PHARMACOLOGICAL STUDIES ON 6-AMINO-2-FLUOROMETHYL-3-(O-TOLYL)-4(3H)-QUINAZOLINONE (AFLOQUALONE), A NEW CENTRALLY ACTING MUSCLE RELAXANT (1)

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Abstract—Neuropharmacological actions of afloqualone were studied in experimental animals. The dose ratio of loss of righting reflex/muscle relaxant activity of afloqualone in mice was larger than those of tolperisone, mephenesin, and chlormezanone. Afloqualone, like tolperisone, mephenesin, and diazepam, inhibited the ipsilateral flexor reflex, but not the patellar reflex. In decerebrate cats, the inhibitory activities of afloqualone and tolperisone on the polysynaptic spinal reflex were reduced to about 1/5 as compared with those in intact preparations, though the activities of diazepam and mephenesin were almost equal in the both preparations. In spinal preparation, the inhibitory activities of afloqualone, tolperisone, and mephenesin were almost equal to those in decerebrate preparations whereas that of diazepam was reduced to about 1/10 as compared with that in decerebrate preparation. Afloqualone and tolperisone inhibited not only the efferent γ-discharges but also the increment and decrement of patellar reflex by stimulation of the lateral and medial reticular formation. These results suggest that the mode of action of afloqualone is different from the actions of mephenesin, chlormezanone, and diazepam. However, afloqualone, unlike tolperisone, showed no influence on the neuro-muscular transmission and inhibited little the muscle spindle discharges.

In early studies (1, 2) we found that 6-amino-2-fluoromethyl-3-(o-tolyl)-4(3H)-quinazolinone, afloqualone, has potent muscle relaxant activity and is lower in neurotoxicity, as compared with its analogues.

In this paper, further pharmacological investigations were made in order to elucidate the site and mode of action of afloqualone.

MATERIALS AND METHODS

Male dd-Y mice weighing 18 to 22 g and cats of either sex weighing 2.2 to 2.8 kg were used. The drugs used here were as follows: mephenesin (Chugai), chlormezanone (Daiichi), tolperisone hydrochloride (Nipponkayaku), baclofen (3), diazepam (Takeda), morphine hydrochloride (Sankyo), chlorpromazine hydrochloride (Shionogi), α-chloralose (Nakarai), and urethane (Katayama). Afloqualone was suspended in 0.5% carboxymethylcellulose sodium salt for p.o. administration or dissolved in physiological saline by adding a proper amount of 1 N-HCl solution for i.v. administration.
Tolperisone was dissolved in physiological saline. Mephenesin and chlormezanone were dissolved in physiological saline by adding a proper amount of polyethyleneglycol (#400). Without special mention, groups of 6 animals were used. ED50 values were calculated by the probit method. Statistical analyses were performed using Student's t-test.

Muscle relaxant activity (motor coordination)

Muscle relaxant activity after p.o. administration of the drugs was studied in mice as follows:

1. Rotating rod test (RR-test): Mice were placed on a horizontal wooden rod with a diameter of 3.5 cm and rotating at the rate of 7.5 r.p.m. This rod was 40 cm above the floor. The mice which remained on the rod for more than 1 min in two successive trials were selected beforehand. ED50 (RR) was calculated from the number of mice which failed to remain on the rod for 1 min at 1 hr after administration of the drugs.

2. Traction test (TR-test): Mice were suspended by their forelimbs on a horizontal, stainless steel bar with a length of 15 cm and a diameter of 1.5 mm. The rod was 30 cm above the floor. The mice which could put either hind paw on the wire within 10 sec were selected previously. ED50 (TR) was calculated from the number of mice which failed to put either hind paw on the wire within 20 sec at 1 hr after administration of the drugs.

3. Inclined screen test (IS-test): Mice were placed on a canvas screen (50×60 cm) inclined at an angle of 30° from the floor. The mice which remained on the screen for more than 1 min in two successive trials were selected beforehand. ED50 (IS) was calculated from the number of mice which failed to remain on the screen for 1 min at 1 hr after the drugs.

Loss of the righting reflex (LRR) activity

Mice were laid gently on their backs on a table 1 hr after p.o. administration of the drugs. ED50 (LRR) was calculated from the number of mice which lost the righting reflex for more than 20 sec.

Spinal reflexes

1. Monosynaptic spinal reflex (patellar reflex): Cats were spinalized (at C1 level) under ether anesthesia and maintained with artificial respiration throughout the experiment. The left hind limb was anchored by a stainless steel bolt through the femur. The knee jerk was elicited mechanically at 30 sec intervals with a rubber hammer operated by magnetic power. The reflex was recorded on a polygraph pen-recorder (Nihon Kohden, RM-150) via a force-displacement transducer (Nihon Kohden, SB-1T). The drugs were injected through a polyethylene tube which had been inserted into the jugular vein. In addition, blood pressure was recorded on a polygraph pen-recorder via a transducer (Nihon Kohden, PR-3) attached to a cannula which had been inserted into the right common carotid. The actual experiments were not started until 2 hr had elapsed after the termination of ether anesthesia.

2. Polysynaptic spinal reflex:

2.1. Ipsilateral flexor reflex: Intact (α-chloralose 60 mg/kg i.p.), spinal (at C1 level), and decerebrate (intercollicular-prepontine) cats (4, 5) were used. The flexor reflex of the anterior tibialis was elicited by electrical stimulation of the ipsilateral peroneal nerve (1 msec, 0.2 Hz, and 1.5 to 2.0 volt). Inhibitory effects of the drugs on the reflex were expressed by the following formula; [1-[(mean amplitude for 3 min in the maximal inhibitory period after the drugs/mean amplitude for 3 min before the drugs)] × 100. Other experimental conditions were the same as mentioned above.

2.2. Ipsilateral hind-limb withdrawal reflex: The spinal cord was transected at the Th10 level, with the mice under ether anesthesia. Twenty four hr later, the mice which showed lifting up or shaking a hind foot immersed
into the water at 50°C were selected and used in groups of eight. The latent period until the appearance of the reflex was measured 1 hr after the drugs. The reflex of the intact mice was also examined similarly. Increment and decrement of the patellar reflex by stimulation of the facilitatory and inhibitory area of the brainstem reticular formation

Cats were anesthetized with α-chloralose (60 mg/kg i.p.) in combination with urethane (0.5 g/kg s.c.) and fixed in a stereotaxic apparatus (Tokyo Univ. Brain Res. Inst.). The left hind limb was anchored by a stainless steel bolt through the femur. The patellar reflex was elicited mechanically 3 times per 10 sec with a rubber hammer operated by magnetic power. Bipolar stimulating electrodes were acutely inserted into the mesencephalic facilitatory [F: 0–3, L (right): 3–4.5, and H: (−3)–2] and medullary inhibitory [P: 10, L (right): 1, and H: (−9)–(−7)] area according to the brain atlas of Snider and Niemer (6). The increment and decrement of the patellar reflex by stimulation (1 msec, 100 Hz, and 3–5 volt for 30 sec) of the facilitatory and inhibitory area were recorded on a polygraph pen-recorder via a force-displacement transducer. The drugs were injected through a polyethylene tube which had been inserted into the jugular vein.

Muscle spindle discharges

The procedure of laminectomy in cats was carried out in the same manner as in the test on the γ-efferent discharges. After laminectomy, the left hind limb was denervated except the nerve innervating the triceps sural muscle and which was stretched using a wt. of 200 g and a string connected to Achilles’s tendon cut away from the calcaneum. After completion of all surgery, the muscle spindle discharges from filaments of L7 dorsal root were recorded as mentioned above.

Neuro-muscular junction

Cats were spinalized (at C1 level) under ether anesthesia and maintained with artificial respiration throughout the experiment. The sciatic nerve trunk was completely severed in the sciatic notch. The insulated bipolar stimulating electrodes were placed on the peroneal nerve. The twitch of the anterior tibialis was recorded on the polygraph pen-recorder (Nihon Kohden, RM-45) via a force-displacement transducer. Double pulses (0.1 msec, 0.2 Hz, and 1.5 to 2.0 volt) were delivered from the electrical stimulator (Nihon Kohden, MSE-40) and interstimulus interval was gradually increased every 0.1 msec from 0.9 msec to maximum 2.0 msec. The refractory period after administration of the
Drugs was compared with those at 5 min before the drugs. All the drugs were injected through a cannula which had been inserted into the right femoral vein. The actual experiments were not started until 2 hr had elapsed after the termination of ether anesthesia.

RESULTS
Effects on motor co-ordination and righting reflex (LRR)
Fifty % inhibitory doses of afloqualone and other drugs in the rotating rod, traction, and inclined screen tests and their 50% effective doses of the loss of righting reflex (LRR) are shown in Table 1. The muscle relaxant activity of afloqualone was more potent than those of other drugs. The ratio of LRR50 to ED50 (RR) of afloqualone was the largest of all.

Effects on spinal reflexes
1. Monosynaptic spinal reflex (patellar reflex): Neither afloqualone nor tolperisone inhibited the patellar reflex at doses up to 50 mg/kg i.v. and at a dose of 20 mg/kg i.v., respectively. Diazepam was also ineffective in a dose of 10 mg/kg i.v. On the other hand, baclofen markedly depressed the reflex at a dose of 5 mg/kg i.v. (Fig. 1).

2. Polysynaptic spinal reflexes
2.1. Ipsilateral flexor reflexes:
Intact cats: As shown in Fig. 2, afloqualone and other drugs inhibited the flexor reflex dose dependently. The doses required to reduce the amplitude of the flexor reflex before the drugs by 50% were approx. 0.8 mg/kg i.v. in afloqualone, 9.5 mg/kg i.v. in mephenesin, 1.4 mg/kg i.v. in tolperisone, and 0.04 mg/kg i.v. in diazepam, respectively (Fig. 2).

Intercollicular-prepontine decerebrate cats: Afloqualone and other drugs inhibited the flexor reflex dose dependently. The inhibitory activities of afloqualone and tolperisone on polysynaptic spinal reflex in intercollicular decerebrate cats were reduced to about 1/5 as compared with those in the intact cats. However, the activities of diazepam and mephenesin were almost equal in the both preparations (Fig. 2).

Spinal cats: Afloqualone and other drugs inhibited the flexor reflex dose dependently. The inhibitory effects of afloqualone, mephenesin, and tolperisone in spinal cats were equal to those in decerebrate cats, respectively. On the other hand, the inhibitory activity of diazepam on polysynaptic spinal reflex in spinal cats was reduced to approx. 1/10 as compared with that in the decerebrate cats (Fig. 2).
Table 1. Muscle relaxant activities of afloqualone and other drugs in mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Muscle relaxant activity ED50 (95% CL) mg/kg p.o.</th>
<th>LRR50 (95% CL) mg/kg p.o.</th>
<th>LRR50 (RR)</th>
</tr>
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<tr>
<td></td>
<td>RR-test</td>
<td>TR-test</td>
<td>IS-test</td>
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<tr>
<td>Afloqualone</td>
<td>22.4(13.8–31.2)</td>
<td>20.7(14.9–28.8)</td>
<td>19.3(14.1–26.4)</td>
</tr>
<tr>
<td>Tolperisone</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Mephenesin</td>
<td>497.8(432.0–542.3)</td>
<td>527.3(480.0–682.0)</td>
<td>551.2(332.4–913.9)</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>221.6(161.2–291.4)</td>
<td>207.5(156.2–278.4)</td>
<td>156.2(128.0–190.5)</td>
</tr>
</tbody>
</table>

RR-: Rotating rod, TR-: Traction, IS-: Inclined screen, CL: Confidence limit, LRR50: 50% Effective dose for loss of the righting reflex, ED50(RR): 50% Effective dose in a rotating rod test. n=6.
Fig. 2. Dose-response relationships for afloqualone and other drugs on ipsilateral flexor reflex in intact (α-chloralose 60 mg/kg i.p.), spinal (at C1 level), and decerebrate (intercollicular-prepontine) cats. Abscissa: dose in mg/kg i.v., ordinate: inhibitory ratio of flexor reflex. Maximum S.E.'s are 26.2% in afloqualone, 22.4% in mephenesin, 29.8% in tolperisone, and 13.7% in diazepam, respectively. n=4

Fig. 3. Dose-response relationships for afloqualone and other drugs on ipsilateral hind-limb withdrawal reflex in intact and spinal (at Th7-10 level) mice. Abscissa: dose in mg/kg p.o., ordinate: the latent period (sec) until the appearance of the reflex. Each vertical line indicates the S.E. *: p<0.05, **: p<0.01. Mephenesin: x 10 dose in mg/kg p.o. n=8

2.2. Ipsilateral hind-limb withdrawal reflex: In intact mice, afloqualone significantly increased the latency of the reflex dose dependently at doses of 10 to 35 mg/kg p.o. Mephenesin and morphine hydrochloride also exhibited the same effect at doses of 350 mg/kg or more p.o. and 5 mg/kg p.o., respectively. In spinal mice, the activity of afloqualone was reduced to about 1/5 as compared with that in intact mice whereas morphine hydrochloride was inactive even at a dose of 35 mg/kg p.o. However, the activity of mephenesin was almost equal in the both preparations (Fig. 3).

Effects on the increment and decrement of the patellar reflex by stimulation of the facilitatory and inhibitory area of the brainstem reticular formation
Afloqualone had no influence on both the increment and decrement of the patellar reflex at doses up to 5 mg/kg i.v. As shown in Fig. 4-a, 10 mg/kg of afloqualone abolished the increment of the reflex, but not the decrement. The marked inhibition of both the increment and decrement of the patellar reflex was observed at 20 mg/kg i.v. of afloqualone and lasted for more than 2 hr.
Tolperisone (20 mg/kg i.v.), mephenesin (20 mg/kg i.v.), and chlormezanone (20 and 50 mg/kg i.v.) also inhibited both the increment and decrement of the reflex (Fig. 4-b). However, the actions of tolperisone and mephenesin were slight and temporary.

**Effects on T-efferent discharges**

Both afloqualone and tolperisone inhibited the T-efferent discharges dose-dependently at doses of 10 to 20 mg/kg i.v. Inhibitory activity of afloqualone was equal or somewhat superior to that of tolperisone. Chlorpromazine hydrochloride which was used for the identification of the T-neuron filament had a long-lasting depression even at a dose of 0.2 mg/kg i.v. (Fig. 5).

**Effects on muscle spindle discharges**

Muscle spindle discharges appeared immediately after the appropriate stretch of the muscle and lasted during the stretch period. Five cats were used at each dose of the drugs. Afloqualone slightly reduced the discharges in 3 cases out of 5 at a dose of 20 mg/kg i.v. whereas tolperisone markedly inhibited the discharges in all 5 cases (Fig. 6).

**Effects on neuro-muscular junction**

The twitch of the anterior tibialis by double stimulation of the peroneal nerve was shown in Fig. 7. The refractory period varied individually between 0.9 and 1.4 msec (mean±S.E.: 1.16±0.03 msec, n=25) before the drugs. Five cats were used for each dose.
of the drugs. In all cases, afloqualone failed to prolong the refractory period, even at a dose of 50 mg/kg i.v. (from 1.28±0.04 to 1.27±0.04 msec). On the other hand, mephenesin, tolperisone, and chlormezanone markedly prolonged the refractory period from 1.14±0.02 msec to 1.28±0.05 msec, from 1.38±0.06 msec to 1.68±0.04 msec (p<0.05), and from 1.10±0.03 msec to 1.30±0.03 msec (p<0.05) at 20 mg/kg i.v., respectively (Fig. 7).

**DISCUSSION**

The dose ratio of loss of righting reflex/muscle relaxant activity of afloqualone in mice was larger than those of tolperisone, mephenesin, and chlormezanone. This suggests that the specificity of muscle relaxing action of afloqualone is considerably high as compared with those of other drugs.

Afloqualone, like mephenesin, tolperisone, and diazepam, inhibited the ipsilateral flexor reflex dose dependently in the intact, spinal, and decerebrate preparations but not the patellar reflex, even at considerably higher doses. On the other hand, baclofen, unlike
these drugs, inhibited both reflexes, to almost the same degree. These results suggest that the mechanism of muscle relaxing action of afloqualone as well as mephenesin (8, 9), tolperisone (10–13), and diazepam (14) is substantially different from that of baclofen (15–20). However, Crankshaw and Raper (21) reported that mephenesin inhibited the

![Fig. 7. Effects of afloqualone and other drugs on the twitch response of the anterior tibialis muscle by double stimulation, at various intervals, applied to the peroneal nerve in spinal (at C1 level) cats. Only afloqualone failed to prolong the refractory period even at a dose of 50 mg/kg i.v. S and figures indicate a single stimulation and stimulation interval (msec), respectively.](image-url)
monosynaptic spinal reflex potential though it did not inhibit the patellar reflex. Therefore, it cannot be concluded from our results that afloqualone has no inhibitory effect on the monosynapse.

In anesthetized intact preparations, the dose required to reduce the amplitude of the flexor reflex before administration of the drugs by 50% was approx. 0.8 mg/kg i.v. in afloqualone, 9.5 mg/kg i.v. in mephenesin, 1.4 mg/kg i.v. in tolperisone, and 0.04 mg/kg i.v. in diazepam, respectively. In the decerebrate preparation, however, the inhibitory activities of afloqualone and tolperisone on the polysynaptic spinal reflex were reduced to about 1/5 as compared with those in the intact preparation, though the activities of diazepam and mephenesin were almost equal in the both preparations. In the spinal preparation, the inhibitory activities of afloqualone, tolperisone, and mephenesin were almost equal to those in the decerebrate preparation whereas that of diazepam was reduced to about 1/10 as compared with that in the decerebrate preparation. In addition, afloqualone and tolperisone inhibited not only the efferent r-discharges but also both the increment and decrement of patellar reflex by stimulation of the facilitatory and inhibitory area (22, 23) of the brainstem reticular formation. These results suggest that afloqualone and tolperisone may also produce the inhibitory action on the polysynaptic pathways in the higher CNS as compared with diazepam (24–26) which dominantly inhibits the polysynaptic pathways in the lower brainstem. As there was no marked difference between intact and spinal preparations in the inhibitory activities of mephenesin on the flexor reflex in cats and on the ipsilateral hind-limb withdrawal reflex in mice supports the idea that the possible main site of the action of mephenesin (27) is on the polysynaptic pathways of the spinal cord.

Tolperisone, when given i.v., produced a marked hypotension at doses which inhibited the polysynaptic reflex, but it neither lowered the blood pressure nor inhibited the reflex when given p.o. even at higher doses (50 to 100 mg/kg). This suggests that the inhibitory action of tolperisone on the polysynaptic reflex, when given i.v., may be secondary to its hypotensive action, though there have been reports which refuted this proposal (11, 28).

Afloqualone, unlike tolperisone (29), mephenesin (21, 29, 30), and chlormezanone (30), had no influence on the neuro-muscular transmission and the muscle spindle discharges, indicating that the mode of action on the peripheral motor system differs from the actions of tolperisone, mephenesin, and chlormezanone.

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REFERENCES


11) Kuga, T., Kaneshige, T. and Sadanaga, Y.: Effects of 1-piperidino-2-methyl-3-(p-tolyl)-propan-3-on on the mechanism of spinal reflex. Folia pharmacol. japon. 58, 170 (1962) (Abs. in English)


28) Suzuki, T., Ohtsu, K., Ogo, T. and Tomiuiga, T.: Pharmacological studies of 1-piperidino-2-methyl-3-(p-tolyl)-propan-3-on (PMP). Folia pharmacol. japon. 59, 28 (1963) (Abs. in English)
