Panax ginseng Extract Improves the Scopolamine-Induced Disruption of 8-Arm Radial Maze Performance in Rats

Hiroyuki Nitta,* Kinzo Matsumoto,* Mineo Shimizu,† Xiao-Hu Ni,* and Hiroshi Watanabe*‡

Division of Pharmacology, Research Institute for Wakan-Yaku (Oriental Medicines),* Laboratory of Pharmacognosy, Department of Medicinal Resources, Faculty of Pharmaceutical Sciences,† Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama-shi, Toyama 930-01, Japan. Received April 6, 1995; accepted June 20, 1995

The effects of Panax ginseng ethanol extract and its water (WSF)- and lipid-soluble (LSF) fractions on the scopolamine-induced disruption of radial maze performance in rats were examined. Ginseng root was refluxed with ethanol, and WSF and LSF were prepared from this ethanol extract. Scopolamine (0.075—0.3 mg/kg, i.p.) dose-dependently impaired the maze performance. However, the oral administration of Panax ginseng ethanol extract and WSF (2—8 g dried root/kg) 90 min before testing improved the maze performance disrupted by scopolamine (0.3 mg/kg) in a dose-dependent manner, but LSF failed to attenuate the disruption. These data suggest that ginseng extract possesses a beneficial effect regarding spatial cognitive impairment and that the water-soluble fraction of ginseng extract mainly contributes to the effect of the ethanol extract.

Key words Panax ginseng; spatial cognition; scopolamine; rat

Panax ginseng has been used for thousands of years as a tonic or a panacea in China, Korea and Japan. We have previously demonstrated that the water extract of Panax ginseng ameliorates the impaired performance of middle-aged rats in the Morris water maze task1) and improves the scopolamine-induced spatial cognitive impairment in the T-maze delayed alternation task in the rat.2) However, it is not yet known which component of ginseng participates in such an ameliorative effect.

Working memory is the memory analogous to recent memory in humans. This type of memory is known to be more severely impaired than remote memory in senile dementia. In the present study, to further clarify the action of ginseng on the working memory process, we investigated the effect of ginseng extract using an eight-arm radial maze task.

MATERIALS AND METHODS

Animals Male Wistar rats (Japan SLC, Shizuoka), weighing 280—390 g were used. Four or five rats were housed in a cage with free access to water in an airconditioned room. Housing conditions were thermostatically maintained at 23 ± 1°C with 60 % humidity, in a 12 h light/dark cycle (light on: 0730—1930). Rats were maintained on a restricted feeding schedule designed to keep their body weight at about 85% of the free feeding level.

Apparatus Each arm (50 x 12 cm) of the eight-arm radial maze extended from an octagonally shaped central hub (30 cm across). The platform was elevated 40 cm above the floor. Black Plexiglas cups (3 cm diameter, 1 cm deep) were placed at the end of each arm as receptacles for reinforcers (45 mg food pellet; Bio-Serv, NJ, U.S.A.). Guillotine doors surrounded the hub.

Procedures Animals were trained as detailed in our previous reports.3—5) Briefly, one daily training trial was performed with rats using food reinforcement. The trial was judged to be completed when the rat had visited all 8 arms or had spent 10 min in the maze. Entry into an arm that the animal had not previously visited was recorded as a correct response and re-entry as an error. The number of correct responses before committing the first error (the number of initial correct responses) was used as an index of radial maze performance. Only the rats which made no errors, or only one error at the eighth choice, for 5 consecutive days were used for the drug tests. Since the effects of scopolamine on radial maze performance are known to vary depending on the training,6) we employed this very high criterion to get a stable performance. To calculate the running time, the total running time in each session was divided by the total number of choices.

Drugs Panax ginseng root (2 kg, Nihon Hunmatsu Yakuhin Co., Ltd., Osaka, Japan) was refluxed three times with 3.0, 2.5 and 2.51 of ethanol, respectively, for 1 h. The combined hot filtrate was evaporated to dryness in vacuo to yield ethanol extract (EE). The extract was suspended in 500 ml of distilled water and extracted three times with 500, 300 and 300 ml of diethylether, respectively, for 1 h. The ether-soluble layer and water-soluble layer were separated and the ether-soluble layer was evaporated to yield lipid-soluble fraction (LSF). The water-soluble layer was freeze-dried to obtain a water-soluble fraction (WSF). Yields of EE, LSF and WSF were 4.6, 0.4 and 3.8% of the dried herb weight, respectively. EE and LSF were suspended in 0.5% CMC-Na solution and WSF was dissolved in distilled water just before the experiments. Each fraction was orally administered 90 min before testing. Doses of each fraction were expressed as the dried herb weight per kg body weight. Scopolamine HBr (Nacalai Tesque, Inc., Kyoto, Japan) dissolved in physiological saline was intraperitoneally injected 30 min before testing.

Statistical Analysis Data were analyzed using the Kruskal-Wallis analysis of variance, followed by the Mann-Whitney U-test for multiple comparison between groups. Differences with p<0.05 were considered statistically significant.

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RESULTS

Scopolamine (0.075—0.3 mg/kg) dose-dependently decreased the initial correct responses in the radial maze performance in rats. The decreasing effect was significant at 0.3 mg/kg (i.p.) \((U=9, p<0.01; \text{Fig. 1})\). From this result, 0.3 mg/kg scopolamine was used in the subsequent experiments. When ginseng EE and WSF (2—8 g dried root/kg) were administered, the maze performance disrupted by scopolamine (0.3 mg/kg) was significantly improved in a dose-dependent manner (8 g/kg EE: \(U=28.5, p<0.05; \text{8 g/kg WSF: } U=21, p<0.05\)). On the other hand, LSF had no statistically-significant effect on the scopolamine-induced disruption of the maze performance (Fig. 2).

As summarized in Table 1, 0.3 mg/kg scopolamine significantly prolonged the running time in one of the three independent experiments and showed a tendency to prolong it in the other two experiments. The ginseng extract, especially WSF, showed a tendency to reverse the running time of scopolamine treated rats to the control level, but the effect was not statistically significant.

DISCUSSION

The present results support our previous data that the ginseng water extract improved scopolamine-induced working memory deficits in the T-maze delayed alternation task in rats, and also give further evidence that such an ameliorative effect is mainly attributed to the WSF of the ginseng extract. In this study, EE and WSF reversed the number of initial correct responses decreased by scopolamine (0.3 mg/kg i.p.).

Consistent with the data reported by Wirsching et al. and Beatty and Bierlay, our preliminary experiment showed that the same dose of scopolamine disrupted only...
Table 1. Effects of Ginseng Root Fractions on Running Time in the Radial Maze Task

<table>
<thead>
<tr>
<th>Drug treatment (g/kg)</th>
<th>n</th>
<th>Running time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>11</td>
<td>16.4 ± 3.3</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>12</td>
<td>85.5 ± 33.4*</td>
</tr>
<tr>
<td>+ ethanol extract 2</td>
<td>11</td>
<td>33.0 ± 6.7</td>
</tr>
<tr>
<td>+ ethanol extract 4</td>
<td>12</td>
<td>48.6 ± 13.5</td>
</tr>
<tr>
<td>+ ethanol extract 8</td>
<td>12</td>
<td>31.7 ± 5.9</td>
</tr>
<tr>
<td>Exp. II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>9</td>
<td>22.4 ± 5.1</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>12</td>
<td>51.7 ± 10.4</td>
</tr>
<tr>
<td>+ water-soluble fraction 2</td>
<td>10</td>
<td>49.2 ± 10.8</td>
</tr>
<tr>
<td>+ water-soluble fraction 4</td>
<td>11</td>
<td>32.1 ± 5.9</td>
</tr>
<tr>
<td>+ water-soluble fraction 8</td>
<td>10</td>
<td>29.6 ± 8.6</td>
</tr>
<tr>
<td>Exp. III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>10</td>
<td>18.2 ± 4.8</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>12</td>
<td>61.0 ± 24.5</td>
</tr>
<tr>
<td>+ lipid-soluble fraction 2</td>
<td>10</td>
<td>21.3 ± 3.0</td>
</tr>
<tr>
<td>+ lipid-soluble fraction 4</td>
<td>12</td>
<td>48.4 ± 17.2</td>
</tr>
<tr>
<td>+ lipid-soluble fraction 8</td>
<td>10</td>
<td>51.3 ± 17.4</td>
</tr>
</tbody>
</table>

Saline or scopolamine (0.3 mg/kg) was intraperitoneally injected 30 min before testing. Ginseng root fractions were orally administered 60 min prior to scopolamine injection. *p<0.01 compared with respective saline control (Mann-Whitney U-test).

the working memory, without affecting reference memory, in the 4-arm baited radial maze performance (unpublished data). Thus, the present results suggest that EE and WSF improved the working memory deficit induced by scopolamine.

We previously reported that the systemic administration of scopolamine prolonged the running time in the radial maze task.3,4) Consistent with the previous observation, 0.3 mg/kg scopolamine significantly prolonged the running time in one of three independent experiments, although the effects were variable in the other two experiments. Moreover, EE and WSF showed a tendency to attenuate the effect of scopolamine on the running time at doses which significantly blocked a scopolamine-induced decrease in the number of initial correct responses. It is not yet clear whether the effect of scopolamine on the running time is mediated by its central action, since methylscopolamine, a peripherally acting scopolamine derivative, reportedly prolongs the running time without affecting the choice accuracy in the radial maze task.9) Thus, the present findings do not completely exclude a possibility that the effects of EE and WSF on the running time may contribute to their ameliorating actions on the spatial cognitive impairment caused by scopolamine.

The effects of ginseng on learning and memory performance have also been studied using negatively reinforced tasks such as passive avoidance3,10,11) and conditioned avoidance tasks.12) In our previous study, the subchronic intake of ginseng water extract improved the impaired performance of middle-aged rats in the Morris water maze task.13) However, in these tasks, rats were subjected to aversive stimuli such as electric shocks or swimming in water. Ginseng is classified pharmacologically as an adaptogen that increases the non-specific resistance of experimental animals against different types of external stress.13) Moreover, ginseng has been reported to have an anti-fatigue effect.14) Therefore, it is difficult to exclude the possibility of the improvement of learning and memory observed in these negatively reinforced tasks is partially due to its adaptogenic or anti-fatigue effects. It should be noted that in the present study, EE and WSF of ginseng improved the performance in the food reinforced task that included no aversive stimuli.

As components of Panax ginseng, ginseng saponins are contained in WSF, while panaxynol, β-sitosterol or linoleic acid are in LSF.15,16) In the present study, WSF improved the working memory deficit induced by scopolamine but LSF failed, suggesting that ginseng saponins play an important role in the improvement of scopolamine-induced spatial cognitive disruption. The mechanisms underlying the memory-enhancing effect of ginseng remain unclear. In in vitro studies, however, ginsenoside Rb1 facilitates the release of acetylcholine from hippocampal slices of rat brain.17) Thus, it is possible that ginseng reveals its ameliorative effect through enhancing the central cholinergic mechanisms. On the other hand, malonylginsenoside Rb1, which has been isolated from a water-soluble fraction of Panax ginseng root, appears to facilitate the generation of long-term potentiation by a weak tetanus stimuli in the dentate gyrus of rat hippocampus.18) Ginseng may be able to affect the synaptic plasticity that may underlie learning and memory. Nevertheless, additional experiments will be necessary to clarify the detailed mechanisms.

In conclusion, the ethanol extract of Panax ginseng improved spatial cognitive impairment induced by scopolamine in the 8-arm radial maze performance, and the water-soluble fraction of ginseng mainly contributes to the ameliorative effect of Panax ginseng.

Acknowledgment This research was supported by a Grant-in-Aid for Scientific Research on Wakan-Yaku and biotechnology from Toyama Prefecture.

REFERENCES


