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MICRO REPORT

Antinociceptive effects of the combined use of butorphanol and buprenorphine in mice

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Abstract

Butorphanol and buprenorphine are mixed opioid receptor agonist-antagonist drugs widely used as analgesics in people and animals. There are few reports concerning the interaction of multiple opioids, and their antinociceptive effects, when combined with other opioids, remain unclear. Therefore, we report the preliminary findings of the antinociceptive effects of the combined use of butorphanol and buprenorphine in C57BL/6JJcl mice. Both drugs were administered either simultaneously or in different orders. Compared with the baseline values, the tail-flick and hot-plate test latencies increased regardless of the order of administration. Furthermore, enhanced latencies were observed on administration of butorphanol followed by buprenorphine. Combined use of these drugs may not attenuate analgesic efficacy. Besides, enhancement of these effects can be obtained by changing the order of the administration of these drugs. It is necessary to further investigate the molecular basis of the underlying mechanism in future definitive studies.

KEYWORDS

antinociception, buprenorphine, butorphanol, mixed opioid receptor agonist-antagonist

1 | INTRODUCTION

Butorphanol and buprenorphine are mixed opioid receptor agonist-antagonist drugs[1] widely used as analgesics in people and animals.[2–5] Both drugs have complex actions on multiple receptors, with conflicting pharmacological effects. In fact, although butorphanol is considered a μ-opioid receptor antagonist,[3,4] it may have agonistic effects on antinociception in some laboratory animals.[6–8] Furthermore, the agonism of the κ-opioid receptor in its analgesic effects has been reported,[9] whereas the role of δ-opioid receptors in the antinociceptive effect remains unclear. Buprenorphine has a unique mechanism of action involving μ- and nociception/orphanin FQ receptors-mediated partial agonistic antinociception as well as the agonism of κ- and δ-opioid receptors,[10–13] while κ-opioid receptor inverse agonism and δ-opioid receptor antagonism have also been reported.[14] Currently, the combined use of buprenorphine and butorphanol is not a common practice in the clinical setting, but there is interest in the antinociceptive effects of the combined use of these drugs in veterinary medical sciences.[6] However, in addition to the conflicting information on the pharmacological profiles of each drug as described earlier, there are few reports on the interaction of multiple opioids[15]; thus, their antinociceptive effects, when combined with other opioids, remain unclear.[11,16] Furthermore, the product information[17] and global veterinary medical guidelines contain statements on the careful administration of this combined therapy.[18] Therefore, we conducted a brief investigation and report preliminary findings of the antinociceptive effects of the combined use of butorphanol and buprenorphine in C57BL/6JJcl mice.

2 | MATERIALS AND METHODS

2.1 | Animals

We purchased male C57BL/6JJcl mice (age: 27-35 weeks) from CLEA Japan, Inc (Tokyo, Japan). All mice were housed in pairs (five to six per cage) in a temperature-controlled animal holding room at 23 ± 1°C, with a relative humidity of 55 ± 10% under a 12-hours/12-hours light/dark cycle (lights on 8:00 AM – off 8:00 PM). They were given ad libitum access to a commercial diet and tap water. All animals were considered healthy based on physical examination.

2.2 | Drugs

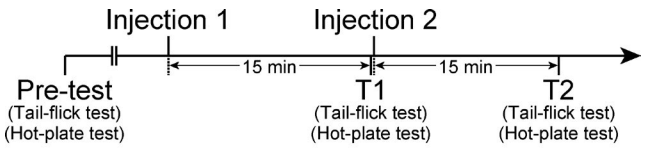
Butorphanol tartrate and buprenorphine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in saline and intraperitoneally injected at 3 mg/kg, with a volume of 100 mL/kg for each administration. Because both drugs reach a plateau at a dose of approximately 1–3 mg/kg,[7,19] we used a dose of 3 mg/kg.

2.3 | Nociceptive tests

For each tail-flick and hot-plate test, the animals were divided into three groups according to the drug administration: simultaneous butorphanol and buprenorphine (Group 1; n = 6); butorphanol followed by buprenorphine (Group 2; n = 6); and buprenorphine followed by butorphanol (Group 3; n = 6). We conducted modified tail-flick and hot-plate tests between 9:00 AM and 6:00 PM, as previously described.[20] Briefly, during the tail-flick test, we focused a light beam on the tail approximately 1-3 cm from the base; subsequently, the latency to flick the tail after the heat stimulus was measured with a 15-second cutoff time using a specific apparatus (Model MK-330A; Muromachi Kikai). We measured the latencies twice per mouse and used the average value. During the hot-plate test, the hot-plate apparatus (Model MK-350A; Muromachi Kikai) was maintained at 52 ± 0.5°C; the latency to lick the hind paws or jump after the heat stimulus was measured using a 60-s cutoff time. The latency measurement was performed once per mouse. After completing the baseline nociceptive test, each test (Test 1 [T1] and Test 2 [T2]) was conducted at 15-minutes intervals (Figure 1).

2.4 | Statistical analysis

The estimated sample size was 5-7 mice per group to detect a 20% effect on tail-flick and hot-plate latencies with a significance level and power of 0.05% and 80%, respectively. Continuous variables were tested for normality using the Shapiro-Wilk test. Antinociceptive effects were evaluated using paired *t* tests. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics software version 24 (IBM Japan Ltd.).



	Injection 1	Injection 2
Group 1	But (3 mg/kg) + Bup (3 mg/kg)	-
Group 2	But (3 mg/kg)	Bup (3 mg/kg)
Group 3	Bup (3 mg/kg)	But (3 mg/kg)

FIGURE 1 Experimental design. But, butorphanol; Bup, buprenorphine; T1, Test 1; T2, Test 2

3 | RESULTS

In Group 1, latency increased compared with baseline values of the tail-flick test ($P = 0.047$ [T1] and $P = 0.010$ [T2]) and hot-plate test ($P = 0.010$ [T1] and $P = 0.046$ [T2]) (Figure 2). In Group 2, latency increased compared with baseline values of the tail-flick test ($P = 0.004$ [T1] and $P = 0.001$ [T2]) and hot-plate test ($P = 0.017$ [T1] and $P = 0.009$ [T2]) (Figure 2). The enhancement of antinociceptive effect on the tail-flick test and hot-plate test was observed in Group 2 ($P = 0.029$ and $P = 0.034$ [T1 vs T2], respectively) (Figure 2). In Group 3, latency increased compared with baseline values of the tail-flick test ($P = 0.006$ [T1] and $P = 0.006$ [T2]) and hot-plate test ($P = 0.006$ [T1]) (Figure 2). None of the mice showed clinically obvious experiment-related adverse events at 24 hours after the nociceptive test.

4 | DISCUSSION

The intraperitoneal administration of combined butorphanol and buprenorphine did not attenuate the antinociceptive effects against a thermal stimulus. Traditionally, the combined use of multiple opioids is controversial in people and animals because of the potential side effects.[3,4,21,22] The combined use of multiple opioids for antinociception is a new concept, but the possible combination of morphine and oxycodone for effective treatment on postoperative analgesia in people has been reported.[21] In the present study, we observed the influence of the order of administration on the antinociceptive effects of this combination of mixed opioids. As described earlier, molecular actions of butorphanol and buprenorphine are still controversial; thus, we could not develop the discussion at this stage. However, we may interpret our findings on the basis of previous reports, which indicated the possible μ-opioid receptor agonistic role of butorphanol with regard to its antinociceptive effects in rats[8] and cats.[6] Furthermore, Ide et al reported that thermal antinociceptive

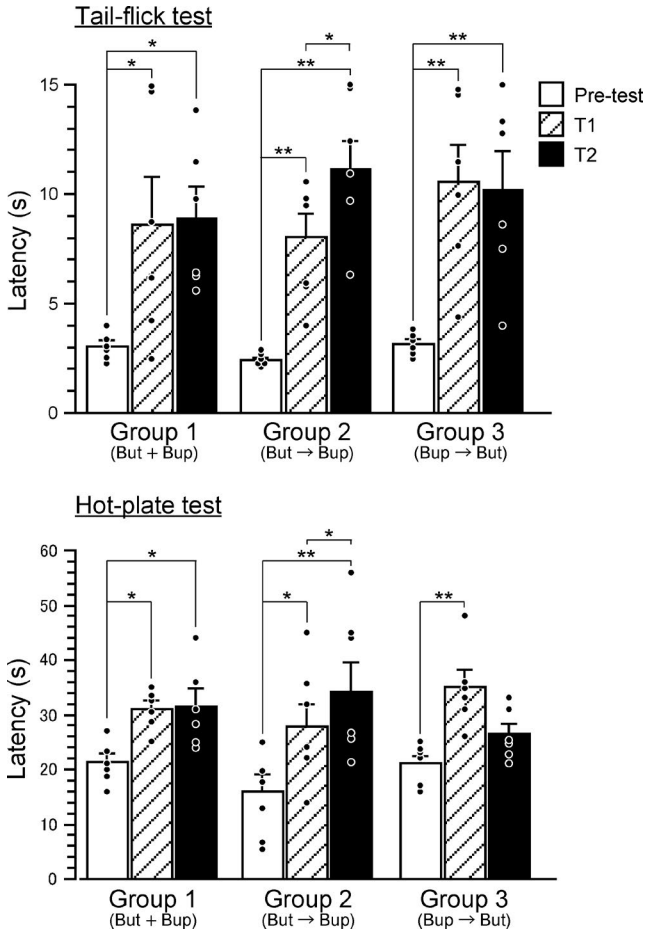


FIGURE 2 Antinociceptive effects of butorphanol and buprenorphine during the tail-flick and hot-plate tests. Nociceptive latencies in seconds for C57BL/6JJcl mice. $^{*}P < 0.05$ and $^{**}P < 0.01$, paired *t* test. But, butorphanol; Bup, buprenorphine; T1, Test 1; T2, Test 2. Each bar represents the mean ± SEM

effects of butorphanol were eliminated in μ-opioid receptor knockout mice.[7] On combining these mixed opioids, the μ-opioid receptor may have a central role in antinociception regardless of agonistic or antagonistic actions to κ-opioid receptors of each drug. We speculate that δ-opioid receptors could contribute to the antinociception, as observed in Group 2 (administration of butorphanol followed by buprenorphine). Although there are few reports on the pharmacological actions of these drugs on the δ-opioid receptors, butorphanol may act as an agonist,[23] whereas buprenorphine is considered antagonistic to these receptors.[10] Although there were no statistically significant differences, decreased hot-plate latencies were observed on the administration of buprenorphine followed by butorphanol (Group 3). Considering the results of the tail-flick test and lower expression levels of δ-opioid receptors in the spinal cord,[24] butorphanol might act on the supraspinal area under the administration order. Additional molecular-based research is required for further clarification.

This study had several limitations. First, the study was not randomized or blinded. Second, the study was conducted at a specific age for male C57BL/6JJcl mice. Third, we only tested thermal stimuli; therefore, further research of other nociceptive stimuli is necessary. Finally, we examined single-administration doses; thus, the dose-dependent antinociceptive effects remain unclear.

In conclusion, the use of combined butorphanol and buprenorphine did not attenuate the antinociceptive effects on spinal reflection against thermal stimuli. However, the administration order of these drugs may diminish their antinociceptive effects on supraspinal response in C57BL/6JJcl mice. Further research to elucidate solid evidence of the combined use of these drugs and to clarify the underlying mechanism is necessary.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

Kazumasu Sasaki involved in conception and design of the study, behavioral test, data interpretation, literature research, and manuscript drafting. Tatsuya Ishikawa involved in interpretation and literature research. Kazutaka Ikeda involved in interpretation and literature research. Shinya Kasai involved in design of study, analyzed the data, data interpretation, and literature research. All authors contributed to the manuscript and approved the submitted version.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEWER BOARD

N/A.

INFORMED CONSENT

N/A.

REGISTRY AND THE REGISTRATION

N/A.

ANIMAL STUDIES

All procedures were reviewed, and approval was granted by the Tokyo Metropolitan Institute of Medical Science Animal Care and Use Committee (no. 20-019). The study was conducted following the *Guide for the Care and Use of Laboratory Animals* (eighth edition; NRC 2011).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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