Midazolam Can Potentiate the Analgesic Effects of Intrathecal Bupivacaine on Thermal- or Inflammatory-Induced Pain

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Epidurally administered midazolam can potentiate analgesia by epidural bupivacaine. However, whether this effect is synergistic or additive is not known. In this study, we investigated the spinally-mediated analgesic interaction between midazolam and bupivacaine by using the tail-flick and formalin tests in rats with chronically implanted catheters. Behavioral effects were also observed. The dose dependency of analgesia and the 50% effective doses of intrathecal midazolam and bupivacaine were determined, and then the interaction of these two drugs was examined with an isobolographic analysis. Both drugs had dose-dependent analgesic effects in both the tail-flick test and the formalin test. The 50% effective dose values of the combination were significantly lower than the calculated additive values in both tests (P < 0.023 in the tail-flick test; P = 0.0325 in Phase 1 and 0.047 in Phase 2 of the formalin test). Behavioral side effects decreased in the combination group compared with each drug alone. In conclusion, intrathecally administered midazolam and bupivacaine had synergistic analgesic effects on acute thermal- or inflammatory-induced pain, with decreased behavioral side effects.


Midazolam produces an analgesic action through the benzodiazepine/γ-aminobutyric acid (GABA)_A receptor complex in the spinal cord (1). Epidurally administered midazolam had an analgesic effect on postoperative somatic, but not visceral, pain in a clinical study (2). When we administered bupivacaine with midazolam to block visceral pain in postoperative epidural analgesia in abdominal surgery, either by bolus (3) or continuous administration (4), epidurally administered midazolam potentiated the analgesic effect of bupivacaine. However, in these clinical studies with epidural administration, it is not known whether the potentiation of the analgesic effects of bupivacaine by midazolam is synergistic or additive. The purpose of this study was to investigate the spinally mediated analgesic interaction between midazolam and bupivacaine in animal experiments.

Methods
After obtaining the approval of the Research Committee of the University of Tokyo, male Sprague-Dawley rats (280–300 g for the tail-flick test and behavioral test and 330–350 g for the formalin test; Nippon Bio-Supply, Tokyo, Japan) were implanted with lumbar intrathecal catheters under sevoflurane (3%) in 100% oxygen. An 8.5-cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ) was inserted caudally to the thoracolumbar level in the intrathecal space through the atlantooccipital membrane. The rostral part of the catheter was plugged with a 28-gauge steel wire and put through to the top of the skull. Only rats with normal motor function and behavior 7 days later were used. After the study, the location of the catheter was confirmed anatomically, and the data of the rats with mal-location of the catheter were excluded. In each dose group, 10 randomly selected rats were used after the exclusion. In total, 140 rats for the tail-flick test and behavioral study and 140 rats for the formalin test were used.

Midazolam (Sigma, St. Louis, MO) and bupivacaine (Sigma) were dissolved in normal saline to make a solution of 1, 3, 10, 30, or 100 μg (midazolam) or 1, 10, 30, or 100 μg (bupivacaine) in 10 μL. For the interaction study, the combination of each 1/2, 1/4, 1/8, or 1/16 of 50% effective dose (ED_{50}) values was adjusted to make...
a 10-µL solution. Normal saline was used as a control. After injection of the drug, the catheter was flushed with normal saline 10 µL to clear the dead space of the catheter (8 ± 0.5 µL).

The tail-flick test was performed with the Tail-Flick Analgesia Meter (MK-330A; Muramachi Kikai Co. Ltd., Tokyo, Japan). Rats were placed in a clear plastic cage with their tails extending through a slot located at the rear of the cage. Thermal stimulation was given by a beam of high-intensity light focused on the tail 2 to 3 cm proximal to the end. The time between the start of the stimulation and tail withdrawal was measured as the tail-flick latency. The cutoff time in the absence of a response was set to 14 s to prevent tissue injury of the tail. The test was performed at 5, 10, 15, 30, 60, 90, 120, 180, 240, and 300 min after drug injection. The data are shown as the percentage of maximum possible effect (%MPE):

$$\% \text{MPE} = \frac{(\text{postdrug latency} - \text{predrug latency})}{(\text{cutoff time} - \text{predrug latency})} \times 100.$$  

The formalin test was performed 10 min after intrathecal drug injection. Fifty microliters of 5% formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30-gauge needle. Immediately after injection, the rats were placed in an open clear plastic chamber, and its flinching or shaking-paw response was observed for 60 min. The number of flinches was counted for 1 min. Usually two phases were observed: Phase 1 during 0 to 6 min after injection and Phase 2 beginning approximately 10 min after injection, with an interval of no flinches between phases.

Side effects were examined and judged as present or absent in rats for the tail-flick test. Agitation was judged as spontaneous irritable movement, vocalization, or both. Allosthenia-like behavior was judged as escape, vocalization, or both induced by lightly stroking the flank of the rat with a small probe. The placing or stepping reflex was evoked by drawing the dorsum of either hind paw across the edge of the table. Normal rats try to put the paw ahead into a position to walk. The righting reflex was assessed by placing the rat horizontally with its back on the table. Normally, rats twist the body to an upright position immediately. Faccidity was judged as muscle weakness by putting the forepaw 3 to 5 cm higher than the hind paw. Normal rats will walk up. The pinna reflex exam was examined with a paper string. When a string is put into the ear canal, rats normally shake their heads.

At first, both the tail-flick test and the formalin test were performed to determine the dose dependency of the analgesic effects and the ED50 of intrathecal midazolam or bupivacaine. The ED50 was obtained by using the maximum effects in the tail-flick test and the area under the curve in the formalin test. Second, to investigate the interaction between midazolam and bupivacaine, an isobolographic analysis was used. The combinations of each 1/2 ED50, 1/4 ED50, 1/6 ED50, or 1/8 ED50 were tested, and the ED50 of the combination was determined. A total fractional dose value was calculated to describe the magnitude of the interaction as follows: (ED50 dose of midazolam in combination)/(ED50 dose of midazolam alone) + (ED50 dose of bupivacaine in combination)/(ED50 dose of bupivacaine alone).

The value was normalized by assigning the ED50 value of each drug given alone as 1. Values near 1 suggest an additive interaction, values >1 imply an antagonistic interaction, and values <1 indicate a synergistic interaction.

Statistical analysis was performed with Student's t-test to compare the calculated ED50 values with the theoretical additive values. A P value <0.05 was considered to be statistically significant.

## Results

Both intrathecally administered midazolam and bupivacaine produced dose-dependent increases of the tail-flick latency (Fig. 1, A and B). The combination of midazolam and bupivacaine also induced dose-dependent increases of the tail-flick latency (Fig. 1C). The ED50 values of the combination were significantly smaller than the calculated additive value (P = 0.023; Fig. 2; Table 1). The total fractional dose value was 0.58 ± 0.10 (mean ± se).

Midazolam, bupivacaine, or their combination produced a dose-dependent decrease in the number of flinches in both Phase 1 and 2 of the formalin test (Fig. 3A–C). The ED50 values in the combination of the formalin test were significantly less than the calculated additive values (P = 0.0025 in Phase 1 and 0.047 in Phase 2; Fig. 4; Table 1). The total fractional dose value was 0.17 ± 0.09 in Phase 1 and 0.71 ± 0.11 in Phase 2 of the formalin test.

Side effects are shown in Table 2. Intrathecal midazolam 3 µg or bupivacaine 30 µg induced agitation or allosthenia, and midazolam 30 µg or bupivacaine 10 µg induced motor disturbances (Table 2). The combination of midazolam and bupivacaine used in this study did not produce any observable side effects.

## Discussion

This study showed that in models of thermal and acute inflammatory pain, midazolam could enhance the analgesic effects of bupivacaine synergistically in intrathecal administration. The side effects decreased in the combination compared with each drug alone.
The GABAergic system plays an important role in the presynaptic inhibition of primary afferents. Benzodiazepine receptor agonists increase the intrinsic efficacy of GABA at the GABA\textsubscript{A} receptor coupling with the benzodiazepine receptor in the spinal cord (5) by increasing Cl\textsuperscript{−} conductance (6), and they reduce the release of glutamate in the spinal cord (7). Intrathecally administered midazolam acts on GABA receptors and induces reversible segmental antinociception (1,8,9).

GABA\textsubscript{A} receptor agonists inhibit the behavioral effects of glutamate such as N-methyl-D-aspartate, quisqualic acid, and kainic acid (10). Thus, GABA\textsubscript{A} receptors might also exhibit a functional coupling with glutamate receptors at postsynaptic sites in the spinal cord with regard to nociceptive transmission. In animal studies, intrathecally administered midazolam synergistically increased the analgesic potency of N-methyl-D-aspartate or \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonists (11). However, midazolam-induced analgesia also has been linked to a non-\(\mu\)-opioid mechanism, possibly...
via κ opioid receptors in the spinal cord (12). Facilitation of morphine binding to spinal opioid receptors was also observed at small concentrations of midazolam (13).

Figure 3. Time-response curves for analgesia by midazolam, bupivacaine, and their combination in the formalin test. Values are means, and bars indicate SE (n = 8).

Figure 4. Isobolograph for the interaction of midazolam and bupivacaine in the formalin test. Bars indicate 95% confidence intervals. The x and y axes show the dose (μg) of midazolam and bupivacaine, respectively. The 50% effective dose (ED50) values of the combination were significantly smaller than the calculated additive value (P = 0.0025 in Phase 1 and 0.047 in Phase 2).

The analgesic effect of intrathecal bupivacaine was potentiated by intrathecal midazolam. In humans, the addition of 1 or 2 mg of intrathecal midazolam prolonged the postoperative analgesic effect of bupivacaine by approximately 2 and 4.5 hours, respectively (14). Bupivacaine blocks sodium currents and rapidly inactivating potassium currents in spinal dorsal horn neurons (15). Although intrathecal midazolam and bupivacaine were synergistic in antinociception in this study, no anatomical interaction has been reported between GABA_A receptors and sodium or potassium channels. Intrathecal bupivacaine significantly potentiated the antinociception produced by intrathecal morphine (16). This facilitation of morphine-induced
Antinociception by bupivacaine was thought to be associated with a conformational change in the spinal opioid receptors induced by bupivacaine (16). This change allows morphine to bind more easily to spinal opioid receptors. The same mechanism might occur at benzodiazepine-binding sites at GABA_A receptors in the spinal cord by bupivacaine, although further studies are necessary to confirm this. Considering the side effects—such as agitation, allodynia, or loss of the pinna reflex—shown in this study, intrathecally administered midazolam and bupivacaine could spread into the brain. Therefore, we cannot deny that some parts of the analgesic effects might be mediated in the brain.

To apply the results of this study to clinical practice, the neurotoxicity of the drugs should be determined. Serious neurologic sequelae have occurred after spinal administration of local anesthetic (17), although the mechanism is not known. Bupivacaine is reported not to be neurotoxic when administered at a concentration of <0.75% (18) or with hypobaric solutions (19). Intrathecally administered midazolam with larger doses (100 µg/d for 20 days or 300 µg) than those used in this study caused damage in the spinal cord (20,21). However, 10 mg of midazolam administered directly to the spinal cord of cats did not induce any histological changes in the spinal cord (22), and other studies with intrathecal midazolam also did not show neurotoxicity (23,24). From these reports, neurotoxicity by both bupivacaine and midazolam appears to occur mainly with large doses, and it is possible that smaller doses are less toxic. Therefore, synergistic analgesic interaction of these two drugs might decrease neurotoxicity. In addition, decreased behavioral side effects are preferable.

In conclusion, intrathecally administered midazolam and bupivacaine had synergistic analgesic effects on acute thermal- or inflammatory-induced pain with decreased behavioral side effects in rats.

### References


