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Immobilization with Ketamine HCl and Tiletamine-Zolazepam in Cynomolgus Monkeys

Jae-il Lee, Sung-hyeok Hong, Soo-jin Lee, Young-suk Kim and Myung-cheol Kim*

Laboratory of Veterinary Surgery, College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea

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Abstract

To compare the effects of ketamine and tiletamine-zolazepam (TZ) drugs widely used for the chemical restraint and immobilization of primates, on various physiological parameters and blood gas values in cynomolgus monkeys (*Macaca facicularis*). Rectal temperature, heart rate, respiration rate and blood gas analysis were measured before treatment and at 1, 10, 20, 30, 40, 50 and 60 min after administration. Additionally, in both groups, induction and maintenance times were compared. Heart rate, respiration rate, rectal temperature, pH and pCO_2 were not significant different in the two groups. However, pO_2 in the ketamine-treated group was significantly lower at 30 and 40 min than in the TZ-treated group. The induction time was short in both groups, and the maintenance time was longer in the TZ-treated group (67.8±6.5 min) than in the ketamine-treated group (42.3±6.7 min). However, decreased rectal temperatures must be watched and prevented following TZ administration to cynomolgus monkeys. It was considered that ketamine may be useful for short duration anesthesia including handling, physical examination, blood sampling and TZ may be useful for prolonged anesthesia including minor surgery and other surgical procedure.

Key words: cynomolgus monkey, immobilization, ketamine HCl, tiletamine-zolazepam

Introduction

The cynomolgus monkey (*Macaca fascicularis*) is used widely in research for respiratory and toxicological work [18]. Non-human primates present special hazards to handlers, particularly the danger of bites and zoonotic infection [18]. Restraint or immobilization is very important to minimize harm incurred to humans or animals when procedures are performed on animals. Restraint is achieved by either physical or chemical methods, and the two are usually used in combination. Usually, under field conditions, we choose chemical method than physical method. When considering the choice of agent for restraint, the following factors should be considered: the welfare of the animal, the safety of the personnel, the facilities and expertise available, the procedure to be performed, the size, condition, temperament and species of animal and the efficacy of the drug [11]. Chemical immobilizers may be administered by oral, intravenous, intramuscular or inhalation routes. However, intramuscular administration is practical for initial immobilization of primates.

Ketamine and tiletamine/zolazepam (TZ) are widely used anesthetics for minor or major surgery in many animals. A low dose of these agents induces short duration anesthesia for restraint or immobilization during handling, blood sampling, tuberculosis testing and simple surgical treatment. Ketamine is usually given intramuscularly but it can be administered intravenously, intraperitoneally or orally [11]. Generally, an intramuscular dose of 10 to 15 mg/kg in primates results in immobilization that is adequate for performing examination, urethral and cardiac catheterization, blood collection, treatment of wounds, tattooing and tuberculosis testing [15].

TZ is a combination of tiletamine HCl and zolazepam HCl. Tiletamine is a dissociative anesthetic agent pharmacologically similar to ketamine, and zolazepam is a benzodiazepine sedative, anxiolytic, and skeletal muscle relaxant pharmacologically similar to diazepam [8]. TZ offers advantages that include small-volume dose requirements, ease of preparation of solutions, rapid induction, dose-related restraint ranging from chemical immobilization to cataleptiform anesthesia, good to excellent muscle relaxation, retention of laryngeal and pharyngeal reflexes, an apparent wide safety margin, and a generally unremarkable recovery [12]. TZ induces minor tranquilizing properties, profound analgesia and cataleptoid anesthesia follow intramuscular injection at 2 to 6 mg/kg [17].

Chemical immobilization is important to prevent hazards, and also investigators should ensure that stress in animal, especially non-human primate, is minimized. Both agents are used in the fields; however, the chemical agents are used by personal preference without any supporting data. Therefore, we investigated the effects of ketamine or TZ on vital signs and blood gas values in the cynomolgus monkey and which

* Corresponding author: Myung-cheol Kim
Laboratory of Veterinary Surgery, College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea
Tel: +82-42-821-6757, Fax: +82-42-822-4216
E-mail: mckim@cnu.ac.kr

is proper in various conditions.

Materials and Methods

Animals

Eight adult cynomolgus monkeys (3 males and 5 females) used in this study were raised in the Philippines and recently moved to the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. Their mean weight was 4.0±0.7 kg (mean±SD) and their ages were approximately 5-6 years. The animals were housed in appropriately sized, stainless steel primate cages in a closed system with limited access. The temperature and relative humidity were kept at 27℃ and 55%. A light cycle of 12 h light and 12 h dark, 07:00 h to 19:00 hr, was maintained. Air was supplied by an Isumi system. Monkeys were fed PMI LabDiet® daily, supplemented with apples, bananas and oranges.

Drug trial

Experiments were performed in the afternoon following an overnight fast. Prior to restraint or immobilization, the animals were squeezed using a cage wall squeezing system. The physically restrained animals were removed from the cages and manually held as close to a dorsally recumbent position as possible. As soon as an animal was restrained, venous blood samples were collected for pH and blood gas analysis. The samples were drawn anaerobically from the saphenous vein, using a 1 ml heparinized disposable tuberculin syringe. After thorough mixing of the sample, it was introduced into a pH/blood gas analyzer. Values for pH, pCO_2 and pO_2 were then recorded at the instrument's temperature of 37°C. Measurements of rectal temperature, respiration rate and heart rate were taken during sampling. In both groups, atropine sulfate in dosages of approximately 0.02 mg/kg was administered by subcutaneous injection to reduce salivation before ketamine and TZ administration. The eight animals were then randomly assigned to receive either ketamine (Ketamine 50 Inj, Yuhan Co, Korea) at an intramuscular dose of 10 mg/kg or TZ (Zoletil 50 Inj, Virbac Laboratories 06516 Carros, France) at an intramuscular dose of 5mg/kg.

Observations

Rectal temperature, heart rate, respiration rate, pH and blood gas analysis (pCO_2 and pO_2) were measured before atropine administration before treatment. And then at 1, 10, 20, 30, 40, 50 and 60 min after drug administration by intramuscular injection of ketamine or TZ. Heart rate was recorded by ECG (Resting ECG, Quintan Q710, Bothell, WA, USA) using lead II at a paper speed of 50 mm/s. The respiration rate was counted as the number of spontaneous breaths per minute. At the same time, the rectal temperature was measured. The pH, pCO_2 and pO_2 were measured with the blood gas analyzer (Blood Gas Analyzer OPTI 1, AVL Scientific Co, USA). The immobilization induction times were measured from the time of drug injection to the disappearance of righting reflex and skin twitch response. Maintenance times were defined as the period from the induction time to the time at which the monkey sat up in its cage. Total restraint or immobilization times were defined as the time from the first injection of anesthetic to the point at which the monkey sat up.

Statistical analysis

Two-factor ANOVA for repeated measures was used to compare groups. Single data points (induction and recovery times) were compared with unpaired *t*-tests and physiological measurements data were compared with analysis of variance using standard statistical software (Statview). And also it was compared with analysis of variance using *t*-tests within groups.

Results

Heart rate/respiration rate/rectal temperature

Both drug regimens caused a rapid decrease in heart rate up to 40 (TZ group) and 20 min (ketamine group) following drug administration. Heart rates did not recover to the basal values until 60 (204 bpm, TZ group) and 50 (220 bpm, ketamine group) min after drug administration (TZ=227, ketamine=246) (Fig. 1). Heart rates in the TZ group were significantly decreased at 10, 20, 30, 40, 50 and 60 min after drug administration ($p<0.05$). Ketamine group were significantly decreased at 10, 20 and 30 min after administration, compared to basal values ($p<0.05$). Heart rates were not significant difference between the two groups.

The respiration rate was decreased in the TZ group, until 50 min post-drug administration. However, the respiration rate was not vary significant different in the ketamine group over time (Fig. 2). The respiration rate was significantly decreased in the TZ group, from 20 to 60 min post-drug administration. The respiration rate in the two groups was not significantly different.

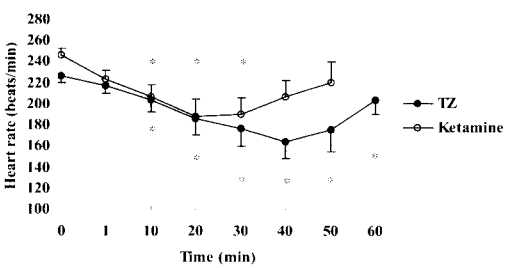

Fig. 1. Mean values for heart rate in cynomolgus monkeys pretreatment, 1, 10, 20, 30, 40, 50 and 60 min after chemical immobilization with tiletamine-zolazepam (TZ) 5 mg/kg (n = 4) and ketamine 10 mg/kg (n = 4). Atropine sulfate was administered before ketamine and TZ injection. *Significantly ($p<0.05$) different from baseline.

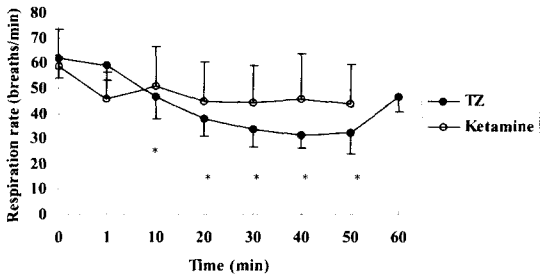

Fig. 2. Mean values for respiratory rate in cynomolgus monkeys pretreatment, 1, 10, 20, 30, 40, 50 and 60 min after chemical immobilization with tiletamine-zolazepam (TZ) 5 mg/kg (n = 4) and ketamine 10 mg/kg (n = 4). Atropine sulfate was administered before ketamine and TZ injection. *Significantly ($p<0.05$) different from baseline.

The rectal temperature gradually decreased with time after administration of ketamine and TZ. In the TZ group, rectal temperature was increased at 1 minute after administration, and then rapidly decreased with time (Fig. 3). In the TZ group, the rectal temperature was showed a tendency to decrease at 30, 40, 50 and 60 min (37.37, 36.95, 36.82 and 36.8℃, respectively), as compared to the temperature before administration (38.85℃). In the ketamine group, rectal temperature was showed a tendency decrease at 30 min (37.77℃), compared to the temperature before administration (38.4℃). Rectal temperature was not significant difference between the two groups.

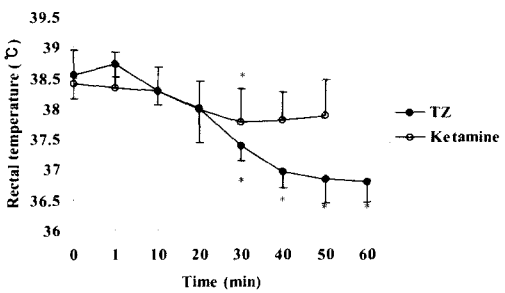

Fig. 3. Mean values for rectal temperature in cynomolgus monkeys pretreatment, 1, 10, 20, 30, 40, 50 and 60 min after chemical immobilization with tiletamine-zolazepam (TZ) 5 mg/kg (n = 4) and ketamine 10 mg/kg (n = 4). Atropine sulfate was administered before ketamine and TZ injection. *Significantly ($p<0.05$) different from baseline.

pH/pCO_2/pO_2

Both drugs caused the pH to gradually increase. The pH significantly increased from 10 to 50 (ketamine group) and 60 (TZ group) min following drug administration. However, pH did not differ significantly in the two groups.

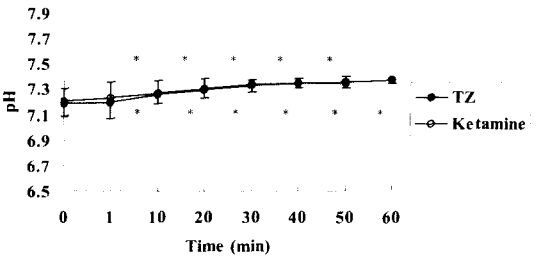

Fig. 4. Mean values for pH in cynomolgus monkeys pretreatment, 1, 10, 20, 30, 40, 50 and 60 min after chemical immobilization with tiletamine-zolazepam (TZ) 5 mg/kg (n = 4) and ketamine 10 mg/kg (n = 4). Atropine sulfate was administered before ketamine and TZ injection. *Significantly ($p<0.05$) different from baseline.

The pCO_2 decreased rapidly in both drugs up to 10 min following administration, before gradually increasing with time (Fig. 5). The pCO_2 significantly decreased at 20, 30, 40, 50 and 60 min after TZ administration and decreased significantly 10 min following ketamine administration, compared to basal levels. The pCO_2 was not significant difference between the two groups.

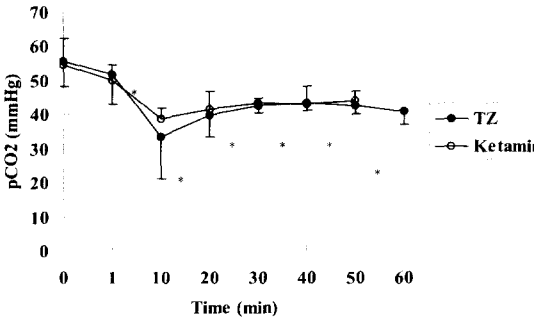

Fig. 5. Mean values for pCO2 in cynomolgus monkeys pretreatment, 1, 10, 20, 30, 40, 50 and 60 min after chemical immobilization with tiletamine-zolazepam (TZ) 5 mg/kg (n = 4) and ketamine 10 mg/kg (n = 4). Atropine sulfate was administered before ketamine and TZ injection. *Significantly ($p<0.05$) different from baseline.

The pO_2 increased rapidly in both groups up to 10 min, before gradually decreasing with time (Fig. 6). The pO_2 significantly increased from 1 to 60 min after TZ administration. pO_2 increased significantly from 1 to 50 min after ketamine administration, compared to basal levels. The pO_2 differed significantly at 30 and 40 min after administration in the two groups.

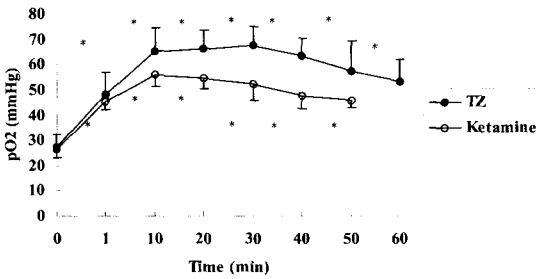

Fig. 6. Mean values for pO_2 in cynomolgus monkeys pre-treatment, 1, 10, 20, 30, 40, 50 and 60 min after chemical immobilization with tiletamine-zolazepam (TZ) 5 mg/kg (n = 4) and ketamine 10 mg/kg (n = 4). Atropine sulfate was administered before ketamine and TZ injection. *Significantly ($p<0.05$) different from baseline.

Duration of induction and maintenance

Induction times was not significantly different following administration of TZ (1.7±0.5 min) or ketamine (1.7±0.8 min). However, maintenance times were significantly longer in the TZ group (67.8±6.5 min) than in the ketamine group (42.3±6.7 min). The total anesthetic time was 69.5±6.6 min following TZ administration and 44±6.6 min following keamine administration (Fig. 7)

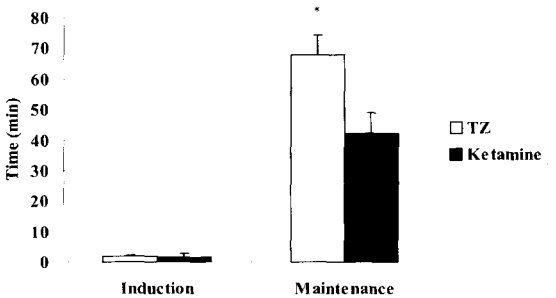

Fig. 7. Duration of induction and maintenance after chemical immobilization with tiletamine-zolazepam (TZ) 5 mg/kg (n = 4) and ketamine 10 mg/kg (n = 4). *Significantly ($p<0.01$) different between the groups.

Discussion

Ketamine and TZ have been used widely for restraint and immobilization of primates [1, 4, 5]. Both agents are cyclohexylamines that induce profound analgesia and cataleptic anesthesia [10]. There are no clinical directions on anesthesia using 2 drugs although both drugs have excellent anesthetic effects to monkeys.

The result of this study demonstrated that ketamine 10 mg/kg treatment decreased heart rates for up to 30 min and quickly recovered heart rates compare to 5 mg/kg TZ. Ketamine has various cardiovascular effects depending on animals. The actions of ketamine in persons [7, 14] and dogs [9, 16] are cardio-stimulatory; however, in non-human primates ketamine causes cardiovascular depression [10]. Ochsner [15] reported that ketamine had cardiovascular depression in rhesus monkey up to 30 min. In this result support the cardiovascular depression in monkey, however, quickly recovered to the base line after 30 min. TZ combination treatment decreased heart rates up to 50 min. Similar responses to TZ administration have been previously observed in macaques [2]. In these result showed ketamine 10 mg/kg had minimum effects to the cardiovascular in anesthesia compare to TZ 5 mg/kg to the monkey.

The respiration rate was significantly decreased from 20 to 60 min following TZ treatment; however, ketamine caused no significant difference compared to respiration rate before administration, although respiration rates were not significantly different between 2 groups. Low dose TZ exerted a respiratory depressant effect in the macaque, as does its analog ketamine, in non-human primates [2, 6]. In this study, the results of TZ treatment group seem to decrease by muscle relaxation of benzodiazepine tranquillizer (zolazepam).

The rectal temperature was lower in the TZ group than in the ketamine group. In animals anesthetized with ketamine, muscle tone is usually adequate to maintain body temperature. However, it is prudent, especially where long procedures are involved, to monitor body temperature and provide supplemental heat when necessary [1]. Our results are consistent in that decreased rectal temperature was slight following ketamine treatment because immobilization is short procedure. Alternatively, TZ treatment caused a rapid decrease in rectal temperature. TZ anesthesia caused rectal temperatures to decrease during anesthesia in dogs, even though the dogs were placed on linen towel covers and heating pads [8].

The values for pH were increased in both groups following drug administration, though not significantly above levels before drug administration. It may be result in reduction of basal metabolism. In other findings, no serious alterations in acid-base balance of the primates immobilized with ketamine and TZ occurred [18]. Temporary changes in pCO_2 and pO_2 were observed at 10 min in both groups during the chemical immobilization. These changes seem to interpose a tissue bed because blood sample obtained in vein or animals are initially stressed by immobilization. In this study, pCO_2 was not significant difference but pO_2 was significant difference between the two groups. It suggests that the difference in pO_2 is merely a reflection of the short acting of ketamine and increasing of metabolism leads to decrease of pO_2 in peripheral tissue and vein.

The induction time was short in both treatment groups, whereas, the maintenance time was significantly prolonged following TZ treatment. These results are similar to the previous report, ketamine and TZ were characterized by rapid induction times, and low dose TZ induced a prolonged

maintenance time and better muscle relaxation than did ketamine [3,12, 15]. Although, two agents are different in combination, in ketamine group, the period of anesthesia was shorted, but the monkeys regained consciousness slowly at the end of the anesthetic period. On the contrary, the anesthetic period of TZ group was prolonged, but the monkey regained consciousness more rapidly at the end of the anesthetic period.

The results presented herein may support the use of 2 drugs in monkeys. We suggest that ketamine is useful for short duration anesthesia including handling, physical examination, blood sampling and TZ is useful for prolonged anesthesia including minor surgery and other surgical procedure.

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