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The influence of intracoronary injection of bone marrow cells on prothrombotic markers in patients with acute myocardial infarction

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ABSTRACT

Introduction: The effects of intracoronary injection of mononuclear bone marrow cells (mBMC) on haemostasis are not clarified. The aim of the present substudy of the autologous stem cell transplantation in acute myocardial infarction (ASTAMI) trial was to investigate the influence of intracoronary injection of mBMC on selected circulating prothrombotic markers.
Materials and methods: One hundred patients with ST-elevation myocardial infarction (STEMI) treated with percutaneous coronary intervention (PCI) on the left descending coronary artery were randomized to receive mBMC (Tx) (median 6 days after the STEMI) or to a control group. Fasting blood samples were drawn the day before Tx (day-1, 4–5 days after the STEMI), and 1 day, 3 days, 2–3 weeks and 3 months after Tx.
Results: No significant differences in changes between the groups were observed from day-1 to any later time points in the levels of TF (tissue factor), F1 + 2 (prothrombin fragment 1 + 2), D-dimer, ETP (endogenous thrombin potential), PAI-1 (plasminogen activator inhibitor 1) or tissue plasminogen activator. However, TF and F1 + 2 decreased from day-1 to the subsequent time points in both groups, except from a small increase of TF at 3 months in the control group. In both groups, D-dimer and ETP decreased from day-1 to 2–3 weeks and 3 months, whereas PAI-1 increased to 2–3 weeks and 3 months.
Conclusions: Intracoronary injection of mBMC did not influence on prothrombotic markers in patients with STEMI. Reduction in several prothrombotic markers from day-1 to 2–3 weeks and 3 months could be demonstrated in both groups indicating decreased hypercoagulability.
This is a substudy of the ASTAMI trial which is registered at www.clinicaltrials.gov, NCT 00199823.

Introduction

Stem cell therapy in patients with acute myocardial infarction (AMI) has been investigated in several trials during the last decade, aiming to improve left ventricular function [1]. In the majority of these studies, patients with acute ST-elevation myocardial infarction (STEMI) treated with percutaneous coronary intervention (PCI), have been given intracoronary injections of autologous mononuclear bone marrow cells (mBMC) within the first week [2–9]. The studies have shown mixed results regarding beneficial effects on infarct size and left ventricular function. In the autologous stem cell transplantation in acute myocardial infarction (ASTAMI) trial we did not find any effects of intracoronary injection of mBMC on global left ventricular function in patients with AMI, as already reported [5]. However, the effects of intracoronary injections of mBMC on prothrombotic markers in patients with AMI have not been examined. Increased levels of prothrombotic markers like soluble tissue factor (TF) and D-dimer have been associated with worse outcome in patients with AMI [10–12]. Therefore, the aim of the present substudy of the ASTAMI trial was to assess the influence of bone marrow aspiration and intracoronary injections of mBMC on selected circulating prothrombotic markers in patients with STEMI.

Materials and methods

The patients demographics, study design, inclusion and exclusion criteria have already been published in 2006 [5]. Briefly, one hundred

Abbreviations: AMI, acute myocardial infarction; ASTAMI, the autologous stem cell transplantation in acute myocardial infarction trial; CK, creatine kinase; ETP, endogenous thrombin potential; F1 + 2, prothrombin fragment 1 + 2; LAD, left anterior descending artery; mBMC, mononuclear bone marrow cells; PAI-1, plasminogen activator inhibitor 1; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction; TF, tissue factor; tPA, tissue plasminogen activator.

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patients participating in the ASTAMI trial were included at Ullevål University Hospital and Rikshospitalet University Hospital, Oslo, Norway. The study subjects were between 40 and 75 years old, both gender with acute anterior wall STEMI and culprit lesion located proximal to the 2. diagonal branch in the left anterior descending artery (LAD). All patients were treated successfully with percutaneous coronary intervention (PCI) and stent implantation within 2 – 12 hours from symptom start and given a loading dose of clopidogrel 300 mg and thereafter 75 mg daily in addition to an initial dose of aspirin 300 mg followed by 75 mg daily.

The investigation conformed with the principles outlined in the Declaration of Helsinki and the Regional Committee for Medical Research Ethics approved the study protocol. Written informed consent was obtained from all the patients. The present study is a substudy of the ASTAMI trial that is registered at www.clinicaltrials.gov, NCT 00199823.

Study intervention

The patients were randomized 1:1 to receive intracoronary injections of mBMC or to a control group. Only the mBMC group was aspirated for 50 ml bone marrow from the iliac crest in local anesthesia 4 – 7 days after the acute PCI. The bone marrow mixed with 10000 IU heparin was centrifuged on a Ficoll density gradient (Axis-Shield, Oslo, Norway) for isolation of mBMC, washed, and resuspended in heparin plasma (heparin 1000 IU/mL). The next day, a median of 6 days (interquartile range 5–6) after the STEMI (day 0), 10 mL of the mBMC suspension containing a median number of 68×10^6 mononuclear cells (0.7×10^6 CD34+ cells) was injected in the infarct related coronary artery (LAD). After administration of heparin 100 IU/kg body weight intravenously, a 0.5 mm oversized over-the-wire balloon catheter was advanced to the proximal part of the stent on the culprit lesion in LAD and inflated with very low pressures (<2 bar) for 90 seconds obtaining no flow. At the same time, one third of the stem cell suspension, followed by 2 mL heparinized saline, was injected distally, followed by deflation of the balloon and reflow for 5 minutes between a total of 3 injections (10 mL). Patients in the control group did not undergo bone marrow aspiration or any further coronary intervention with intracoronary injection of bone marrow cells.

Haemostatic markers

TF is a key factor in the haemostatic system and initiates the extrinsic coagulation cascade by binding free factor VIIa and thereby forming the TF-factor VIIa complex [13]. This complex promotes the conversion of factor X to factor Xa which again converts the inactive prothrombin to thrombin with cleavage of prothrombin fragment 1+2 (F1+2) from prothrombin. Increased levels of endogenous thrombin potential (ETP) and F1+2 are reflecting thrombin generation which catalyzes the formation of fibrin from fibrinogen [14]. Activation of plasminogen to plasmin, fascilitated by tissue plasminogen activator (tPA) and inhibited by plasminogen activator inhibitor 1 (PAI-1), promotes fibrinolysis with degradation of fibrin to end products like D-dimer.

Laboratory methods

Blood samples were obtained by standard venipuncture between 8 and 9 AM after 12 hours fast the day before stem cell transplantation (day-1) and 1 day (day 1), 3 days (day 3), 2–3 weeks, and 3 months after stem cell transplantation (day 0) for determination of circulating levels of TF, F1+2, ETP, D-dimer, PAI-1 activity and tPA antigen. Citrated plasma (0.129 mM citrate in dilution 1:10) was used for all variables. Blood was stored on ice and separated within 30 minutes by centrifugation at 4 °C and $2500\times g$ for 20 minutes to obtain platelet-poor plasma. All samples were stored at −80 °C until analysis and thawed only once.

Enzyme immunoassays

TF was measured by an enzyme-linked immunoassay (Imubind® Tissue Factor, American Diagnostica Inc., US), F1+2 by an enzyme immunoassay (Enzygnost®F1+2, Siemens Healthcare Diagnostics, Marburg, Germany). ETP was determined according to the manufacturer's instruction (Thrombinoscope BV, Maastricht, The Netherlands) and the thrombin generation was measured on the Fluoroscan Ascent® fluorometer (Thermo Fisher Scientific OY, Vantaa, Finland). Fluorescence intensity was detected at the wavelengths of 390 nm and 460 nm for excitation and emission, respectively. A reagent of rTF and phospholipids in addition to a thrombin specific fluorogenic substrate (Z-Gly-Gly-Arg-AMC, Bachem, Bubendorf, Switzerland) in Hepes buffer containg $CaCl_2$, were added to the plasma prior to start to obtain a final concentration of 5pM, 4uM and 2.5 mM each. In order to calculate the final results, plasma was measured along with a thrombin calibrator with known thrombin activity (600nM, Thrombinoscope BV) as a reference. The software program (Thrombinoscope BV, version 3.0.0.29) enabled the calculation of ETP. All the experiments were run in duplicates. D-dimer was measured by enzyme immunoassay (Asserachrom, D-di™, Stago Diagnostica, Asniere, France) and PAI-1 activity by a bio immunoassay (Trinilize PAI-1 activity, Trinity Biotech, plc, Bray, Co. Wicklow, Ireland). The levels of tPA antigen was determined by enzyme immunoassay (TintElize®tPA, Trinity Biotech, Jamestown, NY, US). In our laboratory, the interassay coefficient of variation for TF was 4.4%; F1+2 7.8%; ETP 2.7%; D-dimer 6.5%; PAI-1 4.4%; tPA 3.5%

Statistical analysis

Variables are expressed as proportions, medians with 25,75 percentiles or means with standard deviation as appropriate. Differences between groups were assessed with the Mann–Whitney test. Categorical data were analyzed by the chi-square test. Friedman test was applied for testing the intragroup differences between several

Table 1
Baseline characteristics of the study subjects. Values are presented as proportion, means ± SD, or medians with 25th and 75th percentiles where appropriate. AII antagonist, angiotensin II receptor antagonist; ACE-I, angiontensin-converting enzyme inhibitor; BMI, body mass index; β-blocker, β adrenergic receptor blocker; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction by single-photon emission computed tomography; PCI, percutaneous coronary intervention; SBP, systolic blood pressure. P value<0.05 was considered to indicate statistically significant differences between the groups.

Characteristics of the total ASTAMI population (n = 100)	
Age (years)	57.4 (9.1)
Sex (% female)	16
Hypertension (%)	34
Diabetes (%)	9
Smokers (%)	44
BMI (kg/m^2)	26.7 (3.7)
SBP/DBP (mmHg)	132/83 (22/16)
Total cholesterol (mmol/L)	4.4 (3.8, 5.0)
LDL cholesterol (mmol/L)	2.9 (2.3, 3.4)
HDL cholesterol (mmol/L)	1.0 (0.8, 1.3)
Triglycerides (mmol/L)	1.3 (1.0, 1.6)
Symptom start to PCI (min)	210 (180, 330)
LVEF (SPECT) (%)	41.9 (11.0)
Peak CK-MB (μg/L)	369 (220, 444)
Thrombolysis before PCI (%)	29
Medication at discharge	
Aspirin (%)	100
Clopidogrel (%)	100
ACE-I/AII antagonist (%)	100
Warfarin (%)	11
Beta blocker (%)	99
Statin (%)	100
Diuretics (%)	37