Studies on the Muscle Relaxation Effects of Ethyl Loflazepate (CM6912) and Evaluation as an Anti-Anxiety Drug

Yutaka SAKAI and Misako NAMIMA
Department of Pharmacology, National Defense Medical College, Tokorozawa, Saitama 359, Japan
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Abstract—A new anti-anxiety drug, CM6912 (ethyl loflazepate, ethyl 7-chloro-2,3-dihydro-5-(2-fluorophenyl)-2-oxo-1H-1,4-benzodiazepine-3-carboxylate), was investigated for its effects on the α- and γ-motor systems and on the cooperative muscular motions and for its interactions with other CNS drugs. The results obtained are as follows: Muscular discharges (EMG) induced by decerebrate rigidity were unaffected by 10 mg/kg (p.o.) of CM6912, but the amplitudes of the EMG were reduced by 50% for 3 hr by 30 mg/kg of CM6912 at 30 min after administration. Diazepam (10 mg/kg) also decreased the amplitudes of the EMG even at 5 min after administration, indicating that diazepam had a stronger than CM6912. Both monosynaptic spinal reflex (MSR) and polysynaptic spinal reflex (PSR) were unaffected by CM6912 (100 mg/kg). Dorsal root reflex potential was slightly enhanced by CM6912 (100 mg/kg), but not at a dose of 30 mg/kg. Diazepam (10 mg/kg) did not decrease MSR, but slightly reduced PSR. Dorsal root reflex potential was almost doubled by diazepam. The frequency of spontaneous discharges of Gla spindle afferent fiber of the extensor muscle of the hindlimb of anesthetized cats was unchanged by 10 mg/kg CM6912, but was suppressed by diazepam at the same dose while at a dose of 30 mg/kg, it was reduced mildly by CM6912, and markedly by diazepam. ED50 values for the antagonistic action on bemegride-induced convulsions were 0.30 mg/kg for CM6912 and 0.49 mg/kg for diazepam at 1 hr after administration, and they were 0.30 mg/kg and 0.67 mg/kg for CM6912 and diazepam, respectively, at 4 hr. The potentiating action of CM6912 on chlorprothixene-induced anesthesia was far weaker than that of diazepam. The suppressive potency of CM6912 on the adaptability to the rotarod was about half that of diazepam, and the muscle relaxant action of CM6912, examined by the inclined board test and the hanging test, was found to be similar to that of diazepam. These results suggest that CM6912 is less potent than diazepam in reducing muscular tone and in inducing sleep, while it has a stronger and longer-lasting anti-anxiety activity than diazepam.

The antianxiety activity (minor tranquilizing effect) of a new benzodiazepine, CM6912 (ethyl loflazepate, ethyl 7-chloro-2,3-dihydro-5-(2-fluorophenyl)-2-oxo-1H-1,4-benzodiazepine-3-carboxylate, Fig. 1), has been well demonstrated by the results of experiments with laboratory animals (1, 2) and results of clinical studies (3, 4) including double blind tests.

We have also performed neurochemical and electrophysiological investigations using in vitro experimental systems and found that CM6912 displaced 3H-diazepam from binding to specific benzodiazepine receptors in rat cerebral membranes. We also found that CM6912 augmented the inhibitory action of GABA on spontaneous spike firings of Purkinje cells in guinea pig cerebellar slices, and CM6912 enhanced the high K+-evoked release of 3H-GABA from guinea pig cerebral cortical slices (Y. Sakai and M. Namima, unpublished observations).
In the present study, for the purpose of further characterizing the pharmacological properties of CM6912 as an anti-anxiety drug, the antagonistic potency of CM6912 on bemegride-induced convulsions (the best experimental index for the anti-anxiety activity of minor tranquilizers (5)), its interactions with other centrally acting drugs and its muscle relaxing activity, which may be thought to be one of the side actions of benzodiazepines, were investigated with particular attention to the distinction between the α- and the τ-motor systems (6).

**Materials and Methods**

**A. Effects of CM6912 on motor functions**

Adult male cats (2.8-4.5 kg) were used. CM6912 or diazepam was suspended in 0.5% CMC solution and administered directly into the stomach via an attached gastric tube.

1. **Decerebrate rigidity:** Bilateral common carotid arteries were ligated under ether anesthesia, and then the cat was fixed on a brain-spinal-stereotaxic apparatus. Two holes were made (between A: 10 and P: 10) bilaterally on the cranial bone, and decerebration was carried out by aspiration visually at the level of the tip of the superior colliculus. The cut surface was covered with a piece of oxidized cellulose (Oxycel®, Sankyo), and ether anesthesia was discontinued after bleeding was confirmed to be stopped. Then, a pair of needle-electrodes were impaled into the neck muscle to derive EMG discharges which were amplified and recorded on a pen recorder. Throughout the experiment, the animal body was kept at 37–39°C by warming with a heating pad beneath the abdomen, and the blood pressure was monitored. Recording of EMG discharges was started at least 3 hr after the discontinuation of ether anesthesia.

2. **Spinal reflexes:** An air-duct catheter was attached to a cat under ether anesthesia, and the cat was fixed on a brain-spinal-stereotaxic apparatus. The spinal cord was cut at the level between T13 and L1 under local anesthesia with procaine (1%). Then, the region of L5-S1 of the spinal cord was exposed by laminectomy. The ventral root L7 was cut off as distally as possible and was fixed on a pair of platinum wire electrodes. Similarly, the dorsal root L6 was also fixed on another pair of the electrodes. The exposed area of the spinal cord was covered with liquid paraffin which was warmed to 37–39°C with a lamp. Then, an incision was made on the femoral area of an ipsilateral hindlimb to expose the sciatic nerve which was attached to a pair of bipolar electrodes. Exposed sciatic nerve was warmed with liquid paraffin in a similar manner as that for the spinal cord. Another pair of the electrodes were attached to the ipsilateral saphenous nerve to evoke polysynaptic reflex, and a single square pulse (supramaximal intensity, 0.01–0.1 msec duration, 0.3 Hz) was applied.

Blood pressure was continuously monitored from the contralateral femoral artery. The radial vein was cannulated for the injection of drug solutions. After all these operations were completed, ether anesthesia was discontinued, and the animal was immobilized with pancuronium bromide and kept under artificial respiration.

Spinal reflex signals were amplified and displayed on an oscilloscope screen. The images of mono- and polysynaptic reflexes were directly photographed from the oscilloscope screen. As for dorsal root reflexes, 10 responses were averaged using a data processor (ATAC-350, Nihon Kohden, Tokyo).
3. The activities of the γ-motor neuron:
After a tracheal cannula was attached under pentobarbital anesthesia (40 mg/kg, i.p.), the cat was fixed on a brain-spinal-stereotaxic apparatus. The region of L5-S3 of the spinal cord was exposed by laminectomy. All spinal roots, except for right ventral roots, L6-S1, were cut-off bilaterally. The L7 and S1 dorsal roots on the right were cut-off as proximally as possible to isolate a single fiber. For this, a fine filament was isolated and placed on one pole of paired platinum wire electrodes, and crushed filaments of a similar size were placed on another pole of the electrodes as a reference. The ankle extensor muscles of the ipsilateral hindlimb were separated into 4 nerve bundles, namely, M. gastrocnemius lateralis and medialis, M. soleus and M. plantaris. The tendon of each of the muscle bundles was ligated with silk threads to give constant stretch. All nerves, except for those innervating the extensor muscles, were cut off, and a pair of electrodes were attached to the sciatic nerve. The exposed spinal cord and the hindlimb were covered with warmed liquid paraffin, and the temperature was kept at 36-38°C with a heating lamp.

The right L7 or S1 filament was separated into single fibers, and the discharge of a Gla spindle afferent fiber was monopolarly derived, amplified and displayed on an oscilloscope screen. Isolated Gla spindle afferent fiber was identified (6) as follows: 1. appearance of a pause in the resting discharge during muscle contraction caused by a single shock to the peripheral nerve, moreover, the discharge rate transiently increased immediately after the termination of the muscular contraction; 2. the potential evoked by tibial nerve stimulation was in an all or none manner, and 3. the rate of conduction of the evoked potential was more than 70 m/sec.

The γ-activity of this kind was processed to give interval histograms using ATAC-350 in a similar manner as that for spinal reflexes.

B. Interactions with other CNS drugs
In the following 6 experiments, all drugs used were suspended in 0.3%-CMC-containing physiological saline and orally administered. The value of Ed50 was estimated by means of the Litchfield-Wilcoxon method.

1. Antagonistic action on bemegride-induced convulsions in mice: Ten male mice of the ddY strain (20–26 g) were used as one test group, and 4–5 such groups were used for each dose of a drug. Bemegride, 35 mg/kg, was subcutaneously injected at 30 min, 1 hr and 4 hr after the administration of a test drug, and the development of clonic convulsions was observed for a period of 30 min. The values of Ed50 were estimated from the percentage suppression of convulsions in each group.

In this series of experiments, the actions of 2 major metabolites of CM6912 (2), i.e., CM6913 and CM7116, were also compared with that of CM6912. The potencies of CM6912 and diazepam were compared in an experiment carried out on the same day, and those of CM6912, CM6913 and CM7116 were compared in a test performed on another day.

2. Potentiating action on thiopental-induced anesthesia in mice: Ten male mice of the ddY strain (20–26 g) were used as one test group. One hour after the oral administration of a test drug, thiopental, 30 mg/kg, was intravenously injected, and the duration of the disappearance of the righting reflex was measured. The dose of a drug which doubled the duration of anesthesia induced by thiopental alone was estimated and expressed as an Ed50 value.

CM6912 and diazepam were compared in one experiment, and CM6912 and its metabolites were compared in another experiment.

3. Potentiating action on chlorprothixene-induced anesthesia in mice: Male mice of the ddY strain (26–28 g, 6 weeks old) were used. Immediately after the oral administration of 25 mg/kg of chlorprothixene (Chlothixen®, Yoshitomi), CM6912 was orally given. Then, the suppression of the righting reflex (within a 10 sec duration) was observed 1 hr later.

C. Effects of CM6912 on spontaneous behaviors
1. Suppressive action on the adaptability of mice to the rotarod: Male mice of the ddY strain (20–25 g) were first screened on the rod (3 cm in diameter) rotating at a rate of 10 r.p.m., and the mice which could stay on
the rod for more than 1 min were selected. Ten such mice were used as one test group, and 4–5 such groups were prepared. Similar tests on the rotarod were performed 30 min, 1 hr, 2 hr and 4 hr after the oral administration of a test drug, and the value of ED50 for the suppressive action on the adaptability was estimated from the number of fallen mice. CM6912 and diazepam were compared in the same experiment.

2. Suppressive action on the adaptability of mice to the inclined board: Ten male mice of the ddY strain (20–24 g) were used as one test group, and they were placed on a board inclined at an angle of 45 degree, at 30 min, 1 hr and 4 hr after the administration of a test drug. When a mouse slipped down within 10 sec, this was judged as a suppressed case. ED50 values were estimated from the number of such fallen mice.

3. Suppressive action on the hanging behavior of mice: At 30 min, 1 hr and 4 hr after the oral administration of a test drug, each mouse was hung on a horizontal metal wire (1 mm in diameter) by hooking the forelimb on the wire, and the mouse was observed for a period of 20 sec. When the mouse fell down from the wire within 20 sec, it was judged as a case of suppression. ED50 values were estimated from the number of such fallen mice.

Results

A. Effects of CM6912 on motor functions

1. Decerebrate rigidity

Representative results from 3 experiments are shown in Fig. 2. CM6912 at 30 mg/kg reduced the amplitude of EMG discharge induced by decerebrate rigidity by about 50% at 30 min after its administration (compare B with A). This decreasing action of CM6912 was more pronounced at 60 min (C) and continued to be so until at least 3 hr after administration (observation was not made over 3 hr). This was confirmed in 2 cats. Similar suppressive actions were also observed at a dose of 50 mg/kg in 2 cats tested. At a dose of 10 mg/kg, however, CM6912 did not affect at all the EMG discharge induced by decerebrate rigidity (tested in 2 cats). On the other hand, diazepam started decreasing the amplitude of EMG at 5 min after its administration at a dose of 10 mg/kg, indicating that CM6912 was less potent than diazepam.

2. Spinal reflexes

Typical results obtained with CM6912 and diazepam are shown in Figs. 3 and 4, respectively. As shown in Fig. 3, CM6912 had no effect at 60 min after administration on the amplitude of MSR at a dose of either 50 mg/kg (3 cases tested) or 100 mg/kg.
(compare A with D). Moreover, PSR was also little affected by CM6912 (compare B with E). The dorsal root reflex, on the other hand, was mildly enhanced by CM6912 (compare F with C), this being confirmed in 2 cats. At a dose of 30 mg/kg, however, CM6912 did not alter the dorsal root reflex, this being also confirmed in 2 cats. Observation made 3 hr after the administration of CM6912 showed no time-dependent potentiation of the action of this drug. The blood pressure was little changed by this compound even after administration of a large dose such as 100 mg/kg (p.o.).

Diazepam at a dose of 10 mg/kg, slightly decreased PSR as shown in Fig. 4 (compare E with B), while it little affected the amplitude of MSR (compare D with A). The most outstanding finding with diazepam was the nearly two-fold increase of the dorsal root reflex potential (compare F with C).

3. The activity of γ-motor neurons

The effects of CM6912 upon lumbosacral γ-motor neurons were compared with those of diazepam. Typical results are shown in Fig. 5, in which firing intervals (in msec) of about 1,000 spikes are plotted on the abscissa, while spike numbers are on the ordinate. In the case shown in Fig. 5, spontaneous discharges of the Gla spindle afferent fiber originating from the medial gastrocnemius showed 70–80 msec intervals (about 12.5–14 Hz) (A). Sixty minutes after the administration of CM6912, 30 mg/kg, the interval was slightly prolonged up to 80–100 msec (10–12.5 Hz) (B), indicating reduced discharge rates. This mild suppression by CM6912 was unchanged even 3 hr after its administration. No effect of CM6912 was observed at a dose of 10 mg/kg.

On the other hand, diazepam markedly suppressed the spontaneous activity of the γ-motor neuron. In the case shown in Fig. 6, the discharges of the Gla spindle afferent fiber originating from the medial gastrocnemius had intervals between 15 and 125 msec (an interval of 60 msec occurred most frequently) before the administration (Fig. 6A). However, 1 hr after the administration of diazepam (10 mg/kg, p.o.), the interval ranged between 35 and 425 msec (an interval of 110 msec was most popular) (Fig. 6B). This effect began to be observable at 30 min and attained a maximum at 60 min after the administration of diazepam. These
observations with diazepam indicate that this drug has a remarkably strong suppressive action on the spontaneous discharge of γ-motor neurons. These suppressive actions of CM6912 and diazepam were confirmed using 2 cats for each. According to these observations, it may be judged that the blocking action of CM6912 on the activity of the γ-motor system is weaker than that of diazepam.

Fig. 6. Effects of diazepam on the spontaneous activity of the lumbosacral γ-motoneurons in the cat anesthetized with pentobarbital. Interval histograms were obtained as described in Fig. 5. The number of the unit to make the histogram was 1,000 for both A and B. A: before, and B: 60 min after the administration (p.o.) of 10 mg/kg of diazepam. Abscissa scales: inter-spike intervals (msec). Ordinate scales: spike numbers.

Table 1. ED50 values for the antagonistic actions of CM6912 and diazepam (exp. A) and the metabolites of CM6912 (B) on bemegride-induced convulsions in mice

| Exp. | Compound | ED50 mg/kg, p.o. (95% Confidence limits) | 0.5 | 1.0 | 2.0 | 4.0 |
|------|----------|---------------------------------------|-----|-----|-----|-----|
|      |          |                                       |     |     |     |     |
| A    | CM6912   | 0.33 (0.26–0.42)                      | 0.30 (0.24–0.38) | 0.30 (0.24–0.38) | 0.30 (0.24–0.38) |
|      | Diazepam | 0.39 (0.32–0.49)                      | 0.49 (0.32–0.53) | 0.67 (0.51–0.87) |
|      | CM6912   | 0.22 (0.17–0.28)                      | 0.29 (0.24–0.35) | 0.32 (0.25–0.40) | 0.43 (0.34–0.53) |
| B    | CM7116   | 0.17 (0.14–0.21)                      | 0.26 (0.21–0.33) | 0.29 (0.24–0.37) | 0.32 (0.25–0.40) |
|      | CM6913   | 0.18 (0.14–0.22)                      | 0.29 (0.24–0.36) | 0.32 (0.25–0.39) | 0.53 (0.43–0.65) |

Experiments A and B were carried out separately.

B. Interactions with other CNS drugs

1. Antagonistic action on bemegride-induced convulsions

CM6912 and diazepam: ED50 values for CM6912 and diazepam are listed in Table 1A. There was no difference between them in the onset of their actions, and there was a little difference between them in their potencies at 30 min after administration. At 1 hr and 4 hr after administration, however, CM6912 showed stronger antagonistic action than diazepam. Moreover, CM6912 exhibited similar values of ED50 at 1 hr and 4 hr after administration, indicating its long lasting action.

CM6912 and its metabolites: ED50 values for CM6912, CM7116 and CM7116 are shown in Table 1B. CM7116 showed the highest potency among them at all observation times. The potency of CM6913 was weaker than that of CM7116 and not significantly different from that of CM6912.

2. Potentiating action on thiopental-induced anesthesia

CM6912 and diazepam: As shown in Table 2A, the potentiating action of CM6912 was similar to that of diazepam at 30 min after administration, while it was stronger than diazepam at 1 hr and 4 hr after administration. Moreover, CM6912 showed no decline in ED50 values at 1 hr and 4 hr, whereas the potency of diazepam decreased at 4 hr.

CM6912 and its metabolites: The results
obtained 1 hr and 4 hr after administration are shown in Table 2B. At 1 hr after administration, the metabolites of CM6912, i.e., CM6913 and CM7116, showed stronger potentiating actions than CM6912; thus their potencies were the order of CM7116>CM6913>CM6912.

3. Potentiating action on chlorprothixene-induced anesthesia

The ED50 value of CM6912 was found to be 0.56 mg/kg (0.4-0.76 mg/kg). Although ED50 values for other benzodiazepines were not measured simultaneously with CM6912 in the present study, previously reported values of ED50 may be referred for the purpose of comparison. The ED50 value for diazepam was reported to be 0.36 mg/kg (0.17-0.79) (Y. Sakai, unpublished observation), while those for nitrazepam and haloxazolam were 0.041 mg/kg (0.023-0.074 mg/kg) and 0.061 mg/kg (0.034-0.11 mg/kg), respectively (7). Thus, the potency of CM6912 is weaker than that of diazepam, and it is markedly weaker than those of either nitrazepam or haloxazolam. On this basis, it is inferable that CM6912 may not be a sleep inducing drug as nitrazepam.

C. Spontaneous behaviors

1. Adaptability to the rotarod

As shown in Table 3A, the suppressive potency of CM6912 was about half that of diazepam at the times of 30 min, 1 hr and 4 hr.

The results obtained simultaneously for

Table 2. ED50 values for the augmenting actions of CM6912, diazepam, CM7116 and CM6913 on thiopental-induced anesthesia in mice

| Exp. | Compound | 0.5  | 1.0  | 2.0  | 4.0  |
|------|----------|------|------|------|------|
| A    | CM6912   | 3.4  | 2.2  | 2.0  |      |
|      |          | (2.4-4.8) | (1.7-2.8) | (1.3-3.2) |      |
|      | Diazepam | 3.3  | 3.2  | 5.5  |      |
|      |          | (1.7-4.3) | (2.4-4.2) | (4.2-7.3) |      |
|      | CM6912   | 1.3  | 1.5  |      |      |
|      |          | (0.82-1.28) | (0.79-2.85) |      |      |
| B    | CM7116   | 1.0  | 1.5  |      |      |
|      |          | (0.81-1.94) | (0.69-2.85) |      |      |
|      | CM6913   | 1.1  | 1.7  |      |      |
|      |          | (0.66-1.68) | (1.09-2.66) |      |      |

Table 3. ED50 values for the inhibitory actions of CM6912, diazepam, CM7116 and CM6913 on the adaptability to the rotarod in mice

| Exp. | Compound | 0.5  | 1.0  | 2.0  | 4.0  |
|------|----------|------|------|------|------|
| A    | CM6912   | 18.6 | 15.3 | 15.6 |      |
|      |          | (14.3-25.2) | (11.1-20.6) | (12.5-19.5) |      |
|      | Diazepam | 7.0  | 7.2  | 8.9  |      |
|      |          | (5.1-9.5) | (5.4-9.5) | (6.9-11.3) |      |
|      | CM6912   | 14.4 | 12.8 | 17.1 |      |
|      |          | (11.7-17.7) | (10.3-15.9) | (13.7-21.2) |      |
| B    | CM7116   | 11.7 | 15.5 | 16.4 | 19.1 |
|      |          | (9.6-14.3) | (12.4-19.3) | (14.3-24.1) |      |
|      | CM6913   | 11.2 | 12.8 | 11.7 | 23.3 |
|      |          | (8.9-14.1) | (10.2-16.1) | (9.3-14.6) | (18.9-28.7) |
CM6912 and its metabolites are shown in Table 3B, which shows no particular difference among them.

2. Adaptability to the inclined board

As shown in Table 4, there was no significant difference between CM6912 and diazepam.

3. Hanging behavior

CM6912 and diazepam showed similar values of ED50 at 1 hr, and there was no significant difference between them in general (Table 5).

### Discussion

1. Effects on α- and τ-motor systems:

The monosynaptic reflex (MSR) and the polysynaptic reflex (PSR) are indices for the functions of the α-motor system, while the decerebrate rigidity and the discharges of Glia spindle afferent fiber are indices for the activity of the τ-motor system.

The finding that CM6912 had no effect on either MSR or PSR even at a high dose of 100 mg/kg, therefore, indicates that CM6912 inhibits neither α-motor neurons nor interneurons. Namely, the contraction and relaxation of muscles and the generation of muscular motions may not be affected by CM6912. It is generally known that both MSR and PSR are unaffected by benzodiazepines unless their doses are extremely high. In comparison with diazepam, much greater doses of CM6912 were required to show some effect on MSR or PSR. Thus, it is thought that the α-motor system is not blocked by CM6912 at expected clinical doses. This indicates the high safety of this drug. This concept may also be supported by the finding that the dorsal root reflex potential, which reflects the pre-synaptic inhibition (8), was enhanced by diazepam at a low dose of 10 mg/kg, while it was only slightly enhanced by 100 mg/kg of CM6912.

CM6912 is not devoid of actions on the τ-motor system. This was suggested by the finding that CM6912 decreased EMG discharges induced by decerebrate rigidity and prolonged the inter-spike intervals of discharges by the Glia spindle afferent fiber. The decreased decerebrate rigidity caused by CM6912 is not considered to be due to the suppression of respiration, though this has not been tested yet. This may be inferred from the fact that estazolam (Takeda) which inhibits decerebrate rigidity much more strongly than diazepam did not suppress the spontaneous respiration (Y. Sakai, unpublished observation).

Decerebrate rigidity is thought to be caused by the functional facilitation of the
Since such facilitation can also be induced by anxiety and tension, the suppressive action of CM6912 on the r-motor system may be considered as an advantageous property of this drug. As regards to the mechanism of this inhibitory action of benzodiazepines in general on the r-motor system, we would like to suggest the posterior hypothalamus as a site of action, rather than the direct inhibition of the r-loop. Descendingly, the posterior hypothalamus intrinsically controls the r-motor system in a facilitating manner while ascendingly, it plays important roles in the psychotropic actions of drugs. The activation of the r-motor system by the posterior hypothalamus, as its descending activating function, is thought to be suppressed by benzodiazepines. Therefore, as far as benzodiazepines have the site of action in the posterior hypothalamus, it is reasonable for benzodiazepines to show psychotropic actions as an ascending component and to affect descendingly the function of the r-motor system, namely muscular tone. The extent which one of these actions, ascending and descending, is predominant is related to the pharmacological characteristics of each benzodiazepine.

Based on this concept, it is natural for benzodiazepines to have some inhibitory action on the r-motor system. However, it may be said that when the inhibitory action is weaker, the reduction of muscular tone is smaller, and clinically speaking, the dizziness, weakness and weariness are milder.

Comparing CM6912 with diazepam in this context, CM6912 decreased EMG discharges induced by decerebrate rigidity by about 50% at a dose of 30 mg/kg, while diazepam exhibited similar inhibition at 10 mg/kg, indicating the stronger action of diazepam and a quite weak descending action for CM6912. Assuming that the psychotropic potency of CM6912 is equivalent to that of diazepam, it may be inferred that the separation of the ascending action from the descending one might be quite good in CM6912.

2. Interactions with other centrally acting drugs: It is now widely accepted that the antagonistic activities (ED50) to bemegride-induced convulsions of benzodiazepines are nearly in parallel to their clinical potencies as minor tranquilizers. We have also demonstrated previously that this anticonvulsive potency is the best index for the minor tranquilizing activity of benzodiazepines. It has also been reported by the present author that the potentiation of chlorprothixene-induced anesthesia (the blockade of the righting reflex) may serve as the best index for the sleep inducing action of benzodiazepines. Thus, these two experimental systems are thought to be highly reliable for characterizing individual benzodiazepines. On this basis, as a minor tranquilizer, CM6912 may be ranked to be as potent as diazepam.

In such an experiment with mice, however, it is common to experience that ED50 values tend to vary from experiment to experiment. Therefore, comparisons of the data obtained on different days or under different experimental conditions, even if differences are minor, are not appropriate in general.

Comparing CM6912 with diazepam, it is common to experience that ED50 values tend to vary from experiment to experiment. Therefore, comparisons of the data obtained on different days or under different experimental conditions, even if differences are minor, are not appropriate in general. However, summarizing previous data, it may be said that CM6912 is much more potent than chlor Diazepam and oxazolam, weaker than cloxazolam or bromazepam, and as potent as diazepam. It also seems that CM6912 is several times less potent than lorazepam.

As demonstrated by Cautreels et al., CM7116 and CM6913, formed rapidly after the oral administration of CM6912, and CM7116 and CM6913 have stronger psychotropic activities than CM6912 as reported in the present study. Therefore, CM6912 may be expected to show long-lasting pharmacological actions because of the in vivo formation of these metabolites.

Based on the values of ED50 for the potentiation of chlorprothixene-induced anesthesia, on the other hand, it may be said that CM6912, whose ED50 value is 0.56 mg/kg, is completely devoid of properties as a sleep inducer. The ED50 values of nitrazepam and haloxazolam being as small as 0.041 mg/kg and 0.061 mg/kg, respectively.

Potentiating actions on thiopental-induced anesthesia can not be correlated to the sleep inducing potencies of benzodiazepines in general. However, as shown in Table 2, CM6912 exhibited longer lasting action than
diazepam, and a metabolite, CM7116, was more potent than CM6912.

The rotarod test, the inclined board test and the hanging test are all designed to examine cooperative muscular motions. Therefore, the suppression of the adaptability to these experimental conditions is thought to serve as an index for muscular relaxation. According to the over-all results of these experiments in this study, the suppressive action of CM6912 on cooperative motions of muscles may be judged to be similar to or slightly weaker than that of diazepam.

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