



CLINICAL AND HEMATOLOGICAL EFFECT OF ACEPROMAZINE, MIDAZOLAM, KETAMINE AS GENERAL ANESTHETIC PROTOCOL IN RABBITS

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ABSTRACT

This study was performing to evaluate the efficiency of Acepromazine, as tranquilizer and efficiency of using mixture of midazolam combination with ketamine as a general anesthetic protocols in rabbits. Ten adult local breed rabbits from both sexes were use in this study. Animals were anesthetized by intramuscularly injection of acepromazine 0.1 mg/kg B.W. five minutes later, animals were giving mixture of midazolam 1 mg / Kg B.W and ketamine 30mg/Kg B.W, in same syringe. Physiologic parameters evaluated in zero time as control and each 15 minutes interval until 90 minutes. It was including body temperature, respiratory rate, muscle relaxation, analgesia and stages of anesthesia (Induction of anesthesia, surgical anesthesia and recovery time). Hematological was study by giving 0.5 ml from marginal ear vein for Hg, PCV, WBCs, RBC, MCHC, MCV and MCH in zero time before any injection, 1st hr after anesthesia, 2nd hr and 24 hrs. The results of this combination was provide good general anesthesia regimen with individual variation in anesthetic time with little effect on physiological parameters. Hematological results were showed marked decrease in (Red blood cells) RBC count 1st and 2nd hours, and increased in (White blood cells) WBC count 1, 2, and 24 hours after administration of acepromazine ,midazolam ,ketamine, respectively .

KEYWORDS: ACEPROMAZINE, midazolam, ketamine, general anesthesia, rabbits.

INTRODUCTION

Anesthesia is the provision of relief from pain during surgery or other procedures likely to cause pain. Rabbits are used for experimental studies in different research organizations all over the world and frequently need to be anaesthetized. However, very high peri-anaesthetic mortality (1 in 72) has been reported in rabbits^[1]. The relatively small size and high metabolic rate may be related to high mortality in this species^[2]. Furthermore, rabbits are hind gut fermenters and may be prone to gut stasis during and after anesthesia, if adequate analgesia is not provided^[3]. Many investigators have studied the effects of different anesthetic drugs in rabbits but there appears to be an increasing popularity of ketamine-based combinations for rabbit anesthesia, considering the safety and good analgesic properties of the drug^[2,4]. Acepromazine, a phenothiazine derivative, is a potent neuroleptic agent with relatively low toxicity. It induces mild to moderate tranquilization, muscle relaxation and a decrease in spontaneous activity attributable principally to central dopaminergic antagonism^[5]. Acepromazine possesses antiemetic, anticonvulsant, antispasmodic, hypotensive and hypothermic properties^[6]. It has antiarrhythmic effects and protects against adrenaline-induced fibrillation and this property must be an advantage when this drug is use for pre anesthetic medication^[7]. The benzodiazepines are popular drugs used in different animals' species. These drugs were reported to have minimum effect on respiratory system, heart rate, rectal temperature and cause good muscle relaxation and can be

used to cure convulsion. Midazolam is a short-acting benzodiazepine with hypnotic, anticonvulsant, muscle-relaxant and anxiolytic properties. In clinical practice, it is used for the induction of anesthesia^[8]. The sedative and hypnotic effects of midazolam are dose-dependent as well as dependent on route of administration. Midazolam has been widely used for premedication, for perioperative sedation, and for induction of anesthesia^[9]. Ketamine is a phencyclidine derivative that produces a dissociative state of anesthesia. Dissociative anesthesia was characterized by dissociation between the thalamo-cortical and limbic system^[10]. It produces dose-related unconsciousness and analgesia. Ketamine is often administered in combination with other drug agents such as Xylazine, diazepam, and acepromazine. Although ketamine is considered to have a wide margin of safety, it can cause significant respiratory depression when given in excessive doses or too rapidly^[11]. The purpose of this study was to determine the quality of sedation, general anaesthesia obtained after sedation of rabbits with midazolam ketamine combinations proceed by acepromazine and study the hematological effect of this combination.

MATERIAL & METHODS

The study was conducted on ten apparently healthy adult rabbits of both sexes, weighing 1.6 -2.0 kg. The rabbits were housed indoors at same condition, fed concentrated food and green grass. Before anesthesia, the animals had free access to water. Baseline data of respiratory rate and temperature were obtained prior to injection of any drugs.

Rabbits were received acepromazine as premedication at dose rate of 0.1mg/kg BW (Kella Belgium 2%). Five minutes later a mixture from midazolam (Durum, 5 mg in 1 ml, Alsaad pharmaceuticals, Syria) and ketamine (Ketamine, 50 mg in 10 ml, Alsaad pharmaceuticals, Syria) were used for induction of anesthesia at a dose rate of 1mg/kg and 30 mg/kg B.W., respectively. The rabbits were placed on an operation table after the injection of pre-anesthetic and were observed for the parameters. The time from the injection of pre-anesthetic to the onset of signs of drowsiness was recorded once weakly. The time from the injection of the midazolam- ketamine to the time when the animal was unable to attain sternal recumbency was recorded as a righting reflex. Status of the pedal reflex was recorded as intact or abolished at 3 minute intervals up to 15 minutes and each 15 minutes by applying pressure to finger and hind paw. Time of pedal reflex was recorded after loss of righting reflex. The time of loss of pedal reflex was considered as the time of onset of analgesia as mentioned by^[12]. Clinical parameter was measured at prior to injection of any drug (zero minute), and then at 15, 30, 45, 60, 75 and 90 minutes post injection of pre-anesthetic drug. Respiratory rate was measured directly by costobdaminial movement, Body temperature was recorded using a digital thermometer from rectum and heart rate was estimated by auscultation the heart by stethoscope. The nature of recovery was observed from the time of reappearance of the reflex until complete return to normal position. Hematological was estimate before injection as control, in one hour, 1day and 3 days by given 0.5 ml blood sample from marginal ear vein in vials containing EDTA as anticoagulant and the main parameters were, RBC count, WBC count, Hb concentration , PCV, Red cell indices according to^[13]. The

results were expressed as means (M) \pm stander error (SE). Parametric data were analyzed by one ways Analysis of Variance (ANOVA) continued with Least Significant Difference (L.S.D.), and $p < 0.05$ was considered significant. Statistical Package for Social Sciences (SPSS) was used (14).

RESULTS

The combination of acepromazine as premedication and the use of a mixture of midazolam and ketamine in this protocol were providing a clinically good general anesthesia. Induction occurred without excitation or apnea, it was smooth. The righting reflex was lost within 3.00 ± 1.00 minutes. The combination was provided 54 ± 5.33 minutes as anaesthetic time; the anaesthesia was optimum at 15 minutes after injection of the mixture and the pedal reflex was disappeared at this time. The recovery was smooth free of convulsion, the return of pedal reflex at the end of anesthesia was marked as time of recovery but animal at this time still at lateral recumbency for 15.00 ± 2.00 minutes, then the animal attempts to raise head while remaining quiet for 35.00 ± 5.00 minutes, after this time the animal return to sternal position. The respiratory rate was sharply decreased after the injection of midazolam and ketamine. All rabbits were showed tachypnea before induction. In addition, there were significant differences with other time to the end of recovery (Table 1). The changes in heart rate in the animals were non-significant except for a significant increase in heart rate at the 30, 45 and 60 min with other time. Temperature was show significant decrease at the level of $P < 0.05$ after anesthesia, it was continues decrease until 75 minutes than increase in 90 minutes but stall below normal.

TABLE 1: Effect of general anesthesia regime induced by acepromazine, midazolam and ketamine on clinical parameters in (10) rabbits

parameter	Time minutes						
	0	15	30	45	60	75	90
RR. rate / min	129.7 \pm 8.643A	84.8 \pm 13.499 B	52.1 \pm 7.409 C	62.4 \pm 8.516 C	66.8 \pm 8.641 C	63.8 \pm 5.623 C	84.2 \pm 9.438B C
HR Beat/ min	200 \pm 19.334 A	190 \pm 19A	159 \pm 12.378 B	154 \pm 10.211B	165 \pm 10.741AB	163 \pm 14.437AB	174 \pm 12.732A
TEMP °C	39.2 \pm 0.260 A	38.7 \pm 0.31 AB	38.5 \pm 0.78 B	38.3 \pm 0.4 B	38 \pm 0.40B	37.9 \pm 0.362B	37.9 \pm 0.27B

Different in the capital letters refers to significant differences ($P < 0.05$) between time.

HR: Respiratory rate; HR: heart rate; TEMP: body temperature

The total erythrocytes counts were significantly decrease during the 1st hr, 2nd hr after anesthesia in comparison with zero time (table, 2). The hemoglobin value did not showed any significant changes during the study. The level of packed cells volume were significant decreased during the

1 h, 2 h after anesthetic in comparison with zero time, and during the 24 h post anesthetic the level of packed cells volume showed non-significant changes in comparison with the zero time.

TABLE 2: Effect of general anesthesia regime induced by acepromazine, midazolam and ketamine on hematological parameters in (10) rabbits

Parameter	Time			
	Control	1hr	2 hr	24 hr
RBC 10^6 /ul	4.7 \pm 0.368 A	4.3 \pm 0.328 AB	4.2 \pm 0.216 AB	4.6 \pm 0.195A
WBC 10^3 /ul	6731 \pm 520 AB	6950 \pm 654 A	7637 \pm 690 A	8011 \pm 510 A
Hb g/dl	10.9 \pm 0.481	10.28 \pm 0.420	10.01 \pm 0.450	10.17 \pm 0.374
PCV%	32 \pm 1.558 A	30.1 \pm 1.328 AB	29.8 \pm 1.301 B	30.6 \pm 1.238 A
MCV Fl	71.42 \pm 6.17 A	77.82 \pm 6.49A	68.89 \pm 1.68 AB	62.66 \pm 1.94AB
MCH Pg	24.78 \pm 2.38 A	26.51 \pm 2.27 A	23.88 \pm 0.62 A	21.31 \pm 0.74 AB
MCHC g/dl	29.43 \pm 0.14	29.32 \pm 0.10	28.86 \pm 0.31	29.23 \pm 0.25

Different in the capital letters refers to significant differences ($P < 0.05$) between time.

Total white blood cells count was increased after 1, 2 and 24 hours in comparison with zero time. The mean corpuscular volume was significantly decreased at a level of ($p < 0.05$) at the time 24 h post anesthetic in comparison with 2 hr post anesthetic. The mean corpuscular hemoglobin was significantly decreased at a level of ($p < 0.05$) in comparison between the level of 1 h, 2 h post anesthetic. The corpuscular hemoglobin concentration did not showed any significant changes.

DISCUSSION

The present mixture caused delay in down time and time to loss of righting reflex^[2]. The increase in time of anesthesia by this combination may be due to the synergistic inhibition effects of CNS mediated by effect of combination induced by deep sedative effect of acepromazine and midazolam and anesthetic effects of ketamine. The increase in the recovery time may be due to use of high dose of acepromazine and the sedative and hypnotic effects of midazolam^[12]. Midazolam was caused smooth recovery when use in combination with ketamine because its half-life longer than ketamine^[15]. Although midazolam has the shortest recovery, profile compared with other benzodiazepines^[16]. The first 3 hours of termination of the anaesthesia was the most common time for rabbit die^[17]. For all that combination of acepromazine, midazolam and ketamine was safe with a documented no death of any animal. The result of respiratory rate was agreed with^[18]. Tachypnea may be ascribed to frightening and excitement of the animal; these phenomena caused elevation in the respiratory rate when compared with normal. All anaesthetic techniques commonly used in rabbit anaesthesia depress ventilation^[2]. In addition, midazolam causes bradypnea when associated with ketamine because this drug depresses the respiratory centers of the brain^[19]. According to Van Praag^[20] and Flecknell^[21] depression of respiration below 30 breaths/min was leads to hypoxia, hypercapnea, acidosis and mortality. No mortality was recorded during this study, probably because the experimental rabbits were healthy and did not undergo surgery. The result of heart rate was agrees with Amarpal *et al.*, 2010^[12] we reported that using mixture of acepromazine and ketamine was little effects on heart rate. In addition, midazolam has mild cardiovascular effect^[6]. In general, anaesthesia was causes decrease in body temperature due to reduction in metabolic rate and limited movement of animal^[22]. However' mild hypothermia in rabbits is improves survival and cardiac systematic function and protects lung from injury^[23]. The result of total erythrocytes counts, heamoglobin and packed cells volume can be explained by the alpha-adrenergic blocking effect of acepromazine which might cause relaxation to the spleen and consequently led to splenic sequestration of erythrocytes^[24]. Pact cell volume PCV decreased over time after the administration of acepromazine^[25,26]. Hematological parameters including RBC, PCV and Hb were lower in blood samples collected from skunks anesthetized with the combination of aceprazine / ketamine^[27]. The result total white blood cells count was agreed with^[28] who reported an increase in the WBC of rabbits after intra peritoneal injection of amylobarbitone sodium. Total and

differential leukocyte counts may be altered by stress events, such as capture and handling. Epinephrine-induced physiologic leukocytosis in ruminants is characterized by neutrophilic leukocytosis with increases in lymphocytes and monocytes with a mild eosinopenia. Corticosteroid-induced changes include amature neutrophilia, lymphopenia, eosinopenia, and monocytosis^[29]. In domestic animals, neutrophilia and lymphopenia peaks appear after 4–8 hr of exposure to stress^[24,30]. The observed changes in the erythrocyte indices of rabbits are consistent with finding of previous research (31) we suggested that acepromazine reduction in PCV and RBC of rabbits.

CONCLUSION

The results of the present study suggested that the acepromazine, midazolam and ketamine combination could be useful for sedation, muscle relaxation, premedication and general anesthesia in healthy rabbits and letal effect on hematological parameter.

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