Original Article
Protective effect of neovibsanin B on spatial cognitive functions of rats with cerebrovascular hypoperfusion

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Abstract: Neovibsansins are believed to be promising candidates for the development of novel therapeutic agents to treat neurological diseases like Alzheimer’s disease. It has been shown that chronic cerebral hypoperfusion is linked to neurodegenerative disorders and their subsequent cognitive impairment. In the present study effect of neovibsanin B (NVB) on spatial cognitive functions of rats with lobal cerebrovascular hypoperfusion was investigated. The cerebrovascular hypoperfusion rat model was prepared by bilateral common carotid arteries occlusion (2VO). Morris water maze (MWM) test was employed to examine the effect of NVB on spatial cognitive function before and after 2VO intervention. The animals were divided into two groups; long-term memory (LTM) and short-term memory (STM) groups. Each of the groups was subdivided into 3 subgroups: control, untreated and NVB treated groups. After ten weeks of the surgery, all the subgroups were tested with MWM. The results of working memory test for both control and NVB treated groups revealed that escape latency time and total distance travelled were significantly lower compared to untreated group. Similarly, the maze test performance was observed to be significantly improved for control and NVB treated groups. Moreover, the probe memory test performance for control and NVB treated groups was markedly better than untreated group. Thus NVB has a significant effect on the spatial cognitive preservation in rats with chronic cerebral hypoperfusion. Thus NVB can be a promising agent for the spatial cognitive functions improvement.

Keywords: Cerebral hypoperfusion, escape latency time, cognitive impairment, neovibsanin B

Introduction

Despite advances in chemotherapeutics the treatment strategies to prevent or stop neurodegeneration are unmet [1]. It has been shown that there is no relation between amyloid plaque density and the degree of memory and learning impairment [2, 3]. Removal of the plaques could neither prevent the disease symptoms nor increase the survival rate of the patients [4]. Taking cue from these investigations other causes of neurodegenerative changes in AD were examined [5]. Presently, AD is considered to be a brain vascular disease [6] due to chronic reduction in cerebral blood flow leading to decrease in glucose and oxygen supply to cerebral neurons and finally neurodegeneration and cognitive decline [7]. For investigation of the neurodegenerative disorders two vessel occlusion (2VO) rat model of cerebral hypoperfusion is frequently used. In this model permanent bilateral ligation of common carotid arteries is performed [7]. It leads to cerebral hypoperfusion and then neurodegeneration of the pyramidal hippocampal neurons which control spatial learning and memory [8]. The widely used model to evaluate the spatial reference and working memory impairment is the Morris water maze (MWM) test [9, 10]. The memory used for learning new information about the surrounding space constitutes the spatial working memory (WM). On the other hand, spatial reference memory denotes the brain activity used to recall consolidated positions and places [11, 12]. One of the earliest symptoms of AD is the progressive weakening of spatial memories [13]. It is reported that 2VO operated rats show a significant poor MWM performance in learning as well as memory after 2VO surgery compared to healthy control rats [14].

Neovibsansins are the novel polyfunctionalized diterpenoid compounds isolated from the shrub Viburnum awabuki by Fukuyama et al. [15].
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These natural products are reported to enhance neurite outgrowth activity in PC12 cells. Thus neovibsanin-type compounds could be promising candidates for the development of novel therapeutic agents to treat neurological diseases [16]. Williams et al. in 2009 reported neurite outgrowth activities of unnatural 4, 5-bi-epineovibsanins and their synthetic intermediates. They found minor effect of stereochemistry in the neovibsanin skeleton on biological activity [17, 18]. Imagawa et al. revealed the neurite outgrowth activity of racemic mixture of neovibsanins [19, 20]. In the present study, effect of neovibsanin B (Figure 1) treatment on spatial reference long-term memory (LTM), short-term (STM) and spatial working memory (WM) of cerebrally hypoperfused rats was examined.

Materials and methods

Animals and drug

Male Sprague-Dawley rats, 23 weeks old, were obtained from Beijing Vital River Experimental Animal Technology Co., Ltd. Animals were housed in the Experimental Animal Center of the Capital Medical University under standard conditions. All procedures for animals were performed in agreement with the SIBS Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of the Beijing Institutes for Biological Sciences. All efforts were made to minimize animal suffering and the number of animals used.

Treatment strategy

The rats were randomly assigned into two groups, long-term memory (LTM) and the short-term memory (STM) and working memory (WM) group with 30 animals each. LTM was given MWM training before 2VO surgery and retested 10 weeks after 2VO surgery. The STM and WM test group rats were naïve to MWM at the time of 2VO surgery and tested at the 10th week after operation. The two groups were subdivided into 3 subgroups with 10 rats each: (1) Control group; The rats operated were neither double ligated nor NSO treated, (2) Untreated group; rats were operated with bilateral double ligation but not NVB treated and (3) NVB treated group; rats were operated, double ligated and NVB treated. The oral NVB treatment was started 1 week before 2VO surgery and continued with the daily oral dose of 1 mg/kg for further 70 days (10 weeks) after 2VO surgery.

2VO procedure

Ketamine and xylazine at the dose of 85 mg/kg and 8 mg/kg, respectively were administered as anesthetic dose to the rats. In each rat a 2 cm skin incision was made just above the sternal bone to identify carotid sheath. The carotid arteries were separated carefully from the vagus nerve and then doubly ligated via silk suture just below the bifurcation into internal and external carotid arteries and the arteries were cut between the two ligatures.

Apparatus

The apparatus used was MWM with circular black fiberglass tank (diameter 2, height 60 cm) was filled with water up to 30 cm. A black escape platform (EP) was used as rescue island.

Figure 1. Structure of neovibsanin B (NVB).

Figure 2. The four hypothetical random starting points of MWM pool for the test.
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for the rats. The water was changed periodical-
ly and its temperature was maintained at 26°C.
The walls around the pool were pasted colored
posters to allow animals build up their spatial
memory. ANY-maze video tracking software
(Stoelting Co., USA) was employed to record
and analyze swimming time, distance and spe-
ed of the rats. The tests were performed from 9
am and 5 pm in the pool divided hypothetically
into 4 equal imaginary quadrants (SW, SE, NW
and NE) (Figure 2). Each day the test was start-
ed from different quadrant for each animal. On
the day 5 and 4 before surgery and day 61 and
62 after surgery habituation training was per-

Figure 3. Differences in escape latency time and total distance travelled during the three successive days in MWM acquisition test before surgery.

Figure 4. Differences in escape latency time and total distance travelled among control, NVB treated and untreated groups during LTM test on 68th day after surgery.
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formed for LTM and STM groups respectively. In 2 successive days each animal was given a four trial per day MWM training and each trial lasted for a maximum of 120 seconds. The animals on reaching EP were left there for 30 seconds and then helped out to adapt to the water tank and the surrounding extra-maze visual cues before the actual acquisition test was performed. For LTM and STM groups the acquisition test was performed on day 1-3 before operation and on day 62-63 after operation, respectively. The EP was set in the SW zone of the pool in this phase and the level of water was increased 2 cm above the EP surface to make it invisible (Figure 2). Four trials each for 120 seconds followed by a 4 minute break were given to each rat for 4

Figure 5. Differences among study groups in time spent in the target (SW) zone and number of annulus crossings retention probe LTM test.

Figure 6. Differences between the study groups during the STM test in escape latency and total distance travelled.
consecutive days. For each trial time required and the distance travelled was calculated for each rat. On the day 68 after operation LTM test, encompassing 4 trials for each animal during one test day was performed. On day 69 and 66 after operation, retention (probe) memory test was performed for LTM and STM groups, respectively. A 60 second single swimming trial was given to each animal starting from the NE pole after EP was removed (Figure 2) to calculate the time spent and the number of annulus crossings in the target zone.

On the 67-69, working memory test (WMT) was performed for STM groups in which EP position was changed daily to different zones (Figure 2).
Each rat was given 4 trials each for 120 seconds followed by 1 min interval per day. After the last test trial, cued version test starting from the SE pole was performed. In this test platform was raised 1 cm above the water surface and a yellow flag 10 cm in height was placed on it. Each panel of the flag was 1 cm × 5 cm in dimensions.

Statistical analysis

Total distance travelled and swimming speeds were analysed by two way analysis of variance (ANOVA). One-way ANOVA was used for retention probe memory test data. The results presented are the mean of mean ± SEM. The differences were considered statistically significant at $P < 0.05$.

Results

Mortality and blindness rates after the operation

Out of the 30 rats in untreated group 16 died after surgery (mortality rate = 53%) and 2 of the remaining 14 rats got blindness on the day 3 of operation (blindness rate = 6.6%). The rats with blindness were excluded from the MWM study. Among 30 rats of the NVB treatment group 2 died (mortality rate = 6.6%) and no one suffered from blindness. On the other hand, none of the animals from the control group either died or suffered from the blindness.

LTM test

The results obtained from the animals in all the three groups were similar during pre-training days. The escape latency time and the total distance travelled to reach the EP zone were 0.73 and 0.96, respectively (Figure 3). After the operation, the animals in the control and NVB treated group showed significantly short mean escape latency time compared to the animals in the untreated group. The values of the mean escape latency time for control, NVB treated and untreated group were 16.23 ± 2.23, 18.19 ± 2.76 and 92.45 ± 4.65 sec, respectively (Figure 4). The mean time spent in the target (SW) zone by the animals in control and NVB treated group was significantly loner compared to the animals of untreated group. The mean time spent in the target (SW) zone was 38.67 ± 2.74, 18.23 ± 1.16 and 15.74 ± 1.12 s, respectively by the animals in control, NVB treated and untreated groups (Figure 5). There was also a significant difference in the average number of annulus (EP zone) crossings. This number was higher for control and NVB treated group compared to untreated group (Figure 5).

STM test

The acquisition test of MWM performed on the day 62-65 after operation showed a significant difference in the mean escape latency time and total distance travelled by control and NVB treated groups compared to untreated group (Figure 6). The mean time spent in the target zone by control, NVB treated and untreated group was 40.23 ± 2.23, 14.03 ± 1.34 and 12.34 ± 1.89 s, respectively (Figure 7). The average number of annulus crossings in the EP zone within 60 seconds was 3.64 ± 0.28, 1.12 ± 0.18 and 0.28 ± 0.23 for control, NVB treated and untreated groups (Figure 7).

WMT results

Comparison of the mean escape latency results on 3 successive days for control and NVB treated revealed significant difference from the untreated groups (Figure 8). The mean distance travelled by control and NVB treated groups exhibited significant difference from that of untreated group. Additionally, the difference in swimming distance was significant between control and NVB treated group too ($P < 0.05$) (Figure 8).

Discussion

The present study was designed to investigate the effect of NVB on cognitive learning and memory performance in live animals. The animals were given pre-anesthetic atropine doses to prevent them from respiratory distress. Optic tract acute ischemic injury induced blindness within the 7 days after 2VO surgery is a commonly observed complication [21-23]. We observed a significantly higher mortality rate in untreated 2VO group compared to NVB treated
group. This suggests that the rats could not escape the acute ischemic injury to the vital brain centers. The significantly lower mortality rate in NVB treated group compared to untreated 2VO group suggests the possible neuroprotective effect of NVB preventing cerebral neurons from oxidative stress and neuroinflammation associated with acute ischemia. The 2 days habituation training followed by cued version MWM task test revealed no significant differences among all the tested animals suggesting intact sensory-motor function of the animals. Before surgery, the difference for escape latency and swimming distance in all the animals were negligible because the animals were in cognitively healthy state. However, the animals of untreated 2VO group showed significant deterioration in LTM test performance compared to NVB treated animals. The results from retention memory test revealed a marked loss of remote spatial memory in untreated 2VO group. It is reported that cerebral hypoperfusion becomes chronic between 8th and 12th week after 2VO surgery. There after the flow of blood gets normal but MWM performance continues to deteriorate indicating neurodegeneration [24].

Treatment with NVB led to a significant improvement in 2VO induced LTM impairment showing marked difference from the untreated 2VO group. The improved LTM water maze performance by NVB treated group was confirmed by the probe memory test. This test excludes spatial bias, adoption of non-spatial strategies and possibility that rats reached EP by chance [25]. The results observed for the NVB treated group were comparable to those of the sham control group suggesting the role of NVB to preserve remote spatial reference memories. The average number of annulus crossing for NVB treated rat group was also comparable to that of sham-C. In NVB treated group the improved results of MWM performance are believed to be due to adenylate cyclase (AC) enzyme. It is reported that AC 1 isoenzyme plays a vital role for maintaining intact hippocampal impulse transmission for reference memory [25]. For working memory, AC 8 isoenzyme is believed to play a crucial role presynaptically [26]. Therefore, the neuroprotective effect of NVB was more pronounced at the postsynaptic than the presynaptic neurons creating a significant improvement in the reference memory and only an intermediate enhancement of spatial working memory.

In conclusion, the NVB treatment to 2VO operated animals modulates neurotransmitters within the CNS thereby enhancing the cognitive function.

Disclosure of conflict of interest

None.

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