Transplantation of Neural Stem Cells and Peripheral Blood Endothelial Progenitor Cells in the Treatment of Ischemic Cerebrovascular Diseases

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Abstract: Objective: To investigate the effect of transplantation of neural stem cells and vascular endothelial progenitor cells on ischemic cerebrovascular diseases. Methods: 120 SD rats were randomly divided into observation group and control group. In the observation group (n = 60), the cerebral ischemia model was established by suture-occluded method in SD rats, and neural stem cells and peripheral blood endothelial progenitor cells were transplanted. The control group was treated with conventional drugs (n = 60). The clinical effects, hemorheology and serum inflammatory factors before and after treatment were compared between the two groups. Results: ①After transplantation, the clinical effect of the observation group was higher than that of the control group ($P < 0.05$); ②After treatment, the whole blood viscosity, plasma viscosity, fibrinogen and hematocrit of the two groups decreased ($P < 0.05$), and the observation group was lower than that of the control group ($P < 0.05$); ③Serum homocysteine (HCY), interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor -α(TNF-α)and the level of neuron specific enolase (NSE) after treatment in the observation group was lower than that in the control group ($P < 0.05$). Conclusion: Transplantation of neural stem cells and vascular endothelial progenitor cells is effective in the treatment of ischemic cerebrovascular diseases. It can effectively regulate serum inflammatory factors and improve hemorheology ($P < 0.05$), which has significant curative effect on ischemic cerebrovascular diseases.

1. Introduction

The role of neural stem cells and endothelial progenitor cells transplantation in the treatment of ischemic cerebrovascular diseases has been clearly identified. But in clinical practice, neural stem cells and endothelial progenitor cells transplantation is still in the observation stage. The cell transplantation of SD rats is helpful to the treatment of ischemic cerebrovascular diseases. The incidence rate of ischemic cerebrovascular disease is increasing year by year. It has a high probability of cerebral infarction and stroke, and has become one of the diseases with high mortality and disability rate in cardiovascular and cerebrovascular diseases in China. Due to the very limited self-healing ability of nerve cells in the brain after injury, the effect of routine treatment is not obvious [1]. It is found that good results can be achieved through the transplantation of neural stem cells and peripheral endothelial progenitor cells. This experiment is reported as follows.

2. Materials and Methods

2.1 Materials

①Experimental animals: 120 male SD rats, 12 weeks old, with a body weight of 250-300g. Before the experiment, they were raised in the animal experiment and SPF environment of our hospital. ②Reagent and instrument: DMEM / F12 medium (Zhejiang Xinglin Medical Technology Co., Ltd.); epidermal growth factor (Shanghai Beihai Biotechnology Co., Ltd.); B27 (Shanghai

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Fuxing Biotechnology Co., Ltd.); 5-BrdU monoclonal antibody (Beijing Huijie Biotechnology Co., Ltd.); microinjector (Beijing Hongyuan Medical Technology Co., Ltd.); carbon dioxide incubator (Shanghai Huifang Biotechnology Co., Ltd.); aseptic workbench (Shanghai Yuanhua Biotechnology Co., Ltd.); centrifuge (Ningbo aibor Biotechnology Co., Ltd.); stereo locator (Shanghai Zhenhua Biological Instrument Equipment Co., Ltd.).

2.2 Experimental Method

①Experimental grouping: 120 SD rats were randomly divided into two groups with 60 rats in each group: observation group and control group. The observation group (n = 60) established the cerebral ischemia model by suture-occluded method, and was treated by transplantation of neural stem cells and peripheral blood endothelial progenitor cells. The control group was treated with conventional drugs (n = 60). ②Cell culture: 1) Neural stem cell culture [3]: After anesthesia, the hippocampal tissue of newborn SD rats within 1 day was extracted and blown manually by cutting. After filtration, it was centrifuged for 5 minutes, and the supernatant was removed. It was carried out in DMEM / F12 medium, including epidermal growth factor and B27. It was placed in CO2 incubator for moderate culture. The solution needed to be changed every 2 days, with an average of 48 hours / 1 time. After continuous culture for 7 days, the subsequent experiments were carried out. Neural stem cells were identified by immunohistochemical method and Nestin staining. 2) Culture of vascular endothelial progenitor cells: Take 15ml of SD rat arterial blood, after centrifugation for 5min, absorb the second layer of monocytes for processing, prepare cell suspension, inoculate in plastic petri dish, add DMEM / F12 medium, place it in CO2 incubator for effective and moderate culture, change the solution every 4 days, and subculture after continuous culture for 7days. The third generation cells were collected and the corresponding follow-up experiments were carried out. ③The co construction of vascular endothelial progenitor cells and neural stem cells is established. Now it is added to the complete neural stem cell culture medium (DMEM / F12 medium, epidermal growth factor, B27) and cultured continuously for 4-6 hours. After observing that all neural stem cells adhere to the wall, suck out the culture medium, spread it by coating a thin layer of extracellular matrix, and then inoculate the endothelial progenitor cells with the same concentration as above, add the complete neural stem cell culture medium. Within 24 hours, if it is observed that the inoculated endothelial progenitor cells adhere to the wall and become flat oval or irregular shape, take out the co-transplanted complex cells and store them for standby. ④ Preparation of ischemia-reperfusion rat model: The focal cerebral ischemia-reperfusion model was prepared by suture-occluded method. After the model was prepared, the animal was sober and scored for nerve defect. If the rat turned to the hemiplegic side during walking, the model was regarded as successful. The control group was treated with drugs without other treatment. ⑤Group intervention: Six hours after the successful preparation of the model, the observation group was intervened by neural stem cells and endothelial progenitor cells. The control group was treated with routine drug intervention.

2.3 Main Observation Indexes

① At different time points after transplantation, the clinical effect of the observation group was higher than that of the control group ($P < 0.05$); ② After treatment, the whole blood viscosity, plasma viscosity, fibrinogen and hematocrit of the two groups decreased ($P < 0.05$), and the observation group was lower than that of the control group ($P < 0.05$); ③ Serum homocysteine (HCY), interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor -α(TNF-α) and the level of neuron specific enolase (NSE) after treatment in the observation group were lower than those in the control group ($P < 0.05$).

2.4 Statistical Analysis

SPSS 24.0 was used for t-test statistical treatment. The data were significantly different and had statistical significance, which was represented by $P < 0.05$.

3. Results

3.1 Comparison of Clinical Effects after Treatment

From the results in Table 1, the effectiveness of the observation group was significantly higher than that of the control group, and from the treatment effect, 59 of 60 SD rats were effective, and only one case had no improvement. Compared with 30 effective rats in the control group, the transplantation of neural stem cells and vascular endothelial progenitor cells is more effective in the treatment of ischemic cerebrovascular diseases.

Table 1 Comparison of Clinical Effects between Two Groups [Cases (%)]

Group	Cases	Cure	Markedly Effective	Effective	Invalid	Deteriorate	Death
Group A(observation group)	60	40(66.6)	15(25.0）	4(6.6）	1(1.6）	0(0.0)	0(0.0)
Group B(control group)	60	15(25.0)	5(8.3）	10(16.6)	20(33.3）	4(6.66)	1(1.6)
P	-	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
t/x^2	-	13.1	10.3	9.3	11.2	8.6	5.4

3.2 Comparison of Whole Blood Viscosity, Plasma Viscosity, Fibrinogen and Hematocrit

From the results in Table 2, in the comparison of whole blood viscosity, plasma viscosity, fibrinogen and hematocrit, the treatment effect of the observation group is significantly better than that of the control group, which shows that the transplantation of neural stem cells and vascular endothelial progenitor cells has a positive effect on the change of blood flow in ischemic cerebrovascular diseases.

Table 2 Comparison of Whole Blood Viscosity, Plasma Viscosity, Fibrinogen and Hematocrit between the Two Groups after Treatment ($\overline{X}$ ±s)

Group Case		Whole blood viscosity Mpa·s	Plasma viscosity Mpa·s	Hematocrit (%)	Fibrinogen (g/L)	P value
Control Group 60	Before treatment	6.01±0.66	4.84±0.38	64.83± 2.65	4.85± 0.67	P<0.05
	After treatment	5.52± 0.68	3.82± 0.27	39.32± 2.51	3.34± 0.52	
Observation Group 60	Before treatment	6.04±0.67	4.86±0.41	64.89± 2.52	4.79± 0.55	P<0.05
	After treatment	3.59± 0.34	3.06± 0.33	32.04± 2.31	3.05± 0.32	

Note: Compared with that before treatment, $P < 0.05$; Compared with the control group after treatment, $P < 0.05$.

3.3 Comparison of Hcy,Il-6,Crp,Tnf-A,Serum Factors of Nse

From the results in Table 3, HCY, IL-6, CRP, TNF-α and serum factors of NSE in the observation group is significantly better than that of the control group, which shows that the transplantation of neural stem cells and vascular endothelial progenitor cells has a positive and effective effect on the internal indexes of ischemic cerebrovascular diseases.

Table 3 Serum Homocysteine (Hcy), Interleukin-6 (Il-6), C-Reactive Protein (Crp), Tumor Necrosis Factor -A(Tnf-A) and Neuron Specific Enolase (Nse) Level (g / l) in Two Groups

Group Case		HCY	IL-6	CRP	TNF	NSE
Control group 60	Before treatment	25.21±1.66	8.34±0.68	32.85± 1.17	2.74±0.68	16.57± 1.05
	After treatment	16.58± 1.21	5.44± 0.27	9.34± 0.26	0.84±0.68	6.58± 1.06
Observation group 60	Before treatment	26.14±1.56	8.44±0.51	32.57± 1.05	2.94±0.68	16.77± 1.06

	After treatment	13.29± 1.44	3.16± 0.13	5.80± 0.12	0.34±0.68	5.57± 0.15
P value	P<0.05					
T value	6.32					

Note: TNF -α: Tumor necrosis factor -α; IL-6: interleukin-6; IL-10: interleukin-10; Compared with that before treatment, $^{a}P < 0.05$; Compared with the control group after treatment, $^{b}P < 0.05$

4. Discussion

Neural stem cells are the fundamental driving force for the formation and development of the nervous system [2], which are mainly involved in the repair of nervous system injury and the renewal of normal cells after apoptosis. Transplantation into the damaged central nervous system through neural stem cell culture can effectively stimulate its own repair ability, so that the necrotic nerve cells can effectively secrete neurotrophic factors and return to reconstruct the neural pathway, so as to meet the purpose of treating diseases [3]. Endothelial progenitor cells are pluripotent stem cells, which widely exist in bone marrow and peripheral blood. They have a strong signal effect on injury, so that endothelial progenitor cells can gather at the site of vascular injury and form a large number of effective endothelial cells with intervention ability through active differentiation, so as to participate in vascular regeneration and repair [4]. At the same time, endothelial progenitor cells also have paracrine function, which can improve the living conditions of neural stem cells after transplantation to a certain extent, and is of great value in promoting the proliferation and survival of neural stem cells. From this point of view, the transplantation of neural stem cells and vascular endothelial progenitor cells can play a better role.

With the development of aging society in China, the incidence rate of cerebrovascular diseases has increased year by year. The proportion of ischemic cerebrovascular diseases is more than 70%. At present, the pathogenesis of ischemic cerebrovascular disease is not clear. It is generally recognized in academic and medical circles that it is related to hemorheological disorder and high expression of serum factors such as HCY. Through the experiment of SD rats, it is further proved that when cerebral ischemia occurs, its blood flow will be reduced, and the blood components will be abnormal, and finally local to overall hypoxic ischemia of brain tissue will be formed, and HCY, IL-6, CRP, TNF-α and the level of serum factors such as NSE are also closely related to the occurrence of ischemic cerebrovascular disease. At the same time, it has also become an important evaluation index of ischemic cerebrovascular disease. The co-implantation of vascular endothelial progenitor cells and neural stem cells is significantly effective for the improvement of ischemic encephalopathy.

In conclusion, the co-construction of vascular endothelial progenitor cells and neural stem cells has a significant effect on cerebral ischemic vascular disease, but it is also observed that drugs also have a certain therapeutic effect. Therefore, from the perspective of overall treatment, drug treatment should not be abandoned. Experiments show that vascular endothelial progenitor cells not only promote neovascularization through secreted factors, but also provide a good ecological environment for the survival of transplanted neural stem cells. The co-construction of the two really realizes the consistent therapeutic effect of vascular regeneration and neural cell synergy.

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