Original Article
Effect of granulocyte-macrophage colony stimulating factor treatment on myocardial perfusion and heart function in patients with coronary artery disease

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Abstract: Aim: Here we aimed to evaluate the therapeutic effect of granulocyte-macrophage colony stimulating factor (GM-CSF) on the patients with coronary artery disease (CAD). Method: Total 216 patients admitted between October 2008 and November 2010 were randomly assigned to receive conventional therapy or different doses of GM-CSF (5.0 µg/kg, 2.5 µg/kg, 1.0 µg/kg) (n = 54). Subsequently, patients with reduced blood perfusion in each group were selected to evaluate whether the GM-CSF treatment improved the myocardial ischemia and cardiac function that were assessed by semi-quantitative perfusion score (QGS) and left ventricular ejection fraction (LVEF) under the emission computed to myography. Furthermore, the mortality and side effects were recorded. Results: Compared with pre-treatment, QGS score was significantly decreased after GM-CSF intervention, but not in the conventional group. Further comparison also indicated the significant improvement extent of QGS score after GM-CSF compared with conventional therapy (1.0 µg/kg: 3.30 ± 2.51 vs. 1.09 ± 1.75; 2.5 µg/kg: 2.85 ± 2.79 vs. 1.09 ± 1.75; 5.0 µg/kg: 5.25 ± 3.02 vs. 1.09 ± 1.75; P = 0.000). Similarly, LVEF was obviously increased after GM-CSF, but not in the conventional group. However, only treatment with 5.0 µg/kg GM-CSF achieved a significant difference in LVEF (61.71 ± 12.46 vs. 53.54 ± 12.12, P = 0.010). The mortality and the incidence of side effects (e.g. leukocyte increase and fever) were also lower in GM-CSF group than that in the conventional group (4.17% vs. 14.29%; 6.25% vs. 16.7%). Conclusions: GM-CSF (5.0 µg/kg) may be an effective and safe intervention for patients with CAD.

Keywords: Emission computed tomography, blood perfusion, prognosis

Introduction

Coronary artery disease (CAD), also known as atherosclerotic cardiovascular disease [1], is a common type of heart disease. CAD is caused by the thickening of heart arteries that blocks the blood flow to the heart, resulting in angina pectoris, arrhythmia or heart failure [2]. It is reported that CAD is the leading cause of death in most industrialized societies [3]. The prevalence of CAD rapidly increases in developing countries [4]. Although the developments of angiography and revascularization technology provide new clinical approaches for patients with CAD, the presences of complications such as myocardial ischemia are not effectively controlled by current therapies, including percutaneous coronary intervention, coronary artery bypass graft surgery and medical therapy [2, 5]. Therefore, it is essential to explore the new managements for the treatments of CAD.

Granulocyte-macrophage colony stimulating factor (GM-CSF), a well-known cytokine, can mediate inflammatory response, modulate the proliferation of hematopoietic progenitor cells and regulate the function of mature hemopoietic cells [6, 7]. In a previous study, Sacramento et al. [8] have found that the administration of GM-CSF promotes arteriogenesis and increases capillary density in the murine model of acute limb ischemia, while the mice with deficiency of GM-CSF may develop atherosclerosis [9]. In murine brain ischemia model, Sugiyama et al. [10] also find that GM-CSF reduces infarct volume and enhances collateral artery growth.
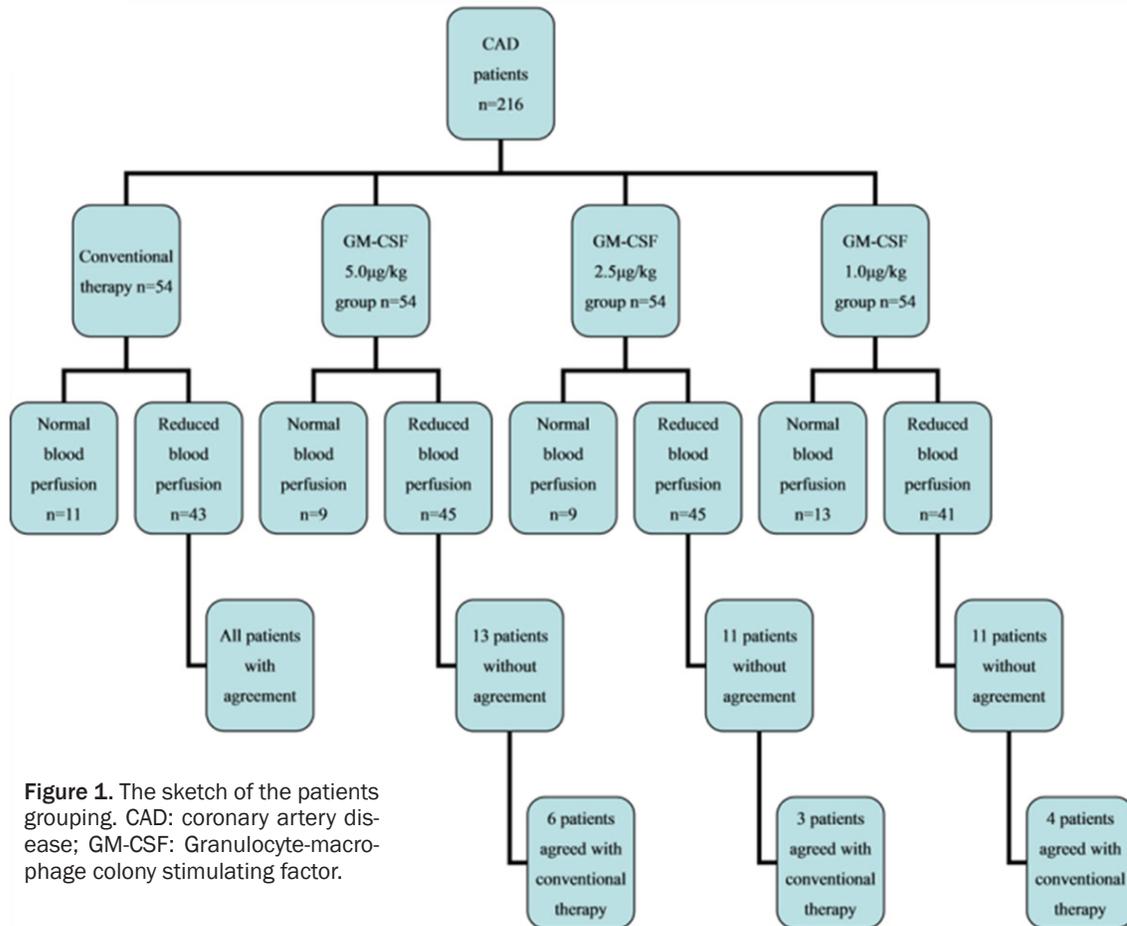
Figure 1. The sketch of the patients grouping. CAD: coronary artery disease; GM-CSF: Granulocyte-macrophage colony stimulating factor.

Besides, GM-CSF may have multiple direct and indirect effects on cardiovascular disease, including promoting neovascularization of ischemic myocardium and reducing the extent of myocardial damage after infarction [11]. The underlying pathogenesis of CAD has been proved to be associated with the disorder of lipid metabolism and abnormal immune response, which result in coronary arterial stenosis and chronic inflammation of the arterial wall [12]. All these studies suggest that GM-CSF may play an important role in CAD. Currently, some clinical studies have shown that GM-CSF can effectively mobilize the CD34 positive cells [13] and promote the growth of collateral circulation vessels in patients with CAD [14]. However, because of the increased incidence of acute coronary syndrome after the intervention of GM-CSF, drug safety remains to be further observed [14]. In previous clinical and animal studies, the dose of GM-CSF are 10 µg/kg/day and the sample size is less than 20 [13, 14]. Therefore, it is necessary to investigate the

clinical applications of GM-CSF with low dose and large sample size in patients with CAD.

In this paper, total 152 patients with decreased blood perfusion were treated with conventional therapy and different doses of GM-CSF (5.0, 2.5, and 1.0 µg/kg) [10], respectively. Emission Computed Tomography (ECT) testing was performed for patients before and after treatment. In the present study, we aimed to examine which concentration of GM-CSF treatment was appropriate in promoting blood perfusion and cardiac function and evaluate the safety of GM-CSF in patients with CAD.

Materials and methods

Patients

Total 216 patients (male: 101; female: 115) admitted into the Cardiology Department of our hospital were included in this study between October 2008 and November 2010. The

Table 1. The basic characteristics of patients in normal blood perfusion group and reduced blood perfusion group

Index	Normal blood perfusion group	Reduced blood perfusion group	P value
LVEF (ECT) (%)	63.46 ± 11.11	50.33 ± 16.50	0.000
BNP (ng/ml)	188.4 ± 205.0	494.9 ± 865.6	0.002
pro-BNP (ng/ml)	40.4 ± 28.8	1912.6 ± 220.7	0.007
atrial premature (mm)	73.0 ± 82.37	707.1 ± 1974.9	0.02
fasting blood glucose (mmol/L)	5.77 ± 1.84	5.26 ± 1.15	0.007
CR (μmol/L)	75.5 ± 19.1	92.3 ± 41.9	0.035
LVESD (mm)	25.6 ± 3.4	29.5 ± 8.4	0.023
LVEDD (mm)	40.78 ± 4.5	44.8 ± 8.77	0.026
minimal heart rate (bpm)	54.6 ± 11.9	49.8 ± 8.0	0.053
blood uric acid (μmol/L)	312.3 ± 110.7	353.4 ± 121.4	0.09
blood coagulation TT (s)	20.9 ± 4.1	19.6 ± 3.0	0.086
AIRC	4.98 ± 0.47	4.77 ± 0.41	0.078
left atrial internal diameter (mm)	38.8 ± 5.3	41.2 ± 6.4	0.086
LVEF (echocardiographic) (%)	66.3 ± 6.8	61.9 ± 11.1	0.066

LVEF, Left ventricular ejection fraction; BNP, Brain natriuretic peptide; ECT, Emission Computed Tomography; CR, Creatinine; LVESD, Left ventricular end systolic diameter; LVEDD, Left ventricle end-diastolic diameter; AIRC, Aggregation index of blood rheological cells.

patients diagnosed with stable CAD were included in this study according to the strict inclusion criteria: (1) patients presented with coronary stenosis (> 50% diameter stenosis) based on the coronary angiography or computed tomography testing; (2) patients underwent myocardial infarction (ST-segment or non-ST-segment elevation myocardial infarction); (3) patients performed with percutaneous coronary intervention or coronary artery bypass grafting surgery. Patients who had acute coronary syndrome, acute cardiac insufficiency, severe liver and renal damage, type 2 respiratory failure in chronic obstructive pulmonary disease, malignancy, severe anemia or apoplexy sequel were excluded from this study.

Grouping

As shown in **Figure 1**, before treatment, 216 eligible patients were randomly and equally divided into four groups with 54 cases in each group: conventional therapy group, GM-CSF 5.0 μg/kg group, GM-CSF 2.5 μg/kg group, and GM-CSF 1.0 μg/kg group. Then, based on the ECT testing, patients in these four groups were, respectively, divided into the normal blood perfusion group and the reduced blood perfusion group. But among the patients with reduced blood perfusion, 22 cases disagreed to participate in this study and were excluded from this study, while 13 cases who agreed with the conventional therapy were included in conventional therapy group. Eventually, 42 patients (male: 15 and female: 27) were included in normal blood perfusion group and 152 patients (male: 78 and female: 74) were included in reduced blood perfusion group. In these patients with reduced blood perfusion, there were 56 cases in the conventional therapy group, 32 cases in the GM-CSF 5.0 μg/kg group, 34 cases in the GM-CSF 2.5 μg/kg group, and 34 cases in the GM-CSF 1.0 μg/kg group. The basic characteristics of the patients were recorded.

Drug intervention

In the conventional therapy group, patients were treated with essential drugs against myocardial ischemia. The drugs contained statins (Atorvastatin; 20 mg/day), β-receptor blocker (Metoprolol; 20 mg 3 times a day), nitrates (Isosorbidemononitrate; 40 mg once a day) and antiplatelet drugs (Trimetazidine; 20 mg 3 times a day). Patients with high blood pressure were treated with amlodipine (Norvasc) and/or valsartan (Diovan) capsules prior to other drugs. Patients with diabetes were given gliclazide sustained-release tablets (Gliclazide), insulin or metformin hydrochloride tablets. In addition to the conventional treatment, patients in GM-CSF groups were injected subcutaneously with GM-CSF of 5.0 μg/kg, 2.5 μg/kg or 1.0 μg/kg, respectively. The treatment was performed once every other day for the consecutive 2 weeks.

Follow-up

The follow-up period ranged from 6 to 36 months. After intervention, the ECT testing was reexamined. The effects of GM-CSF on myocardial ischemia and cardiac function was evaluated. Mortality and side effects of patients were recorded to assess the drug safety.
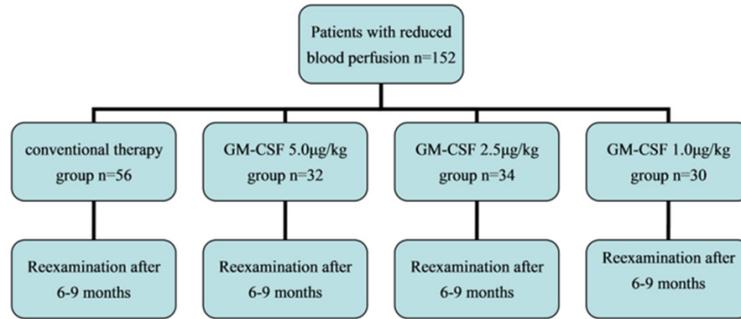
Figure 2. The final groups of patients with coronary artery disease (CAD) in this research. GM-CSF: Granulocyte-macrophage colony stimulating factor.

Myocardial perfusion and cardiac function evaluation

Before and after intervention, the blood perfusions of patients were measured by ECT testing. The myocardial single photon emission computed tomography (SPECT) and gating cardiac blood-pool imaging (GCBP) were examined for all the patients. In myocardial SPECT (E. COM ECT, Siemens, Germany), lipo meal was given at 30 min after intravenous injection of technetium-99-methoxy-isobytyl isonitrile, then the myocardial SPECT was performed after 1.5 h. The collection conditions of image were as followed: 32 original images were collected in 180; the cross-sectional images including short-axis, vertical long-axis and horizontal long-axis of heart were reconstructed after being disposed by computer to observe radioactive distribution of left ventricular. For the GCBP, Tc-99m-pyrophosphate-red blood cell 25 mci was intravenously injected and GCBP was performed in left-front position after 20 min. All the images were analyzed by two experienced nuclear physicians in double blind condition. Based on GCBP, left ventricular wall motion was observed by phase analysis and the left ventricular ejection fraction (LVEF) was calculated. Myocardial perfusion was semi-quantitatively evaluated by myocardial perfusion gated-SPECT (QGS) system. The changes of the myocardial perfusion degrees were calculated as follows: the degree = |QGS score (after being intervened by GM-CSF) - QGS score (before intervention)|.

Statistical analysis

All the data were displayed as mean ± SD (standard deviation). Statistical analysis was performed by SPSS 13.0 software. Comparisons between two groups were analyzed by t-test and chi-square test. $P < 0.05$ was considered to be significant.

Results

Patients

Comparing the clinical characteristics between normal blood perfusion group and reduced blood perfusion group, no differences were observed in gender, age, and the medical history of high blood pressure, diabetes of the patients ($P > 0.05$). The baseline information of patients is shown in **Table 1**. Compared with the normal blood perfusion group, some indexes in the reduced blood perfusion group were significantly decreased, such as LVEF ($P = 0.000$), brain natriuretic peptide (BNP) ($P = 0.002$), pro-BNP ($P = 0.007$), atrial premature in dynamic electrocardiogram ($P = 0.02$), the fasting blood glucose ($P = 0.007$), serum creatinine (CR) ($P = 0.035$), left ventricular end systolic diameter (LVESD) ($P = 0.023$), and left ventricle end-diastolic diameter (LVEDD) ($P = 0.026$), which suggested that patients with reduced blood perfusion might have an increased risk of atrial arrhythmia compared with patients with normal blood perfusion.

Then, patients in the reduced blood perfusion group were divided into 4 subgroups (**Figure 2**). The average age of patients in conventional therapy, GM-CSF of 5.0 μg/kg, 2.5 μg/kg and 1.0 μg/kg group was 64.2 ± 10.3, 65.1 ± 10.4, 63.4 ± 9.8 and 64.8 ± 11.0 years, respectively and the sex ratio of patients (male/female) was 28/28, 17/15, 18/16 and 15/15, respectively, which suggested that the baseline information of the subgroup patients was similar.

Effects of GM-CSF on myocardial perfusion and cardiac function

The intervention effect of GM-CSF was detected in the reduced blood perfusion group based on the QGS score and LVEF (**Tables 2** and **3**). After intervention with GM-CSF, the QGS score of 5.0, 2.5 and 1.0 μg/kg GM-CSF groups were significantly decreased ($P < 0.05$). How-

Table 2. The changes of QGS score before and after intervention by GM-CSF

Group	Conventional therapy	GM-CSF 5.0 μg/kg	GM-CSF 2.5 μg/kg	GM-CSF 1.0 μg/kg
Before intervention	8.21 ± 6.07	8.78 ± 5.43	7.56 ± 3.90	8.37 ± 5.85
After intervention	7.98 ± 6.73	4.63 ± 3.21	4.71 ± 3.56	5.07 ± 4.75
P1 value	0.861	0.000	0.002	0.048
Changed degrees	1.09 ± 1.75	5.25 ± 3.02	2.85 ± 2.79	3.30 ± 2.51
P2 value	—	0.000	0.000	0.000
—	—	P3 = 0.178	P4 = 0.890	—
—	—	P5 = 0.167	—	—

P1 value: Compared with the QGS score between before intervention and after intervention; P2 value: Compared with conventional therapy; P3: Compared improvement degree between GM-CSF 5.0 μg/kg group and GM-CSF 1.0 μg/kg group; P4: Compared improvement degree between GM-CSF 2.5 μg/kg group and GM-CSF 1.0 μg/kg group; P5: Compared the improvement degree between GM-CSF 5.0 μg/kg group and GM-CSF 2.5 μg/kg group. GM-CSF, Granulocyte-macrophage colony stimulating factor.

ever, there was no difference in the conventional therapy group before and after intervention. In addition, the degree of improvement on myocardial blood perfusion was further analyzed. After being treated with GM-CSF of 1.0 μg/kg (3.30 ± 2.51), 2.5 μg/kg (2.85 ± 2.79) or 5.0 μg/kg (5.25 ± 3.02), myocardial blood perfusion was significantly improved compared with the conventional therapy group (1.09 ± 1.75) (P = 0.000) (**Table 2**). However, there was no obvious dose-dependent manner for the improvement on myocardial blood perfusion after GM-CSF treatment and no significant difference was observed among three GM-CSF groups (P > 0.05).

After intervention, the LVEF of 5.0 μg/kg GM-CSF group was significantly improved (53.54 ± 12.12 vs. 61.71 ± 12.46, P < 0.05). In addition, there was an obvious improvement in LVEF of 2.5 (51.54 ± 15.14 vs. 58.31 ± 16.33, P = 0.081) and 1.0 μg/kg GM-CSF group (52.08 ± 15.88 vs. 57.21 ± 16.48; P = 0.224), while the LVEF was not improved in the conventional treatment group (60.52 ± 14.22 vs. 57.52 ± 15.44, P = 0.324) (**Table 3**).

Prognosis

In the follow-up period, the mortality of conventional therapy group was 14.29% (8/56). Six patients died from cardiovascular and 2 patients from other factors. The mortality of GM-CSF treatment groups was 4.17% (4/96). Total 4 patients died after intervention by GM-CSF,

including 1 patient in 5.0 μg/kg group, 1 case in 1.0 μg/kg group, and 2 ones in 2.5 μg/kg group. The mortality of patients was significantly declined after GM-CSF intervention (P < 0.001).

Besides, there were some complications in patients with CAD in our study. Total 16 cases presented with the increase of leukocyte ($\geq$ 10 × 10⁹), including 6 cases in the 5.0 μg/kg GM-CSF group, 6 ones in the 2.5 μg/kg GM-CSF group and 4 ones in the 1.0 μg/kg GM-CSF group. Fever occurred in 6 patients including 2 cases in the 5.0 μg/kg group, 3 ones in the 2.5 μg/kg group and one in the 1.0 μg/kg group. The incidences of the increase of leukocyte and fever were 16.7% (16/96) and 6.25% (6/96), respectively. In addition, no cases underwent acute coronary syndrome and other complications in the GM-CSF treatment groups.

Discussion

CAD has been a health burden all over the world. Although many studies have contributed to explore the new strategies of prevention and treatment for cardiovascular disease [15, 16], the high morbidity and mortality for CAD is alarming. Currently, GM-CSF has attracted many attentions for its promising potential in artery disease treatment. In the present study, we reported that GM-CSF administration at different concentrations (5 μg/kg, 2.5 μg/kg, and 1.0 μg/kg) might have an improved effect on myocardial perfusion and cardiac function in patients with CAD.

The well development of collateral circulation termed arteriogenesis had been proved to be a potent strategy to prevent cardiac mortality and myocardial infarction in occlusive artery disease [17]. Previous studies proposed that GM-CSF could promote the collateral circulation by releasing pluripotent monocyte (stem-) cells [18]. The clinical study showed that the treatment of GM-CSF led to the collateral arteries growth, thereby significantly prevented infarction in patients with cerebrovascular dis-

Table 3. The changes of LVEF before and after intervention by GM-CSF

Group	Conventional therapy	GM-CSF 5.0 μg/kg	GM-CSF 2.5 μg/kg	GM-CSF 10 μg/kg
Before intervention (%)	60.52 ± 14.22	53.54 ± 12.12	51.54 ± 15.14	52.08 ± 15.88
After intervention (%)	57.52 ± 15.44	61.71 ± 12.46	58.31 ± 16.33	57.21 ± 16.48
P value	0.324	0.010	0.081	0.224

GM-CSF, Granulocyte-macrophage colony stimulating factor; LVEF, Left ventricular ejection fraction.

ease [19]. In addition, GM-CSF had been demonstrated to increase collateral flow in patients with CAD [20]. The significant myocardial perfusion improvement in CAD patients of our study might result from the collateral flow stimulated by GM-CSF.

Previous clinical study indicated that low-dose GM-CSF intervention (10 μg/kg/day for consecutive 7 days) could promote the collateral circulation development in mice and the high-dose GM-CSF (20 μg/kg body weight daily) increased the number of Mac-2+ monocytes/ macrophages on the dorsal surface of the brain [21]. Long-term GM-CSF intervention (42 days) in animals of sequential bilateral carotid artery occlusion model contributed to the restoration of impaired cerebral hemodynamics [22]. Different from the previous findings, we applied 3 different doses of GM-CSF (all less than 10 μg/kg) to treat the CAD patients once every other day for the consecutive 2 weeks and the results suggested that 5 μg/kg GM-CSF had the most improvement for the myocardial perfusion of patients. We proposed that the low dose of GM-CSF could also increase collateral flow in CAD patients. In our work, the GM-CSF was administered every other day for 7 times and showed the significant effect of GM-CSF on myocardial perfusion improvement. It was similar with the previous results that both the continuous and intermittent GM-CSF infusion regimens stimulated the formation of collateral vessels [23]. Moreover, Zbinden et al. [14] also found that after treatment with GM-CSF (10 μg/kg/day) every other day for 2 weeks in patients with CAD, significant improvements were observed in electrocardiographic signs of myocardial ischemia and collateral flow index during coronary balloon occlusion. Therefore, we speculated that these benefits might be caused by cytokine-induced angiogenesis or changes in collateral vascular tone.

Besides, evidence showed that GM-CSF could mobilize the stem cells and increase the C-

reactive protein levels that showed efficacy in repairing injured heart of patients with acute myocardial infarction [13]. It was found that the bone marrow stem cells (BMSCs) could differentiate into cardiomyocytes in injury site of heart that were responsible for heart function improvement [24, 25]. In addition, the cardiac function was found to be improved in patients with myocardial infarction after BMSCs transplantation [26]. In this paper, we also found that the cardiac function of CAD patients was improved after GM-CSF intervention, which suggested that the GM-CSF might repair the heart function by mobilizing the stem cells in CAD patients. Furthermore, the cardiac function of patients with 5.0 μg/kg GM-CSF intervention was significantly improved. The 5.0 μg/ kg GM-CSF also led to significantly improved myocardial perfusion. Thus, we speculated that 5.0 μg/kg might be an appropriate dose of GM-CSF for treating CAD patients, but a large number of studies were warranted.

The safety of GM-CSF (10 μg/kg) was questionable regarding to the high morbidity of an acute coronary syndrome during the treatment period [14]. In this paper, no cases underwent acute coronary syndrome after GM-CSF. The safety of GM-CSF might be related with the low dose of GM-CSF in our work. In addition, it was speculated that the occurrence of acute coronary syndrome might be associated with the pro-inflammatory and pro-coagulant effects of GM-CSF [27]. In this paper, patients in GM-CSF groups were treated with GM-CSF combined with anti-inflammation and anti-coagulation drugs (such as statins and aspirin), which might also reduce the risk of suffering acute coronary syndrome. A previous study also indicated that other side effect of GM-CSF included low fever, weakness, skin rashes, headache, and nausea [28-30]. In our work, 16.7% (16/96) patients suffered leukocyte increase and 6.25% (6/ 96) had fever. No other complications were observed in patients treated with GM-CSF. Still,

the safety of GM-CSF for the clinical application needed further investigation.

Conclusions

Our work demonstrated that GM-CSF intervention could significantly improve myocardial perfusion and cardiac function of patients with CAD. The therapy of low dose GM-CSF (5.0 µg/kg) showed significant effect and less complications for CAD patients. GM-CSF might provide promising treatment strategy for patients with CAD. However, the clinical application of this drug still needs to be further defined.

Acknowledgements

Project supported by the independent innovation in Science and Technology Committee of Shanghai Putuo District, the key project (No.: PTKW08-B03).

Disclosure of conflict of interest

None.

Authors' contribution

YGX, QLL and DKH participated in the design of this study, and they all performed the statistical analysis. DWZ, YZB and HY carried out the study and collected important background information. ZHL, XLH and MMX drafted the manuscript. All authors read and approved the final manuscript. The raw data were collected and analyzed by the Authors, and are not ready to share their data because the data have not been published.

Abbreviations

GM-CSF, granulocyte-macrophage colony stimulating factor; CAD, coronary artery disease; QGS, semi-quantitative perfusion score; LVEF, left ventricular ejection fraction; SPECT, single photon emission computed tomography; CR, serum creatinine; LVESD, left ventricular end systolic diameter; LVEDD, left ventricle end-diastolic diameter; BMSCs, bone marrow stem cells.

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