Pharmacological Studies on a New Dihydrothienopyridine Calcium Antagonist, S-312-d
5th Communication: Anticonvulsant Effects in Mice

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ABSTRACT—S-312, S-312-d, but not S-312-1, L-type calcium channel antagonists, showed anticonvulsant effects on the audiogenic tonic convulsions in DBA/2 mice; and their ED₅₀ values were 18.4 (12.8-27.1) mg/kg, p.o. and 15.0 (10.2-23.7) mg/kg, p.o., respectively, while that of flunarizine was 34.0 (26.0-44.8) mg/kg, p.o. Although moderate anticonvulsant effects of S-312-d in higher doses were observed against the clonic convulsions induced by pentylenetetrazole (85 mg/kg, s.c.) or bemegride (40 mg/kg, s.c.), no effects were observed in convulsions induced by N-methyl-D-aspartate, picrotoxin, or electroshock in Slc:ddY mice. S-312-d may be useful in the therapy of certain types of human epilepsy.

Keywords: S-312-d, Calcium antagonist, Anticonvulsant effect (DBA/2 mice)

Using the cerebral microvessels of rats, Dessy et al. (1) further characterized the calcium antagonistic effects of the enantiomers of S-312, methyl 4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)thieno-(2,3-b)-pyridine-5-carboxylate, following the studies of Ninomiya et al. (2) and Mihara and Fujimoto (3). In this study, [³H]-(+)-PN200-110 specific binding was competitively displaced by the two enantiomers in depolarized cerebral microvessels. The calculated Kᵢ values for S-312-d and S-312-1 were 0.12 and 2.4 nM, respectively. The antihypertensive effect of S-312-d in spontaneously hypertensive rats (SHR) was approximately 100 times stronger than that of S-312-1 (4). In addition, a strong prophylactic effect on the occurrence of strokes in stroke prone SHR (SHRSP) and moderate therapeutic effects in diseased SHRSP were also observed by the daily oral administration of S-312-d (5). In these studies, the viability of the pyramidal cells in the hippocampus of surviving SHRSP was significantly increased by S-312-d. S-312-d seems to prevent the occurrence of cell death following a cerebral stroke by its calcium antagonistic effect. Since S-312-d seems to easily pass through the blood brain barrier because of its high lipophilicity (log P = 5.2, n-octanol/water), it should modify some physiological events related to the central nervous system. The present study examined the anticonvulsant effects of S-312-d in several convolution models in mice.

Male and female DBA/2 mice 3 weeks after birth at Aburahi and weighing 7 to 11 g were used in the experiments. Each group consisted of 5 male and 5 female mice. Audiogenic stimulation (70-90 db) caused by an ultrasonic cleaner (B-32; Bronson, Danbury, CT, USA) was applied to each mouse for 1 min at 1 hr after the oral administration of the test compounds. Usually, following a wild running phase, generalized myoclonus and then tonic flexion and extension of mice were caused by an auditory stimulation. Respiratory arrest of mice was observed after the occurrence of tonic extension. The anticonvulsant effect was determined by recording the tonic extension. ED₅₀ values were calculated by the probit method. Anticonvulsant effects of S-312, S-312-d, S-312-1, nimodipine, nicardipine and nilvadipine, which were synthesized at Shionogi Research Laboratories, were compared with those of flunarizine (Sigma, St. Louis, MO, USA). S-312 and S-312-d, but not S-312-1, had anticonvulsant effects in DBA/2 mice. Dose-dependent anticonvulsant effects were observed after the oral administration of S-312, S-312-d, nilvadipine and flunarizine (Fig. 1). Table 1 shows the ED₅₀.
values of these drugs.

To observe the anticonvulsant effects on drug-induced or electroshock convulsions in mice, 10 male Slc:ddY mice (Japan SLC, Inc., Hamamatsu), 4 to 6 weeks after birth, were used as one group. One hour after the oral administration of the test compounds, pentylenetetrazole (PTZ, Sigma) at 85 or 125 mg/kg, s.c.; bemegride (Tanabe, Osaka) at 40 mg/kg, s.c.; N-methyl-D-aspartate (NMDA, Sigma) at 200 mg/kg, i.p.; or picrotoxin (Sigma) at 10 mg/kg, s.c. was given, and the clonic or tonic convulsion was recorded. Electro-shock convulsions were induced by the application of rectangular currents (50 mA, 1 msec, 100 Hz; ECT unit, Ugo Basile, Varese, Italy) to the eyelids of mice for 0.2 sec at 1 hr after the oral administration of the test compounds. Although S-312-d did not have an anticonvulsant effect against the tonic convulsions induced by a higher dose of PTZ, S-312-d at an oral dose of 30 or 100 mg/kg significantly inhibited the clonic convulsions induced with a smaller dose of PTZ or bemegride (Table 2). No anticonvulsant effects of S-312-d were observed on the convulsions induced by picrotoxin, NMDA or electro-shock.

Although almost no anticonvulsant effects were observed with nicardipine or nimodipine, dose-dependent anticonvulsant effects against the audiogenic convulsion in DBA/2 mice were observed with the oral administration of S-312, S-312-d, nilvadipine and flunarizine. Since S-312-1 had no anticonvulsant effect and S-312-d had a stronger effect than S-312, the racemate, these anticonvulsant effects seem to be derived from their calcium antagonistic effects. Comparing the anticonvulsant effects of various calcium antagonists against the audiogenic convulsions in DBA/2 mice, De Sarro et al. (6) observed that the anticonvulsant effect of flunarizine was several times stronger than that of some dihydropyridine calcium antagonists such as nicardipine, nimodipine, nifedipine and nitrendipine. Although the anticonvulsant effect against the drug-induced or electro-shock convulsions was weak, the anticonvulsant effects of S-312-d on the audiogenic convulsions in DBA/2 mice were stronger than those of flunarizine. Jones et al. (7) suggested the activation of the glutamatergic system in the audiogenic convulsions in DBA/2 mice, because these convulsions were inhibited by a phosphonate dipeptide. Although S-312-d could not inhibit the drug-induced convulsions caused by the direct activation of the respective nerve receptors, it might be able to suppress the audiogenic convulsions by the inhibition

**Table 1. Anticonvulsant effects of calcium antagonists on the audiogenic tonic convulsions in DBA/2 mice**

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED50 mg/kg, p.o. (95% confidence limits)</th>
</tr>
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<tbody>
<tr>
<td>S-312-d</td>
<td>15.0 (10.2–23.7)</td>
</tr>
<tr>
<td>S-312</td>
<td>18.4 (12.8–27.1)</td>
</tr>
<tr>
<td>S-312-1</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Nilvadipine</td>
<td>37.6 (22.9–57.1)</td>
</tr>
<tr>
<td>Flunarizine</td>
<td>34.7 (26.0–44.8)</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>&gt;80</td>
</tr>
</tbody>
</table>

Audiogenic stimulation was applied to each mouse at 1 hr after the oral administration of calcium antagonists, which were dissolved in polyethyleneglycol 400.
of the massive release of glutamate upon audiogenic stimulation. Recently, Gemba et al. (8) found that S-312-d has marked neuronal protective effects against ischemic injury in SHRSP by the inhibition of massive releases of glutamate. It is also interesting to note that S-312-d dose-dependently suppressed the tonic convulsion in spontaneously epileptic rats (9). Recently, Sasa et al. (10) observed that treatment with repeated oral administration of S-312-d was more effective in inhibiting this convulsion.

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REFERENCES