**Clinical research**

**Coronary heart disease**

**Intramyocardial injection of vascular endothelial growth factor-A₁₆₅ plasmid followed by granulocyte-colony stimulating factor to induce angiogenesis in patients with severe chronic ischaemic heart disease**

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**Aims**

To assess the safety and effects of combined treatment with vascular endothelial growth factor-A₁₆₅ plasmid (VEGF-A₁₆₅) and granulocyte-colony stimulating factor (G-CSF) mobilization of bone marrow stem cells in patients with severe chronic ischaemic heart disease (IHD).

**Methods and results**

Sixteen patients with severe chronic IHD were treated with intramyocardial injections of VEGF-A₁₆₅ plasmid followed 1 week later by G-CSF (10 µg/kg/day for 6 days). Two control groups included (i) sixteen patients treated with intramyocardial injections of VEGF-A₁₆₅ plasmid and (ii) sixteen patients treated with intramyocardial injections of placebo. In the G-CSF group, circulating CD34⁺ stem cells increased almost 10-fold compared with the control groups (P < 0.0001). After 3 months, there was no improvement in myocardial perfusion at single photon emission computerized tomography in the VEGF-A₁₆₅ and G-CSF treated group, and clinical symptoms were unchanged. There were no side effects to the gene and G-CSF therapy.

**Conclusion**

Intramyocardial VEGF-A₁₆₅ gene transfer followed by bone marrow stem cell mobilization with G-CSF seemed safe. However, a significant increase in circulating stem cells did not lead to improved myocardial perfusion or clinical effects suggesting a neutral effect of the treatment. To improve homing of stem cells, higher doses of VEGF-A₁₆₅ and/or use of SDF-1 transfer might be considered.

**KEYWORDS**

Cardiovascular diseases; Gene therapy; Stem cell; Angiogenesis; G-CSF

**Introduction**

Several vascular growth factors have the potential to induce angiogenesis in ischaemic tissue.¹⁻³ However, only vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) have been tested in clinical studies of patients with occlusive coronary artery disease (CAD).⁴⁻¹⁰ Small and unblinded gene therapy studies of intramyocardial delivered genes encoding VEGF-A₁₆₅ or VEGF-A₁₂₁ have been performed in patients with severe CAD and results have been encouraging, demonstrating both clinical improvement and evidence of angiogenesis.⁵,⁸⁻¹⁰ However, in the first large double-blind randomized placebo-controlled study, we could not demonstrate significant improvement in myocardial perfusion, when compared with placebo.⁴

Haematopoietic stem cells from the bone marrow have the potential to induce vasculogenesis in animals with an acute myocardial infarction.¹¹,¹² Recent human studies indicate that mononuclear cell solutions aspirated from the bone marrow can induce vasculogenesis both in acute and chronic myocardial ischaemia.¹³⁻¹⁹ However, it remains unknown, whether the vasculogenesis is induced by the few (2–3%) stem cells within the mononuclear cells suspension¹⁷ or by cytokines released from the leucocytes. It has been demonstrated that treatment with granulocyte-colony stimulating factor (G-CSF), in order to mobilize stem cells from the bone marrow, does not induce vasculogenesis in patients with chronic myocardial ischaemia²⁰,²¹ or following acute myocardial infarction.²² Animal studies suggest that a combination of treatment with VEGF-A gene transfer followed by G-CSF mobilization of stem cells might be superior to either of the therapies.²³

The aim of the present study was, in a clinical phase I safety and efficacy study, to evaluate the safety and clinical
effect of VEGF-A165 gene transfer followed by bone marrow stimulation with G-CSF to induce myocardial vasculogenesis in patients with severe occlusive CAD.

Methods

Population

Patients were eligible in the study if they had (i) significant reversible myocardial ischemia on an adenosine stress single photon emission computerized tomography (SPECT) as judged by an experienced nuclear physician blinded to all other patient data, (ii) at least one remaining large coronary vessel from which new collaterals/vessels could be supplied, (iii) age between 18 and 75 years, (iv) stable angina pectoris and Canadian Cardiovascular Society (CCS) class ≥ 3. Excluded were patients with (i) unstable angina pectoris, (ii) acute myocardial infarction within the last 3 months, (iii) diabetes mellitus with proliferative retinopathy, (iv) diagnosed or suspected cancer, (v) chronic inflammatory disease, and (vi) fertile women.

All patients received oral and written information, and signed a written informed consent. The study followed the recommendations of the Helsinki II Declaration and was approved by the Ethics Committee of Copenhagen (KF 01-130/01) and the Danish Drug Agency (2612-1490). The study has been registered in clinicaltrial.gov (NCT00135850).

Study design

We prospectively treated 16 patients with severe chronic CAD and no option for revascularization with open-label VEGF-A165 gene transfer followed by G-CSF bone marrow stem cell mobilization (15 men, 1 woman, mean age 62 years, Table 1). Patients were treated with direct intramyocardial injections of the VEGF-A165 plasmid followed one week later by in-hospital daily subcutaneous injection of 10 µg/kg body weight G-CSF (Neupogen®) for 6 days. We (Rigshospitalet, Copenhagen) have previously randomized a total of 32 patients (16 active and 16 placebo in the double-blind Euroinject One multicentre trial.4 These patients were used as controls: (i) sixteen patients treated with VEGF gene transfer alone (15 men, 1 woman, mean age 62 years, Table 1). Patients were treated with direct intramyocardial injections of the VEGF-A165 plasmid followed one week later by in-hospital daily subcutaneous injection of 10 µg/kg body weight G-CSF (Neupogen®) for 6 days. We (Rigshospitalet, Copenhagen) have previously randomized a total of 32 patients (16 active and 16 placebo in the double-blind Euroinject One multicentre trial.4 These patients were used as controls: (i) sixteen patients treated with VEGF gene transfer alone (15 men, 1 woman, mean age 62 years, Table 1). Patients were treated with direct intramyocardial injections of the VEGF-A165 plasmid followed one week later by in-hospital daily subcutaneous injection of 10 µg/kg body weight G-CSF (Neupogen®) for 6 days. We (Rigshospitalet, Copenhagen) have previously randomized a total of 32 patients (16 active and 16 placebo in the double-blind Euroinject One multicentre trial.4 These patients were used as controls: (i) sixteen patients treated with VEGF gene transfer alone (15 men, 1 woman, mean age 62 years, Table 1). Patients were treated with direct intramyocardial injections of the VEGF-A165 plasmid followed one week later by in-hospital daily subcutaneous injection of 10 µg/kg body weight G-CSF (Neupogen®) for 6 days.

Before inclusion, patients had a screening of blood tests, urinary tests, stools for occult blood, and chest X-ray. Patients with diabetes mellitus had an ophthalmoscopy, and a mammography was performed in all women. All patients were scheduled to undergo clinical examination, exercise test, stress SPECT, coronary angiography, and cardiac magnetic resonance imaging (MRI) before the gene transfer and 3 months after. In addition, a clinical examination was performed after 1 month. Peripheral circulating stem cells (CD34+ cells) and biochemistry controls were measured before and on day 7, 14, 21, and 30 after treatment. Circulating CD34+ progenitor cells were determined by flow cytometry and plasma VEGF-A165 and SDF-1 by ELISA.

A total of 73 patients were screened for inclusion; 50 of these were eligible for inclusion but two refused consent resulting in a patient population of 48 (16 in each group). All patients in all three groups (except one patient in the placebo group who died) went through the 3 months follow-up as planned.

Safety assessment

Each patient was carefully examined and interviewed about possible side-effects to the treatment by a physician the day after the gene/placebo injection, after the G-CSF treatment, and at the 1 and 3 month visits. All adverse and serious adverse events (SAEs) within the 3-month follow-up period were recorded in all groups. SAEs are defined as any adverse events that result in any of the following outcomes: death, life-threatening conditions, hospitalization or prolongation of existing hospitalization, persistent or significant disability/incapacity, medically significant event (includes serious laboratory abnormalities), and any new cancer.

Efficacy assessment

The pre-specified efficacy endpoint was changes in perfusion defects at stress SPECT from baseline to 3 months follow-up. Secondary exploratory endpoints were change in (i) CCS angina class, (ii) Seattle Angina Pectoris Questionnaire (SEQ) scores, (iii) frequency of angina attacks, (iv) nitroglycerine consumption, (v) exercise capacity, and (vi) left ventricular volumes measured by SPECT and MRI.

Single photon emission computerized tomography

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), combined with a sub-maximal exercise test except in patients with left bundle branch block.24,25 Care was taken to perform the stress tests at the inclusion and at the follow-up studies with identical cumulative adenosine doses and identical sub-maximal exercise loads. Gated (eight frames) imaging was performed with a two-headed Millennium GE gamma camera, with a Gadolinium interleaved attenuation-scatter correction. A disk with investigations of nine patients belonging to VEGF-A165 and 10 patients to the placebo group was accidentally destroyed and was technically unreadable. Therefore, these data are missing in the comparison analyses.

An independent core laboratory (Bio-Imaging Technologies B.V., Leiden, The Netherlands) performed blinded readings of the SPECT investigations.

NOGA®—electromechanical mapping of left ventricle

Electromechanical evaluation of the left ventricle was performed with the NOGA® system (Biosense Webster A/S, Cordis, Johnson & Johnson) as previously described.4,26,27 The diagnostic NOGA® catheter was introduced percutaneously via the groin into the left ventricle. A sensor at the tip of the catheter in contact with the endocardial surface registered the local myocardial unipolar voltage and regional wall motion/contraction (local shortening) within the left ventricle, and created a three-dimensional colour image of the left ventricle as described previously.4

Intramyocardial injections

The intramyocardial injections were done with the 8-french-sized Myostar® mapping-injection catheter (Biosense Webster A/S, Cordis, Johnson & Johnson) inserted into the left ventricle via the groin.4 Ten 0.3 mL injections were given around and within the area with reversible ischaemia with a total dose of 0.5 mg VEGF-A165 or placebo plasmid. The injections were performed slowly (30–40 s) and only to areas with unipolar voltage above 5 mV and with a thickness of the ventricular wall on echocardiography exceeding 6 mm.

Quality control of the injections included (i) the catheter’s tip perpendicular (+30°) to the left ventricular wall in two planes, (ii) the loop stability at the same level as during the mapping procedure, if possible <2 mm, and (iii) one or more ectopic extraventricular beats should appear in the exact moment of the protrusion of the injection needle into the myocardium.

Plasmid VEGF-A165 and placebo plasmid

The plasmid contained a cytomegalovirus promoter/enhancer to drive VEGF-A165 expression. The placebo plasmid was identical to
of 4, on the basis of previous results.4

level of 0.05 and an assumed standard deviation (of the differences) per fusion defects at stress SPECT, with a two-sided significance detect a difference of 30% from baseline to 3 months follow-up in G-CSF group was calculated to yield an expected power of 0.8 to immediately after intravenous injection of gadopentetate dimeglu-
single position in the true short axis of the left ventricle starting
Myocardial perfusion was assessed as the change in MR signal inten-
reach steady-state concentration during 2 min initial infusion.
Statistical analysis
For comparisons between baseline and follow-up data within the
groups, we used Wilcoxon signed-ranks test, whereas between
group comparisons were done using one-way ANOVA. All data were
analysed using SPSS statistical analysis program (SPSS version 12.0, SPSS Inc., Chicago, Il, USA). To account for multiple testing, a difference was only considered statistically significant if two-sided


table 1 Demographic data for patients treated with placebo, plasmid VEGF-A165, or plasmid VEGF-A165 and G-CSF at baseline

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 16)</th>
<th>VEGF-A165 (n = 16)</th>
<th>VEGF-A165 + G-CSF (n = 16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 ± 9</td>
<td>61 ± 7</td>
<td>62 ± 9</td>
<td>0.9</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>(14/2)</td>
<td>(15/1)</td>
<td>(14/2)</td>
<td>0.8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29 ± 5</td>
<td>28 ± 3</td>
<td>29 ± 4</td>
<td>0.7</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>1 (6)</td>
<td>4 (25)</td>
<td>2 (13)</td>
<td>0.5</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>1 (6)</td>
<td>4 (25)</td>
<td>7 (44)</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>6 (38)</td>
<td>9 (56)</td>
<td>7 (44)</td>
<td>0.7</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>16 (100)</td>
<td>16 (100)</td>
<td>16 (100)</td>
<td>1.0</td>
</tr>
<tr>
<td>Previous CABG, n (%)</td>
<td>14 (88)</td>
<td>16 (100)</td>
<td>16 (100)</td>
<td>0.3</td>
</tr>
<tr>
<td>Previous PCI, n (%)</td>
<td>10 (63)</td>
<td>11 (69)</td>
<td>9 (56)</td>
<td>0.8</td>
</tr>
<tr>
<td>Previous STEMI, n (%)</td>
<td>11 (69)</td>
<td>7 (44)</td>
<td>6 (38)</td>
<td>0.3</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>53 ± 11</td>
<td>57 ± 9</td>
<td>57 ± 10</td>
<td>0.4</td>
</tr>
<tr>
<td>CCS</td>
<td>3.0 ± 0.0</td>
<td>3.1 ± 0.3</td>
<td>3.0 ± 0.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Number of patients with two or three-vessels disease, n</td>
<td>2/14</td>
<td>3/13</td>
<td>4/12</td>
<td>0.9</td>
</tr>
<tr>
<td>Occluded vessel segments (LM/LAD/LCX/RCA), n</td>
<td>4/12/13/14</td>
<td>2/13/11/12</td>
<td>2/14/13/12</td>
<td>1.0</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction measured by ventriculography; CABG, coronary artery bypass grafting; CCS, Canadian Cardiovascular Society angina classification; LM, left main artery; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery.

Values are expressed as mean ± SD or n (%).

Magnetic resonance imaging
MRI was only performed in the VEGF-A165 + G-CSF group and was not feasible in six patients due to claustrophobia or obesity. Myocardial function and perfusion were examined by MRI before and 3 months after VEGF-A165 and G-CSF treatment. The examinations were performed with 1.5 T (Siemens Vision Magnetom, Siemens AG, Erlangen, Germany) using a standard phased-array chest coil. Left ventricular volumes and systolic function were derived from successive short axis slices. Volumes were determined by planimetry and myocardial mass was determined by applying a density factor of 1.05 g/cm³.

Perfusion estimates were obtained from images acquired in a single position in the true short axis of the left ventricle starting immediately after intravenous injection of gadopentetate dimeglumine (Magnevist®, Schering AG) (0.1 mmol/kg) during intravenous infusion of adenosine (0.14 mg/kg/min), which was allowed to reach steady-state concentration during 2 min initial infusion. Myocardial perfusion was assessed as the change in MR signal intensity as a function of time during the first pass (initial slope).28

The investigations were analysed blinded to all patient-data, using the CMRtools software.

Statistical analysis
The sample size of 16 patients in the combined VEGF-A165 gene and G-CSF group was calculated to yield an expected power of 0.8 to detect a difference of 30% from baseline to 3 months follow-up in perfusion defects at stress SPECT, with a two-sided significance level of 0.05 and an assumed standard deviation (of the differences) of 4, on the basis of previous results.4

Baseline characteristics were compared using Fischer’s exact test for categorical or one-way ANOVA for continuous variables.

For comparisons between baseline and follow-up data within the groups, we used Wilcoxon signed-ranks test, whereas between group comparisons were done using one-way ANOVA. All data were analysed using SPSS statistical analysis program (SPSS version 12.0, SPSS Inc., Chicago, Il, USA). To account for multiple testing, a difference was only considered statistically significant if two-sided P < 0.01.

Results
The baseline characteristics of the patients are depicted in Table 1. All patients had severe stable chronic angina pectoris and limited exercise capacity. They were all on maximal tolerable anti-angina therapy with short- and long-lasting nitroglycerin, calcium antagonists, beta-blockers, and ACE-inhibitors. All had previously been treated with at least one coronary artery bypass surgery or percutaneous coronary intervention (PCI).

Safety data
There were no major side effects during the combined VEGF-A165 gene and G-CSF treatment or in the follow-up period. During G-CSF treatment, two patients had slight muscular discomfort, which disappeared after NSAID treatment. One patient with a previous history of a gall bladder stone abdominal pains developed acute abdominal pains and increase in plasma liver parameters. Symptoms and liver test normalized immediately after cessation of G-CSF treatment. No change was seen in liver function tests in the remaining patients.

Five patients in the placebo group had SAEs: one ST-elevation myocardial infarction (STEMI) with third-degree AV-blockade, the patient progressed into cardiogenic shock despite implantation of a pacemaker and subsequently died 2 months after the placebo treatment; two uncomplicated STEMI; one non-STEMI; and one newly developed coronary in-stent stenosis, which needed treatment with PCI. In the plasmid VEGF-A165 group, three patients developed new coronary artery in-stent stenoses with symptoms of unstable angina.
Single photon emission computerized tomography

The combined VEGF-A165 and G-CSF treated group had no changes in myocardial perfusion at rest and stress between baseline and follow-up, and they had identical summed difference perfusion scores (Table 2). Left ventricular end-diastolic (EDV) and end-systolic volumes (ESV), and ejection fraction showed no significant difference in any of the three groups from baseline to follow-up, and there were no differences between changes in these parameters between groups (Table 3). In addition, regional wall thickening and motion were unchanged from baseline to follow-up in the group treated with VEGF-A165 and G-CSF (Table 4).

Clinical outcome

There was no significant difference in changes in CCS classification, angina pectoris attacks, NTG consumption, or exercise time between the three groups (Table 5). This pattern was unchanged, also after classifying patients in the VEGF-A+G-CSF group into CD34+ stem cell responders and non-responders. The SEQ scores demonstrated improvement in physical limitation, angina stability, and angina frequency in all groups, and improvement in treatment satisfaction and disease perception in the VEGF gene transfer group only.

Magnetic resonance imaging

Both EDV and ESV increased, and the change in ESV from 40 to 46 mL was statistically significant (P = 0.009, Table 6). However, the increase in ESV did not change the left ventricular ejection fraction significantly, and there was no difference in left ventricular mass. There was no significant increase in perfusion in the region treated with gene injections (Table 6). Representative images of the myocardial perfusion are shown in Figure 1.

Plasma VEGF-A165, SDF-1, and CD34+ progenitor cells

Circulating CD34+ stem cells from the bone marrow increased significantly during the G-CSF treatment, with
Table 4  Regional wall thickening and motion at rest measured by SPECT

<table>
<thead>
<tr>
<th>Placebo (n = 6)</th>
<th>VEGF-A165 (n = 7)</th>
<th>VEGF-A165 + G-CSF (n = 11)</th>
<th>Difference from baseline to follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>P-value</td>
</tr>
<tr>
<td>Apex (%)</td>
<td>37 ± 12</td>
<td>44 ± 18</td>
<td>0.2</td>
</tr>
<tr>
<td>Lateral wall (%)</td>
<td>20 ± 8</td>
<td>21 ± 9</td>
<td>0.7</td>
</tr>
<tr>
<td>Intraventricular septum (%)</td>
<td>22 ± 3</td>
<td>25 ± 8</td>
<td>0.3</td>
</tr>
<tr>
<td>Inferior wall (%)</td>
<td>16 ± 4</td>
<td>17 ± 8</td>
<td>0.8</td>
</tr>
<tr>
<td>Anterior wall (%)</td>
<td>26 ± 3</td>
<td>29 ± 6</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Mean ± SD.

Table 4  Regional wall thickening in % from end-diastole to end-systole

<table>
<thead>
<tr>
<th>Placebo (n = 6)</th>
<th>VEGF-A165 (n = 7)</th>
<th>VEGF-A165 + G-CSF (n = 11)</th>
<th>Difference from baseline to follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apex (mm)</td>
<td>5.1 ± 1.1</td>
<td>5.9 ± 1.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Lateral wall (mm)</td>
<td>7.5 ± 1.6</td>
<td>7.8 ± 1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Septal wall (mm)</td>
<td>1.9 ± 1.0</td>
<td>2.4 ± 1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Inferior wall (mm)</td>
<td>3.6 ± 0.7</td>
<td>4.2 ± 2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Anterior wall (mm)</td>
<td>7.5 ± 1.0</td>
<td>7.9 ± 0.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Mean ± SD.

Table 5  Changes in angiina and exercise time in patients treated with placebo, plasmid VEGF-A165, or plasmid VEGF-A165 + G-CSF

<table>
<thead>
<tr>
<th>CCS class</th>
<th>Placebo</th>
<th>VEGF-A165</th>
<th>VEGF-A165 + G-CSF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>1a</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
</tbody>
</table>

Mean ± SD.
treated groups and in the placebo group 1 week after the intramyocardial injections (Figure 2C).

Discussion

We present the first, single-centre, clinical safety and efficacy trial, in which patients with chronic reversible myocardial ischaemia have been treated with intramyocardial injections of the gene encoding VEGF-A 165, followed by treatment for 6 days with G-CSF stem cell mobilization from the bone marrow. This treatment was well tolerated with no increase in adverse events, when compared with gene transfer or placebo or G-CSF treatment alone.4,20,21 Several small and uncontrolled clinical studies have indicated that growth factor gene transfer might be safe and have the potential to improve myocardial perfusion.5,8–10 In the first large double-blind placebo-controlled study, we could not demonstrate improved myocardial perfusion after VEGF-A 165 gene transfer compared with placebo in patients with severe CAD.4 However, the study may have been underpowered since we found a significant improvement within the VEGF-A 165 group. Also, in a comparable group of patients, we found that G-CSF mobilization of stem cells from the bone marrow did not improve myocardial perfusion or symptoms.20 Animal studies have suggested that the combination of gene transfer for VEGF-A 165 and G-CSF mobilization of stem cells from the bone marrow induce angiogenesis more effectively than gene therapy alone.23

In the present study, we could not demonstrate a significant improvement in myocardial perfusion or symptoms in patients with chronic reversible myocardial ischaemia after the combined therapy with gene transfer of VEGF and subsequent bone marrow stimulation with G-CSF. The discrepancy between animal research and studies in patients, as the present one, might be related to the definition of chronic ischaemia. In animal studies, chronic ischaemia will include components of acute and subacute ischaemia as well. Most animal studies induce chronic myocardial ischaemia, using an ameroid constrictor around the circumflex or anterior descendent artery. Four to five weeks later the myocardium is often called chronic ischaemic myocardium. However, the intracellular milieu is probably not equivalent to patients' myocardium suffering from chronic ischaemia for several years. In patients with acute myocardial infarction, plasma concentrations of the vascular growth factors VEGF and b-FGF, and the stem cell homing factor SDF-1 increase gradually above control levels with maximum ~3 weeks after the infarction. This could indicate that it takes some time to initiate the transcription of the genes for the cytokine production.29

Furthermore, transfection of cells with the VEGF gene after intramyocardial injection is probably similar in chronic human or pig ischaemic myocardium. However, the transcription of the transferred VEGF gene and thus the induced VEGF production might be different within the human cells after prolonged ischaemia and in animal cells after short-term experimental ischaemia.

Recently, it has been speculated if the VEGF production is already increased within chronic ischaemic human myocardium, thus attempts to further stimulate angiogenesis via an additional VEGF gene stimulation would potentially be without effect. However, we recently studied biopsies from human chronic ischaemic myocardium and found identical quantities of VEGF mRNA in chronic ischaemic myocardium compared with non-ischaemic normal perfused myocardium in the same patient.30 Thus, it seems that VEGF-A 165 gene therapy can potentially increase the local production of the growth factor stimulating the growth of new blood vessels. For safety reasons, however, we chose...
a low VEGF-A165 plasmid dose. A higher dose should be considered for further studies.

We could not confirm the hypothesis, that the combination therapy would increase local production of VEGF and the number of circulation endothelial progenitor cells homing into the ischaemic myocardium, suggested by experimental animal studies, in this clinical study of patients suffering from severe, chronic CAD.

SDF-1 has been found essential for stem cell mobilization/homing after arterial injury. In a recent study, it has been demonstrated that SDF-1 gene transfer increased the homing of bone-marrow-derived stem cells in infarcted myocardium but not in normally perfused myocardium, and induced both vasculogenesis and angiogenesis. Moreover, blockade of VEGF prevented all such SDF-1 effects. We have found that there is no difference between the SDF-1 mRNA levels in normally perfused and chronic ischaemic human myocardium. Therefore, the missing effect of combined gene therapy and stem cell mobilization might be due to a low SDF-1 level in the chronic ischaemic tissue resulting in poor engraftment of stem cells despite an increased number of circulating stem cells as seen during G-CSF treatment.

The efficacy results of the combined treatment have a potential limitation, as (i) it was not the primary endpoint and (ii) missing data potentially introduced a selection bias. However, the patients with missing data were random (due to a disc error) thus minimizing the risk of selection bias. Still, these results should be interpreted with caution.

In conclusion, combined VEGF-A165 gene transfer and bone marrow stem cell mobilization with G-CSF in patients with chronic myocardial ischaemia is safe but, despite a significant increase in circulating stem cells, there were no signs of clinical effects or improved myocardial perfusion of the ischaemic area. Evaluation of homing of circulating stem cells in the clinical setting needs further studies. Higher VEGF-A165 gene doses and the addition of SDF-1 gene transfer might be considered.

Acknowledgements

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Conflict of interest: none declared.

References

Myocardial Doppler tissue velocity improves following myocardial gene therapy with VEGF-A165 plasmid in patients with inoperable angina pectoris. 


Mesquita ET, Willerson JT. Comparison of the left ventricular electromechanical map before percutaneous coronary stent revascularization and at one-month follow-up in patients with a recent ST elevation infarction. 


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