短　報

Effects of trazodone on motor coordination and balance alterations following thiopental-induced anesthesia in mice

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Abstract: Recently, trazodone, an antidepressant which is less likely to induce delirium, has been used off-label as a drug with sleep-inducing effect, but its effect on motor function after awakening has not been sufficiently investigated. Here, we evaluated the effect of trazodone, brotizolam and zolpidem on motor function in mice after awakening. The rota-rod test was performed after the recovery of mice from a loss of righting reflex induced by thiopental sodium (20 mg/kg) administration. Brotizolam 0.075 mg/kg and trazodone (3 and 5 mg/kg) decreased motor coordination and balance alterations 15 min after recovery from the loss of righting reflex. These drugs without thiopental did not affect on motor coordination and balance alterations. These results suggest that caution is necessary early after waking with brotizolam or trazodone to prevent falls.

Keywords: fall, motor function, after awakening, trazodone

Introduction

Fall-related injuries in patients are a serious concern in hospitals. Besides reducing the activities of daily living or quality of life of patients by trauma or fracture, inpatient falls extend hospitalization and increase medical expenses[1, 2)]. The incidence of inpatient falls is reported to be 1.89% in a university hospital in Japan[3)]. Studies have indicated the involvement of hypnotic use, especially benzodiazepines, in falls[4)]. Furthermore, benzodiazepines are a risk factor for delirium development[5)]. Recently, trazodone, a serotonin-2 antagonist and reuptake inhibitor used as antidepressant, has been frequently used off-label as a hypnotic agent to avoid delirium[6)]. However, the risk of falls related to trazodone administration after awakening based on behavioral pharmacological examination has not been sufficiently investigated. It has been reported that thiopental induced anesthesia model is useful to evaluate the side effects of hypnotic agent after awakening[7)]. Thus, here, we analyzed and compared the effect of trazodone on motor function in mice following thiopental-induced anesthesia.

Material and Methods

Materials

Trazodone hydrochloride and brotizolam were procured from FUJIFILM Wako Pure Chemical Corporation (Osaka). Zolpidem was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Trazodone hydrochloride was dissolved in water, and brotizolam and zolpidem were dissolved in dimethyl sulfoxide (DMSO). These drugs were diluted with 0.5% carboxymethylcellulose (CMC) sodium solution (the final DMSO concentration was adjusted within 1.0%). Thiopental sodium (RAVONAL®) was bought from Nipro ES Pharma Co., Ltd. (Osaka).

Animals

Four-week-old male ICR mice were obtained from SHIMIZU Laboratory Supplies Co., Ltd. Experiments were carried out in accordance with the Regulation for Animal Experimentation at Shujitsu University and were approved by the Animal Experimentation Committee of Shujitsu University (042-001).

Induction of loss of righting reflex

Brotizolam, zolpidem, and trazodone were intraperitoneally administered to the mice 15 min before the injection of thiopental sodium, an ultra-short-acting barbiturate. Thereafter, to induce hypnosis, the mice were intravenously administered thiopental sodium (20 mg/kg). Loss of righting reflex, which was defined according to a previous study[7], was induced immediately after the injection of thiopental sodium.

Rota-rod test

The rota-rod test, used to assess motor coordination and balance alterations in mice[7], was performed at 15, 30, and 60 min after the recovery of mice from the loss of righting reflex. We used a rotating rod (3 cm diameter) apparatus (Ugo Basile, Comerio, Italy) for the test. Each mouse was placed on a rod rotating at 20 rpm for 180 s, and the duration each mouse was able to maintain its balance while walking on the rod was measured. Before the experiment, the mice were trained for 3 days, and the mice that could walk on a rod rotating at 20 rpm for 180 s on day 3 were used in the analysis.

Statistical analysis

Data are the mean ± SEM. One-way analysis of variance followed by Dunnett's test, or Tukey–Kramer test were used to evaluate differences. $P < 0.05$ was considered significant.

Results

Loss of righting reflex

The administration of brotizolam, zolpidem, and trazodone significantly increased the duration of righting reflex loss induced by thiopental in a dose-dependent manner (Table 1). Furthermore, the loss of righting reflex induced by the combination of thiopental and trazodone plateaued at the trazodone dose of 3 mg/kg.

Table 1. Duration of the loss of righting reflex

Treatment	Dose (mg/kg)	Number	Duration of the loss of righting reflex (s)
0.5% CMC	10 mL/kg	5	125.6 ± 10.1
Brotizolam	0.050	8	214.0 ± 63.4
	0.075	5	735.2 ± 98.7 *
0.5% CMC	10 mL/kg	8	114.5 ± 16.6
Zolpidem	1	6	231.8 ± 69.9
	2	6	460.5 ± 60.8*
0.5% CMC	10 mL/kg	6	110.5 ± 16.6
Trazodone	1	5	173.5 ±10.9
	3	7	233.6 ± 25.2*
	5	6	233.5 ± 43.6*

Values are mean ± S.E.M. *P<0.05, v.s. 0.5% CMC

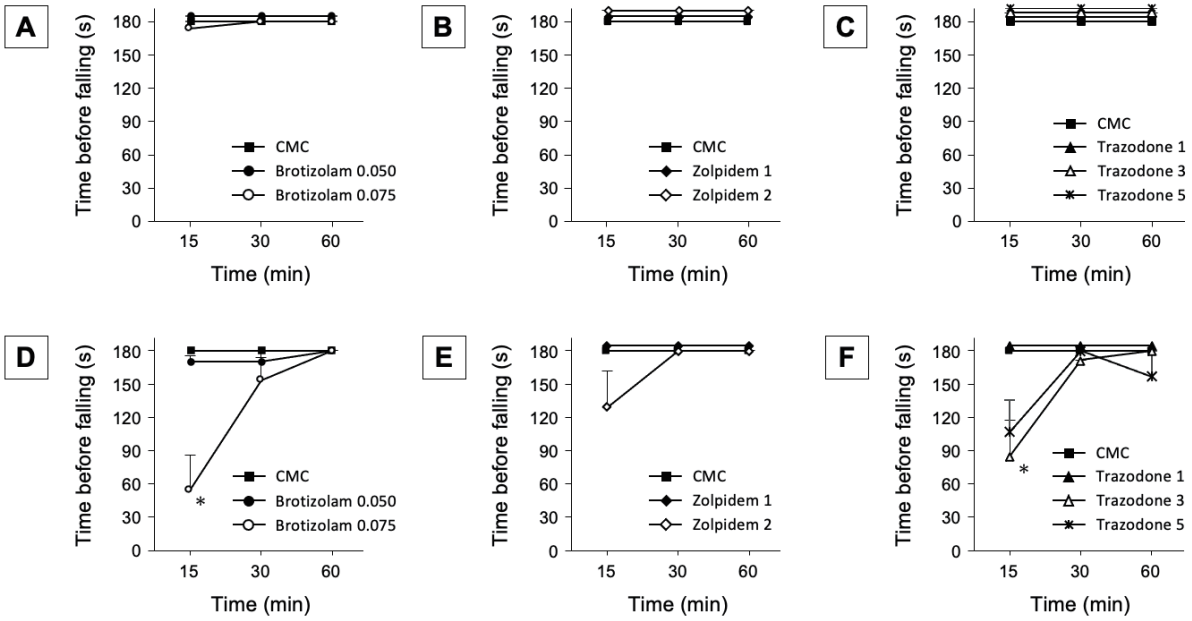

Fig. 1. Behavioral pharmacological profiles after recovery from loss of righting reflex.
Brotizolam (0.050 and 0.075 mg/kg) (A and D), zolpidem (1 and 2 mg/kg) (B and E), and trazodone (1, 3, and 5 mg/kg) (C and F) were intraperitoneally administered 15 min before the injection of thiopental sodium (n = 5–8). A–C are mice not administered thiopental sodium and D–F are mice administered thiopental sodium. Values are mean ± S.E.M. *P < 0.05 compared with 0.5% CMC.

Rota-rod test

Behavioral pharmacological profiles after the recovery of mice from the loss of righting reflex induced by the combination of thiopental and hypnotics/trazodone are shown in Fig. 1. The administration of brotizolam, zolpidem, and trazodone without thiopental did not affect the time before fall from the rota-rod at any dose tested (Fig. 1A-C). Brotizolam 0.075 mg/kg significantly decreased the duration the mice were able to maintain their balance while walking on the rod at 15 min compared with 0.5% CMC alone (Fig. 1D). In contrast, although zolpidem 2 mg/kg tended to decrease the duration the mice could maintain their balance while walking on the rod at 15 min compared with 0.5% CMC alone, it was not statistically significant (Fig. 1E). Trazodone 3 mg/kg also significantly decreased the duration the mice were able to maintain their balance while walking on the rod at 15 min compared with 0.5% CMC alone, and the same tendency was observed at the dose of 5 mg/kg (Fig. 1F). The effect of either drug on the ability of mice to balance while walking on the rod was decreased at 30 and 60 min after recovery from the loss of righting reflex.

Comparison of the effect on the duration before falling of mice administered brotizolam, zolpidem, and trazodone are shown in Fig. 2. In this experiment, the dose of each drug was used at a dose that significantly increased the duration of the loss of righting reflex induced by thiopental administration. The duration before falling of mice administered brotizolam 0.075 mg/kg was significantly shorter than that of mice administered zolpidem 2 mg/kg. The duration before falling of mice administered trazodone 5 mg/kg was likely shorter than that of mice administered zolpidem 2 mg/kg. The drugs were administered at doses at which they significantly increase the duration of righting reflex loss induced by the administration

of thiopental.

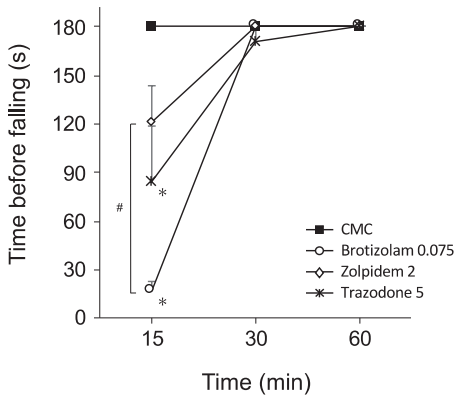

Fig. 2. Comparison of behavioral pharmacological changes after recovery from loss of righting reflex. Brotizolam 0.075 mg/kg, zolpidem 2 mg/kg, and trazodone 5 mg/kg were intraperitoneally administered 15 min before the injection of thiopental sodium (n = 5–7). Values are mean ± S.E.M. *P < 0.05 compared with 0.5% CMC and #P < 0.05 compared with zolpidem 2 mg/kg.

Discussion

We investigated the effect of brotizolam, zolpidem, and trazodone on motor coordination and balance alterations after awakening using thiopental-induced anesthesia. Among the drugs examined, brotizolam strongly decreased motor coordination and balance alterations. This phenomenon was not observed for zolpidem. This result can be explained by the ω receptor selectivity of these drugs. On the basis of the results, we recommend the use of zolpidem rather than brotizolam to prevent falls. Furthermore, the dose-dependent effect of brotizolam on motor coordination and balance alterations following thiopental-induced anesthesia was sharper than that of the others. This suggests that unintentional overdosage of brotizolam caused by decreased metabolic function in elderly patients may easily affect motor function compared with zolpidem or trazodone.

The administration of trazodone decreased motor coordination and balance alterations following thiopental-induced anesthesia at 15 min, and this effect was not observed at 30 and 60 min. Therefore, patients who are administered trazodone would need careful support to prevent falls early after awakening. In contrast, as it did not significantly affect the motor function at 30 and 60 min, trazodone may be relatively safe with respect to the duration of action. It has been reported that the hypnotic action of trazodone involve inhibition of 5-HT_{2A} receptors, H_1 histamine receptors, and α_1 adrenergic receptors[8]. Among these, trazodone of the dose clinically used as hypnotic (25 mg) shows mainly inhibition of 5-HT_{2A} receptors[9]. In this study, trazodone 3 and 5 mg/kg significantly increased the duration of righting reflex loss induced by thiopental, and decreased motor coordination after awaking. Furthermore, higher doses are needed for trazodone to act on other receptors and show antidepressant effects[8, 9]. For these reasons, the hypnotic action and the effect on motor coordination by trazodone in this study would responsible to the inhibition of 5-HT_{2A} receptors.

A clinical study has reported that trazodone has a longer sleep latency than zolpidem[10]. This clinical evidence supports that a loss of righting reflex following thiopental administration might plateau, as observed in this study. Thus, high-dose or repeated administration of trazodone, used as a hypnotic agent to achieve sleep beyond its ability, may cause motor dysfunction. A recent clinical study has shown that the combination of low-dose trazodone and other hypnotics decreases postoperative insomnia compared with low-dose trazodone alone to improve postoperative insomnia[6]. This

strategy may also improve the safety of trazodone by reducing motor dysfunction if the hypnotics used in combination are safe. On the contrary, in the present study, trazodone did not show an apparent decrease in motor function compared with zolpidem, and further investigation is necessary to determine whether trazodone or zolpidem is safe from the perspective of falls.

With the aging society, the use of sleeping pills should be further assessed in terms of safety. We demonstrated behavioral pharmacological profile of brotizolam, zolpidem, and trazodone after recovery from loss of righting reflex in mice, and pointed out the trazodone-related motor function change as the risk of fall. Our study provides behavioral pharmacological information of trazodone, which is used off label in Japan with expected sleep-inducing effect, and we believe it can be useful for safe use of trazodone and the prevention of falls.

Conflict of Interest

The authors declare no conflicts of interest.

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