HOKKAIDO UNIVERSITY

Title	Anesthetic effect of a mixture of medetomidine, midazolam and butorphanol in chickens with antagonism by atipamezole
Author(s)	Inoue, Atsuko; Ochi, Takehiro
Citation	Japanese Journal of Veterinary Research, 67(1), 133-137
Issue Date	2019-02
DOI	https://doi.org/10.14943/jjvr.67.1.133
Doc URL	https://hdl.handle.net/2115/72753
Type	departmental bulletin paper
File Information	133-137 Takehiro Ochi.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Japanese Journal of Veterinary Research 67(1): 133–137, 2019

JJVR

SHORT COMMUNICATION Experimental Research

Anesthetic effect of a mixture of medetomidine, midazolam and butorphanol in chickens with antagonism by atipamezole

Atsuko Inoue[1)] and Takehiro Ochi[2,*)]

[1)] Department of Pharmacotherapeutics, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Higashimura-cho, Fukuyama, Hiroshima 729–0292, Japan

[2)] Department of Biomedical Science, OT Pharma Research Laboratory, Kamihamuro, Takatsuki, Osaka 569–1044, Japan

Received for publication, May 16, 2018; accepted, August 6, 2018

Abstract

The anesthetic effect of a mixture of medetomidine, midazolam and butorphanol (Me/Mi/Bu), administered subcutaneously, was evaluated in healthy chickens. The Me/Mi/Bu mixture dose-dependently induced the duration of anesthesia. However, treatment with only one drug (Me 0.64 mg/kg, Mi 4.8 mg/kg or Bu 6.4 mg/kg) did not induce an anesthetic effect during the tests. The anesthetic effect of the Me/Mi/Bu mixture (Me/Mi/Bu: 0.64/4.8/6.4 mg/kg) was abolished by the α_2-adrenoceptor antagonist atipamezole. No marked changes in the body weight were observed in the chicken administered with these drugs. Taken together, these results suggest that the Me/Mi/Bu mixture exhibits reversible anesthetic effect and is safe to be used in studies involving chickens.

Key Words: anesthetic combination, chicken

Sodium pentobarbital has been available as anesthetic agent in animals. However, sodium pentobarbital is no longer used because of its poor analgesic activity and narrow safety margins[1)]. Therefore, general anesthesia with a single administration of sodium pentobarbital is not recommended. Recently, balanced anesthesia consisting of a mixture of medetomidine, midazolam and butorphanol (Me/Mi/Bu) has been clinically used in some animal species, such as mice[6)], rats[5,8)], beagle dogs[4,14)] and monkeys[10)]. The Me/Mi/Bu mixture that produced both sedative and analgesic effect is now in the spotlight for this new aspect. To the author's knowledge, there are no studies assessing the anesthetic effect of the Me/Mi/Bu mixture in birds. In this report, we assessed the effect of the Me/Mi/Bu mixture in chickens. The present paper concerns the anesthetic effect and safety of the Me/Mi/Bu mixture in chickens.

All animal experimental procedures performed in this study were approved by the Institutional Animal Care and Use Committee of OT Pharma Research Laboratory (approval no. 2016-BM-006). The animals were treated humanely throughout this research, and maximum care was taken to minimize pain on experimental animals. Every effort was made to minimize the number of

*Corresponding author: Takehiro Ochi, Department of Biomedical Science, OT Pharma Research Laboratory, Kamihamuro, Takatsuki, Osaka 569–1044, Japan
Phone: +81–72–693–9261. E-mail: blueocean_ty_ochi@maia.eonet.ne.jp
doi: 10.14943/jjvr.67.1.133

animals used and their degree of suffering.

Healthy female Brown poultry chickens (Mie-Hiyoko, Tsu, Japan) weighing 1.6–2.0 kg, at 7 months, were used in this study. The chickens were reared in a natural lighting conditioned chicken farm in a field environment. Water and poultry feed (Excellent 17, JA Nishinihon Kumiai Shiryo, Kobe, Japan) were provided *ad libitum*. Thirteen chickens received different treatments, at the rate of one treatment per week, in a randomized order. The present study was carried out at a temperature of 20–25°C and a humidity of 30–70%.

Medetomidine injection (1 mg/mL), midazolam injection (5 mg/mL) and butorphanol injection (5 mg/mL) were mixed in the same syringe just before the usage at the volume ratio of 2 : 3 : 4, respectively. Tonicity agent and pH adjuster were added in this drug combination. A subcutaneous (s.c.) administration was made in the leg of each animal at volumes of 0.09, 0.18, 0.36, 0.72, 1.44, 2.88 mL/kg for Doses 1–6, respectively. The s.c. administration of the Me/Mi/Bu mixture was followed by the s.c. administration of atipamezole, into the contralateral leg of chickens, after 30 min. The anesthetic effect of the drugs was evaluated based on the animal posture, which was evaluated according to the following criteria: Score 0, normal; Score 1, sedated but able to stand; Score 2, sterna recumbency; Score 3, lateral recumbency with apparent spontaneous movement (head and/or limb); Score 4, lateral recumbency with subtle spontaneous movement (twitching and/or blink); Score 5, lateral recumbency without spontaneous movement and unable get up[3]. Add to posture scoring in animals with Score 5, we tested body-righting reflexes, skin reflexes on sensitive part of body and hind paw reflexes[9]. Chickens with Score 5 occurred with the lack of body-righting reflexes, skin reflexes on sensitive part of body and hind paw reflexes were regarded as showing positive anesthesia. In all experiments, chickens were maintained on hot water mats to stabilize body temperature while under anesthesia.

The following drugs were used in this study: medetomidine hydrochloride (Domitor Injection®, Nippon Zenyaku Kogyo, Fukushima, Japan), midazolam (Dormicum Injection®, Astellas Pharma, Tokyo, Japan) and butorphanol tartrate (Vetorphale Injection®, Meiji Seika Pharma, Tokyo, Japan).

The following antagonist was used in this study: atipamezole hydrochloride (Antisedan Injection®, Nippon Zenyaku Kogyo, Fukushima, Japan).

The results obtained are expressed as means ± standard error of the mean (SEM). The statistical significance was analyzed using the one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. The difference among the groups was considered statistically significant when $P < 0.05$.

Fig. 1 shows the anesthetic effect of s.c. administered the Me/Mi/Bu mixture (Doses 1–6) in chickens, as assessed by scoring. Drug administration was conducted at the following doses: Dose 1, Me 0.02 mg/kg-Mi 0.15 mg/kg-Bu 0.2 mg/kg; Dose 2, Me 0.04 mg/kg-Mi 0.3 mg/kg-Bu 0.4 mg/kg; Dose 3, Me 0.08 mg/kg-Mi 0.6 mg/kg-Bu 0.8 mg/kg; Dose 4, Me 0.16 mg/kg-Mi 1.2 mg/kg-Bu 1.6 mg/kg; Dose 5, Me 0.32 mg/kg-Mi 2.4 mg/kg-Bu 3.2 mg/kg; Dose 6, Me 0.64 mg/kg-Mi 4.8 mg/kg-Bu 6.4 mg/kg. Low doses of the Me/Mi/Bu mixture (Doses 1–3) did not induce lateral recumbency during the test; however, high doses of the Me/Mi/Bu mixture (Doses 4–6) rapidly induced immobilization and lateral recumbency i.e. anesthesia, in a dose-dependent manner. The duration of anesthesia for the Me/Mi/Bu mixture (Doses 4–6) was as follows: Dose 4 (5 ± 5 min), Dose 5 (42 ± 4 min) and Dose 6 (107 ± 9 min) (Table 1). Conversely, the treatment with only one drug (Me 0.64 mg/kg, Mi 4.8 mg/kg or Bu 6.4 mg/kg) of Dose 6 did not induce sternal recumbency (score: > 2) during the test.

The anesthetic effect caused by the s.c. administration of the Me/Mi/Bu mixture (Dose 6) was reversed when atipamezole was injected at doses of 0.64, 1.28 or 2.56 mg/kg (Fig. 2). The

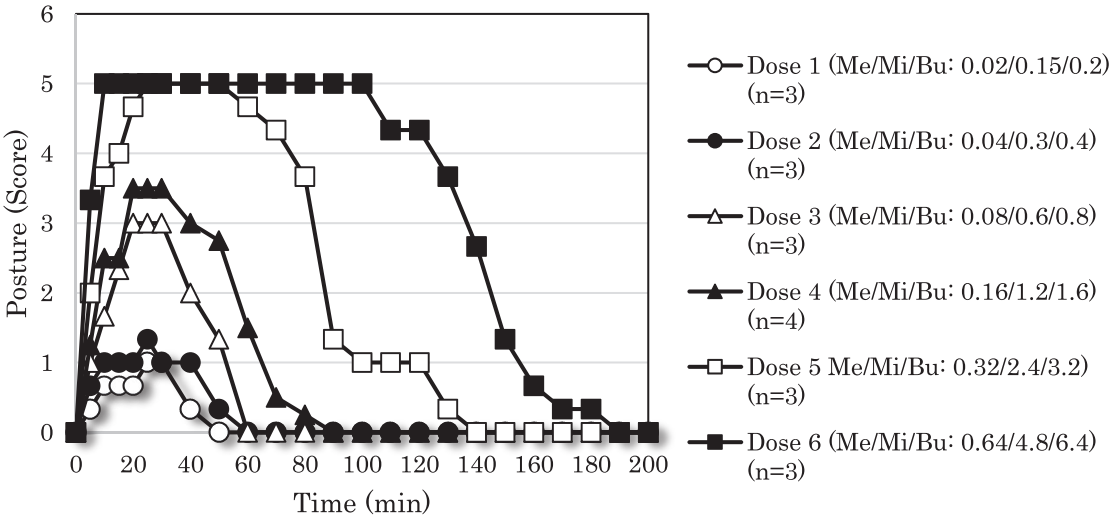

Fig. 1. Anesthetic effect of the medetomidine/midazolam/butorphanol mixture (Doses 1–6; s.c.) in chickens, as assessed by scoring. Each symbol indicates the mean value.

Table 1. Duration time of positive anesthesia for the medetomidime/midazolam/butorphanol mixture

Treatment (mg/kg; s.c.)	Number of animals	Duration time of positive anesthesia (min)
Dose 1 (Me/Mi/Bu:0.02/0.15/0.2)	3	0 ± 0 (NE)
Dose 2 (Me/Mi/Bu:0.04/0.3/0.4)	3	0 ± 0 (NE)
Dose 3 (Me/Mi/Bu:0.08/0.6/0.8)	3	0 ± 0 (NE)
Dose 4 (Me/Mi/Bu:0.16/1.2/1.6)	4	5 ± 5
Dose 5 (Me/Mi/Bu:0.32/2.4/3.2)	3	42 ± 4**
Dose 6 (Me/Mi/Bu:0.64/4.8/6.4)	3	107 ± 9**++

Chickens with Score 5 occurred with the lack of body-righting reflexes, skin reflexes on sensitive part of body and hind paw reflexes were regarded as showing positive anesthesia.
Data are presented as the means ± SEM. Abbreviation; NE, no effect. $^{**}P < 0.01$, as compared with Dose 4 group. ++ $P < 0.01$, as compared with Dose 5 group.

duration of anesthesia for the Me/Mi/Bu mixture (Dose 6-control) was 118 ± 12 min. As additional data, chickens administered only with atipamezole 2.56 mg/kg s.c. were in normal condition.

None of the chickens died during the anesthetic effect, and there were no significant differences of body weights in chickens administered with these drugs (Table 2). Added to this, no serious abnormality was detected on the general physical examination in any chicken.

Herein, we examined the anesthetic effect of the Me/Mi/Bu mixture in chickens and observed that it rapidly induced lateral recumbency without spontaneous movement and reflex responses. After the s.c. administration in chickens, the Me/Mi/Bu mixture exerted a dose-dependent anesthetic effect that was completely reversed by α_2-adrenoceptor antagonist atipamezole.

Medetomidine is a potent α_2-adrenoceptor agonist that stimulates presynaptic adrenoceptors in the CNS, producing sedative and analgesic effects[12]. Midazolam is a benzodiazepine receptor agonist that causes sedation through the GABA neurotransmitter[11]. Butorphanol, that exhibits partial agonist and antagonist activity at opioid receptors in the CNS, blocks pain impulses in

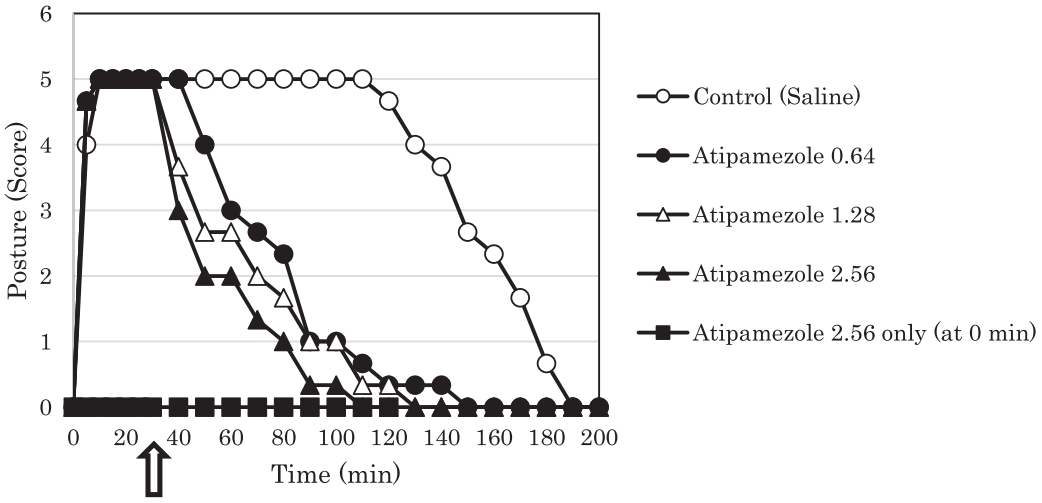

Fig. 2. Effect of atipamezole on the medetomidime/midazolam/butorphanol mixture (Dose 6; s.c.)-induced anesthesia in chickens, as assessed by scoring. Atipamezole was administered subcutaneously 30 min after the medetomidine/midazolam/butorphanol mixture injection. An arrow shows atipamezole injection. Each symbol indicates the mean value (n = 3).

Table 2. Change in the body weight before and after drug administration

Treatment (mg/kg; s.c.)	Number of animals	Body weight (kg)	
		Before administration	3 days after administration
1) Dose response			
Dose 1 (Me/Mi/Bu:0.02/0.15/0.2)	3	1.87 ± 0.07	1.87 ± 0.07
Dose 2 (Me/Mi/Bu:0.04/0.3/0.4)	3	1.87 ± 0.07	1.87 ± 0.07
Dose 3 (Me/Mi/Bu:0.08/0.6/0.8)	3	1.93 ± 0.07	1.93 ± 0.07
Dose 4 (Me/Mi/Bu:0.16/1.2/1.6)	4	1.85 ± 0.05	1.85 ± 0.05
Dose 5 (Me/Mi/Bu:0.32/2.4/3.2)	3	1.73 ± 0.07	1.80 ± 0.00
Dose 6 (Me/Mi/Bu:0.64/4.8/6.4)	3	1.73 ± 0.07	1.80 ± 0.00
2) Antagonism			
Dose 6 + Control (saline)	3	1.93 ± 0.07	1.87 ± 0.07
Dose 6 + Atipamezole 0.64	3	1.87 ± 0.07	1.87 ± 0.07
Dose 6 + Atipamezole 1.28	3	1.93 ± 0.07	1.93 ± 0.07
Dose 6 + Atipamezole 2.56	3	1.80 ± 0.00	1.80 ± 0.00

Data are presented as the means ± SEM.

postoperative patients[2]. Thus, each drug included in the Me/Mi/Bu mixture shows different pharmacological mechanisms and effects.

Effective doses of the Me/Mi/Bu mixture in mice, rats, dogs, and monkeys are 0.3/4/5, 0.15/2/2.5, 0.04/0.5/0.3, and 0.04/0.3/0.4 mg/kg, respectively[4,6,8,10]. In the present study, the anesthetic effect of the Me/Mi/Bu mixture in chickens, at doses of 0.64/4.8/6.4 mg/kg (Dose 6), was maintained for long duration. Conversely, administration of the Me/Mi/Bu mixture (Dose 2), which induces anesthetic effect in monkeys, did not showed a positive anesthetic effect in chickens. This data shows that there are differences regarding the anesthetic potency of the Me/Mi/Bu mixture among species, such as observed for monkeys and chickens.

Atipamezole is an α_2-adrenoceptor antagonist

that reverses the effect of medetomidine[13]. It is reported that the anesthetic effect of a drug combination including medetomidine, such as the Me/Mi/Bu mixture, is antagonized by atipamezole in laboratory animals[7,8,10,14]. In chickens, injection of atipamezole had also the same recovery from anesthesia induced by the Me/Mi/Bu mixture.

Regarding the acute toxicological study of the Me/Mi/Bu mixture, we estimated the body weight of the chickens during treatment with these drugs. Three days after the administration of these anesthetic drugs, there was no body weight loss comparing the pre-dosing case in the way of one toxicological parameter. Additionally, none of the chickens died during the anesthetic effect.

In conclusion, the Me/Mi/Bu mixture, which provided a longer anesthetic effect and was fully reversible, is acceptable for usage in chickens.

References

1) Erhardt W, Hebestedt A, Aschenbrenner G, Pichotka B, Blümel G. A comparative study with various anesthetics in mice (pentobarbitone. Ketamine-xylazine, carfentanyl-etomidate). Res Exp Med 184, 159–169, 1984
2) Gupta R, Kaur S, Singh S, Aujla KS. A comparison of epidural butorphanol and tramadol for postoperative analgesia using CSEA technique. J Anaesth Clin Pharmacol 27, 35–38, 2011
3) Hayashi K, Nishimura R, Yamaki A, Kim HY, Matsunaga S, Sasaki N, Takeuchi A. Comparison of sedative effects induced by medetomidine, medetomidine-midazolam and medetomidine-butorphanol in dogs. J Vet Med Sci 56, 951–956, 1994
4) Itamoto K, Hikasa Y, Sakonjyu I, Itoh H, Kakuta T, Takase K. Anaesthetic and cardiopulmonary effects of balanced anaethesia with medetomidine-midazolam and butorphanol in dogs. J Vet Med A Phaysiol Pathol Clin Med 47, 411–420, 2000
5) Kageyama A, Tohei A, Ushijima H, Okada K. Anesthetic effects of a combination of medetomidine, midazolam and butorphanol on the production of offspring in Japanese field vole, *Microtus montelli*. J Vet Med Sci 78, 1283–1291, 2016
6) Kawai S, Takagi Y, Kaneko S, Kurosawa T. Effect of three types of mixed anesthetic agents alternate to ketamine in mice. Exp Anim 60, 481–487, 2011
7) Kirihara Y, Takechi M, Kurosaki K, Kobayashi Y, Saito Y, Takeuchi T. Anesthetic effects of a three-drugs mixture—comparison of administrative routes and antagonistic effects of atipamezole in mice—. Exp Anim 64, 39–47, 2015
8) Kirihara Y, Takechi M, Kurosaki K, Kobayashi Y, Saito Y, Takeuchi T. Effect of an anesthetic mixture of medetomidine, midazolam, and butirphanol in rats—strain difference and antagonim by atipamezole. Exp Anim 65, 27–36, 2016
9) Nakamura T, Ichii O, Irie T, Hosotani M, Dantsuka A, Nakamura S, Sato S, Sotozaki K, Kouguchi H, Yoshiyasu T, Nagasaki K, Kon Y. Usefulness of an anesthetic mixture of medetomidine, midazolam, and butorphanol in cotton rats (*Sigmodon hispidus*). Jpn J Vet Res 64, 273–276, 2016
10) Ochi T, Nishiura I, Tatsumi M, Hirano Y, Yahagi K, Sakurai Y, Matsuyama-Fujiwara K, Sudo Y, Nishina N, Koyama H. Anesthetic effect of a combination of medetomidine-midazolam-butorphanol in cynomolgus monkeys (*Macaca fascicularis*). J Vet Med Sci 76, 917–921, 2014
11) Reves JG, Fragen RJ, Ronald Vinik H, Greenblatt DJ. Midazolam: Pharmacology and uses. Anesthesiology 62, 310–324, 1985
12) Vainio O, Väha-Vähe T, Palmu L. Sedative and analgesic effects of medetomidine in dogs. J Vet Pharmacol Ther 12, 225–231, 1989
13) Vainio O, Väha-Vähe T. Reversal of medetomidine sedation by atipamezole in dogs. J Vet Pharmacol Ther 13, 15–22, 1990
14) Verstegen J, Petcho A. Medetomidine-butorphanol-midazolam for anaesthesia in dogs and its reversal by atipamezole. Vet Rec 132, 353–357, 1993