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Evaluation of Tramadol-Midazolam-Ketamine Anaesthesia in Rabbits

Oguntoye C. O.*, Oyewande O.A. and Afolabi O.O.
Department of Veterinary Surgery and Radiology, University of Ibadan, Ibadan, Nigeria.

Summary: Selected anaesthetic indices of, and the heart rate, respiratory rate and rectal temperature responses of 6 healthy rabbits to the intramuscular administration of 2mg/kg midazolam and 25 mg/kg ketamine alone (MK) and combined with 4mg/kg tramadol (MKT) were evaluated over a 60-min observation period. Time to loss of righting reflex with MKT (1.7±0.3min) was significantly ($p<0.05$) shorter than with MK (4.2±1.5min). Duration of recumbency with MKT (76.8±5.1min) and MK (77.8±3.6min) were similar. Time to standing with MKT (9.3±1.1min) was shorter than with MK (15.2±2.4 min). Mean heart rates ranged from 204.7±13.0 to 257.5±3 beats/min with MK, and from 207.3±4.6 to 238.8±8.7 beats/min with MKT. Mean respiratory rates ranged from 33.8±6.2 to 64.3±15.0 breaths/min with MK; from 36.2±2.5 to 54.0±8.6 breaths/min with MKT. Mean temperature ranged between 38.0±0.2 and 38.9±0.2^{o}C with MK and between 37.9±0.3 and 39.1±0.1^{o}C with MKT. Neither MK nor MKT produced analgesia. It was concluded that although the inclusion of tramadol did not produce analgesia, it produced a faster onset of action than midazolam-ketamine alone. Midazolam-ketamine-tramadol will be useful for non-painful procedures where rapid drug action is needed.

Keywords*:* Analgesia, Anaesthesia, Ketamine, Midazolam, Rabbits, Tramadol.

**Address for correspondence*: wumcel06@gmail.com

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INTRODUCTION

Injectable anaesthesia involving the use of ketamine drug combinations are currently popular in rabbit anaesthesia because of ease of administration, low cost and relative safety of ketamine (Henke *et al.*, 2005; Orr *et al.*, 2005; Grint and Murison, 2008) and the practical problems associated with inhalation anaesthesia in this species (Flecknell, 2009). The combination of an alpha 2 adrenergic agonist with ketamine is a commonly used anaesthetic protocol for rabbit surgery (Longley, 2008) with xylazine being the most combined agent with ketamine (Lipman, 2008). Xylazine-ketamine combination produces surgical anaesthesia (White and Holmes, 1976; Lipman *et al*., 1990) but it is accompanied by significant side effects, including cardiovascular and respiratory depression (Flecknell, 1984; Longley, 2008). Diazepam-ketamine is another popular ketamine combination (Flecknell, 1998; Kilic, 2004; Oguntoye and Oke, 2014). Diazepam-ketamine combination produces good muscle relaxation and complete immobilization (Longley, 2008; Flecknell, 2009). In addition, diazepam decreases only respiratory rates but not heart rates while ketamine stimulates the cardiovascular system thereby making this combination relatively safer than ketamine combinations with alpha 2 agonists (Longley, 2008). However, many studies have found little or no analgesia with the diazepam-ketamine combination (Kazemi, 2002; Redah, 2011; Oguntoye and Oke, 2014) making it unsuitable for surgery or any painful procedure. Current anaesthetic techniques entail polypharmacy to ensure balanced anaesthesia which is surgical anaesthesia produced by a combination of two or more drugs or techniques with each contributing its own pharmacologic effects including tranquilizers, opioids, nitrous oxide, muscle relaxants and inhalants (Hall *et al.*, 2001; Muir *et al.*, 2013). Thus, the addition of an analgesic to the diazepam-ketamine combination may make it useful for painful procedures. Indeed, many anaesthetic protocols involving the rabbit include the opioids for analgesia (Longley, 2008). The addition of butorphanol to both xylazine-ketamine and medetomidine- ketamine (Marini *et al*., 1992; Longley, 2008) increases duration of anaesthesia and produces analgesia. Another opioid, fentanyl in combination with fluanisone combined with diazepam is a good injectable anaesthetic in rabbits but its usefulness is limited by the associated prolonged recovery (Harcourt-Brown, 2002). However, the traditional opioids are associated with certain side effects including respiratory depression, mental depression, hypothermia, bradycardia and sometimes reduced GIT motility in rabbits (Longley, 2008). Tramadol is a relatively new analgesic with mixed opioid and non-opioid activities (Garrido *et al*., 2000). The pharmacokinetics of tramadol has been

well studied in humans and is reported to cause less respiratory depression compared with morphine or other opioid analgesics (Bhattacharya *et al*., 2005; Natalini *et al.,* 2007). Tramadol may be a good alternative to the traditional opioids with diazepam-ketamine for anaesthesia in rabbits. In addition, tramadol is not under strict regulation and control, is cheap and readily available in developing countries (Ajadi *et al.,* 2009). Midazolam, another benzodiazepine, has some advantages over diazepam. It causes minimal haemodynamic and respiratory changes, is water soluble and therefore can be mixed with other water-soluble substances in a single syringe (Henke *et al*., 2005). Its water solubility property also makes it non-irritating to tissues making it more suitable for intramuscular administration. In addition, it is reportedly to be twice as potent as diazepam (Muir *et al*., 2013). The addition of tramadol to the midazolam-ketamine combination may be able to produce analgesia and longer duration of anaesthesia than midazolam-ketamine alone. The aim of this study, therefore, was to evaluate the anaesthesia produced by midazolam-ketamine-tramadol in rabbits.

MATERIALS AND METHODS

Experimental Animals

The animals used were adult New Zealand x Chinchilla rabbits consisting of three (3) bucks and three (3) does with weight range between 1.2kg to 1.9kg. The rabbits were acquired from a local breeder and were housed singly in cages that provided ample space for movement. They were fed ad libitum with grower's mash which was supplemented with *Tridax procumbens*. Water was also supplied *ad libitum* in their cages. They were stabilized for two weeks, to enable them become familiar with their new home, human restraint, feeding regime and to observe any possible health problem. At the first week of their arrival they were dewormed with ivomec® and given multivitamins (multinon®). The rabbits were judged to be healthy based on complete physical examination done before the commencement of the experiments.

Drugs Used

1. Midazolam (Dormicum®, Hoffmann-La Roche Ltd., Basel, Switzerland) supplied as 5 mg/ml in 2ml ampoule for injection.
2. Ketamine hydrochloride (ROTEXMEDICA, Trittau, Germany) supplied as 5mg/ml solution in 10ml vial for injection.
3. Tramadol hydrochloride (Tramaden®, Laborate pharmaceutical, India) supplied as 100mg in 2ml vial for injection.

Study Design

Two series of randomized experiments were carried out on each rabbit with an interval of one week allowed between both experiments to allow for complete metabolism and excretion of the drugs used.

In the first series of experiments each of the six rabbits was pre-medicated with midazolam 10 minutes before the injection of ketamine then in the second series, each of the six rabbits was pre-medicated with midazolam, followed by the concurrent injection of ketamine and tramadol 10 minutes later.

Experimental Procedures

The rabbits were allowed free access to feed and water until the time of drug administration. In the first series of the experiment, 2mg/kg midazolam was administered intramuscularly followed 10 minutes later by intramuscular injection of 25mg/kg ketamine hydrochloride.

For the second series, 2mg/kg midazolam was intramuscularly administered 10 minutes before the concurrent administration of 25mg/kg ketamine hydrochloride and 4mg/kg tramadol hydrochloride.

Following loss of righting reflex, rabbits were placed in right lateral recumbency and attached to a multiparameter patient monitor (Grady Vet 9200, China).

Measurements

The heart rate (HR), respiratory rate (RR), and rectal temperature (RT) (baseline) data were recorded and subsequently at 5 minutes interval for a period of 60 minutes. Haemostatic forceps closed to the first ratchet was applied on the inter-digital space of the hindlimb to test for analgesia every two minutes throughout the course of the experiments.

HR (beats/min) and RR (breaths/min) were determined using the patient monitor while rectal temperature (degrees Celsius) was determined using digital clinical thermometer. The anaesthetic indices calculated in the course of the trials were:

a) Time to loss of righting reflex: The time interval (in minutes) between the injection of ketamine and loss of righting reflex by the rabbit.
b) Duration of recumbency: Time interval (in minutes) between the loss of righting reflex and the assumption of sternal recumbency by anaesthetized rabbit.
c) Time to stand: Time interval (in minutes) between the assumption of sternal recumbency and standing posture by the anaesthetized rabbit.

Statistical Analysis

All values of data (heart rate, respiratory rate, and rectal temperature) at each time interval in the two series of experiments were expressed for the six (6) rabbits as mean ± standard error of mean. The means of anaesthetic indices (time to loss of righting reflex, onset of analgesia, duration of recumbency and time to standing) were compared using student T test for paired data. The mean values of the measured physiological parameters were compared using

analysis of variance (ANOVA) for repeated measures followed as appropriate by Dunnett's test when a significant difference was indicated. A value of $P < 0.05$ was considered statistically significant for all the comparisons (Dawson & Trapps, 2004).

RESULTS

Observation

No side effects were observed in any of the treated rabbits but they all reacted to pain on application of haemostatic forceps pressure to their hindlimbs throughout the anaesthetic period.

Anaesthetic Indices

The anaesthetic indices calculated for rabbits administered with Midazolam/Ketamine (MK) and Midazolam/Ketamine/Tramadol (MKT) are shown in table 1. Time to loss of righting reflex by the rabbits given MKT (1.7±0.3min) was significantly ($p<0.05$) different from those given MK (4.2±1.5min). The duration of recumbency with MKT (77.8±3.6min) was similar to that of MK (76.8±5.1min). Time to standing with MKT (9.3±1.1min) was not significantly ($p>0.05$) different from that of MK (15.2±2.4min).

Physiological Variables

Mean heart rates ranged from 207.3±4.6 to 238.8±8.7 beats/min with MKT and 204.7±13.0 to 257.5±15.3 beats/min with MK. The mean heart rate values at the 30th, 35th and 40th minutes were significantly different ($p<0.05$) between the MKT and MK treated rabbits (Fig 1).

Table 1. Selected anaesthetic indices of the intramuscular administration of Midazolam/Ketamine alone and with Tramadol in 6 rabbits.

Anaesthetic Indices	TREATMENT	
	MK	MKT
Time to loss of righting reflex	4.2±1.5	1.7±0.3*
Duration of recumbency (min)	76.8±5.1	77.8±3.6
Time to standing(min)	15.2±2.4	9.3±1.1

Data are expressed as means ± standard error of mean (SEM) of 6 rabbits. * $p<0.05$

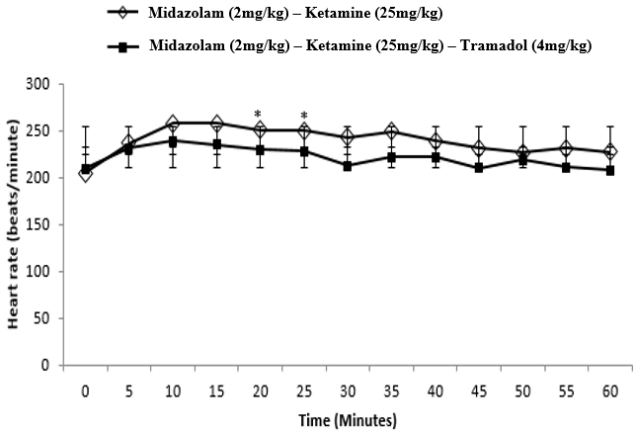

Figure 1: Mean heart rates of 6 rabbits administered with Midazolam/Ketamine (MK) alone or combined with Tramadol (MKT). * $p<0.05$

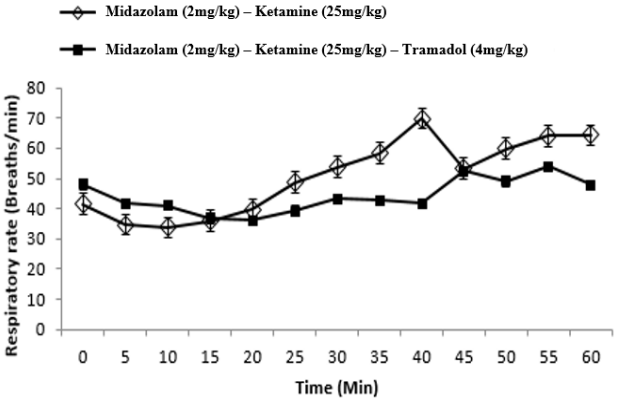

Figure 2: Mean respiratory rates of 6 rabbits administered with Midazolam/Ketamine (MK) alone or combined with Tramadol (MKT).

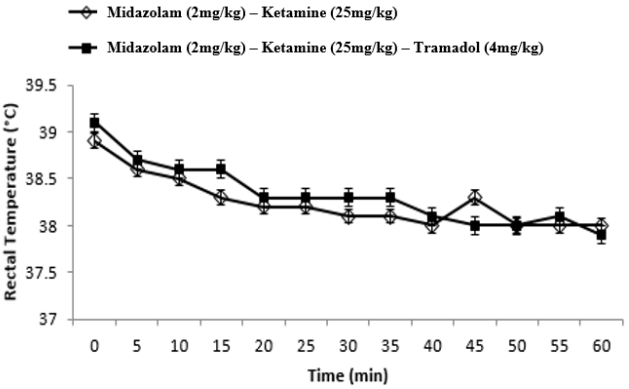

Figure 3: Mean rectal temperatures of 6 rabbits administered with Midazolam/Ketamine alone (MK) or combined with Tramadol (MKT).

DISCUSSION

The results of this study showed that Midazolam-ketamine-tramadol did not produce analgesia in the studied rabbits. It is surprising that the inclusion of tramadol did not confer analgesia on the rabbits in this study since a similar study with diazepam/ketamine and pentazocine reportedly produced analgesia (Adetunji *et al*., 2009). However, a clinical trial involving laparotomy in rabbits to evaluate the use of pentazocine in combination with diazepam and ketamine for surgical anaesthesia reported no analgesia (Udegbunam *et al*., 2017). It may be that a higher dosage of ketamine would be needed in the combination or just a function of strain variation in drug response because Adetunji and others (2009) employed 60mg/kg ketamine whereas Udegbunam and others (2017) used ketamine at a dosage of 15mg/kg and 25mg/kg body weight was used in the present study. Some studies have also shown that there is difference in response to various anaesthetic agents between different rabbit strains (Avsaroglu *et al*., 2003) and individual rabbits (Aeschbacher, 2001). Nonetheless, tramadol inclusion resulted in a faster onset of drug action evidenced by a significantly ($P<0.05$) shorter time to loss of righting reflex by the rabbits in the MKT group. The duration of recumbency

and time to standing were similar in both the MK and MKT treated groups of rabbits. Time to stand was shorter in the MKT but not statistically so (Table 1). The longer duration of recumbency of 76.8±5.1 obtained in the MK rabbits in this study (Table 1) is higher than previous similar studies of 27 min (Bellini *et al*., 2014) and 42min (Dupras, 2001). This observation may be due to the higher dosage of 2mg/kg used in this study compared with 1mg/kg for midazolam in the other studies. However, the lack of analgesia with MK is consistent with results of other similar studies (Dupras, 2001; Bellini *et al*., 2014).

Although the mean heart rates of the MKT treated rabbits were generally lower than those of the MK group, this difference was only significant from the 30th to 40th minute of anaesthesia. Nonetheless, heart rates of the rabbits in the two groups which ranged from 204.7±13.0 to 257.5±15.3 beats per minutes in the MK group and from 207.3±4.6 to 238.8±8.7 beats/min in the MKT group (Fig 1) fell within the normal range of 130 to 325 beats per minutes accepted for awake rabbits, (Harkness and Wagner, 1989; Harcourt brown., 2002).

There was no statistically significant difference in the mean respiratory rates between the MK and MKT treated rabbits. MK group had lower respiratory rates only in the first 15minutes of anaesthesia but subsequently, the MKT rabbits had lower respiratory rates which may be attributed to tramadol. Opioid's major side effect is respiratory depression (Borer-Weir, 2014) but tramadol does not cause significant respiratory depression if normal doses are not exceeded (Vickers *et al*., 1992).

Mean rectal temperature range of 37.9±0.3 and 39.1±0.1 for the MK group and 38.0±0.2 and 38.9±0.2 for the MKT group all fell within the normal range of 38 to 40 (°C) described for awake rabbits, (Harkness and Wagner, 1989; Harcourt – Brown, 2002).

It was concluded that although MKT produced a faster onset of action than MK, it did not produce analgesia. However, both MKT and MK appear safe as they were not associated with any significant cardiorespiratory depression in healthy rabbits not undergoing any clinical procedure. MKT will be useful for non-painful procedures where a rapid drug action is needed.

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