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INTERACTION BETWEEN MIDAZOLAM AND BACLOFEN IN SPINALLY MEDIATED ANALGESIA IN RATS

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ABSTRACT

Background: We investigated the interaction between γ-aminobutyric acid $(GABA)_A$ receptor agonist, midazolam and $GABA_B$ receptor agonist, baclofen on analgesic effects in acute thermal and formalin induced pain models of rats. **Methods:** Male Sprague-Dawley rats implanted with lumbar intrathecal catheters were given intrathecal combination of 1/2, 1/4, 1/8, and 1/16 50% effective doses (ED50s) of midazolam and baclofen, then tail flick test or formalin test was performed. Isobolographic analysis was done using ED50s, and total fractional dose values were calculated. **Results:** Intrathecal combination of midazolam and baclofen showed dose dependent increase of tail flick latency and decrease of flinch response in the formalin test. The ED50s of midazolam and baclofen in combination were significantly lower than each agent alone and additive values. Total fractional dose values were 0.37 (0.17 – 0.81, 95%CI), 0.39 (0.12 – 0.95), and 0.13 (0.03 – 0.75) in the tail flick test, phase 1, and phase 2 of the formalin test, respectively. Behavioral side effects observed in each agent were not seen in the combination treatment. **Conclusions:** Intrathecally administered combination of midazolam and baclofen had synergistic analgesia in thermal induced acute pain and formalin induced acute and facilitated pain without any behavioral side effects.

KEYWORDS: Analgesia, $GABA_A$ receptor, $GABA_B$ receptor, Spinal cord.

INTRODUCTION

The γ-aminobutyric acid (GABA) receptors in the spinal cord have great roles in analgesia. In the dorsal horn of the spinal cord, both $GABA_A$ and $GABA_B$ receptors are located at primary afferent terminals originating from dorsal root ganglion.[1] We have already shown that midazolam, acting on benzodiazepine binding site of $GABA_A$ receptors, [2] and baclofen, a $GABA_B$ receptor agonist,[3] when administered intrathecally, had analgesic effects in acute thermal pain, and formalin induced acute and facilitated pain in the rat models. However, the interaction of intrathecal midazolam and baclofen were not studied well. Therefore, this study investigated analgesic interaction of intrathecal midazolam and baclofen expecting synergistic analgesia with decreasing side effects using the same rat models as the previous studies.[2,3]

MATERIALS AND METHODS

After obtaining the approval of the Research Committee of the University of Tokyo, male Sprague-Dawley rats (280-300 g; Nippon Bio-Supply, Tokyo, Japan) were implanted with lumbar intrathecal catheters under halothane (2 %) anesthesia. The experiment procedures are the same as our previous study. [4] Briefly, an 8.5 cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ) was inserted caudally to the thoracolumbar level in the intrathecal space through atlanto-occipital 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 and increase in body weight seven days later were used for experiments. After the study, rats were euthanized under halothane 5% and the location of the catheter was confirmed anatomically and the data of the rats with mal location of the catheter was excluded and another rat was added to fill the number of each group.

Drug preparation

The ED50s of midazolam[2] and baclofen[3] were derived from our previous studies. The combination of 1/2, 1/4, 1/8, and 1/16 ED50s of midazolam (Sigma, St. Louis, MO) and baclofen (Sigma, St. Louis, MO) were dissolved in 10 μL saline.

Nociceptive test
According to our previous study, [4] we used the same two well-known methods as nociceptive tests.

Tail-flick test
The tail-flick test was performed with the Tail-Flick Analgesia Meter (MK-330A; Muromachi Kikai Co. Ltd., Tokyo, Japan). Rats were placed in a clear plastic cage with their tails extending through a slot located of 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 response was measured as a tail-flick latency. The cut-off time in the absence of a response was set to 14 seconds to prevent tissue injury of the tail. The test was done at 5, 10, 15, 30, 60, 90, 120, 180, and 240 minutes after drug injection. The data were shown as the % of maximum possible effect (% MPE): % MPE = (post-drug latency – pre-drug latency at time 0) X 100 / (cut-off time (14 sec) – pre-drug latency at time 0.

Formalin test
The formalin test was performed 10 minutes after intrathecal drug injection. Fifty μL of 5 % formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30 G needle. Immediately after injection, the rat was placed in an open clear plastic chamber and their flinching or shaking paw response was observed for 60 minutes. The number of flinches was counted for 1 minute. Usually two phases were observed: phase 1, during 0 to 6 minutes after formalin injection; and phase 2, beginning about 10 minutes after injection with the interval of no flinches between both phases.

Side effects
Side effects were examined and judged as present or absent. Agitation was judged as spontaneous irritable movement, vocalization, or both. Allodynia-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. Flaccidity was judged as muscle weakness by putting the forepaw 3 to 5 cm higher than the hind paw. Normal rats will walk up. Pinna or corneal reflex was examined with a paper string. When a string is put into the ear canal or touches the cornea, rats normally shake their heads. Behavioral side effects were checked simultaneously with the tail flick test.

Protocol
Each drug combination was administered intrathecally and after injection of the drug, the catheter was flushed with normal saline 10 μL to clear the dead space of the catheter.

The ED50 was obtained using the maximum effects in the tail flick test and the area under the curve of the number of flinches in the formalin test. A total fractional dose value was calculated to describe the magnitude of the interaction as follows: (ED50 dose of drug 1 in combination) / (ED50 dose of drug 1 alone) + (ED50 dose of drug 2 in combination) / (ED50 dose of drug 2 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 implies an antagonistic interaction, and values < 1 indicate a synergistic interaction. To compare the theoretical additive point with experimentally derived ED50, isobolographic analysis was used.

Data analysis
The data are shown as mean ± standard deviation or 95% confidential interval (CI). Statistical analysis was performed with the factorial analysis of variance (ANOVA) to compare the calculated ED50 values with theED50 of each agent alone and the theoretical additive values. A p value less than 0.05 was considered to be statistically significant.

RESULTS
Intrathecal midazolam and baclofen showed dose dependent increase of tail flick latency and decrease of flinch response in the formalin test (Fig. 1, 2). The ED50s of midazolam and baclofen in combination were significantly lower than each agent alone (Table 1). Total fractional dose values were 0.37 (0.17 – 0.81, 95%CI), 0.39 (0.12 – 0.95), and 0.13 (0.03 – 0.75) in the tail flick test, phase 1, and phase 2 of the formalin test, respectively. The ED50s of the combination were significantly lower than the additive values in the tail flick test and both phases of the formalin test (Fig.3,4,5). Side effects observed in each agent were not seen in the combination treatment (Table 2).

Table 1. ED_{50}.

	Tail flick test	Formalin test phase 1	Formalin test phase 2
Midazolam (μg)	1.6 (0.3 – 4.9)	1.3 (0.3 – 4.2)	1.2 (0.2 – 5.6)
Midazolam (combination) (μg)	0.30 (0.22 – 0.39)*	0.15 (0.12 – 0.21)*	0.08 (0.04 – 0.15)*
Baclofen (μg)	0.32 (0.25 – 0.40)	0.0062 (0.001 – 0.034)	0.013 (0.002 – 0.08)
Baclofen (combination) (μg)	0.059 (0.045 – 0.078)*	0.0017 (0.001 – 0.002)*	0.00084 (0.0005 – 0.0015)*

ED50: 50 % effective dose, Mean with 95% confidence interval in the parenthesis
*: P < 0.05 vs. the value of each agent alone

Table 2: Side effects.

	Saline	Midazolam (μg)[2]					Baclofen (μg)[3]				Midazolam + Baclofen (of ED_{50})			
Dose		1	3	10	30	100	0.1	0.3	1	3	1/2	1/4	1/8	1/16
Agitation	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Allodynia	0	0	1	1	1	1	0	0	0	0	0	0	0	0
Loss of Pinna reflex	0	0	0	0	0	1	0	0	0	2	0	0	0	0
Flaccidity	0	0	0	0	1	3	0	0	4	8	0	0	0	0
Ambulation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asymmetry	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Disturbance of righting reflex	0	0	1	0	2	6	0	0	4	8	0	0	0	0
Disturbance of placing and stepping	0	0	0	0	1	4	0	0	4	8	0	0	0	0

The number of rats showed each side effect was indicated. Total number tested was 8 in each dose.
Data of midazolam and baclofen were derived from previous studies.[2,3]
ED_{50}: 50 % effective dose

Figure legends

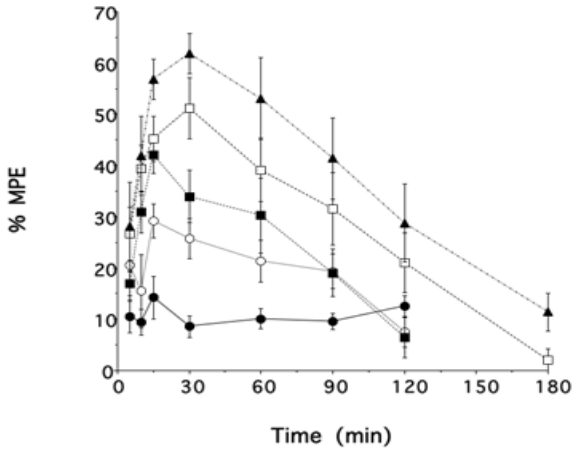

Figure 1: Tail flick test with midazolam + baclofen.
%MPE, % of maximum possible effect; Bars indicate standard deviation. closed circle, saline; open circle, 1/16ED50; closed square, 1/8ED50; open square, 1/4ED50; closed triangle, 1/2ED50

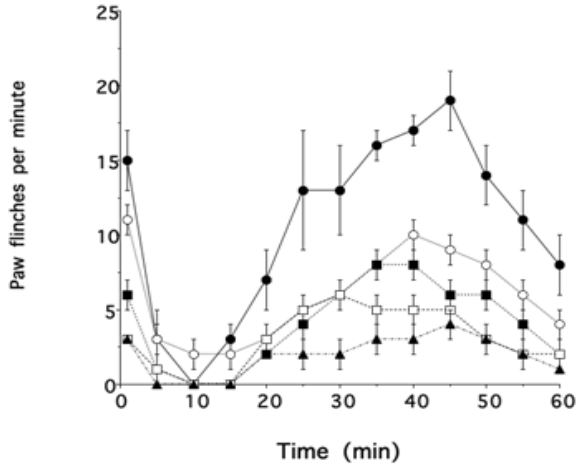

Figure 2: Formalin test with midazolam + baclofen.
Bars indicate standard deviation. closed circle, saline; open circle, 1/16ED50; closed square, 1/8ED50; open square, 1/4ED50; closed triangle, 1/2ED50

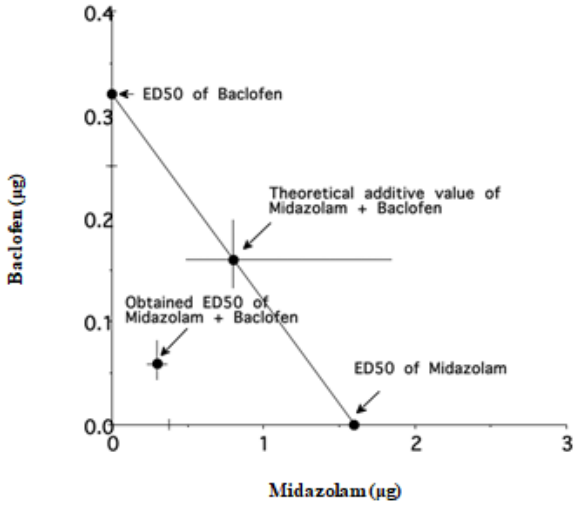

Figure 3. Isobolograph of the tail flick test with midazolam + baclofen Bars indicate 95 % confidence interval.

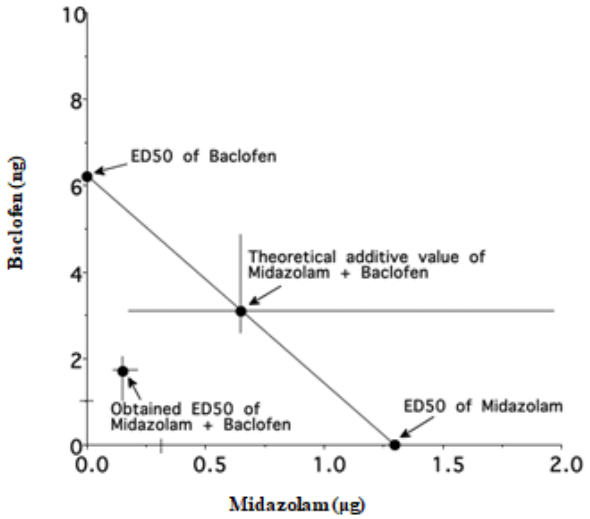

Figure 4: Isobolograph of the formalin test phase 1 with midazolam + baclofen Bars indicate 95 % confidence interval.

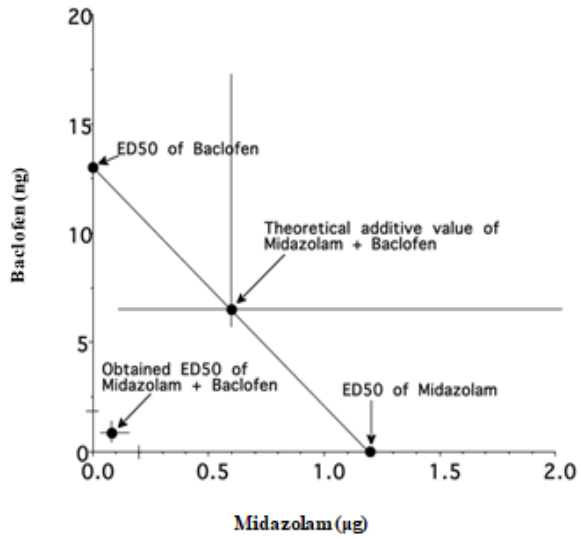

Figure 5: Isobolograph of the formalin test phase 2 with midazolam + baclofen Bars indicate 95 % confidence interval.

DISCUSSION

The present study showed that intrathecally administered midazolam and baclofen had synergistic analgesia in the tail flick test and both phases of the formalin test. No behavioral side effects were observed in the combination.

Midazolam would not activate $GABA_A$ receptor itself, but would increase the effect of endogenous GABA by binding to the benzodiazepine site coupling with $GABA_A$ receptors.[5] Midazolam augmented both the duration of GABA-mediated synaptic current and the amplitude of GABA-induced current.[6] Midazolam reduces excitatory transmitter release presynaptically and excitatory activity postsynaptically in spinal dorsal horn neurons via benzodiazepine-$GABA_A$ receptors.[7]

Presynaptic and postsynaptic $GABA_B$ receptors inhibit synaptic transmission through regulation of synaptic glutamate release.[8] Activation of presynaptic $GABA_B$ receptors by intrathecal baclofen decreases glutamate release from primary afferents,[8] and downregulates N-methyl-D-aspartate (NMDA) receptor expression in both mRNA and protein levels in the spinal cord.[9] Activation of postsynaptic $GABA_B$ receptors suppresses Ca2+ permeability of NMDA receptors and reduces NMDA receptor activity.[10] Thus, activation of both $GABA_A$ and $GABA_B$ receptors decrease excitatory amino acid release and activity.

$GABA_A$ receptor agonists depolarize primary afferent, thus inhibit sensory transmission at the spinal level, while baclofen depresses excitatory synaptic transmission by another pre-synaptic mechanism involving a decrease in the release of excitatory amino acids and neuropeptides from primary afferent neurons.[11] Mechanisms to decrease excitatory transmission were different between $GABA_A$ and $GABA_B$ receptors. Therefore, combination of midazolam and baclofen might be expected to induce additive or synergistic analgesia.

Low concentrations of midazolam act as an agonist via the benzodiazepine site, whereas higher concentrations may interact with another site distinct from that for benzodiazepines on $GABA_A$ receptors as an antagonist.[12]

The doses used in our previous study[2] were already enough small to have analgesic effects, but the lower doses in the combination in the present study were better not to induce anti-analgesic effects.

Intrathecal midazolam induces analgesia not only by acting on $GABA_A$ receptors, but also by acting as a direct agonist at κ and δ opioid receptors in the spinal cord.[13] Spinal analgesic effects of baclofen are in some parts via endocannabinoid modulation.[14] These different mechanisms might also work for additive or synergistic effect.

In conclusion, intrathecal combination of midazolam and baclofen induced synergistic analgesia in acute thermal and formalin induced acute and facilitated pain.

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