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High-Dose Plasmid-Mediated VEGF Gene Transfer is Safe in Patients With Severe Ischemic Heart Disease (GENESIS-I). A Phase I, Open-Label, Two-Year Follow-up Trial

Liliana Favaloro,[1*] MD, Mirta Diez,[1] MD, Oscar Mendiz,[1] MD, Gustavo Vera Janavel,[2,3] MD, PHD, León Valdivieso,[1] MD, Roxana Ratto,[1] MD, Guillermo Garelli,[3] MD, Fabián Salmo,[1] MD, Marcelo Criscuolo,[3] MSC, Andrés Bercovich,[3] PHD, and Alberto Crottogini,[2] MD, PHD

Objectives: We aimed to assess safety and, secondarily, the efficacy of intramyocardial high-dose plasmid-vascular endothelial growth factor (VEGF) 165 (pVEGF165) gene transfer in no-option patients with coronary artery disease (CAD). **Background:** Controlled trials of pVEGF165 in CAD have shown little benefit. One possible reason is shortness of dosage. We have shown in large mammalian models of chronic myocardial ischemia and acute myocardial infarction that intramyocardial pVEGF165 at doses significantly higher than those used in recent phase II trials is safe and efficacious on myocardial perfusion, left ventricular function, and infarct size limitation. **Methods:** Using an injection catheter, 10 patients with severe CAD not amenable for revascularization received 10 intramyocardial injections of 0.38 mg (total dose, 3.8 mg) pVEGF165 in zones exhibiting myocardial ischemia, as assessed by combined stress 99mTc-sestamibi single-photon emission computed tomography and stress echocardiography. **Results:** No serious adverse events related to either VEGF or the injection procedure occurred over the 2-year follow-up. One patient suffered femoral artery thrombosis after a follow-up coronary angiography, successfully resolved with medical treatment. Six patients suffered uncomplicated coronary ischemic events during the second year follow-up. Angina functional class decreased from 2.6 ± 0.2 to 1.2 ± 0.3 (mean ± SEM, $P < 0.05$), quality of life increased from 56.9 ± 3.2 to 82.6 ± 2.4 ($P < 0.05$), the summed difference score of myocardial perfusion decreased from 13.4 ± 2 to 7.7 ± 1.8 ($P < 0.04$), and stress ejection fraction did not change (44.2 ± 3.6% to 47.8 ± 3.1%, P = NS). **Conclusions:** High-dose intramyocardial pVEGF165 is safe at 2 years follow-up in patients with severe CAD. The efficacy results observed must be taken cautiously given the uncontrolled, open-label study design.

Key words: angiogenesis; gene therapy; myocardial ischemia

INTRODUCTION

Early studies on percutaneous intramyocardial transfer of plasmids encoding vascular endothelial growth factor (VEGF) in small groups of patients with symptomatic coronary artery disease (CAD) refractory to maximum medical therapy and not amenable for conventional revascularization yielded encouraging results consisting mainly of clinical symptoms relief and improved myocardial perfusion (1,2).

[1]Favaloro Foundation University Hospital, Buenos Aires, Argentina
[2]Department of Physiology, Favaloro University, Buenos Aires, Argentina
[3]Bio Sidus, Buenos Aires, Argentina

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*Correspondence to: Liliana Favaloro, MD, Favaloro University Hospital, Av. Belgrano 1746, C1093AAS Buenos Aires, Argentina. E-mail: lfavaloro@ffavaloro.org

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However, later trials carried out on larger cohorts and designed on a randomized, double-blind, placebo-controlled basis showed little, if any, benefit (3,4). In the Euroinject Trial (3), in which 0.5 mg of a plasmid encoding VEGF165 was administered, no significant improvement in stress myocardial perfusion was observed at 3 months follow-up. Neither did the clinical symptoms differ between the VEGF-treated and control groups. However, local linear myocardial shortening, as assessed by NOGA, was better in the VEGF gene transfer group than in the placebo group, suggesting a certain degree of anti-ischemic effect. On the other hand, in the NORTHERN trial (4), a fourfold higher dose of plasmid VEGF did not result in significantly different anginal symptoms, myocardial perfusion or treadmill walking time between VEGF-treated and placebo-treated patients either at 3 or 6 months follow-up. To our knowledge, results for longer follow-up periods have not been reported, nor have higher doses been tested.

Over the last 10 years, we have been working with a plasmid encoding human VEGF165 developed in Argentina (pVEGF165) and have shown that, at a total dose of 3.8 mg (i.e., twofold the highest dose used so far) divided into ten 0.38-mg intramyocardial injections in a porcine model of chronic myocardial ischemia (5–7) and an ovine model of acute myocardial infarction (8), it induced angioarteriogenesis, reduction of collagen deposition, myoblast proliferation, and adult cardiomyocyte mitosis. These effects, in turn, resulted in improved stress myocardial perfusion and reduced infarct size. Importantly, the treatment was safe, as indicated by neither gene expression nor undesired angiogenesis in organs remote to the heart.

On these bases, we undertook a phase I, open-label, uncontrolled study to assess the midterm (6 months) and long-term (24 months) safety (primary objective) and efficacy (secondary objective) of high-dose intramyocardial pVEGF165 transfer in 10 patients suffering chronic, symptomatic CAD with documented myocardial ischemia, maximally treated, and not amenable for conventional revascularization.

MATERIALS AND METHODS

The present study was conducted according to the Helsinki II recommendations. The protocol was approved by the National Administration for Drugs, Food and Medical Technology of Argentina, the Ethics Committee of the Favaloro Foundation University Hospital, and the Research and Teaching Department of the Favaloro Foundation University Hospital.

All patients, accompanied by a close relative, were provided detailed oral and written information about the study. In a subsequent encounter, in which further information, if requested, was provided, the patient and his/her relative signed the written informed consent.

Participants

Between April 2007 and March 2009, 10 patients with Canadian Cardiovascular Society (CCS) class 2–3 angina pectoris despite maximal medical treatment, exhibiting a reversible ischemic defect on myocardial stress 99mTc-sestamibi single-photon emission computed tomography (SPECT) and/or myocardial viability by SPECT or echocardiography, and not amenable for conventional revascularization were included. Other inclusion criteria were age between 18 and 80 years, stenosis $\geq$70% of at least one major epicardial coronary artery angiographically diagnosed within the last 3 months, and left ventricular (LV) ejection fraction (EF) $\geq$30%. We excluded patients with unstable angina pectoris, acute myocardial infarction within the last month, stroke within the last 3 months, prosthetic aortic valve, intraventricular thrombi, left ventricular wall thickness $<$5 mm in zones to be injected, proliferative retinopathy, history or current evidence of malignancy, active infections, positivity for HIV, autoimmune or collagen diseases, psoriasis, inflammatory bowel disease, chronic decompensated liver disease, alcohol or drug abuse, allergy to contrast media, and major psychiatric disease. We also excluded premenopausal women and patients with baseline serum creatinine $>$2.5 mg/dl, serum transaminases above twofold the normal upper limit, serum bilirubin above threefold the normal upper limit, hemoglobin $<$10 g/dl, neutrophil count $<$1500 per mm^3, platelet count $<$100,000 per mm^3, prothrombin time $<$50%, and activated partial thromboplastin time $>$ 50 sec.

Description of Procedures

Screening. All patients underwent the following screening procedures prior to inclusion: clinical examination, routine blood and urinary tests, cardiac and liver enzymes, chest x-ray, electrocardiogram (ECG), abdominal echography, gastroenterological examination including video-colonoscopy, dermatological examination, gynecological examination including colposcopy, mammogram, and Papanicolaou, urological examination including serum PSA, and ophthalmologic examination including fluorescein angiography of the retina (RFG). Screening serological tests comprised VDRL, Huddleson's reaction, irregular antibodies, and antibodies against *Tripanosoma cruzi*, HIV and HCV, and HBV surface antigen. The screening for detection of malignancies followed the guidelines of the American Cancer Society.

Endpoints. Safety endpoints were as follows: (1) clinical signs or symptoms of pVEGF and/or VEGF

protein side effects, as assessed by clinical examination (daily until day 3, on weeks 1, 2, 4, 8, 12, 16, 20, and 24, and on month 24); (2) ECG changes (new arrhythmias, new Q waves, and ST-T modifications) on same time points except weeks 8, 16, and 20; (3) myocardial necrosis, as assessed by cardiac enzymes on the same time points as ECG; (4) changes in routine laboratory tests including complete blood count, hemoglobin, erythrocyte sedimentation rate, hematocrit, platelet count, glycemia, blood urea nitrogen, ionogram, bilirubin, liver enzymes, prothrombin time, activated partial thromboplastin time, and urinalysis (daily until day 3, on weeks 1, 4, 12, and 24, and on month 24); (5) plasma human VEGF165 (daily until day 3, days 7 and 10, weeks 2, 3, 4, and 24, and month 24) by ELISA (R&D Quantikine assay, Minneapolis, MN); (6) retinal complications, as assessed by ophthalmological examination with RFG on weeks 4 and 24 and without RFG (unless indicated) on month 24; and (6) injection procedure complications, as assessed by bidimensional echocardiography during injection and 3 hr postinjection.

Efficacy endpoints were as follows: (1) CCS angina functional class (CCS-FC); (2) percent quality of life according to the Seattle Angina Questionnaire (SAQ-QoL) (9) on weeks 4, 8, 16, 20, and 24 and on month 24; (3) stress LVEF by Doppler echocardiography on weeks 12 and 24 and on month 24;(4) summed difference score (SDS) of myocardial perfusion by stress 99mTc-sestamibi SPECT on weeks 12 and 24 and on month 24; and (5) Rentrop index of collateral circulation, as assessed by coronary angiography on week 12.

Plasmid. The eukaryotic expression vector (pVEGF165, deposited as pBSVEK3 at *Deutsche Sammlung von Mikroorganismen und Zellkulturen*, accession number DSM 14346) is a 3930 bp plasmid that includes the human VEGF165 coding gene, whose transcription is regulated by the cytomegalovirus promoter/enhancer. A SV40 terminator is located 3′ to the VEGF165 coding gene. Preparation, purification, and quality control analyses of the plasmid from transformed *Escherichia coli* cultures were performed under GMP conditions (Bio Sidus S.A., Buenos Aires, Argentina). One-milliliter vials containing 1.9 mg of the purified plasmid in PBS were stored at −80°C; pVEGF165 was provided by Bio Sidus.

Injection procedure. After isocentering with the c-arm in two orthogonal views [right anterior oblique (RAO) and left anterior oblique (LAO)], two left ventriculograms (one in RAO and one in LAO) were performed, and the contours of both were superimposed and traced with a board marker on a transparency adhered to the screen. The position of the table and the patient remained fixed during the whole procedure. Then, a 10-Fr JR 3.5 guiding catheter (Cordis Endovascular Systems, Miami Lakes, FL) was inserted through the femoral artery and advanced to cross the aortic valve over a 0.035-in. guide wire. An 8-Fr deflectable injection catheter with a retractable needle at its distal end (MyoCath; BioHeart, Miami, FL) was advanced through the guiding catheter. The use of a guiding catheter permitted advancing the injection catheter in an easier and safer fashion, prevented traumatizing the aortic wall and valve and increased steerability significantly. In addition, the guiding catheter permitted axial rotation of the injection catheter, thus allowing targeting the anterior, posterior, and lateral walls, while the deflectable distal portion of the injection catheter allowed orientating the injections toward the base and the apex. Before insertion, the injection catheter's dead space was filled up with 0.5 ml of injectate. Under fluoroscopic guidance, the catheter was advanced retrogradely to the target zone until the tip contacted the endocardium in a perpendicular fashion with respect to the LV wall. The site was marked on the transparency using a number code for the RAO projection and a letter code for the LAO projection. The operator confirmed the correct position of the catheter tip on the target area in the two views and verified that the site selected was different from that of previous injections. Prior to needle insertion into the myocardium, the thickness of the LV wall was assessed by echocardiography (Vivid 7 dimension TM equipment; General Electric, Vingmed, Horten, Norway), and the puncture depth was set using the needle protrusion regulation device (range: 3–6 mm). Ten 0.2-ml injections containing 0.38 mg of pVEGF165 were performed (total volume: 2 ml; total pVEGF165 dose: 3.8 mg). Marking each injection site on the superimposed ventriculogram silhouettes, along with the possibility of combining axial rotation (provided by the guidance catheter) and longitudinal deflection (provided by the injection catheter), permitted avoiding the risk of repeated punctures on same sites. Successful needle penetration was confirmed by the occurrence of ventricular ectopy.

Follow-up. At the end of the procedure, the patient was transferred to the coronary care unit, and 3 hr later a control bidimensional ECG was performed. On the third day postinjection, the patient was discharged. In-hospital follow-up included daily clinical examination, ECG, cardiac enzymes, routine laboratory tests, and plasma VEGF165 quantification. Outpatient follow-up started on day 7 after injection and continued on day 10, weeks 2, 3, 4, 8, 12, 16, 20, and 24, and month 24 after injection. The tests underwent on each time point are described above. During the period elapsed between the 6-month and 24-month time points, all

patients were contacted weekly or bimonthly by phone and every adverse event was recorded and followed up. In addition, all patients had out-of-protocol clinical visits with anamnesis, physical examination, and control of the medication.

Echocardiogram. Bidimensional Doppler echocardiography at rest and under dobutamine challenge was performed at 12 and 24 weeks and at 24 months post-injection. LV volumes at end diastole and end systole and LVEF% applying the modified Simpson's method were recorded. As explained above, echocardiographic monitoring was done during the injection procedure to assist catheter positioning and to confirm needle penetration, and a control echocardiogram to rule out post-injection complications (particularly pericardial effusion) was performed 3 hr after the procedure.

Pharmacological stress protocol. A dobutamine challenge protocol was used in nine patients. One patient, who had a permanent pacemaker, underwent a dipyridamole stress protocol.

Dobutamine (250 mg) in 5% dextrose solution was infused at a rate of 2.5 μg/kg/min during 5 min, 5 μg/kg/min during 5 min, and 10 μg/kg/min during another 5 min to evaluate myocardial viability. Thereafter, the infusion rate was increased to 20, 30, and 40 μg/kg/min every 3 min until 85% of expected maximum heart rate (220 beats/min minus age in years for men and 210 beats/min minus age in years for women) was achieved. If this heart rate value was not reached, intravenous atropine (up to 1 mg) was administered. At peak stress, the radiotracer for SPECT myocardial perfusion assessment was injected.

Single-photon emission computed tomography. SPECT studies were performed using a 2-day protocol, with a dual-head camera (ADAC Vertex; Philips, Milpitas, CA) and 99mTc sestamibi (15–25 mCi for each day depending on body weight). The gated stress images were acquired 1 hr after sestamibi injection. The rest gated images were performed using a standard nitrate-enhanced protocol to maximize detection of ischemia (10). After that, an ECG-gated SPECT was performed during a low-dose infusion of dobutamine (5 mg/kg/min) administered intravenously to evaluate abnormal motion segments. Acquisitions were gated for 8 frames per cardiac cycle, with 100% beat acceptance. Finally, short-axis images were reconstructed semiautomatically with manual adjustments, using AutoSPECT Plus (Pegasys X software, version 4.20; Philips). Semiquantitative visual interpretation was performed by use of 17 segments for each image set (11). Segments were scored by consensus of two experienced observers using a 5-point scoring system (0, normal; 1, mild; 2, moderate; 3, severe hypoperfusion; and 4, absence of detectable tracer uptake).

The summed rest score (SRS), summed stress score (SSS), and SDS were calculated by adding the scores in all segments.

Coronary angiography. Diagnostic coronary angiography (Philips Allura Xper FD20/10) was performed in patients who had not undergone such procedure within the last 3 months before enrollment and in all patients at the 12th week follow-up.

Right and left coronary artery angiograms were recorded after nitroglycerine injection (200 μg) in RAO and LAO, left lateral and cranial anteroposterior projections. Additional projections (caudal RAO and cranial and caudal LAO) were obtained for the left coronary artery. Identical projections were used for the follow-up angiography. Acquisition velocity was at least 25 frames/s. Collateral circulation was evaluated using the Rentrop index (12).

Statistical Analysis

For categorical variables (CCS-FC and SDS), we compared baseline versus 24 months values using the Wilcoxon signed-rank test for paired comparisons. Numerical variables (SAQ-QoL, EF%) were compared between baseline, 6, and 24 months by λ Wilks multivariate analysis. Dichotomous variables are expressed as percentages and continuous variables as mean ± SEM. P values under 0.05 were considered to indicate statistically significant differences. The R Development Core Team software was used for statistical computing (R Foundation, Vienna, Austria).

RESULTS

Of 14 patients assessed for eligibility, two were excluded for not meeting inclusion criteria (one was considered by the interventional cardiology team to be amenable for percutaneous transluminal coronary angioplasty and the other had EF% < 30) and two for presenting exclusion criteria (one had a history of allergy to iodine-based contrast media and the other had moderate-to-severe aortic stenosis). As a result, nine men and one woman, aged 60 ± 2.3 years (range: 46–71 years) were included in the study. All of them completed the 24-month follow-up. Table I shows the clinical data of the patients. Of a total of 100 injections, 46 were performed in the anterior wall, 31 in the lateral wall, and 23 in the posterior wall of the LV. Needle penetration depth was 3 mm in 14 injections, 4 mm in 85 injections, and 6 mm in one injection. One hundred percent of the planned injections were successfully performed. However, in each injection, a

TABLE I. Patient Demographics and Clinical Data Before VEGF Gene Transfer

Age, yr	60.3 ± 2.3[a]
Gender, male/female	9/1
CCS class	2.6 ± 0.2[a]
Diabetes, *n* (%)	5 (50)
Hyperlipidemia, *n* (%)	9 (90)
Smoking, *n* (%)	10 (100)
Hypertension, *n* (%)	7 (70)
Peripheral vascular disease, *n* (%)	4 (40)
Previous MI, *n* (%)	8 (80)
Stroke/TIA, *n* (%)	0 (0)
LVEF (%)	43.7 ± 3.6[a]
CABG, *n* (%)	9 (90)
Reoperated, *n* (%)	2 (20)
PCI, *n* (%)	5 (50)

[a]Values are expressed as mean ± SEM. CABG, coronary artery bypass graft; CCS, Canadian Cardiovascular Society; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; TIA, transitory ischemic accident.

small amount of the injected volume may have been spilled into the LV cavity by systolic squeezing.

Safety

No procedure-related adverse events occurred, except for ventricular ectopy (mainly isolated extrasystoles, bigeminy, and self-limited ventricular tachycardia without hemodynamic decompensation) at the moment of needle penetration. In-hospital follow-up was uneventful in all patients.

No change was detected in laboratory tests, either during hospitalization or follow-up.

Plasma VEGF165 protein increased from 145.9 ± 34.8 pg/ml to 389.9 ± 74.4 pg/ml ($P < 0.04$) at 10 days after injection and decreased to baseline levels at 15 days and thereafter (Fig. 1). Given that VEGF165 protein level was established as a safety parameter, this increase was recorded as a plasmid-related adverse effect. However, no complications that could be attributable to high plasma VEGF (malignancies, proliferative retinopathy) occurred during the 24-month follow-up.

One serious adverse event, unrelated to either the plasmid or the injection catheter but related to a follow-up procedure, occurred. This was thrombosis of the right femoral artery after the coronary angiogram corresponding to the 12th follow-up week. The patient presented functional limitation and ischemic pain lasting 24 hr. The event was classified as "serious" following the terminology criteria of the National Cancer Institute (13). Treatment consisted of cilostazol 200 mg/d during 3 months, followed by buflomedil 300 mg/d. Three months after the event, the patient was asymptomatic and with normal lower limb functional capacity.

Unrelated acute coronary events requiring hospitalization were non-ST acute myocardial infarction in two patients (at 11 and 15 months posttreatment) and unstable angina pectoris in four (at 12, 15, 15, and 16 months posttreatment). In no cases, hemodynamic decompensation or other complications were observed, and all patients were discharged after medical treatment.

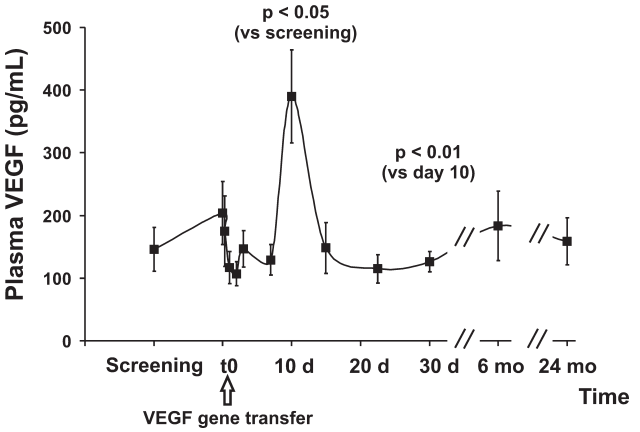

Fig. 1. Time course of plasma VEGF levels. Plasma VEGF165 protein displayed a significant threefold increase 10 days after gene transfer and returned to baseline at later time points.

TABLE II. Clinical and Left Ventricular Function Parameters

	Baseline	6 months	24 months
CCS-FC	2.6 ± 0.2	1.2 ± 0.3[a]	1.2 ± 0.3[a]
SAQ-QoL	56.9 ± 3.2	82.1 ± 2.4[a]	82.6 ± 2.4[a]
%EF (rest)	52.0 ± 2.9	54.3 ± 2.5	48.6 ± 2.4
%EF (stress)	44.2 ± 3.6	51.6 ± 3.6[b]	47.8 ± 3.1

Values are expressed as mean ± SEM.
[a]$P < 0.05$ vs. baseline.
[b]$P < 0.02$ vs. baseline. CCC-FC, Canadian Cardiovascular Society functional class; EF, left ventricular ejection fraction; SAQ-QoL, Seattle Angina Questionnaire quality of life.

Efficacy

Clinical and LV function parameters. Table II lists the values for CCC-FC, SAQ-QoL, and EF% at baseline and at 6 and 24 months of follow-up. As can be seen, CCC-FC decreased and SAQ-QoL improved significantly at both time points. Stress EF%, on the other hand, improved at 6 but not at 24 months, with resting EF% staying unchanged throughout. It should be noticed that the degree of stress reached was similar, as indicated by the double product (baseline: 18,686 ± 1,112; 6 months: 19,129 ± 1,718; 24 months: 18,342 ± 1,222; P = NS). These double product values were calculated for $n = 9$, because, as explained above, one patient with a permanent pacemaker underwent dipyridamole challenge.

Myocardial perfusion. Figure 2 shows myocardial perfusion results. At 6 months, SDS decreased from 13.4 ± 2 at baseline to 9.5 ± 2.4 ($P < 0.04$). This resulted from an almost selective reduction in stress-

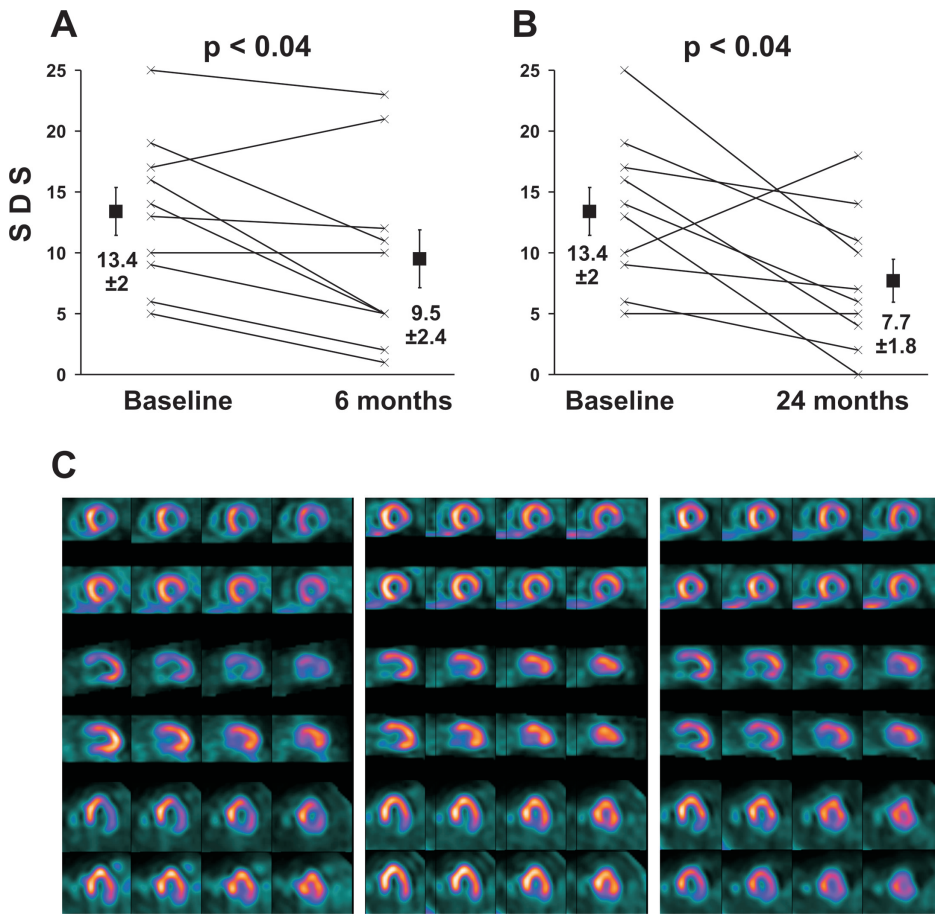

Fig. 2. Individual and group evolution of myocardial ischemic burden from baseline to 6 months (panel A) and from baseline to 12 months (panel B). The zero value corresponds to a patient who suffered non-ST myocardial infarction 11 months after gene transfer. Panel C shows 99mTc-sestamibi single photon emission computed tomography images at rest and stress of one patient at baseline (left), at 6 months (middle), and at 24 months (right) after intramyocardial VEGF gene transfer. A noticeable improvement in myocardial perfusion can be observed. SDS, summed differences score.

induced ischemia (SSS: from 19 ± 2.6 to 14.9 ± 3.2, $P < 0.01$) with no significant change in resting perfusion defect (SRS: from 5.9 ± 1.6 to 5.4 ± 1.5, $P =$ NS). The same happened at 2 years follow-up: SDS was 7.7 ± 1.8 ($P < 0.04$ vs. baseline) with almost unchanged SRS (6.9 ± 1.8, $P =$ NS vs. baseline) Examples of SPECT-sestamibi images are illustrated in Fig. 2c.

Collateral circulation. Epicardial collateral circulation did not differ between baseline and 12 weeks of follow-up, as indicated by the Rentrop index (baseline: 2.2 ± 0.3, 12th week: 2.4 ± 0.2; $P =$ NS).

DISCUSSION

The primary objective of the present study, designed on an open-label, uncontrolled basis, was to assess the safety of the intramyocardial transfer of 3.8 mg of a plasmid encoding human VEGF165 in no-option patients with symptomatic ischemic heart disease. Our results show that the treatment is safe, with no adverse effects attributable to either the injection procedure or the plasmid occurring over 24 months follow-up.

The only serious complication related to a follow-up procedure, though not to the plasmid or the injection catheter, consisted of femoral artery thrombosis following a control coronary angiography performed 12 weeks after gene transfer. The event was appropriately resolved with medical treatment. With regard to the transient increase in plasma VEGF165, it was not associated with the development of tumors, proliferative retinopathy, or any other condition that could be fostered by VEGF.

As concerns efficacy, which was our secondary objective, we observed a significant improvement in the clinical outcome, as indicated by a decrease in the angina functional class and an increase in quality of life. These changes were associated with significantly reduced myocardial ischemia. Improved LV function during pharmacological stress accompanied these

variations at 6 months follow-up, but not at 2 years, as indicated by a LVEF that was not significantly different from that at baseline. At rest, neither myocardial perfusion nor LVEF changed significantly over time. It should be noted that part of the decrease in SDS was due to previously ischemic segments becoming necrotic in two patients. However, the extent of these new non-ST infarcts was small, as indicated by a SRS increasing from 3 to 4 in one patient and from 8 to 10 in the other.

The threefold increase in plasma human VEGF might be reflecting a significant degree of transgene expression, which, in turn, could underlie the improvements observed in myocardial perfusion, LV function, and clinical outcome. Moreover, the time after injection at which plasma VEGF peaked is consistent with the expression kinetics of plasmid-mediated VEGF gene transfer reported for pigs and sheep with experimental coronary artery disease (5,8) and rabbits with hind limb ischemia (14). Transfection efficiency, which is known to be low for plasmids as compared with viral vectors (15), may have been favored in our case by the small size (3930 bp), high supercoiled rate (>90%), and optimal purity (less than 10 endotoxin units/ml) of the plasmid used.

In the Euroinject trial, using 0.5 mg pVEGF, a twofold increase in plasma VEGF levels was observed (3). However, the same happened in the placebo-treated group, raising the question of whether the injection procedure itself may induce VEGF expression. Data from Freedman et al. support this speculation, on account that in patients with CAD receiving intramyocardial plasmid-VEGF165 by minithoracotomy displayed increased plasma VEGF levels as early as 4 hr postprocedure, far before transgene expression could have taken place (16). In our preclinical studies on pigs, sheep, and rabbits, in no case did placebo-treated animals exhibit human VEGF expression, but the possibility exists that species-specific VEGF had been expressed.

In the NORTHERN trial (4), using 2 mg of plasmid, the levels of circulating VEGF165 were neither different at any time point between the treated and placebo groups, nor was there any significant change over time.

It can be speculated that the most likely explanation for the beneficial effects observed in our patients is the dose used, which was eightfold higher than that of the Euroinject trial and almost twice as high as that used in the NORTHERN trial.

Importantly, we had previously shown in pigs with chronic myocardial ischemia (5) and sheep with myocardial infarction (8) that the intramyocardial delivery of 3.8 mg of pVEGF165 exhibited a good safety profile, with neither undesired angiogenesis nor transgene expression (except for a positive RT-PCR reaction in a skeletal muscle sample of one pig) occurring in organs remote to the heart (gonads, retina, liver, lungs, kidney, and skeletal muscle).

The spill of small amounts of the injectate into the LV chamber as a consequence of systolic squeezing may raise the concern of the recirculating plasmid inducing undesired transgene expression in remote organs. However, it has been shown that, when introduced systemically, plasmid DNA is degraded by serum nucleases and eliminated by the liver (17). This makes most improbable that the increased VEGF protein levels observed in our patients came from tissues other than the myocardium.

Although the large dose used may likely be the major reason for the efficacy results, enhanced transfection efficiency due to plasmid characteristics described above, especially the small size, may have played a contributive role. In fact, the particle size of the DNA complex has been shown to affect gene delivery and expression (18).

Nevertheless, however interesting the efficacy results may appear, the lack of a control group is a major limitation that prevents from drawing any conclusions, especially taking into account that controlled trials on proangiogenic strategies have shown a strong placebo effect (19–24).

The main contribution of the present study is showing that the intramyocardial transfer of a high dose of plasmid-VEGF, duplicating that applied in the most recent phase II clinical trial, is safe over a 2-year follow-up time. This is of clinical interest, considering the fact that one of the reasons for the paucity of positive therapeutic effects in these trials could be an insufficient dose regime.

In conclusion, in patients with severe CAD, intramyocardial high-dose (3.8 mg) plasmid-mediated VEGF165 gene transfer is safe. This finding, along with preliminary efficacy results showing a beneficial effect, encourages testing this treatment on larger patient populations using a double-blind, randomized, placebo-controlled protocol design.

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