

CLINICAL RESEARCH

Clinical Trial

Direct Intramyocardial Plasmid Vascular Endothelial Growth Factor-A₁₆₅ Gene Therapy in Patients With Stable Severe Angina Pectoris

A Randomized Double-Blind Placebo-Controlled Study: The Euroinject One Trial

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OBJECTIVES	In the Euroinject One phase II randomized double-blind trial, therapeutic angiogenesis of percutaneous intramyocardial plasmid gene transfer of vascular endothelial growth factor (phVEGF-A ₁₆₅) on myocardial perfusion, left ventricular function, and clinical symptoms was assessed.
BACKGROUND	Evidence for safety and treatment efficacy have been presented in phase I therapeutic angiogenesis trials.
METHODS	Eighty “no-option” patients with severe stable ischemic heart disease, Canadian Cardiovascular Society functional class 3 to 4, were assigned randomly to receive, via the NOGA-MyoStar system (Cordis Corp., Miami Lakes, Florida), either 0.5 mg of phVEGF-A ₁₆₅ (n = 40) or placebo plasmid (n = 40) in the myocardial region showing stress-induced myocardial perfusion defects on ^{99m} Tc sestamibi/tetrofosmin single-photon emission computed tomography.
RESULTS	No differences among the groups were recorded at baseline with respect to clinical, perfusion, and wall motion characteristics. After three months, myocardial stress perfusion defects did not differ significantly between the VEGF gene transfer and placebo groups (38 ± 3% and 44 ± 2%, respectively). Similarly, semiquantitative analysis of the change in perfusion in the treated region of interest did not differ significantly between the two groups. Compared with placebo, VEGF gene transfer improved the local wall motion disturbances, assessed both by NOGA (p = 0.04) and contrast ventriculography (p = 0.03). Canadian Cardiovascular Society functional class classification of angina pectoris improved significantly in both groups but without difference between the groups. No phVEGF-A ₁₆₅ -related adverse events were observed; however, NOGA procedure-related adverse events occurred in five patients.
CONCLUSIONS	The VEGF gene transfer did not significantly improve stress-induced myocardial perfusion abnormalities compared with placebo; however, improved regional wall motion, as assessed both by NOGA and by ventriculography, may indicate a favorable anti-ischemic effect. This result should encourage more studies within the field. Transient VEGF overexpression seems to be safe. (J Am Coll Cardiol 2005;45:982–8) © 2005 by the American College of Cardiology Foundation

Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) have been used in clinical trials to achieve better perfusion through angiogenesis in patients

with chronic myocardial ischemia based on occlusive coronary artery disease (1). After intracoronary infusion of adenoviral vectors encoding VEGF-A₁₆₅ or FGF-4, controlled trials reporting improved perfusion (2,3) and a trend toward an increase in exercise capacity (4) have been encouraging. The efficacy of percutaneous intramyocardial transfer of VEGF encoding genes was explored in small phase I/II studies. The results suggest clinical benefit and improved myocardial perfusion (5,6).

The aim of the present phase II randomized double-blind placebo-controlled multicenter study was to explore the angiogenic and clinical efficacy of direct percutaneous intramyocardial plasmid phVEGF-A₁₆₅ transfer in patients with severe coronary artery disease not appropriate for conventional revascularization.

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Abbreviations and Acronyms

CCS	= Canadian Cardiovascular Society
FGF	= fibroblast growth factor
PCI	= percutaneous coronary intervention
ROI	= region of interest
SD/cord	= standard deviation/cord
SPECT	= ^{99m} Tc sestamibi/tetrofosmin single-photon emission computer tomography
VEGF	= vascular endothelial growth factor

METHODS

The study followed the recommendations of the Helsinki II Declaration and was approved by the national ethics committees and the national drug agencies in the countries of the six participating centers. All patients received verbal and written information about the study and gave their written and signed informed consent. Centralized randomization was performed according to international guidelines by the hospital pharmacy at the Karolinska University Hospital. Numbered vials containing the plasmid (VEGF or placebo) were sent to each center. The code was not revealed until after all core lab analyses had been performed.

Study protocol. Patients with symptomatic severe coronary artery disease that could not be revascularized further were included if adenosine stress ^{99m}Tc sestamibi/tetrofosmin single-photon emission computerized tomography (SPECT) showed a significant reversible perfusion defect as estimated by two independent experienced observers. Patients also were included if they: 1) had at least one large epicardial coronary artery with <70% stenosis remaining from which new collaterals/vessels could be supplied; 2) if they were between 18 and 75 years of age; and 3) if they had stable angina pectoris and a Canadian Cardiovascular Society (CCS) classification ≥ 3 . We excluded patients with an ejection fraction <0.40, unstable angina pectoris, acute myocardial infarction within the last three months, diabetes mellitus with proliferative retinopathy, diagnosed or suspected cancer, or chronic inflammatory disease. We also excluded premenopausal women.

Before inclusion, the following screening tests were performed: routine blood tests, plasma tumor markers (carcinoembryonic antigen, prostate-specific antigen), urinary tests, stools for occult blood ($\times 3$), and chest X-ray. Proliferative retinopathy was excluded by ophthalmoscopy in patients with diabetes mellitus. A mammogram was performed in all women.

The prespecified end points at three months follow-up were: 1) the efficacy of the intramyocardial gene transfer, measured by change in myocardial perfusion defects at stress and rest between inclusion and three-month follow-up SPECT studies (primary end point); 2) the safety of the percutaneous intramyocardial gene therapy, including risks related to the intramyocardial injection procedure and to the VEGF administration; 3) changes in wall motion disturbances at NOGA endocardial mapping and contrast left

ventriculography; 4) CCS angina pectoris class; 5) the frequency of anginal attacks; 6) nitroglycerin consumption; 7) patient score on the Seattle Angina Pectoris Questionnaire, and 8) exercise capacity (secondary end points). Current medication was not changed until follow-up was completed. Additional clinical follow-up was performed six months after the intramyocardial injections. Signs of VEGF expression (Quantkine; R&D Diagnostics, Minneapolis, Minnesota), inflammation in terms of C-reactive protein, and VEGF-induced recruitment of CD34⁺ stem cells (flow cytometry) were determined from successive blood determinations.

Plasmid VEGF-A₁₆₅ and placebo plasmid. The plasmid contained a cytomegalovirus promoter/enhancer to drive VEGF-A₁₆₅ expression. The placebo plasmid was identical to the plasmid VEGF-A₁₆₅ except for the VEGF-A₁₆₅ gene, which had been cut out. Both plasmids were sequenced according to Good Medical Practice (GMP) standards. The placebo plasmid did not contain any VEGF DNA. Both the phVEGF-A₁₆₅ and the placebo plasmid were produced at the Gene Therapy Center, Huddinge University Hospital (Stockholm, Sweden).

Single-photon emission computed tomography and NOGA: electromechanical mapping of the left ventricle.

Single-photon emission computed tomography studies were conducted using a two-day protocol (500 to 700 MBq ^{99m}Tc-sestamibi or tetrofosmin at each study) (7) with combined low-level exercise-adenosine infusion during the course of 4 to 6 min (0.14 mg/kg/min by infusion pump). Patients received 0.25 mg of nitroglycerin before the rest scan. Care was taken to perform the stress tests at inclusion and at the follow-up studies at the same time of day with identical exercise loads and cumulative adenosine doses.

With the NOGA system (i.e., NOGA mapping catheter and MyoStar injection catheter; Cordis, Johnson & Johnson, Miami Lakes, Florida), diagnostic three-dimensional maps of the left ventricle were generated for the locally measured voltage values (voltage map) and the systolic-diastolic movement of the catheter tip (local linear shortening map) (8-10). There were two criteria for acceptance of the NOGA map: at least three edited points at each of the 12 segments of the polar map and a minimum of 50 points on the edited NOGA map.

Intramyocardial injections. On the basis of the localization of the ischemic region assessed by SPECT and the local linear shortening map, the region of interest (ROI) was delineated on the NOGA map, and the injection catheter was navigated into this area. Ten 0.3-ml intramyocardial injections were given with the 8-F-sized MyoStar mapping injection catheter within the delineated area with a total dose of 0.5 mg phVEGF-A₁₆₅ or placebo plasmid. The injections were performed slowly (30 to 40 s) and only to areas with a unipolar voltage >5 mV and with a thickness of the ventricle wall at echocardiography exceeding 6 mm.

Quality control of the injections included the following: 1) the catheter's tip perpendicular ($\pm 30^\circ$) to the left ven-

tricular wall in two planes; 2) loop stability at the same level as during the mapping procedure, if possible <2 mm; and 3) ectopic extraventricular beats at the exact moment of the protrusion of the injection needle into the myocardium.

Analysis of myocardial perfusion images. For semiquantitative and visual scoring core lab SPECT analysis, the treated area (ROI) on the SPECT images was determined on the basis of the NOGA polar plot images with the delineated treated areas provided by each center for its own patients. Because the NOGA treatment area did not completely fit the SPECT-displayed reversible ischemic areas, the usual SPECT segmental analysis was inadequate. Therefore, a three-point scoring system was used. First, the severity of the reversible and irreversible perfusion defects at baseline was scored as defects present or defects not present as consensus readings by three experienced nuclear medicine specialists. Second, to assess changes between baseline and three-month follow-up studies, these were read together as pairs in a randomized order, which was blinded to the readers. Changes were scored as deterioration (-1), unchanged (0), and improved (+1). Two reasons that excluded SPECT maps were death during follow-up (n = 1, placebo) and not interpretable as the result of poor image quality, as assessed by the core lab (n = 4, placebo; n = 1, VEGF).

Computer-based quantitative core lab SPECT analysis was made on global left ventricular perfusion because the ROI could not be exactly determined as a result of the uncertain concordance between SPECT and NOGA maps. Transaxial files of the baseline and follow-up rest and stress SPECT images (provided in DICOM format from all centers) were transferred to an image-analysis workstation (Onyx; SGI, Mountain View, California), and polar map analyses were performed using Munic-Heart software (Munich, Germany). The polar maps were then subdivided into 460 segments. The extent of severe (normalized tracer uptake ≤50%) and moderate (normalized tracer uptake between 51% and 70%) and the summarized (sum of severe and moderate) extent of rest and stress-induced perfusion defects were determined automatically and expressed as percentage of the entire myocardium.

Analysis of NOGA endocardial maps. In the quantitative core lab NOGA analysis, the ROI was delineated blinded on the basis of the injection maps at baseline and at the three-month follow-up. Researchers performed blinded quantitative assessments of the baseline and follow-up maps as mean voltage and as local linear shortening values of the ROI and remote regions.

Analysis of digitized left ventricular angiography. At the blinded core lab analysis, ventricular volumes were digitized and a centerline was constructed midway between the two contours (Quantcor LVA, Siemens, Munich, Germany), and 100 equally spaced cords were drawn perpendicular to the centerline between the contours. The normalized motion for each chord was displayed automatically (11). The severity of the abnormality of the regional wall motion within the left anterior descending, left circumflex coronary

artery, and right coronary artery areas was computed as the mean standardized motion of contiguous chords, and it was assessed as the average standard deviation per chord (SD/chord).

Statistical analysis. Sample size (n = 80) with alpha < 0.05 and beta = 0.80 was calculated on the basis of previously reported results on myocardial perfusion after gene therapy with phVEGF-A₁₆₅ or phVEGF-C in open phase I/II trials (5,12). The analysis was performed on the basis of intention to treat. Changes within groups between baseline and follow-up were tested using Wilcoxon's two-sided test for paired data and between groups with the two-sided Mann-Whitney U test or the exact Mann-Whitney U test. To assess differences between repeated measures between the placebo and VEGF groups, two-way analysis of variance was used. A difference was considered statistically significant at p < 0.05. Values are presented as mean ± SEM.

RESULTS

No demographic baseline differences were found between the two groups (Table 1). The following are the treated ischemic areas for the patients in the VEGF and placebo groups, respectively: anterior region (in 18% and 23%), lateral (in 25% and 15%), posterior (in 33% and 30%), septal (in 13% and 13%), and mixed (in 11% and 19%).

Safety data. Five procedure-related complications occurred, with three occurring in the VEGF group. During the injection procedure, one patient developed a pericardial tamponade, which was treated surgically without sequelae; one patient developed temporary loss of vision; and one patient was successfully treated for sepsis. Within the placebo group, one patient developed third-degree atrioventricular block and received a permanent pacemaker, and one patient developed an ST-segment elevation myocardial infarction in the injection area. In addition, during diagnostic NOGA before randomization at baseline, one patient developed pericardial tamponade and died as the result of an acute myocardial infarction during emergency surgery.

Table 1. Baseline Demographic Data in Patients Treated With Either Placebo or VEGF Gene Transfer

	Placebo	VEGF	p Value
Age, yrs	61 ± 2	61 ± 2	0.97
Gender, female/male	5/35	8/32	0.36
Diabetes, n (%)	8 (20)	7 (18)	0.77
Previous MI, n (%)	27 (68)	24 (60)	0.49
Prior CABG, n (%)	30 (75)	31 (78)	0.79
Prior PCI, n (%)	21 (52)	17 (42)	0.37
LVEF (%)	62 ± 11	61 ± 11	0.68
CCS class	3.0 ± 0.3	3.1 ± 0.3	0.55

Mean ± SEM. p value refers to differences between placebo- and VEGF transfer-treated groups.

CABG = coronary artery bypass surgery; CCS = Canadian Cardiovascular Society classification of angina pectoris; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PCI = percutaneous coronary intervention; VEGF = vascular endothelial growth factor.

Seventeen major cardiac complications occurred during the six-month follow-up. In the VEGF group, four patients required percutaneous coronary intervention (PCI) because of progression of stenosis at three months' follow-up. Three patients developed unstable angina pectoris, of which one required PCI. In the placebo group, acute myocardial infarction occurred in three patients, of which one died from cardiogenic shock two months after treatment. Three patients developed unstable angina pectoris. One patient required PCI because of progression of stenosis at the three-month follow-up. One patient developed third-degree atrioventricular block and received a pacemaker, and two patients were treated for heart failure. The independent safety committee found no serious adverse events related to the gene transfer. No patient developed clinical retinopathy, and no clinical or biochemical evidence of cancer was found.

Efficacy data. MYOCARDIAL PERFUSION ANALYSIS. The computer-based quantitative analysis of size of severe (normalized tracer uptake $\leq 50\%$) and moderate (normalized tracer uptake between 51% and 70%) perfusion defects at rest did not reveal significant differences between the groups neither at baseline nor at the three-month follow-up (Fig. 1). The extent of the severe and moderate defects during stress decreased nonsignificantly in both groups, and no difference at baseline and follow-up was found between the groups (Fig. 1).

Also, the size of the global (severe plus moderate) perfusion defects (normalized tracer uptake $\leq 70\%$) at stress did not differ between the VEGF gene transfer and placebo groups (expressed as percentage of the left ventricle: baseline, $48 \pm 3\%$ and $49 \pm 2\%$, $p = 0.73$; at three months, $38 \pm 3\%$ and $44 \pm 2\%$, $p = 0.18$, respectively), even if the global perfusion abnormality during stress improved significantly ($p = 0.04$, but not normalized) from baseline to follow-up in the VEGF group but not in the placebo group ($p = 0.22$).

Semiquantitative analysis of the ROI in SPECT showed no significant difference between the VEGF gene transfer and placebo groups in stress perfusion defects between inclusion and three months' follow-up, but a significant improvement was found from baseline to three-month follow-up after VEGF gene transfer, with improvement in 44% of the patients, no change in 41%, and impaired perfusion in 15% ($p = 0.02$). Corresponding fractions with placebo were 28%, 56%, and 16% (0.37), respectively.

NOGA mapping. The number of the acquired points of the ROI in the VEGF and placebo groups was 18 ± 1 and 22 ± 5 (total mapping points 100 ± 5 and 102 ± 5), respectively. The mean voltage values of the ROI did not differ at baseline or at the three-month follow-up between the groups (Fig. 2) and were close to normal (cut-off value 12.0 mV) (9). Similarly, the mean voltage values in the remote myocardium did not differ between the groups. The local linear shortening of the ROI was similarly impaired at baseline ($7.0 \pm 1.1\%$ and $7.2 \pm 1.0\%$ in the VEGF gene transfer and placebo groups, respectively). At the three-

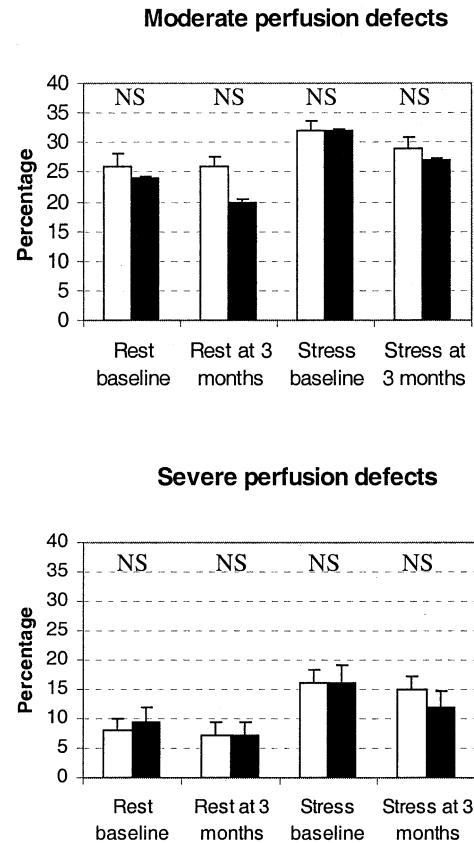


Figure 1. Computer-based quantitative analysis of myocardial perfusion. Fraction (%) of total segments (460) at polar map rest and stress single-photon emission computed tomography with severe (normalized tracer uptake $\geq 50\%$; upper panel) and moderate perfusion defects (normalized tracer uptake between 51% and 70% ; lower panel) at baseline and at follow-up in placebo (empty bars) and vascular endothelial growth factor-treated groups (filled bars). No differences between the groups were observed. Values are given as mean \pm SEM.

month follow-up, the local linear shortening was better in the VEGF gene transfer group than in the placebo group ($12.6 \pm 0.9\%$ vs. $9.9 \pm 0.9\%$, $p = 0.04$) (Fig. 2). Compared with the baseline, an improvement was observed in both groups (VEGF, $p < 0.001$; and placebo, $p = 0.05$). The mean local linear shortening of the remote myocardium did not change (VEGF, from $9.8 \pm 0.8\%$ to $10.0 \pm 0.7\%$, $p = 0.35$; placebo, from $9.3 \pm 0.7\%$ to $10.6 \pm 0.5\%$, $p = 0.28$). Figure 2 shows improved local linear shortening at the three-month follow-up in a patient treated with VEGF gene transfer.

Left ventriculography. Baseline global ejection fraction did not differ between the VEGF gene transfer group and placebo ($61 \pm 1.3\%$ and $62 \pm 1.3\%$, respectively) and was unchanged at follow-up ($61 \pm 1.5\%$ and $59 \pm 1.6\%$, respectively). The severity of local wall motion disturbance did not differ at baseline (-1.9 ± 0.1 SD/cord and -2.1 ± 0.1 SD/cord, respectively), whereas at follow-up it was better ($p = 0.03$) after VEGF gene transfer compared with placebo (-1.5 ± 0.1 SD/cord and -2.0 ± 0.2 SD/cord, respectively) (Fig. 3).

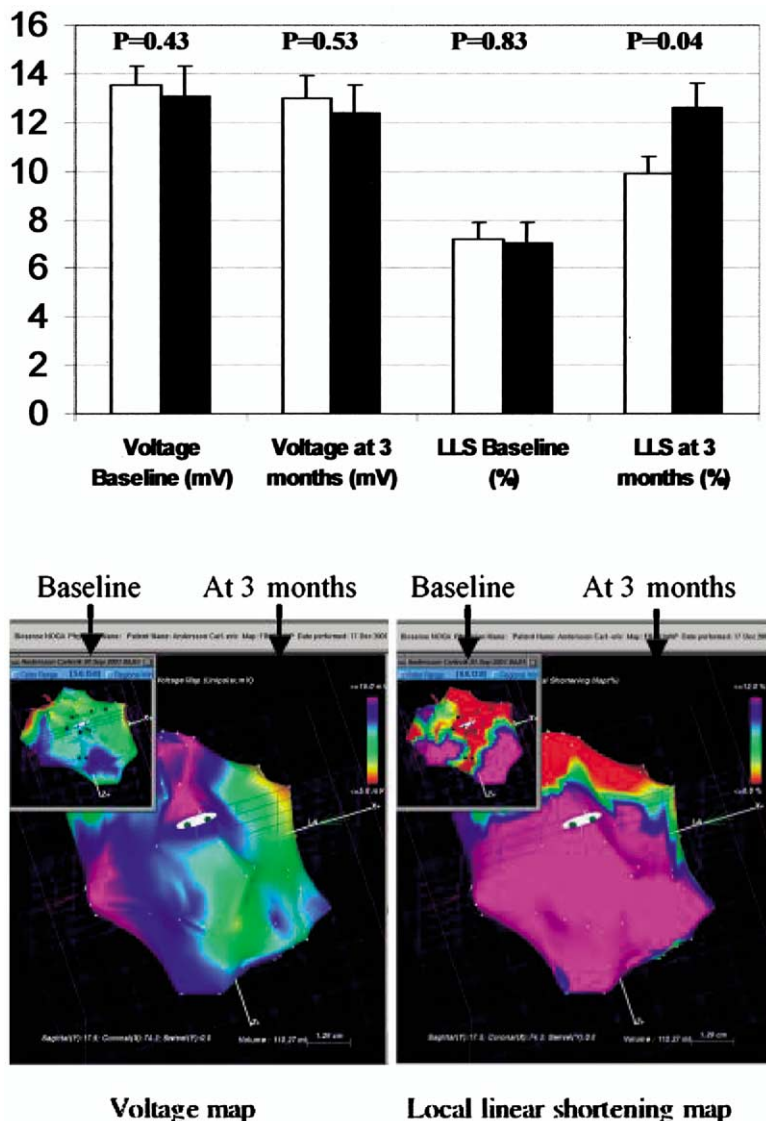


Figure 2. (Upper panel) Mean voltage and local linear shortening values of the injected area at baseline and at the three-month follow-up. No changes were observed in voltage values. Significantly better local linear shortening (LLS) in the injected area in the vascular endothelial growth factor group (**filled bars**) versus the placebo group (**empty bars**) at the three-month follow-up was noted. Values are given as mean \pm SEM. **(Bottom panel)** NOGA mapping from a 56-year-old patient with chronic occlusion of the left circumflex coronary artery. The patient received intramyocardial injection of plasmid encoding vascular endothelial growth factor-A₁₆₅. Baseline registrations with injection points are shown in the small figure in the upper left corner of the NOGA map. The larger figures show three-month follow-up registrations. No change was observed in the voltage map, whereas improved local linear shortening was observed in the injection area.

Clinical outcome. At three-month follow-up, the CCS angina pectoris classification improved significantly in both groups (VEGF, from 3.0 ± 0.04 to 2.2 ± 0.1 , $p < 0.001$; placebo, from 3.1 ± 0.05 to 2.3 ± 0.1 , $p < 0.001$), with no significant difference between the groups and with no further change after six months (2.3 ± 0.2 and 1.9 ± 0.1 , respectively). At the three-month follow-up, no significant differences between the VEGF- and placebo-treated groups were observed regarding peak exercise capacity (108 ± 6 W and 112 ± 8 W, $p = 0.69$) with no further change after six months. Furthermore, at the three-month follow-up, no significant differences between the VEGF and placebo groups were observed regarding nitroglycerin consumption (11 ± 3 tablets/week and 10 ± 4 tablets/week, $p = 0.15$)

and in the Seattle angina questionnaire scores (units): physical limitation (46 ± 3 and 46 ± 4 , $p = 1.00$), angina stability (69 ± 5 and 74 ± 5 , $p = 0.48$), angina frequency (57 ± 4 and 52 ± 5 , $p = 0.44$), disease perception (57 ± 4 and 57 ± 4 , $p = 1.00$), and treatment satisfaction (89 ± 2 and 83 ± 3 , $p = 0.06$).

Plasma VEGF-A, C-reactive protein, and circulating CD34⁺ stem cells. Plasma VEGF-A increased in both groups after treatment, reaching a peak value after one week (VEGF, from 69 ± 14 ng/l to 140 ± 30 ng/l, $p < 0.001$; placebo, from 70 ± 20 ng/l to 140 ± 42 ng/l, $p < 0.001$), but without a difference between the groups. CD34⁺ stem cells tended to be increased in the VEGF group three weeks after treatment (VEGF, from 2.8 ± 0.4 cells/ 10^6 /l to $4.3 \pm$

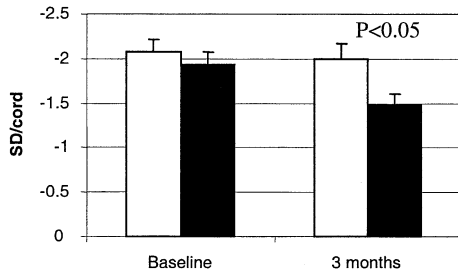


Figure 3. Segmental ventricular function of the injected area expressed as standard deviations (SD)/cord as assessed from digitized left ventriculography. Significantly better local wall motion was recorded in the vascular endothelial growth factor group (filled bars) versus the placebo group (empty bars) at the three-month follow-up. Values are given as mean \pm SEM.

0.6 cells/ 10^6 /l, $p = 0.07$; placebo, from 3.2 ± 0.6 cells/ 10^6 /l to 3.7 ± 0.4 cells/ 10^6 /l, $p = 0.25$), with no difference between the groups. C-reactive protein increased in the VEGF gene transfer group 24 h after treatment (VEGF, from 6.4 ± 1.2 mg/l to 8.5 ± 2.0 mg/l, $p = 0.03$; placebo, from 6.2 ± 1.1 mg/l to 7.8 ± 1.9 mg/l, $p = 0.98$) with no difference between the groups.

DISCUSSION

The Euroinject One trial is the first phase II multicenter, double-blind, randomized placebo-controlled trial investigating the therapeutic effect of percutaneous intramyocardial plasmid VEGF gene transfer in patients with severe stable chronic myocardial ischemia. This study also is the first to use the gene transfer vehicle, a placebo plasmid, for treatment in the control group.

The VEGF gene transfer did not significantly improve the stress-induced myocardial perfusion abnormalities compared with placebo. However, compared with the placebo group, both NOGA-determined left ventricular local linear shortening and the regional wall motion of the treated ischemic area (assessed by left ventriculography) were significantly better in the VEGF group at the follow-up.

The variability in myocardial perfusion at stress SPECT images was great in both groups. This variability was not anticipated on the basis of previous studies (5,12) and could be the result of both methodologic and biologic variability in patients with diffuse severe myocardial ischemia, with a mixture of scar tissue, hibernating myocardium, and still-viable myocardium but without a capacity to improve after revascularization.

In addition, the study revealed that the NOGA treatment area could only be approximately transferred to the SPECT maps. Consequently, only visual semiquantitative SPECT analysis could be performed of the treatment area (ROI), and computer-based quantitative SPECT analysis could only be conducted as a global analysis of the perfusion scintigraphic images in contrast to the exact analysis of the treated area in the NOGA mapping. This discordance between SPECT and NOGA analysis might explain the discrepancies between the significant improvement in local

wall motion and the nonsignificant improvement in perfusion at follow-up.

The improved local linear shortening at NOGA and the improved angiographic wall motion might be due to improved local perfusion of hibernating myocardium with a change to a more contractile cardiomyocyte phenotype. However, a criticism of the NOGA method is that the ischemic ROI was delineated on the baseline map, and although the points of registration were similar at the three-month follow-up map, these points could not be exactly the same as those at the baseline and thus might have included registrations from surrounding nonischemic myocardium, a situation that resulted in a systematically higher mean local linear shortening in the placebo group at the follow-up investigation. Because this study used a double-blind design, such a bias should not have influenced the difference between placebo and VEGF gene transfer treatments.

Advantages of the NOGA-MyoStar injection system are that it provides diagnostic means of studying the therapeutic effect in terms of effects on local left ventricle shortening and unipolar voltage as assessed on three-dimensional maps and the exact localization of the gene injection site, making possible the delivery of genes into the ischemic myocardium. The criteria for a successful injection were developed in pig experiments before initiation of the study, experiments enabling us to conclude that >95% of the injections were successful with myocardial deposition of contrast agent and ink (unpublished observations) and a successful gene expression after intramyocardial injection of a VEGF-A₁₆₅ encoding plasmid (13). As a reflection of the injection trauma, a transient increase in the plasma levels of C-reactive protein and VEGF was observed.

Some end points also were improved in the placebo group (1). The reason for the placebo effect is complex and unclear. Some factors could be the special care and attention the patient receives in a gene therapy trial, inflammation and scar formation after the NOGA procedure, or an inflammatory reaction to the gene transfer vehicle.

Our study was designed with the main end points (stress SPECT, NOGA mapping, left ventricular angiography) performed at the three-month follow-up to avoid confounding factors that might occur during a longer follow-up and are associated with the natural course of coronary artery disease in these severely ill and compromised patients. In our previous trial (12), we used 0.25 mg of phVEGF-A₁₆₅ injection via thoracotomy. Because later feasibility was reported also for 0.5 mg (14), we used 0.5 mg of plasmid distributed in 10 injections, even if the highest effective dose is not known.

The Kuopio Angiogenesis Trial (KAT) and Angiogenic Gene Therapy (AGENT) phase II trials (2,3) have been encouraging, suggesting that intracoronary adenoviral vectors expressing VEGF or FGF-4 may have therapeutic efficacy. Our results indicate that plasmid gene transfer via directed percutaneous intramyocardial injection could be

operative. One drawback of the NOGA system we used is the risk of perforation of the left ventricle wall. Development of the technique and softer catheters may minimize this risk.

Conclusions. Apart from catheter-related risks, percutaneous intramyocardial plasmid gene transfer of VEGF is safe and well tolerated. Although no significant treatment effect was observed at stress-induced perfusion defects at SPECT, there was a trend for favorable anti-ischemic effects. These findings indicate that further studies are required to clarify the future significance of VEGF gene therapy in the treatment of patients with severe ischemic heart disease without any option for conventional revascularization.

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APPENDIX

Safety Committee: Jensen, Sweden; Thayssen, Denmark; Klein, Austria. **SPECT Core Laboratory:** Tägil, Edenbrandt, Sweden; Hesse, Buhl, Denmark; Gyöngyösi, Khorsand, Sholeh Zamini, Austria. **NOGA Core Laboratory:** Gyöngyösi, Austria, Jørgensen, Denmark. **Database and Safety Monitoring:** Rück, Sweden.

The Euroinject One study sites, number of included patients and co-investigators except authors: Sweden, Stockholm (n = 7): Uchto, Nowak, Christensen, Lindvall; Denmark, Copenhagen (n = 32): Carstensen, Gøtze, Kofoed, Johnsen. Denmark, Aarhus (n = 10): Steen Nielsen, Søgaard, Hvidtfeldt; Austria, Vienna (n = 10): Strehblow, Sperker, Maurer, Khorsand Graf, Sochor, Mundigler; Poland, Warsaw (n = 11): Chojnowska, Kukula, Dabrowski, Witkowski, Chmielak, Teresińska; Poland, Krakow (n = 10): Rzeszutko, Heba, Hubalewska, Sowa-Staszczak.