

Gene therapy with a plasmid expressing the VEGF₁₂₁ for the treatment of critical ischemic cardiopathy

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ABSTRACT

Here we report, for the first time in Cuba, the evaluation of the positive therapeutic action and safety in a pilot clinical trial for ischemic cardiac arrhythmia, of a gene therapy using a plasmid expressing the vascular endothelial growth factor isoform 121 gene (pVEGF₁₂₁). Six patients irrespective of sex or race, with ages between 49 and 71, were included. They were refractory to conventional medical treatment and discharged as candidates for surgical revascularization procedures or interventionist cardiology. The pVEGF₁₂₁ was administered as a single dose of 0.5 mg/mL, by intramyocardial route in four sites of the ischemic area (250 µL/site), previously defined by SPECT imaging by the surgical team and during a minimal left thoracotomy. Six months later, patients improved their clinical status after therapy, with partial regression in the treated ischemias, increasing quality of life and life expectancy. Plasmid transfection and VEGF expression were also evidenced. Two deaths were the adverse events reported, by myocardial infarction, probably due to perioperative complications derived from surgical risks and the critical status of the patients. Several parameters indicated that pVEGF₁₂₁ administration did not cause adverse events per se, demonstrating that less invasive myocardial treatment alternatives are required to evaluate this product in other clinical phases.

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RESUMEN

Terapia génica con un plásmido que expresa el VEGF₁₂₁ para el tratamiento de la cardiopatía isquémica crítica. La terapia génica para el tratamiento de la cardiopatía isquémica crítica, se usó por primera vez en Cuba en un estudio clínico piloto con seis pacientes de uno y otro sexo, cualquier raza, entre 49 y 71 años de edad, refractarios al tratamiento médico convencional, y no candidatos a procedimientos de revascularización quirúrgica o de cardiología intervencionista. El objetivo era evaluar alguna evidencia positiva de la acción terapéutica de un plásmido (pVEGF₁₂₁) que expresa la isoforma 121 del factor de crecimiento del endotelio vascular (VEGF, del inglés vascular endothelial growth factor) y valorar su seguridad. El pVEGF₁₂₁ se administró en una dosis única de 0.5 mg/1 mL, por vía intramiocárdica en 4 puntos (250 µL/sitio) en la zona isquémica, previamente definida por tomografía computarizada de emisión de fotón único (SPECT), en el curso de una toracotomía mínima izquierda. A los seis meses, los pacientes evaluados tuvieron mejoría clínica y mostraron regresión parcial de las isquemias tratadas: un mejor estado de salud, mayor calidad de vida, y mayores posibilidades de sobrevivir. Hay evidencias de los procesos de transfección y expresión del gen del VEGF. Los principales eventos adversos graves fueron dos fallecimientos por infarto del miocardio, probablemente debido a complicaciones perioperatorias por el riesgo quirúrgico y el estado crítico de estos pacientes. Varios elementos indican que la administración del pVEGF₁₂₁ por sí sola no causa eventos adversos graves, lo cual coincide con otros autores. El tratamiento a estos pacientes debe ser muy delicado, por lo que es necesario encontrar alternativas de tratamiento intramiocárdico menos invasivas que permitan la evaluación de este producto en todas las etapas.

Palabras clave: terapia génica, factor de crecimiento del endotelio vascular, estudio clínico

Introduction

Ischemic cardiopathy is the main cause of death in developed countries and in Cuba. This condition is treated by pharmacological therapy, or transluminal percutaneous coronary angioplasty and coronary bypass surgery. Although efficacious in 30 to 35% of patients, they commonly suffer from restenosis in coronary vessels or incomplete revascularization, with transient improvement only [1]. Besides, most of the patients suffering from coronary arterial disease do

not qualify as candidates for these treatments. Instead of receiving pharmacological therapy, some of them remain handicapped by frequent angina episodes. They are mainly patients at functional stages III or IV, with a precarious quality of life, limited to carry out normal physical activities with physical discomfort. Deterioration of their cardiovascular system is a resident risk of vascular accident with fatal consequences. At final cardiac failure stages, these patients have no other

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therapeutic option than heart transplantation, which is not always possible due to the unavailability of a compatible donor. Even though, they require being under prolonged immunosuppressive therapy to avoid graft rejection, although regarded as not the most adequate treatment. Therefore, new treatment alternatives are required.

Angiogenesis is a physiological process occurring in response to ischemia or hypoxia, involving endothelial cell activation and formation of new blood vessels from the existing ones [2, 3]. Several factors are involved in this process, including: the vascular endothelial growth factor, angiopoietin, fibroblast growth factor type 2 (FGF-2), the platelet-derived growth factor isoform BB (PDGF-BB), nitric oxide and inflammatory cytokines as interleukin 6 (IL-6) [1].

Several growth factors are used for therapeutic angiogenesis, to stimulate growth of collateral blood vessels in ischemic tissues. The induced neovascularization is intended to amplify the biological response by exogenously delivering proteins or genes either in naked DNA or viral vectors.

Several studies described angiogenic therapy with VEGF. The administration of recombinant VEGF improves blood flow into the ischemic myocardium in animal models [4] and in patients with ischemic cardiopathy [5].

Several genes coding for VEGF isoforms have been successfully employed for therapeutic angiogenesis. The DNA gene transfer of plasmids coding for VEGF has promoted the development of collateral vessels in the ischemic myocardium, in preclinical [6] and clinical [7-19] studies, with positive clinical results, such as: improvement in the functional quality of patients, decreased frequency and intensity of angina episodes and therefore, decreased consumption of anti-angina drugs. Imaging evaluations have shown decreased extension and severity of perfusion defects, increased collateral blood flow into ischemic areas and improved ventricular function parameters. In spite of these encouraging results, all the benefits have not been achieved in all the patients [20], with the extensive use of gene therapy remaining to be approved for massive application in humans [1].

Gene therapy with a plasmid coding for the VEGF isoform 121 (pVEGF₁₂₁) previously demonstrated as inducing angiogenesis in animal models of hind limb [21] and myocardium [22] ischemia, was assayed in a pilot clinical trial at the Center for Genetic Engineering and Biotechnology (CIGB), Havana, Cuba [21].

Materials and methods

Patients

Six patients attending the cardiology service of the Hermanos Ameijeiras Hospital with critical ischemic cardiopathy were recruited, irrespective of sex or race, into this non-randomized pilot clinical trial. They were considered as refractory to conventional medical treatment and discharged as candidates for myocardial revascularization procedures; but with other myocardial areas able to be clinically improved, as evidenced by single photon emission computed tomography (SPECT). The protocol was design complying with

the Declaration of Helsinki, and approved by the respective Ethics Committees and the National Regulatory Authority.

Inclusion criteria

1. Patients clinically and angiographically diagnosed with coronary vascular lesion (ischemic cardiopathy), functional class III or IV, according to the Canadian Cardiovascular Society Functional Classification of Angina.
2. Viable myocardial areas as determined by imaging techniques
3. Patients refractory to conventional medical treatment
4. Coronary angiography: Stenosis of coronary arteries of more than 70% narrowing of the vessel lumen
5. Patients discarded as candidates for myocardial revascularization (revascularization surgery and interventional cardiology)
6. Age between 18 and 75 years
7. Agreement of the patient expressed by signing the informed consent

Exclusion criteria

1. Ejection fraction below 30%
2. Severe and sustained arrhythmia
3. Diabetic retinopathy or of any other type
4. Evidence of cancer, cardiac aneurism, severe ventricular dilation or signs of advanced ventricular remodeling
5. Laboratory parameters out of range

Plasmid DNA

A plasmid coding for the VEGF isoform 121 gene under the control of the human cytomegalovirus (CMV) immediate/early promoter was used. All these elements are inserted into a pUC19 vector, containing an origin of replication in *Escherichia coli*. The pVEGF₁₂₁ has a size of 4039 bp. The plasmid was prepared and purified at the CIGB.

Administration of the pVEGF₁₂₁

A single dose of 0.5 mg of pVEGF₁₂₁ was administered in 1 mL of saline, with 1 mL syringes by intramyocardial route at the 4 sites specified (250 µL/site) in the ischemic area during a minimal left thoracotomy, as defined by SPECT imaging by the surgical team.

Patient progression

Safety parameters

1. Clinical evaluation: Patients were questioned and physically examined prior to treatment for the first seven days and in the consultation after 1, 2, 3 and 6 months. The patients and investigators were constantly in contact, to facilitate consultation of any event during the trial.
2. Clinical laboratory parameters: differential leukogram, hemoglobin, haematocrit, erythrocyte sedimentation rate, platelet count, aspartate aminotransferase (AST), total direct and indirect bilirubin, alkaline phosphatase, albumin, cholesterol, glycemia, creatini-

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ne, urea, lactate dehydrogenase (LDH). Prothrombin time, blood type and Rh factor were determined and kaolin assay was carried out as complementary tests for surgery.

3. Enzymes: Creatin kinase (CK) and Troponin T were determined; baseline, and at 2, 7, 30 and 60 days after the administration of the product.

4. Electrocardiogram before and after surgery, for the first 7 days and at 30, 60, 90 and 180 days after the administration of the product.

5. Fundoscopy, to rule out severe retinopathy before and after the administration of the product.

6. Breast exam, rectal examination, abdominal ultrasound and thoracic radiography to discharge pre-existing tumors.

Data related to the safety of the product were obtained by exhaustive interrogation of the patient, being documented in a record of primary data and also in the Case Report Form (CRF) of each patient.

Parameters to assess the effect

1. Clinical evaluation specifying the number and intensity of angina episodes and nitroglycerin requirement.

2. Myocardial perfusion: A MIBI-SPECT was carried out prior to, and at 30, 60 and 180 days after, pVEGF₁₂₁ administration. The left ventricle was divided into 17 segments for the SPECT imaging study. The analysis was circumscribed to those segments covering the viable ischemic areas, to estimate the magnitude of the pVEGF₁₂₁ administration effect, since pVEGF₁₂₁ shows no effect in infarction areas. Besides, segments showing normal perfusion prior to therapy were excluded, except in one case in which declined during the study. Ischemic segments were classified according to their progression toward a normal perfusion ($\leq 70\%$), a deficient but increased perfusion when compared to baseline perfusion (improvement to less than 70%) or a perfusion without changes during the study (no improvement).

3. Ventricular function: A MIBI-SPECT was carried out prior to surgery, and at 30, 60 and 180 days after pVEGF₁₂₁ administration.

Determination of VEGF in blood

Blood levels of VEGF were determined by an immuno-enzymatic method (Quantiquine, R&D Systems. Human VEGF Immunoassay). Blood samples were taken prior to gene transfer and at 2, 4, 7 and 15 days after treatment.

Results

Characteristics of the patients

Most of the patients were male (83%); 67% white and 33% mixed race. Age ranged from 49 to 71 years, with a mean of 59.2 years and a standard deviation of 7.1 years.

Eighty three percent of the patients had previously suffered from acute myocardial infarction, 83% with previous records of smoking; and 55% with records of arterial hypertension. Thirty three percent showed hyperlipidemia, with 17% diabetic and 67% bearing factors predisposing to ischemic cardiopathy and vascular disorders.

Perioperative course

Surgical interventions lasted between 50 and 110 min, with a mean of 81 ± 23 min, the intramyocardial pVEGF₁₂₁ injection lasting between 2 to 10 min with a mean of 5 ± 3 min.

Safety evaluation

Description of adverse events

During the anesthetizing process, one patient with previous records of arterial hypertension and stress manifested bradycardia with hypotension, related to irritation while locating the intracoronary catheter. The patient rapidly recovered after receiving atropine. A small aneurism, not previously seen in the examinations, was detected in the apex during the surgical procedure. Thus, injections were applied in sites distant from the aneurism. At the end of the intervention, the electrocardiogram and the levels of troponin T were normal. Intubation was removed six hours later. After 12 hours, this patient suffered a diaphragmatic acute myocardial infarction, with an extension in the right ventricle, unrelated to the product administration site. It was followed by a systolic dysfunction in the left ventricle and pulmonary edema, also resisting a cardiac arrest and recovering from it. This patient suffered a cardiogenic shock until death, four days after the surgical intervention. An ischemic complication after surgery, with an ischemic cardiopathy class III-IV as pre-existing condition implying high risk for a surgical intervention, was reported as death cause. Death was not correlated to the product, without elements sufficient to establish a factual relationship with the product, even when any interventionist procedure could generate complications in patients with critical conditions and lead to hemodynamic misbalance.

Another patient had an acute myocardial infarction two hours after the surgical intervention, but presenting arterial hypotension, sinus tachycardia and electrocardiographic changes indicative of sub-endocardial ischemia in the anterolateral wall, pulmonary edema and cardiogenic shock. An enzymatic increase compatible with an acute myocardial infarction was observed. It was tried to implant a balloon catheter for intraluminal angioplasty (BCIA), unaccomplished due to an obstruction at iliac and femoral levels. The patient finally died of post-surgical myocardial infarction (29 h). Necropsy evidenced a severe complicated atheromatosis in coronary arteries, aorta and its efferents. These data indicated the patient suffered from pre-existing conditions more severe than clinically evidenced, and by laboratory tests, the electrocardiogram and SPECT. The acute myocardial infarction appeared immediately after concluding surgery, triggering the rest of complications in the patient.

In one case arrhythmia occurred prior to the administration of the product, related to the perioperative procedure and unrelated to the product and its administration. In another case arrhythmia appeared while administering the product. Ventricular arrhythmias have been reported among events attributable to pVEGF₁₂₁ [7, 23].

Clinical laboratory parameters

Laboratory parameters remained unaffected after the administration of pVEGF₁₂₁.

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Determination of VEGF in blood

Blood levels of VEGF were determined as baseline and at 2, 4, 7 and 15 days after administering the product. Transfection and gene expression were evidenced, required for therapeutic action of the product. Adverse events were unrelated to VEGF concentrations in blood, occurring at the perioperative period and previous to VEGF expression and distribution through the systemic circulation.

Two days after treatment, the blood levels of VEGF remarkably increased as indirect evidence of expression of the transferred gene. The VEGF level peaked at 4.6 ± 3.8 times baseline levels, between days 4 and 15 after treatment. The high variability in VEGF levels and the magnitude and duration of the therapeutic response were in agreement with reports from other research groups [24].

The highest mean level of VEGF was over $1 \mu\text{g/mL}$ and was reached between days 2 and 4 after treatment, with a decreasing tendency above baseline levels until the last determination at day 15.

Parameters for effect assessment

In spite of being a pilot study evaluating a very small sample of patients, in which only four patients were followed during six months, in this report we describe results based on the therapeutic effect according to some variables.

Clinical evaluation

Cardiac angina episodes: The weekly episodes of cardiac angina drastically decreased one month after treatment, with slight oscillations after three and six months (Figure 1).

Nitroglycerin tablets consumption: Nitroglycerin consumption was reduced from one to six months after therapy; although at two months, one patient reported consumption to treat a cardiac angina episode refractory to rest.

Cardiac angina functional classification: Cardiac angina was functionally classified based on patient interrogation. One month after treatment, the functional class tended to improve almost until class I, a tendency increased at month two and sustained until the sixth month (Figure 2). Patients showed some cardiac angina episodes after intramyocardial therapy with pVEGF₁₂₁, while eliminating nitroglycerin consumption almost completely. Thus, cardiac angina decreased in intensity, since chest pain resolved without pharmaceutical treatment, occasionally occurring at specific situations such as: postprandial, under stress and after exercise of certain intensity, implying an improvement in the functional classification. These variables are highly relevant to reflect the clinical status and, therefore, the patient's quality of life.

At the beginning of the study, patient HA01 suffered from seven episodes of cardiac angina every week, only alleviated with 14 tablets of nitroglycerin per week. One month after the therapeutic study, this patient had no angina episodes, not consuming nitroglycerin until three months after the study. Six months after, the patient referred an angina crisis per week, responsive to rest and without any anti-angina treatment, and also showed a gradual increased in walking. Six months after the study, this patient reduced the

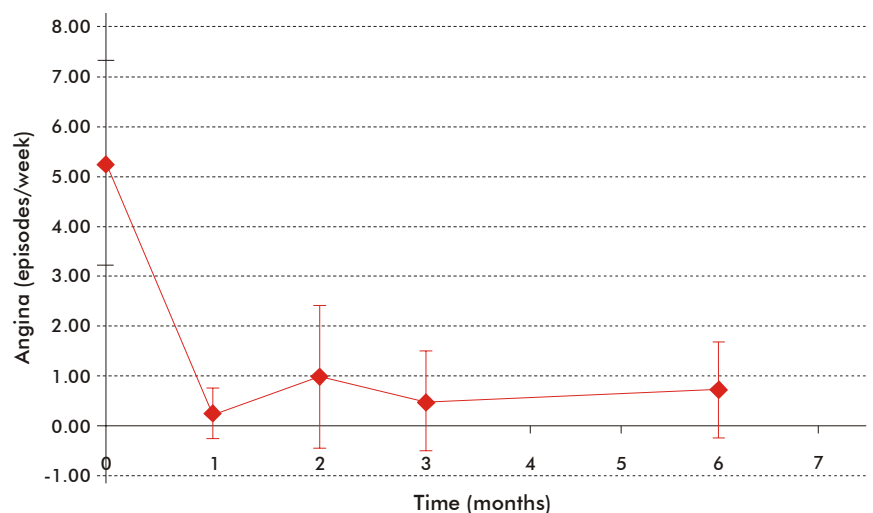


Figure 1. Cardiac angina episodes in four patients following intramyocardial gene therapy with pVEGF₁₂₁. Data are presented as the mean $\pm$ standard deviation.

walking distance without pain, compared to the distance walked after three months, but still being ten times the distance walked without pain before treatment. This result corresponded to a recurrence of chest pain. In the final evaluation of this patient (HA01), it was determined a clinical improvement considering the improvement in the functional class of cardiac angina and the suppression of nitroglycerin consumption.

In the case of patient HA02, cardiac angina was classified as functional class III, maintained one month after intramyocardial therapy with pVEGF₁₂₁. Two months later it was classified as functional class II, which was maintained at month sixth. The change in the functional class of cardiac angina was representative of slight limitations in ordinary life activities for the patient, with angina only occurring during physical exercise, postprandial or under emotional stress conditions. At the beginning of the study, this patient commonly suffered from four angina episodes

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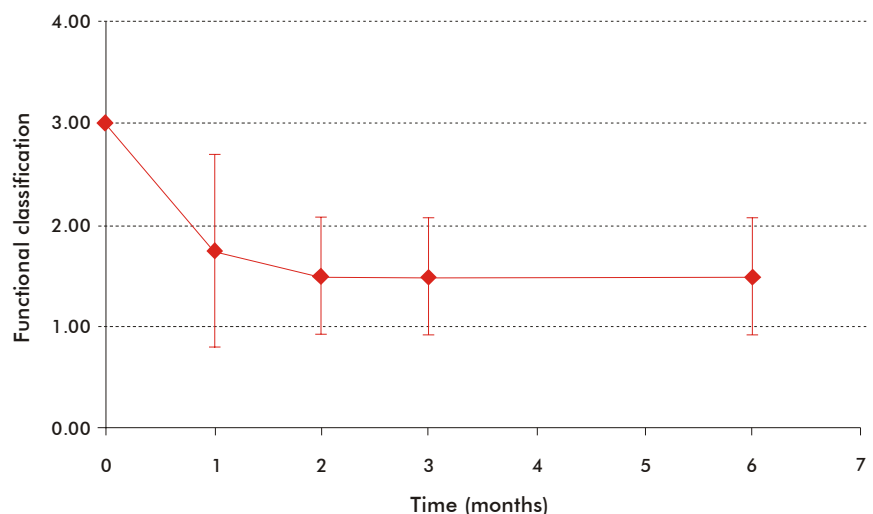


Figure 2. Functional classification of cardiac angina in four patients following intramyocardial gene therapy with pVEGF₁₂₁. Data are presented as the mean $\pm$ standard deviation.

per week, sometimes alleviated with nitroglycerin treatment (1 tablet/week). Angina episodes fluctuated all over the study, two three and six months, with crises responsive to rest, without nitroglycerin treatment. Walking was not estimated, because this patient presented peripheral arterial insufficiency interfering with the evaluation by causing pain in hind limbs. This patient was considered as clinical improvement, based on recovery in the functional class of cardiac angina and suppression of nitroglycerin consumption.

Patient HA03 formerly showed a functional class III angina, being classified as class II one month after treatment, what remained unchanged after six months. This change in the functional class represented slight limitations in ordinary life activities, with angina only appearing after physical exercise, at postprandial or under emotional stress conditions. At the beginning, this patient used to suffer from seven angina crises every week, alleviated with nitroglycerin treatment (7 tablets/week). One month after therapy, angina disappeared, stopping nitroglycerin consumption. After two months, the patient referred one angina episode per week, requiring one tablet of nitroglycerin. On month three and until the end of the study, the patient did not refer any other angina episode nor nitroglycerin treatment. This patient also suffered from peripheral arterial insufficiency, with pain in hind limbs that interfered in the evaluation of the walking distance without chest pain, avoiding an objective estimation. This patient was evaluated as clinical improvement, based on recovery in the functional class of cardiac angina and suppression of nitroglycerin consumption.

When included in the study, patient HA05 had cardiac angina functional class III, which evolved to functional class I one month after treatment, remaining until the end of the study. This classification represented an improvement in ordinary physical activity, such as: walking or climbing stairs without chest pain, which only appeared after intense, fast or prolonged exercise. At the beginning, the patient used to suffer three angina episodes per week, responsive to nitroglycerin treatment. One month later, angina episodes and nitroglycerin consumption stopped, a status that remained until the end of the study. The patient showed a progressive increase in walking along the study up to 2 km. This patient was evaluated as clinical improvement, based on recovery in the functional class of cardiac angina and suppression of nitroglycerin consumption.

Evaluation of myocardial perfusion with MIBI SPECT

Severe and moderate perfusion defects progressively evolved to mild, with all the defects being evaluated as mild, indicating improvement after myocardial perfusion.

An alternative analysis of the progress of cardiac segments involved in the viable ischemic areas was implemented, considering that the variables analyzed were qualitative and that categories they included were inflexible enough to exclude small variations in the progress of patients.

Along the study, when analyzing changes in ischemic segments after perfusion (Figure 3), six out of 17 segments (35.3%) were normal one month after

perfusion, five (29.4%) were improved without reaching normality and six (35.3%) remained unchanged. Two months after therapy, some segments showed recurrence to ischemic conditions. After six months, five segments (29.4%) were normal after perfusion and seven (41.2%) improved below 70% of perfusion. The remaining five segments (29.4%) showed no improvement. Summarizing, the number of segments showing any sign of improvement increased, meanwhile the number of unchanged segments tended to decrease.

The highest perfusion response was achieved one month after treatment, still higher than baseline after six months, although lowered, as previously reported by other research groups [8-10, 12, 14, 17].

Patient HA01 presented mild inferior ischemia when included in the study, remaining unmodified one month after treatment, with complete remission of ischemia after two months. However, four months later, perfusion declined in the ischemic region from 100% to 70%. The positive response occurred after two months and slightly decreased after six, but maintaining improvement compared to baseline. The patient was evaluated as partial regression of ischemia.

Patient HA02 presented at the beginning a mild to moderate apical anteroapical ischemia, which improved to mild with recovered perfusion in the apex, one month later as maximum response. It was reported as partial regression of ischemia, which deteriorated after two months but remained higher than baseline, since the defect did not compromise the apex as at the beginning. Ischemia improved to mild after six months, without reaching levels attained one month after treatment. The patient was finally evaluated as partial regression of ischemia.

Patient HA03, who from a severe inferior wall ischemia, was included in the study. One month after therapy, the defect improved to moderate. However, a new inferior wall lateral apical ischemia appeared, unrelated to the pVEGF₁₂₁ administration areas. This new defect could be associated to the baseline ischemic cardiopa-

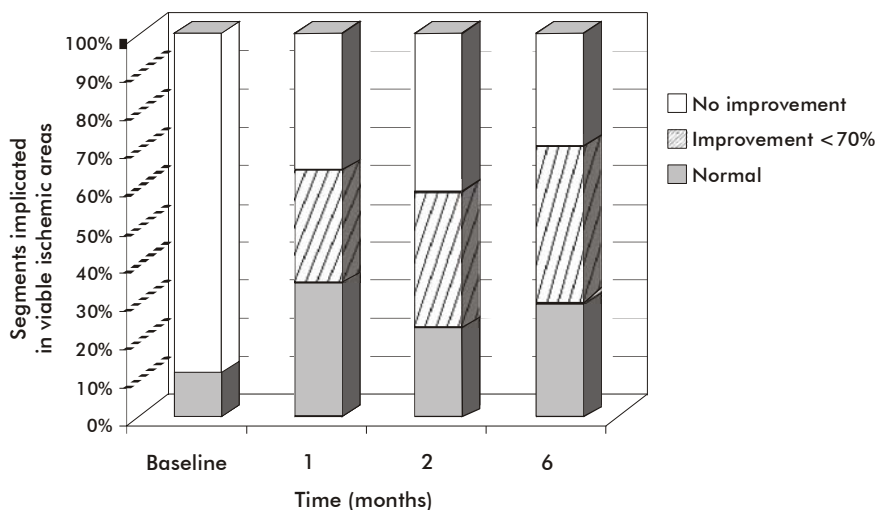


Figure 3. Changes in viable ischemic areas following intramyocardial gene therapy with pVEGF₁₂₁. Data are presented as percentage of all the segments in the four patients. The normal progression after perfusion refers to values higher than 70%.

thy shown in this type of patient, which continues acting on the cardiac vasculature, in spite of therapy. Three months later, the inferior wall ischemia continued improving to mild, while the inferior wall lateral apical ischemia remained. After six months, both effects were reported as mild, with a significant improvement compared to baseline. The highest response was achieved since three months until the end of the study, being evaluated as a partial regression of ischemia.

Patient HA05, included in the study with a mild anteroapical ischemia, remained steady two months after treatment. Some improvement was reported in the treated area compared to baseline after six months, the time point of highest response. The patient was evaluated as partial regression of ischemia.

One month after therapy, improvement was reported and remained until the end of the study in the inferior wall lateral region, unrelated to the area injected with pVEGF₁₂₁. It was probably due to connection of new developed vessels to pre-existing networks irrigating other areas in the myocardium, distal to the pVEGF₁₂₁ injection site. Therefore, the blood flow was improved in these areas, in addition to those chosen by the researchers [8].

The paracrine action of VEGF once expressed and secreted by cardiomyocytes is another possible mechanism to stimulate angiogenesis in distal regions from the injection site. Similar effects were observed in patients HA02 and HA03. The first one received the injection in the anteroapical area, experimenting improvement in the inferior mid-zone. The second one received treatment in the inferior wall, showing improvement in the anteroapical basal and inferior wall lateral basal areas. These improvements were additional to those found in the treated areas.

Noteworthy, non-viable necrotic areas corresponding to previous infarctions suffered by the patients did not improve.

Another aspect denoting improvement and followed during the study was the cardiac segments implicated in viable ischemic areas. Patient HA01 started the study with an ischemic segment, presenting normal perfusion after six months. Patient HA02 was included with five affected segments, two of them normalizing, one improving below 70% of perfusion and the other two showing no improvement after treatment. Patient HA03 had two segments with normal perfusion, which converted into ischemic; other five segments were initially affected, one of them normalized and the other four with some improvement below normality. Patient HA05 started with four ischemic segments, one normalizing, two improving below 70% and one remaining ischemic. Improvement was evidenced in all the cases, with normalized perfusion.

These results were circumscribed to the progression of patients until six months, remaining to be undertaken comprehensive studies with more patients, evaluating their progression for longer periods of time.

It should be noticed that VEGF₁₂₁ generates a collateral neovascularization that improves irrigation in the ischemic area, with weak vessels. Besides, risk factors (arterial hypertension and diabetes) predisposing to arteriopathy, remain in spite of therapy, probably accounting for the temporary improvement of some

segments which progress over time into the ischemic condition.

Evaluation of ventricular function by MIBI SPECT

No changes were observed in the ventricular function instead of clinical and imaging evidences of patient improvement, similar to other studies evidencing no significant changes in the ejection fraction of the left ventricle [7, 15].

Remarkably, ventricular function is a global variable, comprising all the zones in the left ventricle, not only the ischemic ones, but also necrotic from previous infarctions.

The lack of improvement in the global function of the ventricle could be determined by perfusion defects of great extensions and severity of infarctions refractory to the therapeutic action of the product, even improving the treated ischemic area.

Discussion

The administration of pVEGF₁₂₁ to patients suffering from critical ischemic cardiopathy was carried out during thoracotomy, an invasive procedure requiring anesthetics administration as any surgical intervention and other surgery-related risks. Considering the delicate hemodynamic status of these patients, any surgical procedure implies a significant risk.

The selection of patients is one of the essential aspects influencing gene therapy results [1]. Their deteriorated health conditions, refractory to conventional medical treatment, imply that any surgical intervention, even by thoracotomy, a risky procedure implying perioperative complications as explained in the informed consent to the patient.

Preclinical studies supporting safety and efficacy aspects for using growth factors to develop new blood vessels are carried out in healthy young animals, without risks of cardiovascular diseases. This is not the real scenario, where patients have no therapeutic alternatives, thus in many cases suffer from advanced atherosclerosis [1].

Some authors have evaluated the beneficial effect of VEGF combined with transluminal percutaneous angioplasty or coronary bypass [1, 9]. In any case, the patient subjected to some of these surgical options should always receive benefits that surpass the surgical risk.

The less invasive catheter-based system is used to administer growth factors to groups of patients in more advance evaluation phases (II and II). Through this system the product is delivered to the ischemic site and blind trials are carried out, to avoid complications of thoracotomy [25].

Improved clinical parameters are the main purpose of gene therapy. Any changes in these variables bring an idea on the efficacy of the product, due to the initial health conditions of the patients. Evidences found in this study of clinical improvement by using VEGF are in agreement with previously reported similar studies [7-10, 12, 14-17, 19].

The product administered dose is one of the most discussed aspects concerning the effect. Administration of different doses has been studied and at this

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moment, patients are being recruited for a study administering 2 mg of VEGF, trying to increase the beneficial effects [1] obtained in the EUROINJET-1 trial [25] by injecting 500 mg of VEGF₁₆₅.

The administration route is also relevant. Delivery into the ischemic tissue (intramyocardial) rendered best results, maybe due to a synergic effect with the endogenous ischemia-induced revascularization which reinforces the effect of the product [1].

Future strategies point toward concerted stimulation of angiogenesis and arteriogenesis to treat ischemic diseases. Neovascularization of ischemic muscles require coordinating both processes [26]. New blood vessels developed by angiogenic processes from pre-existing vessels in the ischemic tissue are not robust enough to compensate the incidence of risk factors accompanying vascular diseases, the patients being affected ultimately.

Arteriogenesis comprises the development or enlargement of collateral arterioles as adaptative response to arterial occlusion, by a *de novo* process starting from circulating endothelial precursors. Unlike angiogenesis, arteriogenesis does not depend on ischemia and hypoxia [27, 28], but occurring in regions proximal to tissues in active angiogenesis [26]. Several proteins seem to mediate this effect: monocyte chemoattractant protein 1 [29, 30], transforming growth factor beta [31], granulocyte macrophage colony-stimulating factor [32], FGF-1 and FGF-2 and PDGF-BB [33]. Therefore, it is plausible to consider that strategies for combined administration of arteriogenesis- and angiogenesis-promoting factors could be more successful [34].

Therapies using circulating endothelial progenitor cells have been suggested as alternatives to repair endothelial lesions and also induce angiogenesis [35]. When administered in animal models of myocardial infarction, the endothelial progenitor cells induced a

significant neovascularization and reduced apoptosis, improving cardiac function. The different bone marrow-derived cellular subsets secrete angiogenic factors that exert a vital function in neovascularization, such as: VEGF, FGF, hepatocyte growth factor and angiopoietin 1. The final cardioprotective effects and the neovascularization rate achieved depend on the number of cells delivered [36].

Cuba shows a technological development sufficient to produce the pVEGF₁₂₁ and an adequate health infrastructure to carry out this project. At this moment, problems reported by other groups are being studied, but is essential to find strategies to determine if gene therapy is a real alternative for cardiovascular disorders, to treat the first cause of morbidity and mortality in Cuba and in developed countries.

Conclusions

The main adverse events in this study of gene therapy with a plasmid expressing the VEGF₁₂₁ to treat critical ischemic cardiopathy were related to the administration procedure and severity of the pre-existing disease in patients. Plasmid transfection, expression of the VEGF gene and effects after administration were evidenced, with clinical improvement of patients in the evaluated period and partial regression in their treated ischemias. Nevertheless, their ventricular function did not improve. The administration of VEGF₁₂₁ by other routes could bring about more conclusive evidences on this type of therapy.

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