Tetramethylpyrazine Improves Spatial Cognitive Impairment Induced by Permanent Occlusion of Bilateral Common Carotid Arteries or Scopolamine in Rats

Jian-Wei Ni, Kinzo Matsumoto and Hiroshi Watanabe*

Division of Pharmacology, Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

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Abstract—Effects of tetramethylpyrazine (TMP), a major constituent of Ligusticum chuanxiong, on spatial cognitive impairment induced by permanent occlusion of bilateral common carotid arteries (2VO) and scopolamine were investigated using 8-arm radial maze performance in rats. Permanent 2VO produced a severe learning deficit in non-pretrained rats. Daily administration of TMP (3 - 10 mg/kg, i.p.) from the 3rd day after permanent 2VO significantly improved the learning deficit. TMP did not influence the impairment of the retention task in the pretrained permanent 2VO rats, but it tended to reduce the number of errors elevated by 3-min delay interposition in these rats. In the scopolamine model, scopolamine (0.3 mg/kg, i.p.) significantly decreased the initial correct response and increased the number of errors. Single administration of TMP (1 - 3 mg/kg, i.p.) dose-dependently reversed the scopolamine-induced impairment of the maze performance. These results suggest that TMP has therapeutic potential for the treatment of dementia caused by cholinergic dysfunction and/or decrease of cerebral blood flow.

Keywords: Tetramethylpyrazine, Permanent occlusion (bilateral common carotid arteries), Scopolamine, Ischemia, Learning

Tetramethylpyrazine (TMP) is a constituent isolated from Ligusticum chuanxiong, which has long been used in China as a traditional medicine to treat many patients suffering from stroke and some cognitive impairments. TMP is known to increase the cerebral blood flow (1) and to have protective action against severe acute ischemic attacks, resulting in an increase of the survival rate after the cerebral ischemia procedure in Mongolian gerbils (2). Moreover, TMP produces a vasodilatory action by inhibiting both Ca²⁺ influx and intracellular Ca²⁺ release (3) in dog mesenteric arterial preparation. These pharmacological profiles of TMP give rise to the possibility that its administration may improve the cognitive impairment caused by a decrease in cerebral blood flow and/or protect against the neuronal damage following cerebral ischemia in rats.

We previously reported that a chronic mild hypoperfusion induced by permanent occlusion of bilateral common carotid arteries (2VO) caused learning deficits in the early stage (within 1 month) and memory deficits in the late stage (4 months after the 2VO operation) and that this progressive cognitive deficit paralleled the progress of neuronal damage (4). This animal model is advantageous for examining whether drugs prevent progressive cognitive impairment and neuronal damage induced by chronic cerebral hypoperfusion. On the other hand, it is well known that central acetylcholine (ACh) neurons play an important role in cognitive function (5, 6) and that the function of the cholinergic system can be damaged by cerebral ischemia (7 - 9). In the present study, we investigated the effect of TMP on spatial cognitive impairment using two animal models: permanent 2VO-induced learning deficit and scopolamine-induced working memory impairment.

Materials and Methods

Animals

Male Wistar rats (7 weeks to 9 months old; Japan SLC, Inc., Shizuoka) were used. The animals were housed in groups of 3 - 4 per cage, and the housing conditions were maintained at 24±1°C and 55±5% relative humidity,
with a 12-hr light/dark cycle (lights on 07:30 – 19:30). During the eight-arm radial maze task, the animals were maintained on a restricted feeding schedule designed to keep their body weight at approximately 85% of the free-feeding level. Water was available ad libitum.

Apparatus

The apparatus consisted of an octagonal platform (30 cm across) and eight arms (50 x 12 cm) radially extended from the platform. The maze was elevated 40 cm above the floor. Small black cups (3 cm in diameter and 1 cm in depth) were mounted at the end of each arm as receptacles for reinforcer (45 mg pellet; Bio-Serv., Frenchtown, NJ, USA). Guillotine-type doors surrounded the platform and controlled access to each arm.

Permanent 2VO-induced spatial cognitive impairment test

Surgical procedure: Bilateral common carotid arteries were occluded as previously described (4). Briefly, under sodium pentobarbital (40 mg/kg, i.p.) anesthesia, the bilateral common carotid arteries of the rat were carefully separated from the cervical sympathetic and vagal nerves through a ventral cervical incision. Then, the arteries were doubly ligated with silk sutures simultaneously in the permanent 2VO group. The rats that received the same surgical operation without carotid artery ligation served as the sham-operated controls.

Radial maze learning task: Each animal was handled for 5 – 10 min daily for 2 days and was given 5 days adaptation to the maze under the restricted feeding condition. Thereafter, these non-pretrained animals received permanent 2VO or sham operation. Three days after the surgical operation, the maze learning was started. Each rat was placed individually on the central platform with all guillotine doors closed. After 5 – 10 sec, all of the doors were opened simultaneously to allow the animal to access the arms freely. The rat was judged to have made a choice when all four paws entered an arm. Entry into an arm that the rat had not previously visited was recorded as a correct choice, and re-entry was counted as an error. The trial was judged complete when the rat had visited all 8 arms or had spent 10 min on the maze. Each rat was tested once daily for 5 days a week. TMP (3 or 10 mg/kg; Aldrich Chem., Milwaukee, WI, USA) was intraperitoneally injected every morning from the second day of the learning trial. We adopted this schedule because transient increase in cerebral blood flow was observed immediately after administration of TMP (unpublished data).

Retention and interposed delay tests of radial maze performance: Training trials were carried out once daily until the rats made no more than one error per session for 3 consecutive days. Then the pretrained rats received the permanent 2VO or sham operation. Three days after the operation, the retention tests were carried out once daily for 6 days. Thereafter, a 3-min delay was interposed between the fourth and fifth choices (during delay, the rat was kept on the central platform with the guillotine doors closed). TMP (10 mg/kg) was intraperitoneally injected every morning from the second day of the retention test for 5 days. The day following the retention test, these animals were injected with TMP, and the interposed delay test was carried out.

Scopolamine-induced spatial cognitive impairment test

Daily training trial was conducted for each rat as described above. Rats that made no errors or only one error at the eighth choice per session for 5 consecutive trials were used for drug tests. The number of errors and the number of initial correct responses (the number of correct responses before committing the first error) were used as the measures of radial maze performance. Scopolamine hydrobromide (0.3 mg/kg; Nacalai Tesque, Kyoto) or TMP (1 and 3 mg/kg) was intraperitoneally injected 30 min before testing.

Statistical analyses

Behavioral data were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney U-test. Differences with P < 0.05 were considered statistically significant.

RESULTS

Effects of TMP on the permanent 2VO-induced disruption of maze performance

Consistent with our previous data (4), the rats, which had not been pretrained, failed to learn the radial maze performance 3 – 21 days after the permanent 2VO operation (the rats received 15 learning trials). The number of errors in the non-pretrained permanent 2VO rats did not decrease with repeated training. Significant differences between the permanent 2VO and sham-operated group were observed from the 5th to final trial (Fig. 1). Administration of TMP (3, 10 mg/kg, i.p.) partially but significantly attenuated the permanent 2VO-induced impairment of the maze learning task.

In the retention task, the permanent 2VO slightly impaired the maze performance of the pretrained rats. Treatment of the permanent 2VO rats with TMP at 10 mg/kg did not attenuate this impairment (Fig. 2).

Following recovery to the level of the sham-operated controls at the 8 – 9th postoperative days, a 3-min delay was interposed. The interposition of delay significantly increased the number of errors after but not before the delay period in the pretrained permanent 2VO group (Fig. 3). No marked difference in the performance be-
Fig. 1. Effect of TMP on the permanent 2VO-induced disruption of the 8-arm radial maze learning performance in the non-pretrained rats. Each point represents the mean of errors. A horizontal black bar indicates daily administration of either saline or TMP to the permanent 2VO rats 4 hr before each trial. •: sham (n=13); ▲: 2VO+vehicle (n=10); ○: 2VO+TMP, 3 mg/kg (n=8); △: 2VO+TMP, 10 mg/kg (n=11). *P<0.05 and **P<0.01 vs sham-operated group. 

Fig. 2. Effect of TMP on the permanent 2VO-induced disruption of the 8-arm radial maze retention task in the pretrained rats. Each point represents the mean ± S.E.M. A horizontal black bar indicates daily administration of either saline or TMP to the permanent 2VO rats 4 hr before each trial for 5 days. ○: sham (n=16); ◆: 2VO+vehicle (n=13); ■: 2VO+TMP, 10 mg/kg (n=13). *P<0.05 and **P<0.01 vs sham-operated group.

Fig. 3. Effect of TMP on the performance in the 8-arm radial maze task with or without a 3-min-delay interposition in the pretrained rats. When the maze performance of the permanent 2VO rats reached the same level as the sham group, the delay interposition test was carried out. Either saline or TMP (10 mg/kg, i.p.) was daily administered to the permanent 2VO rats for 6 days before the delay interposition. In this test, either 0- or 3-min delay was interposed between the forth and fifth choices. Each column represents the mean of total errors with S.E.M. Open, dotted and hatched columns represent the sham (n=16), 2VO (n=13) and 2VO+TMP (n=13), respectively. **P<0.01 vs sham-operated group.

Effects of TMP on scopolamine disruption of maze performance

As shown in Fig. 4, scopolamine (0.3 mg/kg, i.p.) significantly increased the number of errors and decreased the number of initial correct responses. Acute administration of TMP (1 and 3 mg/kg, i.p.) significantly decreased the number of errors and increased the number of initial correct responses in the rats injected with scopolamine.

DISCUSSION

The present results demonstrate that TMP has beneficial effects on spatial cognitive impairment in rats. The radial maze is one of the most commonly used tasks for testing spatial learning and memory in rodents as well as other experimental animals (10). The memory used in this task is usually considered as working memory that is analogous to recent memory in humans (10, 11). This type of memory is known to be more severely impaired than remote memory in human dementia (12). The present results that rats with permanent 2VO exhibited severe deficit in the learning of new information on the 8-arm radial maze task agree well with our previous data (4) and those reported on the memory loss in normal aged hu-
mans and dementia patients (12, 13). In this study, we found that daily treatment with TMP from the second day after the learning trial significantly improved this learning deficit. To date, a number of studies have demonstrated using ischemic models that chemicals produced beneficial effects when administered prior to the ischemic operation, but only a few compounds were reported to have effects after ischemia insult (14). The efficacious treatment with TMP 3 days after permanent 2VO suggests the potential therapeutic availability of TMP for treatment of dementia derived from cerebral hypoperfusion and/or cerebral ischemia.

Our previous data suggested that permanent 2VO rats could retain newly acquired information for only a very short period (no more than 3 min) and that such limited ability might contribute to the learning impairment caused by permanent 2VO (4). In the present study, although the effect was not statistically significant, TMP administration tended to decrease the number of errors elevated by the 3-min delay interposition. Thus the ameliorating effect of TMP administration on the learning deficits of permanent 2VO rats may be partly due to the improvement of the process for consolidating newly acquired information. It is of interest to note that TMP not only improved the cognitive impairments induced by permanent 2VO, but also reversed the scopolamine-induced amnesia. It is well known that the memory impairment is a cardinal symptom of Alzheimer’s disease and that scopolamine produces memory impairment similar to Alzheimer’s disease (AD) (15–17). The present results are strong evidence supporting the idea that TMP might have some beneficial effects on cognitive impairment in clinical cases.

The mechanisms underlying the ameliorating effects of TMP on the spatial cognitive impairment remain unclear. The memory loss in AD is thought to be related, to some degree, to central cholinergic system dysfunction (18–20). Scopolamine, a competitive muscarinic-receptor blocker, is known to be largely dependent on its anticholinergic property to produce cognitive deficits in humans and experimental animals (10, 12, 21, 22). On the other hand, the central cholinergic system is reportedly damaged by transient (7–9, 14) and permanent 2VO (Ni et al., unpublished data) in rats. Thus, a possible explanation is that the enhancement of central cholinergic function is commonly involved in the ameliorating effects of TMP on the spatial cognitive deficits caused both by permanent 2VO and by scopolamine injection.

The present results, however, do not exclude the possibility that different mechanisms participate in the actions of TMP on permanent 2VO- and scopolamine-induced disruption of radial maze performance. Kwan et al. (3) and Wu et al. (23) have demonstrated that TMP attenuates both Ca\(^{2+}\) influx from the extracellular space and Ca\(^{2+}\) release from the internal stores. Such properties of TMP may contribute to its beneficial effects on the cognitive deficits caused by permanent 2VO, since Ca\(^{2+}\) plays an important role in the process inducing neuronal dysfunction and histological damage following cerebral ischemia (24).

Nevertheless, although the exact mechanism underlying the improving effect of TMP on the learning deficits remains to be clarified, the present findings suggest the therapeutic potential of this amide alkaloid for the treatment of dementia caused by cholinergic dysfunction and/or decrease of cerebral blood flow.
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