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KETAMINE AS A SINGLE GENERAL ANESTHETIC AGENT FOR ORAL SURGICAL PROCEDURES IN WISTAR ALBINO RATS- AN EXPERIMENTAL STUDY

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Abstract: Experimentally induced periodontitis forms the basis for trials of novel drugs and therapeutics. Ketamine, an N- amino-D- aspartate (NMDA) receptor antagonist is considered to be safe dissociative anesthetic agent. But, the literature on the use of Ketamine as an effective general anesthetic is conflicting. We had to determine, safe and effective dose range of intra-peritoneal Ketamine hydrochloride injection in Wistar albino rats for the placement of ligatures in the oral cavity. 50 rats of the age group (greater than 4 months) and weight of160g-350g were selected for the study from the Department of Pharmacology, Yenepoya University, and Mangalore. Intraperitonial injection was administered using Tuberculin syringe. The optimal dose for each animal was tabulated and log dose was calculated. Nonlinear regression analysis was done. 95% confidence limit and ED_{50} was calculated from the graph was 101.9mg/kg (68.1-152.6mg/kg) correlation coefficient was 0.4946. Profound and effective anesthesia was obtained at a dose of 60mg/kg-275mg/kg. The dose range of Ketamine alone from 60-275mg/kg i.p.route produced profound reproducible anesthesia. Minor oral surgical procedures could be carried out without discomfort. Mortality and morbidity due to anesthesia was low.

Keywords: Anesthesia, Ketamine, Wistar albino rats,

INTRODUCTION

In order to carry out dental procedures in the mouth of rats, like intraoral examination of the oral cavity, minor procedures like inducing periodontitis or extra oral major surgical procedures like placement of bone grafts or orthodontic procedures, a profound and safe general anesthesia is a must. [1] Anesthesia is a state of unconsciousness induced in an animal. The three components of anesthesia are analgesia (pain relief), amnesia (loss of memory) and immobilization. Most of the previous studies in animals have used combination of injectable and inhalational anesthetics. [2] Use of inhalational anesthesia and the equipment hamper the visual field and maneuverability during oral surgical procedures. Using single drug intraperitoneally to achieve all three components of anesthesia will make the procedure easy. This study was carried out in order to standardize optimal dose range of ketamine for anesthesia lasting 1-3 hours in Wistar rats to carry out ligation around upper maxillary first molars to induce periodontitis and evaluate the retention of sutures.

The dissociative anesthetic agents include ketamine (Vetalar, Ketaset, Aneket) and tiletamine (Telazol). Ketamine, a cyclohexamine, was used in the current study as it is easy to use and has a greater margin of safety for most of the laboratory animals. [3] High doses of ketamine induce catalepsy and are not accompanied by central nervous system depression.

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Respiratory functions are depressed, but cardiovascular function is maintained. The swallowing reflex is maintained which helps to prevent aspiration pneumonia if the animal regurgitates. But, this may not be complete. Fasting and intubation is recommended. The animal's eye remains open and the cornea should be protected with a layer of ophthalmic petrolatum or other suitable ointment. [4] Ketamine-Xylazine anesthesia is generally safe. But rats administered effective systemic doses of Ketamine and Xylazine developed acute reversible lens opacities. [5] We found variable response to the recommended ketamine dose in Wistar albino rats. Hence an attempt was made to determine a safe and effective range of the anesthetic dosage of ketamine alone in the Wistar albino rats for oral procedures.

To determine a safe and effective dose range of intra-peritoneal dosage of Ketamine hydrochloride injection in Wistar albino rats for placement of silk ligatures around molars in the oral cavity.

MATERIAL AND METHODS

50 Wistar albino rats of the age group (greater than 4 months) and weight of160g-350g were selected for the study. The rats were obtained from the Department of Pharmacology, Yenepoya University, and Mangalore. The animals were housed under standard conditions in the animal house facility in the department of Pharmacology, Yenepoya University.

The institutional board approved all the procedures in this study. The Ketamine (Anaket) and Tuberculin syringe (Braun Co.) were obtained from pharmacy, Yenepoya Hospital, Mangalore.

No feed was given to the animals for 12 hours prior to administration of anesthesia. They were weighed prior to administration of ketamine anesthesia. Thorough physical examination of the rats was done and vital signs noted. The reference dosage of Ketamine/ml/kg was calculated. We chose the intraperitonial route of administration as it was technically less demanding. Using single drug intraperitoneally to achieve all three components of anesthesia will make the procedure easy. Intraperitonial injection was administered using Tuberculin syringe. The site of Intraperitonial injection was limited to the right caudal region to avoid ceacum.

Titration of Dose:

5 rats weighing 160-300g were taken in a poly propylene cage each day of the experiment for 10 days. The initial loading dose for the first set of animals was given as per the literature i.e. 25 mg/kg body weight. A stop watch was used to record the duration of anesthesia. Onset of action of ketamine was 10-20 minutes. The dose was increased incrementally by 1mg/kg body weight till the rats attained complete anesthesia. It was monitored by assessing the corneal reflexes and tail clip method. [6] Then the rats were placed in empty individual polypropylene cages without bedding. Bedding was avoided to prevent animals from swallowing the bedding material. The rats were kept under constant observation.

Once the full plane of anesthesia was achieved, the mouth opening was achieved using a modified mouth prop on a surgical table with temperature regulation. Later pre periodontal examination of rats was done and the upper second molars were ligated using a sterile braided silk suture (5-0). The rats were placed back into the poly propylene cages. Then the stop clock was stopped once the animal showed visible signs of mobility. The animals were placed into their original cages later when the symptoms of anesthesia ceased. They were given food and water ad-libitum 12 hours after they recovered. The entire procedure was repeated after 1 week. There were no signs of any adverse reactions during the procedure.

RESULTS

The optimal dose for each animal was tabulated and log dose was calculated log dose-percentage response graph was plotted. Nonlinear regression analysis was done. 95% confidence limit and ED_{50} was calculated from the graph was 101.9mg/kg (68.1-152.6mg/kg) correlation coefficient was 0.4946 [Table 1, 2, fig 1].

Table.1: Number of animals anesthetized at a specific dose was converted to percentage using the formula given below, Dose was converted to log dose to plot log DRC (Percentage) in order to Interpolate ED50 Value.

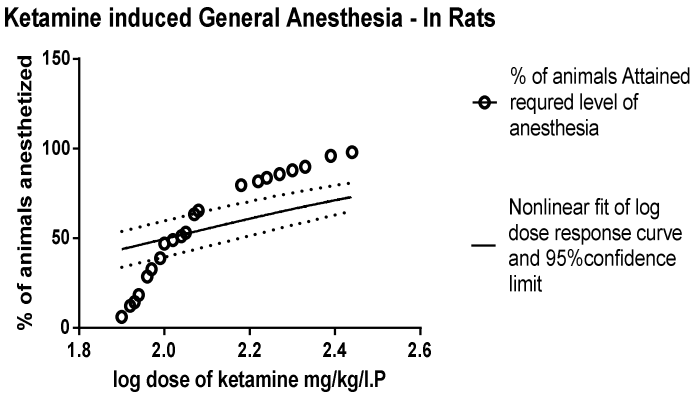
Ketamine dose/kg/i.p	log dose of ketamine	% of animals responded (Anesthetized)
80	1.9	6.12
83	1.92	12.24
85	1.93	14.29
87	1.94	18.37
89	1.95	24.49
91	1.96	28.57
93	1.97	32.65
95	1.98	36.73
98	1.99	38.78
100	2	46.94
105	2.02	48.98
110	2.04	51.02
112	2.05	53.06
115	2.06	55.1
117	2.07	63.27
120	2.08	65.31
126	2.1	71.43
141	2.15	73.47
148	2.17	77.55
151	2.18	79.59
166	2.22	81.63
174	2.24	83.67
186	2.27	85.71
200	2.3	87.76
214	2.33	89.8
229	2.36	91.84
240	2.38	93.88
245	2.39	95.92
275	2.44	97.96

$$\%\ \text{of Animals anesthetized at the Dose} = (\text{Number of animals anesthetised at the dose} \div \text{Total number of Animals}) \times 100$$

Table.2: Summary: Nonlinear Regression Analysis of the Log Dose response curve (log dose of Ketamine vs. % animals anesthetized) and Interpolation of ED50 from the Graph.

Summary	log(agonist) vs. normalized response -% of animals responded (Ketamine induced general Anaesthesia)
Best-fit values	
ED50	101.9 mg/kg/ I.P
95% Confidence Intervals	
ED50	68.10 to 152.6 mg/kg/ I.P
Goodness of Fit	
Degrees of Freedom	20
R square	0.4946
Number of points Analyzed	21

Figure.1: Log dose response (Percentage of animals anesthetized) curve of Ketamine induced anesthesia in Rats. Nonlinear fit of the curve with 95% confidence limit is also shown in the figure.



DISCUSSION

Selecting an appropriate anesthetic agent to induce general anesthesia in the rodents is a challenging procedure, while performing oral surgical procedures. General anesthesia and analgesia is a must for even simple procedures like pre periodontal examination, silk suture ligation or for major oral surgical procedures in rodents. We noticed that the recommended drug dosage in literature may not be applicable to all the rodent species due to many variables like interspecies variation, climatic variations and general health status of the animals.

Dissociative anesthetics have a wide margin of safety for most laboratory species. Ketamine, a dissociative anesthetic is an N- methyl- D- aspartate (NMDA) receptor antagonist.[4] In this study the primary objective was the placement of silk 3-0 sutures around the maxillary second molars to induce periodontitis. But to achieve this we needed to have an effective and safe dose of ketamine anesthesia. We found that induction time of Ketamine anesthesia was about 5- 20 minutes. This was in agreement with the current guidelines. [2]However the dosage and duration of anesthesia in our study varied within animals. In general the duration of anesthesia was approximately 3-4 hours. We had to optimize the dosage and duration of general anesthesia using Intraperitonial route to carry out minor surgical procedures. The dosage ranged from 25mg/kg to 275mg/kg. This dosage is much higher than the conventional dosage mentioned in the literature. The laryngeal and pharyngeal reflexes were maintained and this was advantageous as it minimized the chances of death of animal due to aspiration.

The optimized dosage and duration of anesthesia allowed for convenient pre periodontal examination, suture ligation and intra papillary injection of lipopolysaccharides. Subsequent administration of Ketamine after a washout period produced reproducible general anesthesia. The animal recovery was comparatively quicker. We lost only two animals during the first examination. One animal was lost after 1 week administration. The exact causes of the death of the animals were not known. The probable reason could be unknown infection or pain caused due to periodontal inflammation post suture placement. But the mortality rate was quite low. Ketamine dosage was the same for individual rats after 1 week. We could achieve reproducible anesthesia.

We propose that owing to the profound anesthesia obtained in the Wistar Albino rats, certain major surgical procedures related to the oral cavity like placement of bone grafts in critical sized defects, trials on placement of novel surgical orthodontic appliances and implants can be carried out safely using the dosage range of 60mg/kg- 275mg/kg. Dosage has to be optimal and administered in an incremental manner till profound anesthesia is obtained. However, this has to be validated by further trials.

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