Effects of basic fibroblast growth factor microspheres on angiogenesis in ischemic myocardium and cardiac function: analysis with dobutamine cardiovascular magnetic resonance tagging

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Abstract

Objective: Therapeutic angiogenesis with angiogenic growth factors has described as one of the promising methods for collateral formation in the treatment of ischemic heart diseases. The purpose of this study is to assess the value of intramyocardial injection of slow-released basic fibroblast growth factor microspheres on angiogenesis and cardiac function in the early period of acute infarcted myocardium with dobutamine cardiovascular magnetic resonance tagging. Methods: Acute myocardial infarction was made by ligation of the left anterior descending coronary artery distal to its first diagonal branch. Immediately after coronary artery occlusion, 1 ml of saline containing 100 mg of basic fibroblast growth factor microspheres was injected into peri-infarct myocardial area in the basic fibroblast growth factor group, whereas only gelatin hydrogel microspheres with 1 ml of saline was given in control dogs. Cardiac function was evaluated by cine magnetic resonance imaging. Dobutamine cardiovascular magnetic resonance was performed at rest and during low doses of dobutamine to assess regional wall motion. Immunohistochemical study with von Willebrand factor was performed to observe angiogenesis. Results: Left ventricular ejection fraction improved markedly 10 and 17 days after treatment in the basic fibroblast growth factor group. The basic fibroblast growth factor group had more viable myocardium. Microvessel density was higher in the basic fibroblast growth factor group than in the control group except the first day after treatment. Conclusions: Intramyocardial administration of basic fibroblast growth factor microspheres can promote the growth of microvessels and improve left ventricular function and myocardial viability in the early period of acute myocardial infarction.

Keywords: Angiogenesis; Myocardial infarction; Basic fibroblast growth factor; MR tagging

1. Introduction

The induction of coronary collateral growth seems to be a novel and potential hope for patients who are not candidates for standard revascularization such as coronary angioplasty (PTCA) or bypass surgery (CABG) [1]. Now, the effects of vascular growth factors on reducing myocardial infarct size and improving cardiac function have evoked more interest, for these factors have the potential to speed and increase collateral circulation [2]. Basic fibroblast growth factor (bFGF), which is a potent mitogen that regulates angiogenesis during growth and development, has been successfully used to stimulate cardiac angiogenesis to improve myocardial blood flow through various routes of administration in a variety of animal models of myocardial ischemia [3]. However, the biological effects of growth factors in its protein free form are very limited, because its half-time in vivo is too short [1,3].

Recently, a biodegradable hydrogel composed of acidic gelatin was developed to enable slow and continuous release of bFGF with a resultant prolonged action. Several studies have reported biodegradable gelatin microspheres incorporating bFGF (bFGF microspheres) for ischemic heart disease [1,3,4]. However, to our knowledge, the effects of bFGF microspheres in the early period of acute myocardial infarction have no reports until now. In the present study, we used biodegradable hydrogel microspheres as a release carrier biomaterial to enable the simultaneous sustained release of bFGF, and detected the effects of bFGF microspheres on angiogenesis, cardiac function and myocardial viability in the early period of acute infarcted myocardium.

2. Materials and methods

2.1. Preparation of bFGF incorporating gelatin hydrogels

Human recombinant bFGF and gelatin were supplied by Boster Bioengineering Company, Wuhan, China. bFGF
incorporating gelatin hydrogels were prepared through glutaraldehyde crosslinking in the dispersed state of a gelatin aqueous solution in the oil phase [3]. Briefly, 10 ml of aqueous solution of acidic gelatin (10 wt.%) preheated at 45°C was added dropwise into 375 ml of olive oil while stirring at 450 rpm at 45°C for 10 min to yield a water-in-oil emulsion. The emulsion temperature was decreased to 15°C, followed by further continuous stirring for 30 min to induce spontaneous gelation of the gelatin aqueous solution. After the addition of 100 ml of acetic acid, the emulsion was further stirred for 1 h. The resulting microspheres were washed with acetone and isopropyl alcohol, centrifuged at 5000 rpm for 5 min, and dried. The non-crosslinked and dried gelatin microspheres (50 mg) were placed in 0.1 wt.% of Tween 80 aqueous solution containing 3 mmol/l of glutaraldehyde (10 ml) and stirred at 4°C for 15 h to facilitate their crosslinking. After collection by centrifugation at 5000 rpm for 5 min at 4°C, the microspheres were further agitated in 100 ml of 10 mmol/l glycine aqueous solution at 37°C for 1 h to block residual aldehyde groups of unreacted glutaraldehyde. The microspheres were finally washed with distilled water, centrifuged at 5000 rpm for 5 min, and freeze-dried. bFGF was incorporated into the gelatin microspheres by dropping 5 mg/ml of bFGF solution (20 μl, totally 100 μg of bFGF) onto 2 mg of freeze-dried gelatin microspheres, which were then left at room temperature for 1 h. The solution (20 μl) was completely absorbed into the microspheres during swelling, since the solution volume was less than that theoretically required for the equilibrated swelling of microspheres. The saline microspheres were prepared similarly, using saline without bFGF.

2.2. Animal model preparation

All animals received humane care in compliance with the European Convention on Animal Care and the study was approved by our institutional ethics committee. Twenty-four healthy adult mongrel dogs (15—20 kg, provided by Xijing Hospital Animal Center, Fourth Military Medical University, China) were randomized into the control group and the bFGF group, each with 12 animals. They were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg). Under sterilization and artificial respiration, left thoracotomy in the fifth intercostal space was performed to expose the heart. Then acute myocardial infarction was made by ligation of the left anterior descending coronary artery distal to the first diagonal branch with a 6.0 polypropylene suture. In the bFGF group (n = 12), immediately after ligation of each coronary artery, 1 ml of saline bearing 100 μg of bFGF microspheres was injected with a fine syringe at five points around the peri-infarct area, each point 20 μg of bFGF. In the control group (n = 12), each animal received an intramyocardial injection of gelatin hydrogel microspheres with 1 ml of saline in the same way. There were no changes in blood pressure or heart rate associated with the injection of bFGF or saline, and no obvious adverse effects, such as anaphylactic reaction, in dogs throughout the experiment. After hemodynamic stability the pericardium and chest were closed. All animals after surgery were watched closely to assure success. Then dogs in each group were randomized into four sub-groups and examined 1, 3, 10 and 17 days after surgery, respectively.

2.3. Experimental protocol

2.3.1. MRI protocol

All cardiac magnetic resonance imaging (MRI) scans were performed on a superconduct 1.5-T whole-body magnetic resonance (MR) scanner (Intera Master, Philips Medical System) with 30 mT/m single axis gradient strength and 150 mT/(m s) slew rate using a five-element cardiac synergy coil for signal reception, vectorcardiogram and sensitivity encoded technique. Under general anesthesia, the animal was then positioned in the isocenter of the magnet, the long and short axes of the heart were identified with scout images, and cine MR images in short-axis orientations were acquired with multi-slice gradient-echo sequence covering the entire left ventricle. Left ventricular short-axis MRI scanning included eight slices to observe ventricular wall motion and evaluate cardiac function. Imaging parameters include repetition time 3.0 ms, echo time 1.5 ms, flip angle 50°, field of view 35—48 mm, matrix 192 × 256, slice thickness 8 mm, phase layer 16 with 8 slices each layer. Left ventricular ejection fraction (LVEF) was acquired from the heart software package provided by the imaging system. Tagged images were acquired with an electrocardiogram (ECG)-triggered, segmented k-space spoiled gradient recalled pulse sequence with spatial modulation of magnetization (SPAMM). Contiguous stacks of short-axis images were prescribed to cover the entire heart from base to apex. Imaging parameters were as follows: tag separation 6 mm, field of view 320 mm, slice thickness 8 mm, matrix size 192 × 256, TR 6.5 ms, TE 2.3 ms, flip angle 15°, and temporal resolution 32 ms. The scans were repeated at rest and at all stress levels and evaluated visually on the scanner console to check for wall motion abnormalities. After baseline acquisitions, dobutamine was infused intravenously using a digital pump injector situated outside the scanner room. Infusion was started with 5 μg/(kg min), after which the dose of dobutamine was increased to 10, 20 μg/(kg min). Imaging began 6 min after each dose increase and required 3 min per dose increase. Imaging consisted of acquiring three short-axis cine images (basal, midventricular, and apical) without tagging and two short-axis cine-images (basal and midventricular) with tagging. During the infusion of dobutamine, the radiologist and cardiologist were present in the MR suite to monitor the condition of the dogs and to evaluate the images directly. MRI analysis of tagged images: short-axis images were divided into multiple segments with six segments in the basal and midventricular images and four segments in the apical image. All the images were scored by using a four-point scale, according to the guidelines of the American Society of Echocardiography. Per segment, the wall motion was graded as 1 = normal or hyperkinesia, 2 = hypokinesia, 3 = akinesia, and 4 = dyskinesia. Wall motion score index (WMSI) was derived as the mean score of all segments (n = 16) of all short-axis images (wall motion score index = total scores of all segments/total numbers of all segments) [5].

2.3.2. Microvessels density

Immediately after obtaining the small fresh myocardial blocks, the hearts were fixed in 10% buffered formalin and
five peri-infarct portions treated by saline or bFGF were cut out. With strepavidin–biotin–peroxidase complex (SABC) method, the tissue sections were immunostained with von Willebrand factor (Boster Bioengineering Company, Wuhan, China) to identify endothelial cells. Angiogenesis was assessed by counting the number of microvessels, including capillaries (diameter < 20 μm) and arterioles (diameter > 20 μm with tunica media), which were defined as round or ellipse structures with a central lumen lined by cells staining positively to von Willebrand factor and whose diameter was less than 100 μm [6]. Microvessels of the peri-infarct area were found under low power lens with optical microscope of Japanese Olympus company, then the number of microvessels in unit area (a field of vision with 200 times, 0.442 mm²) with high power lens (20 objective × 10 ocular) was determined by the mean of the number of microvessels in five high power field. The number of microvessels in each section was counted by two investigators blinded to other data and used for statistical analysis.

2.3.3. Statistical analysis

Data were analyzed by SPSS 13.0 software. The data of every group were tested for normal distributions first. Then statistical analysis was performed with independent-samples t test and paired-samples t test. The results were presented as mean ± SD. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Left ventricular function and myocardial viability

The motion of the left ventricular anterior wall weakened or disappeared after ligation of coronary artery. LVEF had no significant difference between the bFGF and control groups before surgery (0.65 ± 0.03 vs 0.66 ± 0.02, P = 0.530), however, it was markedly decreased 1 day after surgery in the bFGF and control groups (0.42 ± 0.02 vs 0.43 ± 0.03, P = 0.768) and was comparable 3 days after surgery (0.44 ± 0.04 vs 0.40 ± 0.02, P = 0.20). The disparity between LVEF in the bFGF and control groups was significant, with a markedly difference 10 days (0.53 ± 0.03 vs 0.31 ± 0.03, P = 0.001) and 17 days (0.58 ± 0.02 vs 0.39 ± 0.03, P = 0.001) after treatment (Fig. 1).

This study showed that a total of 24 dogs had abnormal wall motions located at the anterior and anteroseptal walls of the left ventricle, including hypokinetic and akinetic motions. After stress, some myocardial regions with resting motion abnormalities showed functional improvement. There was a significant difference between the mean wall motion score index of the control group without dobutamine and that with dobutamine (1.44 ± 0.16 vs 1.32 ± 0.16, P = 0.002). So was between the mean wall motion score index of bFGF group without dobutamine and that of bFGF group with dobutamine (1.30 ± 0.10 vs 1.21 ± 0.10, P = 0.002). The mean wall motion score index of the bFGF group with dobutamine was 1.21 ± 0.10, compared with 1.32 ± 0.16 of the control group with dobutamine (P = 0.049).

2.3.2. Immunohistochemistry

Immunohistochemical staining of myocardium for von Willebrand factor was performed after sacrifice and the number of the stained capillaries was counted. As shown in Fig. 2, the microvessel density increased markedly after surgery. The numbers of microvessels 10 and 17 days after surgery in the bFGF group were significantly higher than those in the control group (130.0 ± 6.0 vs 91.7 ± 7.1, P = 0.002 and 152.7 ± 5.0 vs 90.0 ± 9.2, P < 0.001, respectively). The number of capillaries 3 days after surgery in the bFGF group tended to be higher compared with that in the control group (98.0 ± 4.0 vs 79.7 ± 8.4, P = 0.027).

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4. Discussion

The presence of collateral vessels which develop following coronary artery occlusion has been familiar to clinicians for many years [7]. Blood flow through coronary collaterals may be sufficient to preserve wall motion and prevent ischemia at rest and may reduce or prevent ischemia during stress. An increase in the number of collateral vessels might occur as a result of the enlargement of the preexisting vessels (vasculogenesis) or as a result of the creation of the new vessels (angiogenesis) [9]. Recently, therapeutic angiogenesis has emerged as a complementary strategy for the treatment of the ischemic myocardium.

bFGF is one of the powerful angiogenic growth factors regarded as an agent to promote angiogenesis. However, the biological half-life of bFGF is too short and was reported as less than 50 min [10]. Thus, the biological effects of bFGF in its protein free form are very limited. Recently, a biodegradable hydrogel composed of acidic gelatin was developed to enable bFGF to be released at the site of action for an extended time period [1,3,4]. Although they showed an increase in collateral development and cardiac function, the studies were all involved in the chronic infarct model. In clinical practice, acute myocardial infarction is also a common disease. In the present study, we used biodegradable hydrogel microspheres as a release carrier biomaterial for the simultaneous sustained release of bFGF, and detected the effects of bFGF microspheres on angiogenesis and cardiac function in the early period (1, 3, 10 and 17 days after ligation of coronary artery) of acute infarcted myocardium.

Kawasuji et al. [8] injected 100 µg of bFGF protein into the border zone of the acute canine infarct model. The intramyocardial injection improved LVEF 7 days after infarction and increased angiogenesis 28 days after infarction in the bFGF group. And there was no significant difference in LVEF 14 and 28 days after surgery. However, in the present study, the disparity between LVEF in the bFGF and control groups was significant, with a markedly difference 10 and 17 days after treatment. Also, there was no significant difference of the number of microvessels between the bFGF group and the control group 1 day after surgery. On the three other observing times (3, 10 and 17 days after surgery), the number of microvessels in the bFGF group was higher than in the control group, respectively. The reason of the increase of microvessel density since 3 days after surgery may be that the initial response of endothelial cells begins almost 1 day after stimulation of bFGF [11] and the microspheres give a sustained release.

Research in biology and applications of growth factors in coronary artery disease has progressed considerably over recent years. However, with its higher sensitivity and specificity indices for identification of coronary artery disease, CMR has not been extensively used in trials of angiogenic therapies [12]. It allows for the non-invasive assessment of the heart anatomy and precise function in one imaging session without exposure to ionizing radiation. Cine-MRI allows for accurate time-resolved imaging of global and segmental left ventricular function with high spatial resolution [13]. For the investigation of cardiac motion and myocardial viability, myocardial tagging has been shown to be most useful [14]. Dobutamine-stress CMR has emerged as a highly accurate and safe diagnostic modality. Myocardial regions with resting akinesia–dyskinesia, which showed functional improvement following low-dose dobutamine, are viable myocardium. Myocardium was considered nonviable in case of unchanged wall motion [15]. Recently, the use of dobutamine-CMR in combination with the myocardial tagging technique has been reported, with excellent diagnostic results [16]. To our knowledge, no reports were about therapeutic angiogenesis using bFGF microspheres that precisely estimated cardiac function and myocardial viability with MRI in the early period of infarction. Therefore, we estimated myocardial viability by dobutamine MR with tagging and left ventricular function by cardiac cine-MR.

Kawasuji et al. [8] performed echocardiography to assess cardiac function. Yamamoto et al. [3] implanted 100 µg of bFGF microspheres in a canine model of chronic myocardial ischemia and cardiac function detected by coronary angiography did not change significantly 2 weeks after the treatment. In our study, LVEF improved significantly 10 days after the injection of bFGF microspheres. The result was mainly in accordance with Kawasuji’s results, though they used bFGF protein free form [8]. Now that study subjects (dogs), delivery routes (intramyocardial), delivery sites (border zone between the left anterior descending coronary artery and the proximal left circumflex artery), forms of bFGF administration (protein microsphere) and administration doses and frequencies (100 µg, once) were the same with Yamamoto’s study [3], we speculated that the discrepancies may have come from differences in study models (acute vs chronic) and function exam methods (MRI vs coronary angiography).

Kuijpers et al. [17] reported that dobutamine-CMR with myocardial tagging detected more wall motion abnormality compared with dobutamine-CMR without tagging. Our study showed that a total of 24 dogs had abnormal wall motions located at the anterior and anteroseptal walls of the left ventricle, including hypokinetic and akinetic motions. In the bFGF and control groups, the number of wall motion abnormality at rest was higher than the number at all stress levels. The results demonstrated that low dose dobutamine plays effects on the recovery of the contractile function of the ischemic myocardium. The result was in accordance with the previous report [18]. This study also showed that the bFGF group had more viable myocardium than the control group.

This study demonstrated that intramyocardial administration of slow-release bFGF increased angiogenesis in the early period of canine acute infarction. This was the first study that used myocardial tagging with low-dose dobutamine-CMR to detect wall motion abnormality in order to demonstrate the effects of bFGF microspheres on angiogenesis in the infarcted myocardium. And the present results indicated the therapeutic efficacy of the gelatin-microspheres incorporating bFGF on the cardiac function and myocardial viability in the myocardial ischemia.

The main limitation of the present experiment was that bFGF microspheres were injected immediately after acute myocardial infarction, while in clinical practice, most patients receive surgical procedures over the course of hours, who belong to chronic infarct. Several studies have reported the effects of slow-release bFGF in chronic infarct models [1,3,19]. However, with the development of medical condition, more and more patients will receive the
immediate treatment of acute myocardial infarction. Therefore, this study proved the efficacy of bFGF micropheres on myocardial angiogenesis and cardiac function in the early period of acute infarction.

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References


Editorial comment

Growth factor therapy grows, despite limited insight

In this issue, Ying Liu and coworkers [1] report on the effects of slow release FGF-2 in acute myocardial infarction in dogs. The authors incorporated FGF-2 in gelatin microspheres and injected them immediately after experimental infarction into the adjacent myocardium. Functional outcome was determined using the tagged cardiac magnetic resonance imaging method at rest and under dobutamine stress. FGF-2 therapy resulted in a higher L VEF (171% and 170% of controls after 10 and 17 days, respectively) and an improved wall motility score index at rest and with dobutamine after 10 and 17 days. Congruently, a higher number of capillaries and arterioles was found (142% and 140% of controls after 10 and 17 days, respectively).

Growth factor therapy aims at restoration of regional circulation and function in ischemic myocardium and is considered a new option for patients with severe coronary disease who are currently not amenable to surgical or interventional revascularization. Various growth factors have been studied in almost multitudinous animal experiments. However, the success in clinical trials is still limited. For example, the growth factor employed in the paper, FGF-2, has been used in the FGF initiating revascularization trial (FIRST) [2] to treat severe myocardial ischemia. The patients received a single intracoronary infusion of recombinant FGF-2 in a double-blind, randomized fashion. The therapy did not improve exercise tolerance or myocardial perfusion in long-term observation.

One of the reasons for the failure of FIRST has been discussed to be the rapid degradation of the protein in circulation. Retarded FGF-2 application could improve the outcome. We found in a porcine model of chronic myocardial ischemia that growth factor gene therapy, which also results in prolonged delivery, is superior to a single protein injection of basic fibroblast growth factor. In a double-blind, randomized fashion. The therapy did not improve exercise tolerance or myocardial perfusion in long-term observation.

Another limitation of clinical usage of growth factor therapy is that we still not know which growth factor — or growth factor combination — is the best one. Both development of arterial vessels (arteriogenesis) and new capillaries (angiogenesis) contribute to the revascularization of ischemic regions [4]. In a recent experiment, we