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ASSESSMENT OF THE EFFECT OF DIFFERENT ANAESTHETICS AND PAIN RESPONSE AFTER LAPAROTOMY IN RABBIT

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ABSTRACT
This study was carried out for the assessment of various anesthetic combinations on pain management after laparotomy and to find out the best combination of premedication and anesthetic drugs. Fifteen rabbits of 5-6 months weighing 1.5-2.0 kg in average were allocated into three equal groups. The treatment groups were the Midazolam-Ketamine-Xylazine (MKX), Midazolam-Acepromazine-Ketamine (MAK) and Acepromazine-Diazepam-Fentanyl (ADF). The levels of analgesia and wound tenderness were assessed by a dynamic interactive visual analog scale (DIVAS). Parametric variables were analyzed using one-way ANOVA in the appropriate cases. Postoperative pain and wound tenderness scores increased significantly ($p<0.01$ and $p<0.05$) irrespective of groups. Pain decreased significantly ($p<0.01$ and $p<0.05$) in MKX group compared to other two groups. Similarly, wound tenderness decreased significantly ($p<0.01$) in MKX group at each observation compared to other two groups (MAK and ADF). Midazolam-Ketamine-Xylazine (MKX) combination has significantly ($p<0.01$) lower induction period, higher duration of anesthesia and rapid recovery. It was demonstrated that the Midazolam-Ketamine-Xylazine group provided excellent analgesia compared to MAK and ADF.

1. INTRODUCTION

Surgery is an important practice intended for the correction of abnormalities or treatment of diseases. In the case of surgical procedures, some degrees of postoperative pain will be present (Lascelles et al., 1998). Laparotomy is the most common surgical procedure performed with the aim of getting information that is not available via clinical and diagnostic methods. The word laparotomy explains the exploration of the abdomen and proceeds further according to the cause identified (Natalie and Semchyshyn, 2009). General anesthesia is required for laparotomy procedure. General anesthesia in rabbits can be induced by using a variety of drugs and techniques of injection. To produce all the required features of general anesthesia combination of two drugs or more can be given. For successful surgical procedures balanced anesthesia is

mandatory with the aim to achieve desirable hypnosis, analgesia and muscle relaxation.

Very high peri-anaesthetic mortality (1 in 72) has been reported in rabbits (Brodbelt et al., 2005). The reason for high mortality in this species may be due to the relatively small size and high metabolic rate (Grint and Murison, 2008). Rabbits are hindgut fermenters and may be prone to gut stasis during and after anesthesia if adequate analgesia is not provided (Stasiak et al., 2003). Many investigators have studied the effects of different anesthetic drugs in rabbits but there appears to be increasing popularity of ketamine-based combinations for rabbit anesthesia, considering the safety and good analgesic properties of the drug (Grint and Murison, 2008). Thus it is a crying need to explore suitable anesthetic combinations for surgery in rabbits. Midazolam is a short-acting benzodiazepine with a hypnotic, muscle relaxant, anticonvulsant and anxiolytic properties. Ketamine is a phencyclidine derivative that produces a dissociative state of anesthesia. Ketamine can be considered as the most versatile drug which is used for anesthesia and many other medicine (Jonkman et al., 2017). As a dissociative anesthetic, ketamine induces deep analgesia with amnesia (Sean et al., 2016). Xylazine, 2-(2,6-dimethyl phenyl amino)-H-5, 6-dihydrochloride, a potent, non-narcotic, sedative, analgesic and muscle relaxant. Xylazine induces respiratory depression, bradycardia, bradyarrhythmias and hypotension in most animals depending on species and dose (Short, 1987).

Acepromazine, a phenothiazine derivative, is a potent neuroleptic agent with relatively low toxicity which produces mild to moderate tranquilization, muscle relaxation and a decrease in spontaneous activity attributable principally to central dopaminergic antagonism (Vesal et al., 2011). Diazepam is the oldest derivative of 1,4 benzodiazepines (BD), with a high therapeutic index and many properties such as: anxiolytic (Wilson et al., 2004; Loiseau et al., 2003), myorelaxant (Sheriff et al., 1994), sedative and anticonvulsant.

Fentanyl citrate, a highly potent synthetic μ agonist, rapidly crosses the blood-brain barrier which produces rapid onset and short duration of action. Due to different routes of administration fentanyl is a good choice in emergency situations (Ducharme, 2016). The current attitude about animal welfare has increased the importance of pain management in a rabbit. Attitudinal changes toward animal suffering have necessitated an understanding of pain modulation by the veterinarian and willingness of the owners to incur extra cost in order to comfort animals (George, 2003). Therefore, the present study was designed to evaluate various anesthetic combinations on pain management after surgery and to assess the best anesthetic combinations.

2. MATERIALS AND METHODS

The study was designed to assess the effect of different anesthetics and pain responses after laparotomy in a rabbit. To complete the research work following steps were followed-

2.1 Study Period and Area

The study was conducted over a period of six months from July-December, 2018 under the Department of Medicine, Surgery and Obstetrics, Faculty of Veterinary and Animal Science, Hajee Mohammed Danesh Science and Technology University, Dinajpur, Bangladesh.

2.2 Selection of Animals

Fifteen (15) adult healthy rabbits of 5-6 months weighing 1.5-2.0 kg in average were randomly assigned to three treatment groups i.e. Group I, Group II and Group III, each group having five (05) rabbits. All of the experimental rabbits were healthy, disease-free and physiologically sound.

2.3 Experimental design

The animals were divided equally into three groups as Midazolam-Ketamine-Xylazine (MKX), Midazolam-Acepromazine-Ketamine (MAK) and Acepromazine-Diazepam-Fentanyl (ADF).

Sl. No	Groups	No of Replicates	Drugs	Dosage (per kg b.wt.) and route
1.	I	5	Midazolam + Ketamine + Xylazine	2 mg/kg b.wt. IM + 35 mg/kg b.wt. IM + 5 mg/kg b.wt. IM
2.	II	5	Midazolam + Acepromazine + Ketamine	2 mg/kg b.wt. IM + 1 mg/kg b.wt. IM + 50 mg/kg b.wt. IM
3.	III	5	Acepromazine + Diazepam + Fentanyl	1 mg/kg b.wt. IM + 2.5 mg/kg b.wt. IM + 20 µg/kg b.wt. IM

2.4 Anaesthetic procedure

Before proceeding with the anesthetic procedure, the base parameters were recorded. The rabbits were subjected to the following three treatments. Group I- Animals were premedicated with midazolam (Hypnofast®, Incepta Pharmaceuticals Ltd.) @ 2 mg/kg body weight intramuscularly, after 15 minutes ketamine (Ketalar®, Pfizer Ltd.) was given @ 35 mg/kg body weight intramuscularly where xylazine (Xylaxin®, Indian Immunologicals Ltd.) was injected intramuscularly @ 5 mg/kg body weight immediately after ketamine administration. Group II- Rabbits were premedicated with similar drug used in Group I. After premedication, rabbits were injected with ketamine (Ketalar®, Pfizer Ltd.) @ 50 mg/kg body weight immediately followed by acepromazine (Combistress®, Kela N.V., Belgium) @ 1 mg/kg body weight. Both ketamine and acepromazine were administered intramuscularly. Group III- In this group acepromazine (Combistress®, Kela N.V., Belgium) was used @ 1 mg/kg body weight. After 4 minutes, diazepam (Diazepam®, Wockhardt UK Ltd.) was injected @ 2.5 mg/kg body weight. Then rabbits were injected with fentanyl @ 20 µg/kg body weight. All three agents, acepromazine-diazepam-fentanyl, were administered intramuscularly.

2.5 Assessment of clinical parameters

Required parameters were recorded after premedication and induction of anesthesia. The clinical parameters that were assessed after different treatments are- a) Onset of sedation/analgesia, b) Degree of analgesia, c) Duration of anesthesia, d) Complete recovery, e) Pain response: Pain response was measured using dynamic interrupted visual analog pain scale (DIVAS). f) Wound tenderness, g) Complications if any.

DIVAS

	Before Surgery		
Behavioral Category	Expression	Score	Remarks
Posture	Normal Relaxed	0	
	Rigid	1	
	Tense	2	
	Praying Position	3	
Locomotion	Comfortable	0	
	Awake Anxious	1	
	Restless	2	
	Thrasing	3	
Attention to wound	Ignoring	0	
	Licking	1	
	Chewing	2	
	Self mutilation	3	
Wound Palpation	No Response	0	
	Looking	1	
	Tries to bite	2	
	Pulling	3	
Physiological change	Normal RR or HR	0	
	Increase by 50%	1	
	Increase by 100%	2	
Total			

2.6 Assessment of physiological parameters

Physiological parameters viz. rectal temperature, heart rate, respiration rate, were studied at 0 min before premedication, 10 min after premedication and at 10, 20, 30, 40, 50 and 60 minutes post anesthesia.

2.7 Statistical analysis

Statistical analysis of parametric data was performed using one-way analysis of variance (ANOVA) followed by Duncan's test when appropriate. A repeated measure analysis of variance with time and treatment as factor were used to compare physiologic values. Pain scores were analyzed with the help of dynamic interrupted visual analog pain scale (DIVAS). All results were expressed as mean ± SEM and differences were considered significant at $p<0.05$ and $p<0.01$. Statistical analyses were performed using SPSS Version 20.0.

3. RESULTS

3.1 Heart rate, respiration rate and rectal temperature

The mean value of heart rate (HR), respiration rate (RR) and rectal temperature (RT) was 238.8-267.0/min, 47.8-66.8/min and 100.7°F-103.4°F respectively.

3.2 Onset of analgesia, duration of anesthesia and complete recovery

Onset of analgesia, duration of anesthesia and complete recovery were presented in Table 1. Rapid onset of analgesia was found in MKX group (4.23±0.67 min) than other two groups. Duration of anesthesia was

longest in MKX group (71.35±3.98 min) and shortest in ADF group (42.45±2.29 min). Complete recovery from anesthesia was faster in MKX group (44.13±2.58 min).

3.3 Dynamic interactive visual analogue scale (DIVAS)-pain scores

There were significant differences in DIVAS pain scores at each assessment time with low values for pain occurring at 0.5 hours and gradually increasing through other observation periods and reached the peak at 1.0 hours postoperatively in all the groups (Table 2). The pre-operative pain scores were 0 in all groups. There were significant differences ($p<0.01$) among the groups in terms of pain score after an operation in each assessment except 10 minutes. In MKX group, DIVAS-pain scores were (in millimeter) 5.61±0.13, 11.59±0.34, 14.35±0.32, 19.51±0.65 and 27.27±1.07 at 20 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes after operation, respectively (Table 2). Comparatively, MKX treated rabbits had significantly ($p<0.05$) lower pain score after surgery than MAK and ADF treated rabbits. In case of MAK treated rabbits, DIVAS-pain scores were 10.99±0.47, 13.80±0.46, 18.00±0.37, 25.57±0.99 and 31.70±0.64 at 20 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes post-operatively, respectively (Table 2). The MAK group had a significantly ($p<0.01$) higher pain score than MKX but significantly ($p<0.01$) lower pain score than ADF group. In ADF group, DIVAS-pain scores were 13.72±0.32, 17.32±0.50, 23.01±0.68, 31.25±0.95 and 39.93±0.97 at 20 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes after operation, respectively (Table 2) and were significantly higher ($p<0.01$) than the rest of the treatment groups. This may reflect the idea that pain started after the animal become conscious from the general anesthesia and the local analgesic used was short-acting.

3.4 Dynamic Interactive Visual Analogue Scale (DIVAS)-wound tenderness score

Table 3 shows the wound tenderness scores of Midazolam-Ketamine-Xylazine (MKX), Midazolam-Acepromazine-Ketamine (MAK) and Acepromazine-Diazepam-Fentanyl (ADF) at individual assessment time. There was an increase in wound tenderness scores across each time among MAK, ADF and MKX groups with a low value occurring at 10 minutes and gradually increasing through other assessment time and reached the peak at 1.0 hour postoperatively. DIVAS wound tenderness at the preoperative period was 0 in all the treatment groups. At 20, 30, 40, 50 and 60 minutes after operation, MKX group had significantly ($p<0.01$) lower wound tenderness scores than each of MAK and ADF groups.

4. DISCUSSIONS

There were no significant differences among groups regarding heart rate, respiration rate and rectal temperature. These parameters were within the normal physiological ranges. This is in agreement with the findings of Wyatt et al., (1989) who did not find any significant changes in body temperature after ketamine-xylazine administration in a rabbit.

Induction time, duration of anesthesia and recovery time varied significantly ($p<0.01$) among the groups. MKX group required less induction time having a higher duration of anesthesia and rapid recovery than other groups. This may be due to use of a high dose of midazolam and synergistic inhibition effects of CNS mediated by effect of combination induced by deep sedative effect of xylazine and midazolam and anesthetic effects of ketamine. Rapid recovery in MKX was due to midazolam which caused smooth recovery when used in combination with ketamine as half-life of midazolam is longer than ketamine (Stegmann and Bester, 2001). Ketamine induces bronchodilation status, allowing for most secure induction of anesthesia in patients with life-threatening asthma and acute bronchial constriction (Gao et. al., 2016). Fentanyl has a rapid onset of action of 2-3 minutes, a short duration of action of 60 minutes with minimal

hemodynamic effects (Anand, 2017). It is widely used to provide rapid short-lived pain relief during surgery. But like other opioids, fentanyl have a wide variety of side effects, including nausea, dizziness, constipation, weakness, hypopnea, respiratory depression, etc., which limits their clinical application (Cao et al., 2017; Imam et al., 2018). Gupta et al., (2019) found that the intravenous administration of fentanyl analogs can cause varying degrees of fentanyl-induced cough in some patients (up to 65%).

The result of this study demonstrated that Midazolam-Ketamine-Xylazine (MKX) group had significantly ($p<0.05$) lower pain scores compared to Midazolam-Acepromazine-Ketamine (MAK) and Acepromazine-Diazepam-Fentanyl (ADF) groups at each postoperative assessment time. The present study is in agreement with the study of Kehlet and Dahl (1993) & Slingsby and Waterman-Pearson (2001) who suggested a multimodal or balanced analgesic agent for the best pain control. Ali (2013) also stated that, increase in time of anesthesia and lower pain score may be due to use of a high dose of midazolam and synergistic inhibition effects of CNS mediated by effect of combination induced by deep sedative effect of xylazine and midazolam and anesthetic effects of ketamine. Ketamine exerts a pain-modifying effect via its N-methyl-D-aspartate receptor antagonist actions (Richebe et al., 2005). Subanesthetic ketamine constant rate infusion (CRI) in humans prevents pain and has antihyperalgesic and antiallodynic effects (Bell et al., 2006). It is also reported that ketamine is especially preferred for patients with high pain scores, such as surgery patients who experience high levels of postoperative pain (Bell et al., 2018). Combination of acepromazine with diazepam and fentanyl have a little analgesic effect (Kaemi et al., 2002) leading to higher pain scores as fentanyl requires a long period for induction of anesthesia.

Overall, the pain scored was not particularly high in this study. The pain score was an average in of 39.93 out of 100 on the DIVAS in Acepromazine-Diazepam-Fentanyl (ADF) group. The DIVAS pain score was subjective but would fall about halfway between ‘no pain’ and ‘worst imaginable pain’ so it might be considered as moderate pain. This moderate level of pain corresponds with the expected findings of Zimmerman (1986) who reported that there was a mild to moderate level of pain after abdominal surgery in farm animals. The responsive and interactive behaviors after surgical wound palpation were recently considered a vital determinant for assessing the degree of postoperative pain. Here the DIVAS was used which differs visual analogue scale (VAS) only due to incorporated behaviours (Hashim, 2004). The responsive of interactive behavioral manipulation of surgical wound in the present study varied according to the severity of pain and the most dominant are hereby include biting of the wound, painful crying, falling on the ground on the opposite side of the wound, regaining of the standing position and posture. These findings were consistent with the result of Wright and Woodson (1990). In this study, Wound tenderness was significantly lower (($p<0.01$) in Midazolam-Ketamine-Xylazine (MKX) group compared to MAK and ADF groups. This is an aggrement with the findings of Pratap et al., (1997) and Kumar et al., (1999).

5. CONCLUSIONS

All three anesthetic combinations have effects on physiological and clinical parameters in various degrees. Midazolam-Ketamine-Xylazine (MKX) combination has significantly lower induction period, higher duration of anesthesia and rapid recovery. MKX has also significantly lower pain score and wound tenderness compared to remaining two combinations. Pain management after surgery is more likely to be achieved from multimodal approach by using Midazolam-Ketamine-Xylazine. In this study we use some anesthetic agents but there are more drugs available in the market which can be used for further research. Therefore, further study is recommended using other anesthetic agents for a multimodal approach to find

out more anesthetic combinations.

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Conflict of interests

The authors declare no conflict of interest.

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TABLES

Table 1. Onset of analgesia, duration of anesthesia and complete recovery in various groups

Groups (n=5)	Onset of analgesia (min)	Duration of anesthesia (min)	Complete recovery (min)
MKX	4.23±0.67[a]	71.35±3.98[c]	44.13±2.58[a]
MAK	9.20±0.69[b]	54.66±3.26[b]	81.37±3.13[b]
ADF	13.17±1.75[c]	42.45±2.29[a]	111.15±4.87[c]
Level of significance	**	**	**

n = Number of rabbits in each group, [a, b, c] Mean values with different superscripts within the same column differ significantly (p<0.01); ** = Highly significant (p<0.01), MKX = Midazolam-Ketamine-Xylazine, MAK = Midazolam-Acepromazine-Ketamine and ADF = Acepromazine-Diazepam-Fentanyl.

Table 2. Pre- and post-operative DIVAS-pain scores in different groups

Groups (n=5)	Pain scores (millimeters) (Mean±SEM)							
	Premedication		Post operation					
	Before	After 10 minutes	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
MKX	00±00	00±00	5.26±0.11	5.61±0.13[a]	11.59±0.34[a]	14.35±0.32[a]	19.51±0.65[a]	27.27±1.07[a]
ADF	00±00	00±00	6.18±0.14	13.72±0.32[c]	17.32±0.50[c]	23.01±0.68[c]	31.25±0.95[c]	39.93±0.97[c]
MAK	00±00	00±00	5.81±0.28	10.99±0.47[b]	13.80±0.46[b]	18.00±0.37[b]	25.57±0.99[b]	31.70±0.64[b]
Level of significance	NS	NS	NS	*	**	**	**	**

n = Number of rabbits in each group, [a, b, c] Mean values with different superscripts within the same column differ significantly (p<0.05 and p<0.01); ** = Significant at p<0.01, * = Significant at p<0.05, MKX = Midazolam-Ketamine-Xylazine, MAK = Midazolam-Acepromazine-Ketamine and ADF = Acepromazine-Diazepam-Fentanyl.

Table 3. Pre- and post-operative DIVAS-wound tenderness in different groups

Groups (n=15)	Wound tenderness (millimeters) (Mean ± SEM)							
	Premedication		Post operation					
	Before	After 10 minutes	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
MKX	00±00	00±00	3.56±0.51	4.29±0.51[a]	9.79±0.79[a]	12.01±0.61[a]	16.01±0.99[a]	19.24±1.57[a]
ADF	00±00	00±00	5.16±0.39	10.16±0.31[c]	17.04±1.04[c]	21.05±1.19[c]	24.30±1.35[c]	28.80±1.27[c]
MAK	00±00	00±00	4.36±0.61	7.14±0.50[b]	12.84±0.91[b]	16.03±0.64[b]	21.13±0.70[b]	23.08±0.47[b]
Level of significance	NS	NS	NS	**	**	**	**	**

n = Number of rabbits in each group, [a, b, c] Mean values with different superscripts within the same column differ significantly ($p<0.01$); ** = Significant at $p < 0.01$, MKX= Midazolam-Ketamine-Xylazine, MAK = Midazolam-Acepromazine-Ketamine and ADF = Acepromazine-Diazepam-Fentanyl.