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Analgesic Effect of Pentazocine on Midazolam-Ketamine Anaesthesia in Rabbits

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ABSTRACT

The anaesthetic indices of intramuscular administration of 2mg/kg midazolam followed 10min later by 25mg/kg ketamine (MP) were compared with similarly administered 2mg/kg midazolam/ 25mg/kg ketamine / 10mg/kg pentazocine (MKP) in 6 rabbits not undergoing any surgical procedure. Heart rates (HR), Respiratory rates (RR) and Rectal temperature (RT) responses of the rabbits were monitored at 5 minutes' interval over a one-hour period of anaesthesia. The time to loss of righting reflex by the anaesthetized rabbits with MKP (1. 2 ±0. 2min) was significantly ($p<0.05$) shorter than that with MK (4. 2± 1. 5 min). The duration of recumbency with MKP (86. 0±4. 4) min was not significantly ($p>0.05$) different from that with MK (76.8 ±5. 1 min). Standing time with MKP (18. 5± 0. 8) min was also not significantly different from that with MK 15. 2 ±2. 4 min. Neither MK nor MKP produced analgesia in the studied rabbits. Mean HR range of 190.7±7.3 to 232.0±8.2beats/min (MKP) was lower than 200.7±13.0 to 257.5±16.0 beats/min with MK. Mean RR range of 26.5± 2.1 to 39.0±7.7 breaths/min with MKP was also lower than the range of 33.8±6.2 to breaths/min with MK. Mean RT range of 37.9±0.3 to 39.1±0.1 °C (MKP) was similar with MK (38.0±0.2 to 39.1± 0.2°C). It was concluded that the addition of pentazocine to midazolam/ketamine anaesthesia at the recommended doses employed produced only chemical restraint without analgesia and clinically insignificant cardiorespiratory depression.

Keywords: Analgesia, ketamine, midazolam, pentazocine, rabbit

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1. INTRODUCTION

Rabbits are raised commercially as a source of cholesterol free animal protein for human consumption and as laboratory animal models for research (Harkness,1987). These small mammals are also popular as pets and are often presented to veterinarians for assessment for medical and surgical treatment. Anaesthesia in exotic pets is required for many diagnostic and surgical procedures; however, this is associated with higher perioperative risk in rabbits compared with dogs and cats (Wenger, 2012). Rabbits are often considered as difficult laboratory animals to anesthetize successfully. They have strong reflexes which are difficult to suppress during general anesthesia apparently due to the sensitivity of their respiratory centre to an anesthetic agent and a variety of other observed secondary effects related to stress including death (Borkowski *et al.,*1999, Kilic 2004).

Although inhalation anaesthesia has the advantages of providing a controllable anaesthetic plane for protracted procedures, physiologic support for the patient and fast recovery, its use in rabbits is associated with some unique challenges chief of which is the delivery apparatus. Endotracheal intubation is difficult in this species because of the peculiar nature of the oral cavity (Evans, 1992).

The alternative use of an induction chamber is associated with the risk of injury to an unprotected animal and induction by face mask requires premedication in addition to the exposure of operating room personnel to anaesthetic waste (Flecknell, 1991). These challenges with the use of gaseous anaesthesia in rabbits, has made injectable anaesthesia more attractive in this species. In this regard, ketamine- based combinations, incorporating an alpha$_2$ agonist, phenothiazine or benzodiazepam appear to be the most popular in rabbit anaesthesia (Dupras *et al*., 2001; Hedenquist and Roughan, 2001; Henke *et al*., 2005; Orr *et al*., 2005, Grint, 2008; Murphy *et al*., 2010; Bellini *et al*., 2014; Oguntoye *et al*., 2014). Ketamine hydrochloride is a drug in the group of cyclohexylamines which is used in rabbits due to its rapid onset of action with minimal cardiovascular and respiratory depressant effects (Dupras *et al,* 2001, Avsaroglu *et al*., 2003, Bellini *et al*., 2014).

Midazolam, a short-acting benzodiazepine with hypnotic, anticonvulsant, muscle-relaxant and anxiolytic properties, is often combined with ketamine in order to counteract the side effects produced with the sole use of ketamine. When ketamine is used alone in rabbits it tends to cause hypertonus, poor muscle relaxation, persistent pain reflex responses and violent recovery from anaesthesia (Green, 1981; Clarke *et al*., 2014). Both ketamine and midazolam can be administered through the intramuscular route (Longley, 2008; Clarke *et al*., 2014) and therefore easy to administer. However, only few studies have reported analgesia with ketamine benzodiazepam combinations (Oguntoye *et al*., 2014). Many anaesthetic protocols for rabbits include analgesics for the provision of analgesia (Longley, 2008). Opioids are commonly used for this purpose but they are associated with side effects that are dose related and thus these side effects can be minimized by synergestic combination with other drugs. Indeed, the addition of butorphanol to ketamine and medetomidine reduces the doses needed of the latter two agents (Longley, 2008).

Pentazocine Hcl is the first synthesized agonist-antagonist opioid introduced into clinical practice for analgesia (Henderson, 2008). In rabbits, pentazocine has been found to be an effective analgesic agent (Flecknell and Liles,1990). There is however, a paucity of information on the effect of the concurrent administration of this partial opioid agonist with midazolam/ketamine anaesthesia in literature.
The purpose of this study was therefore to evaluate the analgesic effect of intramuscular injection of pentazocine on ketamine/midazolam anaesthesia in rabbits not undergoing any surgical procedure.

2. MATERIALS AND METHODS

Animals

The experimental animals consisted of 6 adult Nigerian breed of rabbits (3 males and 3 females) with a mean body weight of 1.5 ±0.06kg (mean± sem). They were purchased from a local market in Ibadan, Oyo State, Nigeria. The rabbits were housed in an indoor cage constructed with wood and wire net at the experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan. The cage was constructed in a way that facilitated proper faeces disposal thereby preventing ammonia build up in the cage. They were kept for two weeks to acclimatize them with the new environment, to get them acquainted with human handling and to observe them for any deviation from normal state of health before the commencement of the experiments. The rabbits were fed grower's mash supplemented with freshly cut leaves of *Tridax Procumbens* once daily and drinking water ad-libitum.

Drugs

The drugs used for this study were:

1. Midazolam (Dormicum^R, Miloz®, Novell pharmaceuticals limited, United states.) supplied as a 5mg solution for injection in 1ml ampoule
2. Ketamine hydrochloride (Ketalar®, Parke-Dais Pharmaceuticals Limited, India.) supplied as 50mg solution for injection in a 10ml multidose vial.
3. Pentazocine lactate (Talwin®, Sanofi Aventis, Canada) supplied as a 3% aqueous solution for injection in 1ml ampoule.

3. EXPERIMENTAL DESIGN

Two sets of randomized trials were carried out on each rabbit at one-week interval. In the first series of trial, each of the rabbits was premedicated with midazolam, followed by induction of anaesthesia by the administration of ketamine 10 minutes later (MK group). In the second series of the trials, each of the rabbits was premedicated with midazolam followed 10 minutes later by simultaneous administration of ketamine and pentazocine (MKP). In the course of each trial, physiological parameters were monitored every 5 minutes and the selected anaesthetic indices were recorded.

Experimental Procedure

Rabbits were allowed free access to feed and drinking water up to the time of drug administration. The weights of the rabbits were determined using a top loading weighing balance for accuracy in the calculation of the required dosage. The first series of trial involved the administration of 2mg/kg body weight of midazolam intramuscularly followed ten minutes later by intramuscular administration of ketamine at the dose rate of 25mg per kg body weight. The second series of the trials involved intramuscular administration of 2mg/kg body weight of midazolam followed ten minutes later by concurrent intramuscular administration of ketamine and pentazocine at the dose rate of 25mg and 10mg per kg body weight respectively. The rabbits were placed on right lateral recumbency on a hydraulic table following the loss of righting reflex and haemostatic forceps closed to the first ratchet was applied on the inter-digital space (of the hindlimb) to test for pedal withdrawal reflex as a means of assessing analgesia. This was done every two minutes throughout the course of anaesthesia. The physiological parameters including heart rate, respiratory rate and rectal temperature were monitored every five minutes throughout the course of the experiments.

Measurement of Physiological Variables

Following the loss of righting reflex by the rabbits, the heart rate (HR), respiratory rate (PR) and rectal temperature (RT) were determined, and thereafter at 5 minutes' interval over a 60-minute period. The heart rates (in beats per minute) and respiratory rates (in breaths per minute) were determined using a multi parameter veterinary patient monitor (Grady Vet 9200, China) while the rectal temperature was determined using a digital clinical thermometer.

Calculations

The selected anaesthetic indices were calculated as follows:

- **a)** Onset of anaesthesia (or drug action): time interval (in minutes) between ketamine injection and loss of the righting reflex by the rabbits.
- **b)** Duration of recumbency: time interval (in minutes) between loss of the righting reflex and assumption of sternal posture by the rabbits.
- **c)** Standing time: time interval (in minutes) between assumption of sternal and standing postures by the rabbits.

4. ANALYSIS OF DATA

Values of the heart rates, respiratory rates and rectal temperature at each time interval in each trial were expressed as means ± standard error of mean (SEM) of the 6 rabbits. The means of the anaesthetic indices were compared using the student's 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 significant.

5. RESULTS

Observation**:** No side effects were observed in the treated rabbits, except for one rabbit which daefecated during the course of the experiments with MK andanother one with MKP. All the rabbits reacted to pain on application of haemostatic forceps pressure to their hindlimbs throughout the anaesthetic period.

Anesthetic Indices

The selected anesthetic indices that were calculated are shown in Table 1. Time to loss of righting reflex by the anaesthetized rabbits with Midazolam/ketamin/Pentazocine (MKP) 1.2±0.2 min was significantly ($p<0.05$) shorter than that with Midazolam/Ketamine (MK) 4.2±1.5 min. Duration of recumbency with MKP (86.0±4.4 min) was not significantly different from that with MK (76.8±5.1 min). Standing time with MKP 18.5±0.8 min was also not significantly different from that with MK 15.2±2.4 min.

Table1: Selected anaesthetic indices of the intramuscular administration of midazolam/ketamine[a] alone and combined with pentazocine[b] in rabbits.

Anaesthetic Indices	Treatment Groups	
	MK	MKP
Time to loss of righting reflex	4.2±1.5	1.2±0.2*
Duration of recumbency	76.8±5.1	76.8±5.1
Time to standing	15.2±2.4	18.5±0.8

Data are expressed as means ± standard error (SEM) of the 6 rabbits.

a. 2mg/kg of Midazolam / 25mg ketamine hydrochloride

b. 2mg/kg of Midazolam / 25mg ketamine hydrochloride / 10mg/kg Pentazocine
*P<0.05

Physiological parameters

The HR, RR and RT responses of the experimental rabbits to the intramuscular administration of MK and MKP are shown on Table 2. The mean HR with MKP ranged between 190.7±7.3 and 232.0±8.2 beats/min and from 204.7±13.0 to 257.5±15.3 beats/min with MK. The mean RR with MKP ranged from 26.5±2.1 to 39.0±7.7 breaths/min while the control values with MK ranged between 33.8±6.2 and 69.8±14.5 breaths/min. Mean RT with MKP ranged between 37.9±0.3 and 39.1±0.1^{o}C and from 38.0±0.2 to 39.1±0.2^{o}C with MK. There were no significant differences in mean RT between the two groups.

Table 2: Heart rate, respiratory rate and rectal temperature responses to intramuscular administration of midazolam-ketamine[a] alone and combined with pentazocine[b] in rabbits.

Time Interval (min)	Heart Rate (Beats/minute)		Respiratory rate (Breaths/minute)		Rectal Temperature (°C)	
	MK	MKP	MK	MKP	MK	MKP
0	204.7±13.0	225.3±8.5	41.5±5.6	39.0±7.7	38.9±0.2	39.1±0.1
5	236.3±22.2	232.0±8.2	34.7±4.1	30.8±3.4	38.6±0.2	38.7±0.2
10	257.2±16.0	226.5±8.6*	33.8±6.2	26.5±2.1	38.5±0.2	38.6±0.2
15	257.5±15.3	225.8±6.4*	36.0±6.4	28.5±1.5	38.3±0.2	38.6±0.2
20	250.3±15.5	218.8±5.7*	39.8±6.5	28.2±1.9*	38.2±0.3	38.3±0.3
25	250.0±11.4	215.0±5.5*	48.7±9.8	28.0±1.9*	38.2±0.3	38.3±0.2
30	242.7±10.7	203.2±5.3*	53.8±13.0	28.8±1.1*	38.1±0.2	38.3±0.3
35	249.2±8.0	198.8±5.2*	58.3±16.3	30.2±2.2*	38.1±0.2	38.3±0.3
40	238.5±7.0	197. 8±5.9*	69.8±14.5	31.0±1.6*	38.0±0.2	38.1±0.3
45	231.3±10.2	194.0±6.7*	53.3±12.0	29.7±1.3*	38.3±0.2	38.0±0.3
50	226.0±8.2	195.0±6.5*	59.8±13.4	32.7±2.3*	38.0±0.2	38.0±0.4
55	230.8±7.1	193.0±5.7*	64.0±16.1	38.7±6.4*	38.0±0.2	38.1±0.3
60	227.2±8.0	190.7±7.3*	64.3±15.0	38.3±4.4*	38.0±0.2	37.9±0.3

Data are expressed as means ± SEM of 6 rabbits

a. 2mg/kg of Midazolam / 25mg ketamine hydrochloride

b. 2mg/kg of Midazolam / 25mg ketamine hydrochloride / 10mg/kg Pentazocine
*P<0.05

6. DISCUSSION

Each experimental rabbit used in this study served as its own control so as to eliminate the effects of individual variations in drug response. An interval of one week was allowed in between trials to allow for the washout of drugs used in the previous trial. In this study, the addition of pentazocine produced a faster onset of drug action evidenced by the shorter time to loss of righting reflex (1.2± 0.2min) in the MKP rabbits than in the MK rabbits (4.2±1.5 min) ($p<0.05$). A longer duration (though not statistically significant $P>0.05$) of recumbency was also produced by MKP (Table 1), indicating a synergistic effect although the standing time with both MKP and MK were similar (Table 1). It was surprising that the inclusion of pentazocine in the MKP protocol did not produce analgesia. It may be that a higher dose of either midazolam or ketamine would be needed, or could be the variation in the drug responses of the rabbit strains used. A similar study that reported analgesia with diazepam combination gave ketamine at a higher dosage of 60mg/kg body weight (Adetunji *et al*., 2009) than the 25mg/kg body weight used in this study. However, some previous studies have established that there are differences in response to various anaesthetic agents between both different rabbit strains (Avsarogh *et al,* 2003) and individual rabbits (Aeschbacher, 2001). The dosages of 2mg/kg (midazolam) and 25mg/kg (ketamine) and 10mg/kg (pentazocine) are doses that have also been used in rabbits in literature (Longley, 2008).

Heart rate and respiratory rates were significantly ($P<0.05$) lower in the MKP group than in the MK group (Table 2). This finding of lower heart and respiratory rates of rabbits in the MKP group than in the MK group was not surprising. Some of the common side effects with the use of opioids are respiratory depression & bradycardia (Longley, 2008). Nonetheless, the heart and respiratory rate responses of the rabbits to MKP and MK fell within the respective normal ranges of 130-325 beats/minute and 30-60 breaths/minute acceptable for awake, resting rabbits (Harkness and Wagner,1989). There was no significant difference between the rectal temperature in the two treatment groups (Table 2). The range of the rectal temperature also fell within the normal values of 38 to 40℃ described for awake rabbits (Harkness and Wagner, 1989; Harcourt-Brown, 2002). The abilities of the rabbits in this study to maintain normal temperature is probably because factors that predispose to a fall in body temperature were not present.

Some of these factors include hair clipping, surgical opening of a major body cavity, use of surgical scrub solutions, and infusion of cold intravenous fluids (Haskins, 1981). In conclusion, the addition of pentazocine to midazolam/ketamine only produced chemical restraint and not analgesia in the rabbits studied at the dosages used. The inclusion of pentazocine however resulted in a shorter onset of action and a longer duration of recumbency with some clinically insignificant degree of cardio-respiratory depression in healthy rabbits not undergoing any surgical procedure.

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