Effects of ketamine/xylazine premedication on emulsified isoflurane general anaesthesia in swine undergoing embryo transplantation

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ABSTRACT: Cardiorespiratory effects were assessed during ketamine/xylazine premedication followed by emulsified isoflurane anaesthesia in swine undergoing experimental embryo transplantation. Ketamine (10 mg/kg) and xylazine (3.5 mg/kg) were premedicated intravenously, followed by continuous administration of intravenous emulsified isoflurane (2.8 ml/kg/h). Cardiorespiratory parameters, including heart rate, respiratory rate, mean arterial blood pressure, arterial oxygen saturation, and rectal temperature, were recorded in sows undergoing surgical embryo transplantation. Ketamine/xylazine premedication resulted in anaesthetic induction and lateral recumbency within 1 minute without any adverse effects. The physiological changes observed after drug administration remained within biologically acceptable limits. In conclusion, the combination of ketamine/xylazine provided anaesthetic induction, muscle relaxation, and analgesia sufficient for emulsified isoflurane intravenous anaesthesia. There were no adverse events in the experimental animals. This finding supports the use of emulsified isoflurane following ketamine/xylazine premedication in pigs.

Keywords: premedication; emulsified isoflurane; physiological parameters; general anaesthesia; pig

Embryo transplantation (ET) is an effective and practical procedure for reducing disease risk in new genetic stock that replaces herd animals (Martinez et al. 2004). Although previous research showed that non-surgical ET could be performed in pigs (Polge and Day 1968), the pregnancy rate was reduced compared to surgical methods (Martinez et al. 2004), and further improvements are needed to increase fertility. Surgical ET in pigs requires chemical restraint. Swine are widely used in biomedical research, despite being more difficult to immobilise and anaesthetise (Linkenhoker et al. 2010), and sedatives are required to restrain pigs for management.

Inhalant anaesthetics, including commonly used modern agents such as sevoflurane and isoflurane, are used to anaesthetize pigs (Haga et al. 2001; Ishida et al. 2002). However, inhalant anaesthetics require specialised delivery equipment, such as a source of oxygen, a vaporiser, a breathing circuit, and a mechanism to scavenge waste anaesthetic gases. Because of legal restrictions, as well as technical and economic limitations, volatile anaesthetics, e.g. halothane, isoflurane, and sevoflurane, cannot be used in many countries (Haga et al. 2002). Emulsified isoflurane is an unsaturated lipid emulsion preparation of isoflurane, and one preparation is 30% intralipid, a sterile, non-pyrogenic fat emulsion for intravenous administration (Xi'an Libang Pharmaceutical Co, Ltd., Xi'an, China). Several preliminary studies have validated the intravenous administration of emulsified halothane and isoflurane without adverse effects in animals (Musser et al. 1999; Zhou et al. 2006). Xylazine is a typical α_2 adrenoceptor agonist, relatively inexpensive, and is used to immobilise pigs in combination with other anaesthetics (Lu et al. 2011). Ketamine, a dissociative anaesthetic, has been a

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widely used component of anaesthetic regimens in veterinary patients (Boschert et al. 1996; Sakaguchi et al. 1996). It acts as a sympathetic stimulant and counteracts some of the vagotonic effects of α_2 agonists, while α_2-agonists minimise the muscle hypertonicity associated with ketamine (Kerry et al. 1996). In addition, ketamine can be administered both intravenously and intramuscularly, making it a practical intravenous premedication in animals.

There are no known reports investigating the use of emulsified isoflurane (EI) anaesthesia during ET in pigs. The aim of this study was to evaluate the cardiopulmonary, haemodynamic responses, and body temperature stability using the following anaesthetic protocol: premedication using a combination of ketamine/xylazine, followed by maintenance with emulsified isoflurane, in swine undergoing embryo transplantation.

MATERIAL AND METHODS

Animals. This study was approved by the Animal Care and Use Committee of Northeast Agricultural University, Harbin, China. Eight adult Bama miniature sows weighing 28.4 ± 5.1 kg received embryo transplantation. The sows were fed dry food twice daily and had free access to water. They were allowed a 2-week acclimatisation period before the start of the experiment.

Preoperative assessment. To ensure that the animals were in good health, a basal clinical examination (rectal temperature (RT), heart rate (HR), respiratory rate (RR), and thoracic auscultation), and a routine physical examination (complete blood count, biochemical profile, and electrocardiography) were performed preoperatively. The clinical parameters were within normal ranges for all subjects, and all appeared healthy and exhibited no clinical disease.

Anaesthetic premedication and maintenance. The subjects were fasted for 12 h and water was withdrawn 6 h before administering anaesthesia. Two hours preoperatively, the animals were transported to the laboratory to acclimatise to the environment, and the baseline physiological parameters were measured and recorded for each. Then, atropine (0.02 mg/kg) was administered intramuscularly to avoid excessive salivation during anaesthesia. Anaesthetic induction comprising ketamine/xylazine (10/3.5 mg/kg) was administered intravenously (*i.v.*), and once the animals were in a lateral decubitus position (within 1 min), they were moved to the surgical table. An 18 G intravenous catheter (Jinhuan Co., Ltd., Shanghai, China) was placed into the middle ear vein at the bilateral dorsal aspect of the ear under sterile conditions. The catheter was secured to the skin using surgical tape and connected to an *i.v* line using a three-way stopcock to administer fluids and drugs as required. Each subject was administered emulsified isoflurane 2.8 ml/kg/h intravenously using an infusion pump. The sows were not intubated and breathed ambient air.

Physiological monitoring. The heart rate (HR), respiratory rate (RR), rectal temperature (RT), mean arterial blood pressure (MABP), and arterial oxygen saturation (SPO_2) were recorded at the beginning of anaesthesia (T0), at 10 (T10), 20 (T20), 30 (T30), 40 (T40), and 50 (T50) min, and at the end of anaesthesia, defined as the time the infusion pump was switched off (T anaesthesia end). The end-tidal carbon dioxide ($ETCO_2$) was recorded at T10, T20, T30, T40, T50 and at T anaesthesia end. The respiration rate was measured by observing or palpating the thoracic tidal excursions for 1 min. Heart rate was determined by auscultation of the heart at the lower left lateral thoracic wall for 1 min. Subjects were monitored non-invasively (Datex-Ohmeda S/5; Datex-Ohmeda Division Instrumentarium Corp. Helsinki, Finland). The SPO_2 values were deemed acceptable only if the pulse rate derived from the pulse oximeter matched the auscultated heart rate. The non-invasive patient monitor was used to measure blood pressure by placing an adjustable blood pressure cuff around the left antebrachium. The rectal temperature was measured using a digital thermometer and displayed by the monitor. $ETCO_2$ was measured using a Datex-Engstrom Capnomac Ultima Anaesthesia Gas Monitor (Datex-Engstrom Division Instrumentarium Corp.).

Experimental embryo transplantation model. Experimental transplantation was performed using quality morphous embryos that were transferred into straws and packaged with sterile foil. Natural oestrous sows were selected; food, but not water, was withheld for 12 h prior to ET. Cloned embryos were surgically transferred to the recipient's cornua uteri through the oviduct umbrella using a technique that enabled a clear view of the surgical manoeuvres and adequate exteriorisation of the ovary, oviduct, and uterus.

End of anaesthesia and postoperative analgesia. Ten minutes before the pump was switched

off, the subjects were administered tramadol (2 mg/kg) for postoperative analgesia. Once the surgery was complete, the end of anaesthesia was achieved by switching off the pump. The sows were allowed to recover on the floor and were housed individually under controlled light and temperature conditions during postoperative recovery. Animals were allowed water and were administered Lactated Ringer's Solution 10 ml/kg/h (500–1000 ml) intravenously on the first postoperative day. Normal oral feeding with commercially available dry food was resumed on the second postoperative day. During the first two days, the general health, consciousness, hydration, respiratory rate, water and food intake, urination, and defecation of each subject was monitored.

Statistical analysis. The data were calculated as the mean ± SD and analysed using the statistical software SPSS v13.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were analysed using one-way ANOVA followed by the Student-Newman-Keuls test. Differences were considered significant at $P < 0.05$.

RESULTS

Ketamine/xylazine rapidly induced sedation-anaesthesia and lateral recumbency within 1 min. None of the subjects vomited during anaesthetic induction, and there were no signs of CNS excitement during the transition to lateral recumbency. The mean anaesthetic duration, designated as the duration from EI administration to the time the infusion pump was stopped, was 57 ± 3 min; the surgery lasted 44 ± 4 min, measured from skin incision until the last suture.

As summarised in Table 1, the heart rate significantly decreased at 10 and 20 min, compared with the beginning of anaesthesia ($P < 0.05$). No additional changes occurred from 30 min onwards. The respiratory rate also significantly decreased at 10 and 20 min, compared with the baseline ($P < 0.05$). SPO_2 decreased significantly compared with the baseline ($P < 0.05$). The mean arterial blood pressures were significantly lower from 10 min to 20 min of anaesthesia onward ($P < 0.05$); the mean SpO_2 at 30 min was significantly lower than at 20 min ($P < 0.05$). During the procedure, $ETCO_2$ decreased from T10 to the end of anaesthesia after treatment. From T30 to anaesthesia end, mean $ETCO_2$ was significantly lower than in T10. A significant decrease in body temperature was observed beginning at 10 min throughout the anaesthetic period ($P < 0.05$). Moreover, the mean body temperatures at 20 min, 30 min, 40 min, 50 min, and at the end of anaesthesia were significantly lower than the mean temperature at 10 min. Furthermore, compared with the mean at 40 min, the mean body temperature was decreased at 50 min from the start of anaesthesia. However, the body temperature remained within the normal range throughout the procedure; therefore, this decrease lacked clinical relevance. There was no significant increase in mean heart rate or mean arterial blood pressure, indicating the absence of signs of pain during the procedure. There were no adverse events in the experimental animals, and no instances of awakening intraoperatively. Experimental embryo transplantations were successfully performed in all animals.

DISCUSSION

The primary route of elimination following *i.v.* emulsified isoflurane administration is through the lungs; after administration, the pulmonary

Table 1. Mean (±SD) physiological parameters in pigs undergoing ketamine/xylazine anaesthetic induction followed by emulsified isoflurane anaesthesia

Time (min)	T (°C)	HR (beats/min)	RR (beats/min)	SPO_2 (%)	MABP (mmHg)
0	39.1 ± 0.2^{a}	122 ± 8^{a}	44 ± 5^{a}	98.2 ± 1.7^{a}	89 ± 8^{a}
10	38.5 ± 0.3^{b}	100 ± 13^{c}	32 ± 6^{b}	91.3 ± 3.2^{b}	80 ± 11^{b}
20	38.0 ± 0.2^{c}	103 ± 11^{c}	35 ± 7^{b}	90.7 ± 2.1^{b}	76 ± 8^{bc}
30	37.7 ± 0.3^{c}	109 ± 9^{b}	39 ± 5^{ab}	92.3 ± 1.9^{b}	73 ± 6^{c}
40	37.4 ± 0.2^{c}	118 ±5^{ab}	37 ± 6^{b}	91.0 ± 2.6^{b}	78 ± 7^{bc}
50	36.9 ± 0.3^{d}	117 ±7^{ab}	40 ± 8^{ab}	93.4 ± 3.2^{b}	77 ± 6^{bc}
anaesthesia end	37.0 ± 0.3^{d}	117 ± 6^{ab}	39 ± 6^{ab}	93.6 ± 2.2^{b}	75 ± 6^{bc}

^{a,b,c,d}indicates a significant difference ($P < 0.05$) between the time points in each column

blood isoflurane concentration is higher than in the alveoli, and some of the isoflurane diffuses into the alveoli. The isoflurane is excreted through the lungs (Yang et al., 2006), and in our experiments pigs breathed room air spontaneously without intubation. Therefore, to prevent human exposure, anaesthetic gas adsorbers (Hakko 493-11, Hakko, Tokyo, Japan) should be placed near the animal's respiratory passages. For a safe anaesthesia it is important to administer pre-anaesthetic medications. When used appropriately, these medications induce a desirable state of calm or sedation (Vesal et al. 2011). Ketamine and xylazine were selected because they achieved rapid and deep sedation which was reported to be more effective in several animal species (Green et al. 1981). This study demonstrated that the combination of ketamine and xylazine was effective and safe for intravenous induction. Intravenous ketamine and xylazine premedication resulted in the loss of consciousness within 1 min which lasted long enough to permit intravenous catheterisation. Anaesthetic induction was predictable and smooth, and muscle relaxation was good throughout immobilisation.

Xylazine is an α_2 adrenergic receptor agonist, sedative, and muscle relaxant widely used for analgesia and for improving the well-being of animals preoperatively, as it induces a sleep-like state characterised by decreased respiratory and heart rates (Hewson et al. 2007). Ajadi et al. (2009) reported a decreased heart rate after the administration of a combination of ketamine and xylazine. The heart rate decreased in all the pigs during anaesthesia. A significant HR decrease is believed to reflect vagal activity after administration of an α_2-agonist in animals (Ruffolo et al. 1993). However, isoflurane can increase heart rate by decreasing the cardiac vagal tone (Picker et al. 2001). Thus, the significant HR decrease may have been caused by the simultaneous decompression reflex of isoflurane and the bradycardic effect of xylazine. Mean arterial blood pressure decreased during anaesthesia, which may be due to the effect of isoflurane on the sympathetic nervous system (Eger 1984). There was a transient decrease in mean blood pressure during the procedure, which was then corrected, but values remained significantly lower than the baseline values at the end of anaesthesia. Although the mean blood pressure decreased significantly, this variable was still within the clinically acceptable physiological range. The results confirmed that *i.v.* isoflurane in a lipid emulsion promoted haemodynamic stability (Mathias et al. 2004). Similar to the heart rate, RR decreased after anaesthesia was induced. The present results are likely due to the respiratory depression effects of α_2-adrenoceptor agonists (Hewson et al. 2007). During the procedure, the SPO_2 decreased, while conversely, the $ETCO_2$ increased after administration of anaesthetic agents. Changes in SPO_2 and $ETCO_2$ may be caused by changes in respiratory and heart rates. After the induction of anaesthesia, we observed a decrease in respiratory and heart rates. This may have changed the tidal volume and the minute ventilation, and decreased the exchange of oxygen and the discharging of CO_2. In combination, xylazine and ketamine reportedly provide a short duration of analgesia. In this study, despite the lack of intraoperative analgesia, EI anaesthesia promoted cardiac and haemodynamic stability, with values remaining within normal ranges during the entire procedure and no evidence of pain. The protocol provided appropriate muscle relaxation without the need for neuromuscular blocking agents while allowing spontaneous respiration that maintained normocapnia throughout the procedure. Tramadol was administered for analgesia during the immediate postoperative period, with the first dose administered intravenously. Tramadol is an analgesic that acts centrally with several mechanisms of action (Choi et al. 2011); it is a μ-opioid agonist, as well as an inhibitor of serotonin and norepinephrine synaptic re-uptake and facilitates spinal modulation of pain (Nossaman et al. 2010; Lu et al. 2014). This effect promotes analgesia and avoids the adverse effects of xylazine, such as respiratory depression. Without an insulating thermal blanket, body temperature decreased after anaesthetic administration; this may have been caused by the abdominal surgery (Carli et al., 1982), or by the potential effects of isoflurane on the thermotaxic centre (Sessler et al., 1991). General anaesthesia inhibits metabolism, leading to a decreased metabolic rate and the inhibition of skeletal muscle activity. Overall, the combination of ketamine/xylazine anaesthetic premedication in swine undergoing experimental embryo transplantation enabled a safe and effective reduction in the requirements for emulsified isoflurane during general anaesthesia. Although arterial pressure and temperature decreased secondary to anaesthesia and surgery, all parameters remained within normal ranges throughout the study.

In conclusion, a combination of ketamine/xylazine provided sedation, muscle relaxation, and

analgesia during intravenous emulsified isoflurane anaesthesia in swine undergoing experimental embryo transplantation. There were no adverse events in the experimental animals in this study. These findings support the use of emulsified isoflurane following ketamine/xylazine premedication in pigs.

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