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Anesthesia for Cardiovascular Interventions and Magnetic Resonance Imaging in Pigs

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Large animal models are still required for many experimental purposes. The aim of the current study was to define a viable narcotic procedure for experimental cardiovascular interventions and imaging in pigs. A total of 32 domestic pigs were used. Animals received propofol, midazolam, and fentanyl as continuous intravenous infusion anesthesia for complex vascular interventions, angiographic X-ray imaging, and magnetic resonance imaging (MRI). Anesthesia was maintained for 6 to 10 h. The initial hourly doses were 2.29 mg/kg of propofol, 1.14 mg/kg of midazolam, and 0.009 mg/kg of fentanyl, with controlled ventilation. Anesthesia, interventions, imaging, periods of apnea of as long as 2 min, and transportation were well-tolerated. Stress-induced arrhythmias were not noted, and artifact-free imaging was achieved. The combination of propofol, midazolam, and fentanyl is well-suited for experimental angiographic interventional studies, experimental cardiovascular MRI, and MR-guided interventions in pigs.

Abbreviations: MRI, magnetic resonance imaging

During the last decade, interventional radiology, including magnetic resonance imaging (MRI)-guided angiography, has become a promising field of research after many recent hardware and software developments. Pigs, due to their size, anatomy, and physiology, are a suitable large animal model for many kinds of interventional techniques requiring similarity to human beings. The marked morphologic and functional similarities between the organ systems of humans and pigs have been described previously.[8,10,19,20] In addition, experiments in interventional radiology require intraoperative transportation, deep sedation and the possibility of investigator-induced intermittent breath-hold episodes to ensure sufficient image acquisition without motions artifacts. In light of our experience of narcosis in more than 400 cases, pigs have poor tolerance to stress as well as a high tendency to develop malignant arrhythmias; both of these conditions frequently interfere with ECG-triggered imaging that demands a stable heart rhythm.

Due to the complex issues regarding handling inhalation anesthesia devices, particularly in nonspecialized facilities that potentially contain ferromagnetic materials, as well as the high risk of circulatory insufficiency and malignant hyperthermia associated with inhalation anesthesia, the use of inhalation anesthetics may not be viable in interventional radiology, at least as far as MRI experiments are concerned. The aim of our experiments was to develop an effective anesthesia protocol using controlled ventilation for vascular interventions and imaging in a large animal model. In particular, our anesthetic protocol for performing various vascular interventional procedures, including MRI-directed placement of guidewires and catheters, accommodates transportation of the animals between the animal laboratory, catheterization laboratories, and the MR scanner.[3,10-13]

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Materials and Methods

Animals. The study population comprised 32 domestic pigs (German Landrace Universal; DL[U]; weight, 40 to 125 kg) that were used according to the policies and guidelines for the humane care and use of animals; all procedures were approved by the regional and institutional animal care and use committee (Duesseldorf, Germany). Animals had standard laboratory food and water provided ad libitum. No solid food was given 12 h prior to induction of anesthesia. Routine laboratory diagnostics were done to exclude potential infectious contamination. Animals were acclimated to the facility and personnel for at least 2 wk and ensured to be free of specified infectious diseases that might have had an adverse effect on the experimental protocol.

Anesthesia. Sedation of the animals was achieved by intramuscular injection of ketamine (30 mg/kg), azaperone (2 mg/kg), and atropine (0.025 mg/kg BW) vertically into the neck.[8] Adequate sedation was achieved when the animal failed to withdraw after stroking of its snout. The animals then were transferred to the catheterization laboratory. Intravenous access was achieved by insertion of an 18-gauge cannula into an ear vein. After intravenous bolus injection of propofol (2 mg/kg) and fentanyl (0.005 mg/kg), animals in prone position on the operation table underwent endotracheal intubation (inner diameter of the tubes: 5.0 to 8.0 mm) if the swallowing reflex was completely absent. After the pig was preoxygenated with 100% O_2, its tongue was carefully put into a forward position. When palate and palatal velum became visible, the tube was directed toward the epiglottis and carefully pushed forward while being rotated around its axis. The animals were ventilated by use of a respirator (Oxylog, Dräger, Lübeck, Germany) and controlled ventilation with 50% oxygen and a ventilation rate of 10 and 12 breaths per minute. The tidal volume varied between 350 and 1000 ml, depending on the weight of the pig. Preoxygenation using 100% oxygen was performed prior to each induction of apnea. Monitoring included heart rate and electrocardiography via external leads (ECG monitor, Siemens Medical Solutions, Erlangen, Germany), oxygenation by use of a peripheral oxygen

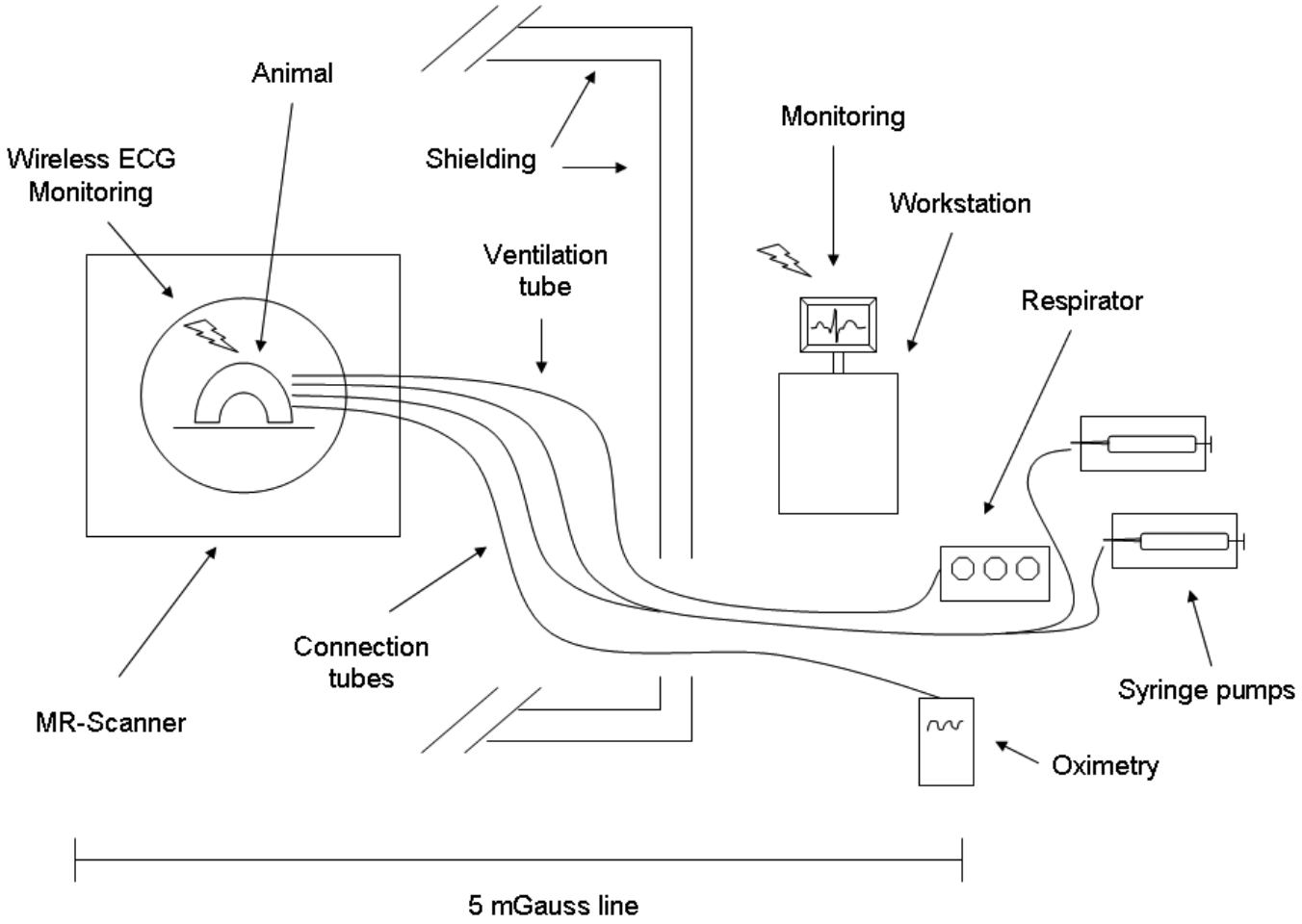

Figure 1. Schematic diagram of the ventilation and monitoring setup for experiments in the MR scanner room.

sensor (Vet/Ox 4404 Pulse Oximeter, Heska Animal Health Products, Clayton, Australia) on the pig's tongue, and respiration via external chest sensors. Anesthesia was maintained by continuous intravenous infusion of propofol (2.29 mg/kg/h), midazolam (1.14 mg/kg/h), and fentanyl (0.009 mg/kg/h) delivered via infusion pump (Compact Perfusor, Braun, Melsungen, Germany). For our experiments we infused a mixture of fentanyl and midazolam (0.04 mg/ml fentanyl and 5 mg/ml midazolam in a 50-ml perfusion syringe) in 1 syringe pump and provided propofol (10 mg/ml) through a separate 50-ml syringe pump. If necessary, the dose was increased according to the reflex status of the pig (palpebral reflexes and tone of jaw and anal sphincter muscles). For MR procedures, the potentially ferromagnetic infusion pumps and respirator were placed beyond the 5-mGauss line by use of a 6-m ventilation tube (Figure 1).

Vascular interventional procedures. A paramedian incision of the left groin to expose the left iliac artery was performed alone (n = 24) or in combination with a longitudinal incision on the left side of the neck for additional preparation of the carotid artery (n = 8). Briefly, the cervical and iliac muscles were dissected to reveal the underlying vessels (iliac artery, carotid artery). Catheter sheaths (8- to 14-French) were implanted, tunneled subcutaneously into the neck or groin area, and fixed. All skin lesions and the bluntly dissected muscles were closed by single stitch technique. The animals then were transported to the MR scanner (Figure 2). During anesthesia, pigs underwent various vascular interventional procedures, such as guidewire and catheter placement with subsequent selective catheterization of various vascular territories[10,12,13] (n = 23) and aortic stent–graft placement[3,11] (9 pigs), under real-time MR imaging guidance. After all experiments, animals were euthanized by intravenous bolus injection of pentobarbital (80 mg/kg) without recovery from anesthesia, as mandated by the experimental protocol and animal use guidelines.

Results

Anesthesia. Administration of ketamine (30 mg/kg), azaperone (2 mg/kg), and atropine (0.025 mg/kg) resulted in adequate sedation of the pigs. Spontaneous breathing was maintained. Venous access was obtained easily through the ear vein. After bolus injection of propofol or fentanyl or both agents, intrinsic reflexes disappeared, and all pigs were intubated without noteworthy complications shortly after suspension of spontaneous breathing. In addition, all animals tolerated the anesthetic procedures without any complications.

The duration of anesthesia ranged from 6 to 10 h. Anesthesia by continuous intravenous infusion of propofol (2.29 mg/kg/h), midazolam (1.14 mg/kg/h), and fentanyl (0.009 mg/kg/h) resulted in deep sedation, sufficient analgesia, and stable hemodynamic status, thus allowing the required manipulation. If necessary due to reoccurrence of the palpebral reflex, the dosage was increased to a maximum of 5.42 mg/kg/h propofol, 2.71 mg/kg/h midazolam, and 0.021 mg/kg/h fentanyl. Muscle relaxants were not needed. Oxygen saturation and blood pressure remained stable during the surgical, imaging, interventional, and transportation procedures. As required for electrocardiogram-triggered MRI sequences, no cardiac arrhyth-

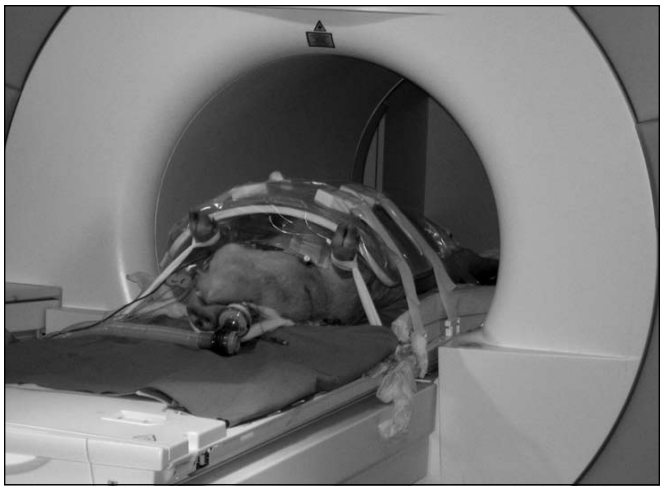

Figure 2. Photograph of the experimental setup with a pig inside the MR scanner.

mias that interfered with image acquisition occurred.

Vascular interventional procedures. Catheterization was performed in the catheterization laboratory or at the MR scanner, depending on the requirements of the experiment. Blood loss during these interventions was less than 100 ml in all cases, and all surgical and interventional procedures were uneventful as well. Heart rate remained stable without any noteworthy arrhythmias in all animals. In the case of MR-guided interventions, apnea (maximum duration, 2 min) was achieved without any complications.

Transportation. Transportation between the animal facilities and catheterization laboratory without external ventilation of the pigs was possible after injection of ketamine, azaperone and atropine injection to achieve appropriate sedation. Transportation between the catheter laboratory and the MR scanner was performed by use of the described combination of propofol, fentanyl, and midazolam for anesthesia. The maximum time of transportation during anesthesia was 5 min, during which all animals had stable cardiopulmonary function. The iliac access was dislocated in 1 pig while it was being moved onto the MR scanner table, resulting in a 500-ml blood loss that compensated by rapid infusion of colloidal solution (Haes-steril 10%, Fresenius, Bad Homburg, Germany) and prompt surgical repair and reinsertion of the catheter sheath. Otherwise, transportation procedures were uneventful in all animals. The vital signs indicated no evidence of increased stress during transportation.

MRI. MR image acquisition lasted for as long as 6 h. The long (6 m) ventilation tube with its increased volume of dead space did not hamper oxygenation. Intermittent periods without ventilation were possible to ensure image acquisition without motion artifacts. Maximum duration of apnea was 2 min without signs of hypoxia. Electrocardiograms remained stable throughout imaging of all pigs, and no experiment had to be terminated prematurely due to cardiopulmonary instability.

Discussion

Many experimental studies require prolonged anesthesia for large animal angiographic and MRI procedures. Our experimental requirements included transportation of narcotized and intubated pigs from the catheter laboratory to the MR scanner, prevention of cardiac arrhythmia, and induction of breath-hold phases.

In general, sufficient anesthesia results in a well-balanced combination of sedation, analgesia, hypnosis, and muscle relaxation. Selection of an appropriate anesthetic regimen requires consideration of the goals of the investigation as well as the possible side effects of the chosen anesthetic drugs.[7,18] Anesthesia for interventional procedures in animals can be achieved through inhalation or intravenous routes or a combination thereof. Anesthesia by infusion is possible using pentobarbital, ketamine, xylazine, azaperone, propofol, midazolam, and fentanyl.[4,5,8,21] Other drugs including sevoflurane, sufentanil, and remifentanil also can be used for large animal investigations but are relatively expensive.[6,14,15]

A leading advantage of intravenous anesthesia is the simplicity of the equipment required.[18] Therefore, intravenous anesthetics may be a reasonable choice in complex experimental settings that include the need for transportation during the investigation. Furthermore, intravenous anesthetics are superior to inhalation anesthetics in terms of maintaining anesthesia for MRI, because infusion pumps and the ventilator can be placed the necessary distance from the scanner. Further, periods of apnea of up to 2 min are possible. Although the ideal intravenous anesthetic agent has yet to be developed, a wide spectrum of drug combinations can be used. Intravenous anesthesia typically involves the use of a combination of sedative-hypnotic, analgesic, and neuromuscular relaxant drugs.[21] To maintain sufficient analgesia and sedation of our pigs, we used a combination of propofol, fentanyl and midazolam for prolonged interventions and imaging procedures. These anesthetic agents were administered intravenously via syringe infusion pumps. This combination of injectable anesthetics provided optimal conditions for intervention and transportation without any notable side effects, whereas no single drug alone met all requirements. Propofol and fentanyl provide good control of anesthetic depth and minimal hemodynamic changes and, if necessary, also provide the advantages of rapid onset and recovery.[1,2,16]

In humans, propofol promotes relaxation and sleep, reducing anxiety and tension. Propofol also provides loss of awareness for short diagnostic tests and surgical procedures or may supplement other types of prolonged anesthetics.[8] Midazolam has a rapid onset of action and high efficacy for reliable hypnosis, amnesia, and strong antianxiety effects.[17] Although midazolam is hypnotic-amnesic during maintenance of general anesthesia, it cannot be used alone for interventional procedures because it lacks analgesic potency. Fentanyl has been used to maintain prolonged anesthesia in surgical experiments and has high analgesic activity without significant cardiopulmonary adverse effects.[7,9] Selection of the most appropriate route, dosage, and depth of anesthesia depends on the type of experiments, sites and techniques of interventions, and expected duration of procedures.

The studies we describe had several limitations. All of the trials reported were nonsurvival, and detailed invasive cardiovascular measurements were not used. However, since 2005, we routinely have performed additional measures, including blood gasses (pH, base excess, arterial oxygen and carbon dioxide concentrations), blood pressure, and capnometry. Results from recent experiments incorporating electrocardiography, monitoring of ventilation through chest sensors, and pulse oximetry confirm the stable cardiovascular status of pigs anesthetized as described (data not shown).

Because breath-hold MRI requires periods of apnea of as long as 2 min, continued use of ketamine with midazolam and fentanyl does not seem to be promising, as spontaneous breathing commonly remains in pigs under ketamine narcosis. Increasing the doses of midazolam or fentanyl or both sufficiently to abolish spontaneous respiration increases the risk of side effects of these agents. The combination of propofol and

fentanyl might be suitable for breath-hold MRI in pigs, but again the need for high doses may lead to side effects, such as profound hypotension.

In conclusion, the present studies evaluated an intravenous anesthesia protocol for MRI investigations in pigs that combined propofol, midazolam, and fentanyl. This protocol could be used safely in a MRI environment; was suitable in terms of the need to transport anesthetized, absence of spontaneous breathing, and ease of use near an MR scanner; and led to stable heart rates without any significant arrhythmias for prolonged periods (as long as 10 h) of anesthesia.

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