Prolonged Anaesthesia in Minipigs

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Prolonged anaesthesia may be required in minipigs undergoing terminal toxicological, pharmacological or microbiological studies in which the onset of action of test substances or infective agents is slow. It is also necessary in studies using pigs as models for the human intensive care patient. Being able to maintain anaesthesia for as long as possible is desirable because it maximizes the data volume that may be collected from individual pigs. Maintaining a stable physiological state minimizes data variability and increases study power. Both will contribute to *reducing* the number of animals required in a given project. Maintaining physiological variables within appropriate limits is particularly important for recovery experiments in which deviations from normality may compromise convalescence rate and welfare and so undermine attempts at *refinement*. The principles of reduction and refinement underpin the justification for the ongoing use of animals in biomedical research (Russell and Burch 1959).

A biomedical literature review reveals considerable variation in the definition of the term "prolonged"; see Table 1. However, describing "prolonged" in units of time ignores the term's implicit link with increased anaesthetic risk. Risk arises from the experiment itself, and from the skills and experience of those responsible for the anaesthetic. As the former is a "fixed effect" it is appropriate that any definition of "prolonged" should embody a sense of challenge to the latter. One option is to define a "prolonged anaesthetic" as one whose duration is expected or planned to exceed the anaesthetists' previous experience by a pre-determined factor.

Previous work (table 1) has focused on the anaesthetic drugs used to produce prolonged anaesthesia. However, diligent attention to non-pharmacological factors (Table 2) is important in maintaining stable conditions and preventing unexpected complications in prolonged procedures. Such attention becomes even more important in survival experiments where the goals are to minimize postoperative morbidities while restoring normal function as rapidly as is consistent with the animal's welfare. The following article is based on personal experiences and concentrates on the non-pharmacological aspects of prolonged anaesthesia in minipigs.

Table 2.
Factors requiring attention in prolonged anaesthetics in pigs

Subject selection: pre-operative examination
Pre-operative preparation
Drugs / technique selection
Depth of anaesthesia monitoring
Venous access and surgical cleanliness
Airway management
Anaesthetic breathing system selection
Inspired O2 levels
Ventilation mode
Temperature management
Fluid and blood loss management
Urinary bladder distension
Glucose and electrolyte management
Body position
Eye protection
Oral hygiene
Monitoring & tedium - Maintaining vigilance

1) Subject selection: pre-operative examination

The potential for problems arising during and after prolonged anaesthetics (in the case of survival procedures) is reduced by ensuring subjects are in optimum health. The animal's recent medical history, including body mass changes, coupled with a physical examination establishing health is a minimum require-

Table 1. Publications on pig anaesthesia incorporating the term "prolonged" in the title.

Citation	Pigs				Duration (hours)
	n	breed	mass (kg)	sex	
Cummings et al (1972)	67	Yorkshire, Landrace, Hamphshire, Duroc crosses	165 - 323	♀	5 - 13
Holmes et al (1990)	8	Landrace crosses	15 - 20	♀	7 - 9
Mutch et al (2000)	20	?	20 - 30	?	7
Nosser (2003)	20	Pietrain X Hampshire	36 - 50	♀	7
Kilic & Erhardt (2004)	9	?	30 (approx)	?	4
Eddleston et al (2012)	> 100	Gottingen minipigs	15 – 30 kg	♂♀	24 - 48

?: undisclosed

ment. Haematological and biochemical testing may be justified at this stage in providing baseline values that may prove useful in monitoring recovery and convalescence, rather than indicators of health.

2) Pre-operative preparation

No pre-operative preparation is required in healthy pigs beyond some degree of food and water deprivation. Water should be withheld once pre-anaesthetic medication has been given. However, the appropriate degree of food deprivation required is controversial. In our laboratory, pigs are fed 500 g per pig per day, once per day in the morning. The pellets are spread on the floor so the pigs take a few hours to find them amongst the straw. They are not fed on the morning of surgery until the animals scheduled for anaesthesia are removed. However, some pigs eat the straw upon which they bedded when they appear to become hungry in the early morning. This may be related to what appears to be the initial stages of necrotizing colitis seen at necropsy in a proportion of pigs after 24 - 48 hours of anaesthesia, and which appear to be associated with a full large bowel. Whilst some pigs spontaneously defecate during anaesthetics, it is possible that anaesthetic drugs and/or surgical stimulation exert constipative effects in others. That this may lead to impaction and associated problems in survival experiments prompts the consideration of aggressive gastro-intestinal evacuation measures, such as purgatives, enemas and extensive fasting before prolonged procedures. However, such measures are probably stressful and may adversely affect glucose homeostasis.

3) Drugs / technique selection

The anaesthetics chosen must produce experimental conditions whilst having minimal – or at least predictable effects on study variables. Side-effects should be manageable. In recovery experiments, anaesthetics must have non-cumulative properties. While volatile agents and total intravenous techniques have distinct advantages and disadvantages, modern practice favours a hybrid technique in which anaesthesia is produced using low inspired concentrations of insoluble volatile anaesthetics like sevoflurane or isoflurane, whilst analgesia is provided by constant rate infusions (CRIs) of short-acting opioids like fentanyl or alfentanil.

4) Depth of anaesthesia monitoring

Monitoring and controlling the depth of anaesthesia is necessary if rapid recoveries are to occur because these depend on the administration of the lowest doses of anaesthetic consistent with the production of experimental conditions. Furthermore, a constant depth of anaesthesia is often necessary because changes in anaesthetic depth resulting from changes in anaesthetic dose will invariably be associated with unstable physiological conditions. Constant dosing is possible with injectable techniques, e.g., CRIs and target-controlled infusions (TCI), or when volatile agents are delivered to produce constant end-tidal concentrations. Unfortunately, constant dosing techniques do not assure stable levels of anaesthesia, because other factors affect depth, the most important of which is probably body temperature. Consequently, in some experiments it may be more appropriate to produce a constant level of central nervous depression rather than the constant administration of anaesthetic. Unfortunately, signs of anaesthetic depth in pigs are rarely "scalable": corneal reflexes are either present or absent, and do not become sluggish as anaesthesia deepens. Jaw tone is an exception to this generalisation. Whilst evidence for the effectiveness of electroencephalographic-based methods of anaesthetic depth monitoring, e.g., the bi-spectral index (BIS) has been difficult to establish experimentally, we have found BIS monitoring to be near-invaluable in prolonged minipig anaesthetics (figure 1).

Figure 1

5) Venous access

Venous access may be established in the marginal auricular vein of the sedated pig for the purpose of inducing anaesthesia with intravenous agents. In prolonged procedures, it is necessary to ensure higher levels of surgical cleanliness when cannulating blood vessels and performing other "routine" instrumenting procedures, because infection becomes more likely the longer implants are in place. There is a greater justification for prophylactic antibiosis.

6) Airway management

The airway must be protected when anaesthetics eliminate protective airway reflexes. The intubation of a tracheal stoma provides a secure airway in acute procedures although oro-tracheal tubes are used when recovery is planned. Both cuffed endotracheal and tracheostomy tubes left *in situ* for prolonged periods may lead to traumatic and/or ischaemic tracheitis. The latter is more likely when cuffs are over-inflated. The cuff should be inflated to a pressure just in excess of the highest pressure required to produce lung inflation when positive pressure is imposed (see below). In prolonged operations, the cuff should be periodically deflated and re-inflated after the tube is repositioned 1 – 2 cms craniad or caudad. Sterile (new) endotracheal tubes should be used in pigs undergoing prolonged anaesthetics.

The prolonged inspiration of anaesthetics carried in dry medical gases (and the expiration of gases saturated with water vapour)

leads to dehydration of the tracheobronchial mucosa which paralyses the mucociliary carpet and viscidifies airway secretions. Accumulated airway secretions may occlude distal airways and by increasing intrapulmonary shunt lower blood O_2 tensions. Consequently, thought may be given to the intraoperative humidification of inspired gases, and in survival cases, performing endobronchial suction before tracheal extubation, providing post-operative expectorants and, or mucolytics, humidified air and thoracic percussion. The post-operative use of antitussive drugs must be considered as these will retard the expectoration of airway debris.

The prolonged loss of water vapour from the tracheobronchial tree also leads to considerable heat loss. This may be conserved with suitable breathing systems or the use of heat and moisture exchangers (HMEs) interposed between the breathing system and the endotracheal tube connector (figure 2).

Figure 2

7) Anaesthetic breathing systems

The choice of anaesthetic breathing system is critically important when pigs are expected to breathe spontaneously during the procedure, but selection is not straightforward. Non-rebreathing systems, e.g., the Bain system, offer little resistance to breathing and so minimize the work of breathing over extended periods. However, they do not conserve expired water vapour and their capacity to warm inspired breath is questionable. Moreover, the high gas flows required may prove expensive. Rebreathing systems like the circle and "to-and-fro" are regarded as high resistance systems but are more effective at conserving respiratory heat and moisture. The choice is academic when the lungs are mechanically ventilated.

8) Inspired O_2 levels

Prolonged exposure to high concentrations of inspired O_2 adversely affects respiratory function in several ways. However, information on safe exposure levels in minipigs does not appear to exist. In dogs it is recommended that exposure to 100% O_2 is limited to no more than 24 hours, or to 48 hours when 60% O_2 mixtures are breathed. In prolonged procedures, O_2 concentrations from the anaesthetic machine may be reduced by dilution with medical air. Unfortunately, many veterinary anaesthetic machines do not possess the capacity to deliver air, but information on simple modifications is available (Clutton et al 2012)

9) Ventilation mode

Allowing pigs to breathe spontaneously throughout a prolonged anaesthetic confers some advantages: respiratory pattern and rate are useful indicators of anaesthetic depth and the approach is straightforward – no equipment beyond a suitable breathing system is required. However, the work of breathing is provided entirely by the animal; respiratory "fatigue" is possible in prolonged anaesthetics, particularly if resistance to breathing is increased. During spontaneous breathing minute for minute changes in the inspired gas volume will result in the uptake of different levels of inhalant anaesthetic and contribute to unstable anaesthetic depth. Spontaneously breathing animals will hypoventilate (if adequately anaesthetized) which will elevate blood CO_2 levels and lower pH. Furthermore, during spontaneous breathing dependent lung tends to collapse if not periodically inflated. Areas of diffuse atalectic lung fails to oxygenate blood and by increasing venous admixture will lower blood O_2 levels. The progressive collapse of dependent lung tissue is retarded by "sighing" – the imposition of a supra-normal lung inflations at 3 – 5 minute intervals. However, diffuse pulmonary microatalectasis is best prevented by controlling ventilation throughout anaesthesia.

By imposing constant gas volumes at a fixed frequency, controlled ventilation maintains stable blood gas values & pH and stabilizes "depth". It also preserves metabolic energy. However, the use of respiratory pattern as a "depth" indicator is lost and there are adverse physiological effects: a reduction in cardiac output and accelerated heat loss being the most important. Prolonged controlled ventilation is synonymous with mechanical ventilation and the use of mechanical ventilators, because the other option, manual inflation, is infeasible in operation lasting more than several hours. The prolonged inappropriate use of mechanical ventilators is associated with ventilator associated lung injury (VALI).

Ventilator associated lung injury results from excessive pressures (barotrauma), excessive distending volumes (volutrauma), alveolar damage resulting from transient and repeated closure and reopening of alveoli during the respiratory cycle (atalectrotrauma) and biotrauma, in which the altered magnitude and pattern of lung stretch changes gene expression and cellular metabolism in a way that produces an overwhelming inflammatory response - even in the absence of structural damage. The latter phenomena, known as systemic inflammatory response syndrome (SIRS) has been investigated in the porcine model and can be fatal within 12 – 24 hours of a relatively brief period (8 – 12 hours) of injudicious (excessive tidal volumes over prolonged periods) lung ventilation. The risk of SIRS is reduced by using the ARDSnet protocol, a lung ventilation pattern established by syndicated United States health agencies committed to the treatment of acute inflammatory lung disease. The ASRDSnet protocol, which is detailed online (http://www.ardsnet.org/system/files/Ventilator%20Protocol%20Card.pdf) involves the delivery of low tidal volumes (6 – 8 mL kg^{-1}) at relatively high respiratory rates. Unfortunately, the complex ventilatory

patterns required by the ARDSprotocol requires intensive care, rather than anaesthetic ventilators. The former are more complicated, expensive and less available than the latter (figure 3).

Figure 3

10) Temperature management

Anaesthetized pigs inevitably lose heat because anaesthetics and surgery promote heat loss; while compensatory behavioural and physiological responses to hypothermia are impaired by anaesthetics. In the absence of interventions, e.g., externally applied heat, temperature loss is a function of time and the ambient temperature. Hypothermia is undesirable in all operations because it affects numerous physiological variables and so contributes to variability in collected data. It contributes to central nervous depression and so augments the effects of anaesthetics; it may contribute to relative overdose and prolonged recoveries. It reduces ventilation and cardiac output, which will affect the uptake and distribution of inhaled anaesthetics and retard their elimination. In terms of tissue oxygenation, its adverse effects on cardiac contractility are augmented by increasing blood viscosity and a left-shift in the oxyhaemoglobin dissociation curve. At core temperatures of approximately 28^{o}C ventricular fibrillation is near-inevitable.

In recovery procedures, the hypothermic mammal whose capacity for thermoregulation returns with recovery from anaesthesia mounts a hypermetabolic heat generating response that in humans is unpleasant, and which may exhaust limited glucose and O_2 reserves.

Hypothermia is more appropriately prevented than treated. Numerous strategies are feasible including the use of high ambient

Figure 4

(laboratory) temperatures or creating warm "local" conditions (bubble wrap insulation with hot air blankets and/or heater pads; see figure 4). During invasive procedures, irrigation fluids should be warmed to 37 - 38^{o}C. The anaesthetist minimizes heat loss by avoiding high doses of (vasodilating) a_1 antagonist drugs, e.g., azaperone, using positive pressure ventilation judiciously, favouring "low-low" re-breathing systems and using HMEs.

In recovery experiments "cold" pigs which are slow to recover must be given O_2 by mask whenever shivering is present. Core temperature can be raised in numerous ways but over-enthusiastic warming must be avoided as the risk of burns is greater in animals with impaired thermoregulatory reflexes and cutaneous blood flow. Recovering animals should be thoroughly dried (if wet) with towels and hair dryers. Topical heat may then be judiciously applied using 40 watt light-bulbs, radiant infra-red lamps or insulated hot-water bottles. Topical internal heating methods, i.e., peritoneal, pleural, gastric or rectal lavage, are highly effective but each has specific disadvantages, e.g., peritoneal and pleural lavage may exert significant dialysing effects. Haemolysis will occur if intravenous fluids are warmed > 40^{o}C.

11) Fluid and blood loss management

Fluid balance is extremely important in prolonged anaesthetics. If the simple principle that fluids lost must be replaced on a "mL-for-mL" and "like-for-like" basis is ignored then either fluid overload or hypovolaemia will ensue. Overinfusion of crystalloid solutions will result in 3rd space losses, including pulmonary oedema. The excessive use of colloids will result in systemic and pulmonary hypertension, and ultimately ventricular failure. The net loss of circulating blood volume over time will result in signs of hypovolaemic shock, e.g., arterial hypotension, oliguria and depressed mentation.

Water and electrolyte losses are inevitable in the anaesthetized pig because urine production *should* continue while free water is lost by evaporation in the lung. These losses are compounded by surgical blood loss. The latter is easily quantified:

mLs blood lost = (mass [in g] of N bloody swabs - mass of N dry swabs) / 1.3

The volume of blood lost to surgical suction in suction jars can also be quantified:

mLs blood lost = (PCV in suction jar / pig PCV) X mls blood in suction jar.

Water and electrolyte losses (and crystalloid requirements) are more difficult to quantify although empirically, infusing polyelectrolyte solutions at 5 - 15 ml kg^{-1} $hour^{-1}$ during minor - major operations usually maintains cardiac preload and sustains urine output without obvious adverse effect.

12) Urinary bladder distension

Continued urine production - which is desirable - will result in progressive urinary bladder distension and a rise in intravesicular pressure - which is not desirable: excessive pressures will cause post-renal failure and cystitis. In recovery experiments full bladders cause extreme discomfort. Neither minipig boars nor sows seem able to void full bladders passively under anaesthesia and so some method of urine evacuation is required. The digital introduction of a urethral catheter is occasionally - but not always achievable *per vaginum* in sows. In prolonged anaesthetics in minipigs of both sexes, a surgical cystotomy is recommended. This must be performed under sterile surgical conditions as ascending catheter associated urinary tract infections are not uncommon.

13) Glucose

During prolonged anaesthetics blood glucose levels frequently fall from 2.7 - 4.3 mmol L^{-1} to < 2.0 mmol L^{-1} over 18 or more hours. This progression can be retarded by infusing 4.3% dextrose 0.18% saline solutions at 5 - 15 ml kg^{-1} $hour^{-1}$. When blood glucose falls < 2.0 mmol L^{-1}, dextrose (50%) injections are recommended at 0.5 mL kg^{-1}.

14) Body position

Intra-operative pig position should be optimized for surgery or experiment but without adversely affecting blood flow and ventilation. Ties must be used carefully (figure 5); when overtight, they exert a tourniquet-like effect and cause tissue and nerve damage. The imposition of abnormal positions for prolonged periods may also lead to nerve and/or muscle damage.

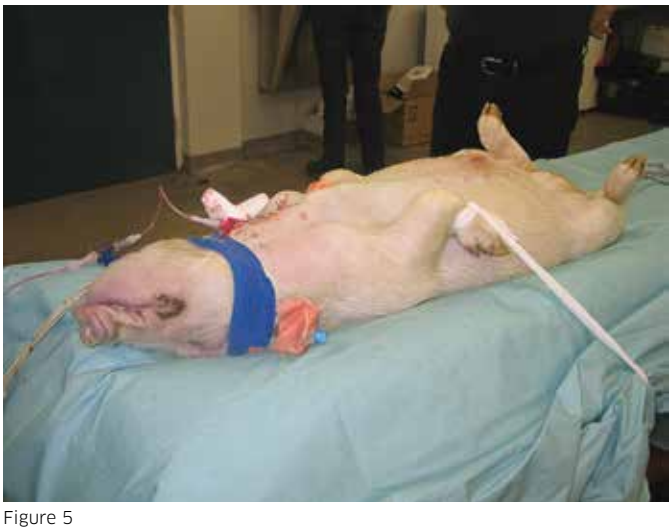

Figure 5

When arthritis is present, severe discomfort can be expected after prolonged joint flexion in (normally) intolerable positions. This is extremely undesirable in animals involved in recovery experiments. Periodic position changes (if feasible) and some simple physiotherapeutic manipulations may be considered in pigs recovering from prolonged experiments.

15) Eye protection

Prolonged anaesthetics in which the eyes remain partly or fully open will lead to corneal dessication, post-operative discomfort and possible corneal damage. The ocular surface must be kept moistened and/or protected by taping or bandaging the eyes shut, or applying artificial tears, e.g., "Lacrilube ", at frequent intervals.

16) Oral hygiene

The proliferation of bacteria in the oral cavity over time contributes to ventilator-associated pneumonias in humans undergoing intensive care. In pig models, cleaning the teeth three times daily and applying 0.2% chlorhexidine gel afterwards may prevent similar problems, and in any case is justified as it contributes to a "truer" model.

17) Monitoring & tedium - Maintaining vigilance

Prolonged anaesthetics in our laboratory involves individual shifts of 8 hours. At least two people are present at all times, but especially at night. Food and drink are available immediately outwith the laboratory area. When data loggers are used, continued surveillance and care of the unconscious pig can be promoted by designing studies that demand periodic attention to the animal and the manual recording of data.

Conclusion

Prolonged anaesthesia in pigs for biomedical research is challenging and success depends as much on critical attention being paid to non-pharmacological factors as it does to the anaesthetic technique. Attention to these factors becomes critically important in recovery experiments because it will affect the comfort and post-operative welfare of the animal.

References

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