

A clinical study on the effects of recombinant human colony stimulating factor on the expression of Bcl-2 in serum of patients with basal ganglia hemorrhage and its clinical significance

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Abstract. – **OBJECTIVE:** We investigated the effects of the colony-stimulating factor (CSF-1) on Bcl-2 expression in serums of patients with basal ganglia hemorrhage and subsequently, its clinical significance.

PATIENTS AND METHODS: The expression levels of Bcl-2 in serums of patients with basal ganglia hemorrhage were analyzed, and the effects of the CSF-1 on Bcl-2 expression were observed. Samples of peripheral blood were taken from 120 patients with basal ganglia hemorrhage admitted to the Neurology Department and 120 healthy people undergoing a physical examination at Xiangyang Central Hospital between May 2013 to December 2014. The detection of Bcl-2 levels in serums of patients was performed using the ELISA method, and patients were divided into two groups, the colony-stimulating factor (CSF-1) group and the control group. The CSF-1 group was treated with recombinant human granulocyte colony-stimulating factor after routine treatment, while the control group was treated only with routine treatment. The two groups of patients were followed up for observation of treatment effects.

RESULTS: Before treatment, serum Bcl-2 levels in both the CSF-1 and control group showed no significant differences; however, their levels were significantly higher than those of the healthy cohort ($p<0.05$). After treatment, serum Bcl-2 levels of the CSF-1 group were significantly higher than those of the control group ($p<0.05$). However, compared to the healthy control group, the levels remained significantly higher and the differences were statistically significant ($p<0.05$). When compared to the recovering conditions of patients in the CSF-1 group and the control group, we found that the average hospitalization time and occurrences of com-

plications in the CSF-1 group were significantly less than those in the control group ($p<0.05$).

CONCLUSIONS: CSF-1 is clinically effective in improving the serum Bcl-2 levels after a basal ganglia hemorrhage, and it can be used as adjuvant therapy in the treatment of basal ganglia hemorrhage.

Key Words:

Basal ganglia hemorrhage, Colony stimulating factor, Bcl-2.

Introduction

In recent years, with the improvement of the dietary structure, the morbidity associated with basal ganglia hemorrhage has been increasing year after year^{1,2}. Many researchers have studied and found that after a basal ganglia hemorrhage and early hypoxia-ischemia of neuronal cells, multiple stage cascade reactions were activated, whose occurrence and development were closely related to the release of a lot of inflammatory factors and inflammatory cells³. Moreover, hypoxia-ischemia generated a large amount of oxyradicals, which could further increase the apoptosis of nerve cells⁴. As an inhibitor of the apoptosis gene, Bcl-2 is located on the human chromosome 18q21⁵. It is mostly expressed in stem cells of special human tissues, including skin basal collagen cells and cells at the bottom of small intestinal crypts. Through the inhibition of cell apoptosis, the cell types mentioned above will have sufficient

time to transform from stem cells into well-differentiated cells⁶. Bcl-2 is closely related to the occurrence and development of lymphoma, rectal cancer, breast cancer, cervical cancer, thyroid cancer and various nervous system tumors as well as patients' prognosis^{7,8}. As one of the main cellular factors in the regulation of granulocytes in bone marrow hematopoiesis, recombinant colony stimulating factor can be selectively used for granulocyte hemopoietic progenitor cells to promote their proliferation and differentiation and to increase the function of undifferentiated granulocyte cells. It plays a protecting role in the apoptosis of tissue cells⁹ and is used for the treatment of hematological diseases. In recent years, it was found that recombinant colony-stimulating factor also played a certain role in the tumor, metabolism and other diseases¹⁰⁻¹². In this study the effects of recombinant colony stimulating factor on Bcl-2 levels of patients with basal ganglia hemorrhage and its mechanism of action were further studied to provide theoretical support for its establishment as a therapeutic treatment.

Patients and Methods

Experiment Reagents

ELISA kit (Elisa Biotech Co., Ltd., Shanghai, China) and 0.9% stroke-physiological saline solution (Otsuka Pharmaceutical, Tokyo, Japan) were obtained commercially.

Experimental Instruments

Ultra micro ultraviolet-visible spectrophotometer (Shanghai Metash Instruments Co., Ltd., Shanghai, China), low temperature table centrifuge (Shanghai Tocan Bio-Technology Co., Ltd., Shanghai, China), micropipettes with various ranges (Eppendorf, Hamburg, Germany), small-sized table centrifuge (Sigma-Aldrich, St. Louis, MO, USA), electronic balance (Hangzhou Wantai Balance Instrument Co., Ltd., Hangzhou, China), Real-time fluorescent quantitative PCR (ABI, Foster City, CA, USA), -80°C low temperature refrigerator (Sanyo, Tokyo, Japan), automatic double pure water distillatory (Beijing Jolyc Technology Co., Ltd., Beijing, China), glass homogenizer (Shanghai Charm Instrument Technology Co., Ltd., Shanghai, China) and vertical electrophoresis tank (Bio-Rad, Hercules, CA, USA) were obtained commercially.

Methods

Enzyme-linked immunosorbent assay¹⁰⁻¹¹

(1) Plasma pretreatment was conducted as follows: EDTA (Yangtze Pharm. Co. Ltd., Taizhou, China) or heparin (Yangtze Pharm. Co. Ltd., Taizhou, China) could be selected as an anticoagulant. Within 30 min after being collected, samples were treated for 30 min under the conditions of 2-8°C and 2000 r/min or stored at -20°C or -80°C. Samples were stored at either -20°C or -80°C, paying attention to avoid repeated freezing-thawing treatment. (2) The battens required for the experiment were placed evenly at room temperature for 20 min. They were taken out of aluminum foil bag and then rest battens were sealed with valve bag and stored at 4°C. (3) Standard substance holes and sample holes were set and 50 µL of standard substance with different concentrations was added to standard substance holes. (4) A 50 µL sample to be tested was added into the sample holes, while no treatment was done for blank holes. (5) 100 µL detection antibody marked with HRP (horseradish peroxidase) was added into various standard substance holes and sample holes. The reaction holes were sealed with microplate sealers and treated in incubator or water bath kettle at 37°C for 60min. No treatment was conducted for blank holes. (6) The liquid was discarded and patted dry with absorbent paper. 350 µL of scrubbing solution was added into all the holes. After being placed for 1 min, scrubbing solution was removed and patted dry with absorbent paper. The operation was repeated 4-5 times. (7) All holes were added with 50 µL A and 50 µL B and were stored in the dark at 37°C for 15 min. (8) 50 µL stop buffer was added into all holes and its absorbance (OD value) was measured at the wave length of 450 nm within 15 min. (9) The OD values of blank holes were removed from the OD values of all standard substances and samples. The horizontal axis was set as the concentration and the vertical axis was set as the light absorption value. The relevant software was used to draw the standard curve as well as to calculate the index level to be tested.

The serum concentrations of indexes to be tested were measured through the ELISA principle. Moreover, the operation was conducted strictly according to the instructions marked on kit. Before the experiment, all reagents were mixed well. A large number of bubbles were avoided by all means so as to avoid deviation of sample adding.

Recombinant Colony Stimulating Factor Injection

The course of treatment was 3 weeks. The injection dose was 300 g/m, once a day, and was administered as a subcutaneous injection.

Statistical Analysis

Statistical treatment and analysis were conducted by applying the SPSS19.0 software (Version X; IBM, Armonk, NY, USA). The ANOVA test and X^2 test was applied for normal distribution data. Fisher's exact test was applied for a four-layer table data that failed to meet conditions. The t-test or X^2 test was applied for the comparison between skewed distribution data. $p < 0.05$ was considered to be statistically significant.

Results

Comparison of General Clinical Data Between the two Groups of Patients

We conducted sorting and statistics for the general clinical indexes and clinical manifestations of the enrolled patients. The baseline data of patients in the two groups had no significant statistical differences ($p > 0.05$), as shown in Table I.

Expression of Bcl-2 in the Serum of Patients with Basal Ganglia Hemorrhage and Healthy Control

To further study the expression levels of the Bcl-2 protein in the serum of patients with basal ganglia hemorrhage and healthy controls, we used the ELISA method to conduct detection. The results were shown in Table II and bar chart was shown in Figure 1. The expression quantity of Bcl-2 protein in serum of patients with basal ganglia hemorrhage was significantly higher than that in healthy controls without basal ganglia hemorrhage; differences were statistically significant ($p < 0.01$). Bcl-2 and OD value in patients had a linear relation, $y = 483.51x - 63.126$; $R^2 = 0.9731$, as shown in Figure 2.

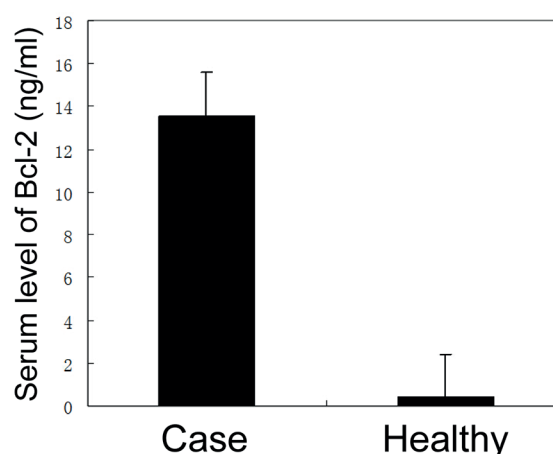


Figure 1. Compared with healthy group, Bcl-2 level in serum of patients in case group increased obviously and the difference had statistical significance ($p < 0.05$).

Effects of Colony Stimulating Factor on the Expression levels of Bcl-2 in Serum of Patients with Basal Ganglia Hemorrhage

We conducted further treatment for patients in both groups. Recombinant colony stimulating factor adjuvant therapy was applied, with the treatment course of 3 weeks. The results showed that among patients in the CSF-1 group, the Bcl-2 levels were significantly higher than that before treatment. Compared to the control group, differences were statistical significance ($p < 0.05$), as shown in Table III and Figure 3.

Comparison of Clinical Prognosis of Patients in the two Groups

We studied the clinical prognosis of patients in both groups and compared the average stay in hospital time and complications of patients in both groups. The statistical results showed that the average stay in hospital time of patients in the CSF-1 group was significantly less than that of control group. Moreover, the occurrence rate of complications within the one-year follow-up

Table I. General clinical indexes of patients in two groups (U/L).

	Age (years old)	Disease course (years)	BMI (kg/m ²)
Case group	35.3±5.5	1.33±0.44	18.8±1.2
Control group	32.8±4.7	1.27±0.89	19.27±0.87
T value	0.98	1.22	0.87
p-value	>0.05	>0.05	>0.05

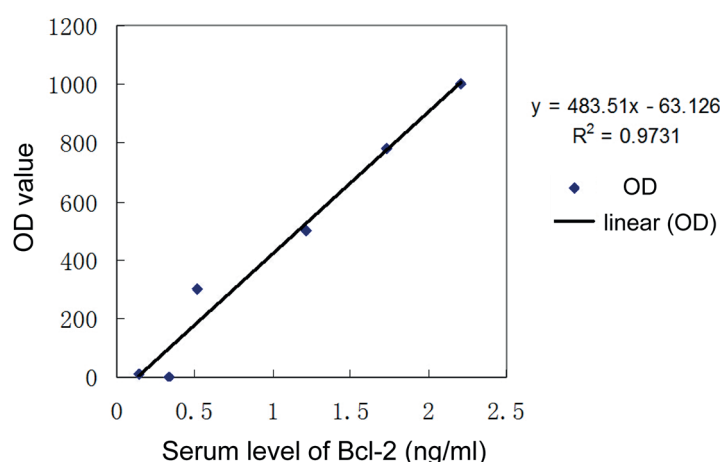


Figure 2. Bcl-2 and OD value in patients had a linear relation, $y = 483.51x - 63.126$; $R^2 = 0.9731$.

was decreased significantly and the difference was statistically significant ($p < 0.05$), as shown in Table IV and Table V.

Discussion

As the most common pathogenesis of non-traumatic intracranial hemorrhage, hypertensive cerebral hemorrhage is hypertension accompanied by lesions of small brain arteries and is caused by arteriorrhesis due to the sudden rise of blood pressure. However, among patients with basal ganglia hemorrhage, putamen and thalamus were the two most common parts of hypertensive ce-

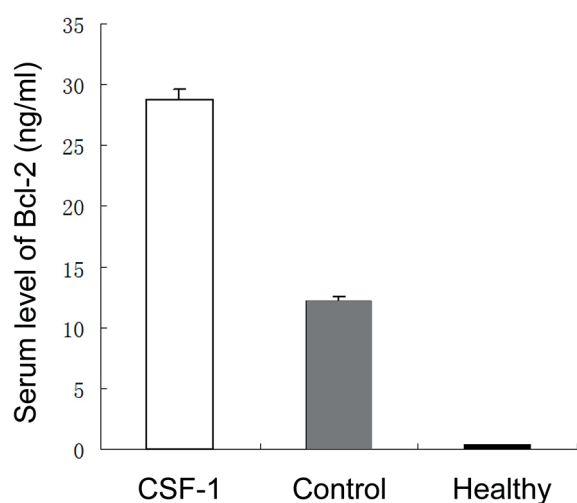


Figure 3. The Bcl-2 level in serum of patients in CSF-1 group was obvious higher than that of control group and the difference had statistical significance ($p < 0.05$).

Table II. Bcl-2 level in serum of patients ($\bar{x} \pm s$).

Group	Cases	Bcl-2ng/ml
Case group	120	$13.57 \pm 11.64^*$
Healthy control group	120	0.39 ± 0.4

Note: Compared with control group, $^*p < 0.01$.

Table III. Bcl-2 level in serum of patients after treatment ($\bar{x} \pm s$).

Group	Cases	Bcl-2 ng/ml
CSF-1 group	60	$28.7 \pm 12.5^{a,b}$
Control group	60	12.2 ± 9.7
Healthy control	120	0.39 ± 0.4

Note: Compared with control group, $^a p < 0.01$; compared with healthy control group, $^b p < 0.01$.

rebral hemorrhage¹³. Patients with basal ganglia hemorrhage have different clinical manifestations and early treatment of hemiplegia for patients have important clinical significance for the prevention and treatment of complications¹⁴. Studies¹⁵⁻¹⁹ have shown that the inhibiting effects of Bcl-2 on cell apoptosis were characterized by the following three points: 1. Channel proteins were formed to inhibit the release of mitochondrial apoptotic protein through permeability transition of the cell membrane and finally to inhibit cell apoptosis; 2. It improved the anti-oxidative effects of body cells and removed oxygen free radicals to inhibit cell apoptosis; 3. It generated a retardation effect on the transmembrane flowing of calcium ions and inhibited cell apoptosis

Table IV. Comparison of clinical prognosis of the two groups after operation.

Group	Cases	Stay in hospital time
CSF-1 group	60	8.2±2.7
Control group	60	12.6±9.5

through the regulation of the concentration of calcium ions in cells. Through our studies, we have found that the expression levels of Bcl-2 protein in serum of patients with basal ganglia hemorrhage were significantly higher than that in the serum of healthy controls without the occurrence of basal ganglia hemorrhage; differences were statistically significant ($p < 0.01$). Bcl-2 and OD value in patients had a linear relation, $y = 483.51x - 63.126$; $R^2 = 0.9731$. In literature, it has been reported that Bcl-2 is an inhibitor of the apoptosis gene¹¹. It is mostly expressed in stem cells in special tissues of the human body and it could ensure that various types of cells have sufficient time to finish the conversion from stem cells to well-differentiated cells through the inhibition of cell apoptosis¹². We observe that among patients with basal ganglia hemorrhage, hypoxia-ischemia and a series of subsequent cascade reactions could cause apoptosis of neuronal cells. Therefore, through the expression of Bcl-2 with the effects of feedback regulation, the Bcl-2 levels in serum of patients with basal ganglia hemorrhage were significantly higher than those of healthy controls. Moreover, we used recombinant colony stimulating factor injection in the hopes of treating patients with basal ganglia hemorrhage from the perspective of inhibiting apoptosis of neuronal cells. We found that, after using recombinant colony stimulating factor as an addition, the Bcl-2 levels in serum significantly increased ($p < 0.01$), compared to the control group. With regards to this phenomenon, although there were relevant reports to support that the human colony-stimulating factor could increase the levels of Bcl-2, the protective effects of colony-stimulating factor on cell apoptosis and the effects of promoting cell proliferation have been widely recognized. For instance, among patients with blood diseases, the utilization of the human colony stimulating factor could significantly increase granulocyte hemopoietic progenitor cells, promote their proliferation and differentiation, and increase the function of granulocytes, finally leading to undifferentiated cells²⁰⁻²². We held that CSF-1 might have no

Table V. Comparison of complications of the two groups.

Group	Cases	Occurred	Not occurred
CSF-1 group	60	3	57
Control group	60	18	42
X2 value	-		31.47
p-value	-		0.002

direct stimulating effects on Bcl-2 and it plays a part in various signaling pathways and cascade reactions of inflammatory factors. However, this conclusion needs to be further proven by animal experiments.

Conclusions

We hold that CSF-1 has excellent clinical effects on improving Bcl-2 levels in serum after basal ganglia hemorrhage and it could be used as auxiliary treatment drug after basal ganglia hemorrhage.

Conflict of interest

The authors declare no conflicts of interest.

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