Int J Clin Exp Pathol 2015;8(7):8692-8698
www.ijcep.com /ISSN:1936-2625/IJCEP0009393

Original Article

Role of neovibsanin scaffold in preservation of spatial cognitive functions of rats with chronic epilepsy

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Received April 20, 2015; Accepted June 26, 2015; Epub July 1, 2015; Published July 15, 2015

Abstract: The effect of neovibsanin scaffold (NS) on the spatial cognitive functions of rats with lobal cerebrovascular hypoperfusion was investigated. Rats were divided into long-term memory (LTM) and short-term memory (STM) groups with 15 rats in each group. The groups were subdivided into 3 groups: control group comprised of 5 rats without surgery, untreated group of 5 rats left without treatment after 2OV, and NS treatment group with 5 rats receiving 5 mg/kg daily for 12 weeks of 2V0 operation. NS-treatment caused a marked decrease in the escape latency time and total distance travelled in the treatment group compared to untreated group which was evident from the working memory test. The animals of treatment group also showed significant improvement over that of untreated group in maze test performance. Furthermore, NS treatment also resulted in significant improvement in the probe memory test performance in treatment group compared to untreated group. These findings suggest that NS exhibits therapeutic effect on the spatial cognitive preservation in rats with chronic epilepsy.

Keywords: Chronic epilepsy, Morris water maze, cognitive, escape latency, working memory test

Introduction

In 1996 Fukuyama's research group isolated novel polyfunctionalized diterpenoid compounds, neovibsanin A and B from the shrub Viburnum awabuki [1]. Neovibsanins possess the ability to induce neurite outgrowth activity in PC12 cells therefore can be potent candidates for the treatment Alzheimer's disease [2, 3]. In 2009, the first total synthesis of (±)-neovibsanin B using an intramolecular Diels-Alder reaction and a subsequent oxy-Michael addition-lactonization reaction was reported [4]. It was also revealed that the neurite outgrowth activity of racemic mixture is almost the same as that of natural (+)-neovibsanin [4]. In the present study, effect of neovibsanin scaffold (NS, **Figure 1**) on spatial reference long-term memory (LTM) and short-term (STM) of cerebrally hypoperfused rats was examined.

Alzheimer's disease (AD) is characterized by defective decomposition of amyloid peptide. Plasmin is the enzyme having the ability to degrade amyloid peptide and its poor activity has been reported in areas of AD human brains [5]. It has been demonstrated that plasmin blocks β-amyloid neurotoxicity which indicates that plasmin exhibits physiological role in this disease [6, 7]. The level of ß-amyloid is maintained within physiological range by its degradation which is performed by plasmin. Enhanced expression of β-amyloid induces tPA inhibition and thereby suppresses activity of plasmin and promotes plaque formation [8].

Alzheimer's disease (AD), a progressive cognitive dysfunction caused by extracellular deposition of *β*-amyloid leads to 100000 deaths per year and affects about four million people in USA [9-12]. Earlier, the treatment strategies used for AD were antioxidant therapy, acetylcholinestrase inhibitors, nicotinic and muscarinic agonists, estrogen, nerve growth factor, low molecular lipophilic compounds that can activate neurotrophic factor signaling pathway [11]. AD is associated with chronic reduction in cerebral blood flow leading to reduction in glucose and oxygen supply to cerebral neurons and finally neurodegeneration and cognitive

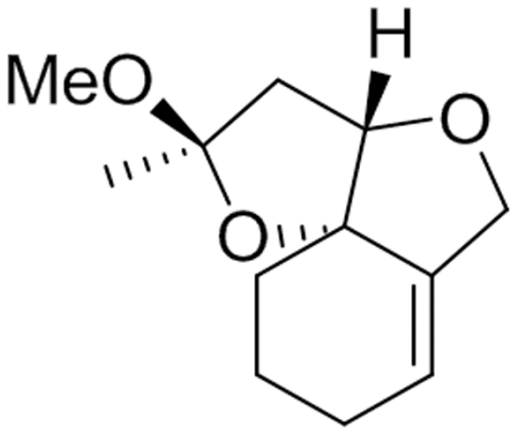

Figure 1. Structure of neovibsanin scaffold (NS).

decline [8]. Thus AD is considered to be a brain vascular disease and for its study two vessel occlusion (2VO) rat model which involves permanent bilateral ligation of common carotid arteries is used [8]. There is cerebral hypoperfusion followed by neurodegeneration of the pyramidal hippocampal neurons which control spatial learning and memory [13]. The spatial reference and working memory impairment is evaluated by employing Morris water maze (MWM) test [14, 15]. The spatial reference memory denotes the brain activity used to recall consolidated positions and places [16, 17]. One of the earliest symptoms of AD is the progressive weakening of spatial memories [18]. The 2VO operated rats show a significant poor MWM performance in learning as well as memory after 2VO surgery compared to healthy control rats [19].

Materials and methods

Animals and drug

18 week old male Sprague Dawley rats, 30 in number were obtained from Beijing Vital River Experimental Animal Technology Co., Ltd. The animals were acclimatized to lab conditions for one week before the start of experiment. The study was approved by the Committee for the Care and Use of Laboratory Animals of the Beijing Institutes for Biological Sciences. All the animal procedures were performed according to their instructions and efforts were made to minimize animal suffering and the number of animals used.

Treatment strategy

Thirty rats were divided into long-term memory (LTM) and short-term memory (STM) groups with 15 rats in each group. The groups were sub-divided into 3 groups: control group comprised of 5 rats without surgery, untreated group of 5 rats left without treatment after 2OV, and NS treatment group with 5 rats receiving 5 mg/kg daily for 12 weeks of 2VO operation. The STM test group rats were naïve to MWM at the time of 2VO surgery and tested at the 12th week after operation. NS was administered intraperitoneally at the dose of 5 mg/kg daily starting at 1 week before 2VO surgery and continued for further 12 days following 2VO surgery.

2VO procedure

Isoflurane was used to anesthetize the rats after treatment with NS for 12 weeks following 2VO surgery. Into the skull of anesthetized rats a 3 mm skin incision was made immediately above the sternal bone of each rat and the carotid sheath was identified. The carotid artery and the vagus nerve were carefully separated. After separation, the carotid artery was doubly ligated using silk suture and the arteries were cut between the two ligatures.

Apparatus

Modified Morris water maze (MWM) apparatus consisting of black colored circular fiberglass tank was used to perform the memory tests. The diameter and height of the MWM was 2 and 60 cm, respectively. Half height of the apparatus was filled with water and a black escape platform (EP) at its centre served as the rescue island for the rats. Water was changed every day and was maintained at 26°C temperature. Colored posters were pasted on the walls of the pool and the swimming time, distance and speed of the animals was recorded and analyzed using ANY-maze video tracking software (Stoelting Co., USA). Into the four imaginary quadrants (SW, SE, NW and NE) of the pool the tests were performed from 9 am to 5 pm starting from different quadrants each day. On day 5 and 4 prior to surgery and day 61 and 62 following surgery, respectively for LTM and STM groups the habituation training was carried out. Each rat received four trials of MWM training daily each for 2 minutes. The animals

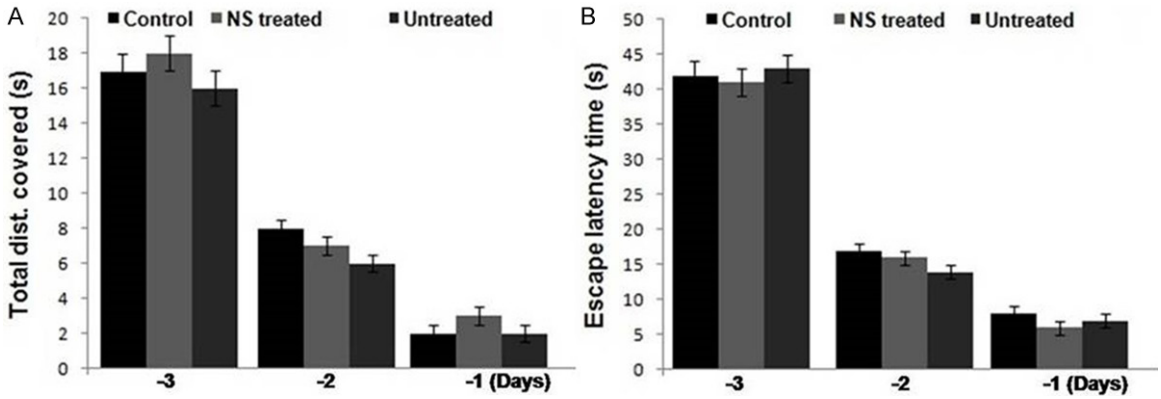

Figure 2. Differences in escape latency time and total distance travelled during the three successive days in MWM acquisition test before surgery.

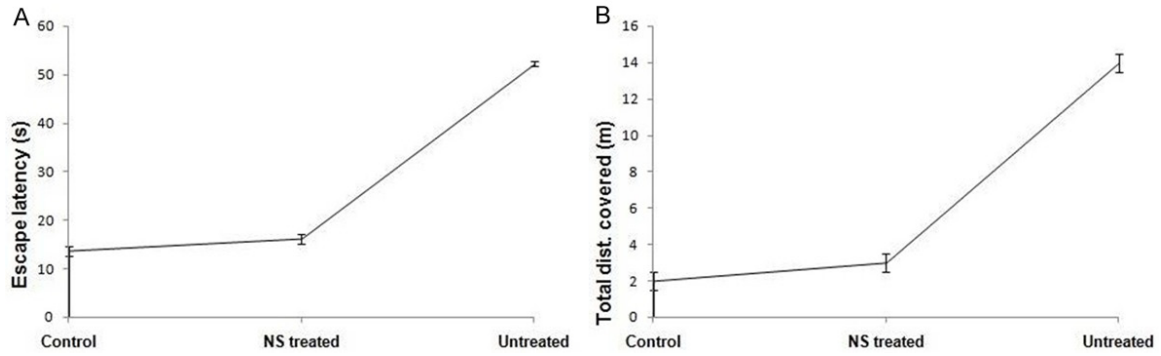

Figure 3. Differences in escape latency time and total distance travelled among control, NS treated and untreated groups during LTM test on 72th day after surgery.

were allowed to rest on EP for 30 second after reaching to it. The acquisition test was performed on the day 1-3 before and 62-63 after surgery for LTM and STM groups, respectively. The location of EP during this period was SW zone and height of waster was increased 2 cm above the EP surface. Four trials each for 2 minutes with 4 minute break following every trial was performed for 4 consecutive days. During this training, the distance travelled and time taken was calculated for each rat. On day 68 LTM test was performed and retention (probe) memory test was performed for LTM and STM groups on day 69 and 66 after operation, respectively. To each animal a 1 minute single swimming trial was given from the NE pole in absence of EP. The time spent and the number of annulus crossings in the target zone was calculated.

The working memory test (WMT) was carried out for STM group after changing the position of EP every day to different zones from day 67-69. Four trials each of 120 seconds with 1 minute intervening period were performed every day. The final test trial was followed by cued version test beginning with the SE pole. During the test EP 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

The distance covered by rats and their swimming speeds were analyzed using two way analysis of variance (ANOVA) as retention probe memory test data was investigated by one-way ANOVA. The results expressed are the mean ± SD. The differences were considered statistically significant at $P < 0.05$.

Results

Mortality and blindness rates after the operation

Among the animals of untreated group 8 died (mortality rate = 53%) and one got blindness

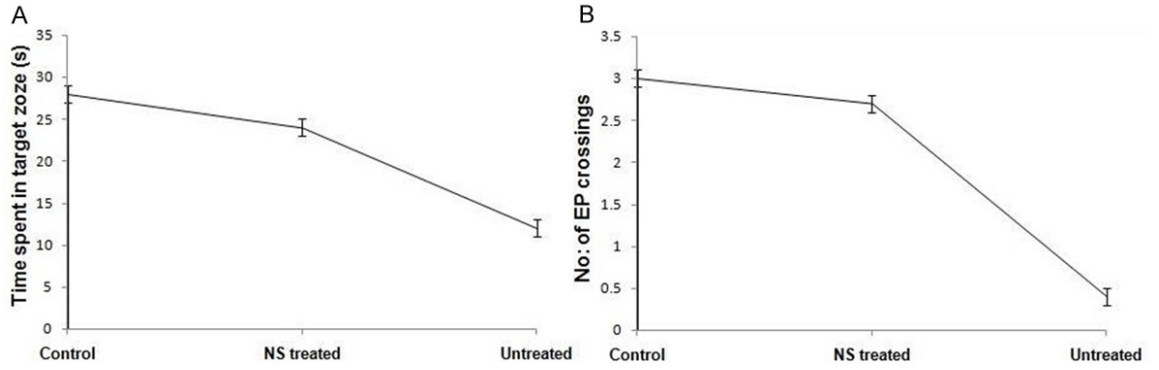

Figure 4. Differences among study groups in time spent in the target (SW) zone and number of annulus crossings retention probe LTM test.

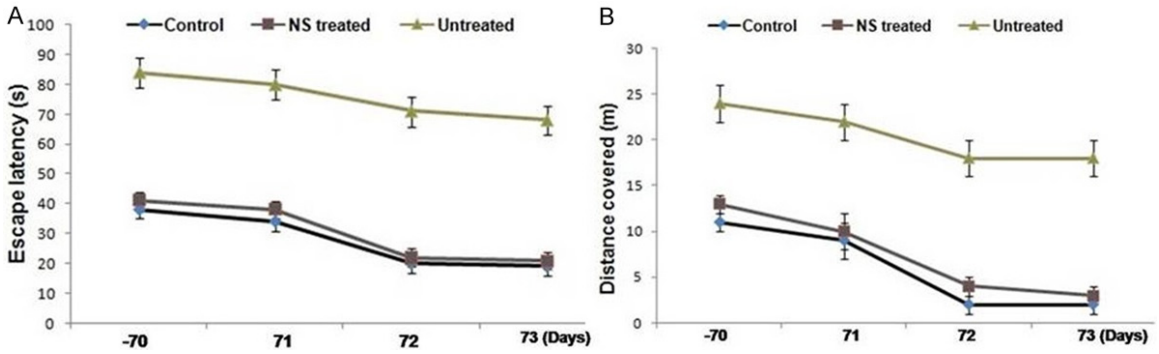

Figure 5. Differences between the study groups during the STM test in escape latency and total distance travelled.

after the surgery (blindness rate = 6.6%). In NS treatment group only one rat died (mortality rate = 6.6%) and in control group no death was found.

LTM test

The animals received training before 2VO surgery and were then examined for latency time and the distance covered for reaching the EP zone. The results revealed that mean escape latency time and the distance covered were same for all the animals under study before surgery was performed (**Figure 2**). However, after surgery NS treated group showed shorter mean escape latency time compared to untreated group which was close to that of control group. For control and NS treated groups the mean escape latency time was 13.71 ± 2.11 and 16.21 ± 2.43 sec, respectively where as in untreated group the value was 52.23 ± 4.32 sec (**Figure 3**).

For control and NS treated groups the distance covered during swimming were 4.8768 ± 0.87 and 5.3537 ± 0.85 m, respectively which was markedly shorter than that of untreated group with the value of 15.2537 ± 2.54 m (**Figure 3**). Furthermore, the time spent in the EP target zone was significantly more for control and NS treated groups compared to untreated group. For control, NS treated and untreated groups the mean time spent in the EP zone was 54.65 ± 4.46, 47.39 ± 4.22 and 19.32 ± 2.08 s, respectively (**Figure 4**).

STM test

After surgery on days 70-73 acquisition test of MWM was performed which showed marked differences in mean escape latency time and total distance covered by the three groups of animals (**Figure 5**). The values for mean time spent in the EF zone during this period were 33.43 ± 2.45, 29.34 ± 2.11 and 11.23 ± 1.87 s for control, NS treated and untreated groups, respectively (**Figure 6**).

WMT results

The mean escape latency analyzed on the days 74-76 showed marked differences between

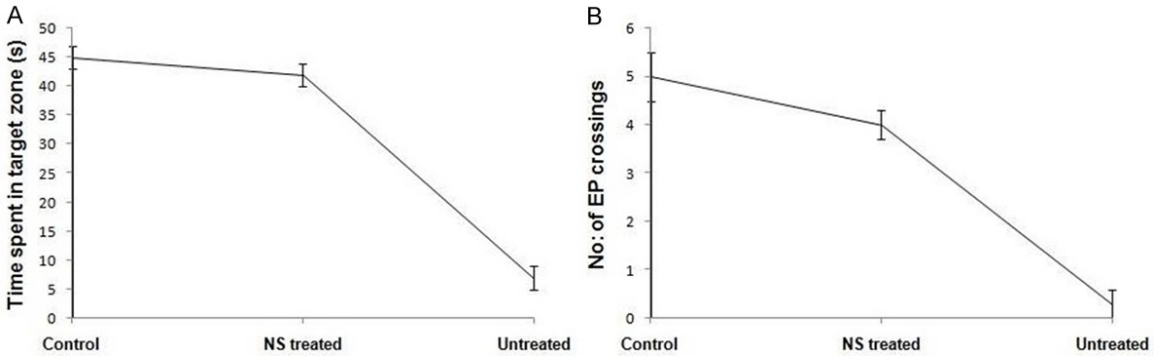

Figure 6. Differences among time spent in the target (SW) zone and number of annulus crossings.

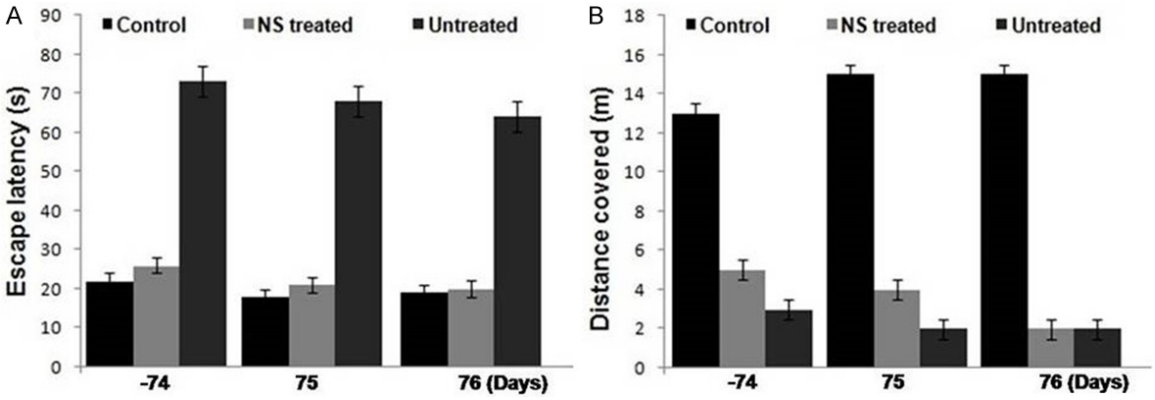

Figure 7. Differences in the escape latency time and total distance travelled with daily changing of EP location.

control, NS treated and untreated groups (**Figure 7**). Similar results were observed for the distance travelled during swimming.

Discussion

Neovibsanins possess the ability to induce neurite outgrowth activity in PC12 cells therefore can be potent candidates for the treatment Alzheimer's disease [2]. It was also revealed that the neurite outgrowth activity of racemic mixture is almost the same as that of natural (+)-neovibsanin [4]. The present study was aimed to investigate the effect of NS, the core of neovibsanins on the cognitive learning and memory performance in the rats. The rats were subjected to bilateral common carotid arteries occlusion (2VO) surgery for obtaining the cerebrovascular hypoperfusion rat model. 2VO operation induces optic tract acute ischemic injury which then results in loss of eyesight within one week of the operation [19-21]. Our results showed that in untreated group the number of deaths after 2VO operation was significantly higher compared to NS treated group. Acute ischemic injury in the vital brain centers may be the cause leading to deaths in the untreated group. This suggests that NS exhibits neuroprotective effect in the rats which is consistent with the earlier reports that neovibsanins induce neurite outgrowth activity [10].

Initially all the animals showed similar results for escape latency time and distance travelled during swimming in habituation training and cued version MWM task test. This indicated that all the animals had intact sensory-motor function before the surgery and were in cognitively healthy state. However, the results from LTM test revealed that the rats left untreated after 2VO surgery were in deteriorated state compared to NS treated group. Similarly, in retention memory test significant decrease in remote spatial memory was observed in untreated rats. Usually the cerebral hypoperfusion assumes chronic stage after 56th day of the 2VO operation and the MWM performance of animals deteriorates leading to neurodegeneration [20].

Treatment of rats with NS after 2VO surgery resulted in marked improvement in LTM impairments. This was further confirmed by LTM water maze performance and probe memory test. Our results demonstrated that NS preserves remote spatial reference memories of the 2VO operated rats. Thus, NS significantly improved the reference memory and enhanced the spatial working memory.

Conclusion

The NS treatment to 2VO operated animals modulates neurotransmitters within the CNS thereby enhancing the cognitive function.

Acknowledgements

This work was supported by Shandong Province Natural Science Foundation (No. ZR2011HM-087).

Disclosure of conflict of interest

None.

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