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Effect of mobilization of bone marrow stem cells by granulocyte colony stimulating factor on clinical symptoms, left ventricular perfusion and function in patients with severe chronic ischemic heart disease☆

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Abstract

Objectives: A phase I safety and efficacy study with granulocyte colony stimulating factor (G-CSF) mobilization of bone marrow stem cells to induce vasculogenesis in patients with severe ischemic heart disease (IHD) was conducted.
Design, patients and results: 29 patients with IHD participated in the study. Thirteen patients were treated with G-CSF for 6 days and 16 patients served as controls. G-CSF treatment was without any serious adverse events. Four patients were "poor mobilizers" with a maximal increase in CD34+ cells to 5,000±700/mL blood (mean±S.D.) compared to 28,900±5,100/mL blood in "mobilizers". At the follow-up, G-CSF treated had improved in CCS classification, NTG consumption and angina attacks, but the controls only in CCS classification. No difference was seen between the two groups. The decline in NTG consumption tended to be significant in "mobilizers" compared to controls. Myocardial perfusion was unchanged at adenosine stress single photon emission computerized tomography (SPECT) or magnetic resonance images (MRI). Left ventricular ejection fraction decreased from 57% to 52% ($p<0.01$, MRI) and from 48% to 44% ($p=0.07$, SPECT) in G-CSF treated, but was unchanged measured with echocardiography.
Conclusions: Treatment by G-CSF improved symptoms but not signs of myocardial ischemia in patients with severe IHD. The effects seemed related to mobilization of stem cells. An adverse effect on ejection fraction could not be excluded.

Keywords: Stem cells; Vasculogenesis; Ischemic heart disease; Left ventricular ejection fraction; G-CSF

1. Introduction

Cardiovascular drug therapies and revascularization with coronary artery angioplasty and by-pass surgery have reduced the mortality and morbidity in patients with coronary artery disease. However, occlusive coronary artery disease is still one of the leading causes of morbidity. Animal and human studies suggest that stem cells (endothelial progenitor cells) from the bone marrow have the potential to differentiate into endothelial cells and create new blood vessels [1–5]. Therefore, treatment with

Abbreviations: G-CSF, granulocyte colony stimulating factor; IHD, ischemic heart disease; SPECT, single photon emission computerized tomography; MRI, magnetic resonance images; CCS, Canadian Cardiovascular Society angina classification; SEQ, Seattle Angina Pectoris Questionnaire; NTG, nitroglycerine.

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these cells constitutes a potential, new therapeutic option for development of blood vessels in patients with occlusive coronary artery disease. Tracer techniques have demonstrated that endothelial progenitor cells participate in the formation of new blood vessels in the myocardium after an acute myocardial infarction [6]. Therefore, these cells constitute a potential, new clinical treatment regime for the development of new blood vessels in patients with myocardial ischemia.

Recently, small clinical safety studies with direct intramyocardial or intracoronary injections of mononucleated cell suspensions from the bone marrow have in most studies suggested a beneficial effect on myocardial function and symptoms after an ST-elevation myocardial infarction or in chronic myocardial ischemia [7–13]. Treatment with granulocyte colony-stimulating factor (G-CSF) has been used for many years in clinical haematology to mobilize bone marrow stem cells in patients with leukaemia treated with bone marrow transplantation [14]. A recent preliminary publication indicated that G-CSF treatment in patients with acute myocardial infarction could aggravate the in-stent restenosis rate [15].

We hypothesised that G-CSF mobilized stem cells from the bone marrow will home in ischemic myocardium and induce vasculogenesis and improved perfusion. The aim of the present study was in a clinical phase I safety and efficacy study to evaluate the safety and clinical effect of stimulation with G-CSF to induce myocardial vasculogenesis on symptoms and signs of myocardial ischemia in patients with severe occlusive coronary artery disease.

2. Materials and methods

We prospectively treated 13 patients with severe occlusive coronary artery disease with G-CSF (11 men, 2 women, mean age 63 years) and 16 identical patients receiving placebo treatment in a parallel study Euroinject One served as controls (14 men, 2 women, mean age 62 years). These controls were treated with placebo injections directly into the ischemic myocardium in the left ventricle [16]. Inclusion criteria were in both studies identical: reversible ischemia at an adenosine stress single photon emission computerized tomography (SPECT), a coronary arteriography demonstrating at least one main coronary vessel from which new collaterals/vessels could be supplied, age above 18 years, Canadian Cardiovascular Society angina classification (CCS)≥3. Excluded were patients with unstable angina pectoris, acute myocardial infarction within the last three months, diabetes mellitus with proliferative retinopathy, diagnosed or suspected cancer disease, chronic inflammatory disease and fertile women. According to the decisions of cardiac surgeons and cardiologists, none of the patients could be treated further by conventional revascularization.

Before inclusion, patients were screened for haematological and biochemical abnormalities, occult blood in stools×3 and by chest X-ray; patients with diabetes mellitus had an ophthalmoscopy and a mammography was performed in the women. Patients received oral and written information and signed a written informed consent.

The study was approved by the national Ethical Committee (02-053/01) and Federal Drug Agency (2612-1782).

2.1. Study protocol

Patients were treated in-hospital with one daily subcutaneous injection of 5 μg/kg body weight G-CSF (Neupogen®) for 6 days. In the same period, they performed light bicycle exercise for 15 min three times daily in order to induce myocardial ischemia. In the follow-up period, the patients were encouraged to perform moderate, but daily physical exercise, although this was not controlled. Peripheral circulating stem cells (CD34+ cells) and biochemistry controls were measured before and on days 2, 7, 14 and 28 after treatment.

Prescriptions of anti-angina or vasoactive medications were not changed during the study period. Drug related adverse effects were recorded during the treatment and follow-up period. The patients were followed once a week the first months and then at months 2 and 6 to control for side-effects and safety issues.

Two months after treatment, the patients were investigated for changes in myocardial ischemia as assessed by SPECT, global and regional left ventricular function measured by echocardiography and magnetic resonance imaging (MRI), angina pectoris class according to the CCS classification and Seattle Angina Pectoris Questionnaire (SEQ), frequency of angina attacks and nitroglycerine (NTG) consumption per week and exercise capacity.

2.2. Single photon emission computerized tomography (SPECT)

SPECT studies were performed as a 2-day protocol (500–700 MBq 99mTc-sestamibi at each study) with adenosine infusion over 4–6 min (0.14 mg/kg/min by infusion pump), if possible combined with a sub-maximal exercise test [17,18]. Care was taken to perform the stress tests at the inclusion and at the follow-up studies with identical cumulative adenosine doses and identical submaximal exercise loads. Gated (8 frames) imaging was performed with a two-headed Millennium GE gamma camera, with a Gadolinium interleaved attenuation-scatter correction [17].

Blinded, visual analysis according to a 17 segment model of the SPECT images (myocardial slices in three planes) was performed as consensus readings by 2 experienced nuclear medicine specialists, using an eNTEGRA working station (GE Medical). Polar plots with and without blackout