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The efficacy of therapeutic angiogenesis using basic fibroblast growth factor in patients with coronary artery disease (CAD): A double-blind, placebo-controlled study

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Abstract

Background: Complete revascularization is not possible in up to 37% of patients with coronary artery disease (CAD), these patients may benefit from the stimulation of neovascularization.

Aim: We evaluated the efficacy of therapeutic angiogenesis using basic fibroblast growth factor in patients with coronary artery disease (CAD).

Methods: in this double-blind, placebo-controlled Study eighteen patients with a severe diffuse atherosclerotic disease along the left anterior descending artery (LAD) who were a CABG candidate with at least one graftable coronary artery and the presence of ischemia and viable areas along the LAD were enrolled. During the CABG procedure in 10 patients, 10 FGF-2/alginate-heparin-sepharose microcapsules, each contains 100 mcg FGF-2, were implanted in the subepicardial layer of the diffusely defective LAD territory via 2-3 mm stab incisions.

Results: at least 7 patients in each group were followed for a period of 24 months. The result of left ventricular evaluation with echocardiography and perfusion scans showed significant improvement in FGF-2 receiving group with no significant change in controls, 3 and 6 months after the intervention. NYHA class was significantly lower in the intervention group (1.43±0.535 vs. 2.57±.535, P=0.002) and they remained free of angina 24 months after the intervention while 3 patients in the control group were hospitalized due to the acute chest pain ($P > 0.05$).

Conclusion: We revealed that FGF-2 improves the outcomes of patients with CAD undergoing CABG, without serious adverse effects.

Keywords: Therapeutic Angiogenesis, Fibroblast Growth Factor, Coronary Artery Disease, CABG

Introduction

Coronary artery disease (CAD) imposes a high burden in Iran and it is including 50% of deaths in this country (1). Despite the recent advances in the therapeutic methods, the morbidity and mortality of CAD are increasing mostly due to its rising prevalence [2]. Some of the patients may benefit from the restoration of the blood flow, using coronary angioplasty and stents, or coronary artery bypass graft (CABG). CAD patients who achieve complete revascularization by CABG may enjoy a higher quality of life and survival rate [3]. However, the benefits of CABG are limited since complete revascularization is not possible in up to 37% of cases because of the diffuse arterial involvement or small size of the arteries [3]. Stimulation of the neovascularization as a biological bypass of the atherosclerotic vessels may improve the symptoms in patients with the severe ischemic cardiac disease who cannot benefit from the conventional percutaneous coronary intervention or CABG [2]. Thus far, numerous inducers of angiogenesis have been identified, including the members of the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) [4], transforming growth factor-α and -β (TGF-α and -β) [5], chemokines, interleukins, and the members of the fibroblast growth factor (FGF) family [6]. Studies reported that basic fibroblast growth factor, also known as fibroblast growth factor-2 (FGF-2) have a strong affinity for the extracellular matrix and basal lamina and also paracrine, autocrine and intracellular modes of action [7]. It is reported that FGF-2 may improve the survival rate in patients with the ischemic heart disease if it is administered before, during or after the injury [8]. Furthermore, exogenous administration of FGF in different animal models of chronic myocardial ischemia or acute infarction showed increased myocardial perfusion and preserved left ventricular function [9, 10]. In this double-blind, placebo-controlled study we evaluated the effectiveness and safety of angiogenic protein therapy by implantation of sustained-release alginate- heparin-sepharose microspheres capsules of FGF-2 in the ischemic myocardium of patients who underwent CABG.

Material and methods

Patient selection

Eighteen patients who were eligible for CABG were included in this study, which was performed at the Tehran Heart Center

(Tehran, Iran) between February 2007 and July 2009. The inclusion criteria consisted of severe diffuse atherosclerotic disease along the left anterior descending artery (LAD), being a CABG candidate with at least one graftable coronary artery, and the presence of ischemia and viable areas along the LAD. The patients were excluded if there were a need for concomitant valve repair or replacement, malignant arrhythmia, history of malignancy, hemangioma, or diabetic retinopathy, renal insufficiency (serum creatinine > 3 mg/dL), abnormal liver function tests, and an estimated post-operation mortality risk higher than 25%.This study was approved by the ethics committee of Tehran University of Medical Sciences. The detailed process of this study, including the diagnostic and therapeutic procedures was explained to the patients and informed consent was obtained from all of the participants. Eighteen patients were enrolled sequentially, randomized into 2 groups to either undergo CABG and simultaneous FGF-2 therapy or CABG without FGF-2 therapy. For each patient, a questionnaire containing demographic information and detailed medical history was completed as well.

Preparation of FGF-2 / Alginate – heparin - sepharose microcapsules

FGF-2 was purchased from PeproTech (Princeton Business Park, Rocky Hill, United States), and heparin-sepharose and other chemicals from Sigma-Aldrich (Seelze, Germany). The microcapsules were prepared as per the method of Selke *et al* and Edelman *et al* [11, 12] with little modifications. Briefly, 1ml of heparin-sepharose suspension (33.3mg) was sterilized under ultraviolet light for 30 minutes in a disposable sterile petri dish (35mm). Except heparin-sepharose suspension, other solutions were sterilized by 0.22 disposable filters. Sepharose suspension was washed 3 times with sterile water (1:1) and mixed with 1ml of filter-sterilized sodium alginate (1.2%, w/v). After mixing, the slurry was dropped through a fine needle into a beaker containing 500 ml hardening solution of CaCl2 (1.5%, w/v) with continuous mixing using a magnetic stirrer. Immediately, formed microcapsules were incubated in the CaCl2 solution for 5 minutes under gentle mixing, and then for 10 minutes without mixing. The microcapsules were washed three times with sterile water. Afterward, 25 to 30 microcapsules were kept in a microfuge tube containing 0.9% NaCl, 1 mM CaCl2, 0.05% gelatin and 100 µg FGF. Microfuge tube was kept on shaker at 4°C for 16-20 hours. Microcapsules could be stored at 4°C for 3 months in a solution of 150 mM NaCl and 1 mM CaCl2. The procedures were conducted under laminar flow to avoid any contamination. Furthermore, after the completion of each preparation, several microcapsules were evaluated for contamination using the culture media to confirm their sterility. The microcapsules were sent under the sterile condition to the operating room at the Tehran Heart Center on the day of the surgical procedure.

Surgical Procedure and Implantation of FGF-2 Containing microcapsules

Standard hematologic laboratory tests and evaluation of cardiac enzymes for assessment of myocardial ischemia, evaluation of serum creatinine level, liver function tests, urinalysis, baseline electrocardiography, LV echocardiography, Dobutamine Stress Echocardiography (DSE) and myocardial perfusion scintigraphy were performed on all patients as indicated before undergoing the cardiac surgery. All patients were operated on by a cardiac surgery team according to the standardized surgical protocol. Following sternotomy, the patients were heparinized and cannulated. Cardiopulmonary bypass (CPB) was established. Then patients were cooled down to 28°-30 °C. After cross-clamping of the aorta, 0.8 to 1.0 L of cold blood cardioplegic solution was infused into the aortic root with a mean pressure of 50 to 70 mmHg. At first the distal coronary anastomoses were performed. Systemic rewarming was started before the final distal anastomoses were completed. Subsequently, 10 FGF-2/alginate-heparin-sepharose microcapsules each contains 100 mcg FGF-2 were implanted in the subepicardial layer of the diffusely defective LAD territory via 2-3 mm stab incisions.[3] After the implantation, the epicardial incisions were sutured with 5-0 polypropylene. Afterward, proximal anastomoses were performed, the aorta was unclamped and the patients were separated from CPB and the chest was closed normally.

Follow up

After surgery, patients were transferred to the intensive care unit (ICU) for precise monitoring of hemodynamic status and arrhythmia. Indicated blood tests were also performed. After 24-48 hours, patients were discharged from the ICU to the ward if their hemodynamic status was stable. Thereafter, the operation complications were investigated in the patients. Complete blood counts, liver function tests, serum chemistries, urinalysis, and electrocardiography were performed on the 7th day, 3th month and 6th month after the operation. Besides the clinical investigations and blood test evaluations, electrocardiography, New York Heart Association (NYHA) class evaluation and myocardial perfusion scintigraphy were repeated at the 6th month of the follow-up.

In the second phase of this study (For the long term follow up), between September 2008 and February 2010, all patients were contacted by the investigators to assess clinical events (mortality, myocardial infarction, recurrent angina, ICU admission, or any subsequent complications).Perfusion scan and echocardiography were performed in all patients 6 months after surgery. Besides, 18 to 24 months after surgery, they were asked by phone conducted interviews for their clinical symptoms, morbidity, mortality or NYHA functional class.

Statistical analysis

All analyzes were performed by operators blinded to all clinical and other functional data using SPSS version 11.5 software. Data are presented as mean ± standard deviation (SD). Paired samples *t*-test and independent samples *t*-test were used as appropriate. Fisher exact test was performed to compare clinical variables between groups. A *P*-value less than 0.05was considered significant.

Results

Patients' characteristics

Eighteen patients who met the inclusion and exclusion criteria were divided into two groups. Ten patients were allocated to BFGF group and eight patients were allocated to control group. During the process of this study, 2 patients of the bFGF group were excluded, because they refused to participate in the follow up evaluations. Also, two patients (one from each group) were withdrawn from the study since they could not be located for follow up. Finally, 7 patients in each group were investigated till the end of this study. Patients were randomly assigned into two groups and there were no significant demographic differences

between them. Preoperative characteristics of patients are summarized in Table 1. The prevalence of various risk factors and co-morbidities (Diabetes, hypertension and smoking) were similar in both groups.

Table 1: Patients' characteristics and co-morbidities

	FGF-2 (n=7)	Control (n=7)
Age (y)	58.29±6.57	59.57±9.86
Male/Female (n)	6/1	6/1
Diabetes mellitus (n)	3	4
Hypertension (n)	3	2
Smoking (n)	3	3

y: year, n: number

Functional status according to NYHA Classification

NYHA functional class before the intervention was similar between the FGF-2 receiving group and control group (2.29±0.488 vs.2.57±.0535, P=0.317). However, it was significantly lower in the FGF-2 group than control group 6 months after the intervention (1.43±0.535 vs. 2.14±0.378, P=0.014); this significant difference was also observed 24 months after the surgery (1.43±0.535 vs. 2.57±.535, P=0.002).

NYHA functional class was significantly reduced in FGF-2 receiving group 6 and 24 months after the intervention, comparing to the time before the surgery (1.43±0.535 vs. 2.29±0.488, P=0.001, and 1.43±0.535 vs. 2.29±0.488, P=0.001, respectively). NYHA functional class did not significantly change in the control group at 6 and 24 months after the intervention, comparing to the time before the surgery (2.14±0.378 vs. 2.57±0.535, P=0.2 and 2.57±5.535 vs. 2.57±0.535, P=1, respectively). In both groups, NYHA functional class did not change significantly from the 6th month to 24th months of follow up.

Evaluation of left ventricular function with echocardiography

According to the 2 dimensional echocardiography, there was no significant difference between the preoperative (baseline) ejection fraction of the FGF-2 and control groups (35±4.082 vs. 33.14±2.410, P=0.32). However, after the 6 months of follow up, a significantly higher ventricular ejection fraction was observed in the FGF-2 group compared to the control group (46.43±3.78 vs. 30.71±5.345, P<0.001). Ventricular ejection fraction improved significantly in the FGF-2 group after the 6 months of follow up compared to its value before the intervention (35.00±4.082 vs. 46.43±3.780, P<0.001). In contrast, this value did not change significantly in the control group after the 6 months of follow up comparing to the time before the surgery (30.71±5.345 vs. 33.14±2.140, P=0.18).

Perfusion scans

According to myocardial perfusion scan and uptake of radioactive material we had three types of segments as listed here; 1.normal uptake segment, 2.mild to moderate uptake segment, 3. Segment with severely decreased to absent uptake. Nine segments were defined for the LAD related area. There were improvements in perfusion scores within the FGF-2 group while this finding was not observed in the control group. This improvement included increases in the number of segments with normal uptake and reduction in the number of segments with mild to moderate or severely decreased or absent uptake (P<0.001, P<0.001 and P=0.003 respectively). We compared changes in perfusion scan between 2 groups which is summarized in Table 2. There was a significant difference between the FGF-2 treated and control group in the changes of the number of severely decreased to absent and normal uptake segments (P= 0.03, P=0.01). This finding was not observed before the intervention. Comparison of changes in perfusion scores between the 2 groups is shown in Figure 2.

The mean Summed Stress Score was not significantly different between FGF-2 and control groups before the intervention (17.571±3.20 vs. 17.85±6.01, P=0.138). However, patients in the FGF-2 group had significantly lower score, 6 months after the surgery (5.428±7.299 vs. 14.857±3.976, P=0.011). This score was significantly reduced in the FGF-2 group after 6 months of follow up (5.428±7.299 vs. 17.571±3.20, P=0.001) in contrast to the control group who did not show a significantly lower score 6 months after the intervention (14.857±3.976 vs. 17.857±6.011, P=0.156).

Table 2: The result of myocardial perfusion scintigraphy. Myocardial segment uptake before and after surgery in two groups

Uptake segment	FGF-2 treated group			Control group			Changes of perfusion scores between two groups
	Before surgery	6 month after surgery	P value	Before surgery	6 month after surgery	P value	P value
Normal uptake	3±1	7.29±2.36	<0.001	1.71±2.43	3.29±2.13	0.052	<0.05
Mild to moderate uptake	1.43±0.53	0.14±0.37	<0.001	2.86±2.41	2.14±2.26	0.283	0.385
Severe to absent uptake	4.57±0.97	1.57±2.07	0.003	4.43±1.90	3.57±1.39	0.225	<0.05

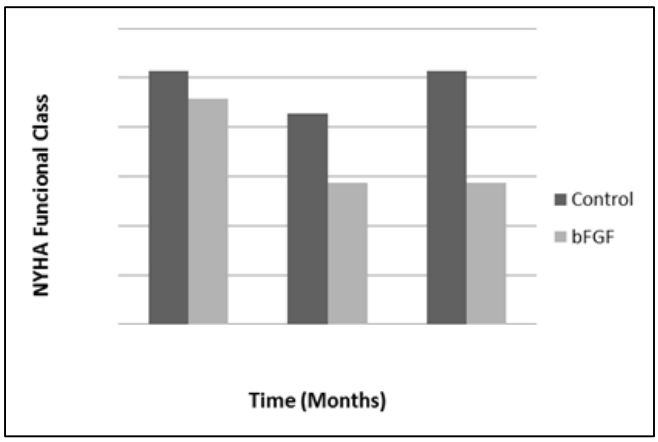

Fig 1: Functional class (NYHA) before operation, 6 and 24 months after the operation

Post operation side effects

No serious adverse reactions associated with FGF-2 were observed. During the 24 months of follow up no post-operative side effects (bleeding, myocardial infarction, cardiac arrest, chest pain…) were seen in the FGF-2 group and all cases in this group remained free of angina. However, 3 patients (42.85) of the control group were admitted to hospital because of the acute chest pain (P = 0.192, Fisher's exact test).

Discussion

Up to 37% of patients with ischemic heart disease, suffer from a diffuse and severe CAD that neither can undergo percutaneous coronary intervention nor surgical bypass to restore the normal blood flow [13]. Therapeutic angiogenesis can be considered as an option in the management of these patients. Protein therapy poses some advantages since it can be delivered directly to the target area, its quantity can be controlled more accurately, can immediately affect the target tissue and probably causes minimum inflammatory and immune adverse effect compared to the other methods [14, 15]. More importantly, it does not require complicated methods of gene therapy or the exposure to foreign genetic materials with unknown long-term outcomes [16]. The aim of this study was to evaluate the effectiveness and the safety of administering FGF-2 for therapeutic angiogenesis in ischemic myocardium of patients who undergo CABG. Administration of FGF-2 at the onset of ischemia is reported to reduce injury and attenuate heart enzymes [17, 18]. It can be a promising choice of pretreatment in coronary artery bypass grafting to reduce the ischemic injury of cross-clamp time. Nakamae *et al*. reported that FGF-2 enhanced vascular density in necrotic iliac bone in rabbits [19]. Previous studies showed the angiogenic effects of FGF-2 in limb ischemia [20, 21] Yanagisawa-Miwa *et al* improved cardiac function by intracoronary administration of FGF-2 in infarcted canine heart model; also FGF increased the number of arterioles and capillaries in the infarcted area [18]. Several other studies also have applied FGF-2 for inducing angiogenesis in cardiac ischemia. Scheinowitz *et al*. administered FGF-2 via peritoneum after coronary artery occlusion in rats for a six week period but found no significant recovery in the ischemic tissue [22]. This might be related to the short time of arterial occlusion or peritoneal delivery of the drug. The first trial designed to examine the safety, pharmacokinetics, and efficacy of FGF-2, in which 0-, 0.3-, 3-, or 30- µg/kg doses of FGF-2 delivered intracoronary to 337 patients that were not suitable candidates for percutaneous or surgical revascularization [23]. No significant differences were seen between treatment and control groups in exercise tolerance test (ETT) time assessed after 90 or 180 days [23]. Angina frequency significantly reduced at 90 days, but the difference was no longer existed after 180 days [23]. Ruel *et al*. also used different doses of FGF-2 in patients with ischemic heart disease and reported a higher ejection fraction of the left ventricle and lower sum of stress perfusion found with FGF-2 group [24].The severity of symptoms in our population study was evaluated using NYHA Classification. It showed significant improvement in FGF group 6 and 24 months after intervention while in the control group, improvement was not significant. This finding was in agreement with the result of the study performed by Simons *et al*. who reported a better physical well-being 3 and 6 months after the intervention in FGF-2 receiving group. However, this improvement was not significantly higher in the FGF-2 group compared to the control group [23]. Most of the previous studies reported FGF-2 increased ventricular ejection fraction in patients receiving FGF compared to control group [25]. In line with these findings, in the present study, left side ventricular ejection fraction improved significantly in FGF group 6 months after the surgery while this finding was not observed in the control group. Myocardial perfusion scan was performed 6 months after the intervention in all patients. In the FGF group, segments with impaired uptake significantly decreased while this finding was not significant in the control group.

In a phase I clinical trial by Sellke *et al*. the result of perfusion scan 3 months after surgery of 7 patients receiving FGF showed enhanced perfusion in 3, minimal changes in 3 and the appearance of new small defects in 1 patient [12]. However, Simons *et al*. reported no significant difference in myocardial perfusion of patients in FGF-2 group and the control group after 6 months of follow-up [23]. A limitation in FGF therapy in cardiovascular patients is its relatively short serum and tissue half-life [26, 27], which would cause the need of repetitive administration or higher doses. High doses of these factors can cause vasodilatation and hypotension. In addition, a long-term administration of high-dose FGF can lead to membranous nephropathy [28]. It was observed that endothelial cell growth factors showed a significant affinity for heparin [29]. The FGF family group contains heparin/heparan sulfate binding domains [30]. Heparin alginate capsules are an appropriate choice for FGF delivery, which is also used in our study. Other studies have used fibrin glue for drug delivery of growth factors [31-33], that has shown enhancing the effects of VEGF [31]. Also, it can serve as a temporary matrix for the granulation tissue development [31]. However, it is preferred to use recombinant human fibrinogen since it may prevent antibody reactions or possible infection transmission via human or animal derived fibrinogen [34, 35]. Laham, *et al*. in 1999 showed that local perivascular implantation of heparin-alginate pellets containing 10 or 100 µg of bFGF or placebo in ischemic and viable but ungraftable myocardial territories in patients undergoing CABG are safe and feasible in patients with viable myocardium that cannot be adequately revascularized. Also, reduction in the target ischemic area in the 100-mg bFGF group was shown via magnetic resonance assessment. [3] Another concern regarding the use of FGF-2 is its safety due to its probable side effects, including acute severe hypotension and renal insufficiency [16, 36]. According to the previous studies, single local administration of FGF-2 seems to be well-tolerated and safe [12, 16, 23]. In our study,

no patient showed any serious side effect. However, since 2 patients in the FGF-2 group did not participate in the follow-up evaluations after the intervention, and 2 patients, one from the FGF-2 group and another from control group could not be located till the end of the follow-up period, accurate evaluation of side effects and mortality could not be performed.

Limitations

The lack of double blinded placebo controlled trial and a few findings supporting the efficacy of administering FGF-2 for angiogenesis proposes in CAD patients have prevented the usage of this therapeutic method at the clinical setting. Further double blinded, placebo-controlled studies are needed to establish the benefits of this therapeutic method at the clinical setting.

Conclusion: We revealed that FGF-2 improves the outcomes of patients with CAD undergoing CABG, without serious adverse effects.

Conflict of Interests

The authors declare no conflict of interest.

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