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RESEARCH ARTICLE

EVALUATION OF FENTANYL- KETAMINE- MIDAZOLAM COMBINATION FOR ANESTHESIA OF NEW ZEALAND WHITE RABBITS: A RANDOMIZED CONTROLLED STUDY

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ABSTRACT

Different combinations were examined for rabbit's anesthesia. To the best of our knowledge, the combination of ketamine-midazolam-fentanyl has never been used in rabbits. We assumed this will provide several advantages compared with the previously used combinations. The aim of this study was to evaluate the effects of three different anaesthetic combinations (Ketamine/ Midazolam/ Fentanyl, Ketamine/ Xylazine/ Midazolam and Ketamine/ Xylazine) on nociceptive reflexes, circulatory and respiratory functions, and stress markers, in a New Zealand White rabbit; a commonly used model in experimental urological procedures. The rabbits were randomly assigned into 3 groups (n=24 rabbits): Ketamine/ Midazolam/ Fentanyl (K-M-F) group by (35, 1, 0.02 mg/kg respectively), Ketamine/ Xylazine/ Midazolam (K- X- M) group by (35, 5, 1mg/kg respectively) and ketamine/ Xylazine (K- X) group by (50, 10 mg/kg respectively). Sleep time was significantly earlier and longer in K-M-F but surgical anesthesia was poorest. Bradycardia produced by K-M-F was insignificant in contrast to the other two groups. The effect on stress markers was insignificant. In coclusion, although the addition of fentanyl to ketamine-midazolam combination did not improve the anesthetic quality, but, may be advantageous in anesthesia of circulatory compromised cases, otherwise, other anesthetic combinations should be considered.

INTRODUCTION

Rabbits have been generally regarded as "unsafe" during anesthesia (1). They are easily stressed and show high plasma catecholamine concentration during induction of anesthesia (2). Moreover, they display wide strain and sex-specific differences and inter-individual variability in response to anesthetics (3). The most common complications encountered during anesthesia are due to respiratory depression (2) and prolonged hypotension especially when anesthesia lasts longer than one hour (4). Ketamine is a short-acting dissociative anesthetic with variable analgesic effects. It is used for chemical restraint and for the induction and maintenance of anesthesia in a number of species (5). Unlike many anesthetics, ketamine usually stimulates cardiovascular function in normal animals, causing increase in heart rate (HR) and mean arterial pressure (MAP) (6). However, the use of ketamine as a sole anesthetic has been limited by muscle hypertonicity, convulsions that occasionally occur (7) and also mild respiratory depression **(**8**).** In an attempt to counteract these undesirable effects, ketamine has been used in combination with various drugs including benzodiazepines and alpha-2 agonists. A combination of ketamine with xylazine was long used for anesthesia in veterinary practice. Xylazine is an α_2-agonist that has been reported to confer analgesic, sedative and muscle relaxant actions in many species including rabbits (9). However, mild respiratory depression occurs and may be attributed to the use of xylazine (10). If it is used for surgery longer than 20 minutes, animals will likely require additional anesthetic. Re-dosing with ketamine rather than the combination is usually safer, as the cardiovascular depression of xylazine is often longer-lasting than the sedation or analgesia produced (11). Thus, other drug combinations should be considered. Midazolam is a benzodiazepine with sedative, amnesic, anxiolytic, muscle-relaxant and anticonvulsant properties. Midazolam has a faster onset but shorter duration of action than other benzodiazepines (12). To the best of our knowledge, the combination of ketamine-midazolam-fentany (K-M-F) has never been used in rabbits. We assumed that replacement of xylazine by fentanyl will provide several advantages compared with the previously used combinations. Fentanyl is short-acting synthetic opiate agonist which has potent analgesic properties. It causes only minimal changes in circulatory variables although it can cause marked respiratory

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depression in large dose (13). Fentanyl reduced the time needed for midazolam to induce anaesthesia (14) Dose-response studies have found combinations of midazolam with fentanyl (15) to be synergistic. In the present study, we designed a randomized controlled trial to evaluate the effect of addition of fentanyl to ketamine-midazolam compared with other drug combinations in a New Zealand White (NZW) rabbit.

MATERIALS AND METHODS

Experimental animal

Seventy two healthy male NZW rabbits weighed 2.5-3 kg aged were used. They were kept in a controlled environment in the Animal Research Facility Unit of Mansoura Urology & Nephrology Centre. The rabbits were fed standard pelleted rabbit food and had free access to tap water. One day before the experiment, each animal was weighed. Physiological variables [Respiratory rate (Rs.R.), heart rate (H.R.) and rectal temperature (R.T.)] and reflexes [Ear pinch, pedal withdrawal and righting reflexes (R.R.)] were evaluated. Only clinically healthy animals were accepted to pass the study. The protocol was approved by the ethical committee of Mansoura Faculty of Veterinary Medicine.

Preanesthetic preparation

All anesthetic procedures were performed between 9 am – 2 pm. EMLA cream (EMLA; AstraZeneca, Luton, UK) was applied on left ear, a venous line (24-SWG cannula) was inserted in the auricular vein for fluid therapy administration (lactated's Ringer solution at a rate of 10 ml/kg/hr intra-operative). While, another venous line was inserted in auricular vein of right ear to collect venous samples for estimation of serum glucose and cortisol levels at the start and termination of the experiment. Also, an arterial line (22-SWG cannula) was inserted in the central auricular artery for collection of arterial blood samples for estimation of blood gases. The selected reflexes (Righting, ear pinch and pedal withdrawal reflexes) and physiological variables (Rs.R. and H.R.) were recorded as a baseline before anesthetic administration (time = 0 minute) followed by injection of anesthetics which were served in one syringe.

Study design

The animals were assigned into three groups (n=24 rabbits) by blocked randomization into: 1) K-X group: received a combination of ketamine (50 $mg.kg^{-1}$) and xylazine (10 $mg.kg^{-1}$) (16); K-X-M group: received a combination of ketamine (35 $mg.kg^{-1}$), xylazine (5 mg kg^{-1}) and midazolam (1 $mg.kg^{-1}$) (17); and 3) K-M-F group: received a combination of ketamine (35 $mg.kg^{-1}$), midazolam (1 $mg.kg^{-1}$) and fentanyl (0.02 $mg.kg^{-1}$) (18).

Assessment of sleep time and surgical anesthesia

The spontaneously breathing animal was then positioned on the operating table and rabbit was gently tilted every 30 seconds to assess the time of loss of righting reflex at which sleep time starts (= No ability to regain sternal recumbency after positioning on back) (19). Surgical anesthesia (S.A.) was judged to be present if there was an absence of response to ear pinch (absence of reaction to clamping the ear margin) and absence of pedal withdrawal reflex (absence of pelvic limb withdrawal on clamping the inter-digital space between third and fourth digits) (18). All rabbits underwent the same surgical procedure in the form of abdominal incision, ligation of the left ureter then closure of the abdominal incision. Local anesthetic xylocaine 2% (5$mg.kg^{-1}$) was injected subcutaneously in site of incision.

Collection of samples and Physiological observations

Arterial blood samples were withdrawn two times (10 and 30 minutes post injection of anesthetic combination) for blood gas analysis (pH, HCO_3, Pa CO_2 and Pa O_2) using ROCHE OMNIS GAS ANALYZER.Venous blood samples were withdrawn two times (before the injection of anesthetics and after the surgical procedure) the blood sugar was measured by ACCU-CHEK Active and cortisol was detected by enzyme linked immunosorbant assay (ELISA) technique. Physiological variables and reflexes were recorded at t_0 and every 15 minutes throughout the period of experiment (2 hours). A pulse oximeter was used to measure heart rate. Respiratory rate (Rs.R) was counted by thoracic movements.

Post-anesthetic care

For post operative pain Buprenorphine 0.02 $mg.kg^{-1}$ SC t.i.d / 3 days post-operative was used. The drug was used following full recovery of the animal (45 - 120 minutes post-operative) from anesthetic regimen. The analgesic therapy must be delayed to avoid interaction with drugs used in anesthesia for fear of CNS or respiratory depression (20).

Statistical analysis

Blood gases and clinical observation data were analyzed to ascertain whether the changes occurred were significant using One-Way Analysis of Variance (ANOVA). If there was a significant difference, multiple comparisons vs. K-X group (Tukey's method) were done. A t-test was used to determine whether mean values at selected sample times differed between groups. The results are expressed as the mean (SEM) and considered significant when $P < 0.05$.

RESULTS

Surgical anesthesia & sleep time

Regarding the anesthetic quality provided by the three used combinations, surgical anesthesia (indicated by loss of ear pinch and pedal withdrawal reflexes) was poorest in K-M-F group (12 out of 24 rabbits) starting significantly later (43.85 ± 12.94 min.) and lasting significantly shorter duration (34.38 ± 35.79 min.) when compared with the other two groups. On the other hand, surgical anesthesia showed no statistical difference between K-X and K-X-M regarding the onset 26.25 ± 12.71min, 25.0 ± 9.56 min respectively and duration (80.63 ± 24.95 min, 83.75 ± 20.23min) respectively of surgical anesthesia. The contrary was true for sleep time that is calculated by the loss of righting reflex. Sleep time in K-M-F group started significantly earlier (3.13 ± 7.64 min.) and lasted significantly longer in duration (116.88 ± 7.64 min) than the other two groups. No statistical difference existed between K-X and K-X- M groups for onset of sleep time (10.0 ± 8.47 min) (9.38 ± 8.64 min) respectively or for duration of sleep time (109.38 ± 9.36min) (110.0 ± 9.56 min).

Physiological variables (HR and Rs.R.)

Regarding the physiological variables: All used combinations produced gradual decrease in heart rate that was almost continuous throughout the whole period of experiment from t_0 to t_{120} (figure 1a). This decrease was insignificant in rabbits anesthetized with K-M-F in contrast to the other two groups which showed significant bradycardia (K-X from 219 ± 15.66 to 173.63 ± 29.86 beats/min. and K-X-M from 219.72 ± 14.34 to 188.16 ± 23.14 beats/min.) from t_0 to t_{120} respectively. Changes in respiratory rate (Rs.R.) produced by the three anesthetic combinations were insignificant when t_0 was compared with t_{120}. However, for all groups, these changes took a characteristic pattern (Figure 1b). At first, Rs.R. decreased to reach the lowest level between t_{15} and t_{30}. Rs.R. of K-M-F was significantly lowest when compared with K-X and K-X-M. At t45, Rs.R. of all groups started to increase again steadily but K-M-F group is still significantly the lowest group. Final values of Rs.R. showed no significant difference between all groups.

Blood gas analysis

In all groups, $paCO_2$ values 10 minutes after injection of the pre-anesthetic (t_{10}) was within the physiological range for healthy rabbits.

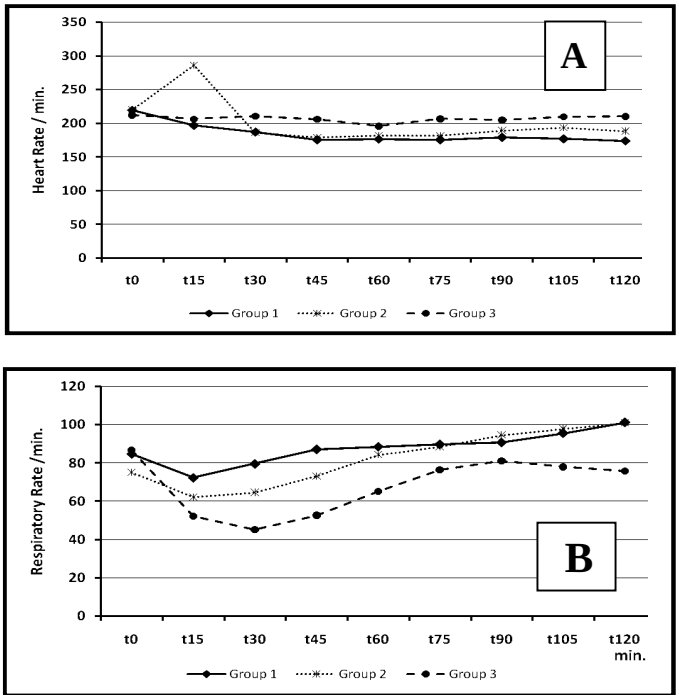

Fig.1. Mean heart rate (A) and Mean respiratory rate (B) in rabbits receiving one of three anesthetic combinations; Group 1 (K-X), group 2 (K-X-M) and group 3 (K-M-F). K-X = Ketamine-Xylazine, K-X-M = Ketamine-Xylazine-Midazolam, K-M-F = Ketamine-Midazolam-Fentanyl

However, values recorded 30 minutes after injection of anesthesia (t_{30}) showed significant increase from its t_{10} value. This elevation was the significantly highest in K-M-F group and lowest in K-X-M group when compared with K-X group. Consistent with this data, significant compensatory elevation of HCO_3^- can be noticed at t_{30} in all the experimental groups when compared with t_{10} values. This elevation was significantly highest in K-X group and lowest for K-M-F group. The recorded values of pH were corresponding to values of $paCO_2$ and HCO_3^- suggesting compensated respiratory acidosis. Although t_{30} values for pH in all groups showed significant decrease when compared with t_{10} values, all values whether t_{10} or t_{30} lies within physiological range (7.35 – 7.44). Values of paO_2 recorded at t_{10} were within the physiological range for the conscious rabbits (85-102 mmHg). Injection of anesthesia was associated with significant reduction of paO_2 (hypoxia) at t_{30}. The hypoxia was more prominent in K-X-M group and K-M-F whereas was slightly less prominent in K-X group.

Plasma glucose and cortisol

The three anesthetic combinations significantly increased plasma glucose and cortisol levels (Table 2) two hours after their injection when compared with that of the control. The elevation of blood glucose produced by K-X group was significantly highest when compared with the other 2 groups whereas no statistical significance existed among the three groups when serum cortisol level was regarded.

DISCUSSION

The margins of safety between anesthetic and lethal doses in rabbit are less than those found in other animal and there is wide individual variation in response to anesthetic and ancillary agents. Also, rabbit has strong reflexes which are difficult to suppress during general anesthesia (21). Establishment of safe but effective anesthetic combination was our goal in this study. The present study showed that surgical anesthesia was prolonged in K-X (80.63± 24.95 min.) and K-X-M (83.75± 20.23 min.) groups in contrast to K-M-F group that failed to produce such prolonged anesthesia. In the current study, sleep started significantly earlier and lasted longer duration in K-M-F group when compared with the other two groups. This may be due to xylazine (α_2 adrenoreceptor agonist) which induce sedation, decreased locomotor activity and suppressed conditioned reflexes (22), beside central inhibition of parasympathetic tone and/or direct stimulation of α_2 adrenoceptors located in C.N.S (23). In contrast, fentanyl (Mu receptor agonist) results in rapid onset of analgesia (1 to 5 min) but of short duration (less than 1 hr) (24). Respiratory depression was noticed in the three regimens tested between 15- 30 min after each regimen administration, with the most prominent effects in K-M-F group. The induced respiratory depression was associated with parallel changes in all arterial blood-gas variables in the form of hypoxia ($\downarrow PaO_2$), (hypercapnia $\uparrow$ $PaCO_2$) with compensatory increase in HCO_3^-. The cause for hypoxia and hypercapnia may be respiratory depression which was more prominent in the presence of fentanyl (25). On the other hand, bradycardia in rabbits anesthetized with K-M-F was insignificant when compared with the other two groups. Whereas xylazine is known to produce marked bradycardia, fentanyl produces a dose related suppression of HR (26). Consistent with their role as markers of stress, glucose and cortisol, increased in all groups. Elevated blood glucose and serum cortisol was insignificant in K-M-F group. On the other hand, hyperglycemia was significant in K-X group when compared with other groups. This acute hyperglycemia was explained largely by xylazine component of the combination (27). Finally, our study pointed that although the new combination of ketamine-midazolam-fentanyl has not added much to anesthetic quality but it may has a privilege in anesthesia of circulatory compromised cases.

Table 1. The effects of the tested anesthesia 30 minutes after injection (t_{30}) on NZW rabbit blood gases variables

Item	K - X		K - X - M		K - M - F	
	10 min	30 min	10 min	30 min	10 min	30 min
pH	7.44 ± 0.01	7.37 ± 0.06 *	7.42 ± 0.03	7.37 ± 0.04 *	7.42 ± 0.06	7.35 ± 0.07 *
HCO_3^-	22.48 ± 3.34	28.63 ±5.58 *	22.94 ± 2.27	24.89 ± 2.97 *	22.56 ± 4.47	23.85 ± 3.93
P CO_2	31.57 ± 2.99	39.76 ± 5.3*	36.37 ± 3.6	42.94 ± 3.07 *	35.53 ± 3.74	45.51 ± 12.38*
P O_2	89.1 ± 8.45	75.8 ± 7.67 *	87.76 ± 1.38	72.41± 3.9 *	89.68 ± 1.33	74.61 ± 5.12 *

pH, bicarbonate concentration (HCO_3^-), carbon dioxide partial pressure ($PaCO_2$), and oxygen partial pressure (PaO_2) compared to 10 minutes after pre-anesthetic medication (t_{10}) (Mean ± SD). * = significant when compared with t_{10} subgroup. K-X = Ketamine-Xylazine, K-X-M = Ketamine-Xylazine-Midazolam, K-M-F = Ketamine-Midazolam-Fentanyl.

Table 2. The effect of (K – X) , (K- X- M) and (K- M- F) on blood glucose and serum cortisol two hours from imtramuscular injection in NZW rabbit

Item	K - X		K - X - M		K - M - F	
	Basal*	Post-2 hours	Basal*	Post-2 hours	Basal*	Post-2 hours
Cortisol (mg/dl)	49.8 ± 1.07	51.32 ± 1.02 [a]	49.74 ± 1.08	50.96 ± 1.05 [a]	49.64 ± 1.2	51.04 ± 1.27 [a]
Blood sugar (mg/dl)	115.73 ± 21.16	279.47 ± 15.52 [a]	117.85 ± 20.87	229.56 ± 12.37 [a,b]	116.88 ± 14.89	241.36 ± 14.33 [a,b]

a = significant when compared with 10 minutes subgroup (significant at $p \leq 0.05$), b = significant when compared with other groups post 2 hours (significant at $p \leq 0.05$), Results are expressed as (mean ± SD).

Also our study was limited by the absence of analgesia assessment score which serve as a surrogate for pain assessment, lack of cytokine measurement (interleukins 1and 6; tumor necrosis factor) which are released at the site of injury and better serves as markers of the extent of tissue injury , extension of serum cortisol or blood sugar measurement the first 24 hours post operative at least to observe the diminution in post opertative serum cortisol and blood sugar and calculate first requirement of additional analgesics.

Conclusion

The addition of fentanyl to ketamine-midazolam may be advantageous in anesthesia of circulatory compromised cases. Otherwise, other anesthetic combinations should be considered.

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