Original article

Effects of mouse nerve growth factor in treating cerebral injury in acute period caused by cerebral hemorrhage

Yang Sun, Xiaobo Zhu, Junling Hou, Kun Hou, Weiwei Jin *

Department of Neurosurgery, No.1 Hospital Branch 2 of Jilin University, Jilin, Changchun, China

A B S T R A C T

Objective: To explore the clinical effects of mouse Nerve Growth Factor (NGF) in treating cerebral injury in acute period caused by cerebral hemorrhage, observe its influences on Natriuretic Peptide (BNP) and NF-kB Level and evaluate its safety and efficiency.
Methods: 96 cases with acute cerebral hemorrhage from January 2016 to January 2017 in our hospital were recruited as this study, they were randomly divided into the control group and the observation group, each 48 cases. The observation group were given NGF on the treatment of the control group. NIHSS, BI score, adverse reactions records were compared in two groups before and after treatment. The clinical effective rate were evaluated. Then BNP and NF-KB Level of patients in two groups before and after treatment were detected by using ELISA.
Results: There were no significant differences in two groups before treatment with respect to NIHSS and BI score ($P > 0.05$). After treatment, NIHSS score in the observation group significantly lower than the control group. BI score in the observation group significantly higher than the control group, differences had obvious significance ($P < 0.05$). The total effective rate in the observation group was 93.75%. The control group was 70.83%. Clinical effective rate of patients in the observation group significantly better than the control group ($P < 0.05$). There were no significant differences of patients in two groups before treatment with respect to BNP and NF-kB Level ($P > 0.05$). BNP and NF-kB Level decreased with different levels in two groups after treatment, and the observation group lower than the control group at the same time ($P < 0.05$).
Conclusion: NGF is benefit for relieving neurological function injury of patients with acute cerebral hemorrhage in acute period, improving living ability of patients. Patients have good tolerance and no adverse reactions. NGF can lower BNP and NF-kB Level. It has a certain function of inhibiting inflammatory injury caused by cerebral hemorrhage, thus protecting neuron. It is worthy of clinical promotion.

1. Introduction

Cerebral hemorrhage is the common and severe acute cerebrovascular diseases with high incidence rate, disability rate and death rate. It has become the leading cause for people's health. According to statistics, incidence rate of cerebral hemorrhage is about 10 to 30 cases in 10 thousand. The death rate up to 30% to 50% (Jiang et al.,). By far, conservative treatment of internal medicine in treating cerebral hemorrhage can absorb cerebral hematoma. However, this hematoma oppresses the adjacent cerebral hematoma, which will cause ischemia, apoptosis and necrosis. It is difficult to recovery, which cause cerebral neurological function recovery a difficulty. It significantly increases disability after cerebral hemorrhage. Fig. 1.Fig. 2.Fig. 3.

The occupied effect of cerebral hematoma in acute period of acute cerebral hemorrhage stimulates cerebral tissue to release vasoactive substances, direct or indirectly induce cerebral tissue injury, thus leading to a series of inflammatory reactions, cell apoptosis and neurological function impairment (Al-Mufti et al., 2017). NGF (Pan et al., 2010) is a kind of regulator factor for neurological cell growth. It has an important effect in maintaining sympathic and sensory neuron, nourishing neuron, restoring after neurological injury and promoting enation. After cerebral hemorrhage

* Corresponding author.
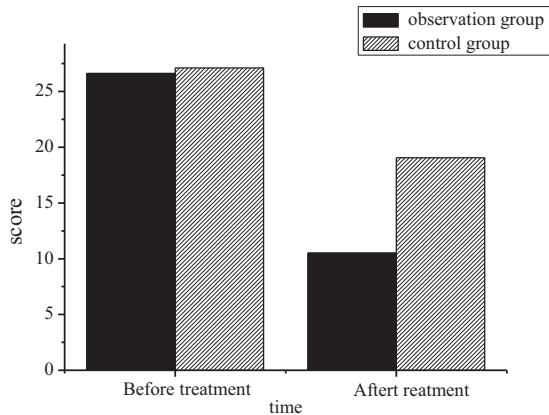E-mail address: jinweiweineuro@126.com (W. Jin).

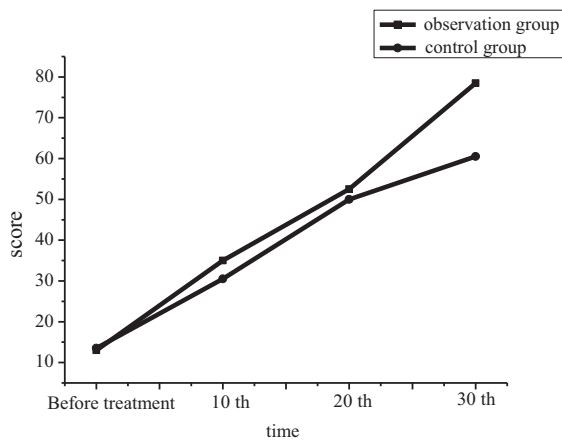**Fig. 1.** Comparison of neurological impairment scores between the two groups.

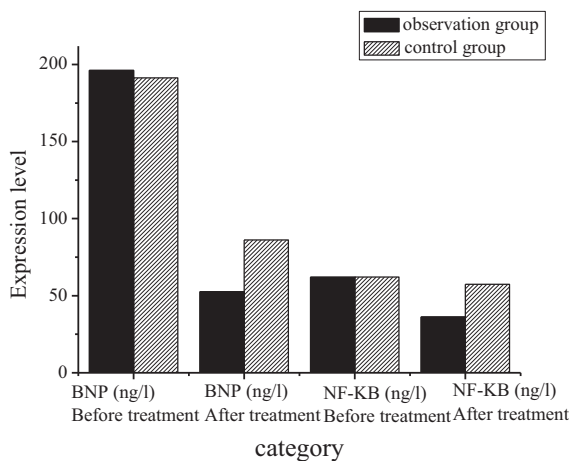**Fig. 2.** Comparison of BI scores between the two groups.

Fig. 3. Changes of BNP and NF-kB levels in two groups before and after treatment.

(Xu, 2016), NGF can play antagonistic cell toxin by entering into central nervous system, promote nerve recovery and protect brain. Therefore, in this study, nerve factors are used to treat acute cerebral hemorrhage, and experimental group and control group are set up to study the therapeutic effect and mechanism of nerve factors on cerebral hemorrhage. After experimental study, it is found that nerve factors as a new type of neuroprotection, nutritional regenerants can effectively treat cerebral hemorrhage, reduce the

level of BNP and NF-kB in patients. This study can provide important theoretical basis and guidance direction for clinical treatment of cerebral hemorrhage injury, which has great significance and value for clinical treatment of cerebral hemorrhage injury.

2. Materials and methods

2.1. General data

Cerebral hemorrhage patients from January 2016 to January 2017 in our hospital were recruited. All were given cerebral CT and MRI for the accurate diagnosis. They met the diagnostic criteria of acute cerebral hemorrhage issued by The Fourth National Cerebrovascular Conference (Chinese cerebral hemorrhage guide of neurology branch in Chinese Medical Association, 2014). Exclusive criteria concerned first, hemorrhage in cerebellum and brain stem; second, hematoma entered into subarachnoid space or hemorrhage in ventricle; patients who had mixed stoke; fourth, patients who had cerebral aneurysm or hemorrhage caused by cerebrovascular malformation and fracture; fifth, patients who had deep coma or brain herniation caused by severe conditions; sixth, patients who had hemorrhage over 30 ml and needed surgery; seventh, patients who had failure of heart, lung and kidney and blood coagulation disorder. This study had been approved by Ethics committee. All patients had signed informed consent form and were willing to accept relevant treatment and examination. Total 96 patients met inclusive patients. There were 48 cases in the observation group, consisting of 27 males and 21 females. Mean age was 76.21 ± 9.28 years. Hemorrhage location: there were 26 cases in basal ganglia, 16 cases in thalamus and 6 cases in brain lobe. There were 48 cases in the control group, consisting of 25 males and 23 females. Mean age was 77.48 ± 3.58.

2.2. Treatment methods

The observation group and the control group were given routine treatment methods (including blood pressure control, intracranial pressure lowering, bleeding stop, infection prevention etc.). Patients in the observation group given 20 μg NGF intramuscular injection on the basis of it (Xiamen Beida Road biotechnology limited company, SFDA approval number S20060052, specification: 18 μg one contingent) about once one day for ten days constantly totaling three courses.

2.3. Observation indexes

First, neurological function impairment of patients before and after treatment were evaluated by using NIHSS (National Institutes of Health Stroke Scale). The lower the score, more normal the neurological function of patients. Clinical treatment effects were judged by improvement rate of NIHSS score. Differences of NIHSS score before and after treatment were compared with NIHSS score before treatment. The decreased degree of neurological function impairment score of patients over 75% meant obvious effects; the decreased degree of neurological function impairment score of patients from 25% to 74% meant valid; the decreased degree of neurological function impairment score of patients below 25% meant invalid. Second, Activities of daily living were given evaluation score by BI. Third, BNP and NF-kB level in two groups before and after treatment were detected by using ELISA. All kits were bought from Shanghai enzyme-linked biotech limited company; fourth, adverse reactions of patients in two groups were recorded during medication process.

In this study, the main indicators of BI score included eating, bathing, dressing, toilet control, bed chair movement, walking, up

and down stairs, etc. Among them, there were four evaluation criteria, which were completely independent; partial help; great help; completely depend. Each criterion had different scoring options.

NIHSS score: the scale included the nervous system examination items that might appear in each major cerebral artery disease. Two items were selected from the Edin-burg-2 coma scale to supplement the mental state examination; 15 items were discussed with The National Institute of Neurological and Stroke researchers to increase sensory function, pupil reflex, and foot reflex, which were used in the treatment of acute stroke to evaluate the neurological deficit. The higher the score was, the more serious the nerve damage was. The classification was as follows: 0–1 for normal or nearly normal; 1–4 for mild stroke / minor stroke; 5–15 for moderate stroke; 15–20 for moderate-severe stroke; 21–42 for severe stroke.

2.4. Detection of ANP gene expression in cardiomyocytes by real-time fluorescence quantitative PCR

After trypsin digestion, the myocardial cells of the two groups were collected and the mRNA levels of ANP and BNP were detected by real-time fluorescence quantitative PCR. The total RNA in the cells was extracted according to the instructions of the total RNA extraction kit, and the cDNA was synthesized according to the instructions of the reverse transcription kit. The synthesized cDNA was used as the template, and the real-time fluorescence quantitative PCR analysis was carried out by IQ5TM Real-Time PCR Detection System of Bio-Rad company. Three independent experiments were repeated and $2^{-\Delta ct}$ was used to calculate the relative expression of ANP and BNP.

2.5. Statistical management

SPSS 22.0 was adopted to do data analysis. Measurement data were represented as mean ± SD. Among them, the number of examples is represented by "number" and percentage. Data before and after treatment were done with paired t test. Statistical significance was assumed at $P < 0.05$.

3. Results

3.1. Comparison of general information between two groups

The bleeding sites of the two groups were basal ganglia 28 cases, thalamus 14 cases, and lobus 6 cases. There was no significant difference between the two groups in age, gender, bleeding volume, and bleeding site (P > 0.05), as shown in Table 1:

3.2. Comparison of neurological function impairment of patients in two groups before and after treatment

Before treatment, there were no significant statistical differences in neurological function impairment of patients in two groups, P > 0.05. It had comparability. After three courses' treatment, NIHSS score decreased in two groups compared with before treatment, score improvement in the observation group

Table 2
Comparison of neurological function impairment of patients in two groups before and after treatment (±s, score).

Group	Number	Before treatment	Aftertreatment
The observation group	48	26.61 ± 1.28	10.51 ± 1.01
The control group	48	27.12 ± 1.68	19.06 ± 3.28
t	/	0.521	12.121
P	/	0.663	0.021

significantly better than before treatment. And compared with the control group, there were significant differences ($P < 0.05$). It is shown in the Table 2.

3.3. Evaluation of clinical effects of patients in two groups

There were 20 obvious effects cases, 25 valid cases and 3 invalid cases in the observation group. The total effective rate was 93.75%; there were 12 obvious effects cases, 22 valid cases and 14 invalid cases in the control and treatment group. The total effective rate was 70.83%. Clinical effective rate of patients in the treatment group significantly higher than the control group, there were statistical differences in two groups ($P < 0.05$). It is shown in the Table 3.

3.4. Comparison of BI score of patients in two groups

After 30 days' treatment, BI score of patients in two groups significantly increased compared with before treatment, differences had statistical significance ($P < 0.05$).BI score in the observation group significantly higher than the control group, differences had significant statistical meaning ($P < 0.05$). It is shown in the Table 4.

3.5. Comparison of adverse reactions of patients in two groups

In the process of clinical observation, there were 5 adverse reaction cases in the observation group, including 3 rash cases, 2 slight nausea and vomiting cases. There were 4 adverse reaction cases in the control group, including 2 rash cases, 2 slight nausea and vomiting cases. There were no dysfunctions in liver and kidney of patients in two groups during treatment process.

3.6. BNP and NF-kB level changes of patients before and after treatment in two groups

There were no statistical differences of patients before treatment in two groups with respect to BNP and NF-kB level ($P > 0.05$). BNP and NF-kB level decreased with different levels after treatment in two groups, and the observation group lower than the control group at the same time, differences had significant statistical meaning ($P < 0.05$). It is shown in the Table 5.

4. Discussion

Cerebral hemorrhage refers to primary hemorrhage in brain parenchyma generally caused by hypertension and cerebral arteriosclerosis. It is a kind of acute cerebrovascular disease with acute

Table 1
Basic information of subjects.

Group	Number	Gender		Mean age (years)	Estimated Hemorrhage volume (ml)	Hemorrhage location (cases)		
		Male	Female			Basal ganglia	Thalamus	Brain lobe
The observation group	48	27	21	76.21 ± 9.28	20 ± 6	30	10	8
The control group	48	25	23	77.48 ± 3.58	20 ± 5	29	12	7

Table 3
Comparison of clinical effects of patients in two groups.

Group	Number	Obvious effects	Valid	Invalid	The total effective rate (%)
The observation group	48	20 (41.67%)	25 (52.08%)	3(6.25%)	93.75*
The control group	48	12(25.00%)	22 (45.83%)	14 (29.17%)	70.83

Compared with the control group, *P = 0.021 < 0.05.

Table 4
Comparison of BI score of patients in two groups (score).

Tine	The observation group (48 cases)	The control group (48 cases)
Before treatment	13.0	13.5
The 10th day after treatment	35.0	30.5
The 20th day after treatment	52.5	50.0
The 30th day after treatment	78.5*#	60.5*

Compared with before treatment, *P < 0.05; Compared with the control group, #P < 0.05

onset, high incidence rate, high disability rate, high death rate. Chronic disability rate of patients with cerebral hemorrhage about 39.1% (Xue et al., 2017), which brings heavy economic burden to family and society. When cerebral injury with hemorrhage occurs, occupied effects of hematoma and direct injury on surrounding tissue caused by hematoma is an important pathological mechanism for cerebral injury. However, in recent years, it is found that (Long et al., 2017 Oct 20) (WenBo et al., 2017 Sep 26) secondary cerebra injury is more severe caused by edema, ishemia and inflammation in surrounding tissue of hematoma. Therefore, this study is to observe the treatment effects of NGF on patients with cerebral hemorrhage in acute period and its influences on inflammatory indexes.

NGF (Zhao et al., 2017 Oct) is nerve growth factor extracted from submandibular gland in mice. After cerebral hemorrhage, blood brain barrier has been destroyed. NGF can play cytotoxicity effects of antagonist doping amino acid, resist free radical, inhibit calcium overload, relieve apoptosis of neuron and promote neurological function recovery by entering into central nervous system. Studies of some scholars find that (Britti et al., 2017) full NGF in early period can protect cerebral neuron of patients with cerebral hemorrhage and help recovery of neurological function recovery. This study selects NIHSS and BI score as detection criteria. NGF has been applied in treating cerebral hemorrhage for three courses. Results show that NIHSS score of patients in the observation group after treatment significantly lower than the control group. Clinical effective rate up to 93.75%. BI score significantly higher than the control group. It shows NGF in treating cerebral hemorrhage, neurological function recovery obviously better than traditional treatment methods, which can significantly improve living ability of patients. In addition, in the process of clinical treatment, adverse reactions of patients in two groups have significant differences. Liver and kidney function of patients before and after treatment

belongs to the normal ranges, it shows NGF in treating patients with cerebral hemorrhage has a certain safety. BNP is a kind of polypeptide composed by 32 amino acid residues (Li et al., 2017). It belongs to neurohormone and has a certain vasoconstrictive effect against RAAS system. It also can inhibit plasminogen activator and release of antidiuretic hormone, promote urination, discharge sodium and dilate vessels. Studies find (Lei et al., 2017) that plasma BNP level increases of patients with acute cerebral hemorrhage, it has correlations with severity of cerebral injury. BNP level detection can be regarded as an important index for judging conditions and prognosis of cerebral hemorrhage patients. In this study, after herbs treatment, BNP level in the observation group significantly decreases, the effects significantly better than the control group. It shows NGF has good effects for lowering BNP level of patients. It can improve conditions of cerebral hemorrhage in acute period, thus promoting prognosis of patients.

NF-kB widely exists in nerve system (Ferrante et al., 2017). Secondary injury in surrounding tissue after cerebral hemorrhage has obvious correlations with constant activation and expression of NF-KB. Hematoma after cerebral hemorrhage induces intracranial hypertension and cerebral edema caused by oppression directly. Blood components out of vessels have inflammatory stimulation on cerebral tissue. It will cause cerebral injury. NF-kB plays a key role in the development of inflammation. NF-kB excitation can promote release and activation of multiple inflammatory medium (Sacchetti et al., 2017), form a complicated network, expand inflammatory reaction constantly and aggravate cerebral injury. This study finds that NF-kB level of cerebral hemorrhage in the early stage is relatively high, after treatment, NF-kB in peripheral blood decreases obviously. After NGF treatment, NF-kB level in the observation group significantly decreases, NF-kB level in the observation group significantly lower than the control group after treatment. It shows that NGF can effectively control inflammatory reactions after cerebral hemorrhage and inhibit further aggravation of cerebral injury.

Through this study, it has been proved that BNDF plays an important role in the protection and repair of cardiovascular system injury, such as hypoxia environment, ischemia–reperfusion and so on. It can effectively inhibit the ANP and BNP mRNA and protein expression level of patients, affect the activation of calcium channel, and reduce calcium overload. It can also antagonize the neurotoxicity of excitatory amino acids, and thus inhibit the apoptosis of neuron cells, so as to reduce or alleviate the secondary brain injury. It has a better curative effect and safety for the treatment of acute cerebral hemorrhage, promotes the recovery of damaged nerve function, and has a significant neuroprotective effect. The experimental results of this study are consistent with the experimental results of many researchers, and achieve the

Table 5
BNP and NF-kB level changes of patients before and after treatment in two groups.

Group	BNP (ng/l)		NF-KB (ng/l)	
	Before treatment	After treatment	Before treatment	After treatment
The observation group	196.2 ± 8.5	52.6 ± 4.1	61.94 ± 3.2	36.15 ± 2.1
The control group	191.3 ± 8.5	86.2 ± 5.2	62.07 ± 4.6	57.36 ± 4.4
t	0.072	2.214	0.146	6.678
P	>0.05	<0.05	>0.05	<0.05

expected results. While the difference is that the level of NF-kB in patients with cerebral hemorrhage is also used as an indicator and content of the study. However, the study did not take the important inflammatory mediators like hs-CRP, IL-8 and TNF-α as the key indicators of the study. In the future research, they can be the key content of the study.

References

Jiang, S., Fei, A., Peng, Y.a., Zhang, J., Lu, Y.-R., Wang, H.-R., Chen, M., Pan, S., Wang, X., ang et al.,. Predictors of Outcome and Hemorrhage in Patients Undergoing Endovascular Therapy with Solitaire Stent for Acute Ischemic Stroke. PLoS ONE 10 (12).

Al-Mufti, F., Amuluru, K., Smith, B., et al., 2017. Early merging Markers Of Delayed Cerebral Ischemia Following Aneurysmal Subarachnoid Hemorrhage. A Literature Review. World Neurosurg., 26

Pan XF, Wan S, Zhan RY. Study progress of inflammatory reactions in surrounding tissue of hematoma after cerebral hemorrhage. Journal of International Neurology and neurosurgery, 2010, 37 (3):263-267.

Xu, Y., 2016. Experimental study of influences of NGF on IL-6 and SOCS-3 expressions of rats after hemorrhage. Southwest medical university.

Chinese cerebral hemorrhage guide of neurology branch in Chinese Medical Association (2014). Chinese Journal of Neurology,2015, 48 (6):435-444.

Xue, Q.X., Zhao, B.H., Bian, Q., Huang, Y.J., 2017. The influences of Linjiap gouteng decoction combined with NGF injection on hemodynamics and inflammatory factors of acute cerebral hemorrhage patients. Modern Journal of Integrated Traditional Chinese and Western Medicine 26 (04), 393–395.

Long, X., Li, M., Li, L.X., et al., 2017 Oct 20. Butyrate promotes visceral hypersensitivity in an IBS-like model via enteric glial cell-derived nerve growth factor. Neurogastroenterol Motil.

WenBo, W., Fei, Z., YiHeng, D., et al., 2017 Sep 26. Human Umbilical Cord Mesenchymal Stem Cells Overexpressing Nerve Growth Factor Ameliorate Diabetic Cystopathy in Rats. Neurochem Res.

Zhao, G., Li, D., Ding, X., et al., 2017 Oct. Nerve growth factor pretreatment inhibits lidocaine-induced myelin damage via increasing BDNF expression and inhibiting p38 mitogen activation in the rat spinal cord. Mol Med Rep. 16 (4), 4678–4684.

Britti, D., Crupi, R., Impellizzeri, D., et al., 2017 Aug 2. A novel composite formulation of palmitoylethanolamide and quercetin decreases inflammation and relieves pain in inflammatory and osteoarthritic pain models. BMC Vet Res. 13 (1), 229.

Li, R., Wu, Y., Zou, S., et al., 2017 Nov. NGF Attenuates High Glucose-Induced ER Stress, Preventing Schwann Cell Apoptosis by Activating the PI3K/Akt/GSK3β and ERK1/2 Pathways. Neurochem Res. 42 (11), 3005–3018.

Lei, J., Feng, F., Duan, Y., et al., 2017 Sep. Intranasal nerve growth factor attenuating the seizure onset via p75R/Caspase pathway in the experimental epilepsy. Brain Res Bull. 134, 79–84.

Ferrante, C., Recinella, L., Locatelli, M., et al., 2017 Aug. Protective Effects Induced by Microwave-Assisted Aqueous Harpagophytum Extract on Rat Cortex Synaptosomes Challenged with Amyloid β-Peptide. Phytother Res. 31 (8), 1257–1264.

Sacchetti, M., Mantelli, F., Rocco, M.L., et al., 2017 Jul. Recombinant Human Nerve Growth Factor Treatment Promotes Photoreceptor Survival in the Retinas of Rats with Retinitis Pigmentosa. Curr Eye Res. 42 (7), 1064–1068.