EVALUATION OF THE USE OF PENTAZOCINE IN COMBINATION WITH DIAZEPAM AND KETAMINE FOR SURGICAL ANAESTHESIA IN RABBITS

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ABSTRACT

The effect of pentazocine on diazepam/ketamine anaesthesia was evaluated in this study. Pentazocine (10 mg/kg) was administered intramuscularly (im) prior to injection of diazepam (2 mg/kg, iv) and ketamine (15 mg/kg, im) in pentazocine /diazepam/ketamine (P/D/K) group. In the diazepam/ketamine group, anaesthesia was induced using diazepam (2 mg/kg, iv) and ketamine (15 mg/kg, im). All rabbits were laparotomized after induction of anaesthesia. Intra operatively, anaesthetic indices, physiologic variables and pain responses of rabbits were studied. Blood glucose and serum cortisol of rabbits were monitored post laparotomy. The durations of analgesia and anaesthesia were significantly shorter (p<0.05) in the P/D/K group. Respiratory rates of P/D/K group were significantly higher (p<0.05) than those of D/K group. Pain scores of P/D/K group were significantly higher (p<0.05) than scores obtained in D/K group. At 30 and 90 minutes post laparotomy, blood glucose and serum cortisol of P/D/K group were significantly higher (p<0.05) than those of D/K group. This study showed that pentazocine/diazepam/ketamine drug combination did not produce surgical anaesthesia in rabbits.

Keywords: Ketamine, Diazepam, Pentazocine, Combined therapy, Surgical anaesthesia, Rabbit

INTRODUCTION

General anaesthesia is a drug induced state of unconsciousness characterized by controlled but reversible depression of the central nervous system and analgesia (Thurmon and Short, 2007). Most often, single anaesthetic agents when used for general anesthesia are unable to produce surgical anaesthesia. Thus in modern veterinary practice, the technique of balanced anaesthesia is often employed. This anaesthetic technique requires concomitant administration of multiple drugs to produce individual components of the anaesthetic state namely unconsciousness, analgesia, muscle relaxation and alteration of autonomic reflexes (Thurmon and Short, 2007). Ultimately, following this practice, surgical anaesthesia, a plane of general anaesthesia which allows painless surgery is attained.

Ketamine is a dissociative anaesthetic notably used for its deep somatic analgesic effect and wide margin of safety (Henke *et al*., 2005; Orr *et al*., 2005; Grint and Murison, 2008). Ketamine however is rarely used alone since it produces a cataleptoid state characterized by poor muscle relaxation, muscle tremors, myotonic contractions, opistotonus and rough recovery (Green *et al*., 1981; Christensen *et al*., 1987; Mohammad *et al*., 1993). Thus to counteract these undesirable effects, various drugs such as xylazine, medetomidine, diazepam and acepromazine have been used in combination with ketamine (Nuh, 2004; Amarpal *et al*., 2010). The agent most widely used in combination with ketamine is xylazine an alpha-2 agonist (Lipman *et al*., 2008).

Ketamine/xylazine combination has been reported to produce surgical anaesthesia in rabbit but with some mortality (White and Holme, 1976). Other ketamine based combinations described for anaesthetizing rabbits include ketamine/medetomidine (Orr *et al*., 2005; Grint and Murison, 2005; Amarpal *et al*., 2010), ketamine/acepromazine (Amarpal *et al.,* 2010; Oguntoye and Oke, 2014) and ketamine/xylazine (Henke *et al*., 2005; Amarpal *et al*., 2010; Oguntoye and Oke, 2014). Diazepam/ketamine combination produces short duration anaesthesia, fair muscle relaxation and minimal cardiopulmonary depression in goats (Ghurashi *et al*., 2009). Although this combination appears ideal for rabbit anaesthesia, it produces inadequate surgical analgesia (Redah, 2011; Oguntoye and Oke, 2014**)**. Therefore, to attain a plane of surgical anaesthesia there may be need for the administration of potent analgesics pre operatively prior to use of diazepam/ketamine for anaesthesia.

Pentazocine is an agonist-antagonist opioid and exerts mild analgesic action by acting on the kappa opioid receptor (Lamont and Mathews, 2007; Lipman *et al.,* 2008). Previous studies carried out using rabbits showed that use of butorphanol (an agonist-antagonist opioid) in combination with xylazine/ketamine improved operating conditions in rabbits (Marini *et al.,* 1992). Despite the aforementioned benefit of concurrent use of this opioid for general anaesthesia, nothing is known of the effect of pentazocine on the quality of anaesthesia produced by diazepam/ketamine drug combination. This study evaluated the effect of prior use of pentazocine on diazepam/ketamine anaesthesia. To ascertain this, the anaesthetic and analgesic effects of pentazocine/diazepam/ketamine combination were compared with those of diazepam/ketamine combination.

MATERIALS AND METHODS

Animals: Twenty 10 months old male New Zealand white rabbits weighing between 1.2 – 1.8 kg were used in this study. They were housed in standard cages and were left to acclimatize for 14 days. During the period of study, they were fed with commercial breeders' diet supplemented with potatoes' leaves and water was provided *ad libitum*. After acclimatization, the rabbits were randomly assigned to two treatment groups of five rabbits, each rabbit represented a replicate. The groups were the diazepam/ketamine (D/K) and pentazocine/diazepam/ketamine (P/D/K) groups respectively. This research was approved by the Animal Ethics Committee, University of Nigeria, Nsukka.

Pre-anaesthetic protocols: Baseline readings of heart rates (HR) and respiratory rates (RR) of rabbits were obtained at 0 minute before anaesthetic induction. Blood samples were collected from jugular veins of rabbits and dispensed into tubes containing ethylene diamine tetra acetate (EDTA) and into test tubes without anticoagulant. Blood glucose values were determined by the glucose oxidase method using a commercial glucose kit (Accu-chek Advantage II, Roche diagnostic, Germany). Serum separated from clotted blood samples were collected within 30 minutes and used for serum cortisol determination using a commercial enzyme linked immunosorbent assay kit.

Induction of anaesthesia: In P/D/K group, 10 mg/kg pentazocine (Pentalab, Laborate Pharmaceutical, India) was injected intramuscularly 30 minutes before intravenous injection of 2 mg/kg diazepam (Valium, Roche, France). After a 5 minutes latency period, 15 mg/kg ketamine (Ketamine, Rotex Medica, Germany) was injected intramuscularly to induce anaesthesia. Rabbits in diazepam /ketamine (D/K) group were injected with 2 mg/kg diazepam intravenously five minutes prior to intramuscular injection of 15 mg/kg ketamine.

Laparotomy: After induction of anaesthesia, rabbits were placed on insulated operating tables and their midventral abdomen shaved and prepared for aseptic surgery. A paramedian laparotomy incision was made 2 cm caudal to the xiphoid region down to 1 cm below the umbilicus through the subcutaneous tissues,

muscle and peritoneum and into the abdomen. Thereafter, the peritoneum, muscles and subcutaneous tissues were sutured with simple continuous pattern using size 2/0 chromic catgut suture. The skin was sutured with size 2/0 silk using simple interrupted suture pattern.

Post-surgical care: On completion of surgery, each rabbit was thoroughly dried and returned to its cage where food and water were provided. Penicillin (0.5 mg/kg body weight) and streptomycin (1 mg/kg body weight) were injected intramuscularly once daily for 5 days post surgery to all the rabbits. After post-operative pain assessments at 90 minutes in both groups, pentazocine (5 mg/kg body weight) was administered intramuscularly after laparotomy and subsequently once daily for another 2 days to rabbits in both groups.

Anaesthetic Variables: The following were studied intra and post operatively.

Induction time: Time from end of ketamine injection to onset of recumbency.

Duration of analgesia: Time from onset of analgesia to return of response to surgical manipulations.

Duration of anaesthesia: Time from loss of righting reflex to return of righting reflex.

Heart and respiratory rates: Heart and respiratory rates of rabbits were measured intra operatively at 10 minutes and post operatively at 20 and 30 minutes.

Pain assessments: Pain responses of rabbits were studied during incision and closure of the skin, subcutaneous tissue and muscles. Pain responses were categorized as mild, moderate and severe. These categories were allotted numerical scores viz: 1 (mild), 2 (moderate) and 3 (severe).

Blood glucose and serum cortisol: Blood glucose and serum cortisol of rabbits were measured intra operatively at 10 minutes and post operatively at 30 and 90 minutes.

Data Analysis: The mean parametric data obtained in the P/D/K group were compared respectively with those of D/K group using T-test. Pain scores were compared using Kruskall-Wallis test. Probability values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Assessment of the duration of analgesia and anaesthesia as presented in Table 1 showed that duration of analgesia and anaesthesia of the P/D/K group were significantly shorter ($p<0.05$) compared to that of D/K group. These results suggested that pre-anaesthetic administration of pentazocine shortened the durations of analgesia and anaesthesia produced by diazepam/ketamine combination. Apparently it appeared that pentazocine shortened the short duration of D/K anaesthesia either due to its stimulant effect on the central nervous system (CNS) or due to its antagonist effect on ketamine. Central nervous system excitation has been reported following the use of opioids in cats, horses, sheep, goats and pigs (Branson *et al.,* 2001). Thus, pentazocine might have counteracted the CNS depressant effect of both diazepam and ketamine thereby shortening the duration of anaesthesia produced by D/K combination.

Heart rates (HR) of D/K and P/D/K groups increased though statistically they were not different ($p>0.05$) at all-times during surgery (Table 2). Respiratory rates (RR) of both D/K and P/D/K groups decreased by 10 minutes post induction of anaesthesia with subsequent increase noted post-operatively (Table 2). The RR of P/D/K group was significantly higher ($p<0.05$) than those of D/K group at all-times during the study. In a study by Booth (1988), diazepam was reported to have minimal cardiovascular effect while in another study; Kumar and Kumar (1984) reported that ketamine increased the heart rate of rats and rabbits. On the other hand, use of pentazocine have been shown to cause dose dependent increase in blood pressure of conscious, curarized-conscious and anaesthetized dogs (Kucukhuseyin, 2003).

Table 1: Anaesthetic variables obtained following administration of drug combinations

Groups	Anaesthetic variables (minutes)		
	Onset of anaesthesia	Duration of analgesia	Duration of anaesthesia
D/K	3.0 ± 0.5	46.4 ± 5.0[a]	76.2 ± 6.4[a]
P/D/K	4.8 ± 0.9	13.2 ± 2.9[b]	25.8 ± 4.0[b]

Different superscript on D/K and P/D/K indicates significantly different means at p<0.05

Table 2: Mean heart (HR) and respiratory rates (RR) of anaesthetized rabbits

Variables	Groups	Baseline t = 0 min	Intra-operative period	Post-operative period	
			t = 10 min	t = 20 min	t = 30 min
HR (beats/min)	D/K	250.8 ± 11.9	267.8 ± 8.5	276.3 ± 9.9[a]	289.9 ± 3.1[a]
	P/D/K	248.0 ± 11.0	268.0 ± 17.9	288.8 ± 13.3[b]	292.8 ± 6.0[b]
RR (breaths/min)	D/K	258.0 ± 8.0	144.0 ± 50.3[a]	171.6 ± 35.5[a]	213.6 ± 17.3[a]
	P/D/K	262.4 ± 11.0	221.1 ± 11.3[b]	228.0 ± 13.3[b]	243.9 ± 13.1[b]

Different superscript on D/K and P/D/K indicates significantly different means at p<0.05

Based on these earlier reports, we suggest that the observed increase in heart rates of rabbits in both groups in the intra operative period might be pain or drug induced. The finding of respiratory depression in both groups was at variance with previous reports documented after injection of diazepam/ketamine (Kul *et al.,* 2000; Ghurashi *et al.,* 2009) and pentazocine (Kucukhuseyin, 2003). The finding in D/K group was however similar to that reported by Oguntoye and Oke (2014) following use of this drug combination in rabbits. Therefore RR of all rabbits might have decreased since rabbits are prone to respiratory depression during anaesthesia (Orr *et al.,* 2005; Peeters *et al.,* 2008). In the post-operative period, both HR and RR of rabbits increased with those of P/D/K group being significantly higher than those of D/K group. This increased in both vital parameters of rabbits post-operatively probably occurred in response to pain. Studies have shown that post-operative changes in these variables might occur in response to pain in the animals (Flecknell, 1987; Scott *et al.,* 1994). According to these researchers, the administration of analgesics prior to the procedures prevented the occurrence of these changes. Furthermore, the higher HR and RR of rabbits in P/D/K group throughout the post operative period suggest that use of pentazocine prior to laparotomy did not significantly reduce laparotomy-induced pain response in rabbits.

Pain scores of P/D/K group were significantly ($p<0.05$) higher than scores obtained in D/K group during skin incision, muscle incision, muscle stitching and skin stitching (Table 3).

Table 3: Intra-operative pain scores in tissue layers of rabbits during laparotomy

Surgical Procedure	Pain scores	
	D/K	P/D/K
Skin incision	2.4±0.8a	2.6±0.8 b
Muscle incision	2.0±0.0 a	2.4±0.4 b
Muscle suturing	1.0±0.0 a	2.0±0.5 b
Skin suturing	1.4±0.2 a	2.4±0.4b

Different superscript on D/K and P/D/K indicates significantly different means at p<0.05

Also, in the intraoperative and post-operative periods, blood glucose and serum cortisol of P/D/K group were significantly higher ($p<0.05$) than those of D/K group (Table 4). The results of the above mentioned assessments suggested that more pain was felt in P/D/K group. Earlier, Lascelles *et al.* (1995) reasoned that failure of pre-emptive drug action might arise from inadequate binding of the analgesic drugs to the relevant receptors and inability of analgesic to remain effective throughout the surgical procedures. It has been proposed that ketamine might be a mu and kappa opioid receptors antagonist (Hirota *et al.,* 1999). Thus since pentazocine has agonist activity at the kappa opioid receptor, we suggest that ketamine might

Table 4: Blood glucose and serum cortisol of rabbits at different time points after laparotomy

Variables	Groups	Baseline t = 0 min	Intra-operative period t = 10 min	Post-operative period t = 30 min	Post-operative period t = 90 min
Glucose (mg/dl)	D/K	160.7 ± 8.3	177.9 ± 1.4[a]	287.5 ± 3.7[a]	258.7 ± 4.5[a]
	P/D/K	148.0 ± 1.3	182.2 ± 0.9[b]	316.6 ± 4.7[b]	304.6 ± 6.3[b]
Cortisol (µg/dl)	D/K	43.9 ± 4.1	85.4 ± 2.1[a]	108.1 ± 1.9[a]	93.5 ± 2.3[a]
	P/D/K	37.6 ± 6.9	98.2 ± 0.9[b]	122.8 ± 2.4[b]	116.7 ± 3.2[b]

Different superscript on D/K and P/D/K indicates significantly different means at p<0.05

have antagonized it thus reducing its intrinsic analgesic activity. Furthermore, this finding highlights the need for further research to evaluate the negative effect of pentazocine and other opioids on ketamine.

Conclusion: Pentazocine at the dose used in this study shortened the duration of analgesia and anaesthesia produced by diazepam /ketamine. Also, its use neither improved intra operative analgesia nor ameliorated laparotomy-induced post operative pain in rabbits. We therefore conclude that use pentazocine in combination with diazepam/ketamine did not produce surgical anaesthesia in rabbits.

REFERENCES

AMARPAL, P., KINJAVDEKAR, P., AITHAL, H., PAWDE, A., SINGH, J. and UDEHIYA, R. (2010). Evaluation of xylazine, acepromazine and medetomidine with ketamine for general anaesthesia in rabbits. *Scandinavian Journal of Laboratory Animal Science*, 37(3): 223 – 227.

BOOTH, N. H. (1988). Psychotropic agents. Pages 363 – 395. *In:* BOOTH, N. H. and MCDONALD, I. E. (Eds.), *Veterinary Pharmacology and Therapeutics,* 6th Edition, Iowa State University Press, Ames, USA.

BRANSON, K. R., GROSS, M. E. and BOOTH, N. H. (2001). Opioid analgesics. Pages 274 – 310. *In:* ADAMS, H. R. (Ed.), *Veterinary Pharmacology and Therapeutics,* 8th Edition, Iowa State University Press, Ames, USA.

CHRISTENSEN, J., FOSSE, R. T., HALVORSEN, O. J. and MORILD, I. (1987). Comparison of various anaesthetic regimens in the domestic fowl. *American Journal of Veterinary Research,* 48(11): 1649 – 1657.

FLECKNELL, P. (1987). Laboratory mammal anesthesia. *Journal of Association of Veterinary Anesthetists*, 14: 111-119.

GHURASHI, M. A., SERI, H. I., BAKHEIT, A. H., ASHWAG, E. A. M. and ABAKAR, J. A. (2009). Evaluation of ketamine/diazepam anaesthesia for performing surgery in desert goats under field conditions. *Australian Journal of Basic and Applied Sciences*, 3(2): 455 – 459.

GREEN, C. J., KNIGHT, J., PRECIOUS, S. and SIMPKIN, S. (1981). Ketamine alone and combined with diazepam or xylazine in laboratory animals: A 10 year experience. *Laboratory Animal*, 15(2): 163 – 170.

GRINT, N. J. and MURISON, P. J. (2008). A comparison of ketamine-midazolam and ketamine-medetomidine combination for induction of anaesthesia in rabbits. *Veterinary Anaesthesia and Analgesia*, 35(2): 113 – 121.

HENKE, J., ASTNER, S., BRILL, T., EISSNER, B., BUSCH, R. and ERHARDT, W. (2005). Comparative study of three intramuscular anaesthetic combinations (medetomidine /ketamine, edetomidine/fentanyl/midazolam, xylazine/ketamine) in rabbits. *Veterinary Anaesthesia and Analgesia*, 32(5): 261 – 270.

HIROTA, K., OKAWA, H., APPADU, B. L., GRANDY, D. K., DEVI, L. A. and LAMBER, D. G. (1999). Stereoselective interaction of ketamine with recombinant µ, κ and δ

opioid receptors expressed in Chinese hamster ovary cells. *Anaesthesiology*, 90: 174 – 182.

KUCUKHUSEYIN, C. (2003). Cardiovascular effects of intravenous pentazocine and cyclazocine in conscious, curarized-conscious and anaesthetized dogs. *Journal of Basic Clinical Physiology Pharmacology*, 14: 235 – 255.

KUL, M., KOC, Y., ALKAND, F. and OGURTAN, Z. (2000). The effects of xylazine/ ketamine and diazepam/ketamine on arterial blood pressure and blood gases in dogs. *Journal of Veterinary Research*, 4: 122-132.

KUMAR, R. and KUMAR, A. (1984). Ketamine with and without premedication in rats and rabbits. *Veterinary Journal*, 61: 372 – 376.

LAMONT, L. A. and MATHEWS, K. A. (2007). Opioids and non steroidal anti-inflammatories and analgesic adjuvants. Pages 241 – 272. *In:* TRANQUILLI, W. J., THURMON, J. C. and GRIMM, K. A. (Eds.), *Lumb and Jones Veterinary Anaesthesia and Analgesia,* 4th Edition, Blackwell Publishing Professional, Iowa, USA.

LASCELLES, B. D. X., WATERMAN, A. E., CRIPPS, P. J., LIVINGSTON, A. and HENDERSON, G. (1995). Central sensitization as a result of pethidine for ovariohysterectomy in rats. *Pain*, 62: 201 – 212.

LIPMAN, N. S., MARINI, P. R. and FLECKNELL, P. (2008). Anaesthesia and analgesia in rabbits. Pages 299 – 333. *In:* FISH, R. E., BROWN, M. J., DANNEMAN, P. J. and KARAS, A. Z. (Eds.) *Anaesthesia and Analgesia in Laboratory Animals*. 2nd Edition, Elsevier, USA.

MARINI, R. P., AVISON, D. L., CORNING, B. F and LIPMAN, N. S. (1992). Ketamine/xylazine/ butorphanol: a new anaesthetic combination in rabbits. *Laboratory Animal Science*, 42: 57 – 62.

MOHAMMAD, F. L., AL-BADRANY, M. S. and AL-HASAN, A. M. (1993). Detomidine-ketamine anaesthesia in chickens. *Veterinary Record*, 133(8): 192. doi:10.1136/vr.133.8.192.

NUH, K. A. (2004). Comparison between medetomidine-ketamine and xylazine-ketamine in rabbits. *Turkish Journal of Veterinary and Animal Sciences*, 28: 921 – 926.

OGUNTOYE, C. O. and OKE, B. O. (2014). A comparison of xylazine/ketamine, diazepam/ketamine and acepromazine/ ketamine anaesthesia in rabbit. *Sokoto Journal of Veterinary Sciences*, 12(3): 21 – 25.

ORR, H. E., ROUGHAN, J. V. and FLECKNELL, P. A. (2005). Assessment of ketamine and medetomidine anaesthesia in domestic rabbits. *Veterinary Anesthesia and Analgesia*, 32(5): 271 – 279.

PEETERS, M. E., GIL, D., TESKE, E., EYZENBACH, V. V. D., BROM, W. E., LUMEIJ, J. T. and DE VRIES, H. W. (2008). Four methods for general anaesthesia in the rabbits: A comparative study. *Laboratory Animals*, 22: 355 – 360.

REDAH, S. A. (2011). A comparative study between using of midazolam-ketamine and diazepam-ketamine combinations as anaesthetic program in sheep. *Al-Qadisiya Journal of Veterinary Medicine Science*, 10(1): 66 – 72.

SCOTT, P. R., HENSHAW, C. J., SARGISON, N. D., PENNY, C. D. and PIRIE, R. S. (1994). Assessment of xylazine hydrochloride epidural analgesia for open castration of rams. *Theriogenology*, 42(6): 1029 – 1034.

THURMON, J. C. and SHORT, C. E. (2007). History and overview of veterinary anaesthesia. Pages 3 – 7. *In:* TRANQUILLI, W. J., THURMON, J. C. and GRIMM, K. A. (Eds.), *Veterinary Anaesthesia and Analgesia,* 4th Edition, Blackwell Publishing, United Kingdom.

WHITE, G. L. and HOLMES, D. D. (1976). Comparison of ketamine and the combination ketamine xylazine for effective surgical anaesthesia in the rabbit. *Laboratory Animal Science*, 26: 804 – 806.