Protective effect of polydatin on learning and memory impairments in neonatal rats with hypoxic-ischemic brain injury by up-regulating brain-derived neurotrophic factor

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Abstract. Polydatin is a key component of *Polygonum cuspidatum*, a herb with medical and nutritional value. The present study investigated the protective effect of polydatin against learning and memory impairment in neonatal rats with hypoxic-ischemic brain injury (HIBI). The unilateral common carotid artery ligation method was used to generate neonatal HIBI rats. Y-maze testing revealed that rats with HIBI exhibited memory impairment, while rats with HIBI treated with polydatin displayed enhanced long-term learning and memory. Of note, polydatin was found to upregulate the expression of hippocampal brain-derived neurotrophic factor (BDNF) in rats with HIBI. BDNF has a role in protecting HIBI-induced brain tissue injury and alleviating memory impairment. These findings showed that polydatin had a protective effect against learning and memory impairment in neonatal rats with HIBI and that the protective effect may be mediated through the upregulation of BDNF.

Introduction

Perinatal hypoxic-ischemic brain injury (HIBI) is a major cause of mortality and neurodevelopmental impairment in newborn infants (1). The increased risk of neurodevelopmental impairment may be due to the rate of survival of premature infants, with survivors displaying neurological sequelae (2,3). Thus, investigations are required to develop effective drugs to protect against neurodevelopmental impairment in newborn infants with HIBI. Polydatin (Fig. 1) is one of the primary bioactive components of *Polygonum cuspidatum* and has been reported to exhibit a neuroprotective effect in the rat brain, as well as protect against learning and memory impairment in a rat model of vascular dementia, alleviate oxygen and glucose deprivation-induced myocardial cell injury and attenuate ischemia-reperfusion-induced cardiac myocyte damage (4-8). However, the protective effect and mechanism of polydatin on perinatal rats with HIBI has yet to be elucidated.

Neonatal hypoxic-ischemic model rats exhibit predictable brain injuries similar to those observed clinically in humans (9-12). In the present study, a neonatal rat model of HIBI was generated using a unilateral carotid artery ligation method on postnatal seven-day-old rats, comparable to the 34-week-old human fetus (13). The protective effect of polydatin was then investigated on neurodevelopmental impairment in the neonatal rats with HIBI. The effect of polydatin on learning and memory in neonatal rats with HIBI was assessed using a behavioral test. Furthermore, the expression of brain-derived neurotrophic factor (BDNF) was assessed in the rat hippocampus following polydatin treatment. The findings of the present study may be useful to determine the potential neuroprotective effect of polydatin in neonatal rats with HIBI and to develop a potential clinical treatment of neonatal hypoxic-ischemic encephalopathy.

Materials and methods

Polydatin and experimental animals. Polydatin dry powder (Yousi Biotechnology Inc., Shanghai, China) was purified, analyzed using high-performance liquid chromatography and the purity was determined to be >99%. A total of 156 seven-day-old, healthy Sprague-Dawley rats, weighing between 12 and 19 g, were selected regardless of gender from the Animal Experimental Center of Dalian Medical University (Animal license no: SYXK20080002; Dalian,

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China). Rats were maintained under a 12-h light/dark cycle at 22˚C with free access to food and water.

Neonatal rats with HIBI, grouping and treatment design. The animal model of HIBI was generated using Rice's method (9). In brief, a midline incision was made at the rat neck and the subcutaneous fat was separated. The left carotid artery was exposed and permanently ligated. The rats were maintained in nitrogen gas containing 8% oxygen in closed containers for 2 h. Following 2 h hypoxia, the rats were allowed to recover. Polydatin dry powder was dissolved in physiological saline and intraperitoneally injected into the rats (10 mg/kg body weight) once a day for 10 consecutive days. A total of 156 experimental Sprague-Dawley rats were randomly divided into the following three groups: Sham-operated (SO; n=40), model (rats with HIBI treated with saline; n=58) and polydatin (PD; rats with HIBI treated with polydatin; n=58). The study was approved by the ethics committee of the Hospital of Maternal and Child Health of Dalian, Dalian, China.

Y-maze learning test. The Y-maze learning test was used to assess the learning and memory of the rats. Ten days following hypoxic-ischemic damage, rats with HIBI were tested using the stochastic restless method. The rats underwent 20 trials every 24 h with the Y-maze parameters (60V; 5 sec duration). The error reaction number (ERN), total reaction time (TRT) and correct avoidance rate (CAR) were measured to assess the learning ability of the rats.

Immunohistochemical staining. Rats were sacrificed using an intracardiac perfusion fixation method. Brains were removed and fixed using 4% paraformaldehyde in phosphate-buffered saline for 48 h. The tissues were dehydrated and embedded in paraffin for immunohistochemistry. The embedded brain tissues were sliced to a thickness of 4 μm. Rabbit anti-mouse BDNF antibodies (H-117; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and a streptavidin-peroxidase immunohistochemistry kit (Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) were used for the staining. Image-Pro Plus 6 software (Media Cybernetics, Inc., Rockville, MD, USA) was used to calculate the average optical density of the staining.

Western blot analysis. Rats were sacrificed using decapitation. The left hippocampal tissues were isolated from the fresh brain tissue and stored in liquid nitrogen for western blot analysis. Total protein was extracted from the hippocampal tissues and measured. Equal aliquots of protein were used for western blot analysis using rabbit anti-mouse BDNF and β-actin antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Gray value analysis software (Quantity One; Bio-Rad Inc., Hercules, CA, USA) was used to analyze the gray value of the protein bands. Relative BDNF protein expression was calculated using the ratio of BDNF gray value to β-actin gray value.

Statistical analysis. SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) was used for the statistical analyses. All experimental data are presented as the mean ± standard deviation for each group. Differences among the groups were

Table I. Effect of polydatin on memory impairment in HIBI rats on day one assessed using the Y-maze test.

Groups	Samples	TRT (%)	ERN (%)	CAR (%)
SO	10	157.92±10.31	2.80±1.23	43.50±5.80
Model	10	201.47±21.96[a]	10.10±0.74	21.00±6.99[a]
PD	10	179.80±11.23[b]	5.60±1.26	29.50±4.38[b]

Data are presented as the mean ± standard deviation. [a]P<0.01 vs. SO group; [b]P<0.01 vs. PD group. HIBI, hypoxic-ischemic brain injury; TRT, total reaction time; ERN, error reaction number; CAR, correct avoidance rate; SO, sham-operated group; PD, polydatin group.

Table II. Effect of polydatin on memory impairment in HIBI rats on day two assessed using the Y-maze test.

Groups	Samples	TRT (%)	ERN (%)	CAR (%)
SO	10	148.94±12.60	2.40±0.52	45.00±7.45
Model	10	198.40±26.69[a]	9.80±1.40	18.00±7.15[a]
PD	10	173.84±9.72[b]	5.00±0.94	31.00±8.76[b]

Data are presented as the mean ± standard deviation. [a]P<0.01 vs. SO group; [b]P<0.01 vs. PD group. HIBI, hypoxic-ischemic brain injury; TRT, total reaction time; ERN, error reaction number; CAR, correct avoidance rate; SO, sham-operated group; PD, polydatin group.
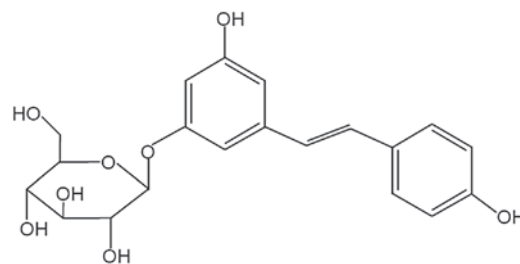

Figure 1. Molecular structure of polydatin.

compared using one-way analysis of variance (ANOVA), or multiple ANOVA followed by least significant difference tests. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of polydatin on memory impairment in rats with HIBI. On days one and two, Y-maze testing revealed that the TRT and ERN of the rats in the model group were significantly increased (P<0.01), while the CAR was significantly reduced (P<0.01) compared with the rats in SO group (Tables I and II). Compared with the rats in model group, the TRT of the rats in the PD and SO groups was observed to be significantly decreased (P<0.01), while the CAR was found to be significantly increased (P<0.01; Tables I and II). These findings suggested that rat memory was significantly impaired
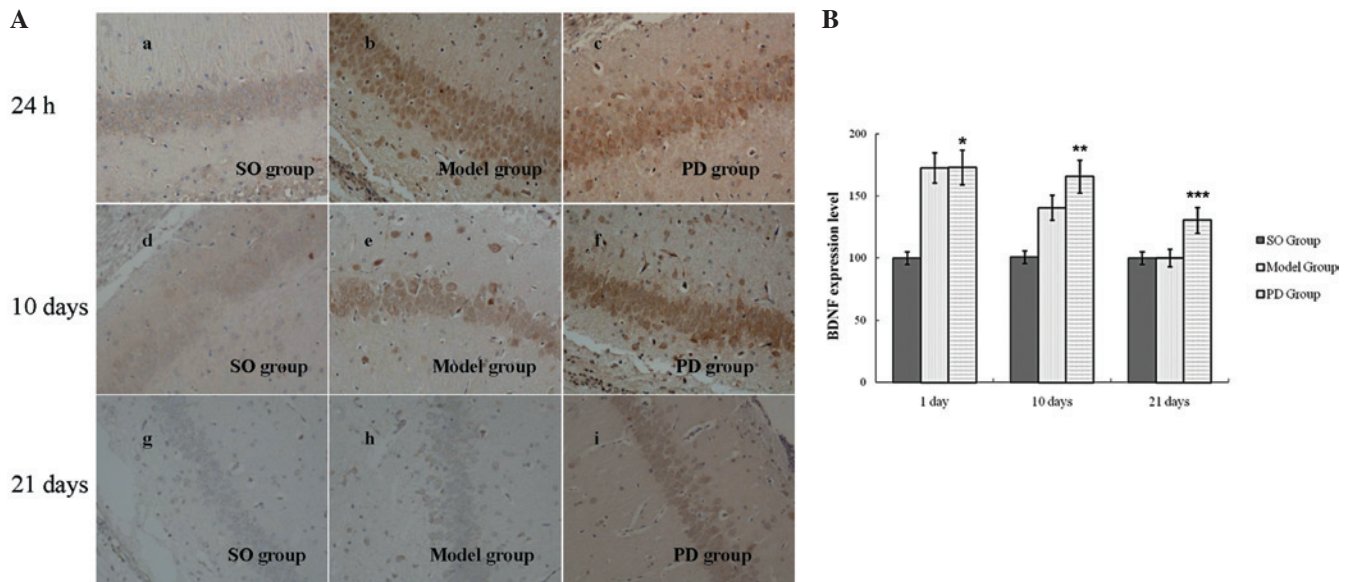
A

B

Figure 2. BDNF expression in the hippocampal CA1 region of the rats detected using immunohistochemistry. (A) Imunohistochemical staining of BDNF expression in the hippocampal CA1 region of HIBI rats in each group on day 1, 10 and 21 after hypoxic-ischemic damage (magnification, x200). (B) Immunohistochemistry scores. BDNF expression was scored between 100 and 200, with BDNF expression in the rats in the SO group set to 100. Data are presented as the mean ± standard deviation of three independent staining experiments.*P<0.01 vs. SO group on day one; **P<0.01 vs. SO group and model group on day 10; ***P<0.01 vs. SO and model groups on day 21. HIBI, hypoxic-ischemic brain injury; BDNF, brain-derived neurotrophic factor; SO, sham-operated; PD, polydatin.

by hypoxia and ischemia and that polydatin ameliorated the HIBI-induced memory impairment.

Polydatin increases BDNF expression in the rat hippocampus. BDNF expression in the rat hippocampus was assessed using immunohistochemistry and western blot analyses. In the rats in the SO group, BDNF was observed to be expressed in the hippocampal CA1 and CA3 regions. At 24 h after hypoxic-ischemic damage, BDNF expression in the left hippocampal CA1 region was found to be significantly increased in the rats in the model group compared with the rats in the SO group (P<0.01; Fig. 2). There was no significant difference in BDNF expression in the rats in the model group compared with those in the PD group (P>0.05; Fig. 2). On days 10 and 21 following hypoxic-ischemic damage, BDNF expression in the hippocampal CA1 region in the rats in the model group was observed to be significantly increased compared with those in the SO group (P<0.01; Fig. 2). Furthermore, BDNF expression in the hippocampal CA1 region of the rats in the PD group was found to be significantly increased compared with the rats in the model group (P<0.05; Fig. 2). BDNF expression in the hippocampal CA3 region exhibited a similar pattern of expression to that in the hippocampal CA1 area (data not shown).

Western blot analysis revealed that at 24 h after hypoxic-ischemic damage, BDNF expression was increased in the hippocampus of the rats in the model group compared with those in the SO group (Fig. 3). Furthermore, BDNF expression was observed to be increased in the rats in the PD group compared with those in the SO and model groups. On day 10 following hypoxic-ischemic damage, BDNF expression in hippocampus of the rats in the model group was found to be reduced compared with that on day one. On day 21 after hypoxic-ischemic damage, there was no significant difference in BDNF expression in the hippocampus of the
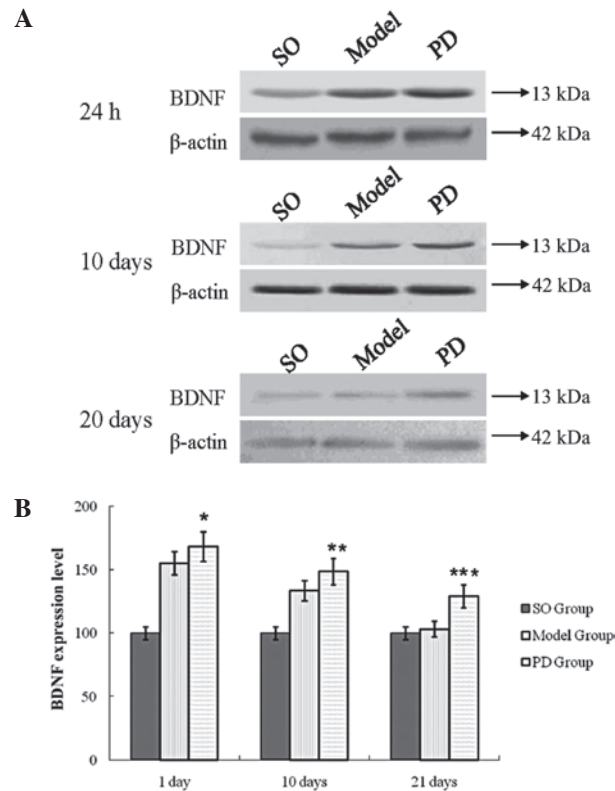
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Figure 3. Western blot analysis of BDNF expression in the hippocampus of the rats in each group. (A) Western blot analysis of BDNF expression in the hippocampus of the rats in each group on day 1, 10 and 21 after hypoxic-ischemic damage. β-actin was used as an internal control. (B) Scoring of western blot analysis data using the ratio of BDNF gray value to β-actin gray value. BDNF expression was scored between 100 and 200, with BDNF expression in the rats in the SO group set to 100. Data are presented as the mean ± standard deviation of three independent experiments.*P<0.01 vs. SO group on day one; **P<0.01 vs. SO and model groups on day 10; ***P<0.01 vs. SO and model groups on day 21. BDNF, brain-derived neurotrophic factor; SO, sham-operated group; PD, polydatin group.

rats in the SO group compared with the model group; however, BDNF expression in the rats in the PD group remained high (Fig. 3). These findings suggested that polydatin induced BDNF expression in the hippocampus of rats and sustained the expression of BDNF in rats with HIBI.

Discussion

Polydatin has been reported to have an important role in neuroprotection against cerebral cell injury induced by focal ischemic-reperfusion (8). In the present study, a neonatal HIBI rat model was used to investigate the effect of polydatin on HIBI-induced learning and memory impairment.

Y-maze tests were used to detect the spatial learning and memory of the rats (14,15). Rats in the maze exhibited a passive avoidance reaction to electrical stimulation. Generally, subsequent to training, rats learn and remember the spatial location of the safe zone within the maze. However, hippocampal damage impairs memory and spatial orientation (12). In the present study, the Y-maze test results reflected HIBI-induced hippocampal neuron damage and memory impairment in the rats. Following polydatin treatment, the Y-maze test revealed that TRT and ERN were reduced, while CAR was increased, compared with the rats in the model group. These findings suggested that hypoxia and ischemia impaired learning and memory in neonatal rats and that polydatin enhanced spatial orientation and memory in rats with hypoxic-ischemic injury, as well as alleviated neurologic sequelae in rats with HIBI.

BDNF is widely distributed throughout the brain to protect against brain injury. Moreover, BDNF expression is increased in cerebral ischemia (16-18). BDNF reduces neuronal damage following ischemia, as well as promotes nerve repair (19-23). In the present study, in the rat model of HIBI, cerebral ischemia and reperfusion injury caused high BDNF expression, which is associated with local neuronal resistance to injury. Of note, BDNF exerted protective effects on spatial memory deficits following neonatal hypoxic-ischemic injury.

The present study showed that hippocampal BDNF expression was increased following HIBI, but gradually decreased to normal levels within 21 days. Furthermore, polydatin was found to have a protective effect against memory impairment in rats with HIBI and significantly increased the expression of BDNF. Thus, while the neuroprotective mechanism of polydatin in HIBI treatment requires further investigation, the high levels and duration of BDNF expression induced by polydatin may have a protective role in neurons against brain damage induced by hypoxia-ischemia. In conclusion, the present study showed that polydatin may have an important role in promoting neuronal survival and may contribute to neuron recovery following injury, as well as HIBI-induced memory impairment. Polydatin was found to increase the expression of hippocampal BDNF, which may be the long-term mechanism underlying the improvements in learning and memory induced by polydatin in rats with HIBI.

Acknowledgements

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