Research Article
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Comparison the effects of ketamine alone and ketamine-diazepam combination in dogs of local breed in Mekelle, Ethiopia

Abstract

An experimental study was conducted to evaluate the effects of ketamine alone and with diazepam combination on anesthetic parameters; on physiological and hematological parameters so as to choose a suitable general anesthetic combination for use in surgical procedures in local breed of dogs in Mekelle, Ethiopia. The experimental study was carried out on ten local breed of dogs and were randomly divided in to two groups with five dogs in each group. Data was collected for analyzing physiological effects of anesthetic combinations; anesthetic effects and hematological effects using physical recording and laboratory analysis. The results of this study showed duration of general anesthesia was (44.8 ± 1.92min) and animal recovered (55.6 ± 3.85min) in diazepam –ketamine combination whereas duration of general anesthesia was (30 ± 1.05min) in ketamine alone. The result also showed that the physiological and hematological parameters remained significantly unchanged during the anesthesia in both groups. Therefore, relation duration of action, diazepam and ketamine combination was a suitable choice for undertaking of surgical operations in dogs of local breed for longer duration of action i.e, (44.8 ± 1.92min). Further studies on several other anesthetic combinations i.e. (acepromazine + xylazine + ketamine and acepromazine + diazepam + ketamine) on local breed of dogs and several other anesthetic combinations may be conducted.

Keywords: diazepam, general anesthesia, hematological parameter, ketamine, local breed of dogs, Mekelle

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Abbreviations: ANOVA, analysis of variance; CSA, central statistical agency; DK, diazepam – ketamine; DLC, differential leukocyte counts; EDTA, ethylene diamine tetra acetic acid; GABAA, gamma-amino butyric acid type A receptors; HbC, hemoglobin concentration; IM, intramuscular; Kg, kilo gram; mg, milligram; PCV, packed cell volume; SD, standard deviation; SPSS, statistical package for social sciences; TEC, total erythrocyte count; TLC, total leukocyte count

Introduction

Ketamine is combined with an alpha-2-agonist (e.g. xylazine), a benzodiazepine (e.g. diazepam) or a phenothiazine tranquillizer (e.g. acepromazine) to enhance muscle relaxation, analgesia, to prevent seizures/convulsions and prolong the duration of anesthetic effect. It is associated with a rapid onset, good to excellent sedation of one to two hours duration, excellent analgesia and smooth recovery. The analgesia and sedation are due to central nervous system depression and the muscle relaxation is due to the central inhibition of intraneural transmission.[1]

Ketamine is poor in visceral analgesia. However, it can be used in combination with xylazine or diazepam to provide good visceral analgesia in case of abdominal surgery (including ovariohysterectomy) and thoracic surgery. Pain is an unpleasant sensory or emotional experience most commonly associated with potential tissue damage. The sensation of pain is a consequence of the activation of specialized receptors and neurological pathways after such pain stimuli.[2,3]

Diazepam is associated with occasional individual variability in response. Moreover, its solvent (propylene glycol) may produce hypotension. Acepromazine has prolonged duration of sedation effect and is associated with hypotension secondary to marked peripheral vasodilation. The combination of diazepam and ketamine is commonly described protocol for induction of general anesthesia in healthy dogs of various ages. It may also be indicated in certain cases with cardiovascular compromises.[4–6] This combination has proved good in providing excitement-free induction of anesthesia in dogs.[7,8]

Studies on acute pain in clinical cases have most often evaluated the effects of surgical trauma on animals, while prevention and pain management are the key issues in anesthesia.[9–10] When pain is not appropriately managed, it is not only an animal welfare issue, but it can also have many detrimental effects which can impact the patient recovery.[11] A variety of physiological changes also occur in response to pain such as increases in heart rate, respiratory rate, blood pressure and body temperature.[12,13]

Ketamine is rarely used alone because of its association with poor muscle relaxation, tachycardia and catalepsy or muscle rigidity. Therefore, it is commonly used in combination with xylazine, diazepam and acepromazine to minimize the adverse effects. Moreover, there are different breed of dogs which require proper anesthetic medicament combination. However, there are limited or no experiments carried out to determine a specific anesthetic combination in relation to the local breed of dogs in the study area. Hence, determining the effects of the ketamine in combination with other sedative agents may help to come out with the safest combination for surgical procedures in local breed of dogs. Therefore, the purpose of this study is to evaluate the anesthetic, hematological and physiological effects of ketamine alone and ketamine-diazepam combination for use in surgical procedures in

local breed of dogs in Mekelle, Ethiopia.

Materials and methods

Study area

The present study was conducted from November 2016 to April 2017 in Mekelle, Tigray, Ethiopia. Mekelle is the capital of Tigray region located about 783kilometers north of Addis Ababa with a total area of approximately 102,000square kilo meters. Its geographic location is 13˚32`N latitude and 39˚33`E longitude with human population of about 215,546. It has an average altitude of 2200meter above sea level with a mean minimum and maximum monthly temperature of 8.7 degree Celsius and 26.8 degree Celsius respectively. The annual average rainfall of Mekelle is 600millimeters and more than 70 percent of it falls between the months July and August. The long dry season extends from October to May.[14]

Study population

The present study was carried out on mature and apparently healthy local breed of dogs weighing between 10-15kg and aged between 2-4 years. Dogs were declared healthy based on physiologically normal parameters i.e. rectal temperature, heart rate, respiratory rate, and capillary refill time.

Sample size

The present study was carried out on ten mature and apparently healthy local breed of dogs (six males and four females).

Sampling technique

Ten dogs were randomly divided in to two groups, an experimental group with five dogs (three males and two females) in each group and one control group with five dogs (three males and two females) in each group.

Experimental design and procedure

Ten (10) local breed of dogs were purchased, of which five dogs were randomly assigned to an induction regimen of ketamine alone (control group) and the other five dogs were assigned to ketamine-diazepam combination (experimental group). The dogs were housed individually in a kennel, fed meat and bread. Prior to anesthesia, each dog was withheld of food and water for 12 and 6hours respectively. These dogs were placed in a quiet kennel and left undisturbed. Heart rate, respiratory rate and temperature were recorded prior to premedication. Blood samples were taken prior to premedication. All dogs were premedicated with atropine sulphate at 0.04mg/kg body weight subcutaneously for the reduction of salivary and bronchial secretions fifteen minutes before induction of anesthesia with ketamine alone and with diazepam.

Administration of drugs

Group 1-control group (ketamine alone): First all dogs were premedicated with atropine sulphate at 0.04mg/kg body weight subcutaneously. After fifteen minutes of premedication, a combination of diazepam and ketamine were administered at two different doses of ketamine at 5mg/kg and 10mg/kg IM with one week interval between trials.

Group 2- experimental group (ketamine –diazepam combination): First all dogs were premedicated with atropine sulphate at 0.04mg/kg body weight subcutaneously. After fifteen minutes of premedication, a combination of diazepam and ketamine at two different doses diazepam and ketamine at 1mg/kg and 0.1mg/kg respectively and again at 2mg/kg and 0.5mg/kg intramuscularly respectively with one week interval between trials.

Monitoring of post intervention

After administration of the ketamine alone and with diazepam dogs of all groups were kept under close observation. Induction period, duration of anesthesia and recovery period were recorded. Rectal temperature, respiratory rate, and heart rate were recorded every 5minutes interval after administration of the anesthetic combinations.

Hematological and physiological parameters

Three ml of blood sample were collected from cephalic vein of each experimental dog prior to administration of the premedication (atropine sulphate) and 30-45minutes after administration of the anesthetic agents; because maximum effects occurred at 30-45minutes. Immediately after collection, the blood samples were transferred in a sterile test tube containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant for estimation of Packed Cell Volume, White blood cells, Hemoglobin concentration, red blood cells and differential leukocyte counts according to the procedures of Orpet & Welsh.[15] Physiological parameters like heart rate, respiratory rate and rectal temperature were measured every five minutes after administration of the anesthetic combinations.

Data collection

Data were collected on physiological effects (heart rate, respiratory rate and rectal temperature), anesthetic effects (induction period, duration of anesthesia, recovery period,) and hematological effects (packed cell volume, red blood cells, white blood cells, hemoglobin concentration and differential leukocyte counts.

Data analysis

The recorded data was entered into Microsoft excel sheet and analyzed to Mean±SD (Standard Deviation) using Statistical Package for Social Sciences (SPSS) version 17.0.[16] Paired t-test was used to compare physiological and hematological parameters taken before and during the administration of the drug combination for each group. One-Way Analysis of Variance (ANOVA) at 95% confidence interval (CI) was used to determine the level of significant difference in mean values among three groups; to compare the means of induction time, duration of anesthesia and recovery time between the groups. Values of $p \leq 0.05$ were considered as statistical significant and Values of $p > 0.05$ was considered as non-significant.

Results

Anesthetic effects of ketamine alone and ketamine-diazepam combination

In this study, the duration of action ketamine alone at a respective dose of 5mg/kg and 10mg/kg body weight given intramuscularly were 30 ± 1.05 minutes, 25 ± 1.05minutes, respectively, whereas the recorded onset of action, duration of action and recovery time of the anesthetic combination of diazepam – ketamine at 0.1mg/kg and 5mg/kg body weight given intramuscular, respectively were 12 ± 2.12, 37.8 ± 1.92 and 47.8 ± 1.92minutes, respectively. The recorded onset of action, duration of action and recovery time of the anesthetic

combination of diazepam – ketamine at 0.5mg/kg and 10mg/kg body weight given IM, respectively were 10 ± 2.12, 44.8 ± 1.92 and 55.6 ± 3.85minutes, respectively. In this study, onset of action was shorter whereas duration of action and recovery time were longer in the anesthetic combination of diazepam – ketamine at 0.5mg/kg and 10mg/kg, respectively when compared to the anesthetic combination of diazepam – ketamine at 0.1mg/kg and 5mg/kg, IM. So in this study, the duration of anesthesia was longer in ketamine -diazepam combination as compared with ketamine alone (Table 1).

Table 1 Effects of ketamine alone (control group), ketamine-diazepam combination (experimental group) on onset of action, duration and recovery time

Anesthetic agents	Doses (mg/kg)	Onset of action (min)	Duration of action (min)	Recovery time (min)
Control group	5mg/kg	1.5 ± 0.5	30 ± 1.05	120 ± 7.25
	10mg/kg	1 ± 0.4	25 ± 1.05	180 ± 7.25
Experimental group	0.1mg/kg and 5mg/kg	12 ± 2.12	37.8 ± 1.92	47.8 ±1.92
	0.5mg/kg and 10mg/kg	10 ± 2.12	44.8 ± 1.92	55.6 ± 3.85

Body reflexes activity

Rightening reflex

In the current study, the different body reflexes activities were assessed during the anesthesia for the sake of assessing the depth of anesthesia. The rightening reflex was elicited by squeezing or pinching a digit of fore limb and observed whether the dog flexes the leg or withdraws the digit from the investigator during the examination after administration of the anesthetic combinations. In ketamine-diazepam combination this reflex was lost at 12 ± 2.12minutes (Table 2).

Palpebral reflex

The palpebral reflex was tested by lightly taping the lateral canthus or medial canthus of the eye and observed whether the dog blinks in response after administration of the anesthetic combinations. In ketamine-diazepam combination this reflex was lost at 12.2 ± 2.12minutes (Table 2). In this study, the palpebral reflex remained unchanged throughout the anesthesia in both groups.

Corneal reflex, eye position and pupil size

Corneal reflex was tested by touching the cornea with a drop of sterile water and noted whether the dog blinks in response and withdraws the eye into the orbital fossa. In this observation, the time for corneal reflex loss was the same as to the time loss for palpebral reflex in all the three groups. In ketamine-diazepam combination this reflex was lost at 12.2 ± 2.12minutes (Table 2).

Pedal reflex

The pedal reflex was elicited by squeezing or pinching a digit of hind limb and observed whether the dog flexes the leg or withdraws the digit from the investigator during the examination after administration of the anesthetic combinations. In ketamine-diazepam combination this reflex was lost at 12.5 ± 2.21minutes (Table 2). In this study, the pedal reflex remained unchanged throughout the anesthesia in both groups.

Physiological effects of ketamine alone and ketamine –diazepam combination

In this study, the heart was decreased non- significantly (P = 0.064) from 30minutes up to 40minutes after administration of the anesthetic combination of diazepam – ketamine on both doses (Table 4). The recorded respiratory rate was decreased non- significantly (P = 0.067) from 10minutes up to 40minutes following administration of the anesthetic combination of diazepam – ketamine on both doses (Table 3). The recorded rectal temperature was also decreased non-significantly (P = 0.065) from 20minutes up to 40minutes after administration of the anesthetic combination of diazepam – ketamine on both doses (Table 4).

Hematological effects of ketamine alone and ketamine –diazepam combination

In the current study, blood samples were taken before and during administration of ketamine alone and the anesthetic combinations of diazepam-ketamine for evaluating of hemoglobin concentration, packed cell volume, total erythrocyte count, total leukocyte count, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

In group 1 hemoglobin concentration (P = 0.066), packed cell volume (P = 0.073), total erythrocyte count (P = 0.069), total leukocyte count (P = 0.079), lymphocyte (P = 0.064), monocyte (P = 0.061), eosinophil (P = 0.074) and basophils (P = 0.084) were decreased non-significantly, Neutrophils (P = 1.000) were increased non-significantly from 58.8 ± 0.39 to 64 ± 0.68 (Table 5).

In group 2 hemoglobin concentration (P = 0.062), packed cell volume (P = 0.065), total erythrocyte count (P = 0.067), total leukocyte count (P = 0.078), lymphocyte (P= 0.084), monocyte (P = 0.071), eosinophil (P = 0.0614) and basophils (P = 0.083) were decreased non-significantly, on the other hand, neutrophils (P = 1.0211) were increased non-significantly from 58.8 ± 0.39 to 66.5 ± 0.34 (Table 5)

Table 2 Loss of body reflexes activity

Loss of body reflexes in minutes					
Anesthetic combinations	**Doses(mg/kg)**	**Rightening reflex**	**Palpebral reflex**	**Corneal reflex**	**Pedal reflex**
Control group	5mg/kg	1.5±0.5	1.6±0.4	1.6±0.4	1.7±0.6
	10mg/kg	1±0.5	1.1±0.4	1.1±0.4	1.5±0.6
Experimental group	0.1mg/kg and 5mg/kg	12± 2.12	12.2±2.12	12.2±2.12	12.5±2.23
	0.5mg/kg and 10mg/kg	10± 2.12	10.2±2.12	10.2±2.12	10.5±2.23

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Table 3 Effects of ketamine alone on heart rate (HR), respiratory rate (RR) and rectal temperature (RT) of the control group

Parameters	Time interval in minutes												
	BA	5	10	15	20	25	30	35	40	45	50	55	60
HR(beat/min)	80.1 ±2.2	85.8 ±2.23	90.5 ±2.24	123.3 ±2.34	128.2 ±2.26	132 ±2.28	137 ±2.34	140 ±2.35	98.5 ±2.41	90.3 ±2.47	81.3 ±2.35	81.4± 2.24	80.8 ±2.23
RR(breadth/ min)	23.7 ±0.7	23.1 ±0.78	18.6 ±0.80	17.4 ±0.91	17.4 ±0.92	16.6 ±1.13	15.7 ±1.24	15.4 ±1.36	15.0 ±1.34	20.2 ±0.77	23.2 ±0.77	23.2 ±0.64	23.5 ±0.53
RT(˚C)	37.8 ±0.77	37.76 ±0.79	37.68 ±0.80	37.64 ±0.85	37.45 ±0.87	37.42 ±0.89	37.4 ±0.912	37.35 ±0.93	37.29 ±0.95	37.182 ±1.02	37.68 ±0.84	37.69 ±0.79	37.78 ±0.69

BA, Before anesthesia; HR, Heart rate; RR, Respiratory rate; RT, Rectal temperature

Table 4 Effects of diazepam and ketamine combination on heart rate, respiratory rate and rectal temperature of experimental dogs

Parameters	Time interval in minutes									
	BA	5	10	15	20	25	30	35	40	45
HR(beat/ min)	80.1±2.22	79.8±2.33	79.5±2.42	79.3±2.46	78.2±2.47	77.4±2.37	74.2±2.1	73.4±2.4	72.5±2.5	79.3 ±2.4
RR(breath/ min)	23.7±0.73	23.1±0.79	18.6±0.82	17.48±0.89	17.43±0.94	16.67±1.32	15.74±1.	15.45±1	15.03±1	23.2 ±0.7
RT(˚C)	37.81±0.77	37.76±0.78	37.68±0.81	37.64±0.86	37.45±0.88	37.42±0.90	37.4±0.9	37.35±0.94	37.29±0.96	37.62 ±1.02

BA, Before anesthesia; HR, Heart rate; RR, Respiratory rate; RT, Rectal temperature

Table 5 Effects of ketamine alone (control group), ketamine-diazepam combination (experimental group) on hematological parameters

Anesthetics		HBC	PCV	TEC	TLC	NTP	LYM	MN	EOS	BAS
Control group	Before anesthesia	14.3±0.25	42.3±0.25	6.4±0.25	10.3±0.25	58.9±0.39	29.9±0.53	7.4±0.25	3.5±0.27	0.9±0.13
	During anesthesia	13.91±0.29	41.72±0.29	5.78±0.25	9.29±0.33	65±0.68	28.1±0.67	6.5±0.16	2.6±0.16	0.5±0.1632
Experimental group	Before anesthesia	14.2±0.25	42.2±0.25	6.2±0.25	10.2±0.25	10.2±0.25	29.8±0.53	7.2±0.25	3.4±0.27	0.8±0.133
	During anesthesia	12.61±0.29	40.59±0.27	4.78±0.20	7.78±0.19	66.5±0.34	26.1±0.38	5.6±0.16	2±0.21	0.2±0.13

HBC, Hemoglobin concentration; PCV, Packed cell volume; TEC, Total erythrocyte count; TLC, Total leukocyte count; NTP, Neutrophil; LYM, Lymphocyte; MN, Monocyte; EOS, Eosinophil; BAS, Basophil

Discussion

Ketamine is rarely used alone because of its association with poor muscle relaxation, tachycardia and catalepsy or muscle rigidity and it is therefore commonly used in combination with xylazine, diazepam and acepromazine to minimize the untoward effects The highest duration of anesthesia was observed in the dogs of Group 2 (experimental group) as compared to group 1 (control group). This might be due to wide-distribution of diazepam and ketamine combination in the body, because they are highly soluble in lipid and can be redistributed into muscles and adipose tissues.[17] This finding difference in the present study from previous studies might be due to difference in breed and physiological status of the dogs or might be due to difference in dose of the anesthetic agents.

The decrease in body temperature after the administration of ketamine alone and the diazepam-ketamine could be explained by blocking of the hypothalamic thermoregulatory center. The decrease in heart rate could be attributed to inhibition of the release of the neurotransmitter noradrenalin or depression of the sympathetic activity.

The decrease in respiratory rate could be attributed to depression of the respiratory center by the ketamine alone, diazepam-ketamine.[18] The non-significant decrease in heart rate, respiratory rate and rectal temperature in the present study when compared to other studies might be due to difference in breed and physiological status of the dogs or might be due to difference in dose of the sedative agents.

In the present study, the average duration of anesthetic induction after administration of diazepam and ketamine at 0.1mg/kg and 5mg/

kg, respectively were 12 ± 2.12 and 37.8 ± 1.92minutes, respectively. This finding is in agreement with the studies by Ferreira[19] reported 35.7minutes of the average duration of anesthesia after administration of diazepam and ketamine combination, but the onset of action was slower in the present finding when compared to observations separated by Ferreira[19] he had reported average 4.2minutes after administration of diazepam and ketamine combination. The slower onset of action in the present finding when compared to the other studies might be due to difference in breed of the dog or due to difference in physiological status of the dog.

In this study, heart rate was increased significantly at 15-35minutes after administration of ketamine alone but decreased non-significantly at 30-40 minutes, respiratory rate was decreased non-significantly at 10-40 and rectal temperature was decreased non-significantly at 20-40minutes after administration of diazepam at 0.5 mg/kg and ketamine at 10mg/kg body weight intramuscularly. Relatively similar finding were reported by White[7] who found decreased respiratory rate at the first 30 minutes after administration of the diazepam at 0.3 mg/kg and ketamine at 5mg/kg body weight intramuscularly on ten healthy dogs.

After administration of ketamine alone, Diazepam –the hemoglobin concentration, packed cell volume, total erythrocyte count, total leukocyte count, lymphocyte, monocyte, eosinophil and basophils were decreased non-significantly, but neutrophils were increased non-significantly.

Pooling of circulating blood cells in the spleen and other reservoirs secondary to decreased sympathetic activity could be the reason for a decrease in hemoglobin concentration, packed cell volume, total erythrocyte count, total leukocyte count, lymphocyte, monocyte, eosinophil and basophils.[20]

The decrease in hemoglobin concentration, packed cell volume, total erythrocyte count, total leukocyte count, lymphocyte, monocyte, eosinophil and basophils after administration of the diazepam and ketamine combination might be attributed to the shifting of fluid from extravascular compartment to intravascular compartment in order to maintain normal cardiac output in the dogs.[21]

This finding is in agreement with the findings of Mahmud[22] who had reported decreased the hemoglobin concentration, packed cell volume, total erythrocyte count, total leukocyte count, lymphocyte, monocyte, eosinophil and basophils and increased neutrophils after administration of diazepam at 0.4mg/kg and ketamine at 10mg/kg combination in dogs.[23–33]

Conclusion

Ketamine is rarely used alone because of its association with poor muscle relaxation, visceral analgesia, tachycardia and catalepsy or muscle rigidity. Therefore, it is commonly used in combination with xylazine, diazepam and acepromazine to enhance muscle relaxation, to provide good visceral analgesia in case of abdominal surgery (including ovariohysterectomy) and thoracic surgery, to prevent seizures/convulsions and to prolong the duration of anesthetic effect. The study was conducted on 10 mature and apparently healthy local breed of dogs which were randomly grouped in to Group I and Group II. All dogs were premedicated using atropine (0.04mg/kg BW, S.C). After 15mints premedication, anesthesia induced with ketamine a lone and diazepam-ketamine (1.0mg/kg/BW+10.0mg/kg BW, I.M) for Group I, and Group II respectively. The anesthetic parameters; induction time, duration of anesthesia recovery period, the physiological parameters; temperature, heart rate, respiratory rate, and the hematological parameters; packed cell volume, total erythrocyte count, total leukocyte count, hemoglobin determination were recorded and analyzed in both groups and all the anesthetic parameters were found statistically significant but the physiological and hematological parameters were statistically non-significant in both groups. The results of the present study concluded that diazepam-ketamine combination is useful anesthetic protocol for rapid induction, prolonged duration of anesthesia; diazepam-ketamine combination is useful anesthetic protocol for short duration of anesthesia and rapid recovery. All drug combinations do not affect the physiological and hematological parameters of the animals during the study time and all of them can be safe for surgical procedures if used safely and appropriately. However, further studies on several other anesthetic combinations i.e. (acepromazine + xylazine + ketamine and acepromazine + diazepam + ketamine) on local breed of dogs and several other anesthetic combinations may be conducted.

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Conflict of interest

Author declares that there is no conflict of interest.

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