European Review for Medical and Pharmacological Sciences 2017; 21: 4431-4436

Effects of dl-3-n-butylphthalide on serum VEGF and bFGF levels in acute cerebral infarction

S.-C. TANG[1], C.-J. LUO[2], K.-H. ZHANG[3], K. LI[4], X.-H. FAN[1], L.-P. NING[1], P. XUE[5]

[1]Department of Rehabilitation Medicine, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong Province, China
[2]Department of Neurology, Jinan Shizhong Distract People's Hospital, Jinan, China
[3]Department of Psychiatry, the 8th Ward of Shandong Mental Health Center, Jinan, Shandong Province, China
[4]Department of Radiotherapy, Zhangqiu People's Hospital, Jinan, Shandong Province, China
[5]Department of Neurology, Liaocheng People's Hospital, Liaocheng, China

Abstract. – OBJECTIVE: To observe the curative effect of dl-3-n-Butylphthalide (NBP) on patients with acute cerebral infarction (ACI) and its effects on levels of serum vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).

PATIENTS AND METHODS: A total of 160 ACI patients treated in our hospital who met the criteria were selected and randomly divided into treatment group (n=80, including 42 males and 38 females) and control group (n=80, including 40 males and 40 females). The control group was treated with routine drug therapy, while the treatment group was treated with butylphthalide on this basis. The curative effect was evaluated using the National Institute of Health Stroke Scale (NIHSS) and the Activity of Daily Life Scale (ADL Scale). The levels of the two factors in serum were measured using enzyme-linked immunosorbent assay (ELISA), and the changes in the levels of the two factors in serum at different time points before and after treatment were compared between the two groups.

RESULTS: After treatment, the levels of the two factors in serum in both groups were significantly increased compared with those before treatment ($p<0.05$), and the increase in treatment group was more significant than that in control group ($p<0.05$). The scores of ADL scale in both groups were significantly increased after treatment compared with those before treatment, and the increase in treatment group was more significant than that in control group ($p<0.05$). The scores of NIHSS in both groups were significantly decreased compared with those before treatment, and the decrease in treatment group was more significant than that in control group ($p<0.05$).

CONCLUSIONS: NBP can improve the expressions of VEGF and bFGF in serum of ACI patients, and its effect is superior to that of conventional drugs.

Key Words:
Acute cerebral infarction, Vascular endothelial growth factor, Basic fibroblast growth factor, NBP.

Introduction

Acute cerebral infarction (ACI) is characterized by acute onset, high morbidity rate, high disability rate and high fatality rate, seriously threatening people's health and life safety, and bringing serious burden to individuals and the country. Once ACI occurs, a series of sequential physiological and pathological responses used to occur in the body. Therefore, improving the cure rate of cerebral infarction will be our main task in the future, and the key to treatment of cerebral infarction is the revascularization and establishment of collateral circulation. A number of studies have shown that basic fibroblast growth factor (bFGF) is a kind of growth factor and active peptide that can promote the revascularization, which can promote the establishment of vascular collateral circulation, promote the revascularization in ischemic penumbra, improve the blood supply in cerebral infarction region and enhance the treatment effect and prognosis of patients with cerebral infarction[1,2]. In addition, Marti et al[3] studied and showed that within 24 h after cerebral ischemia and hypoxia, stress proliferation occurs in a large number of vascular endothelial growth factors (VEGF) and other neurotrophic factors (NF),

Corresponding Author: Ping Xue e-mail: Juziguo0906@163.com
Kaihong Zhang; e-mail: 380133490@qq.com

and many endothelial cells begin to be produced in the ischemic penumbra and expand to infarction region. NF is produced by the body itself, which is a kind of necessary peptide or protein molecule for neuron growth and survival, playing a very important role in the survival and functional maintenance of neural stem cells (NSCs)[4].

In this study, the changes in VEGF and bFGF levels in peripheral blood after cerebral infarction were compared to indirectly reflect the effect of NBP on revascularization of patients with cerebral infarction.

Patients and Methods

Patients

A total of 160 patients with cerebral infarction admitted to our hospital were randomly selected. The onset time was within 72 h, and they met the diagnostic criteria of cerebral infarction specified in the Fourth Academic Conference of Cerebrovascular Disease of the Chinese Medical Association. The patients were diagnosed via the head CT and MRI (as well as DWI if in super acute period), and they had clear consciousness, stable vital signs, hemiplegia and normal swallowing function. The disease had no progression within 48 h. Exclusion criteria: 1) patients aged <18 years old; 2) patients with severe hepatic renal dysfunction; 3) patients with systemic bleeding tendency or hemorrhagic disease; 4) patients with allergic constitution; 5) women during pregnancy or lactation. This study was approved by the Ethics Committee of Liaocheng People's Hospital. Signed written informed consents were obtained from all participants before the study.

Research Methods

A total of 160 patients were enrolled in Liaocheng People's Hospital. The control group (n=80) was treated with conventional therapy, including the control of blood pressure, blood lipid, blood glucose, anti-platelet aggregation (aspirin, Bayer Medicine Healthcare Co., Ltd. Leverkusen, Germany; dose at admission: 300 mg/time; dose after admission: 100 mg/once every night), and protection of brain (Citicoline Sodium Capsules, Qilu Pharmaceutical Co., Ltd. Shangdong, China; 0.2 g/time, 3 times/d), and patients with brain edema were treated with dehydration therapy using 20% mannitol or glycerol fructose to maintain the water-electrolyte balance, etc. The treatment group was treated with Butylphthalide Soft Capsule (trade name: NBP, manufactured by CSPC NBP Pharmaceutical Co., Ltd, Shijiazhuang, China) for 3 times/d (0.2 g/time) for 12 days as one course of treatment.

Detection of Serum VRGF and bFGF Contents

4 mL fasting elbow venous blood was drawn in the early morning from all cases selected at admission and at 2 d, 7 d and 14 d after treatment, placed at room temperature and centrifuged. The supernatant was retained and placed in the cryogenic refrigerator at -80°C for later detection. The levels of VEGF and bFGF were detected by double-antibody sandwich insoluble enzyme-linked immunosorbent assay.

National Institute of Health Stroke Scale (NIHSS) was used for neurological deficit score of patients in both groups at admission and 14 d after treatment. At the same time, activity of daily life scale (ADL Scale) was used to score the activities of daily life of patients in both groups at admission and 14 d after treatment.

Statistical Analysis

Statistical Product and Service Solutions (SPSS Inc., Chicago, IL, USA) 18.0 statistical software was used; measurement data were presented as x±s, and *t*-test or x^2 test was used. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of Serum VEGF Levels Before and After Treatment Between the two Groups

The average levels of serum VEGF at 2 d, 7 d and 14 d after treatment in both groups were significantly higher than those before treatment ($p<0.05$), and the level of VEGF in the acute phase continued to be increased and reached the peak at 7 d, then it was gradually decreased and still remained at a high level until 14 d. In addition, the levels of VEGF in NBP treatment group at 7 d and 14 d after treatment were higher than those in control group, and the differences were statistically significant ($p<0.05$) (Figure 1 and Table I).

Comparisons of Serum bFGF Levels Before and After Treatment Between the two Groups

The level of bFGF at 2 d after treatment in both groups was the highest, began to be decreased at 7

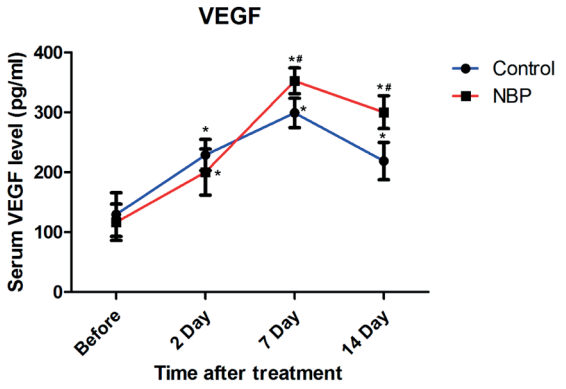

Figure 1. Serum VEGF levels at different time points before and after treatment between the two groups. $^{*}p<0.05$, compared with before treatment; $^{\#}p<0.05$, compared with control group at 7 d and 14 d, respectively.

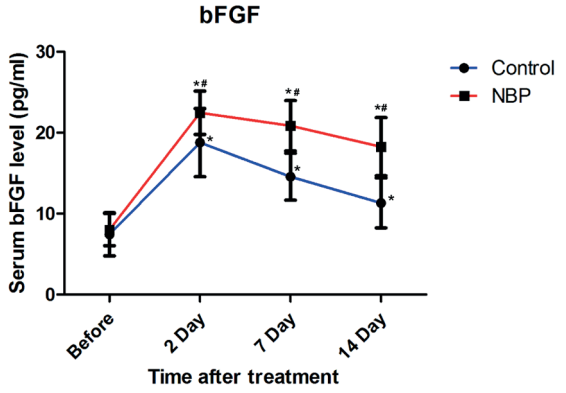

Figure 2. serum bFGF levels at different time points before and after treatment between the two groups ($\bar{x}$±s, pg/ml). $^{*}p<0.05$, compared with before treatment; $^{\#}p<0.05$, compared with control group at 2 d, 7 d, and 14 d, respectively.

d and reached the bottom at 14 d, but it was still higher than that before treatment, and the difference was statistically significant ($p<0.05$). In addition, the levels of bFGF at 2 d, 7 d and 14 d in NBP treatment group were significantly higher than those in control group, and the differences were statistically significant (Figure 2 and Table II).

Comparisons of ADL Scores Before and After Treatment Between the two Groups

The ADL scores before and after treatment between the two groups were compared, and the results showed that the ADL scores of both groups after treatment were significantly increased compared with those before treatment, and it was increased in NBP treatment group more significantly than that in control group ($p<0.05$) (Figure 3 and Table III).

Comparisons of Neurological Deficit Scores Before and After treatment Between the two Groups

The neurological deficit scores before and after treatment between the two groups were compared, and the results showed that the NIHSS scores of both groups after treatment were significantly decreased compared with those before treatment, and it was decreased in NBP treatment group more significantly than that in control group ($p<0.05$) (Figure 4 and Table IV).

Table I. Comparisons of serum VEGF levels at different time points before and after treatment between the two groups ($\bar{x}$±s, pg/ml).

Group	Case	VEGF			
		Before treatment	2 d	7 d	14 d
Control group	80	129.39±36.45	228.91±26.07*	299.06±24.59*	218.68±31.17*
NBP treatment group	80	116.47±30.21	200.25±38.56*	352.59±21.45*#	300.27±27.47*#

$^{*}p<0.05$, compared with before treatment; $^{\#}p<0.05$, compared with control group at 7 d and 14 d, respectively.

Table II. Comparisons of serum VEGF levels at different time points before and after treatment between the two groups ($\bar{x}$±s, pg/ml).

Group	Case	bFGF			
		Before treatment	2 d	7 d	14 d
Control group	80	7.45±2.67	18.78±4.21*	14.56±2.89*	11.32±3.09*
NBP treatment group	80	8.02±1.99	22.46±2.67*#	20.85±3.11*#	18.27±3.58*#

$^{*}p<0.05$, compared with before treatment; $^{\#}p<0.05$, compared with control group at 2 d, 7 d, and 14 d, respectively.

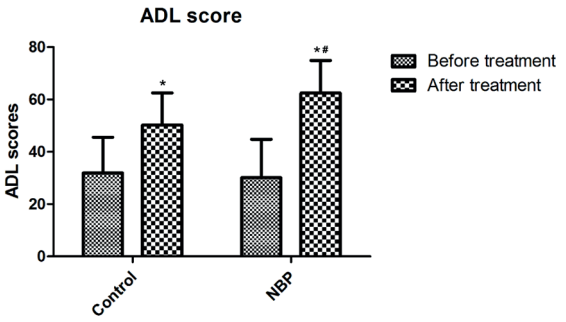

Figure 3. Comparisons of ADL scores before and after treatment between the two groups (points; $\bar{x}$±s). $^{*}p<0.05$, comparison of ADL scores before and after treatment in both groups, respectively; $^{\#}$ $p<0.05$, comparison of ADL scores between two groups.

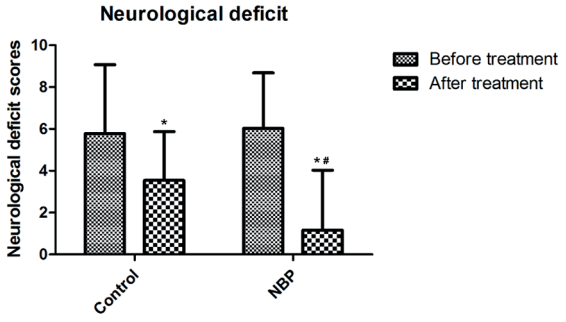

Figure 4. Comparison of neurological deficit scores before and after treatment between the two groups (points; $\bar{x}$±s). $^{*}p<0.05$, comparison of neurological deficit scores before and after treatment in both groups, respectively; $^{\#}p<0.05$, comparison between two groups.

Table III. Comparisons of ADL scores before and after treatment between the two groups (points; $\bar{x}$±s, pg/ml).

Group	Case	Before treatment	After treatment	Increased degree
Control group	80	31.89±13.69	50.23±12.26*	17.56±12.89
NBP treatment group	80	30.13±14.65	62.46±12.47$^{*\#}$	33.85±13.51§

$^{*}p<0.05$, comparison of ADL scores before and after treatment in both groups, respectively; $^{\#}p<0.05$, comparison of ADL scores between two groups.

Table IV. Comparison of neurological deficit scores before and after treatment between the two groups (points; $\bar{x}$±s).

Group	Case	Before treatment	After treatment	Decreased degree
Control group	80	5.78±3.29	3.55±2.32*	2.16±2.89
NBP treatment group	80	6.03±2.65	1.16±2.87$^{*\#}$	4.85±2.51$^{\#}$

$^{*}p<0.05$, comparison of neurological deficit scores before and after treatment in both groups, respectively; $^{\#}p<0.05$, comparison between two groups.

Observation of Adverse Reactions

In the treatment and return visit, there were 2 cases of mild nausea and 1 case of mild rash; they were not treated and the symptoms disappeared spontaneously in treatment group. There were 2 cases of headache in control group and it was relieved after the infusion speed was decreased. There were 3 cases of abnormal liver function in treatment group and 2 cases in control group; but the increases of AST and ALT were 2 times less than the normal values; after 1 month without treatment, the liver function returned to normal. Other indexes (blood routine, renal function electrolyte, coagulation function and ECG, etc.) were normal in the review. Adverse reactions between the two groups were compared using the x^2 test, and the differences were not statistically significant ($p>0.05$).

Discussion

Pathophysiological effects in the occurrence of ACI, such as intracellular Ca^{2+} overload, toxic effect of excitatory amino acid, inflammatory response, oxygen free radical destruction, apoptosis and energy depletion, lead to a series of ischemic cascade reactions in the body and seriously damage the nerve tissues. At the same time, the body protects itself and repairs the nervous system, and many endogenous NSCs existing in the brain in ACI and other acute stress

promote the synthesis of a large number of NF, and NF can induce the proliferation, differentiation and directed migration to the damaged site of endogenous NSCs in turn[4-6], forming a benign cycle. NF can not only reduce the neural degeneration and prevent further damage to the neurological function, but also play a key role in the growth and regeneration of axons[7,8]. VEGF and bFGF are important members of the NF family and play important roles in the treatment of ACI, regeneration, growth, proliferation and differentiation of neurons and development of nervous system[9,10]. Studies have shown that VEGF has the endothelial cell characteristics, which is a kind of potential mitogen that can promote the endothelial cell proliferation and differentiation, improve the neovascularization and recovery of collateral circulation in damaged vessels; moreover, VEGF with nerve cell affinity can promote the angiogenesis and save the tissues in peripheral ischemic area, thus fundamentally improving the prognosis of stroke[11]. bFGF has many target cells in the brain tissue playing a strong neurotrophic effect and an important role in nervous system damage repair and normal function[12]. bFGF can promote the autocrine effect of angiogenesis through releasing vesicles into the intercellular space for signal transduction[13]. Tanaka et al[14] proposed that increasing the blood flow in ischemic penumbra may be related to the therapeutic effect of bFGF. However, the mechanisms of VEGF and bFGF in NSCs remain unknown and require further study.

The main effective component of NBP is the L-butylphthalide that can be extracted from celery seeds and artificially synthesized. As a kind of Class-I new drug, its advantage lies in that it can block a series of pathological damage process in ACI, such as promoting the brain energy metabolism, alleviating the hydrocephalus, anti-free radical damage, reducing the oxidative stress injury, inflammatory response, release of excitatory amino acid and cerebral infarction area[15-17]. Therefore, NBP has a strong anti-cerebral ischemia effect.

Our results also confirmed that the levels of VEGF and bFGF in the blood of patients with cerebral infarction were significantly increased after administration of NBP, which also indirectly confirmed that NBP plays an important role in promoting the revascularization and recovery of neurological function in patients with cerebral infarction.

Conclusions

We found that the average serum VEGF and bFGF levels in patients with cerebral infarction at different time points after treatment were higher than those before treatment, indicating that VEGF and bFGF are involved and play important roles in the early pathological and physiological process of cerebral ischemia reperfusion; VEGF and bFGF levels in the recovery phase were still higher than those before treatment, indicating that they may be involved in the repair process of cerebral infarction. Moreover, this study analyzed the effect of NBP on serum VEGF and bFGF levels in patients with cerebral infarction, and it was found that NBP could increase the VEGF and bFGF levels in peripheral blood of patients with cerebral infarction, and the increased VEGF and bFGF levels played important roles in promoting the regenerations of peripheral vessels and nerve cells in cerebral infarction, which could further improve the rehabilitation of patients with cerebral infarction.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Rosell-Novel A, Montaner J, Alvarez-Sabin J. [Angiogenesis in human cerebral ischemia]. Rev Neurol 2004; 38: 1076-1082.
2) Fu FY, Chen BY, Chen LL, Zhang FL, Luo YK, Jun F. Improvement of the survival and therapeutic effects of implanted mesenchymal stem cells in a rat model of coronary microembolization by rosuvastatin treatment. Eur Rev Med Pharmacol Sci 2016; 20: 2368-2381.
3) Marti HJ, Bernaudin M, Bellail A, Schoch H, Euler M, Petit E, Risau W. Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. Am J Pathol 2000; 156: 965-976.
4) Jeong CH, Kim SM, Lim JY, Ryu CH, Jun JA, Jeun SS. Mesenchymal stem cells expressing brain-derived neurotrophic factor enhance endogenous neurogenesis in an ischemic stroke model. Biomed Res Int 2014; 2014: 129145.
5) Oliveira SL, Pillat MM, Cheffer A, Lameu C, Schwindt TT, Ulrich H. Functions of neurotrophins and growth factors in neurogenesis and brain repair. Cytometry A 2013; 83: 76-89.
6) White RE, Rao M, Gensel JC, McTigue DM, Kaspar BK, Jakeman LB. Transforming growth factor alpha transforms astrocytes to a growth-supporti-

ve phenotype after spinal cord injury. J Neurosci 2011; 31: 15173-15187.

7) Park JW, Kang YD, Kim JS, Lee JH, Kim HW. 3D microenvironment of collagen hydrogel enhances the release of neurotrophic factors from human umbilical cord blood cells and stimulates the neurite outgrowth of human neural precursor cells. Biochem Biophys Res Commun 2014; 447: 400-406.
8) Greenberg DA, Jin K. Vascular endothelial growth factors (VEGFs) and stroke. Cell Mol Life Sci 2013; 70: 1753-1761.
9) Yao RQ, Zhang L, Wang W, Li L. Cornel iridoid glycoside promotes neurogenesis and angiogenesis and improves neurological function after focal cerebral ischemia in rats. Brain Res Bull 2009; 79: 69-76.
10) Wang YQ, Guo X, Qiu MH, Feng XY, Sun FY. VEGF overexpression enhances striatal neurogenesis in brain of adult rat after a transient middle cerebral artery occlusion. J Neurosci Res 2007; 85: 73-82.
11) Sims NR, Muyderman H. Mitochondria, oxidative metabolism and cell death in stroke. Biochim Biophys Acta 2010; 1802: 80-91.
12) Chen SQ, Cai Q, Shen YY, Cai XY, Lei HY. Combined use of NGF/BDNF/bFGF promotes proliferation and differentiation of neural stem cells in vitro. Int J Dev Neurosci 2014; 38: 74-78.
13) Stavri GT, Zachary IC, Baskerville PA, Martin JF, Erusalimsky JD. Basic fibroblast growth factor upregulates the expression of vascular endothelial growth factor in vascular smooth muscle cells. Synergistic interaction with hypoxia. Circulation 1995; 92: 11-14.
14) Tanaka R, Miyasaka Y, Yada K, Ohwada T, Kameya T. Basic fibroblast growth factor increases regional cerebral blood flow and reduces infarct size after experimental ischemia in a rat model. Stroke 1995; 26: 2154-2158, 2158-2159.
15) Li J, Li Y, Ogle M, Zhou X, Song M, Yu SP, Wei L. DL-3-n-butylphthalide prevents neuronal cell death after focal cerebral ischemia in mice via the JNK pathway. Brain Res 2010; 1359: 216-226.
16) Zhang T, Jia W, Sun X. 3-N-Butylphthalide (NBP) reduces apoptosis and enhances vascular endothelial growth factor (VEGF) up-regulation in diabetic rats. Neurol Res 2010; 32: 390-396.
17) Huang JZ, Chen YZ, Su M, Zheng HF, Yang YP, Chen J, Liu CF. Dl-3-n-butylphthalide prevents oxidative damage and reduces mitochondrial dysfunction in an MPP(+)-induced cellular model of Parkinson's disease. Neurosci Lett 2010; 475: 89-94.