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Original article

Constant rate infusion of tramadol in isoflurane-anesthetized pigs undergoing experimental surgery

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Abstract

The purpose of this study was to investigate the effect of tramadol (TM) (2 mg/kg) administered intramuscularly (IM) followed by a constant rate infusion (CRI) of TM (2 mg/kg/h) in pigs. Sixteen pigs undergoing experimental surgery were premedicated IM with a combination of alfaxalone (5 mg/kg) and midazolam (0.5 mg/kg). Anaesthesia was induced with propofol (2 mg/kg) intravenously (IV) and maintained with isoflurane. Pigs were randomly assigned to one of the two following groups: Group 1 (n=8): received a loading dose of TM (2 mg/kg) followed by a CRI of TM (2 mg/kg/h); Group 2 (n=8): a loading dose of TM (2 mg/kg) followed by a CRI of lactated Ringer's solution (2 ml/kg/h). Heart rate (HR), respiratory rate (RR), rectal temperature (RT), haemoglobin oxygen saturation (SpO_2), fraction of inspired oxygen (FIO_2), end-tidal concentration of isoflurane (FE_{ISO}), end-tidal carbon dioxide concentration (FE_{CO2}), pH, arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$) and bicarbonate concentration (HCO_3-) were recorded immediately after loss of righting reflex (T=0 min) and at 15-min intervals over a period of 60 min. Continuous data were analysed using a repeated--measure analysis of variance (ANOVA) and a p-value <0.05 was considered significant.

HR, RR and FE_{ISO} were significantly lower ($p<0.05$) in Group 1 at T30 and T45, which corresponded to the time of the most intense surgical stimulation.

The results suggest that the TM infusion minimizes the HR and RR response, slightly reducing isoflurane requirements and determining a superior perioperative analgesia.

Key words: analgesia, constant rate infusion, pig, tramadol, experimental surgery

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Introduction

Pigs are often used as experimental animal models in medical research because they have significant similarities to humans and a considerable number of experimental surgical studies are conducted under general anaesthesia (Swindle et al. 2012). Moreover, it has now been verified by the scientific community that a failure to provide adequate details with respect to analgesic provision implies an underestimation of the distorting effects of pain on research data (Ulrich-Lai et al. 2006, Karas et al. 2008). Therefore, it is increasingly necessary to study new and effective anaesthetic protocols in order to preserve animal welfare in medical investigation (Calzetta et al. 2014), since little information has been published on pain assessment and pain alleviation in pigs undergoing experimental surgery (Swindle and Sistino 2015, Bradbury et al. 2016). Tramadol (TM) is a synthetic opioid analogue of codeine that produces its antinociceptive and analgesic effects by both an opioid and non-opioid mechanism of action (Raffa 2008). It displays a weak affinity to the μ-opioid receptor and shows an additional mechanism that is different from the pure μ-opioid agonist, inhibiting norepinephrine and serotonin reuptake in the central nervous system (Raffa et al. 1992). The clinical efficacy of TM administration is dependent on its metabolism, due to the different analgesic activities of its metabolites. In fact, O-desmethyl-tramadol hydrochloride (M1), the only active metabolite, has 200 times more affinity for the μ-receptor than the parent drug TM, accounting for most of the clinical effects (Raffa et al. 1992). The pharmacokinetics of TM have been investigated in several animal species including pigs, where one study demonstrated that piglets produce a larger amount of M1 compared with dogs, horses and goats (Vullo et al. 2014). The analgesic effect of TM and its constant rate infusion (CRI) have been exploited to reduce the amount of inhalational agents required, as shown by the reduction in the minimum alveolar concentration (MAC) of volatile anaesthetics in dogs (Seddighi et al. 2009). Moreover, CRI assures a constant level of analgesia, avoiding the intermittent peak plasma concentration associated with intermittent administration, and enables the use of smaller doses, leading to a reduction in side effects (Gudes 2012). Although the efficacy of TM has been evaluated in combination with different analgesic protocols in pigs (Ajadi et al. 2009, Lu et al. 2010, Lu et al. 2011, Ajadi et al. 2012), no studies have yet examined the effect of TM when used in CRI in this species. The aim of the present study was to evaluate the effects of TM in CRI as an additional analgesia on the quality of the anaesthesia and on physiological variables in isoflurane-anesthetized pigs.

Materials and Methods

Study design and animals

Sixteen healthy female pigs (Large White × Duroc) weighing between 25 and 45 kg (34.8±8.7), aged between 60 and 95 days (78±11.50), affected by uncomplicated reducible congenital umbilical hernia were included in the study. The pigs were involved in another experimental study in which surgery 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). 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. 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. Baseline heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were recorded (Table 1). All pigs were premedicated intramuscularly (IM) with a combination of alfaxalone (5 mg/kg, Alfaxan®, Dechra, Italy) and midazolam (0.5 mg/kg, Midazolam®, Ibi, Italy) mixed in the same syringe. After recumbency, a 22-gauge catheter was placed in the auricular vein in order to induce general anaesthesia with propofol (2 mg/kg, PropoVet®, Abbott, Italy) and to receive lactated Ringer's solution (500 mL, Fresenius, Kabi®, Italy) during anaesthesia. A second intravenous (IV) catheter was placed in the auricular vein of the other ear for the loading dose and the CRI administration of TM. Five min after the induction of anaesthesia, the pigs were randomly assigned to one of the two following groups: Group 1 (n=8): received IV a loading dose of TM (2 mg/kg, Altadol®, Formevet, Italy) followed by a CRI of TM (2 mg/kg/h); Group 2 (n= 8): received IV a loading dose of TM (2 mg/kg;) followed by a CRI of lactated Ringer's solution (2 ml/kg/h). The loading dose was given over a 4-min period and the CRI was administered using a syringe pump (Alaris GH; CareFusion, Italy). The investigators involved in the study were blinded as to treatment. The pigs were intubated with a cuffed endotracheal tube following the instillation of lidocaine (Lidocaina 2%®, Esteve, Italy) in the pharyngeal mucosa and connected to a small-animal anaesthetic machine. Anaesthesia was maintained with isoflurane (Isoflo®, Zoetis, Italy) in 100% oxygen delivered via a circle rebreathing system in spontaneous ventilation. The pigs then underwent the surgical treat-

Table 1. Parameters recorded at the different time points in pigs of Group 1 and Group 2.

	Baseline	0 min	15 min	30 min	45 min	60 min
HR						
Group 1	136.5±2.6	123.5±3.6	113.5±3.8	116.6±2.4	118.8±2.3	114.8±2.3
Group 2	138.6±1.6	125±2.4	115.5±4.2	135.5±2.8	137.8±2.6	118.8±4.6
RR						
Group 1	30.5±4.6	28.4±2.2	26.5±1.6	25.0±1.2	25.5±1.6	25.8±1.8
Group 2	29.6±4.2	28.5±1.6	26.5±2.8	34.5±1.0	33.6±1.4	27.5±0.8
RT						
Group 1	38.4±0.8	38.2±0.4	38.0±0.2	37.6±0.5	36.8±0.2	36.4±0.4
Group 2	38.7±0.6	38.5±0.5	38.1±0.3	37.5±0.6	36.9±0.1	36.5±0.5
SpO_2						
Group 1	NA	98.2±0.4	97.8±0.2	98.0±0.1	98.2±0.4	97.8±0.2
Group 2	NA	98.5±0.2	98.2±0.1	97.4±0.2	97.6±0.1	98.0±0.2
FIO_2						
Group 1	NA	85.2±2.8	93.2±2.2	97.6±0.2	98.2±0.2	98.2±0.2
Group 2	NA	87.2±2.4	94.2±1.0	96.2±0.4	98.0±1.2	98.1±0.2
FE_{ISO}						
Group 1	NA	0.80±2.6	1.24±1.2	1.18±1.8	1.18±1.7	1.20±0.7
Group 2	NA	0.82±2.4	1.38±1.8	1.40±1.4	1.41±1.0	1.30±1.0
FE_{CO2}						
Group 1	NA	35.1±2.2	39.1±2.0	44.1±1.2	45.1±2.0	48.1±1.8
Group 2	NA	36.2±1.9	40.0±1.7	45.2±1.4	46.0±0.9	48.8±1.9

NA: not available
Values are given as the mean ± standard deviation (SD)

ment. Depth of anaesthesia was monitored using the following clinical criteria (eye position, degree of palpebral reflex, jaw tone, spontaneous movement). A multiparametric anaesthesia monitor (BeneView T8, Mindray) was used to record HR, RR, RT, haemoglobin oxygen saturation (SpO_2), fraction of inspired oxygen (FIO_2), end-tidal concentration of isoflurane (FE_{ISO}) and end-tidal carbon dioxide concentration (FE_{CO2}). All these parameters were taken immediately after loss of righting reflex (T = 0 min) and at 15-min intervals over a period of 60 min. Arterial blood samples were collected anaerobically from the carotid artery and pH, arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$) and bicarbonate concentration (HCO_3-) were immediately analysed using a blood-gas analyser (i-STAT System, Abbott) over the same time points.

Statistical analysis

The Shapiro-Wilk test was used to confirm that the data were normally distributed. Subsequently, HR, RR, RT, SpO_2, FIO_2, FE_{ISO}, FE_{CO2}, pH, PaO_2, $PaCO_2$ and HCO_3- values of the two Groups were compared using analysis of variance (ANOVA) for repeated measures. A value of $p< 0.05$ was considered significant. The data were reported as the mean ± standard deviation (SD).

Results

The premedication with alfaxalone and midazolam induced weak sedation, requiring physical restraint to perform clipping and to insert the bilateral venous auricular access, as reported in a previous study (Vullo et al. 2019). The induction of anaesthesia with propofol was considered good; apnoea occurred in only one animal, which required assisted ventilation. None of the animals exhibited laryngeal reflex and all showed suitable muscle relaxation for intubation. The administration of the loading dose of TM caused no side effects. There were no significant differences between the Groups in the parameters monitored at T0, T15 and T60; however, HR, RR and FE_{ISO} were significantly lower ($p< 0.05$) at T30 and T45 in Group 1 with respect to Group 2. There was a tendency for the RT to decrease throughout the monitoring period in both Groups, but the reduction was not statistically significant. There were no significant differences in the arterial blood gas parameters between the Groups at any time. The data are shown in Table 1 and 2.

Discussion

Failure to provide effective analgesia to animals in experimental studies infringes the obligation to refine animal experimentation and may decrease the scientific

Table 2. Arterial blood gas parameters recorded in pigs of Group 1 and Group 2.

	0 min	15 min	30 min	45 min	60 min
pH					
Group 1	7.4±0.3	7.4±0.2	7.4±0.2	7.5±0.5	7.5±0.3
Group 2	7.4±0.5	7.3±0.6	7.4±0.3	7.5±0.3	7.5±0.3
PaO_2 (mm/Hg)					
Group 1	82.2±8.2	105.5±12.3	177.5±8.3	240±8.0	325±8.0
Group 2	79.2±9.0	108.5±8.3	179.5±6.3	250±3.0	330±4.0
$PaCO_2$ (mm/Hg)					
Group 1	35.4±0.18	39.2±1.1	42.5±2.1	45.9±2.1	48.4±1.6
Group 2	35.2±0.15	38.0±1.3	40.5±2.3	44.3±3.3	47.4±1.0
HCO_3- (mmol/L)					
Group 1	24.2±3.3	25.1±2.9	28.3±2.2	28.7±2.0	29.3±2.0
Group 2	24.7±1.3	26.1±2.2	27.7±2.2	28.0±2.2	29.9±1.4

Values are given as the mean ± standard deviation (SD)

validity of results (Bradbury et al. 2016). To date, little information has been published on pain assessment and pain alleviation in pigs undergoing experimental surgery (Swindle and Sistino 2015). When considering one drug alone, CRI is more efficacious than intermittent administration. Unfortunately, there are very few studies reported in the literature on TM and its influence on intraoperative conditions in pigs. The present study tested the effects of TM in CRI as an additional analgesia on the quality of the anaesthesia and on physiological variables in isoflurane-anesthetized pigs under experimental conditions.

Previous studies investigated the analgesic effect of TM in pigs. Ajadi et al. (2009) evaluated the influence of premedication with 5 mg/kg of TM on xylazine-ketamine anaesthesia in young pigs. They concluded that TM improved the quality of anaesthetic induction and increased the duration of antinociception without increasing that of anaesthesia. Moreover, it resulted in no additional reduction in HR, RR, SpO_2 and RT. Similarly, in our study, we observed no side effects after the administration of the loading dose of 2 mg/kg of TM, nor were there any modifications in the monitored parameters.

The effects of anaesthesia with a ketamine-tramadol (KT) combination on behavioural, physiological and plasma cortisol changes following surgical castration in pigs, were evaluated and compared to the effects of anaesthesia with ketamine and normal saline (KS). Both KT and KS combinations failed to provide satisfactory intraoperative analgesia (Ajadi et al. 2012). This result was probably due to an insufficient anaesthetic protocol, which involved the use of ketamine alone and did not allow for an adequate level of anaesthesia.

In the present study, a combination of alfaxalone and midazolam was used as premedication. These two drugs are known to have no analgesic effect and, similarly, propofol and isoflurane are respectively an IV and an inhalation anaesthetic with no analgesic properties. Therefore, TM was the only drug with analgesic activity used in this study. The statistically significant higher HR, RR and FE_{ISO} observed at T30 and T45 (during intense surgical stimulation, while inserting the prosthetic mesh) in Group 2 compared with Group 1, which received a constant infusion of TM, suggests that the TM infusion minimized the HR and RR response and slightly reduced isoflurane requirements, determining a superior perioperative analgesia.

Our results show that the CRI of TM is associated with statistically better cardiorespiratory parameters and could be considered a valid analgesic protocol in pigs undergoing experimental procedures. Further studies are needed to evaluate the antinociceptive potential of TM in more aggressive surgeries, possibly at higher doses.

Abbreviations

ANOVA: Analysis of variance; **CRI:** Constant rate infusion; **FE_{CO2}:** End-tidal carbon dioxide concentration; **FE_{ISO}:** End-tidal concentration of isoflurane; **FIO_2:** Fraction of inspired oxygen; **HCO_3-:** Bicarbonate concentration; **HR:** Heart rate; **IM:** intramuscular; **IV:** Intravenous; **KS:** Ketamine-saline; **KT:** Ketamine-tramadol; **M1:** O-desmethyl-tramadol hydrochloride; **NA:** not available; **$PaCO_2$:** Arterial carbon dioxide partial pressure; **PaO_2:** Arterial oxygen partial pressure; **RR:** Respiratory rate; **RT:** Rectal temperature; **SD:** Standard deviation; **SpO_2:** Haemoglobin oxygen saturation; **TM:** Tramadol

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