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Original Article

Effect of granulocyte colony stimulating EPC on cardiac function and myocardial energy expenditure in patients with heart failure after myocardial infarction

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Abstract: Objective: To study the changes of cardiac function and myocardial energy expenditure following treatment with granulocyte colony stimulating factor (G-CSF) in patients with heart failure after myocardial infarction. Methods: Thirty-eight patients with heart failure after myocardial infarction were randomized into G-CSF treatment group and control group. All the patients received conventional treatment (medication and interventional therapy), and the patients in treatment group were given additional G-CSF (600 μg/day) for 7 consecutive days. The plasma level of brain-type natriuretic peptide (BNP) and the number of endothelial progenitor cells (EPC) in the peripheral blood were detected before and at 7 days and 4 months after the treatment. The cardiac functions (LVEF, FS, LVIDs, PWTs, EDV, SV, ET) was evaluated by ultrasonic imaging before and at 2 weeks and 4 months after the treatment. The MEE and circumferential end-systolic wall stress (cESS) were calculated by correlation formula. Results: The number of EPC was significantly higher in the treatment group than in the control group after the treatment especially at 7 days (P<0.01). In both groups, BNP level was lowered significantly after the treatment to recover the normal level (P<0.01). The cardiac functions and myocardial energy expenditure were improved in all the patients at 2 weeks and 4 months after the treatment, and the improvement was more obvious in the treatment group (P<0.05), especially in terms of the MEE and cESS was significantly lowered in the treatment group than in the control group after the treatment at 2 weeks (P<0.01), the LVEF and FS was significantly increased in the treatment group than in the control group after the treatment at 4 months (P<0.01). Conclusion: EPC mobilization by G-CSF can effectively improve the cardiac functions, lessen ventricular remodeling and reduce myocardial energy expenditure in patients with heart failure after myocardial infarction.

Keywords: Endothelial progenitor cells, granulocyte colony stimulating factor, myocardial infarction, heart failure, cardiac function, myocardial energy expenditure

Introduction

Both systole and diastole are an energy expenditure process. The myocardial energetic, ME is a subject to research on the relationship between myocardial energy, metabolism, aerobes and balancing of oxygen supply as well as cardiac functioning. It is an effective method to treat ischemic heart disease and inhibit progress of myocardial by researching metabolism of myocardial energy and conduct effective adjustment as well as improve myocardial energy expenditure level [1, 2]. In recent years, marrow stem cell subset-endothelial progenitor cells (EPC) has become a hot topic of researching on treatment of ischemic cardiovascular diseases. EPC participates not only in the angiogenesis of embryo process, but also in the process of post-natal angiogenesis and damaged endothelial repairing. It has provided new treatment thought for patients with myocardial infarction-MI by improving cardiac flood supplying, cardiac function and myocardial oxygen expenditure with acquaintance of EPC biological features and continuously further researches on MI treatment. This research aims at applying granulocyte colony stimulating factor (G-CSF) to mobilize EPC treatment on myocardial patients after myocardial infarction, analyzing effect of EPC on myocardial energy expenditure (MEE) and systole and diastole index of such patients as well as discussing the relation-

ship between improving myocardial energy expenditure level and myocardial resistance treatment.

Data and methods

Selection of clinical cases

Inclusion criteria: ① Under age 75. ② Has a medical history of definite myocardial infarction for at least three months. ③ A clinical manifestation of gradual cardiac failure and NYHA classification of cardiac function is II to IV level. ④ The left ventricle is expanded, its regional wall motion is abnormal and left ventricle ejection fraction (LVEF) under a resting state is lower than 45%, which is confirmed with the Color Doppler echocardiography.

Removal standard: Patients with a medical history of cardiogenic shock, severe arrhythmia, cardiogenic syncope, liver and renal insufficiency as well as a medical history of malignant tumor and blood system.

Clinical cases and grouping: Thirty-eight patients with myocardial infarction who have received treatment in PLA 303 Hospital from March to September in 2009 are gathered and they are divided into two groups randomly based on routine treatment, nineteen patients for each group. Their age is from forty-six to seventy-five and the average age is (60.89± 7.53). There are twenty-four males and fourteen females. Twenty-five patients suffer from hypertension; twenty patients have a clinical history of diabetes mellitus; twenty-one patients suffer from the hyperlipemia and sixteen patients have a hobby of smoking. The time of myocardial infarction is three months to two years and the average time is (11±8.46) months. Nine patients suffer from antero-septal myocardial infarction; ten are anterior myocardial infarctions, another ten are extensive myocardial infarctions, six are inferior myocardial infarctions and three are rear myocardial infarctions. Classification of NYHA cardiac function as II/III/IV level are ten/twenty-four and four respectively. The number of patients with single-vessel pathology indicated by coronary angiography is six; dual-vessel pathology is fourteen and pathology in more than three vessels is eighteen. The number of patients with affected vessels LAD/LCX/RCA is twenty-three/fourteen/eight respectively. All of them haven't received intervention treatment before. Patients in the two groups have basically balanced situations and they are comparative for their age, gender, complication, time and position of myocardial infarction as well as medical treatment are similar.

Methods

Therapy: The group is provided with the 600 μg/d mobilization agent G-CSF (Trade name: granulocyte colony stimulating factor produced by Hangzhou Jiuyuan Biological Gene Co., Ltd, whose specification is 150 μg/piece) for hypodermic injection based on routine treatment. They are injected for seven days continuously. All patients receives corresponding drug treatment based on clinical needs during their treatment and random visiting, including the right handling of Sodium Nitoprusside, diuretic, nitrate, anti-platelet medicines, ACEI or angiotensin II receptor antagonists (ARB), β receptor retardant, statins for Hyprtlipidemia, lowering blood pressure and blood sugar level.

Concentration determination of brain natriuretic peptide (BNP): Draw blood from the vein in the morning after overnight fasting seven days before and after the mobilization treatment and four months after the mobilization treatment using 100 g/L EDTA anti-coagulants added with aprotinin. The plasma is separated after centrifugation and BNP concentration of the plasma is determined with the enzyme linked immunosorbent assay (ELISA) method.

Method for determining EPC of peripheral blood [3]: Draw peripheral blood from the patients seven days before and after the mobilization treatment and four months after the mobilization treatment. Then separate, cultivate EPC of peripheral blood and determine its amount. Compare the difference between two groups before and after the treatment. Separate mononuclear cells from the peripheral vein of patients tested with the density gradient centrifugation method and analyze the adherent cells cultivated for seven days with the EBM22 medium. Add DiLDL into the adherent cells and fix it with 2% paraformaldehyde after being incubated for one hour. Then add FITC-UEA-I and invert it under the fluorescence microscope for observation after being incubated for one hour. Take in DiLDL in combination of FITC-UEA-I and the dual and positive

chromosome is EPC being separated. Cultivate the EPC in six pore plates and collect non-adherent cells (including EPC) after being incubated for two days. The adjustment density is 1×10^6 cell/mL and inoculate into the twenty-four pore plates and cultivate it for five days continuously. Count the number of clones in each pore of each sample.

Evaluation standard of clinical effect: According to classification standards of NYHA cardiac function, correction of cardiac failure or two levels' cardiac function improvement is regarded as excellent, one level's cardiac function improvement is regarded as effective and no cardiac function improvement or function deterioration is regarded as invalid.

Inspection of cardiac function and myocardial energy expenditure: conduct routine ultrasonic cardiogram inspection with the GE Logig 7 Color Doppler echocardiography apparatus at the period of hospitalization, two weeks and four months after the treatment to determine the left ventricular long axis section, the left ventricular short axis section and cardiac apex four-chamber section. Measurement index of the left ventricular structure is as follow: LVEF, FS, LVIDs, EDV, PWTs, SV and ET. Take the average value of three continuous cardiac cycles for each index. After the ultrasonic inspection, take down the height and weight of patients and determine their systolic pressure with sphygmomanometer. Calculate the metering standard (cESS) [5] of tension at the end of left ventricle systole based on relative reference formula:

$$cESS = \frac{SBP \times (LVID_S/2)^2 \times \{1 + \frac{(LVID_S/2 + PWT_S)^2}{(LVID_S/2 + PWT_S/2)^2}\}}{(LVID_S/2 + PWT_S)^2 - (LVID_S/2)^2}$$

Evaluation of myocardial biological energy consumption with a non-invasive method (MEE) [6]:

$MEE(cal/systole) = cESS(kdyn/cm^2)\times ET(s)\times SV(ml)\times 4.2\times 10^{-4}$.

Statistics handling with a statistical method: The SPSS 11.5 statistical software is applied and the data is indicated as the average value ± standard deviation. The matched t inspection is used for comparison in one group and the ANOVA analysis is used for comparison between groups. If *P<0.05,* the difference is with statistical significance.

Results

Treatment safety

The treatment processes of thirty-eight patients in two groups in this research are safe and no severe complication is occurred. Four patients in the treatment groups (21%) suffer from slight ostalgia and power shortage, but these symptoms are tolerable and disappeared two or three days after drug suspension. Eighteen patients (100%) have a fever phenomenon after receiving G-CSF mobilization treatment and their white blood cells and granular cells are increased to different extents. Patients whose body temperature is lower than 37.5°C receives no special handling and patients with a fever receives the right handling. Their white blood cells and granular cells recover to the previous level gradually after four weeks. No above changes are observed in the matched group. No gastrointestinal tract reactions, such as nausea and vomit, as well as severe arrhythmia and main Cardiovascular events are occurred in the two groups.

Changes of EPC of peripheral blood before and after G-CSF injection

A mononuclear cell acquired from separation will differentiated into fusiform endothelial-like cells after being cultivated for seven days. EPC has the features of endothelia cells. Its color is red and green inverted under the fluorescence microscope after absorbing DiLDL and combining with lectin. Dual colored cells are endothelia cells being differentiated and their color is yellow. More than 98% of the cells are dual colored positive cells.

Changes of EPC of peripheral blood: The number of EPC in both groups is increased. The number of EPC in the treatment group seven days after the treatment is obviously higher than that before the treatment (*P<0.01*) and that in the matched group (*P<0.01*); The number of EPC four months after the treatment is a little lower than that seven days after the treatment, but it still obviously higher than that before the treatment (*P<0.05*). The number of EPC in the matched group seven days after the treatment increased a lot compared with that before the treatment (*P<0.05*) and that four months after the treatment is still higher than that before the treatment. However, the differ-

Table 1. Changes of EPC in peripheral blood before and after G-CSF injection (unit: CFU-end/10^6 PBMCs) ($\bar{x} \pm s$)

Groups	Before the treatment	Seven days after the treatment	Fourth months after the treatment
The treatment group	38.3±5.1	78.2±7.6$^{*,\Delta}$	62.6±5.9*
The matched group	39.1±4.7	50.4±5.3*	43.8±4.7

Note: Compare with that before the treatment $^{*}P<0.05$; compare with the matched group $^{\#}P<0.05$ and compare with another time in the same group $^{\Delta}P<0.01$.

Table 2. Comparison of cardiac function indexes of patients in both groups two weeks and four months after the treatment ($\bar{x} \pm s$)

Observation index	Treatment group			Matched group		
	Before the treatment	Two weeks after the treatment	Fourth months after the treatment	Before the treatment	Two weeks after the treatment	Fourth months after the treatment
SBP (mmHg)	157.8±15.8	140.9±12.5$^{*,\#}$	126.4±10.3$^{*,\#}$	156.5±15.6	145.3±13.3*	133.8±11.4*
LVIDs (mm)	48.4±3.7	40.6±3.4$^{*,\#}$	32.1±3.1$^{*,\#}$	48.6±3.5	45.4±3.5*	38.2±3.3*
PWTs (mm)	11.7±1.5	11.1±1.4$^{*,\#}$	10.5±1.3$^{*,\#}$	11.8±1.6	11.5±1.4*	10.9±1.5*
EDV (ml)	180.2±20.5	172.1±19.3	163.8±18.6	179.5±20.1	172.3±19.6	164.3±18.4
ET (ms)	246.8±28.5	262.2±31.5$^{*,\#}$	280.3±36.4$^{*,\#}$	247.2±28.1	256.9±33.3*	266.3±38.5
SV (mL)	47.3±13.9	48.8±14.5$^{*,\#}$	55.1±15.9$^{*\#}$	47.2±13.3	48.4±15.2*	52.7±15.6*
LVEF (%)	42.3±5.7	49.8±6.2$^{*,\#}$	61.9±6.9$^{*,\#,\Delta}$	42.1±5.4	46.1±6.1*	50.8±6.5*
FS (%)	15.5±2.3	19.3±5.4$^{*,\#}$	32.3±5.7$^{*,\#,\Delta}$	14.8±3.7	17.4±4.3*	26.2±4.3*
cESS (kdyn/cm^2)	322.2±31.7	251.3±29.5$^{*,\#,\Delta}$	186.5±22.1$^{*,\#}$	319.7±32.2	277.6±28.7*	234.1±26.3*
MEE (cal/systole)	1.59±0.48	1.35±0.36$^{*,\#,\Delta}$	1.20±0.24$^{*\#}$	1.57±0.45	1.46±0.41*	1.38±0.38*

Note: Compare with that before the treatment $^{*}P<0.05$; compare with the matched group $^{\#}P<0.05$ and compare with another time in the same group $^{\Delta}P<0.01$.

ence is with no statistical significance (*P>0.05*). See **Table 1**.

BNP level in blood plasma: BNP concentration in blood plasma of patients in both groups is decreased to normal after the treatment and there is a statistical significance compared with that before the treatment (*P<0.01*). BNP level in blood plasma seven days and four months after the treatment is obviously lower than that in the same period in the matched group (*P<0.05*).

Comparison of cardiac function treatment effect: For nineteen patients in the matched group, one is obvious; fourteen is effective and four is invalid. The total efficiency is 78.9%; for nineteen patients in the treatment group, two is obvious; fifteen is effective and two is invalid. The total efficiency is 89.4%. The difference in total efficiency between two groups is with statistical significance (*P<0.05*) and that in the treatment group is obviously higher than that in the matched group.

Inspection results from ultrasonic cardiogram: Cardiac function indexes in both groups after the treatment are improved compared with that before the treatment. LVIDs, PWTs, cESS and MEE are decreased compared with that before the treatment and ET, SV, LVEF and FS are increased compared with that before the treatment (*P<0.05*). For comparison between the treatment group and the matched group, the difference is with statistical significance (*P< 0.05*). cESS and MEE in the treatment group are decreased obviously two weeks after the treatment (*P<0.01*). However, LVEF and FS are increased obviously fourth month after the treatment (*P<0.01*). See **Table 2**.

Discussion

Myocardial cells death or apoptosis comes after myocardial infarction and ventricular reconstruction contributes to cardiac failure. For the treatment of MI, main function of such traditional therapies as drugs, intervention treatment and surgeries is reconstructing blood supply and recover part blood supply in the myocardial infarction area, but they cannot repair damaged myocardial cells or regenerate

new ones. Cell treatment has become a hot topic of researching on treatment of ischemic cardiovascular diseases. EPC is a kind of precursor cell which can differentiate vessel endothelium directly. It not only participates in the angiogenesis of embryo process, but also plays an important role for vessel regeneration of adults [4]. Although it has confirmed that EPC has the ability to greatly promote vessel regeneration, generally speaking, the content of EPC in a circulation is low and the ability of proliferation and differentiation is not strong. Therefore, it cannot meet requirements of treatment. In recent years, the research has focused on utilization of the mobilization agent which is able to mobilize ECP to peripheral blood from the marrow so that EPC in peripheral blood is enough for treatment. It can participate in repairing of dead myocardium and regeneration of vessels with its feature of automatic homing to the myocardial infarction area and differentiation under the myocardial micro-environment. G-CSF is the cell factor firstly found to be able to mobilize EPC in the marrow. In several animal tests that MI treatment with EPC mobilized by G-CSF and some small-scale clinical researches, it has found that cardiac function is improved to some extent. Seiler etc. injects G-CSF10 μg/(kg·d) to the skin of twenty-one AMI patients with wide lesions to which a bypass surgery is suitable every day and the result is that: G-CSF can promote EPC to separate from marrows and enter a blood circulation by reducing molecules affixed on the surface of EPC. After two weeks, invasive collateral flow index increases greatly and the occurrence of myocardial ischemia in the ECG reduces significantly, which promotes vessel reconstruction and improves cardiac function. Japanese Harada [9] etc. has found that MI treatment with EPC mobilized by G-CSF can activate Jak-stat signal path in myocardial cells so as to restraint myocardial apoptosis and prevent ventricular reconstruction by researching. Ohtsuka [10] etc. has found that MI treatment with EPC mobilized by G-CSF has increased vessel density in the myocardial ischemia area so as to improve partial blood supply ability and cardiac function by researching. This research has also found that the cardiac function of patients MI and cardiac function insufficiency is obviously improved with EPC mobilized by G-CSF.

The assumption that "failing heart is energy-starved" points out that abnormal myocardial energy metabolism plays an important role in the heart failure pathogenesis and a new treatment target for CHF. At present, determination indexes of myocardial energy metabolism include Myocardial Oxygen Consumption, MVO_2 and tension time index, TTI. Treatment methods are invasive and complex, which are not largely applied into clinical practices. A research [13] indicates that the tension (stress) generated in the systole period of left ventricle determines myocardial oxygen consumption and the stress generated at the end of the systole period of left ventricle is the tension bore by myocardium in the left ventricle per unit. This research adopts cESS in place of the tension generated in the systole period of left ventricle [6] to reflect myocardial systole ability in the left ventricle and the load after it. Iwashima [14] etc. have confirmed that external function (mechanical function) in the left ventricle is in proportion to the average angiosthenia and cardiac output so that the product MEE by cESS and ET and SV can reflect myocardial biological energy expenditure more comprehensively. LVEF is a sensible index to determine blood ejection function in the left ventricle and expansion in the left ventricle is the predictive factor for the cardiovascular death rate. For patients with reduction of systole function in the left ventricle, reduction of LVEF is related to the increasing of MEE which is an independent predictive factor of cardiac deaths [15]. With this research, it is found that LVEF in the group with EPC treatment mobilized by G-CSF has increased a lot compared with that before the treatment. It points out that blood ejection of patients with MI and cardiac function insufficiency is improved with EPC treatment so as to improve the systole function in the left ventricle and avoid malignant arrhythmia. The result is similar to reports at home and abroad [16]. This research indicates that cardiac function index LVIDs and PWTs are decreased after the treatment, but ET and SV are increased. The difference compared with that before the treatment and the matched group is with statistical significance ($P<0.05$), which indicates that MI treatment with EPC mobilized by G-CSF can prevent chamber expansion, cardiac wall thinning and increasing of cardiac output. However, the difference of EDV before and after treatment is with no statistical significance because

that improvement of cardiac function reflects in improvement of ventricular systole function at first. It cannot reverse bulging and partial ventricular tension function insufficiency is remained [17]. In this research, cESS is greatly decreased as the systole function in the left ventricle is improved gradually, but ET and SV are inclined to increasing. As MEE is the product multiplied by cESS, SV and ET and reduction of cESS offset the increasing of ET and SV, cESS has become is the most important affecting factor of MEE. MEE and cESS in the treatment group have decreased greatly two weeks after the treatment compared with that before the treatment and that in the matched group (*P<0.01*). With EPC treatment, damaged myocardium is repaired; cardiac systole function is improved and cardiac energy expenditure is decreased, which is inconsistent with the report made by Shen Anna [18] etc. that MEE and cESS are the sensible index to evaluate early potential systole function insufficiency in the left ventricle of patients with CVD. In addition, it has also found that EPC keeps a high concentration four months after the treatment and LVEF improves a lot and the long-term effect of cardiac function treatment is satisfied. The reason may be good and solid blood flow reconstruction after EPC treatment in combination of intervention treatment (It is testified by the radiography result of reexamination on some patients). BNP level in blood plasma in the treatment group seven days after the treatment is much lower than that in the matched group (*P<0.01*), which indicates that EPC treatment can delay occurrence and development of cardiac failure. Although BNP level is increased, it is still lower that before the treatment for in combination of the intervention treatment. EPC treatment is considered to apply to clinical practices when it is proper.

In this research, it is found that EPC treatment mobilized by G-CSF for patients with MI and cardiac function insufficiency can improve blood ejection in the left ventricle effectively and prevent chamber expansion, cardiac wall thinning, increasing of cardiac output and effective myocardial energy expenditure. It indicates that this therapy plays an important role in improving cardiac systole and diastole function and myocardial energy expenditure. It is safe and feasible to treat MI and heart failure with EPC treatment mobilized by G-CSF and no thromboembolism, severe arrhythmia and cardiovascular events are occurred. EPC in combination of the intervention treatment cannot only improve the treatment efficiency in a short time, but also in favor of long-term improvement of patients' cardiac function and survival quality. However, its functioning mechanism is still not clearly understood and it needs further research and discussion.

Disclosure of conflict of interest

None.

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