recorded every 10 minutes. Arterial blood gases, glucose and lactate were measured every 30 minutes. During three hours following surgery, HR, f_R and RT were recorded every 15 minutes and arterial blood gases, glucose and lactate concentrations every 45 minutes.

Post-operative care

After completion of surgery, the anaesthesia delivery was discontinued and the oxygen-air mixture was delivered until extubation, after which the rabbits were placed in a heated cage for three hours. Times to extubation and return of the righting reflex were recorded. Buprenorphine (Temgesic, RB Pharmaceuticals Limited, UK) was administered three hours post surgery SC at a dose of 0.05 mg kg^{-1}. The reason for the delay in administration was that a simultaneous study of facial expression for the evaluation of pain according to Keating et al. (2012) was carried out. The study required that the rabbits were scored after they had fully recovered from anaesthesia (not sedated), and before

buprenorphine administration. For scoring, the rabbits were filmed for 20 minutes and photographed in a cage with a Plexiglas front before and one hour after buprenorphine administration. The result of pain scoring will be reported elsewhere. Rabbits that had not recovered the righting reflex within 60 minutes after surgery, or became apnoeic postoperatively, were administered naloxone or atipamezole, depending on the group assignment. Any rabbit that seemed to suffer from more than slight pain (e.g. grinding teeth) was administered buprenorphine for rescue therapy.

For a week following surgery, the rabbits were examined clinically and weighed daily. Rabbits that were not eating normally were hand fed with crushed pelleted feed mixed with water several times a day, until normal food intake returned. Carprofen was administered every 24 hours for two-three days post-operatively at a dose of 5 mg kg^{-1} SC.

After completion of the study, twelve rabbits were euthanized and six were re-homed. For euthanasia, an IV injection of pentobarbital (Apotekets avlivningsvätska, Kronans Droghandel, Sweden) was administered in the auricular vein after sedation with medetomidine 0.1 mg kg^{-1} and application of local anaesthetic cream on the skin over the ear. Rabbits that were not re-homed underwent a complete necropsy at the National Veterinary Institute (Uppsala, Sweden).

Statistical evaluation

After testing for normal distribution (Kolmogorov-Smirnov Test), a Student's *t*-test or Mann-Whitney Rank sum test were used for comparison of non-repeated measures between groups (times to induction, intubation, extubation, and recovery of the righting reflex, duration of anaesthesia and surgery). The anaesthetic period was divided into surgical preparations (PREP) and surgery (SUR), since the dose of anaesthesia was adjusted to the different requirements, and thereby may have had an effect on the variables studied. Moreover, since the time-points for the start of PREP, SUR and the post-operative period (POST) differed in each rabbit, mean values were calculated for every rabbit for repeated measures analyses (HR, SpO$_2$, PaCO$_2$, PaO$_2$, MAP, RT, blood glucose and lactate) during PREP, SUR and POST. Individual means were used to establish group means. Group means for PREP, SUR and POST were compared with values recorded during sedation (SED) in a two way repeated measures analysis

of variance (ANOVA) followed by Holm-Sidak post-hoc test. Postoperative body weights were compared with body weights on the day of surgery in a two way repeated measures analysis of variance (ANOVA) followed by Holm-Sidak post-hoc test. A *p*-value < 0.05 was considered significant. Data are presented as mean ± SD.

Results

There were no differences between groups in body weight before surgery (SUF-MID: 2.1 ± 0.1 kg, SEVO: 2.2 ± 0.1 kg) and none of the rabbits showed any clinical signs of disease. SED values of f_R (88 ± 32 minutes^{-1}), HR (159 ± 31 minutes $^{-1}$) SpO_2 (94 ± 5%), PaO_2, $PaCO_2$ and blood glucose (Table 1) were not different between groups.

Induction was smooth and there were no differences in induction time (SUF-MID: 5 ± 1 minutes, SEVO: 5 ± 2 minutes), intubation time (SUF-MID: 6 ± 7 minutes, SEVO: 8 ± 6 minutes) or induction dose (0.5 ± 0.2 µg kg^{-1} sufentanil and 0.1 ± 0.05 mg kg^{-1} midazolam). The duration of surgery and the total duration of anaesthesia did not differ (SUF-MID: 63 ± 16 and 103 ± 15 minutes, SEVO: 55 ± 11 minutes and 112 ± 13 minutes, respectively).

In group SUF-MID, the mean infusion dose was 1.6 µg kg^{-1} hour^{-1} sufentanil and 0.3 mg kg^{-1} hour^{-1} midazolam during PREP and 3.9 µg kg^{-1} hour^{-1} and 0.8 mg kg^{-1} hour^{-1}, respectively, during SUR. All rabbits in group SUF-MID required mechanical ventilation. The mean end-tidal SEVO concentration was 0.8 ± 0.2% during PREP and 2.1 ± 0.4% during surgery, with a range from 1.6% to 2.6% during SUR. Four SEVO rabbits required mechanical ventilation for 20–50 minutes, one during PREP only and three during SUR.

There were no differences between groups in HR or MAP. In group SUF-MID, HR was higher POST

Table 1 Heart rate (HR), arterial blood pressure (MAP), arterial blood gases, blood glucose and lactate levels (mean ± SD) in Himalayan rabbits undergoing ovariohysterectomy during sufentanil-midazolam (SUF-MID) or sevoflurane (SEVO) anaesthesia

	Time point	SUF-MID (*n* = 9)	SEVO (*n* = 9)
HR in beats minute^{-1}	Sedation	157 ± 24	161 ± 40
	Preparation for surgery	166 ± 21	183 ± 34
	Surgery	170 ± 33	199 ± 29*
	Post-op (0–3 hours)	200 ± 34*	202 ± 19*
MAP in mm Hg	Sedation	74 ± 12	72 ± 9
	Preparation for surgery	67 ± 8	61 ± 11*
	Surgery	82 ± 10	68 ± 13
PaO_2 in kPa (mmHg)	Sedation	10 ± 2 (75 ± 15)	9 ± 1 (68 ± 8)
	Preparation for surgery	20 ± 10 (150 ± 75)	25 ± 9 (188 ± 68)*
	Surgery	24 ± 8 (180 ± 60)	27 ± 10 (203 ± 75)*
	Post-op (0–3 hours)	11 ± 3 (83 ± 23)	12 ± 3 (90 ± 23)
$PaCO_2$ in kPa (mm Hg)	Sedation	5.2 ± 0.8 (39 ± 6.0)	5.4 ± 0.7 (41 ± 5.3)
	Preparation for surgery	7.1 ± 1.7 (53 ± 13)†	9.6 ± 1.3 (72 ± 9.8)*†
	Surgery	6.9 ± 1.5 (52 ± 11)	7.3 ± 1.2 (55 ± 9.0)*
	Post-op (0–3 hours)	6.8 ± 1.2 (6.8 ± 9.0)	5.7 ± 1.1 (43 ± 8.3)
Blood glucose in mmol L^{-1}	Sedation	11.0 ± 3.7	11.0 ± 3.8
	Preparation for surgery	12.0 ± 2.0	14.0 ± 4.6
	Surgery	12.0 ± 2.1	11.0 ± 1.5
	Post-op (0–3 hours)	11.0 ± 2.8	7.9 ± 1.3*
Blood lactate in mmol L^{-1}	Sedation	0.8 ± 0.7	0.9 ± 0.4
	Preparation for surgery	1.0 ± 0.6	0.8 ± 0.8
	Surgery	1.9 ± 0.8†	1.0 ± 0.4†
	Post-op (0–3 hours)	2.0 ± 1.3†	1.3 ± 0.2†

All rabbits in group SUF-MID were mechanically ventilated and four rabbits in group SEVO were hand ventilated as required. Group means for PREP, SUR and POST were compared with values recorded during sedation (SED) in a two way repeated measures analysis of variance (ANOVA) followed by Holm-Sidak post-hoc test. *p < 0.05 in comparison with values in medetomidine premedicated rabbits (Sedation). †p < 0.05 in comparison between groups, two way repeated measures ANOVA (post-hoc test Holm-Sidak).

compared with SED values, whereas in group SEVO, HR was higher both during SUR and POST, compared with SED values (Table 1). In group SUF-MID, MAP did not change between sedation, preparation and surgery whereas in group SEVO, MAP was lower during preparation, compared with SED values ($p = 0.05$, power = 0.9, Table 1, Fig. 1).

It was only possible to get a consistent reading from the pulse oximeter in five animals in group SUF-MID and three animals in group SEVO. In these animals, SpO_2 ranged between 86 and 100%. Arterial blood samples were obtained from all but one animal in group SUF-MID and mean PaO_2 did not differ between groups. It was higher during PREP and SUR compared to SED in group SEVO (Table 1). $PaCO_2$ was higher in group SUF-MID than in group SEVO during PREP, and higher in group SEVO during PREP and SUR compared with SED (Table 1).

Glucose levels did not differ between groups but were lower POST compared to SED in group SEVO. Lactate levels were lower in group SEVO than SUF-MID during SUR and POST (Table 1).

Rectal temperature was lower in group SUF-MID during SUR and POST compared to SED (37.3 ± 0.8 °C and 37.5 ± 0.7 °C *versus* 38.4 ± 0.6 °C). In group SEVO, RT was lower only during POST compared to SED (37.6 ± 0.6 *versus* 38.5 ± 0.6 °C). There were no differences in RT between groups.

Time to extubation did not differ between groups, being 20 (5–30) minutes for group SUF-MID and 6.5 (0–15) minutes for group SEVO [median (range)] in rabbits that did not receive a reversal agent. Time to recovery of the righting reflex was longer in group SUF-MID (62 ± 32 minutes) than in group SEVO (27 ± 22 minutes). Four rabbits in group SUF-MID received 0.06 mg kg^{-1} naloxone (Naloxon B Braun, B. Braun Medical AB, Sweden) IV; three rabbits due to respiratory depression at 9, 35 and 45 minutes after surgery respectively, and one rabbit that had not regained the righting reflex 60 minutes after surgery. One rabbit in group SEVO received 0.25 mg kg^{-1} atipamezole (Antisedan vet, 5 mg mL^1, Orion Pharma Animal Health, Sweden) intramuscularly due to marked sedation 90 minutes after end of surgery.

Body weight was stable in group SUF-MID whereas it decreased 4% during post-operative day 1–4 in group SEVO ($p = 0.05$, power = 1.0, Fig. 2). However, there were no differences of body weight between the groups.

Complications

During recovery from anaesthesia, two rabbits in group SUF-MID and one in group SEVO showed neurological signs, including nystagmus, head tilt, opistotonus and convulsions at arterial blood sampling. The symptoms only lasted a few seconds and therefore no treatment was initiated. One of these rabbits (in group SUF-MID) showed reduced activity and food intake for three days, and a head tilt for five days postoperatively. Two rabbits in group SEVO needed hand feeding on the first post-operative day. In three rabbits, one in group SUF-MID and two in

Figure 1 Mean arterial blood pressure (MAP) in Himalayan rabbits during sedation (0 min), preparation for surgery (PREP), and ovariohysterectomy (SUR) during anaesthesia with sufentanil-midazolam —○— ($n = 7$) or sevoflurane —●— ($n = 9$). The time for start and end of surgery varied between animals and the horizontal bars represent the mean time. Results are mean ± SD.
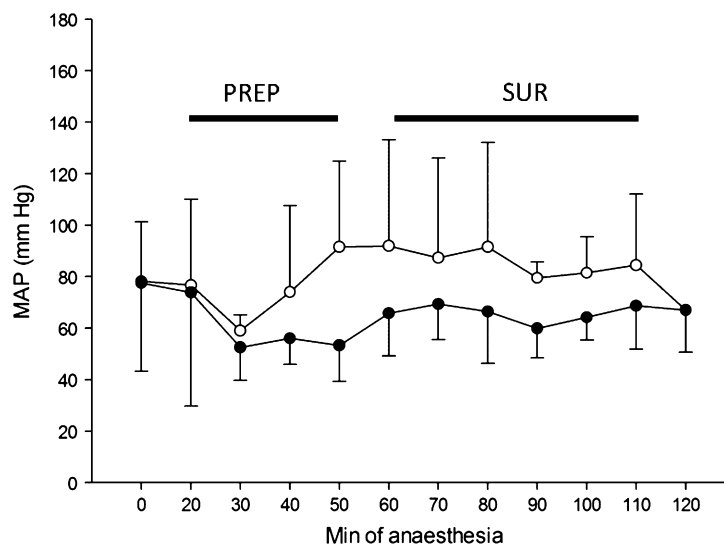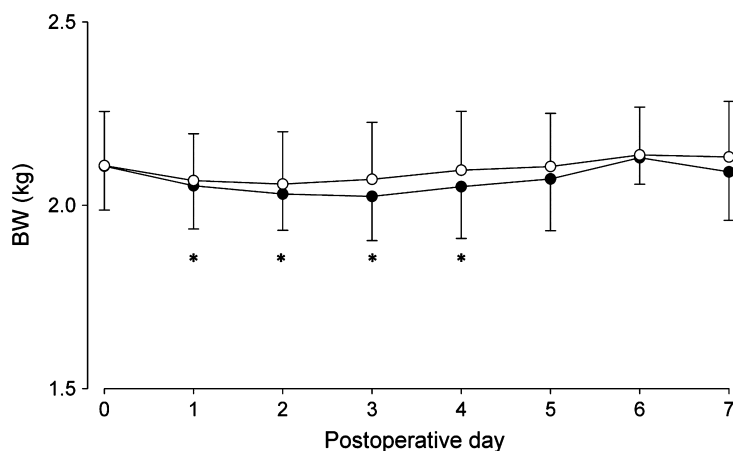
Figure 2 Body weight (BW) (mean ± SD) in Himalayan rabbits during one week after ovariohysterectomy under anaesthesia with sufentanil-midazolam —○— (n = 9) or sevoflurane —●— (n = 9). *p < 0.05 compared with postoperative day 0.

group SEVO, dry necrosis of part of the ear developed in the area of arterial catheter placement within a week after surgery. Two of these rabbits were the same as those showing neurological symptoms. At necropsy an area of coagulative necrosis in the cerebral cortex was observed in the rabbit with the tilted head. In all other rabbits undergoing necropsy, no changes were observed.

Discussion

In healthy rabbits, SUF-MID seems to be at least as efficacious as SEVO for providing anaesthesia during abdominal surgery. The overall quality of anaesthesia was similar for both groups, although SEVO was associated with an increase in HR during and after surgery, and a postoperative body weight loss of 4%. Anaesthesia with SUF-MID, in contrast, was associated with a stable HR during surgery and a stable postoperative body weight. One reason for considering opioid-based anaesthesia, is the limited cardiovascular effects, which has been documented to be the case for SUF-MID in rabbits (Hedenqvist et al. 2014) and dogs (Hellebrekers & Sap 1991) previously. In humans it was shown that sufentanil blunts the stress response during surgery, as well as post-operatively (Desborough & Hall 1989), and that a reduced stress response decreases the risk of cardiac complications during surgery (Wijeysundera et al. 2009).

In the present study, specific-pathogen free rabbits were used. The effects of anaesthesia may be different in rabbits with subclinical or clinical disease, and especially in pet rabbits which frequently have upper respiratory diseases (Rougier et al. 2006).

Whenever full μ-opioid agonist based anaesthesia protocols are used, it is recommended to administer buprenorphine immediately after completion of surgery, to antagonise respiratory depression, decrease time to recovery, and provide long-lasting pain relief. Since the rabbits were additionally part of a pain behaviour study (results reported elsewhere), it was necessary to postpone buprenorphine administration for facial pain scoring to be possible before the administration of further analgesics. Since the first rabbits in group SUF-MID remained sedated for 3 hours, that time point was selected for the scoring. However, all rabbits received carprofen preoperatively; hence, they were not completely devoid of analgesic treatment. Five rabbits were administered naloxone or atipamezole postoperatively, but we anticipate that it did not have an impact on pain scoring 3 hours after surgery.

The righting reflex was not recovered until 30 minutes after end of surgery in group SEVO and 60 minutes in group SUF-MID, which may cause concern. The long duration of surgery (60 minutes) and the delay in buprenorphine administration in group SUF-MID probably contributed to this. In a previous study with SUF-MID anesthesia, buprenorphine was administered immediately after 60 minutes of orthopedic surgery in rabbits, and rabbits recovered within 30 minutes (Hedenqvist et al. 2014). The recommendation is thus for several reasons to administer buprenorphine immediately after completion of surgery.

Rigidity has been described to occur during anaesthetic induction with high doses of opioids e.g. in pigs (Schumann et al. 1994), and in rabbits IV xylaxine-alfentanil-midazolam anaesthesia caused convulsions (Borkowski et al. 1990). Alfentanil has been

shown to induce convulsions more easily in humans compared with sufentanil and fentanyl (Smith et al. 1989). In the present study, anaesthesia was induced slowly to prevent rigidity and convulsions, with a SUF-MID ratio that was established not to cause rigidity in a previous study (Hedenqvist et al. 2013). Rigidity may also have been prevented by the use of medetomidine pre-medication. Medetomidine has been shown to reduce induced seizures in rats (Airaksinen et al. 2012). In more recent studies, sufentanil-midazolam anaesthesia induction has been simplified by using a higher initial infusion rate, and omitting the bolus administration (personal observation, P Hedenqvist).

The seizures that occurred in three rabbits postoperatively were probably not related to anaesthesia (one was in group SEVO), but to flushing of the ear artery catheter. The likely cause was embolization after thrombus formation in the ear artery catheter, which is supported by the result from necropsy of one rabbit, which showed a necrotic area in the cerebral cortex. Physiological saline was used as lock solution in the arterial catheters. which has been described to be satisfactory in studies in humans (Del Cotillo et al. 2008). Ear necrosis, which also occurred in the present study, has been described in small rabbits after catheterization of the auricular artery (Harcourt-Brown 2002). Catheter placement was found more difficult in Himalayan rabbits compared with the larger NZW rabbit. Complications from arterial catheterisation are less likely to occur in the clinic, since blood pressure is not usually measured invasively. Whenever catheter placement is performed however, great care must be taken not to damage the artery and heparinized saline should be used as lock solution.

The mean concentration of SEVO during surgery in the present study was 2.1% in the Himalayan rabbits, which is 40% lower than the reported MAC value of 3.7% in unpremedicated NZW Rabbits (Scheller et al. 1988). This magnitude is similar to the reduction of isoflurane MAC by pre-medication with medetomidine, observed in pigs (Malavasi et al. 2008) and dogs (Weitz et al. 1991). In the present study not only medetomidine, but also the SUF-MID used for induction may have had an effect on the SEVO concentration required for surgical anaesthesia.

Oxygen saturation and arterial partial pressure were satisfactory at almost every measured time point. The lowest values for both parameters were encountered during sedation, before oxygen was delivered. Pulse oximeter measurement was not successful in all rabbits because of difficulties to re-attach an incorrectly placed probe on the tail during surgery. A limitation of the study was that the method of assisted ventilation varied between groups, and probably why SEVO rabbits were hypercapnic during preparation for surgery. During surgery however, normocapnia was present in both groups. It is likely that surgical stimulation contributed to the decrease from PREP to SUR in $PaCO_2$ in group SEVO.

In summary, SUF-MID provided a similar quality of anaesthesia to that of SEVO in medetomidine pre-medicated Himalayan rabbits, and seems to be at least as efficacious as SEVO for providing surgical anaesthesia. Cardiovascular conditions were more stable in group SUF-MID, however, respiratory depression was more pronounced and required IPPV Further, no postoperative body weight loss was seen after SUF-MID anaesthesia.

Acknowledgements

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