A New Protocol of Inhalatory Induction of Anaesthesia in Experimental Swine Surgery. A Pilot Study

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ABSTRACT

Induction of Anaesthesia in swine may prove to be difficult because of the differences in anatomy and physiology. Intubation of large animals is especially difficult due to the length of the snout and the relative difficulty of obtaining adequate relaxation. The present study is aimed to compare two induction techniques for tracheal intubation in pigs. The secondary aim was to assess the effectiveness of a new anaesthetic protocol for experimental surgery. For this, six female pigs underwent two liver surgeries each. Induction of anaesthesia was performed either using intravenous propofol and fentanyl (IV intravenous group) or by using Sevoflurane (inhalatory group). The attending anaesthesiologist was asked to grade the ease of intubation from a scale between 1 and 5. Orotracheal intubation was easier to obtain in the inhalatory group ($p=0.00$) and a smaller number of attempts were necessary ($p=0.00$). A lower End-tidal CO₂ after intubation was observed in the inhalatory group ($p=0.05$). There were no differences in both systolic ($p=0.11$) and diastolic ($p=0.72$) arterial pressure and heart rate ($p=0.16$) after induction of anaesthesia between the two groups. Maintenance of anaesthesia was made using Sevoflurane and boluses of fentanyl for adequate analgesia while no muscle relaxants were used. Intravenous morphine and ketoprofen were used for postoperative analgesia. In conclusion, volatile induction of anaesthesia is safe and offers a better view of the larynx in large swine, while the proposed anaesthetic protocol provides adequate anaesthesia for the surgeons and assures comfort to the animals.

Key words: experimental surgery, Swine surgery, volatile induction, intubation

INTRODUCTION

Anaesthesiologists worldwide are getting more and more involved in animal research studies. The development of multidisciplinary teams of practitioners (1), including surgeons, anaesthesiologists, radiologists and pathologists for experimental surgeries in swine offers new challenges to doctors as both pig anatomy and physiology are different (2).

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From the anaesthesiologist perspective, assuring an adequate airway may prove very difficult due to the length of the snout and of the larynx. A laryngeal mask airway may offer some benefits (3) but it is not adequate for long procedures or laparoscopic surgery. Many studies in humans demonstrated that volatile induction of anaesthesia is associated with a higher cardiovascular stability (4) and improved oxygenation (5). On the other hand cost effectiveness is important in funding for research grants and adequate perioperative intensive care must ensure low costs, adequate operating conditions and animal comfort. As so, the present study focuses on the development of an individualized anaesthetic protocol for experimental swine surgery.

METHODS

Ethical approval for the present study was granted by Fundeni Clinical Institute Ethical Committee, in accordance with principles of the Declaration of Helsinki. We analyzed data obtained from 6 swine that underwent hepatic surgery. Each swine underwent two surgeries: during the first one cryoelectrolysis and electrolytic electroporation was applied and during the second one (24 hours after the previous surgery) a hepatectomy was performed and the pigs were euthanized using a 7.45% potassium chloride solution (1 ml/kg) and 0.2 mg/kg iv morphine while under general anaesthesia.

The primary aim of this study was to compare two induction techniques in term of ease of intubation. The secondary outcome was to assess the effectiveness of an anaesthetic regimen applied in experimental swine surgery.

Six domesticated female pigs were randomly chosen from a commercial farm based on weight (in order to have between 75 and 100 kg). In order to minimize environmental stress, the animals were moved and housed at Fundeni Clinical Institute Centre for Excellence in Translational Medicine for at least 24 hours before surgery. The pigs were housed alone in dedicated rooms with a surface of at least 5 m². The room was heated and humidified appropriately in order to make the animals comfortable. Food was withheld 24 hours before surgery and water before 6 hours.

The following variables were recorded. The swine were weighed and measured (snout length, snout diameter, snout to tail length, snout to vertex, snout to 12th rib). Induction of anaesthesia: the swine were equally divided into two groups: in the first group (iv group) an intravenous induction was applied (propofol

and fentanyl) and in the second group (inhale group) an inhalation technique using 8% Sevoflurane was applied. The swine were intubated with cuffed 7.0 endotracheal tube by using a number 4 Miller blade. After induction of anaesthesia an assistant held the pigs' snout opened using two loops made of gauze. The tongue was firmly held with a gauze as the anaesthesiologist carefully introduced the blade from the right lateral side of the snout and advanced it midline until the epiglottis was visible. Further advancement of the blade made visible the entrance to the larynx. The endotracheal tube was advanced through the larynx and after the tip passed the arytenoids the tube was further advanced by rotation for a further 3 to 5 cm as the larynx in pigs has a much larger length compared to humans (*fig. 1 and 2*).

The anaesthesiologist was asked to grade the ease of intubation from 1 (most difficult/inability to intubate) to 5 (easiest to intubate). The number of attempts until intubation was successful was also noted. Preoperative vital signs (heart rate and blood pressure) were noted at induction and there after at 5 minutes intervals. For maintenance of anaesthesia we used a Primus Anaesthesia workstation (Dräger®, Lubeck, Germany) equipped with a sevoflurane vaporizer. End-tidal CO₂ was noted immediately after intubation.

Figure 1 - Dysection of the larynx in pigs.
Legend: A – trachea, B – larynx, C – opening of larynx, D – first tracheal ring

Figure 2 - Tracheal intubation in pigs. View at laryngoscopy.
Legend: A – hard palate, B – base of tongue, C – epiglottis,
D - cartilagoarytenoidea, E – endotracheal tube

Intraoperative drug requirements and minimum alveolar concentration (MAC) of inhaled anaesthetic agent were noted. During the postoperative period, the swine were kept in separated, well heated rooms and appropriate analgesia was administered.

Statistical analyses were performed using SPSS 19.0 (SPSS Inc®, Chicago, IL, USA). Data are presented as mean $\pm$ standard deviation of the mean or percentage. Ease of intubation and number of attempts were compared between the two groups using Gamma test. Data distribution was examined in order to insure the proper statistical examination. Quantitative data were analyzed with independent samples t-test if normal distributed or Mann-Whitney test if the data did not follow a normal distribution. All p-values are two-tailed. Statistical significance was considered at a p-value < 0.05.

RESULTS

Preanaesthesia and induction of anaesthesia

The anthropometric measures are presented in *table 1*. The swine were sedated using intramuscular 0.5 mg/kg acepromazine and 15 mg/kg ketamine. Venous access was obtained by catheterization of one of the auricular veins using a 20G peripheral venous catheter. Induction of anaesthesia was then performed with the pig in the ventral position. In the iv group the animals were pre-oxygenated using a tight-fitting snout mask with 100% oxygen at a flow rate of 12 L/min for at least 3 minutes. Intravenous fentanyl (1 mcg/kg) and propofol (2 mg/kg) were administered and tracheal intubation attempted. In the inhale group the anaesthetic circuit was primed for 5 minutes using an inspired 8% sevoflurane concentration. The tight fitting mask

Table 1 - Anthropometric parameters

Anthropometric parameter	Value ($\pm$ SD)
weight (kg)	91.7 $\pm$ 7.5
snout length (cm)	20.3 $\pm$ 2.0
snout diameter (cm)	33.6 $\pm$ 3.5
snout to tail length (cm)	131.0 $\pm$ 2.6
snout to vertex (cm)	28.0 $\pm$ 2.0
snout to 12 th rib (cm)	95.6 $\pm$ 2.5

was then applied on the pigs snout and the animal was left to breathe spontaneously for several minutes (*fig. 3*). Intubation was assessed as significantly easier in the inhale group and a smaller number of attempts were made to intubate (*table 2*). There were no statistical differences in hemodynamic parameters between inhale group and iv group although a smaller end-tidal CO₂ was observed in the inhale group probably due to the smaller number of attempts (*table 3*).

A central venous catheter was placed percutaneously in the internal jugular vein. The needle was inserted 3 to 5 cm lateral of the midline of the neck with the tip of the needle facing the midline of the sternum. For better understanding, the anatomy of the internal jugular vein is presented in *fig. 4*.

Figure 3 - Volatile induction of anaesthesia in pigs by using a tight fitting mask on the animals' snout (A) coupled to the anaesthesia machine with the inhaled anaesthetic agent turned on at a concentration of 8% (B) while the animal is breathing spontaneously

Table 2 - Comparison of difficulty of intubation and number of attempts between iv group and inhale group
[data are presented as median (minimum, maximal)]

	IV Group	Inhale group	P value
Difficulty of intubation (1-5 grade)	3 (2,4)	5 (4,5)	0.00
Number of attempts	3.5 (1,5)	1 (1,2)	0.00

Table 3 - Haemodynamic parameters compared between groups

Parameters	All surgeries (n=12)	Inhale group (n=6)	IV group (n=6)	P value
APsyspreind	139.8 ± 17.4	145.6 ± 13.2	134.0 ± 22.0	0.48
APdiapreind	75.8 ± 11.4	82.3 ± 10.7	69.3 ± 9.29	0.18
HR preind	76.8 ± 13.4	80.0 ± 13.0	73.6 ± 15.9	0.62
APsyspostind	126.5 ± 38.7	151.3 ± 38.8	101.6 ± 18.0	0.11
APdiapostind	66.0 ± 13.9	68.3 ± 10.4	63.6 ± 7.0	0.72
HR postind	104.6 ± 38.3	85.0 ± 6.24	123.3 ± 50.4	0.26
EtCO ₂	43.1 ± 4.3	40.0 ± 2.64	46.3 ± 3.05	0.05

Legend: AP - arterial pressure; sys - systolic; dia - diastolic, HR - heart rate, preind - preinduction of anaesthesia, postind- postinduction of anaesthesia, EtCO₂ - end tidal carbon dioxide

Figure 4 - Great vessels of the neck in pigs.
Legend: A - vagus nerve, B - carotid artery, C - internal jugular vein, D - trachea, E - larynx

Maintenance of anaesthesia

After induction of anaesthesia the pigs were mechanically ventilated in volume-control using a tidal volume of 10 ml/kg, a respiratory rate adjusted (12 to 18 breaths per minute) to maintain normocapnia and a PEEP of 3 mmHg was applied. The fresh gas flow (80% oxygen in air) was set to 2 L/min. Anaesthesia was maintained using sevoflurane set to maintain a MAC between 1.5 and 2.5 (EtSev 1.5-2.5 MAC). Further analgesia was achieved using 0.1 mg bolus doses of fentanyl at the anaesthesiologists discretion. No muscle relaxants were used during surgery. Warm Ringer solution was administered for the entire duration of

surgery at a rate of 3 ml/kg/h. At the end of surgery a bolus dose of 250 ml of 10% glucose was administered. For postoperative analgesia 1.5 mg/kg ketoprofen and 0.1 mg/kg of morphine were administered intravenously at skin closure. At the end of surgery the inhaled anaesthetic was discontinued, the pigs were left in spontaneous breathing mode and the trachea was extubated when spontaneous tidal volume was at least 5 ml/kg and the respiratory rate at least 12 breaths/min.

Postoperative care

During postoperative care intravenous 0.1 mg/kg of morphine and 1 mg/kg ketoprofen were administered every 8 hours. In addition 2 mg/kg meperidine was administered intravenously two hours after surgery. The pigs were not allowed to eat but were allowed to drink water during the 24 hours between surgeries.

DISCUSSION

During experimental swine surgery anaesthesia must provide both comfort and safety of the animal and the desired level of anaesthesia and relaxation for the surgical staff. The anaesthetic regimen is of crucial importance especially in cases in which re-intubation is planned and the animals must be safely weaned from mechanical ventilation and adequate comfort and analgesia provided during the postoperative period. The anaesthetic technique proposed is feasible for the purposes mentioned.

Intubation in swine is hard to achieve especially in large animals (6). Studies (7) have already shown that the

ventrodorsal position is recommended for oro-tracheal intubation. Nevertheless, induction techniques vary from center to center (8). To the authors knowledge few papers were published worldwide on this topic. Because of the small number of animals included, the present study lacks statistical power, the highly significant results that favor inhalational induction demonstrates the potential benefits in terms of ease of intubation and hemodynamic stability. Further investigations are needed in order to reach definitive results.

Although some studies favor intravenous maintenance of anaesthesia (9,10), in terms of safety and hemodynamic stability, one must remember that new inhaled anaesthetics such as sevoflurane offer both cardioprotection and ease of administration (11,12). A balanced approach (13) using both intravenous and inhaled agents has been proposed and probably offers the potential benefits of both techniques. Our protocol showed similar results.

As founding for experimental research and surgery is hard to find, cost effectiveness must be addressed. Although most studies (14,15) come from analysis of human surgeries the same principle applies and inhalational maintenance of anaesthesia is more appropriate

from a financial point of view.

Postoperative anaesthesia care is crucial from both an ethical point of view and for the animals' safety. If re-intervention is planned, adequate analgesia, comfort and hydration are essential. Both transdermal and intravenous opioids have been successfully used for pain relief (16). In our opinion, the combination of morphine and non-steroid anti-inflammatory drugs offers successful pain relief at small costs. The effects of hypothermia on different organs and systems are well documented (17). Intraoperative warming using forced heated air is strongly recommended as well as warming the postoperative facility to at least 23-24 degrees Celsius. Postoperative shivering was successfully treated using meperidine.

CONCLUSION

In conclusion, our proposed protocol for anaesthesia (*table 4*) in hepatic surgery in swine is feasible and offers both cost-effectiveness and safety. Inhalation induction of anaesthesia may be superior to classic intravenous protocols but a larger randomized control trial is required in order to reach definitive results.

Table 4 - Anaesthetic protocol in experimental swine surgery

Preoperative care:

- Bring the animals at least 24 hours before surgery in order to accommodate the pigs
- Fasting: 12 hours for solid foods and 6 hours for liquids
- Check the animal general state of health
- Provide comfort (temperature, noise, food)

Induction of anaesthesia:

- Preanesthesia: 0.5 mg/kg Acepromazine and 15 mg/kg Ketamine
- Position the pig on the operating table in a ventral position
- Venous access: 20 G peripheral catheter in an auricular vein
- Prime the anaesthetic circuit with 8% sevoflurane in 100% oxygen at a flow 8L/min
- Use a tight fitting mask for inhalational induction. Assess the corneal reflex for depth of anaesthesia
- Intubation: ask an assistant to open the pigs mouth using two loops made of gauze and pool out the tongue using another gauze. Insert a no 4 Miller blade until the epiglottis is visible. Insert a 7.0 cuffed endotracheal tube into the larynx and advance it by rotation into the trachea

Maintenance of anaesthesia:

- Catheterize the internal jugular vein
- Monitor EKG, heart rate, non-invasive blood pressure and SpO₂
- Ventilate the lungs in volume control with a tidal volume of 10 mL/kg, a respiratory rate between 12-18 and PEEP 3 – 5 mmHg using a FiO₂ of 80% and a fresh gas flow of 2 L/min
- Adjust inhaled sevoflurane to maintain MAC between 1.5 – 2.5
- Administer 3 ml/kg/h ringer solution during surgery and 250 ml 10% glucose at the end of surgery
- Administer boluses of 0.1 mg of fentanyl for pain control
- If muscle relaxants are required use rocuronium 0.6 mg/kg
- At the end of surgery administer 1.5 mg/kg Ketoprofen and 0.1 mg/kg of morphine

Postoperative care:

- Administer 0.1 mg/kg of morphine and 1 mg/kg Ketoprofen q 8 hours for postoperative analgesia
- If shivering or additional analgesia is required administer 0.2 mg/kg meperidine
- Allow drinking of clear fluids after 4 hours and semi-solid food after 12 hours

Conflict of interest

The authors have no conflict of interest to declare.

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