Vinpocetine Improves Scopolamine Induced Learning and Memory Dysfunction in C57 BL/6J Mice

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Vinpocetine is an inhibitor of phosphodiesterase type 1 (PDE1), which has been used for treating stroke for over 40 years. However, according to current clinical dosage and treatment period, its direct effect on memory is unclear. In this study, we investigated whether vinpocetine could reverse the scopolamine (SCO)-induced cognitive deficits in animals. Behavioral experiments, including open field, Y-maze, and fear conditioning tests were used to determine the possible role of vinpocetine on scopolamine-induced memory dysfunction. In the open field and Y-maze tests, there were significant differences between the control (CON) group and SCO group. Vinpocetine (4 mg/kg) administration for consecutive 28 d significantly improved the scopolamine-induced memory dysfunction. In the fear conditioning test, vinpocetine (2, 4 mg/kg) administration had certain beneficial effect on emotional memory. Our results suggest that vinpocetine could improve cognitive function in memory deficient mice and high clinic dosage might be better.

Key words vinpocetine; scopolamine; memory dysfunction; behavior test

Vinpocetine might enhance memory deficit through the modulation of cholinergic functions. Traditionally, scopolamine (SCO) has been used in the field of neuropsychopharmacology as a standard/reference drug for dementia-related cognitive deficits model. Deshmukh et al. found chronic treatment with vinpocetine (5, 10 and 20 mg/kg intraperitoneally (i.p.)) for 21 d following first intracerebro-ventricularis (i.e.v.) streptozotocin infusion significantly improved learning and memory in Morris water maze and passive avoidance paradigms. DeNoble et al. found that vinpocetine (3 mg/kg per os (p.o.)) was also effective in preventing disruption of passive avoidance retention impaired by 7% oxygen-induced hypoxia. These data support the view that vinpocetine can improve cognitive ability on scopolamine and hypoxia-induced memory impairment rats, but whether it could improve memory effects by clinical dosage and treatment period is still unclear.

In the present study, we used behavioral analyses to test the possible role of vinpocetine on scopolamine-induced dementia in C57BL/6J mice by clinical dosage and treatment period, which provide a basis for further clinic administration. Open field was also conducted to analyze spontaneous locomotor behavior. Y-Maze and fear conditioning were conducted to estimate working memory and reference memory.

MATERIALS AND METHODS

Clinical Dose Conversion According to clinical treatment dosage, vinpocetine is used 5/10 mg per time, tid. Conversion to mice dosage is below:

\[
3 \times 5 \text{mg/70} \times 70 \text{kg} \times 0.0026 = 0.02 \text{mg/kg} = 1.95 \approx 2 \text{mg/kg;}
3 \times 10 \text{mg/70} \times 70 \text{kg} \times 0.0026 = 0.02 \text{mg/kg} = 3.9 \approx 4 \text{mg/kg.}
\]

Compound Administration Vinpocetine was suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) (Sigma-Aldrich, Milan, Italy) for oral administration (p.o.) once daily at 9:00 a.m. for 28 consecutive days at a dosage of 2 or 4 mg/kg (n=10 animals per treatment). On the 28th d, scopolamine hydrobromide (10 mg/kg, Sigma-Aldrich) was dissolved in 0.9% saline and administered i.p. to mice prior to vinpocetine administration. Vinpocetine was supplied by the Northeast Pharmaceutical Group; Shenyang 110027, P. R. China.

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Pharmaceutical group, Shenyang, China. All other reagents were purchased from Shandong Yuwang Chemical Reagent Co., Ltd. (Shenyang, P. R. China).

Animals Seventy adult male C57BL/6J mice (20–25g) were randomly divided into seven groups (10 mice/group): Control (CON), 0.5% CMC-Na control (CN-CON), vehicle scopolamine control (SCO, 10 mg/kg), low dose of vinpocetine (2 mg/kg) (L-VIN), high dose of vinpocetine (4 mg/kg) (H-VIN), low dose of vinpocetine combined with scopolamine (L-VIN+SCO), high dose of vinpocetine combined with scopolamine (H-VIN+SCO). The CON and SCO groups received normal saline intraperitoneally. The animals were given the respective drugs at a dose of 0.1 mL/10 g body weight (BW) by oral gavage once daily for 28 d. Doses of vinpocetine and treatment period were based on the clinical dosage in patients.1,2

Behavioral Procedures According to the experiment protocol, learning and memory were assessed by three separate tests: “Open field, Y-maze, and fear conditioning tests.” Training or testing was performed on 26th, 27th, and 28th d between 10.00 and 16.00 h in a light- and sound-controlled room (Table 1). After each test, the behavior equipment was wiped with ethanol–water (50% v/v). All animal procedures were in accordance with the Regulation of Experimental Animal Administration issued by the State Committee of Science and Technology of the People’s Republic of China on November 14, 1988. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University.

Open Field Gross motor ability was measured using a novel open field paradigm. Briefly, mice were placed in a novel, brightly lit, square arena measuring 50×50 cm. A TOPSCAN tracking system (Clever Sys Inc., U.S.A.) was used to quantify the ambulation distance over a 5-min trial period. The task provides measures of not only locomotor activity, but of anxiety in response to a novel environment and habituation by including the number of beams broken in the periphery vs. the corners, and the mid zone areas every 5 min. This allowed us to measure all the required parameters: total distance moved (cm), time spent moving (s), average speed (cm/s), number of entries into different zones and proportion of total time spent in the open field arena in different speed threshold ranges (percentage). Following the experimental session, the mice were carefully removed from the open field, and returned to their home cage. The test equipment was cleaned with 50% ethanol solution and dried between subjects in order to avoid olfactory cuing.

Speed thresholds were correlated to the maximal moving speed (45 cm/s) of the mice. The maximal moving speed of the mice was then halved, to give a slow moving speed group (<22.5 cm/s) and a high moving speed group (>22.5 cm/s).

Y-Maze The Y-maze apparatus consisted of a black plastic maze with three arms of equal size, labeled as A, B, and C, respectively. Each arm was 60 cm long, 15 cm high, and 10 cm wide and was oriented at an angle of 120 from the other two. In the training trial, each mouse was allowed to explore the maze for 5 min with one arm closed (novel arm) and returned to their home cage until the retrieval trial, during which they could explore all three arms of the maze freely for 2 min. The time spent in the novel arm was calculated as a percentage of the total time in all three arms. A 1-min intertrial interval (ITI) was used to confirm the spontaneous novelty exploration and test vision. An arm entry was judged to be completed when the hind paws of the mouse were completely placed in an arm. The arena was cleaned using 70% (v/v) ethanol between trials so as to avoid olfactory cues. The initial arm of the maze was also changed within mice of the same group in order to avoid bias of arm placement.

Fear Conditioning Apparatus The conditioning and extinction chamber (25 cm wide, 18 cm high, and 21 cm deep) had a cage floor made of stainless steel rods connected to an electric shock generator (Med Associates Inc., U.S.A.). It was surrounded by a frame that emitted 16 infrared photo beams. A computer controlled the delivery of electric footshock and recorded the beam interruptions and latencies to beam interruptions (freezing time).

Conditioning Trial On the 26th d, mice were placed in the chamber and allowed to explore for 2 min before exposure to a 30 s, 85 dB acoustic tone (conditioned stimulus, CS) that co-terminated with a 2 s, 0.5 mA electric footshock (unconditioned stimulus, US). The tone plus footshock was repeated three times randomly within each subsequent 2 min epoch. One minute after the last tone footshock delivery, mice were returned to their home cages. The total time in the conditioning chamber was 8 min.

Contextual Trial On the 27th d, 24 h after the reactivation/first extinction trial, the mice were placed in the chamber for 5 min without footshock, and freezing was measured as an indication of contextual fear.

On the 28th d, after SCO administration, the mice were returned to the chambers that had been modified. A flat floor overlay the usual grid, an insert modified the chamber dimensions, and an acetic acid odor was used to reinforce a novel environment. After habituation of the mice within the cham-

Table 1. Groups and Treatments

<table>
<thead>
<tr>
<th>Groups (abbreviation)</th>
<th>Treatment (1st–25th d)</th>
<th>Treatment (26th d)</th>
<th>Treatment (27th d)</th>
<th>Treatment (28th d)</th>
</tr>
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<tbody>
<tr>
<td>0.5% CMC-Na control (CN-CON)</td>
<td>0.5% CMC-Na p.o.</td>
<td>0.5% CMC-Na p.o.</td>
<td>0.5% CMC-Na p.o.</td>
<td>0.5% CMC-Na p.o.</td>
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<tr>
<td>Low dose of vinpocetine (L-VIN)</td>
<td>Vinpocetine (2 mg/kg) p.o.</td>
<td>Vinpocetine (2 mg/kg) p.o.</td>
<td>Vinpocetine (2 mg/kg) p.o.</td>
<td>Vinpocetine (2 mg/kg) p.o.</td>
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<tr>
<td>High dose of vinpocetine (4 mg/kg) (H-VIN)</td>
<td>Vinpocetine (4 mg/kg) p.o.</td>
<td>Vinpocetine (4 mg/kg) p.o.</td>
<td>Vinpocetine (4 mg/kg) p.o.</td>
<td>Vinpocetine (4 mg/kg) p.o. + SCO i.p. (10 mg/kg)</td>
</tr>
<tr>
<td>Low dose of vinpocetine combination with scopolamine (L-VIN+SCO)</td>
<td>Vinpocetine (2 mg/kg) p.o.</td>
<td>Vinpocetine (2 mg/kg) p.o.</td>
<td>Vinpocetine (2 mg/kg) p.o.</td>
<td>Vinpocetine (2 mg/kg) p.o. + SCO i.p. (10 mg/kg)</td>
</tr>
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<td>Vinpocetine (4 mg/kg) p.o.</td>
<td>Vinpocetine (4 mg/kg) p.o.</td>
<td>Vinpocetine (4 mg/kg) p.o.</td>
<td>Vinpocetine (4 mg/kg) p.o. + SCO i.p. (10 mg/kg)</td>
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a) Fear conditioning conditional trial. b) Fear conditioning contextual fear trial, Y-maze training. c) Fear conditioning test, Y-maze test, open field test.
bers for 5 min, a continuous tone (85 db, 4kHz) was applied for 5 min. The percent freezing was recorded.

**Statistical Analysis** Data are expressed as the mean± standard deviation (S.D.). To assess the significance of differences between groups, summed effects of drugs over the course of an experiment were used to compare with the treatment area under the curve by one-way ANOVA followed by Fisher’s least significant difference (LSD) in open field and Y-maze experiments. Data in fear conditioning were analyzed using a two-way ANOVA, followed by Duncan post hoc test. Statistical analyses were performed by SPSS 16.0 software and p<0.05 was regarded as statistical significance.

**RESULTS**

**Locomotor Activity** Spontaneous alternation behavior determined using the open field. Activation was observed in various parameters: total distance moved (Fig. 2A), time spent moving (Fig. 2B), their average speed (Fig. 2C). The effects of repeated treatment of vinpocetine at various doses (2, 4mg/kg) on locomotor activity of mice were examined. Significance difference in total distance moved was found between the control and SCO groups, which were 5241±164.2 and 1946±87.9 cm, respectively (Fig. 2A). After vinpocetine (2, 4mg/kg) treatment, total distance moved increased to 2470±253.8 and 2788±162.9 cm, respectively, which showed significance compared with SCO group animals [F=6.09, p<0.05] (Fig. 2A). Significance difference in moving time was found between the control and SCO groups, which were 182±32.5 and 128±29.2 s, respectively [F=6.45, p<0.05] (Fig. 2B). After vinpocetine (2, 4mg/kg) treatment, moving time reached 147±20.4 and 138±15.7 s, respectively, which showed no significance compared with SCO group animals [F=2.42, 1.98, p>0.05] (Fig. 2B).

Meanwhile, after vinpocetine 4mg/kg administration, the average speed in VIN+SCO 4mg group from 15.2±4.3 cm/s increased to 20.2±5.2 cm/s, which also showed significance compared with SCO group [F=21.85, p<0.01] (Fig. 2C).

**Vinpocetine Treatment Increased Motor Ability of SCO-Treated Mice in Open Field Experiment** In the CON group, the time spent in the border area, intermediate area, and center area were 135±10.5, 85.4±13.5, and 79.6±12.8 s, respectively. In the SCO group, mice spent significantly more time (180.2±21.9 s) in the center area and less time in the border area (49.5±9.7 s), which showed significant difference compared with the CON group. After vinpocetine 4mg/kg administration, the H-VIN+SCO mice spent significant less time in the center area and more time in the border area compared with the SCO mice [F=10.56, p<0.05] (Fig. 3A). Further, in the CON group, the number of entries into these areas was 22.6±5.2 times (border), 61.6±7.6 times (intermed), and 15.8±2.8 (center), respectively [F=14.81, p<0.01] (Fig. 3B). Meanwhile, in the SCO group, the number of entries into these areas was 24.8±15.8 times (border), 48.4±6.2 (intermed), 26.8±3.8 (center), respectively. After vinpocetine 4mg/kg administration, the H-VIN+SCO mice significantly spent more time in the intermediate area and had increased number of entries to the intermediate area compared with the SCO mice [F=7.04, p<0.05] (Fig. 3C).

**Vinpocetine Treatment Increased Motor Ability of SCO-Treated Mice on Y-Maze** We evaluated short-term memory function using Y-maze task. The results obtained with the Y-maze task are shown in Fig. 4. Baseline spatial memory performances in the CON and SCO mice were measured before vinpocetine administration. The parameters measured included percentage of same arm returns (% of structure–activity relationship (SAR)) (Fig. 4A), and number of arm entries (Fig. 4B). As shown in Fig. 4A, scopolamine (10mg/kg, i.p.) significantly decreased % of SAR in novel arm, and this alteration was significantly restored by VIN (4mg/kg) treatment.

Notable differences were observed among the VIN treated groups relative to the SCO 10mg/kg group. The percentage of same arm returns (%SAR) in SCO group was significantly less than CON group. Our results found that %SAR of SCO group in novel arm is 18.3±7.0. After VIN (4mg/kg) treatment, the %SAR was significant increased to 38.3±8.2 [F=13.32, p<0.01 compared with the SCO group] (Fig. 4A). Meanwhile, the number of arm entries in novel arm of CON group is...
Fig. 3. Effect of Vinpocetine (2, 4mg/kg) on the Motor Ability in Open Field Task (n=10)

A: represent time spent in different area; B: represent distance traveled in different area; C: represent number of entries into different zones. Data were analyzed by one-way ANOVA followed by Fisher's LSD. Data represent the mean±S.D. *p<0.05, **p<0.01 compared with CON mice; #p<0.05, ##p<0.01 compared with SCO mice.
25±5.1; which is 13±4.3 in SCO group. After VIN treatment, the number of arm entries in novel arm reached to 19±3.6 \( [F=19.84, p<0.01] \) (Fig. 4B).

**Vinpocetine Treatment Had Beneficial Effects on Fear Conditioning** Fear conditioning was evaluated in the same groups of mice over 3 consecutive days. The key elements in the test schedule are shown in Fig. 5A. On day 1, all mice showed a similar reaction to footshock. Twenty-four hours later, when the mice were returned to the same testing chambers with no tone or footshock application, the SCO mice showed low levels of freezing. This is shown both by repeated measures ANOVA for all measures taken at 30 s intervals. The percent accumulated freezing events in the context fear test were 55±7.36% in the CON group and 22±4.83% in the SCO group. The difference was statistically significant \( [F(6, 63)=9.48, p<0.05] \) (Fig. 5B). During the first 5 min, the percent accumulated freezing events in the two vinpocetine groups reached 46±6.5% and 50±8.9%, respectively, which showed significant difference with the SCO animals \( [F(6, 63)=7.67, p<0.05] \) (Fig. 5B). After 5 min, the percent accumulated freezing events in the two vinpocetine groups did not show any difference with the SCO group \( [F(6, 63)=3.67, p>0.05] \) (Fig. 5B). On day 3, the mice returned to a novel environment. Neither group of mice froze to the novel context during the first ten blocks of recording (0–5 min). In the second half of the recording (5 min), a continuous tone was applied to better detect any possible desensitization in the mice. The tone-conditioning stimulus immediately increased freezing in all groups. There was no difference in the percent freezing to tone \( [F(6, 63)=1.08, p>0.05] \) (Fig. 5C) between all animal groups. Thus, the SCO mice had deficits in the contextual memory, but vinpocetine 2 and 4 mg/kg administration...
had certain beneficial effects on emotional memory.

DISCUSSION

In the present study, we demonstrate that clinical dose vinpocetine administration for consecutive 28 d could ameliorate scopolamine-induced memory impairments. This effect was observed in the open field, Y-maze and fear conditioning tests in mice. In this research, we found that vinpocetine could improve memory retrieval on C57 BL/6J mice.

Vinpocetine increases blood circulation and metabolism in the brain. On clinic, vinpocetine was effective in improving memory and concentration of patients with epilepsy and dementia patients in Nigerian population.11) There are also some experiments proved that vinpocetine could improve memory dysfunction on animals. Gupta et al. found that treatment of vinpocetine reduced chronic cerebral hypoperfusion (CCH) induced learning and memory deficits through Morris water maze test.12) Torres et al. proposed that it is possible that inhibits oligodendroglial precursor cell differentiation thus having a direct negative effect on remyelination which play anti-inflammatory function might partly associate with cognitive improvement.13) Meanwhile, Filgueiras et al. found that vinpocetine (20 mg/kg i.p.) could improve learning and memory in animal models of fetal alcohol spectrum disorders (FASD) on Long Evans rats.14) Also, Chronic treatment with vinpocetine (5, 10, 20 mg/kg i.p.) for 21 d following first i.c.v. streptozocin infusion significantly improved learning and memory in Morris water maze and passive avoidance paradigms in rats.15)

Vinpocetine enhance spatial memory might through the modulation of cholinergic functions. Up to now, there are several mechanisms involved in vinpocetine to improve memory deficits and blood flow.1) Inhibition of phosphodiesterase (PDE) enzyme to enhance second messenger-mediated signaling, and consequently, influence the pathways involved in learning and memory. 1) Type I phosphodiesterases (PDE1) are a family of Ca2+-calmodulin-modulated phosphodiesterases involved in the regulation of both cGMP and cAMP.2,15,16) PDE1 has been reported to show higher expression in neurons of the hippocampus and cortex, which are areas important for memory formation and storage.10) 2) Anti-inflammatory agent that might have a potential role to treatment memory dysfunction. Inhibitor of xB kinase (IKK)/nuclear factor-kappa B (NF-κB) and extracellular signal-regulated kinase (ERK) 1/2 appear to be the pathways inhibited by vinpocetine, by reducing the inflammatory response in vascular smooth muscle cells.17) 3) In addition, vinpocetine enhances the structural dynamics of dendritic spines, and improves memory retrieval in patients who are suffering from mild to moderate psychoses.18) Ameliorate scopolamine induced experimental memory dysfunction, enhance performance in cognitive tests in humans.19) Also, vinpocetine has been reported to increase cerebral metabolism, which is responsible for the utility of this compound in the treatment of cerebral circulatory disorders, such as dementia and acute stroke. Increased cerebral metabolism might be induced through enhanced cerebral flow (vasodilation), increased consumption of glucose and oxygen in the brain, increased production of ATP.20) Apart from a selective increase in cerebral blood flow, the activity of vinpocetine as an inhibitor of platelet aggregation and an activator of erythrocyte deformability lowers the viscosity of blood, thereby making this compound effective in the treatment of ischemic stroke.20) A novel experiment found that vinpocetine locally restricted decrease of nociception, implies an inhibition of the retrograde axoplasmic transport of nerve growth factor (NGF) in peripheral nerves.

These findings indicate that vinpocetine enhances spatial memory through an antioxidant mechanism, the modulation of cholinergic functions and the prevention of neuronal cell damage.9)

In conclusion, our results showed that vinpocetine contributes at least partly to the improvement of cognitive function in memory deficient mice. Due to the lack of sufficient data, currently no conclusion can be drawn for the use of vinpocetine in cognitive function.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


