Rev Bras Cir Cardiovasc 2009; 24(2): 143-149

Effects of therapeutic angiogenesis with plasmid $VEGF_{165}$ on ventricular function in a canine model of chronic myocardial infarction

Efeito da angiogênese terapêutica com $VEGF_{165}$ sobre a função ventricular em infarto do miocárdio crônico

Ana Paula FURLANI[1], Renato A. K. KALIL[2], Iran CASTRO[3], Andrés CAÑEDO-DELGADO[4], Marinez BARRA[5], Paulo Roberto PRATES[6], Roberto T. SANT'ANNA[1], Ivo A. NESRALLA[7]

RBCCV 44205-1069

Abstract

Objective: **Therapeutic angiogenesis is currently under investigation for CABG procedure. This study aimed to verify the angiogenesis induction and functional myocardial improvement by transmural injection of plasmid $VEGF_{165}$, in areas with chronic myocardial infarction.**

Methods: **Left thoracotomy was performed in 10 mongrel dogs, and myocardial infarction induced by ligation of the major diagonal branch of the anterior descending coronary artery. At the 7th postoperative day, left ventricular ejection fraction was assessed by echocardiogram. The animals were divided into 2 groups: Treated (TG) and Control, (CG) and underwent a second procedure, for intramyocardial injection of solution with plasmid $VEGF_{165}$ at 200 mg/mL (TG) or saline solution (CG), injected over 10 points of the ischemic areas. Fourteen days later new echocardiogram was performed, the animals were sacrificed and histological examination was performed.**

Results: **Ejection fraction was maintained in the treated group: initial echocardiogram post-AMI: CG 59.33 ± 4.6% and TG 52.45 ± 15.1% (*P*=0.359). EF after 14 days: CG 39.37 ± 19.43% and TG 48.53 ± 11.74% (P=0.394). Intra-group comparison: negative variation of LVEF in the CG: 59.37 ± 4% to 39.37 ± 19.43% (*P*=0,04) and maintenance if the TG: 52.45 ± 15.1% to 48.53 ± 11.74% (*P*=0,59). In the CG was revealed a non significant increase in capillary vessels number in injected areas, but in all the myocardium. Paradoxically, arterioles were significantly less in number in all areas of treated group.**

Conclusion: **Transmural injection of plasmid $VEGF_{165}$, resulted in minimization of myocardial contractility loss,**

1. Resident Physician.
2. PhD in Medicine; Scientific Director of the Cardiology Institute of Rio Grande do Sul/University Cardiology Foundation. Associate Professor of UFSPA.
3. PhD in Health Sciences: Cardiology; Professor of the Postgraduation Program in Health Sciences of the Cardiology Institute of Rio Grande do Sul/University Cardiology Foundation.
4. PhD in Genetics; Professor of the Postgraduation Program in Health Sciences of the Cardiology Institute of Rio Grande do Sul/ University Cardiology Foundation.
5. Pathologist Specialist; Responsible for the Pathological Anatomy Service of the Cardiology Institute of Rio Grande do Sul/ University Cardiology Foundation.
6. Specialist in Cardiovascular Surgery; Responsible for the Surgical Unit of the Cardiology Institute of Rio Grande do Sul/ University Cardiology Foundation.
7. Full Professor, UFRGS; Head of the Cardiovascular Surgery Service of the Cardiology Institute of Rio Grande do Sul/ University Cardiology Foundation.

This study was carried out at the Cardiology Institute of Rio Grande do Sul/ University Cardiology Foundation – Porto Alegra, RS, Brazil, and Federal University of Heath Sciences of Porto Alegre.

Correspondence address:
Prof. Renato A.K. Kalil. Unidade de Pesquisa do IC/FUC. Av. Princesa Isabel, 370 – Santana – Porto Alegre, RS, Brazil – CEP 90620-001.
E-mail: kalil.pesquisa@cardiologia.org.br/kalil@cardiologia.org.br

This research project was supported by grants from the Ministry of Science and Technology/National Research Council - CNPq, Brazil and from the Research Foundation of the State of Rio Grande do Sul - FAPERGS.

Article received on September 25th, 2008
Article accepted on May 20th 2009

in the chronic-stage of AMI. Histological examination of the vascular network, however, was unable to explain completely these results.

Descriptors: **Angiogenesis inducing agents. Myocardial infarction. Animal experimentation. Myocardial ischemia. Gene therapy.**

Resumo

Objetivo: **Angiogênese por terapia gênica é alternativa ainda experimental para revascularização miocárdica. Este estudo objetivou verificar a indução de angiogênese e melhora funcional miocárdica pela injeção transmural de plasmídeo contendo $VEGF_{165}$ em áreas de infarto crônico do miocárdio.**

Métodos: **Em 10 cães anestesiados, por toracotomia lateral esquerda, foi induzido infarto agudo do miocárdio (IAM) por meio da ligadura do ramo diagonal principal da artéria coronária descendente anterior. Após 7 dias, realizado ecocardiograma para avaliação da fração de ejeção (FE) ventricular esquerda. Os animais foram divididos em dois grupos: Tratado (GT) e Controle (GC) e submetidos a segundo procedimento, para injeção intramiocárdica de solução contendo plasmídeo $VEGF_{165}$ na concentração de 200mg/ml (GT) ou solução salina (GC), distribuída em 10 pontos da área infartada. Após 14 dias, novo ecocardiograma, sacrifício dos animais e retirada do coração para estudo histológico.**

Resultados: **Houve tendência à manutenção da FE no GT e de queda da FE no GC, conforme os valores: FE ao ecocardiograma pós-IAM inicial: GC 59,33 ± 4,6% e GT 52,45 ± 15,1% (*P*=0,359). FE após 14 dias: GC 39,37 ± 19,43% e GT 48,53 ± 11,74% (*P*=0,394). Comparação intra-grupo: Variação negativa da FEVE GC: 59,37 ± 4% para 39,37 ± 19,43% (*P*=0,04) e manutenção GT: 52,45 ± 15,1% para 48,53 ± 11,74% (*P*=0,59). No GT observou-se aumento do número de vasos capilares, mais intenso nas regiões injetadas, porém presente em todo miocárdio. Paradoxalmente, no GT houve redução do número de arteríolas.**

Conclusão: **A injeção transmural de plasmídeo $VEGF_{165}$ resultou em tendência para atenuar a perda de contratilidade consequente ao dano miocárdico, na fase crônica do IAM. O exame histológico da rede vascular, entretanto, não explica completamente os eventos funcionais.**

Descritores: **Agentes indutores da angiogênese. Infarto do miocárdio. Experimentação animal. Isquemia miocárdica. Terapia de genes.**

INTRODUCTION

Gene therapy represents an interesting therapeutic alternative for ischemic cardiomyopathy, using genes encoding angiogenic growth factors to promote the development of new blood vessels or the remodeling of existing vessels [1,2]. In spite of significant developments in the treatment of ischemic cardiomyopathy during the last two decades, some cases of advanced coronary arterial disease are not suitable for conventional therapy, so that alternative therapeutic strategies are necessary. In this regard, neoangiogenesis induced by alternative methods could provide some revascularization in ischemic areas for no-option patients.

Experimental studies of ischemic models have shown that angiogenic interventions represent a valuable approach to improve myocardial perfusion [3,4], as well as left ventricular function [5]. Genes encoding angiogenic agents may be successfully administered associated to liposomes, via viral vectors, or by direct intramyocardial injection of plasmid DNA. Myocardial injection presents some advantages, such as the low level of potential side effects and, depending on the type of transfection employed, the possibility to be used as adjuvant of well established therapeutic protocols. The effect intensity is influenced by route of administration and vector used. There are evidence, however, that naked DNA is sufficiently effective at the target organ and produces less global effects than when viral vectors are used [6].

Vascular endothelial growth factor (VEGF) is one of the best characterized angiogenic agents [7-11], employed alone or in association [12]. Expression of this factor is induced by hypoxia, and its mitogenic activity is specific for endothelial cells, suggesting that it is a natural mediator of angiogenesis in response to ischemia. VEGF is most commonly produced as a homodimer of polypeptides with 165 or 121 aminoacids ($VEGF_{165}$ e $VEGF_{121}$, respectively), and both forms have been shown to improve colateral circulation in experimental models [4,8,10].

In this study, we evaluated the effects of myocardial administration of plasmidial $VEGF_{165}$ on maintenance of ventricular function, possibly due to generation of new

blood vessels (capillaries and arterioles) in a canine model of chronic ischemic cardiomyopathy.

METHODS

Animals

Ten male dogs of unknown breed, weighing between 9 and 12 kg (9.2 ± 2.14 kg), were used. The dogs received care in compliance with the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Academy of Sciences, Washington DC, and the ethical principles for animal experiments of the Brazilian Code for Animal Experimentation (COBEA).

This study was approved by the Internal Ethical Committee in 19/09/2001, under protocol number UP2891.

Production of $pHuVEGF_{165}$

The $pHuVEGF_{165}$ plasmid, a transient and non-viral vector that express the $VEGF_{165}$ gene under the control of the CMV promoter, was obtained from Genentech (San Francisco, CA, USA) and introduced in XL1-Blue *Escherichia coli* by standard heat-shock transformation. Three clones of transformed cells were analyzed with different restriction enzymes, and one of them was selected for further cloning. The resulting plasmid was extracted with the PureLink™ HiPure Plasmid Maxiprep Kit (Invitrogen, USA), which allows the isolation of a large amount of DNA (0.5-1 mg). Plamid integrity was analyzed by electrophoresis in agarose gel stained with ethidium bromide, and DNA was quantified by spectrophotometry at 260-280 nm.

Experimental methods

The animals were anesthetized with intravenous administration of thiopental (3 to 5 mg/Kg), propofol (3 mg/Kg) and pancuronium (0.1 mg/Kg) and were mechanically ventilated with a volume respirator.

A lateral left thoracotomy through the fifth intercostal space was performed, and acute myocardial infarction was made by simple ligation of the main diagonal branch of the left anterior descending coronary artery with 5.0 monofilament propylene suture. The pericardium and chest were closed and the dogs were allowed to recover. The animals were maintained in a veterinary clinic, and seven days later (day 7) were submitted to transthoracic echocardiography for examination of the ischemic area and measurement of left ventricular ejection fraction (LVEF). On the same day, the dogs were submitted to a second surgical procedure, with a transmural injection of 1 mL saline (n=5, control group) or 200 μg of the plasmidial $VEGF_{165}$ in 1 mL saline solution (n=5, treated group), administered in 10 points in and around the ischemic area. The dogs were allowed to recover and remained for another 14 days at the veterinary clinic, when a second transthoracic echocardiogram and measurement of LVEF were performed (day 21). All echocardiograms were independently examined by two investigators, and in case of difference in measurements, mean values were considered.

On the final day the dogs were sacrificed and the hearts were excised and fixed in buffered formalin for histological analysis.

Histology and analysis of vascular density

Myocardial samples were collected from ischemic and transition areas, as well as from the posterior wall of the left ventricle, free from ischemia in both groups. Histological sections size of the ischemic area were 2.59 ± 0.59 cm^2 and 2.38 ± 0.76 cm^2 for treated and control groups, respectively; for the transition area, sections' size were 2.41 ± 0.37 cm^2 and 2.83 ± 0.94 cm^2, and for the posterior wall, 1.33 ± 0.22 cm^2 and 1.52 ± 0.39 cm^2. The sections were embedded in paraffin, cut in 5 micra sections and stained using the hematoxylin-eosin method. Vascular density and vessel size were electronically determined. The mean weight of the samples analyzed was 110.8 ± 16.57 g and 132.4 ± 26.89 g in treated and control groups, respectively. Vessels smaller than 25 mm in diameter were considered to be capillaries, vessels larger than 25 mm and smaller than 100 mm were considered arterioles, and those larger than 100 mm were considered to be arteries [13] (Figure 1).

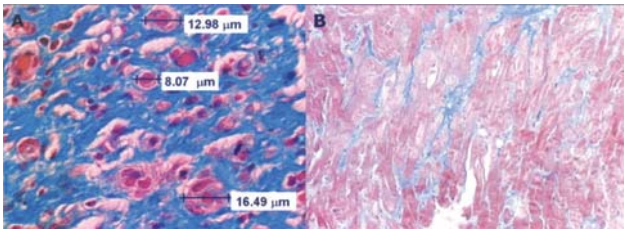

Fig. 1 - (A, left) Histological aspect of canine myocardium treated with plasmidial $VEGF_{165}$, prepared for the evaluation of vessel density. Vessels count and diameter were done through an electronic automated program. There was a significant increase in capillaries, defined as vessels under 25 micra in diameter, in all myocardial areas, but more pronounced in the transition area between ischemic and normal myocardium. Stain: haematoxylin-eosin. (B, right): Histological aspect of infracted canine myocardium, where areas of fibrosis (gray-blue stained areas) are demonstrated. Stain: Masson's trichromic

Statistical analysis

Statistical analysis were done using Student's t test for paired samples between each two areas of control and treated groups and between pre and post values for ventricular function.

RESULTS

Transthoracic echocardiography

There were no significant differences in mean LVEF between treated and control groups during the study period. Mean LVEF on day 7, before treatment, was 59.3 ± 4% in dogs of the control group and 52.45 ± 15.1% in the treated group. On day 21, mean LVEF was 39.37 ± 19.43% and 48.53 ± 11.74% in the control and treated groups, respectively.

Comparison of the evolution within each group, however, showed that mean ejection fraction decreased significantly more in control dogs than in those receiving plasmidial $VEGF_{165}$. When mean LVEF was compared in days 7 and 21, a reduction of 3.9 ± 15% was observed in the treated group (*P*=0.13), whereas in the control group this reduction was 19.9 ± 15% (*P*=0.04) (Figure 2).

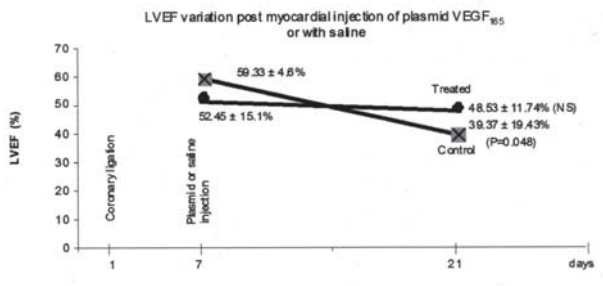

Fig. 2 - Mean LVEF of control (injected with saline) and treated (injected with plasmid $VEGF_{165}$) dogs on day 7 (pre-treatment) and day 21 (14 days after treatment). The LVEF declined significantly in the control group and was maintained in the treated group. LVEF= left ventricular ejection fraction

Vessel Density

As presented in Table 1, mean capillary density was higher in all areas of myocardium analyzed in the treated group when compared with the control group, this difference, however, was not significant. In the treated group, capillary density was higher in the transition than in the posterior wall (*P*=0.029). This difference was not observed comparing the same areas of the control group (*P*=0.480).

An increased number of arterioles was observed in the ischemic and transition area of control when compared to the same areas of the treated group (*P*=0.016 and *P*=0.024, respectively). Control group showed higher arteriolar density in the ischemic area than in the posterior wall (*P*=0.018) .This difference was not observed comparing the same areas of the treated group (*P*=0.201).

DISCUSSION

The main target of VEGF is the endothelial cell. Recent studies in experimental models of myocardial ischemia involving mammals of medium size have shown that VEGF is arteriogenic, supporting their use to promote arteriole growth in patients with severe coronary disease [14]. Other roles have also been suggested for VEGF, such as the ability to induce cardiomyocyte cytokinesis, as revealed by cardiomyocyte hyperplasia in an experimental model for ischemia [15,16].

We have previously reported a canine model of ischemic cardiomyopathy with myocardial perfusion analysis through cintilography, in which the gene encoding the green fluorescent protein was successfully transfected [17]. With a similar model, we were able to induce myocardial angiogenesis by transmural injection of plasmidial $VEGF_{165}$ in areas of acute myocardial infarction (AMI) in dogs [18]. A similar method was used by Kawasuji et al. [13], but in that case intramyocardial basic fibroblast growth factor (bFGF) was administered.

The favorable results obtained in experimental studies, and the absence of adverse effects related to the use of $VEGF_{165}$ in initial clinical trials, indicate the potential clinical use of this therapeutic approach. Recent clinical studies showed that high doses of rhVEGF improve myocardial perfusion in patients with known severe coronary artery disease and provided evidence of a dose-dependent effect [19]. Clinical results obtained with the treatment of patients with stable exertional angina have shown significant

Table 1. Number of capillaries and arterioles per square centimeter in each of the three areas analyzed under histological examination. Capillaries were defined as vessels under 25mm and arterioles those between 25 and 100 mm in diameter. Areas: Ischemic was the center, where some fibrosis could be identified, Transition was between ischemic and normal myocardium, Posterior was the dorsal area of normal myocardium away from the intervention.

Group	Capillaries/cm² Area			Arterioles/cm² Area		
	Ischemic	Transition	Posterior	Ischemic	Transition	Posterior
Control	143 ± 65.05	111.4 ± 79.06	59.4 ± 52.3	183.8 ± 41.94	146.2 ± 48.99	79.2 ± 52.51
Treated	186.4 ± 85.24	199.4 ± 74.75	100 ± 41.92	104.8 ± 40.21	79.6 ± 21.52	59.6 ± 52.4
P<	0.392	0.082	0.213	0.016	0.024	0.571

improvement in angina class and a favorable trend in angina frequency and exercise treadmill test in patients receiving rhVEGF as compared with placebo [20].

The optimal method for administration of VEGF is unclear. Catheter-based local intracoronary transfer of adenoviral VEGF was shown to be safe and to improve myocardial perfusion during percutaneous transluminal balloon angioplasty and after 6-month follow up [21]. On the other hand, direct intramyocardial injection resulted in improved contractility of the ventricular wall as evaluated by NOGA and ventriculography [22]. In this study, the stress-induced myocardial perfusion was not affected, but the results suggest an anti-ischemic effect of the treatment.

The best approaches to evaluate the effects of tissue perfusion are not yet clear. Simple angiography is not able to detect changes at the microvascular level, but contrast myocardial echocardiography, along with the use of radiolabeled microspheres, has been successfully employed to analyze the beneficial effects of $VEGF_{121}$ therapy [11].

Although the initial expectations in the clinical testing of gene therapy with angiogenic factors in ischemic cardiomyopathy have not been fully met, there are evidences of consistent positive results for patients. These results include histological analyses, vascular neoformation and, more recently, myocardial hyperplasia. Improvement of functional parameters related to measurements of left ventricular function and diminished frequency of cardiovascular complications, as well as subjective improvements in symptoms of patients treated for advanced ischemic cardiomyopathy and with no other viable therapeutic options, have also been observed [10,21,22].

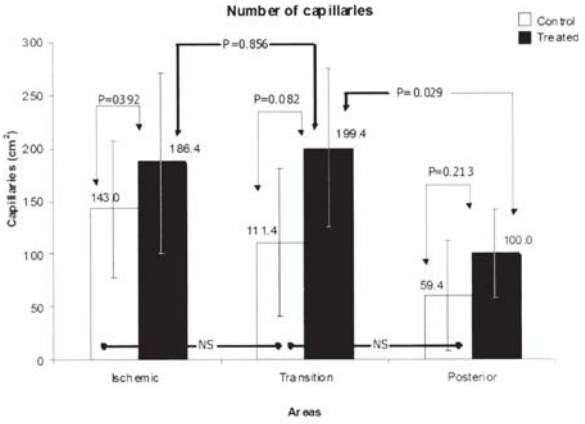

Fig. 3 - Comparison between the number of capillaries/cm² of area in control and treated groups. NS, non significant. Student's t test between each two samples, for expression of differences between areas and groups

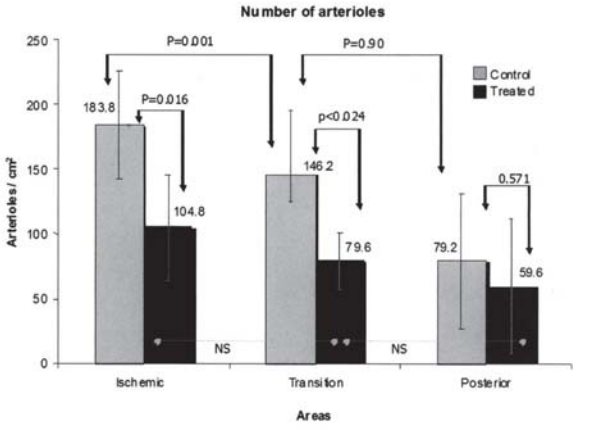

Fig. 4 - Comparison between the number of arterioles/cm²; in control and treated groups. NS, non significant. Student's t test between each two samples, for expression of differences between areas and groups

These results stress the importance of further studies about the effects of angiogenic factors in animal models and in controlled clinical trials.

This experimental model in dogs was established for this purpose and previously tested, as described above [17,18]. Canine models have limitations related to the establishment of abundant collateral circulation, that limits the extension and severity of the infarction. Our model however was adequate, since areas of infarction and transition were clearly identified under direct vision and microscopically. The above mentioned limitation of canine models, however, could be overcome by the control group, since all animals were submitted identical procedures for myocardial infarction production and left ventricular function assessment.

The functional evaluation of global left ventricular function, by echocardiographic measurement of left ventricular ejection fraction, showed stability in treated dogs (absolute reduction of 3.9%, NS) and a decrease in the control group of around 20% (19.9%, $P = 0.04$). So, the role of therapeutic angiogenesis on stabilization of LVEF has been demonstrated in this experiment. In control animals, on the other hand, significant decrease of LVEF was observed until the end of the experimental period. Regional contractility, not investigated in the present study, could give more detailed information about the specific effect of the treatment in the ischemic area.

Although the canine model of myocardial infarction have their recognized limitations, it has been used to evaluate gene therapy, as by Ferrarini et al. [23], who found improvement in cardiac tissue viability and functional

recovery of left ventricle after infarction produced by permanent coronary occlusion in conscious dogs. Their results indicate that $VEGF_{165}$ gene delivery exerts a marked beneficial action by enhancing both arteriologenesis and cardiomyocyte viability in infarcted myocardium.

Histological results showed no difference regarding capillary density in the ischemic and transition areas between treated and control groups. In treated group, capillary density was higher in the transition areas than in other regions. Arteriole density, on the other hand, showed results different from those expected, since a smaller number of arterioles was observed in samples from treated than control dogs, in all examined areas. We could not explain this phenomenon, that deserves further investigation. Some limitations occurred in histological analysis, including using a large area for counting vascular density, which contributed to higher variability between values and the method used for, based on staining with hematoxylin-eosin method and electronically counting, without imunnohistochemistry which is more specific. The small number of samples also limited the statistical analysis.

In a study of efficacy of therapeutic angiogenesis by intramyocardial injection of pCK-$VEGF_{165}$ in pigs, Choi et al. [24] found that at 30 days after, there were no significant differences in segmental perfusion, wall thickening, and wall motion between groups. In the VEGF group, all variables of perfusion, wall thickening, and wall motion were significantly improved at day 60 compared with those at day 30 ($P<0.05$), while there were no differences in the control group. At day 60, perfusion ($P=0.018$), wall motion ($P=0.004$), and wall thickening ($P=0.068$) of the VEGF group were improved compared with those of the control group. Histologic analysis showed that microcapillary density was significantly higher in the VEGF group than the control group ($P < 0.001$) and concluded that intramyocardial injection of pCK-$VEGF_{165}$ significantly augmented neoangiogenesis in the ischemic area and improved regional myocardial function as well as myocardial perfusion.

CONCLUSION

In conclusion, in this canine model of chronic myocardial ischemia, therapeutic angiogenesis induced by intramyocardial injection of plasmidial $VEGF_{165}$ resulted in stabilization and maintenance of left ventricular function. There was simultaneous increase in capillary density in all the myocardium, but particularly in the transition area between infarcted and normal myocardium. A smaller number of arterioles was, unexpectedly, observed in treated animals. These results stress the importance of continuing experimental studies and controlled clinical trials of gene therapy for ischemic cardiomyopathy.

REFERENCES

1. Banai S, Jacklitsch MT, Shou M, Lazarous DF, Scheinowitz M, Biro S, et al. Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. Circulation. 1994;89(5):2183-9.

2. Bull DA, Bailey SH, Rentz JJ, Zebrack JS, Lee M, Litwin SE, et al. Effect of Terplex/VEGF-165 gene therapy on left ventricular function and structure following myocardial infarction. VEGF gene therapy for myocardial infarction. J Control Release. 2003;93(2):175-81.

3. Choi JS, Kim KB, Han W, Kim DS, Park JS, Lee JJ, et al. Efficacy of therapeutic angiogenesis by intramyocardial injection of pCK-VEGF165 in pigs. Ann Thorac Surg. 2006;82(2):679-86.

4. Crottogini A, Meckert PC, Vera Janavel G, Lascano E, Negroni J, Del Valle H, et al. Arteriogenesis induced by intramyocardial vascular endothelial growth factor 165 gene transfer in chronically ischemic pigs. Hum Gene Ther. 2003;14(14):1307-18.

5. Ferrarini M, Arsic N, Rechia FA, Zentilin L, Zacchigna S, Xu X, et al. Adeno-associated virus-mediated transduction of VEGF165 improves cardiac tissue viability and functional recovery after permanent coronary occlusion in conscious dogs. Circ Res. 2007;100(4):e58.

6. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285(21):1182-6.

7. Hao X, Mansson-Broberg A, Blomberg P, Dellgren G, Siddiqui AJ, Grinnemo KH, et al. Angiogenic and cardiac functional effects of dual gene transfer of VEGF-A165 and PDGF-BB after myocardial infarction. Biochem Biophys Res Commun. 2004;322(1):292-6.

8. Hao X, Mansson-Broberg A, Grinnemo KH, Siddiqui AJ, Dellgren G, Brodin LA, et al. Myocardial angiogenesis after plasmid or adenoviral VEGF-A(165) gene transfer in rat myocardial infarction model. Cardiovasc Res. 2007;73(3):481-7.

9. Harada K, Friedman M, Lopez JJ, Wang SY, Li J, Prasad PV, et al. Vascular endothelial growth factor administration in chronic myocardial ischemia. Am J Physiol. 1996;270(5 pt 2):H1791-H802.

10. Hedman M, Hartikainen J, Syvanne M, Stjernvall J, Hedman A, Kivela A, et al. Safety and feasibility of catheter-based local intracoronary vascular endothelial growth factor gene transfer in the prevention of prostangioplasty and in-stent restenosis and in the treatment of chronic myocardial ischemia: phase II results of the Kuopio Angiogenesis Trial (KAT). Circulation. 2003;107(21):2677-83.

11. Hendel RC, Henry TD, Rocha-Singh K, Isner JM, Kereiakes DJ, Giordano FJ, et al. Effect of intracoronary recombinant human vascular endothelial growth factor on myocardial

perfusion: evidence for a dose-dependent effect. Circulation. 2000;101(2):118-21.

12. Henry TD, Abraham JA. Review of preclinical and clinical results with vascular endothelial growth factors for therapeutic angiogenesis. Curr Interv Cardiol Rep. 2000;2(3):228-41.

13. Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. VIVA Investigators. Circulation. 2003;107(10):1359-65.

14. Kalil RA, Teixeira LA, Mastalir ET, Moreno P, Fricke CH, Nardi NB. Experimental model of gene transfection in healthy canine myocardium: perspectives of gene therapy for ischemic heart disease. Arq Bras Cardiol. 2002;79(3):223-32.

15. Kalil RAK. Terapia gênica aplicada à cirurgia cardiovascular. Rev Soc Cardiol Rio Grande do Sul. 2001;3:61-6.

16. Kastrup J, Jorgensen E, Ruck A, Tagil K, Glogar D, Ruzyllo W, et al. Euroinject One Group. Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina pectoris. A randomized double-blind placebo-controlled study: the Euroinject One trial. J Am Coll Cardiol. 2005;45(7):982-8.

17. Kawasuji M, Nagamine H, Ikeda M, Sakakibara N, Takemura H, Fujii S, et al. Therapeutic angiogenesis with intramyocardial administration of basic fibroblast growth factor. Ann Thorac Surg. 2000;69(4):1155-61.

18. Laguens R, Cabeza Meckert P, Vera Janavel G, De Lorenzi A, Lascano E, Negroni J, et al. Cardiomyocyte hyperplasia after plasmid-mediated vascular endothelial growth factor gene transfer in pigs with chronic myocardial ischemia. J Gene Med. 2004;6(2):222-7.

19. Lee LY, Patel SR, Hackett NR, Mack CA, Polce DR, El-Sawy T, et al. Focal angiogen therapy using intramyocardial delivery of an adenovirus vector coding for vascular endothelial growth factor 121. Ann Thorac Surg. 2000;69(1):14-24.

20. Mack CA, Patel SR, Schwarz EA, Zanzonico P, Hahn RT, Ilercil A, et al. Biologic bypass with the use of adenovirus-mediated gene transfer of the complementary deoxyribonucleic acid for vascular endothelial growth factor 121 improves myocardial perfusion and function in the ischemic porcine heart. J Thorac Cardiovasc Surg. 1998;115(1):168-77.

21. Sant'Anna RT, Kalil RAK, Moreno P, Anflor LCJ, Correa DF, Ludwig R, et al. Terapia gênica com VEGF165 para angiogênese no infarto agudo do miocárdio experimental. Rev Bras Cir Cardiovasc. 2003;18(2):142-4.

22. Takeshita S, Zheng LP, Brogi E, Kearney M, Pu LQ, Bunting S, et al. Therapeutic angiogenesis: a single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invest. 1994;93(2):662-70.

23. Vera Janavel G, Crottogini A, Cabeza Meckert P, Cuniberti L, Mele A, Papouchado M, et al. Plasmid-mediated VEGF gene transfer induces cardiomyogenesis and reduces myocardial infarct size in sheep. Gene Ther. 2006;13(15):1133-42.

24. Villanueva FS, Abraham JA, Schreiner GF, Csikari M, Fischer D, Mills JD, et al. Myocardial contrast echocardiography can be used to assess the microvascular response to vascular endothelial growth factor-121. Circulation. 2002;105(6):759-65.