

## Effects of intramuscular alfaxalone-midazolam combination in pigs

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### Abstract

The aim of this experimental study was to evaluate the sedative and cardiorespiratory effects of alfaxalone and midazolam after intramuscular administration in pigs. Fourteen pigs, weighing 18 to 22 kg, aged between 55 and 70 days, American Society of Anaesthesiologists classification 2, affected by congenital reducible umbilical hernia, were included in the study. Alfaxalone (5 mg/kg) and midazolam (0.5 mg/kg) mixed in the same syringe were administered into the neck muscle. Pain on injection, quality of sedation and time to achieve lateral recumbency were recorded. Heart rate (HR), respiratory frequency ( $f_R$ ), and rectal temperature (RT) were recorded at 0 (baseline: before drug administration), 10, 15, and 20 min after the injection. Oxygen saturation of haemoglobin (SpO<sub>2</sub>), arterial blood pH, arterial oxygen (PaO<sub>2</sub>) and carbon dioxide (PaCO<sub>2</sub>) tensions and bicarbonate concentration (HCO<sub>3</sub><sup>-</sup>) were recorded at 10, 15, and 20 min after injection. Continuous data were analysed using a repeated-measure analysis of variance (ANOVA) and a *P*-value < 0.05 was considered significant. Ten animals out of fourteen showed no pain on injection, whereas the remaining four exhibited mild pain. The time from the end of injection to lateral recumbency was 266 ± 40 s. The quality of sedation ranged between good to very good. No significant changes in the variables monitored were observed between the time points. In conclusion, the intramuscular administration of alfaxalone and midazolam in pigs at the doses used induced reliable and fast sedation, without pain on injection and moderate respiratory effects.

*Experimental study, swine model, sedation*

Pigs are often used as experimental animal models in medical research because their organism has major similarities to that of humans (Huter et al. 2004; Gupta et al. 2005). Therefore, it is necessary to study new and effective anaesthetic protocols in order to preserve animal welfare in medical investigation (Calzetta et al. 2014). Physical restraint is difficult in pigs and a source of stress; furthermore, placement of an intravenous (i.v.) catheter is challenging if the animal is not properly sedated (Heinonen et al. 2009; Lee and Kim 2012; Malavasi 2015). Therefore, sedatives are generally administered intramuscularly (i.m.) in pigs (Nishimura et al. 1992; Henrikson et al. 1995). Ideally, the sedative(s) administered should provide a reliable and fast sedation, an adequate analgesia and muscle relaxation, should be painless at injection and produce minimal cardiorespiratory effects (De Monte et al. 2015).

Several drugs have been combined and investigated in pigs for i.m. administration: ketamine, alfaxalone, tiletamine-zolazepam, tranquillizers (such as azaperone) and  $\alpha$ 2-agonists (Sakaguchi et al. 1995; Hall et al. 2001; Keats 2003; Kim et al. 2007; Ajadi et al. 2008; Bettschart-Wolfensberger et al. 2013; Santos González et al. 2013; Santos et al. 2016).

Alfaxalone is a potent neuroactive steroid anaesthetic agent that activates the gamma-aminobutyric acid (GABA)<sub>A</sub> receptor (Lan and Gee 1994). In pigs, alfaxalone produces sedation or induces general anaesthesia depending on the dose, and it can be administered both i.m and i.v. (Keats 2003; Bigby et al. 2017; Santos González et al. 2013).

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Therefore, it could be a good alternative to ketamine or tiletamine-zolazepam previously used in this species (Nishimura et al. 1992; Sakaguchi et al. 1995; Sweitzer et al. 1997; Kim et al. 2007; Ajadi et al. 2008; Heinonen et al. 2009; Lee and Kim 2012; Bettschart-Wolfensberger et al. 2013; De Monte et al. 2015; Santos et al. 2016) that can often cause a rough and stressful recovery (Malavasi 2015).

Midazolam is a benzodiazepine that, acting on the GABA<sub>A</sub> receptor, causes muscle relaxation and sedation with minimal cardiorespiratory effects in pigs (Smith et al. 1991; Bustamante and Valverde 1997). In contrast to diazepam, midazolam is hydrosoluble and is well absorbed after an i.m. injection (Quandt 2013). Even when administered on its own, i.m. midazolam produces sedation, facilitating physical restraint in pigs (Bustamante and Valverde 1997).

The purpose of this study was to investigate the effect of i.m. administration of alfaxalone and midazolam in pigs. We hypothesize that alfaxalone and midazolam will not cause pain on injection and will produce effective and fast sedation with minimal cardiorespiratory effects in pigs.

### Materials and Methods

Fourteen healthy mixed-breed male pigs (Large White × Duroc) weighing 18 to 22 kg, aged between 55 and 70 days and affected by uncomplicated reducible congenital umbilical hernia were included in the study. The pigs were involved in another experimental study in which herniorrhaphy was performed inserting a new absorbable prosthetic mesh (BARD®, Italy) under general anaesthesia. The animals were handled according to European and national regulations on the protection of experimental animals (Directive 2010/63/UE and RD 53/2013) and the study was approved by the Italian Ministry of Health (authorization number 403/2016).

The animals were housed in the Large Animal Facility of the Camerino University, Italy. After an acclimation period of at least 72 h, the pigs underwent a routine pre-anaesthetic physical examination in order to assess their health status which was classified as 2 according to the American Society of Anaesthesiologists. Exclusion criteria were: incarcerated or strangulated hernia, local inflammation or infection due to trauma, presence of systemic symptoms (i.e. cough, nasal discharge, hyperthermia). Food was withheld for 12 h and water for 30 min prior to anaesthesia.

Before the i.m. injection, baseline heart rate (HR), respiratory frequency ( $f_R$ ) and rectal temperature (RT) were recorded (Table 1). The HR was measured by auscultation using a stethoscope,  $f_R$  was measured by observation of thoracic excursions, RT was recorded using a digital thermometer. Alfaxalone (5 mg/kg, Alfaxan®, Dechra, Italy) and midazolam (0.5 mg/kg, Midazolam®, Ibi, Italy) were mixed in the same syringe, and 0.9% sodium chloride was added as necessary to reach a total volume of 14 ml. The mixture was administered i.m. into the neck behind the base of the ear and in front of the angle of the shoulder using a 18 gauge, 3.75 cm long hypodermic needle connected to a line extension (75 cm, 1.3 ml volume, Sidam Medical Device®, Italy). The extension was previously filled with the sedative solution and, to avoid leaving sedative residues inside, it was immediately flushed with 1.5 ml of sterile water for injection. All the injections were performed over a period of 20 s without physical restraint.

Pain on injection was scored using a simple descriptive scale modified from that of Michou et al. (2012) and Santos et al. (2016): score 0 = no pain (the animal is quiet and indifferent to the injection); score 1 = mild pain (movement of tail and turning of head towards injection site); score 2 = moderate pain (light grunts and attempts to remove needle); and score 3 = severe pain (strong vocalization and attempts to escape, requiring vigorous physical restraint). Time from end of injection to lateral recumbency was also recorded.

At 10, 15 and 20 min from i.m. administration HR,  $f_R$ , RT were recorded. Further, the pulsatile oxygen saturation of haemoglobin (SpO<sub>2</sub>; Nellcor™ Portable SpO<sub>2</sub>, Covidien) was recorded applying the probe to the tail. At the same time points, arterial blood samples were collected anaerobically from the femoral artery and immediately analysed (i-STAT System, Abbot) in order to obtain pH, arterial oxygen (PaO<sub>2</sub>) and carbon dioxide (PaCO<sub>2</sub>) tensions and bicarbonate concentration (HCO<sub>3</sub><sup>-</sup>).

Quality of sedation was scored using a 4-point simple descriptive scale modified from Santos et al. (2016) 20 min after drug administration: score 0 = no apparent sedation, pig standing and able to walk; score 1 = pig is quieter but it stands and is reactive to manipulation; score 2 = pig in sternal recumbency and unable to walk; score 3 = pig in lateral recumbency and not reactive to manipulation.

All of the variables and scores were assessed and recorded by the same investigator.

Shapiro-Wilk test was used to confirm that the data were normally distributed. Subsequently, values of HR,  $f_R$ , RT, SpO<sub>2</sub>, pH, PaO<sub>2</sub>, PaCO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> were analysed using a repeated-measure analysis of variance (ANOVA). A *P*-value < 0.05 was considered significant. The data are reported as the mean ± standard deviation (SD).

Pain at injection and sedation score are reported as median (range).

## Results

The sedative solution was administered without any problem in all the pigs. Pain at injection was scored 0: ten animals showed no pain on drug injection (score 0) and four mild pain (score 1). After 20 min from injection, the quality of sedation was scored 3: score 2 in two pigs and score 3 in the remaining twelve. Time from the end of injection to lateral recumbency was  $266 \pm 40$  s. There were no significant differences in HR,  $f_R$ , RT and SpO<sub>2</sub> between the time points (Table 1). Arterial blood pH, PaCO<sub>2</sub> tensions and HCO<sub>3</sub><sup>-</sup> did not change significantly over time, while PaO<sub>2</sub> showed a mild decrease (Table 2). Just before and during recumbency, none of the animals presented excess salivation or apnoea. No pigs showed vocalization, limb paddling or any other local side effect related to the volume injected. Anaesthesia was deepened using isoflurane (Isoflurane Vet®, Merial, Italy) administered via face mask. An i.v. catheter (Terumo®, Demas, Italy) was inserted into the auricular vein and, once the mandibular reflex was lost, the endotracheal intubation was performed. Anaesthesia was maintained with isoflurane vaporized in oxygen.

Table 1. Variables measured in pigs at baseline and at 10, 15 and 20 min following alfaxalone-midazolam administration.

Time	0 min	10 min	15 min	20 min
HR	$174.4 \pm 8.6$	$172.5 \pm 10.2$	$174.5 \pm 7.4$	$178 \pm 9.0$
$f_R$	$48.5 \pm 8.7$	$46.5 \pm 10.2$	$47.5 \pm 12.3$	$46 \pm 10.0$
RT	$39.6 \pm 0.1$	$39.4 \pm 0.2$	$38.9 \pm 0.1$	$38.9 \pm 0.1$
SpO <sub>2</sub>	NA	$94 \pm 3.3$	$94.2 \pm 3.0$	$94.5 \pm 2.2$

HR: heart rate;  $f_R$ : respiratory rate; RT: rectal temperature; SpO<sub>2</sub>: oxygen saturation of haemoglobin; NA: not available. Values are given as the mean  $\pm$  standard deviation. No significant differences in HR,  $f_R$ , RT and SpO<sub>2</sub> between time points ( $P > 0.05$ ).

Table 2. Arterial blood gas indices in pigs at 10, 15 and 20 min following alfaxalone-midazolam administration.

Time	10 min	15 min	20 min
pH	$7.4 \pm 0.3$	$7.4 \pm 0.2$	$7.4 \pm 0.2$
PaO <sub>2</sub> (mmHg)	$72.2 \pm 11.2$	$75.5 \pm 12.3$	$73.5 \pm 8.3$
PaCO <sub>2</sub> (mmHg)	$46.4 \pm 0.18$	$48.2 \pm 1.1$	$48.5 \pm 2.1$
HCO <sub>3</sub> <sup>-</sup> (mmol/l)	$34.2 \pm 3.3$	$32.1 \pm 2.9$	$33.7 \pm 2.2$

pH: hydrogen ion concentration; PaO<sub>2</sub>: arterial oxygen; PaCO<sub>2</sub>: arterial carbon dioxide tensions; HCO<sub>3</sub><sup>-</sup>: bicarbonate concentration. Values are given as the mean  $\pm$  standard deviation. No significant differences in HR,  $f_R$ , RT and SpO<sub>2</sub> between the time points ( $P > 0.05$ ).

In the present study, the i.m. administration of alfaxalone-midazolam produced a reliable sedation with no pain on injection and moderate respiratory effects, as similarly reported in another study (Bigby et al. 2017).

Previous studies investigated the use of midazolam as a sedative in pigs but with conflicting results. After i.m. administration of midazolam (0.1 mg/kg), Smith et al. (1991) described a stable cardiac function with no modifications of blood gases and pH compared to non-sedated pigs, despite a significant decrease in HR and  $f_R$ . The authors concluded that midazolam is an effective swine sedative.

Bustamante and Valverde (1997) administered i.m. 0.25, 0.5 and 1 mg/kg midazolam to determine the most effective sedative dose for handling pigs. They concluded that

## Discussion

Since pigs are highly sensitive to stress, the choice of sedative drugs that decrease the sympathetic tone with minimal alterations in cardiovascular function and respiratory rate plays an important role in the extent of restraint and in the quality of sedation (Henrikson et al. 1995; Malavasi 2015).

the dose of 0.5 mg/kg i.m. of midazolam was the most suitable for sedation in pigs with minimal cardiorespiratory changes. Similarly to our results, no significant decrease in HR and  $f_R$  was documented.

Alfaxalone has been previously used in pigs as a pre-anaesthetic medication, to induce and / or maintain general anaesthesia, either alone or in combination with other drugs (Quandt 2013; Santos González et al. 2013; Santos et al. 2016; Bigby et al. 2017). When administered on its own in non-sedated pigs, alfaxalone is effective but causes muscular twitching (Keates 2003; Santos González et al. 2013; Bigby et al. 2017). Similar results have also been found in other animal species (Keates et al. 2012; Warne et al. 2015; Tamura et al. 2015). Therefore, alfaxalone is often used in combination with other drugs.

The i.m. administration of alfaxalone (5 mg/kg) and diazepam (0.5 mg/kg) in six pigs was investigated by Santos González et al. (2013). The authors reported that the administration resulted in a rapid onset of lateral recumbency ( $232 \pm 45$  s) and in a fair to smooth quality of sedation. One animal showed mild pain during drug injection. In our study, the time to achieve recumbency was longer ( $266 \pm 40$  s) compared to that reported by Santos González et al. (2013). This variation might have been due to the different site of injection chosen in this study (the neck muscle vs the lumbar muscle).

Differently from midazolam, diazepam has a pH of 6.6–6.9, it is poorly water soluble and it is commercialised as a solution containing benzoic acid, ethanol 96%, sodium benzoate, benzyl alcohol and propylene glycol. This may cause pain after i.m. and i.v. diazepam administration. Further, the poor hydrosolubility and the excipient contained in the solution can cause unpredictable systemic absorption after i.m. administration (Meyer and Fish 2008; Rankin 2015). Therefore, the use of midazolam (commercialised as a hydrosoluble solution, with pH < 4, containing sodium chloride, 10% hydrochloric acid, sodium hydroxide) can probably explain the lack of pain and the more profound sedative effect recorded in our study.

Further studies are needed in order to assess the quality of the recovery following the alfaxalone-midazolam administration, since in our work the animals were involved in another experimental research which required the utilization of additional drugs for the induction of the general anaesthesia.

Moreover, the commercially available formulation of alfaxalone is characterized by a low concentration, thus, the resultant volumes of injection limit its i.m. use to small pigs.

Since neither alfaxalone nor midazolam produce any significant analgesia, the use of analgesics should be considered to treat pain prior to the procedural training.

The results of this study suggest that the i.m. administration of alfaxalone in combination with midazolam at the dose used induces good to very good sedation and fast lateral recumbency without significant differences in HR,  $f_R$  or RT compared to baseline values. Moreover, arterial blood pH, PaCO<sub>2</sub> tensions and HCO<sub>3</sub><sup>-</sup> remained within the physiological range, whereas PaO<sub>2</sub> showed a mild decrease.

Although neither alfaxalone nor midazolam are allowed in pigs, their use should be considered in medical training and research, since their combination, at the doses used, resulted in reliable and satisfactory restraint in pigs, which could result in a useful handling protocol. Further studies evaluating the recovery time are required to assess the differences between the combination used in this study and other combinations, such as ketamine-benzodiazepine or tiletamine-zolazepam which can also be administered i.m. to induce anaesthesia in pigs.

#### Conflict of Interest

The authors declare no conflicts of interest.

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