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Inoue, Atsuko; Ochi, Takehiro

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

Atsuko Inoue1) and Takehiro Ochi2,*)

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

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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 α₂-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 margins1). 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 mice6), rats5,8), beagle dogs4,14) and monkeys10). 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
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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 Nishinihan Kumiai Shityo, 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. 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
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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

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

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 α2-adrenoceptor antagonist atipamezole.

Medetomidine is a potent α2-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
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 show 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 \(\alpha_2\)-adrenoceptor antagonist

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

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

Data are presented as the means ± SEM.
that reverses the effect of medetomidine\textsuperscript{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\textsuperscript{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