HUMAN GENE THERAPY METHODS 24:298–306 (October 2013)

DOI: 10.1089/hgtb.2012.221

High Doses of Vascular Endothelial Growth Factor 165 Safely, but Transiently, Improve Myocardial Perfusion in No-Option Ischemic Disease

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Abstract

Gene therapy can induce angiogenesis in ischemic tissues. The aim of this study was to assess safety, feasibility, and results, both clinical and on myocardial perfusion, of gene therapy in refractory angina. This was a phase I/II, prospective, temporal-controlled series, clinical trial. Thirteen patients were maintained for minimum 6 months under optimized clinical management, and then received intramyocardial injections of 2000 μg plasmid vascular endothelial growth factor 165 and were followed by single-photon emission computed tomography (SPECT), treadmill tests, Minnesota quality of life questionnaire (QOL), and New York Heart Association (NYHA) functional plus Canadian Cardiovascular Society (CCS) angina classifications. There were no deaths, early or late. During the optimized clinical treatment, we observed worsening of rest ischemia scores on SPECT ($p < 0.05$). After treatment, there was a transitory increase in myocardial perfusion at the third-month SPECT under stress (pre-operative [pre-op] 18.38 ± 7.51 vs. 3 months 15.31 ± 7.30; $p < 0.01$) and at the sixth month under rest (pre-op 13.23 ± 7.98 vs. 6 months: 16.92 ± 7.27; $p < 0.01$). One year after, there were improvements in treadmill test steps (pre-op 2.46 ± 2.07 vs.12 months 4.15 ± 2.23; $p < 0.01$) and oxygen consumption (pre-op 7.66 ± 4.47 vs.12 months 10.89 ± 4.65; $p < 0.05$), QOL (pre-op 48.23 ± 18.35 vs.12 months 28.31 ± 18.14; $p < 0.01$) scores, and CCS (pre-op 3 [3–3.5] vs.12 months 2 [1–2.5]; $p < 0.01$) and NYHA (pre-op 3 [3–3] vs. 2 [2–2] vs. 12 months 2 [1–2]; $p < 0.01$) classes. Gene therapy demonstrated to be feasible and safe in this advanced ischemic cardiomyopathy patient sample. There were improvements in clinical evaluation parameters, and a transitory increase in myocardial perfusion detectable by SPECT scintigraphy. Clinical Trial Registration: NCT00744315 http://clinicaltrials.gov/

Introduction

It is estimated that about 900,000 individuals in the United States have refractory angina. This syndrome is defined as severe symptomatic coronary artery disease (CAD), not manageable by conventional treatment, including coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI), and optimized clinical treatment (Yang *et al.*, 2004; McGillion *et al.*, 2007). Each year, 25,000–75,000 new cases are diagnosed (Yang *et al.*, 2004; McGillion *et al.*, 2007). With increasing population age and CAD mortality reduction, it is believed that the incidence of refractory angina will increase.

In order to improve quality of life for these patients, alternative revascularization strategies have been evaluated. In this context, given its potential for aiding the development of myocardial angiogenesis and formation of collateral circulation in the ischemic myocardium (Kalil *et al.*, 2010), gene therapy using the vascular endothelial growth factor (*VEGF*) could represent a new therapeutic option (Bokeriya *et al.*, 2005; Kalil *et al.*, 2010). In 1998 was conducted the first clinical trial using gene therapy with *VEGF165* for patients with refractory angina (Losordo *et al.*, 1998). Over a decade later, however, clinical benefit of the therapy and the most effective dose are yet to be established.

Clinical trials of gene therapy with *VEGF165* as the sole therapy for patients with refractory angina have used low gene vector doses (125–400 μg) (Losordo *et al.*, 1998; Symes *et al.*, 1999; Sarkar *et al.*, 2001). Higher doses have been used in some studies, although in combination with well-

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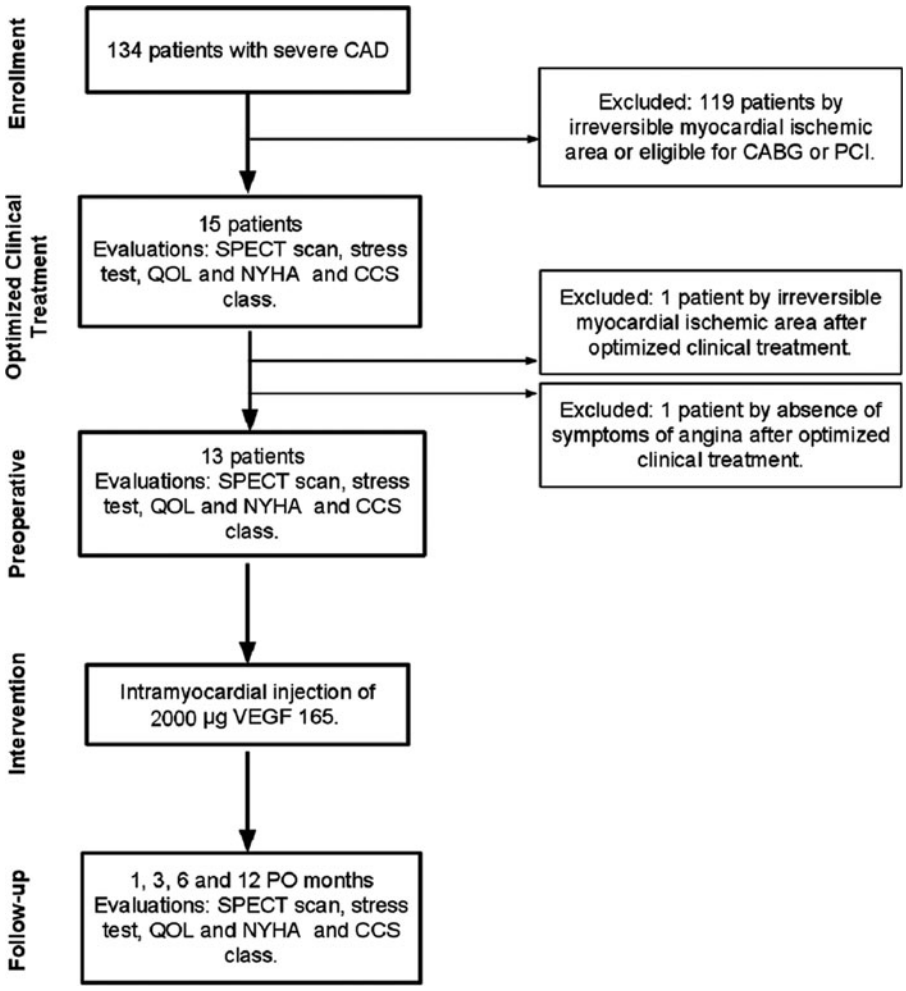

FIG. 1. Study phases diagram. CABG, coronary artery bypass grafting; CAD, coronary artery disease; CCS, Canadian Cardiovascular Society; NYHA, New York Heart Association; PCI, percutaneous coronary disease; QOL, quality of life; SPECT, single-photon emission computed tomography; VEGF165, vascular endothelial growth factor 165.

established techniques of myocardial revascularization, such as CABG and PCI (Vale *et al.*, 2000; Fortuin *et al.*, 2003; Bokeriia *et al.*, 2004; Kołsut *et al.*, 2004; Ruel *et al.*, 2008; Stewart *et al.*, 2009). Such trials were able to demonstrate safety and feasibility of this type of therapy, but benefits over myocardial perfusion and functional capacity were small and inconsistent.

The aim of this study was to assess the safety and feasibility and to evaluate the results, both clinical and on myocardial perfusion, of gene therapy with 2000 μg of a plasmid expressing the *VEGF165* isoform in patients with advanced ischemic heart disease and refractory angina. Our group has previously published the short-term results of this trial (Kalil *et al.*, 2010), and the present report describes the 1-year outcomes in the follow-up of this series of patients.

Methods

Study registrations and approvals: ethical aspects

This study was registered at ClinicalTrials.gov under the registration number NCT 00744315 and was carried out in a reference cardiology hospital in southern Brazil. This is the first clinical trial using gene therapy in Brazil for the treatment of refractory angina patients and the first one to use a vector entirely developed in this country, by Excellion, Petropolis, RJ, Brazil. The project was designed according to the Guidelines and Norms Regulating Research Involving Human Subjects (Declaration of Helsinki World Medical Association, 1964). It was approved by the National Commission of Ethics in Research and Research Ethics Committee of IC/FUC under number 3849/06, as well as by the National Technical Commission on Biosafety (CTNBio) for the surgical center and the supplier of the plasmid vector. All patients signed informed consents. Figure 1 demonstrates the flow diagram for the study phases.

Clinical study design and patient selection

This trial was designed as a prospective, temporal-controlled, clinical series study and enrolled 13 patients with refractory angina selected from 134 candidates screened from a dedicated outpatient clinic during a 2-year period. Of the 13 included patients, 12 were men (92.3%), the mean age was 58.7 ± 5.9 years, and the mean left ventricular ejection fraction was 56.0% ± 14.5%. The sample characteristics are shown in Table 1. All 13 patients had advanced ischemic heart disease, inoperable by CABG or PCI, as assessed by independent cardiovascular surgeons and intervention cardiologists. The inclusion criteria were signs and symptoms of angina and/or heart failure despite maximum medical treatment, myocardial ischemic area of at least 5% as assessed by single-photon

Table 1. Sample Characteristics ($N=13$)

Characteristics	n *(%)*
Age (years), mean ± SD	58.7 ± 5.9
Sex (male)	12 (92.3)
Ventricular ejection fraction (%), mean ± SD	56.0 ± 14.5
Comorbidities	
Hypertension	12 (92.3)
Diabetes	6 (46.2)
Previous vascular diseases	
Myocardial infarction	13 (100)
Stroke	2 (15.4)
Peripheral vascular disease	2 (15.4)
Previous myocardial revascularization	
Percutaneous coronary intervention	12 (92.3)
Coronary artery bypass grafting	11 (84.6)
CCS angina classification	
II	2 (15.4)
III	8 (61.5)
IV	3 (23.1)
NYHA heart failure classification	
III	11 (84.6)
IV	2 (15.4)

CCS, Canadian Cardiovascular Society; NYHA, New York Heart Association; SD, standard deviation.

emission computed tomography (SPECT) scans, and diffuse CAD with vessels unfeasible for CABG or PCI. The exclusion criteria were age over 65 years, left ventricular ejection fraction less than 25%, and diagnosed neoplasms. After selection, patients had their medical treatment optimized following current clinical guidelines and were followed for a minimum of 6 months (259.6 ± 167.3 days) in a dedicated outpatient clinic. Before the planned intervention, all patients underwent a preoperative evaluation that included all of the described parameters, with emphasis on a new SPECT scan. Patients who responded well to medical treatment or who showed improvements in myocardial SPECT scan perfusion were discarded from the study and not submitted to gene therapy.

Plasmid vector

The plasmid backbone used was developed by one of the authors (S.W.H.) at the Federal University of São Paulo and produced commercially by Excellion Serviços Biomédicos in Petropolis, RJ, Brazil. The PEX-HV5 plasmid backbone is composed of cytomegalovirus (CMV) intron 1 (enhancer element) and splicing signals, its promoter, and a bovine growth hormone polyadenylation site (BGH PolyA), besides a pUC origin of replication and kanamycin resistance sequences for propagation in bacteria. The human *VEGF165* cDNA was inserted between the CMV promoter and the BGH PolyA sequence. The solution was considered stable while it was kept under refrigeration for 6 months, and it was discarded after this period.

Hypersensibility test

All patients were tested for hypersensitivity to the *VEGF165* plasmid vector solution. The test consisted of a 0.5 ml subcutaneous injection containing a 500 ng dose of vector PEX-HV5 in 0.5 ml of sodium chloride 0.9% in the left forearm at least 2 weeks before the intervention.

Intervention

The ischemic area of the myocardium previously identified by a SPECT scan was approached through a small anterolateral thoracotomy with an incision of approximately 5 cm in the fourth or fifth left intercostal space, according to the area to be treated, followed by a corresponding T-shaped pericardiotomy. This area received injections of the plasmid vector solution under direct vision, distributed over 10 points of the ischemic myocardium, with a total of 2000 μg of plasmid *VEGF165*, diluted in 5 ml saline, using a 5 ml syringe and a 25 F Butterfly injection needle. Before thoracic closure, a thoracic tube was put in place and maintained for 12 hr. Postoperative pain was treated with intercostal neural block with local marcaine solution and postoperatively with injectable analgesics.

Myocardial perfusion

All patients underwent ECG-gated myocardial perfusion studies. The protocol involved a 2-day (rest/stress) technetium 99m sestamibi imaging approach (SPECT). Patients were submitted to pharmacological stress using dipyridamole (0.56 mg/kg in 4 min). A stress injection of Tc-99m sestamibi was administered at the seventh minute of the beginning of dipyridamole infusion. The images were acquired with a low-energy, high-resolution collimator using a 180° noncircular orbit from 45° right anterior oblique to left posterior oblique, with a 64 × 64 matrix. The rest and stress studies were reconstructed using a Butterworth filter with a critical frequency of 0.5 Nyquist, with an order of 5.

An Emory Cardiac Toolbox (ECTb) was used to identify left ventricular landmarks from short-axis slices, which were then verified by an operator. The ejection fraction and left ventricular volume values were calculated using the ECTb, and the extent and severity of the ischemic area were obtained using Cedars-Sinai QPS software. In this software, the left ventricle is divided into segments and each receives scores between 0 and 4 (0 = normal perfusion and 4 = absence of perfusion). The severity scores obtained were summed stress score (SSS), summed rest score (SRS), and summed difference score (SDS). These scores represent the extent of ischemia during stress phase of the exam with dypiridamole, during rest and the difference between them, respectively. A SSS of <4, 4–8, 4–13, and >13 are considered normal, mildly abnormal, moderately abnormal, and severely abnormal, respectively. The extent of ischemia was calculated by comparing the perfusion of the patient with a database of normal patients as is normally done in clinical practice. The final examination result was defined via a consensus between two experts.

Functional capacity, quality of life, and functional classification assessment

The Minnesota quality of life questionnaire was specifically developed for heart failure, making it closer to reality for this class of patient and was already validated for Portuguese (Carvalho *et al.*, 2009). Scores of angina according to the Canadian Cardiovascular Society (CCS) and heart failure

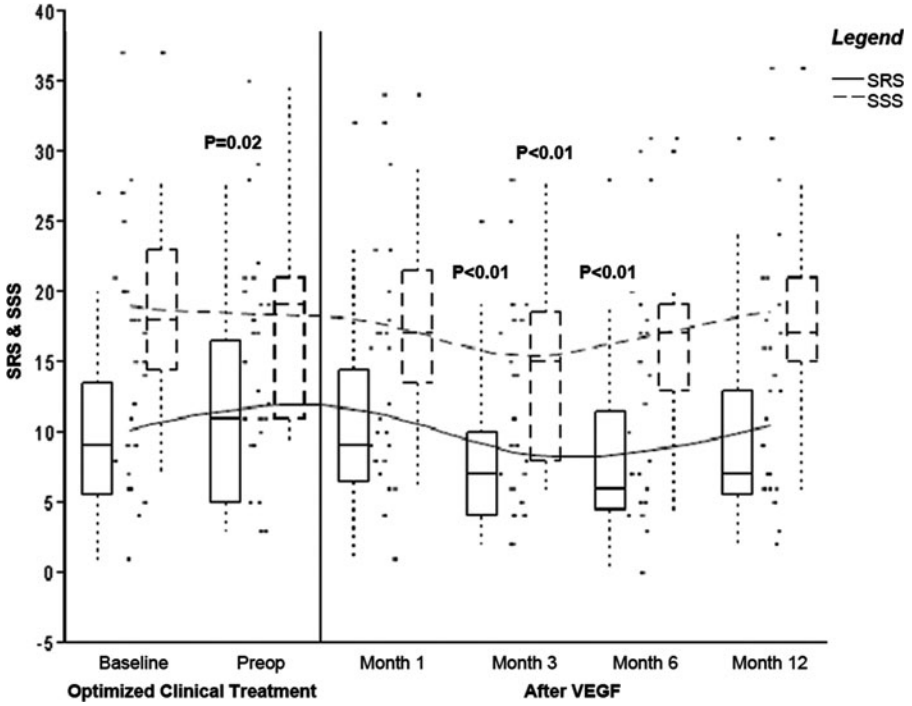

FIG. 2. Ischemia scores in the SPECT. Pre-op, pre-operative; SRS, summed rest score; SSS, summed stress score.

according to the New York Heart Association (NYHA) were assigned at each interval evaluation.

In order to objectively assess functional capacity, a treadmill test with the Naughton protocol was used, which provides an estimated oxygen consumption of 1 MET per stage. This protocol is often used in postmyocardial infarction exercise testing to classify patients into high-risk or low-risk categories and to determine optimal treatment strategies. The protocol begins with a belt speed of 1.0 mph with zero elevation, followed by an increase to 2.0 mph, keeping the elevation zero. From this stage, the rate is fixed at 2 mph and the elevation of the ramp increases in 3.5% increments every 3 min.

Outcomes

The patients were evaluated at the study inclusion, after at least 6 months of optimized clinical treatment (preintervention period), and at 1, 3, 6, and 12 months after intervention, observing the following outcomes.

Primary outcome. Ischemia extension evaluated by serial SPECT scans.

Secondary outcomes. Angina class by CCS classification, heart failure class by NYHA classification, quality of life by the Minnesota questionnaire, and functional capacity and estimated oxygen consumption by treadmill tests.

Statistical analysis

Continuous variables are expressed as means and standard deviation, and categorical variables as absolute and relative frequencies. Friedman's nonparametric test and ANOVA were used to compare the same variables at different times in relation to the same group. Analyses were performed using the SPSS 18.0 statistical package.

Results

Safety and feasibility

There were no deaths or surgical re-interventions during the study period. There was a case of prolonged postoperative hospitalization because of decompensated diabetes. One patient had premature ventricular contractions in the immediate postoperative period. It is noteworthy that this same patient had already presented the same arrhythmia in a previous treadmill test, with spontaneous reversion. Another patient developed bacteremia associated with pneumonia within 12 hr post-op. Microbiological analysis of the plasmid solution used in this patient was negative for bacterial or fungal growth until the fifth day of observation. This same patient required assistance in the emergency unit in the fourth month after the intervention because of stress caused by family illness, but no cardiovascular abnormalities were observed. At the tenth month, this patient presented with thoracic discomfort, was submitted to a new coronary artery cineangiogram that identified a new lesion, and was treated with PCI with good results. Another patient required hospitalization for signs of heart failure and was managed clinically.

Myocardial perfusion

During the previous optimal drug treatment period, there was no difference in stress and differential ischemia SPECT scores. However, an increase in the rest ischemia SPECT score (SRS; baseline 10.23 ± 7.06 vs. pre-op 13.23 ± 7.98; $p < 0.05$) was observed. Three months after VEGF165 gene therapy, analyses of ischemia SPECT scores showed a reduction in the number of ischemic segments under stress (SSS; pre-op 18.38 ± 7.51 vs. 3 months 15.31 ± 7.30; $p < 0.01$) and at rest (pre-op 13.23 ± 7.98 vs. 3 months 8.77 ± 6.82; $p < 0.01$). There was no difference in the differential score in

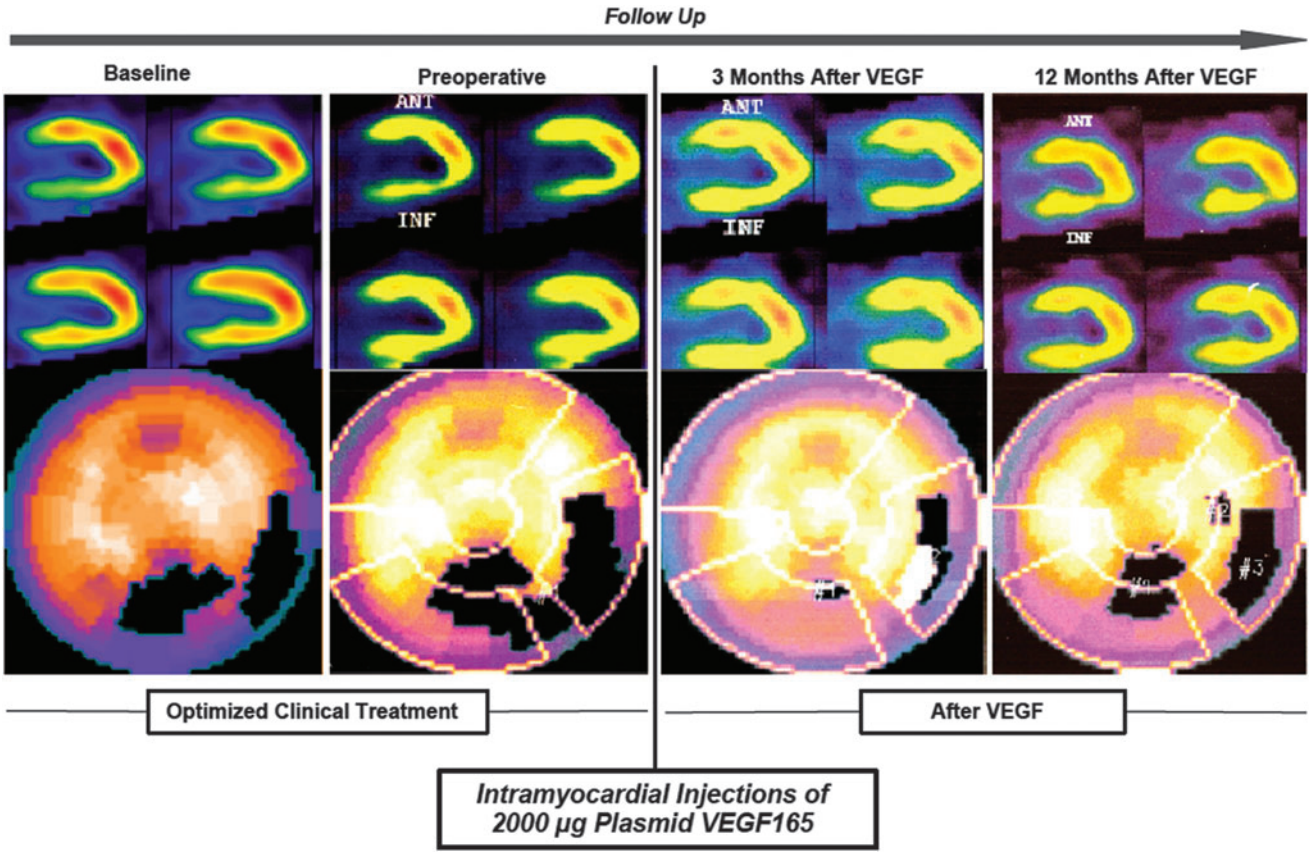

FIG. 3. Variation in myocardial perfusion as assessed by the SPECT scans of one patient included in this study. Color images available online at www.liebertpub.com/hgtb

the same period. Six months after intervention, there was a significant difference only in the rest ischemia score (SDS; 6 months: 16.92 ± 7.27; $p < 0.01$).

One year after gene therapy, the ischemia scores were similar to the preoperative values. Figure 2 demonstrates the ischemia scores throughout follow-up, and Fig. 3 presents the variation in myocardial perfusion as assessed by SPECT scans for one of the patients included this study.

Functional capacity

One month after *VEGF165* gene therapy, there was improvement in the number of steps performed in the treadmill test (pre-op 2.46 ± 2.07 vs. 1 month 3.31 ± 1.75; $p < 0.05$), which was maintained at 1 year after intervention (3 months 3.77 ± 2.13, $p < 0.05$; 6 months 3.92 ± 2.14, $p < 0.05$; and 12 months 4.15 ± 2.23, $p < 0.01$). Oxygen consumption in the patients was improved 3 months after intervention (pre-op 7.66 ± 4.47 vs. 3 months 10.30 ± 4.36; $p < 0.05$), and this effect remained at 1 year after gene therapy (12 months 10.89 ± 4.65; $p < 0.05$). During the optimal drug treatment period, there was no difference in the number of steps (baseline 2.00 ± 2.27 vs. pre-op 2.46 ± 2.07; $p =$ NS) or in oxygen consumption (baseline 6.70 ± 4.54 vs. pre-op 7.67 ± 4.47; $p =$ NS) in the treadmill tests. These results are presented in Fig. 4.

Quality of life

During the optimal drug treatment, there was no improvement in quality of life (baseline 49.77 ± 20.85 vs. pre-op 48.23 ± 18.35; $p =$ NS). One month after *VEGF165* gene therapy, a decrease in these scores was observed, showing an improvement in quality of life (1 month 31.00 ± 15.71; $p < 0.01$). The same decrease was observed 3 (3 months 30.15 ± 20.13; $p < 0.01$), 6 (6 months 27.62 ± 18.47; $p < 0.01$), and 12 months (12 months 28.31 ± 18.14; $p < 0.01$) after gene therapy, indicating an improvement in the quality of life throughout follow-up (Fig. 5A).

Angina (CCS) and heart failure (NYHA) classification

One month after *VEGF165* gene therapy, the patients showed an improvement in angina classification (pre-op 3 [3–3.5] vs. 1 month 2 [2–2]), which remained constant until 1 year after intervention (12 months 2 [1–2.5]; $p < 0.01$). The same results were observed for heart failure classification (pre-op 3 [3–3] vs. 2 [2–2] and 12 months 2 [1–2]; $p < 0.01$). During the optimal drug treatment period, there was no change in either angina (baseline 2.69 ± 0.63 vs. pre-op 3.08 ± 0.64) or heart failure classification (baseline 3 [2–3] vs. pre-op 3 [3–3]). Angina improved in 10 of the 13 patients included in the trial, whereas no improvements were noticed in three patients. There was no case of clinical worsening after gene therapy. During the previous optimal drug treatment period, five patients reported worsening of angina symptoms and eight reported no difference. One year after gene therapy, 11 patients showed improvements in NYHA heart failure classes, whereas 2 patients reported no changes. During the previous optimized clinical treatment period, there were five cases of worsening and eight patients

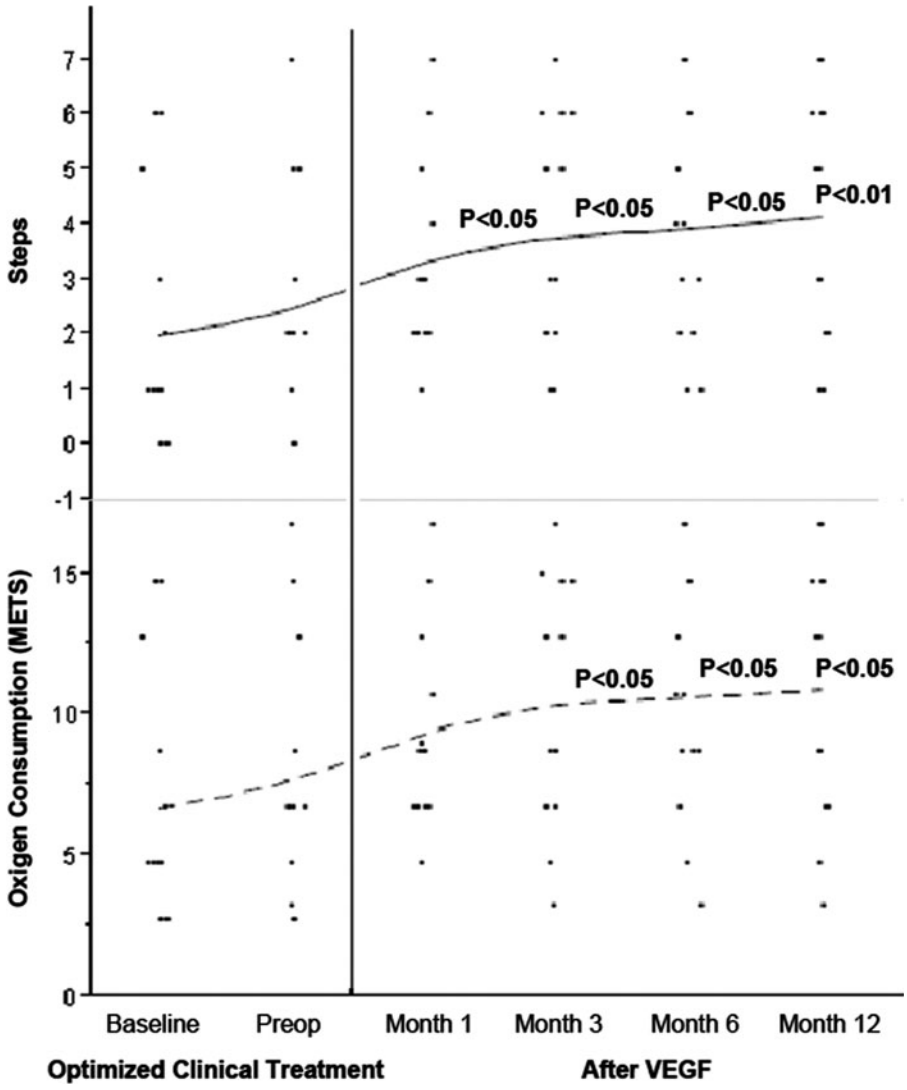

FIG. 4. Steps taken during treadmill tests. The points represent each patient's oxygen consumption (METs), and the lines represent mean values at each point of the study.

reported no difference in relation to NYHA heart failure classes (Fig. 5B).

Discussion

In this trial, we assessed the effect of high doses of *VEGF165* gene therapy for patients with severe CAD and refractory angina after 1-year follow-up. The most effective clinical dose is not yet known. We chose this high dose based on our previous experimental experience, in which injection of 200 μg of plasmidial solution in dogs of 7–10 kg provided significant increase in capillary density of treated areas (Furlani *et al.*, 2009). Furthermore, we believe that since we used a plasmid vector, we would require a higher dose so that the gene could reach the cell nucleus and promote the expression of the proangiogenic factor in heart tissue. Nevertheless, the intervention was shown to be safe and feasible. There were objective and subjective significant changes related to the intervention. A transitory improvement in ischemic SPECT scores and an increase in the number of steps taken and oxygen consumption in the treadmill test, an improvement in quality of life, and angina and heart failure classifications were observed in the patients immediately after treatment.

Only minor complications were observed during the follow-up period of 1 year. Six months after the study began, there were two cases of hospitalization, but only noninvasive management, such as clinical treatment, was necessary to control the situation. There was one case of angina 10 months after gene therapy, which demanded PCI in a new developed coronary lesion. Previous studies have demonstrated the safety and feasibility of high-dose gene therapy in combination with other revascularization techniques (Fortuin *et al.*, 2003).

When the SPECT images were evaluated, we observed an improvement in the perfusion scores in the treated area at 3 and 6 months after the injections of plasmid *VEGF165*, whereas after 1 month an improvement was not yet evident (Kalil *et al.*, 2010). While the scores of segmental ischemia under stress and at rest, SSS and SRS, respectively, which assess both the extent and intensity of myocardial ischemia, showed myocardial perfusion improvements, the differential scores (SDS) for individual observations did not change in this period. The proposed treatment aims to improve

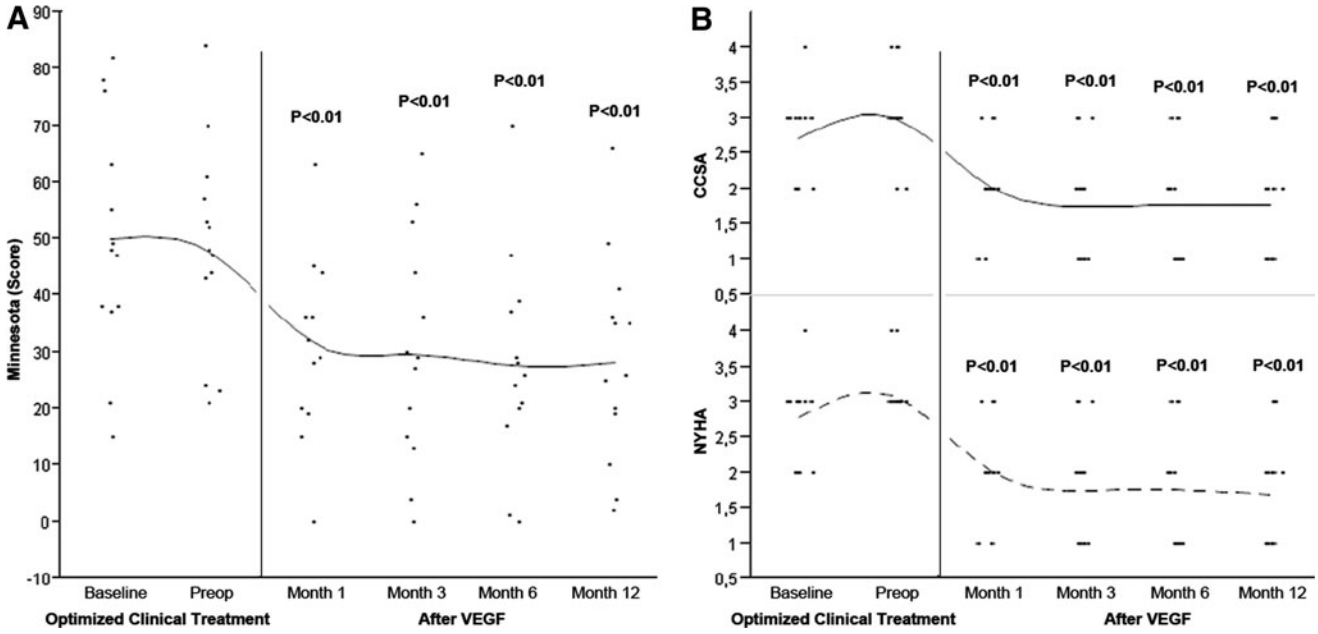

FIG. 5. **(A)** Scores obtained in Minnesota questionnaire ($n = 13$). **(B)** Angina (CCS) and heart failure (NYHA) classification ($n = 13$).

perfusion scores in regions with a viable myocardium rather than recover inactive areas. Thus, an improvement in the scores reflects a real decrease in ischemia severity in the treated areas.

The improvement in the scores of ischemia at rest in this series of patients suggests that myocardial perfusion was also improved, but not enough to provide normal perfusion, so that evidence of stress-induced ischemia remained during stress. One year after gene therapy, the SPECT scores were similar to baseline parameters, showing a transient effect of the treatment. However, no cases of worsening perfusion scores were observed; therefore, considering the severity of the CAD in this class of patients, 1 year without worsening of clinical status may be seen as a favorable result. Gene therapy results in humans have been limited by transitory gene expression, which is caused by several factors, including an immune response from the receiver to the applied vector. For therapeutic angiogenesis, this transitory effect could be enough to create a collateral circulation that would be able to sustain itself, subsequently as suggested by experimental data (Hughes *et al.*, 2004). In the studied sample, with severe CAD and multiple risk factors, whose response to *VEGF* is blunted (Heeschen *et al.*, 2004), this transitory expression might not be enough to ensure significant long-term effects. Also, patient may have experienced natural progression of the CAD during follow-up.

Two previous studies (Losordo *et al.*, 1998; Symes *et al.*, 1999; Sarkar *et al.*, 2001) similar to ours showed different results, with improvements in myocardial perfusion and angina in one, and only an improvement in angina in the other. However, the number of patients in these studies was small; in addition, different doses of *VEGF165* were used. A randomized clinical trial using gene therapy combined with CABG surgery and supplemental oral L-arginine treatment showed promising results for the association of different treatments, also suggesting that endothelial dysfunction may be associated with the pathophysiology of these patients (Ruel *et al.*, 2008).

Two larger studies, using a similar patient profile, compared injection of plasmid *VEGF-165* with placebo using NOGA technique (Kastrup *et al.*, 2005; Stewart *et al.*, 2009). Kastrup *et al.* (2005) used 500 μg of plasmid solution. Three months after the procedure, myocardial stress perfusion defects (primary endpoint) and angina class did not differ significantly between the *VEGF* gene transfer and placebo groups (Kastrup *et al.*, 2005). *VEGF* gene transfer improved the local wall motion disturbances, assessed by both NOGA and contrast ventriculography. No *VEGF*-related adverse events were observed; however, NOGA-procedure-related adverse events occurred in five patients, and one patient died during diagnostic NOGA before randomization. Stewart *et al.* (2009) randomized 93 patients with CCS class 3 or 4 angina, to 2,000 mcg of VEGF plasmid DNA or placebo (buffered saline) delivered via the endocardial route using a percutaneous electroanatomical NOGA guidance catheter. Despite the intramyocardial administration of a high dose of plasmid DNA, there was no benefit of *VEGF* gene therapy at 3 or 6 months for the studied end points (Stewart *et al.*, 2009).

Evaluation of the exercise test results in this series of patients showed improved oxygen consumption at 3 months and up to 1 year after the intervention. For some of the patients, the exercise test had to be interrupted because of claudication and fatigue, which represent a limitation of the test. In a clinical trial using a *VEGF-2* plasmid, Losordo *et al.* (2002) observed an increase in the average duration of physical activity in the treated group compared with the placebo group. A similar study (Stewart *et al.*, 2006), with intramyocardial administration of a *VEGF121* adenoviral vector, showed improvements in exercise time at week 26 after gene therapy in the treated patients, but not in the control patients treated with maximum medical therapy. In this series of patients, quality of life was improved from 1

month up to 1 year after gene therapy with *VEGF165*, but not in the patients receiving maximum medical treatment. A previous study (Losordo *et al.*, 2002) that included patients with angina classes III and IV showed a significant reduction in these classes after 12 weeks of treatment with ph*VEGF2*, which did not occur in the control group. Most of larger therapeutic angiogenesis trials showed nonsubstantial benefit for cardiovascular disease, and the early promising results of preclinical studies are yet to be translated to the clinic (Kastrup, 2010; Zachary and Morgan, 2011).

A possible interference of the placebo effect in this series of patients may change their perception of the symptoms of the disease, representing a confounding variable. However, the improvements observed in both SPECT scans and exercise treadmill tests were objective indications of the relevance of the findings on quality of life and of CCS and NYHA classification. It should also be noted that no improvements were observed in the previous period of optimal medical treatment, which supports the hypothesis that angiogenesis and improved myocardial perfusion were induced by the gene therapy.

It is recognized that the method of a clinical series with prospective temporal control model, instead of a parallel control group, might be a limitation of this study. We chose the temporal control series model, where the patient is his/her own control in the period before treatment, given the heterogeneity and variability of CAD and limitation in sample size. In this case, the method could be appropriate for analysis of the effects of gene therapy. All patients remained under optimal medical treatment for at least 6 months before the intervention, which would be a conventional treatment option for patients who were not undergoing gene therapy. Improvement in symptoms or ischemia parameters such as SPECT during that period was criteria for excluding patients from treatment. It was therefore possible to compare the results obtained with the optimal medical treatment and those after treatment with *VEGF165* by considering each patient as his/her own control. It should be recognized, however, that ischemic heart disease has its own natural history and this could influence the outcomes, irrespective of the treatment options.

We used a more conventional surgical approach instead of the less invasive NOGA technique. There are no randomized studies comparing these two approaches in terms of safety and results. NOGA is attractive since it allows electromechanical mapping of the left ventricle, facilitating injection in appropriate areas, but there is a risk of perforation, which is not negligible as demonstrated by the occurrence of cases of pericardial tamponade requiring cardiac surgery (Kastrup *et al.*, 2005). It is also difficult to be certain that the DNA product was indeed injected into the myocardium. A surgical approach is more traumatic, requiring a 5 cm incision, but allows direct injection and the presence of a thoracic tube reduces the risk of tamponade. With proper SPECT scan analysis and surgical planning, all areas of the left ventricular free wall can be approached through this small incision in the fourth or fifth intercostals spaces. Limitations in this approach are related to the ventricular septum. With modern anesthetic techniques and meticulous patient care, this approach has demonstrated to be safe even in high-risk patients. However, it renders impossible to perform blind, placebo-controlled studies.

Our previous article (Kalil *et al.*, 2010) reported short-term results at 3 months follow-up, and the conclusion at that time was concordant with preclinical and with some small clinical series or trials on *VEGF*-induced angiogenesis: There was a positive effect on reducing myocardial ischemia. In this article, we report that the effect was transitory. We realize this as the main information derived from our findings, very relevant and completely original. The understanding of this transitory effect might explain why gene therapy has had limited clinical application for myocardial ischemia in the clinical setting, contrary to previous experimental reports. Furthermore, it can direct future research, seeking for extending or complementing the initial positive improvement. On the basis of that finding, we are now conducting a preclinical trial associating *VEGF* with angiopoietin, which might contribute for development of more mature vessels, aiming to obtain a more permanent neovascularization.

Gene therapy with the *VEGF165* plasmid was shown to be safe and feasible in this patient group. Three months after gene therapy, the clinical results demonstrated an improvement in the intensity of myocardial ischemia in the treated areas. One year after treatment, the ischemia scores were similar to the preoperative levels, but no patient showed worsening of ischemia during this period. Improvements in quality of life and classes of angina and heart failure were observed in the same 1-month period after gene therapy and were maintained for 1 year after treatment.

Acknowledgments

We would like to acknowledge the staff of the Surgical Unit, Postoperative Unit, and Outpatient Department of Instituto de Cardiologia do Rio Grande do Sul/Fundação Universitária de Cardiologia, where this study took place, for their help in assisting with the patients. We would also like to thank the Research Unit at the same Institution for their efficient assistance throughout this study.

Funding sources: Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; State of Rio Grande do Sul Research Foundation) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; National Council for Scientific and Technological Development).

Author Disclosure Statement

The authors had no relations whatsoever with any laboratory, industry, or company with interests in the subject of this study. The trial was designed and conducted independently by the authors, with financial support from the government agencies cited above (FAPERGS and CNPq).

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Received for publication November 12, 2012;
accepted after revision July 17, 2013.

Published online: August 14, 2013.