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Safe and effective anaesthesiological protocols in domestic pig

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Abstract: *Safe and effective anaesthesiological protocols in domestic pig.* The aim of this study is to establish a neuroleptanalgesic protocol and a general anaesthetic protocol in domestic pigs. The study was approved by approved by the Local Ethical Commission of the University of Warmia and Mazury in Olsztyn (the permit 73/2015). Fifteen juvenile female crossbred gilts were used. The drugs used in intramuscularly injection were: atropine (0.035 mg/kg b.w.), ketamine (7.0 mg/kg b.w.), medetomidine (0.063 mg/ /kg b.w.).The main anaesthetic drug, propofol, was given intravenously for induction and maintenance of general anaesthesia. To achieve the adequate neuroleptanalgesia the animals required in intramuscular injection atropine, ketamine and medetomidine mixing in the same syringe. The average time from muscular injection to the point at which pigs remained laterally recumbent was 1.95 ±0.72 min. The average time from muscular injection to the point at which pigs managed to stand in recovery was 75.80 ±13.14 min. To achieve the general anaesthesia the pigs required atropine, ketamine, medetomidine and propofol. Premedication with mixture of atropine, ketamine and medetomidine was achieved rapidly after intramuscular injection. All pigs became recumbent within 1.20 ±0.19 min without any signs of excitement. The average time from muscular injection to the point at which pigs remained laterally recumbent was 2.06 ±0.67 min. The induction dose of propofol was 4.5 mg/kg b.w. Anaesthesia was maintained with propofol 2.0 mg/kg b.w. During the surgery no complications occurred intraoperatively concerning. Concluding the findings demonstrate that the proposed protocols permits to obtain a safe and an effective neuroleptanalgesia and general anaesthesia in swine and can be useful in biomedical investigations. A short-lasting time for recumbency after intramuscularly injection of atropine, ketamine and medetomidine mixture injection can allow optimizing the workflow of clinical practice in a laboratory animal farm.

Key words: anaesthesia, ketamine, medetomidine, propofol, pig

INTRODUCTION

The pig is an important biomedical model and provides an important resource for further research studies on many diseases of animals and humans. In addition the pigs are still a preferred animal in surgical research for development of new surgical techniques (Kaiser et al. 2006). The porcine models are of interest, as they will allow detailed characterization in an experimental model organism whose physiology is very similar to that of human. One obvious reason why the pigs are so good biomedical models is that they are phylogenetically closer to humans than rodents.

A disproportionate amount of research efforts have focused on rodent biomedical models as opposed to porcine models, despite the advantages of using porcine models, that more closely represent the natural underlying disease in humans. The network connectivity of the central nervous system is different in mammals than in rodents. Sometimes it may not be possible to identify com-

pounds that have identical or even similar affinity for the human versus rodent variants, e.g. calcitonin gene-related peptide (CGRP) receptor antagonists (Salvatore et al. 2008). Analyses of the pig genome extend the potential of using the pig as a biomedical model (Groenen et al. 2012).

The humane and ethical use of animal models plays a critical role both in understanding the physiological and pathophysiological processes in the human body as well as in the development of therapeutic treatments. To achieve these goals it is very important to minimize prevalent pain conditions of experimental animals. That is why animal anaesthesia and analgesia are crucial components of guides of using animals in experiments and selection of the most appropriate anaesthesiological protocol is very important. Therefore, the aim of this study is to establish a neuroleptanalgesic protocol and a general anaesthetic protocol which have proven to be safe and easy to perform in domestic pigs.

MATERIAL AND METHODS

The study was approved by approved by the Local Ethical Commission of the University of Warmia and Mazury in Olsztyn (the permit 73/2015). All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86–23, revised 1985, Washington, DC, US Government Printing Office). All animal procedures were carried out under licence in accordance with the European Communities (Amendment of Cruelty to Animals Act 1876), Regulations 2002 and Directive 2010/63/EU on the protection of animals used for scientific purposes.

Fifteen juvenile female crossbred gilts (Pietrain × Duroc) were used. Pigs were maintained in individual pens under standard laboratory conditions for 2 weeks prior to the experiment in order to allow adaptation to the new environment. All animals were allowed *ad libitum* access to food and water. The experiments took place when pigs were 8–9 weeks of age with body weight 16–18 kg.

All animals, which were judged to be healthy on the basis of physical examination, normal appetite, expected weight gain were used in two experiments. All procedures and the drugs have been managed and administered by a veterinary doctor (DVM, PhD). The drugs used in the study were: atropine (1 mg/ml, Atropinum Sulfuricum Polfa Warszawa S.A., Poland), ketamine (100 mg/ml, Bioketan, Vetoquinol Biowet Sp. z o.o., Poland & Vetoquinol S.A., France), medetomidine (0.85 mg/ml, Cepetor, CP-Pharma Handelsges mbH, Germany) and propofol (10 mg/ml NORBROOK, Northern Ireland, IRL.PN). All drugs were stored at room temperature up to 25°C. All intramuscular and intravenous injections were performed by the same person (DVM, PhD). The heart rate, body temperature and the number of breaths were monitoring during the all procedures. All efforts were made to minimize the number of animals used and their suffering.

Experiment 1: neuroleptanalgesia in domestic pigs

Five clinically healthy juvenile female crossbred gilts were used. Food, but not water was withheld for 12 h before the start

of the experiment. Atropine (0.035 mg/ /kg b.w.), ketamine (7.0 mg/kg b.w.), medetomidine (0.063 mg/kg b.w.) were mixed in the same syringe and injected intramuscularly into the cervical muscles caudal to the ear base at the border between the dorsal and middle third of the animal's neck. After injection, the pigs were left undisturbed but under surveillance, so that the times at which they became recumbent and when they remained so could be recorded.

When animals were in lateral recumbency, an 22GA (0.8 × 25 mm) angiocatheter was placed in the marginal auricular vein through which 10 ml of 0.9% NaCl (sodium chloride 0.9% WET Baxter, 9 g/1000 ml, Baxter Sp. z o.o., Poland) were injected. After intramuscular (i.m.) and intravenous (i.v.) injections, the pigs were left undisturbed but under surveillance to measure the time from i.m. injection to the point at which pigs menaged to stand in recovery.

Follow up after the recovery the pigs were carried out with daily evaluations of behavior and water and food consumption for 8 days. The animals were observed for signs of pain, and some complications after i.m. and i.v. injections.

Experiment 2: medetomidine, ketamine and propofol general anaesthesia in domestic pigs

Ten clinically healthy juvenile female crossbred gilts were used. The pigs were not fed for 24 h before surgery, but had free access to the water. The pigs were premedicated with intramuscular atropine, ketamine and medetomidine injection. Atropine (0.035 mg/kg b.w.), ketamine (7.0 mg/kg b.w.), medetomidine (0.063 mg/kg b.w.) were mixed in the same syringe and injected intramuscularly into the cervical muscles caudal to the ear base at the border between the dorsal and middle third of the animal's neck. After injection, the pigs were left undisturbed but under surveillance, so that the times at which they became recumbent and when they remained so could be recorded.

After the recumbency period, the marginal ear vein was canalized with a 22 standard wire gauge cannula and an intravenous anaesthetic induction was performed with 4.5 mg/kg b.w. propofol keeping spontaneous ventilation. The level of general anaesthesia was assessed by the lid reflex and by the response to nociceptive stimuli (clamping of the interdigital fold with forceps). After the induction of general anaesthesia the endotracheal intubation by 6.0 mm diameter tube was carried out and then animals were supine for laparotomy surgery.

The surgical procedures have been performed by a veterinary doctor (DVM, PhD). The depth of anaesthesia was monitored by testing the corneal reflex and the increase of heart and respiratory rate (Swindle 2007, Calzetta et al. 2014). The general anaesthesia was maintained with propofol (2.0 mg/kg b.w.) for up to an average of 10 to 15 min and after that time 2.0 mg/kg b.w. of propofol was used again. The main anaesthetic drug, propofol, was given intravenously in a fractionated infusion. During the transection surgery procedure, a conventional midline incision of the abdominal wall was made. The cecum and ileum were identified and the ileocecal valve (ICV) were isolated from the abdominal

cavity. The ileocecal valve was gently exposed to administer non-toxic aqueous solution of the fluorescence retrograde neuronal tracer Fast Blue (FB; EMS-Chemie GmbH, Groß-Umstadt, Germany). A total volume of 50 µl 5% aqueous dye FB solution was injected into the wall of the ileocecal valve in multiple injections using a Hamilton syringe with a 26-gauge needle. A great attention was paid to avoiding any contamination of the surrounding tissues with FB due to the hydrostatic leakage from the injection canal. To avoid leakage, the needle was left in each place of FB injection for about tens of seconds. The peritoneum with the transverse abdominal muscles, the internal and external abdominal oblique muscles and the cutaneous muscle with subcutaneous fascia were closed in a simple continuous pattern. The skin was closed in a subcuticular pattern.

After the surgery (lasting approximately 51.8 ±4.52 min) animals received an intravenous drip infusion of 0.9% NaCl solution at 15 ml/kg b.w. and were transferred to the recovery room. The pigs were covered with emergency blanket. During recovery all pigs were monitored for signs of possible cardio-respiratory failure. The time from the muscular injection of atropine, ketamine and medetomidine to the point at which pigs managed to stand in recovery were recorded. Follow up after the recovery was carried out with daily evaluations of behavior and water and food consumption for 3 weeks. During this period the animals were observed for signs of pain, and surgical complications including infection or dehiscence of the surgical site.

The times (minutes) from intramuscular injection to the times at which pigs became recumbent and remained recumbent and managed to stand in recovery were calculated for both experiments. Duration of the surgery was recorded from cutting the skin to the last stitch skin for experiment 2. These data were expressed as means standard deviation (*SD*).

RESULTS AND DISCUSSION

All pigs used in both experiments survived the neuroleptanalgesic, general anaesthetic and surgical procedures without complications. The heart rate, body temperature and the number of breaths all remained in tolerable ranges during the all procedures. Neither vomiting nor nausea was detected. Any events of apnoea was not detected during both experiments. All animals maintained sufficient urinary output.

To achieve the adequate neuroleptanalgesia the animals required atropine (0.035 mg/kg b.w.), ketamine (7.0 mg/kg b.w.), medetomidine (0.063 mg/kg b.w.) in intramuscular injection. The neuroleptanalgesia was achieved rapidly after intramuscular injection of the drugs, and all pigs became recumbent without any signs of excitement. The average time from muscular injection to the point at which pigs became recumbent was 1.27 ±0.21 min. The average time from muscular injection to the point at which pigs remained laterally recumbent was 1.95 ±0.72 min. The neuroleptanalgesia permitted an adequate placement of the catheter in the ear veins of all gilts and intravenous injection of 10 ml of 0.9%

NaCl. The average time from muscular injection to the point at which pigs managed to stand in recovery was 75.80 ±13.14 min. They were not observed any irregularities during 8 days evaluations. The neuroleptanalgesic protocol is good for reduce fear and induce restraint necessary for some small diagnostic procedures and physical examination.

The practical guidelines for general anaesthetic protocol in a 10 kg swine is summarized in Table to simplify the comprehension and the feasibility. To achieve adequate premedication animals required atropine (0.035 mg/kg b.w.), ketamine (7.0 mg/kg b.w.), medetomidine (0.063 mg/ml b.w.) in intramuscular injection. Premedication was achieved rapidly after intramuscular injection of the drugs. All pigs became recumbent within 1.20 ±0.19 min without any signs of excitement. The average time from muscular injection to the point at which pigs remained laterally recumbent was 2.06 ±0.67 min. The preanaesthetic sedation permitted an adequate placement of the catheter in the ear veins of all piglets. After an induction dose of propofol (4.5 mg/kg b.w.), the endotracheal intubation was feasible. The intubation difficulty wasn't observed (spraying of lidocaine on the larynx wasn't necessary). All piglets of trial protocol had a soft and rapid induction of anaesthesia. The maintenance dose of propofol (2.0 mg/kg b.w.) resulted in adequate analgesia and anaesthesia that allowed surgical manipulation in all animals. The average time from muscular injection of atropine, ketamine and medetomidine to the point at which pigs managed to stand in recovery was 126.20 ±24.75 min. No complications occurred intraoperatively concerning the general anaesthetic protocol. All animals survived the operation. There were no complications related to anaesthesia. Medetomidine, ketamine and propofol anaesthesic protocol is good for surgical procedures performed in the abdominal cavity.

The present study demonstrated that the proposed protocols for neuroleptanalgesia and general anaesthesia were safe and effective in domestic pigs thanks to combination of the appropriate dose of drugs. Propofol is a medication for intra-

TABLE. Practical guidelines for general anaesthetic protocol in a 10 kg swine

Steps of general anaesthetic protocol	Drug (active substance)	Dose of the drug per 10 kg b.w. (ml)
General anaesthetic premedication – mix in the same syringe and inject i.m.	Atropinum Sulfuricum (1 mg atropine/ml)	0.35
	Bioketan (100 mg ketamine/ml)	0.7
	Cepetor (0.85 mg medetomidine/ml)	0.75
Induction of general anaesthesia – inject i.v.	Scanofol (10 mg propofol/ml)	4.5
Maintenance of general anaesthesia – inject i.v.	Scanofol (10 mg propofol/ml)	2.0

venous use in swine at 4.0–13.0 mg/kg b.w., with short action beginning and lasting, absence of accumulative effects, quick and calm recovery, so that makes it ideal to induce and conduct general aneasthesia (Muir et al. 2012). Propofol is as a safe anaesthetic even for long anaesthesia for use in porcine experimental models (Kaiser et al. 2003, Gaviria et al. 2007). Ketamine is the most commonly injectable anaesthetic used in a variety of species. However, ketamine used as the sole anaesthetic is not recommended. In most cases, ketamine is used in combination with other injectable agents such as alpha-2 agonists or benzodiazepines to reduce or eliminate many of the less desirable side effects if used alone. Alpha-2 agonists are used for their sedative and analgesic properties in a variety of species. Used as the sole agent, they do not produce an adequate level of anaesthesia for even minor surgical procedures and they have very little therapeutic effect in swine. The degree of muscle relaxation produced by medetomidine seemed to be dose dependent and was stronger than that produced by xylazine. No analgesic effect was produced by xylazine, however moderate analgesia was obtained by medetomidine in swine (Sakaguchi et al. 1992). Xylazine is a potent sedative and central nervous system depressant. Medetomidine is more specific central alpha-2 agonists, resulting in longer, more profound sedation and analgesia than xylazine and fewer adverse cardiovascular side effects. In pigs, the effects of medetomidine are much more potent than those of xylazine (Sakaguchi et al. 1992). Medetomidine is a more selective and full agonist for the central alpha-2--adrenergic receptor than xylazine and has significant dose dependent sedative effects. In combination with ketamine, alpha-2-agonists become much more useful and effective as anaesthetics for surgical procedures. Alpha-2 agonists can be combined with ketamine to produce adequate surgical anaesthesia in many species. Sakaguchi et al. (1996) showed that intramuscular administration of medetomidine, combined with butorphanol and ketamine, provides better anaesthesia than xylazine with butorphanol and ketamine combinations in pigs.

The results of this study suggest that dose combination of atropine (0.035 mg/ /kg b.w.), ketamine (7.0 mg/kg b.w.), medetomidine (0.063 mg/kg b.w.) in administered as an intramuscular injection, has been shown to be one of most effective means of immobilizing pigs. Compared to other researches (Gaviria et al. 2007, Lee et al. 2010) there were the most rapid induction times and lateral recumbency times in both experiments. A short-lasting time for lateral recumbency after i.m. ketamine and medetomidine mixture injection (1.95 ±0.72 min in experiment 1 and 2.06 ±0.67 min in experiment 2) can allow optimizing the workflow of clinical practice in a laboratory animal farm. Gaviria et al. (2007) recommended to combine propofol with fentanyl after premedication with combination of ketamine with xylazine to achieve analgesic effect. Ketamine mixed with medetomidine provides a sedative and analgesic effect much better then combination of ketamine mixed with xylazine. Therefore, it was not necessary to use opioids during propofol anaesthetic-surgical procedures after premedication with medetomidine and ketamine.

It is very important to recognize the response of animals to pain after the surgical procedures. The somatic reflexes, cornealpalpebral reflexes are not a good indicator for evaluating the depth of anaesthesia in swine. Therefore the depth of anaesthesia of this study was monitored by not only testing the corneal reflex but first of all by the increase of heart and respiratory rate. The best way to identify signs of pain after the surgical procedures is to closely observe the appearance and behavior of the animal prior to surgery, and note any changes after surgery. No changed posture or a changed profile of the body of pigs were observed during both experiments. Animals in pain may show altered a behavior (e.g. animals may remain immobile or may exhibit restlessness), may stop eating and drinking, or markedly reduce their intake, resulting in rapid weight loss. None of the above-described behavior was observed during both experiments. Behavior of the animal during postoperative period, and before surgery was not changed. Any clinical or behavioral signs of pain were not observed.

The time that it takes until an animal is fully recovered from anaesthesia will vary depending on the anaesthetic agent, the type and duration of the surgery, and the physiological imbalances induced by the surgery or anaesthesia. Replacement fluid therapy is not usually required for many surgeries, because they do not involve prolonger operative times or result in significant blood loss. However, fluid therapy can be beneficial and aids in the postoperative recovery of the animal, therefore during experiment 2, after the surgery animals received an intravenous drip infusion 250 ml of 0.9% NaCl.

Reed et al. (2015) reported that the age and mass of commercial pigs do not influence the response to using azaperone, ketamine, medetomidine, midazolam in male pigs aged 105–166 days. So protocols of this study can be useful not only for animals weighting 16–18 kg.

CONCLUSIONS

Concluding the findings demonstrate that the proposed protocols permits to obtain a safe and an effective neuroleptanalgesia and general anaesthesia in swine and can be useful in biomedical investigations. This study demonstrated that the use of atropine, ketamine, medetomidine in intramuscular injection and propofol in intravenosus infusion produces the rapid induction of anaesthetic events, good muscular relaxation and adequate analgesia. A short-lasting time for recumbency after intramuscularly injection of atropine, ketamine and medetomidine mixture injection can allow optimizing the workflow of clinical practice in a laboratory animal farm.

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Streszczenie: *Bezpieczne i skuteczne protokoły anestezjologiczne znieczulania świni domowej.* Celem tego badania jest ustalenie protokołu neuroleptoanalgetycznego oraz protokołu znieczulenia ogólnego świni domowej. Badania zostały zatwierdzone przez Lokalną Komisję Etyczna ds. Doświadczeń na Zwierzętach w Olsztynie (zezwolenie 73/2015). Doświadczenie przeprowadzono na 15 loszkach. W iniekcji domięśniowej zastosowano: atropinę (0,035 mg/kg m.c.), ketaminę (7,0 mg/kg m.c.) i medetomidynę (0,063 mg/kg m.c.). Podstawowy środek znieczulający, propofol, podawano dożylnie w celu indukcji i podtrzymania znieczulenia ogólnego. Aby zapewnić odpowiednią neuroleptanalgesię, zwierzętom podano domięśniowo w jednej strzykawce mieszaninę atropiny, ketaminy i medetomidyny. Średni czas od iniekcji domięśniowej do momentu, w którym świnie pozostały w pozycji bocznej leżącej, wyniósł 1,95 ±0,72 min. Świnie odzyskały świadomość średnio po 75,80 ±13,14 min od podania iniekcji domięśniowej. W celu osiągnięcia znieczulenia ogólnego użyto atropiny, ketaminy, medetomidyny i propofolu. Premedykację przy użyciu mieszaniny atropiny, ketaminy i medetomidyny osiągnięto bardzo szybko po iniekcji domięśniowej. Loszki znalazły się w pozycji

leżącej w ciągu 1,20 ±0,19 min, bez jakichkolwiek oznak niepokoju. Średni czas od iniekcji domięśniowej do momentu, w którym świnie pozostały w pozycji bocznej leżącej wyniósł 2,06 ±0,67 min. Dawka indukcyjna propofolu wyniosła 4,5 mg/kg m.c., a podtrzymująca 2,0 mg/kg m.c. Podczas zabiegu nie zaobserwowano jakichkolwiek powikłań śródoperacyjnych. Powyższe badania wskazują, że proponowane protokoły są bezpieczne i skuteczne oraz mogą być użyteczne w badaniach biomedycznych. Krótki czas, w jakim świnie znalazły się w pozycji leżącej po podaniu domięśniowym mieszaniny atropiny, ketaminy i medetomidyny, może pozwolić na zoptymalizowanie przebiegu prac w zwierzętarni.

Słowa kluczowe: znieczulenie, ketamina, medetomidyna, propofol, świnia

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