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Used CD133+ stem cells transplantation to treat erectile dysfunction (ED) by corpus cavernsum angiography for animal model.

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Abstract

This study reported the method of stem cells transplantation through corpus cavernosum angiography surgery, to treat the animal model erectile dysfunction (ED), which constructed by high fat food for eight month. The used formula was mixture of umbilical cord blood stem cells and peripheral blood stem cells from animal. It transplanted that mixture cells by corpus cavernosum angiography surgery technique. After the first phase of surgery, transplantated with CD133+ mixture stem cells via penis vein once. The ICP was an effect evaluation method of the erectile response. Taken ICP detection for them, once three months. There was a process of slowly rising of the scores. Furthermore, there were significant differences in blood citrulline level between before and after transplantation (F=5.36, P=0.004) by analysis of variance. Observed the animal condition of clinical symptoms and laboratory indexes for 6 months. 6 SD rats of ED were in good health, without abnormal reaction or exacerbations. That approach had certain curative effect. It might be a promising approach to repair penile vascular endothelial cells and improve citrulline andnitric oxide cycle.

Keywords: ED; CD133+, Cells transplantation, Corpus cavernosum angiography.

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Introduction

Erectile dysfunction (ED) is defined as the inability to attain and maintain an erection with sufficient rigidity to permit satisfactory sexual intercourse. Vascular diseases, which are correlated with smoking, aging, hyperlipidemia, diabetes and hypertension, are major causes of ED [1-3]. Injury to the cavernous nerves during pelvic surgery, such as radical prostatectomy, comprises an appreciable number of ED cases as well. Other causes of ED include direct trauma to the genitals, endocrine disorders, and fibrosis of the penile vasculature and corporal smooth muscle. The aetiological theme linking both vasculogenic and neurogenic ED is the loss of normal cellular function or apoptosis of the cells themselves. That dilemma would increase the economic burden of their families, especially in developing countries [4,5].

While oral pharmacotherapies, such as phosphodiesterase-5 inhibitors (PDE5is), have clear benefits, their functions are necessarily ephemeral, and treatment is relatively costly. Furthermore, they do not provide a cure, and a number of patients have tissue damage that is so extensive that the response to either oral or local pharmacotherapy is minimal [6-8]. Consequently, researchers have been investigating stem cells as a substitute therapeutic strategy method. Compared with other fields, the application of stem cell-based therapy for ED was relatively cure. The potentially curative nature of stem cells recently prompted a number of studies with large stem cell populations and strategies [9]. Currently, it appears to be one of the most studied potential future treatments for ED. Stem cells were multipotential cells had the ability of self-renewing. A small amount of stem cells could make effective role in diseases about vascular wall. Because peripheral blood stem cell transplantation was used to treatment with many disease, regardless cell transplant reaction, it was a bold attempt to treatment ED. On the other side, umbilical cord mesenchymal stem cells transplantation was used to therapy as extensive as peripheral blood stem cell. The CD133 is one unique molecular marker on cell-surface, being isolation and identification of stem cells. So it selected the CD133 positive stem cells to treat the ED model animals in this study.

The passageway of treatment was through corpus cavernosum angiography surgery on ED model animal. It collected and analyzed their performance data in this study. It also analyzed the changes of their blood citrulline and arginine level. This research was permitted by Animal Ethics Committee in Third Affiliated Hospital of Guangzhou Medical University. It took working experience from Peking University pediatric hematopoietic stem cell transplant center. In our previous studies, it observed the experiment outcome of bone marrow mesenchymal stem cells transplantation in growth of vascular endothelial cell. Our department has been exploring if umbilical cord mesenchymal stem cells transplantation could be attempted to therapy atrophy of endothelial.

Materials and Methods

Establishment of animal model

Hyperlipidemia could cause atherosclerosis, further cause impotence. It used fat diet method to induce the atherosclerosis to construct animal model of ED for SD rats. Experimental beagles were purchased from Southern Medical University laboratory animal center. They were at age of 4 weeks, weight of 210 to 212 g. It taken animal with temperature of 15 to 25°C and fed them with high fat diet, composed with 10% lard, 10% yolk and 2% cholesterol, which had been analysed by our department. After 8 weeks feeding, Arteriosclerosis animal model had been established. Then, 6 rats were diagnosed as erectile dysfunction (ED) by the Guangzhou Medical University.

Detected the Internal Cavernous pressure (ICP)

The ICP was an effect evaluation method of the erectile response. Apomorphine (APO, American, Sigma) was used subcutaneous injection through back of the neck, with dose of 5 mg/kg. Taken intraperitoneal anesthesia by chloral hydrate, Using a multi-channel electrophysiology instrument (purchased from Chengdu Instrument Factory) to detect the internal cavernous pressure of SD rats for 30 min. When they got the maximum degree of erection, it read the pressure value. And it made the baseline values in a state of unconsciousness. Calculated the rate of ICP to assess ED improvement dgree. The control group used physiological saline replace CD133+ transfected stem cells suspension for two SD rats.

Detecting by biochemistry

Amino Acid Analyzer (AAA) was used to detect the amino acids in blood and urine; gas chromatography-mass spectrometry (GC/MS) was used to detect the organic acids in urine. The two indicators, including citrulline below 10 mg/L in blood and orotic acid above 10 mmol/ml in urine, were monitored before and after treatment.

Separation of stem cells

Umbilical cord blood stem cells were collected from healthy new-born SD rats puerperal without bloodborne infectious diseases. According to national health standards (China) of umbilical cord blood collection guideline, collected 10.0 ml cord blood of normal embryo and separated 0.5 ml stem cell suspension with concentration of 2.2×10^7 stem cells by hydroxyethyl starch method (HSM) from that. As to peripheral blood stem cells, mobilized the peripheral blood stem cell by recombinant human granulocyte colony-stimulating factor (rhG-CSF, TeLeJin, Xiamen TeBao biological technology co., LTD) for 6 days by dose of 5 ug/kg/d, firstly. Secondly, collected 120 ml mononuclear cells from the donors by COBE 6.1 Spectra Version (U.S.A Genbro Company) at the sixth day. Thirdly, CD133+ peripheral blood stem cells were separated by cell sorting reagent (Germany Miltenyi Company) with 2.2×10^7 stem cells too, by flow cytometry. Prepared the pre-transplant CD133+ stem cells by 1:1 mixing ratio of stem cells from both sources.

Cell transplantation

6 ED rats had CD133+ mixture stem cells transplantation operation through corpus cavernosum angiography surgery technique. Throughout the operation process, taken the rats supine on the operating table, with local anesthesia by lidocaine. Taken the tourniquet on root of the penis; Injected 6 mg papaverine on one side of cavernosum. After 2 min later, taken off the tourniquet and observed it for 10 min. Under high-pressure syringe, 0.5 ml prepared CD133+ mixture stem cells suspension was smoothly injected into corpus cavernosum, with dropping speed at 10-18 ml/min. It had been keeping the high-pressure syringe under 0.2 kPa/ml/min. Then, it made compression hemostasis for 10 min, to prevent hematoma, after puncture needle injection.

The followed treatment with stem cells

After the first phase of surgery, transplantation with CD133+ mixture stem cells via penis vein performed by next 7 days. Without angiography, another 0.5 ml prepared stem cell suspension was smoothly injected into penis vein on its root.

Physical fitness surveillance

The ED rats were with stable vital signs after surgery, without tissue necrosis. Only one rat got fever after 6 hours transplantation. There were no secondary infection, no bleeding wound, and no abnormal reaction for transplantation. Among 6 rats, penis tissue lesion was detected by B ultrasonic wave, computed tomography (CT) and magnetic resonance imaging (MRI). None of neoplasm or tumor-like lesions was found in their penis for 6 months. By 6 months medical observation, 6 rats of ED were in good health, without abnormal reaction or exacerbations. Taken ICP detection for them, once every three months. There was a process of slowly rising of the scores.

Blood urea nitrogen

Although there was a short-time fast increase trend of blood urea nitrogen (BUN) levels after operation, it was decreased slowly to the normal level in one week, which was still less than 100 μmol/L and comprehensive symptoms slowly improved by 2 weeks later.

Level changes of citrulline and arginine

There were significant differences in blood citrulline level between before and after transplantation ($F=5.36$, $P=0.004$) by analysis of variance. The results of paired t-test demonstrated that the significant differences were existence between them continuing 6 months. It was detected that the level of arginine decreased after surgery, it continued to increase to normal in 2 weeks later. However, citrulline was at higher level of growth than arginine.

Discussion and Conclusions

In 6 rats blood ammonia level decreased evidently after stem cell transplantation, and it was always below 100 μmol/L, though there was a little rebound two weeks after transplantation. According to Wang Ping's research, the stem cells transplanted would be transplanted from blood vessel to adjacent cells through gap junctions in one week, and be scattered all over the liver and combine with liver cells in 4 weeks after transplantation. This conclusion was consistent with our study [10-14].

All 6 rats had CD133+ mixture stem cells transplantation operation by corpus cavernosum angiography surgery technique. Then CD133+ stem cells gradually migrated to replace or repair vascular endothelial cells, so that citrulline could be synthesized smoothly, which leads to long-term steady state of blood ammonia. Biochemical synthesis of citrulline including two pathways: citrulline is the products from ornithine and carbamyl-phosphate in the urea cycle; under the catalysis effect of nitric oxide enzyme, arginine is oxidized to N-hydroxyl-arginine, which is again oxidized to citrulline. It released nitric oxide during this process [14-18]. And nitric oxide can improve sexual function, resulting in smooth muscle relaxation.

The effect of citrulline and arginine increasing to normal level after a short time decreasing could make urea cycle improve with CD133+ stem cells transplantation. It could be inferred that the damage of endothelial cells conducted by some reason has been improved with blood ammonia decreasing. And the ED symptoms have been alleviated than before by analysis of ICP data. There were no adverse effects found during long-term observation of CD133+ stem cells transplant recipients. With regard to transplantation access, the method of corpus cavernous angiography surgery technique was effective and security, which had been long-term clinic practical application. It is worth pointing out that one rat received transplant, whose highest content of blood ammonia was only 9.5 umol/L, without any clinical symptoms of fatigue and spasm. We analyzed that might because transient obstruction of urinary tract. It had returned to normal by rechecking.

In summary, this study used CD133+ stem cells transplantation to therapy the ED model animal by corpus cavernosum angiography surgery. That method had certain curative effect. It might be a promising approach to repair penile vascular endothelial cells and improve citrulline andnitric oxide cycle. Assured its safety and effectively by 6 months observation. However, more research is needed, which could be more reasonable and scientific approach, including the effect of stem cells quantity, transplantation number and transplantation ways. On the other side, the Professor Liu Yanze from Peking University suggested the ED model animals would be treated with oral drugs for supply citrulline and arginine, for the purpose of making blood ammonia at high levels. We reconsidered these views, so that it should be used with caution in the clinical treatment.

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