Effect of Polygala tenuifolia Root Extract on Scopolamine-Induced Impairment of Rat Spatial Cognition in an Eight-Arm Radial Maze Task

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The effects of Polygala tenuifolia root fractions and the acyl groups of its constituents on the retrieval process of spatial cognition in rats were studied using an eight-arm radial maze task. Oral administration of a precipitate fraction (PTB) obtained by concentration of the n-BuOH-soluble portion from the extract of the roots significantly decreased the number of total errors (TEs) and that of working memory errors (WMEs) at doses of 100 mg/kg and 200 mg/kg. However, it caused no significant decrease in the number of reference memory errors (RMEs). In addition, the saponin-rich fraction (PTBM) obtained by purification of PTB also showed significant decreases in TEs and WMEs at a dose of 100 mg/kg. Among the cinnamic acid derivatives present as the acyl groups in the P. tenuifolia constituents, sinapic acid (SNP A) significantly decreased TEs and WMEs at doses of 10 to 100 mg/kg. These results indicated that P. tenuifolia extracts, PTB and PTBM, and SNP A had a beneficial effect on the memory impairment induced by dysfunction of the cholinergic system in the brain. The memory improvement in the scopolamine-induced memory impairment seen in the radial maze performance was due to improvement in the short-term memory. A contribution of some constituents other than SNP A to the memory improvement was also suggested.

Key words Polygala tenuifolia; spatial cognition; eight-arm radial maze task; sinapic acid; saponin

Since senile dementia of the Alzheimer type and multi-infarct dementia accompanying the increase in aging populations has become a social problem, the development of effective drugs for treating these conditions is vital. Among the traditional Chinese natural medicines, Polygala root (Japanese name: onjii)—the root of Polygala tenuifolia WILDL.DEN.W (family Polygalaceae)—may improve memory, since some Chinese prescriptions containing Polygala root have been used to treat amnesia, neurasthenia, palpitation, and insomnia.1) Recent studies revealed that P. tenuifolia roots are effective for memory impairment in rats and mice, and scopolamine-induced impairment of passive avoidance response was improved by oral administration of water extract of Polygala root in mice.2) Park et al. also reported that ethanol extract from Polygala root (BT-11) was effective against scopolamine-induced cognitive impairments in passive avoidance response and water maze tests.3) Tenuifoliside B, one of the saccharides containing acyl groups isolated from P. tenuifolia, had an ameliorative effect on scopolamine-induced impairment in the performance of passive avoidance tasks in rats.4)

The eight-arm radial maze task is regarded as more useful for studying the effects of drugs on the learning process and memory than other methods such as passive and active avoidance tasks5—7) because it estimates the process of spatial cognition. Moreover, drugs used clinically to treat dementia also display effectiveness in the radial maze task.8) Therefore, we studied the effects of a precipitate fraction (PTB) obtained from an n-BuOH extract of P. tenuifolia on the retrieval process of spatial cognition in rats using the eight-arm radial maze with four-arms baited. We also investigated the effects of cinnamic acid derivatives, which are present as the acyl groups of the constituents of PTB, and a purified saponin-rich fraction (PTBM) on scopolamine-induced memory impairment in this experimental system.
lowing day. The test drugs were administered 30 min before the scopolamine treatment, and test trials were undertaken 30 min posttreatment.

The total error (TE) was defined as the number of entries into the unbaited arms. The reference memory error (RME) and working memory error (WME) were defined as the first entry to never-baited arms, and reentry to arms where the pellet had already been taken, respectively.

**Extraction and Fractionation of *P. tenuifolia* Roots**

Dried roots (500 g) of *P. tenuifolia* purchased from Tochimoto-tenkai-do, Osaka, Japan, were pulverized and extracted three times with MeOH at room temperature. The combined crude extract (141 g) was dissolved in H2O and successively extracted with *n*-hexane, EtOAc, and *n*-BuOH (Fig. 1). These solutions were concentrated to give the *n*-hexane extract (10.2 g), EtOAc extract (5.5 g), and a water-soluble portion (63 g). The *n*-BuOH solution was concentrated to give PTB as the precipitate and the precipitate (27 g) was separated from the supernatant (9.6 g) by centrifugation (300×g for 5 min).

**HPLC Analysis of the Hydrolysate of PTB**

The PTB fraction (1 g) was dissolved in 5% NaOH (15 ml) and the solution was refluxed for 1.5 h (98 °C). The reaction mixture was then analyzed by HPLC [column, YMC-pack ODS A-302 (internal diameter×length, 4.6 mm×150 mm); mobile phase, CH3CN–H2O–HCOOH (30 : 70 : 1), or 0.01M H3PO4–0.01 M KH2PO4–MeOH (40 : 40 : 20); column temp., 40 °C; flow rate, 1.0 ml/min; detection, UV 205 nm or 230 nm; instrument, model LC-10AD (Shimadzu, Kyoto, Japan)] to detect the presence of 3,4,5-trimethoxycinnamic acid (TMCA), *p*-methoxycinnamic acid (PMCA), sinapic acid (SNP A), and tenuifolin (= presenegenin 3-*O*-glucoside), the major prosapogenin in the saponins of *P. tenuifolia* roots (Fig. 2, Table 1). The presence of tenuifolin was further substantiated by isolation from the reaction mixture by preparative TLC [Silica gel 60F254 (Merck)] with the upper layer of *n*-BuOH–AcOH–H2O (4 : 1 : 5) to produce a compound (5.6 mg), which was identified as 3,6’-di-*O*-sinapoylsucrose by comparing its 1H- and 13C-NMR spectral data with those in the literature. The identity was confirmed by the electrospray-ionization mass spectroscopic data, and the heteronuclear multiple-bond connectivity (HMBC), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear single quantum correlation (HSQC), and 1H–1H correlation spectroscopy (1H–1H COSY) analyses. The 100% MeOH elute from the Diaion column was concentrated to give a saponin-rich fraction (PTBM, 4.8 g). The PTBM fraction was treated with alkali as in the case of PTB, and the reaction mixture was analyzed by HPLC to show the presence of TMCA, PMCA, and tenuifolin (Table 1).

**Administration of Polygala Root Fractions and Hydroxycinnamic Acid Derivatives**

The PTB and PTBM fractions were dissolved in 0.9% physiological saline and administered orally (p.o.) 60 min before each session. Scopolamine hydrobromide (Nacalai Tesque, Kyoto, Japan) was dissolved in 0.9% physiological saline and 0.5 mg/kg scopolamine was injected intraperitoneally (i.p.) 30 min before the session. TMCA (Sigma, St. Louis, MO, U.S.A.), PMCA (Nacalai Tesque), and SNP A (Tokyo Kasei, Tokyo, Japan) were suspended in 5% gum arabic and administered p.o. 60 min before each session.

**Data Analysis**

Each value is shown as the mean±S.E.M. One-way analysis of variance (ANOVA) with Dunnett’s test or the Mann–Whitney U-test was used for statisti-
Scopolamine-Induced Memory Impairment and Effect of PTB on Radial Maze Performance

The polar PTB precipitate, obtained by concentration of an n-BuOH extract of *P. tenuifolia*, was considered to be a glycoside-rich fraction (Fig. 1), since *P. tenuifolia* contains large amounts of polar glycosides. The presence of glycosides was confirmed by hydrolysis of PTB to yield tenuifolin.

Scopolamine administration (0.5 mg/kg i.p.) increased both RMEs and WMEs in the radial maze task with four-arms baited. Oral administration of PTB improved TEs and WMEs and the decreases in these errors were dose-dependent. At doses of 100 mg/kg and 200 mg/kg, PTB significantly decreased TEs and WMEs, while PTB at a dose of 50 mg/kg produced no significant decreases in TEs and WMEs. Moreover, it showed no significant effect on RMEs at any dose.

Since WMEs and RMEs correspond to short-term and long-term memory, respectively, the results indicated that PTB has an ameliorating effect on short-term memory, but not long-term memory (Fig. 3). This is the first report on the improvement effect of a glycoside-rich fraction from *P. tenuifolia* on the retrieval process in spatial cognition using eight-arm radial maze assay systems. The effects were limited to short-term memory in the experimental system used. Although the ameliorating effects of the crude extract of *P. tenuifolia* on memory in a passive avoidance response experimental system and in a water maze system have been previously reported, these systems did not discriminate between these two types of errors.

The Analysis of the Constituent Acyl Groups of PTB

Since PTB improved WMEs and TEs in the radial maze test, this fraction was analyzed after hydrolysis to determine its constituent acyl groups. HPLC analysis of the hydrolysate showed the presence of TMCA, PMCA, and SNP A, in addition to tenuifolin in the reaction mixture (Table 1). The presence of SNP A in PTB was further verified by isolation of the polar PTB precipitate, obtained by concentration of an n-BuOH extract of *P. tenuifolia* and the isolation of TMCA, PMCA, and SNP A, in addition to tenuifolin in the reaction mixture (Table 1). The presence of SNP A in PTB was further verified by isolation of the polar PTB precipitate, obtained by concentration of an n-BuOH extract of *P. tenuifolia* and the isolation of TMCA, PMCA, and SNP A, in addition to tenuifolin in the reaction mixture (Table 1).

**Effects of Cinnamic Acid Derivatives: the Constituents of *P. tenuifolia* on Radial Maze Performance**

As shown in Fig. 4, a significant improvement on scopolamine-induced memory impairment was observed for SNP A under a wide range of doses: SNP A decreased TEs and WMEs at doses of 10 to 100 mg/kg. These results show that SNP A participated in the cognitive improvement of PTB on TEs and WMEs.

SNP A may enhance neurotransmitters in the central nervous system (CNS), such as the prolongation of hexobarbital sleeping time in mice. were also reported for the cinnamic acid derivatives PMCA and TMCA. However, these compounds caused no improvement in the cognitive impairment in the present experiment systems: PMCA slightly decreased TEs at doses of 20 to 100 mg/kg, but the decreases were not statistically significant. TMCA produced no significant effect on TEs and WMEs at doses of 20 to 100 mg/kg.

**Effect of PTBM Obtained by Purification of PTB on the Radial Maze Performance**

Since tenuifolin was detected in the hydrolysate of PTB, saponin (triterpene glycoside) structures or their aglycone residues other than the acyl groups (i.e., cinnamic acid derivatives) may also contribute to the improvements produced by PTB. PTB was therefore
further purified in a Diaion HP-20 column to give PTBM as the MeOH eluate. The quantitative analysis of the reaction mixture obtained by alkaline hydrolysis of PTBM indicated that the content of tenuifolin was higher than that in the hydrolysate of PTB (Table 1).

The improvement in spatial cognition with PTBM was more pronounced than that observed for PTB: PTBM caused a significant decrease in TEs and WMEs at a dose of 100 mg/kg (*p*/H11021 0.01). By contrast, PTBM showed no significant effect on RMEs at any dose. Analysis of the hydrolysate of PTBM indicated that the major constituent acids were PMCA and TMCA. Therefore, the cognitive improving activity of PTB could be due to a residue or residues other than these acyl groups. However, the effect of PTBM at a dose of 200 mg/kg was weaker than that observed for a dose of 100 mg/kg (Fig. 5).

**CONCLUSIONS**

In this study, PTB from the n-BuOH fraction of *P. tenuifolia* had a significant ameliorating effect on scopolamine-induced memory impairment in the spatial cognition process of rats. The effect was especially pronounced in short-term memory, but not in long-term memory. The HPLC analysis of hydrolysate of PTB suggests that the effect of PTB might be due to highly polar compounds. SNPA, which is present...
Table 2. Effects of *Polygala tenuifolia* Fractions and Compounds Structurally Related to the Constituents on the Running Time per Choice of Rats in Radial Maze Performance

(A) Effect of PTB and PTBM on the Running Time per Choice of Rats in Radial Maze Performance

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Scopolamine</th>
<th>Running time per choice (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>–</td>
<td>–</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>Control (Saline)</td>
<td>+</td>
<td>+</td>
<td>18.8 ± 2.0</td>
</tr>
<tr>
<td>PTB</td>
<td>50</td>
<td>+</td>
<td>13.5 ± 2.6</td>
</tr>
<tr>
<td>PTB</td>
<td>100</td>
<td>+</td>
<td>13.2 ± 1.2</td>
</tr>
<tr>
<td>PTB</td>
<td>200</td>
<td>+</td>
<td>13.2 ± 1.1</td>
</tr>
<tr>
<td>PTBM</td>
<td>50</td>
<td>+</td>
<td>12.5 ± 2.0*</td>
</tr>
<tr>
<td>PTBM</td>
<td>100</td>
<td>+</td>
<td>11.2 ± 1.0**</td>
</tr>
<tr>
<td>PTBM</td>
<td>200</td>
<td>+</td>
<td>11.8 ± 1.4*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. (n=12). *p<0.01 compared to the non-scopolamine-treated group. **p<0.001 compared to the control (scopolamine-treated group).

(B) Effect of Cinnamic Acid Derivatives on the Running Time per Choice of Rats in Radial Maze Performance

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Scopolamine</th>
<th>Running time per choice (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum Arabic</td>
<td>–</td>
<td>–</td>
<td>5.9 ± 1.7</td>
</tr>
<tr>
<td>Control (Gum Arabic)</td>
<td>+</td>
<td>+</td>
<td>17.0 ± 4.9*</td>
</tr>
<tr>
<td>SNPA</td>
<td>100</td>
<td>+</td>
<td>11.6 ± 1.8</td>
</tr>
<tr>
<td>TMCA</td>
<td>100</td>
<td>+</td>
<td>12.4 ± 2.0</td>
</tr>
<tr>
<td>PMCA</td>
<td>100</td>
<td>+</td>
<td>10.4 ± 1.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. (n=12). *p<0.01 compared to the non-scopolamine-treated group.

As the sinapoyl moiety in the glycosides,25) showed a significant improvement in TEs and WMEs. The participation of other parts of the glycosides such as tenuifolin in these improvements was also suggested.

Since the mechanism underlying the memory-enhancing effect of *P. tenuifolia* root extracts was correlated with the induction of choline acetyltransferase activity26) and the inhibition of acetycholine esterase activity,27) the improving effect of PTB on scopolamine-induced memory impairment shown here may be correlated with enhancement of the central cholinergic system. In the early phase of Alzheimer’s disease, the memory deficits are thought to involve short-term, but not long-term memory,28) and the spatial cognitive impairment induced by anticholinergic agents corresponds to dementia of the Alzheimer type. Our results indicate that the memory improvement of PTB in the scopolamine-induced memory impairment seen in the radial maze performance was due to the improvement of the short-term memory, but did not involve long-term memory. Therefore, *P. tenuifolia* may represent a potential treatment for the early phase of Alzheimer’s disease.