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Usefulness of an anesthetic mixture of medetomidine, midazolam, and butorphanol in cotton rats (*Sigmodon hispidus*)

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Abstract

The cotton rat (*Sigmodon hispidus*) is a laboratory rodent used for studying human infectious diseases. However, a lack of suitable anesthetic agents inconveniences the use of cotton rats in surgical manipulation. This study demonstrated that subcutaneous injection of the mixture of medetomidine, midazolam, and butorphanol (0.15, 2.0, and 2.5 mg/kg, respectively), which is a suitable anesthetic agents for mice and rats, produced an anesthetic duration of more than 50 min in cotton rats. We also demonstrated that 0.15 mg/kg of atipamezole, an antagonist of medetomidine, produced a quick recovery from anesthesia in cotton rats. This indicated that the anesthetic mixture of medetomidine, midazolam, and butorphanol, functioned as a useful and effective anesthetic for short-term surgery in cotton rats.

Key Words: anesthesia, cotton rat

Sigmodon hispidus, known as cotton rats, originates from southern part of the United States. Cotton rats are moderate-sized rodents, ranging in overall length from 125 to 200 mm and weight from 70 to 200 g, and are smaller than laboratory rats (*Rattus norvegicus*)[7]. Cotton

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rats are popular models for infectious diseases, owing to its broad susceptibility to human pathogens including viruses, bacteria, fungi, and parasites[7]. Additionally, cotton rats spontaneously develop cardiomyopathy, characterized by degeneration and inflammation, and similar lesions are observed in skeletal muscles[1]. Inbred cotton rats also develop female-dominant enterochromaffin-like cell-derived carcinomas, anemia with renal inflammation and chronic kidney disease[2,9]. Therefore, cotton rats, in addition to mice and rats, may be a useful animal model for biomedical research. Nonetheless, cotton rats are disadvantaged by their lack of appropriate anesthetic agents, and information regarding messenger RNA and amino acid sequences[1,7]. In cotton rats, inhalational anesthesia with halogenated ether such as isoflurane[1] and sevoflurane (unpublished data) have a short induction time, and their usage frequently results in deaths. Furthermore, although the combination of ketamine, xylazine, and acepromazine works well in cotton rats[1], ketamine has been categorized as a narcotic drug in Japan. Pentobarbital is no longer used as an anesthetic owing to its poor analgesic activity and narrow safety margins[8]. This study examined the anesthetic effects of the mixture of medetomidine, midazolam, and butorphanol on cotton rats. This mixture is introduced as an alternative to ketamine, and produces sufficient anesthetic effects in mice and rats[3–6].

Animal experimentation was performed in accordance with the guidelines issued by Hokkaido Institute of Public Health (approval no. K27-03). Cotton rats (*Sigmodon hispidus*) were maintained at Hokkaido Institute of Public Health since 1971, and were inbred by brother-sister mating since 1982[9]. Ten males (age: 1 to 19 months, body weight: 86 to 220 g) and nine females (age: 1 to 17 months, body weight: 67 to 190 g) were used. The anesthetic protocol simulated short-term laparotomy, such as ovariectomy. In brief, medetomidine hydrochloride (Me) (Dorbene®, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan), midazolam (Mi) (Dormicum®, Astellas Pharma Inc., Tokyo, Japan), and butorphanol (B) (Vetorphale®, Meiji Seika Pharma Co., Ltd.) were mixed at doses of 0.15, 2.0, and 2.5 mg/kg body weight of cotton rats, respectively (Me/Mi/B: 0.15/2.0/2.5), were diluted with sterile saline, and were injected subcutaneously at 0.5 ml/100 g. The anesthetic mixture was prepared at time of use. Following the injection, the animals were kept on the wood chip, and each anesthetic score was measured using a grading system described in a previous study[6]. The score was based on 5 reflexes: a front paw reflex, a hind paw reflex, a tail reflex, a corneal reflex, and a body righting reflex. If an animal lacked one of the aforementioned reflexes, it was given a score of 1. If it reacted, it was given a score of 0. The total anesthetic score was graded from 0 to 5. A cotton rat that scored 4 or more was considered to be under anesthetic condition. Induction time was defined as the duration from injected time to the start of anesthetic duration. The duration for which a cotton rat showed a score of either 4 or 5 was considered to be the anesthetic duration. The time required for the anesthetic score to return to 0 from the end of anesthetic condition was defined as the recovery time. Animals that showed an anesthetic duration of more than 80 min had atipamezole (Antisedan®, Nippon Zenyaku Kogyo Co., Ltd., Tokyo, Japan) injected intraperitoneally at a dose of 0.15 mg/kg to assess their recovery from anesthesia. The dose of atipamezole was same as that of medetomidine, which has sufficient effect to recover from the anesthesia in mice[5] and rats[6]. In addition, a two-fold volume of the anesthetic mixture, consisting of a dose of Me, Mi and B at 0.3, 4.0 and 5.0 mg/kg, respectively (Me/Mi/B: 0.3/4.0/5.0), was injected subcutaneously to three cotton rats. The results are expressed as mean ± standard error. The Mann–Whitney *U* test was used to compare data between sexes.

None of the cotton rats died during anesthesia, and all of them showed anesthetic scores of 4 or more. The induction time was 12.9 ± 3.1 min in

Table 1. Evaluation of the anesthetic effect in cotton rats

Sex	Induction time	Atipamezol injection	Anesthetic time	Recovery time
Male	12.9 ± 3.1 (1–31, n = 10)	No	49.5 ± 11.2 (10–77, n = 6)	44.5 ± 8.0 (23–76, n = 6)
		Yes	>80 (n = 4)	2.5 ± 0.6 (1–4, n = 4)
Female	9.4 ± 2.2 (1–25, n = 9)	No	61.5 (n = 2)	50.0 (n = 2)
		Yes	>80 (n = 7)	4.0 ± 1.1 (1–7, n = 7)

Unit: min. Data are presented as means ± standard error. Parentheses represent the range and the sample size. When the sample size is 2, data are presented as a mean. For the animals showing an anesthetic duration of more than 80 min, atipamezole was injected to access their effect on the recovery from anesthesia. Differences between sexes were analyzed using the Mann–Whitney *U* test. A *P* value less than 0.05 was considered to be statistically significant. There were no significant differences between the sexes.

males and 9.4 ± 2.2 min in females, showing no significant difference between the sexes (Table 1). The anesthetic duration was longer than 80 min for four out of 10 males and for seven out of nine females. Animals under anesthetic condition for less than 80 min had a mean time of the anesthetic duration of 49.5 min in males, and 61.5 min in females (Table 1). This suggested that the anesthetic effect was stronger in females than in males. In one male, the anesthetic time was 10 min, which was relatively shorter than other animals. The anesthetic duration showed poor correlation to the age or body weights (data not shown). The mean recovery time without atipamezole injection was 44.5 min in males, and 50.0 min in females (Table 1). For animals that had an anesthetic time longer than 80 min, an intraperitoneal administration of atipamezole, at the same dose as medetomidine, resulted in a quick recovery within 10 min (Table 1). There was no significant difference in the effect of atipamezole between sexes (Table 1). In all cotton rats that received Me/Mi/B: 0.3/4.0/5.0, the anesthetic time was longer than 120 min although no animals died during anesthesia (data not shown).

Generally, the anesthetic effects depend upon the dosage of the anesthetic agents, route of administration, and drug sensitivity of the animals. The dosage of Me, Mi and B shows species-specific differences. For mice, the Me/Mi/B: 0.3/4.0/5.0 mixture results in approximately 40–50 min of anesthetic time[3,4]. While in rats, a half dose of the mixture is sufficient for the same anesthetic effect as mice[6]. Our data demonstrated that the Me/Mi/B: 0.15/2.0/2.5 mixture produced sufficient anesthetic duration for short-term surgical procedures in cotton rats. For the cotton rats showing short anesthetic time as was seen in this study, the additional injection of Me/Mi/B: 0.15/2.0/2.5 mixture might be applicable since the two-fold dose of the anesthetic mixture resulted in no deaths. For longer surgical procedures, the Me/Mi/B: 0.3/4.0/5.0 mixture was also applicable. The Me/Mi/B mixture shows the nearly same anesthetic duration among intravenous, intraperitoneal, and subcutaneous routes in mice[5]. However, cotton rats have no suitable peripheral veins for intravenous drug administration[1]. Although tail veins are the most accessible for intravenous injection in mice and rats, tail skin of cotton rats easily degloves when the tail was picked up[1]. Compared to intraperitoneal route, subcutaneous route results in lower failure rate and less additional stress[5]. Our data indicated that no cotton rats died and that all cotton rats presented an adequate anesthetic time via the subcutaneous injection approach, suggesting that this was a preferable route for anesthesia in cotton rats. In addition, the medetomidine antagonist, atipamezole, was useful for the quick recovery of cotton rats, as well as mice and rats[5,6].

In conclusion, our study indicated that subcutaneous injection of an anesthetic mixture of medetomidine, midazolam, and butorphanol at a dose of 0.15, 2.0, and 2.5 mg/kg, respectively,

produced a favorable anesthetic effect for cotton rats. This anesthetic agent helps in the use of cotton rats in biomedical experiments.

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