Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 322 (2004) 287–291
BBRC
www.elsevier.com/locate/ybbrc

ANP and BNP but not VEGF are regionally overexpressed in ischemic human myocardium

Andreas Rück[a,*], Thomas Gustafsson[b], Jessica Norrbom[b], Jacek Nowak[b], Göran Källner[c], Magnus Söderberg[d], Christer Sylvén[a], Viktor Drvota[a]

[a] Department of Medicine, Section of Cardiology, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden
[b] Department of Physiology and Pharmacology, Section of Molecular Exercise Physiology, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden
[c] Center for Surgical Sciences, Department of Cardiothoracic Surgery and Anaesthesiology, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden
[d] Department of Microbiology, Pathology and Immunology, Division of Pathology, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

Received 12 July 2004

Abstract

Angiogenic gene therapy in angina pectoris has been disappointing so far. Reasons might be that the administered genes already are overexpressed in ischemic myocardium, or that atrial and brain natriuretic peptides (ANP and BNP) are overexpressed, as they have anti-angiogenic effects. Five stable angina pectoris patients without heart failure were studied. Left ventricular biopsies were taken during coronary by-pass surgery from a region with stress-inducible ischemia and from a normal region. Both ANP and BNP but not vascular endothelial growth factor (VEGF) and VEGF-receptor 1 and 2 were overexpressed in ischemic regions compared to non-ischemic regions as measured by real-time PCR. The expression of 15 other angiogenic genes measured by oligonucleotide arrays was not consistently increased in ischemic regions. The overexpression of ANP and BNP suggests an anti-angiogenic effect in ischemic heart disease. The lack of overexpression of angiogenic genes supports the concept of therapeutic overexpression of these genes.

Keywords: Natriuretic peptides; VEGF; Myocardial ischemia; Gene expression; Ischemic heart disease

Ischemic heart disease is the leading cause of death in developed countries and angina pectoris is one of the most common chronic manifestations [1]. As the spontaneously occurring angiogenesis and collateral growth in patients with angina pectoris usually are insufficient to alleviate stress-induced ischemia [2], efforts have been made to therapeutically enhance angiogenesis. It has been possible to induce angiogenesis and decrease stress-induced myocardial ischemia in animal models by transfer of angiogenic genes such as vascular endothelial growth factor (VEGF) [3]. While initial small clinical trials with VEGF gene transfer in angina pectoris patients showed remarkable effects [4], larger randomised trials with VEGF [5,6], and fibroblast growth factor (FGF) [7] have failed to show the same clear effect.

It is well established that ischemia induces expression and stabilisation of VEGF RNA via hypoxia inducible factor-1 (HIF-1α). VEGF is critical in angiogenesis and has also been denoted a rate-limiting step [8].

The natriuretic peptides atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are predominantly expressed in the atrium and the ventricle of the heart, respectively. Their release has been linked to increased stretch and heart failure. Interestingly, ANP and BNP have recently been reported to inhibit the

* Corresponding author. Fax: +46 8 58586710.
E-mail address: andreas.ruck@medhs.ki.se (A. Rück).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.07.114

angiogenic signalling of VEGF and their expression might also be partially regulated by HIF-1α [9,10]. A recent report also suggests that the myocardial expression of BNP might be associated with acute ischemia [11].

Too low doses, inefficient delivery routes or inefficient vectors have been proposed as explanations for the so far weak clinical effect seen in clinical trails of angiogenic agents [12]. Another possibility is that the administered genes already are regionally overexpressed, and thus there would be no rationale for administering, for example, vectors expressing VEGF or FGF. Despite the fact that stable angina pectoris patients typically are enrolled in these trials, the myocardial expression level of angiogenic genes in this patient group is not yet known. A third possibility is that increased expression of ANP and BNP could inhibit angiogenesis. Therefore, the aim of this study was to investigate the expression level of several angiogenesis-related genes and also ANP and BNP in myocardial regions with stress-induced ischemia in stable angina pectoris patients. We combined expression measurement of a larger number of genes by microarray with confirmation of key findings by more precise real-time PCR.

Methods

Subjects. Five patients scheduled for coronary artery bypass surgery were studied. The study was approved by the Institutional Medical Ethical Committee and conforms to the principles outlined in the Declaration of Helsinki. All patients gave written informed consent. All had angina pectoris with stable symptoms since three months to two years. None had heart failure or was on treatment with angiotensin converting enzyme inhibitors. Three were males. The age range was 53–71 years. All underwent a preoperative coronary angiogram and SPECT (single photon emission computer tomography) stress-rest perfusion imaging (Software: HERMES, Nuclear Diagnostics, Stockholm). The Left Ventricular Ejection Fraction ranged from 48% to 69% on resting gated SPECT. As we aimed at including patients with regional stress-induced myocardial ischemia and intact angiogenic capacity, all patients had a chronic occlusion of one major coronary artery with complete filling of the distal part of the same vessel via collaterals (Rentrop score 3) [13].

Myocardial biopsy collection. Two regions in the left ventricle of each patient were selected for biopsy by correlating the coronary angiogram and the SPECT images (Fig. 1). The ischemic region was distal to the chronically occluded vessel on the angiogram and had a significant SPECT uptake defect at stress with normal tracer uptake at rest. Thus, this region had stress-inducible ischemia and no permanent perfusion defect. The non-ischemic region served as control and was located in another part of the left ventricle without critical coronary stenosis and with normal tracer uptake both on rest and stress SPECT (no ischemia, no permanent perfusion defect). Transmural left ventricular biopsies were obtained with a 14-gauge biopsy instrument (Tru-Core II, MD Tech, Gainesville, FL) during coronary artery bypass surgery, before cardioplegia and cross-clamping.

Biopsies were frozen in liquid nitrogen within 20 s and stored at −80 °C.

Histology. A small part of each biopsy was formalin-fixed, paraffin-embedded, and cut and stained with hematoxylin–eosin for routine evaluation. Immunohistochemical staining was performed using monoclonal mouse anti-vimentin and monoclonal mouse anti-human CD45 antibodies (DAKO, Glostrup, Denmark), with subsequent streptavidin-peroxidase incubation.

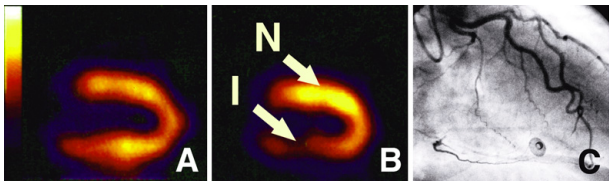

Fig. 1. Example of selected areas for biopsies. (A) Myocardial SPECT perfusion imaging, vertical long axis section, rest image: no perfusion defect. (B) Same section, stress image with perfusion defect. Arrows show the selected areas for the ischemic I and non-ischemic N biopsies. (C) Coronary angiography in the same patient shows filling of the occluded distal right coronary artery via collaterals, explaining the stress-induced ischemia I and confirming no stenosis in the artery to the N area.

RNA extraction. Total RNA was isolated from each biopsy by the acid phenol method [14]. The $OD_{260:280}$ ratio was controlled and visual inspection of the denaturing gels was made.

Real-time polymerase chain reaction. Quantitative real-time PCR was performed with TaqMan probes. VEGF, VEGF receptor 1 and 2, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) were chosen as target genes. One microgram of total RNA from each biopsy was reverse transcribed by Superscript Rnase H reverse transcriptase (Invitrogen, Carlsbad, CA) using random hexamer primers according to the manufacturer's specifications. Real-time polymerase chain reaction (PCR) was performed with an ABI-PRISMA 7700 Sequence Detector (Perkin–Elmer, Foster City, CA). Control experiments revealed approximate equal efficiencies over different starting template concentrations for target genes and B-actin. Target gene and B-actin were amplified in multiplex experiment in triplicate. Amplification mixes (25 μl) contained the sample cDNA diluted, 2× TaqMan Universal PCR Mastermix, forward and reversed primers, and probe. Thermal cycling conditions included 2 min at 50 °C and 10 min at 95 °C before 50 PCR cycles (95 °C for 15 s and 65 °C for 1 min).

For VEGF, forward primer 5′-ACTGCCATCCAATCGAGACC-3′, reversed primer 5′-GATGGCTTGAAGATGTACTCGATCT-3′, and TAMRA-probe 5′-TGGTGGACATCTTCCAGGAGTACCCTGA-3′ were used. For ANP, forward primer 5′-AGCGGACTGGGCTGTAACAG-3′, reversed primer 5′-GCCCAGCCCTGCTTGTC-3′, and dark quencher probe 5′-CGGTACCGAAGATAACAGCCAGGGA-3′ were used. For BNP, forward primer 5′-GAGGAAGATGGACCGGATCA-3′, reversed primer 5′-TGTGGAATCGAAGCAGGTGTCT-3′, and TAMRA probe 5′-TGCAAAGTGCTGAGGCGGCATTAA-3′ were used. For VEGF receptor 1, forward primer 5′-GCATATGGTATCCCTCAACCTACAA-3′, reversed primer 5′-CATCCAGGATAAAGGACTCTTCATTAT-3′, and TAMRA probe 5′-TAACCATAATCATTCCGAAGCAAGGTGTGACT-3′ were used. For VEGF receptor 2, forward primer 5′-CTTCTGGCTACTTCTTGTCATCAT-3′, reversed primer 5′-CCTACGCTTTGGGTTTTCCA-3′, and TAMRA probe 5′-TCTGCACATGGAGCCTTGGTCATCA-3′ were used.

Change in expression was calculated as fold-change, after normalisation for B-actin expression.

Oligonucleotide microarrays. Human Genome GeneChips U95Av2 oligonucleotide arrays were used (Affymetrix, Santa Clara, CA), one for each ischemic and non-ischemic sample. The sequences of all probe sets are available at www.affymetrix.com.

In vitro transcription was carried out with the Enzo BioArray HighYield RNA Transcript Labeling Kit. Fragmentation of biotinylated cRNA, protocols and reagents for hybridisation, and washing and staining followed instructions by Affymetrix [15]. Hybridisation controls, housekeeping controls, noise, and background all showed good data quality.

Microarray data analysis. The ischemic and non-ischemic samples from the same patient were compared (no comparison between patients). Twenty genes (see Results), including ANP and BNP, previously described to be important in myocardial angiogenesis [16] were selected for expression change analysis.

Data were analysed with Affymetrix Microarray Suite version 5.0 software. Only genes on probe sets type "_at" on the microarrays were used, as other probe set types may be more prone to unspecific hybridisation [15]. Global scaling was used with default settings. The reliable detection of each probe set was determined using the "present call" algorithm, where both the absolute expression level and the background noise were taken into consideration. Change in gene expression was independently calculated in two ways: (1) qualitative change or change call ("increase," "no change" or "decrease") and (2) quantitative change (fold-change, i.e., times higher expression in the ischemic compared to the non-ischemic sample). Wilcoxon's signed rank test was used for change call calculation at the default significance value of 0.0025.

Results

ANP and BNP but not VEGF and VEGF receptor 1 and 2 are regionally overexpressed in ischemic myocardium

ANP expression measured by PCR had a mean fold-change of 8.8 (range 0.8–31) when the ischemic sample was compared to the non-ischemic sample (Fig. 2). Four of five patients had a fold-change over two. For BNP the mean fold-change was 23 (range 1.3–70), with the same four patients with a fold-change over two.

VEGF-A expression had a mean fold-change of 0.9 (range 0.6–1.3). For VEGF receptor 1 and 2 the mean fold-change was 0.9 (range 0.4–1.7) and 0.9 (range 0.7–1.2), respectively.

By oligonucleotide microarray measurements, there was qualitative overexpression (change call "increase") in four patients for ANP, all five patients for BNP, one for VEGF-A, and two for VEGF receptor 2 (Table 1). Mean fold-change values by microarray were 4.0 for ANP, 9.9 for BNP, 1.1 for VEGF, and 1.4 for VEGF receptor 2. VEGF receptor 1 had present call "absent" in all patients due to low signal and therefore expression change measurement was not possible by microarray Table 1.

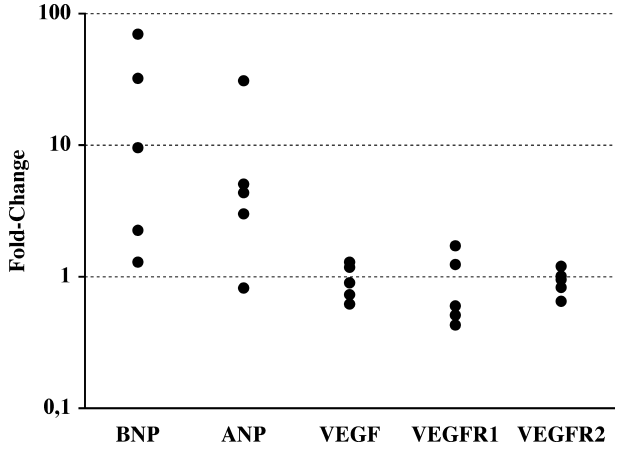

Fig. 2. Expression increase (fold-change) in the ischemic sample compared to the non-ischemic sample for five genes, measured by real-time PCR.

Expression of 15 other angiogenesis-related genes

The expression level of 15 additional genes of importance in angiogenesis was measured by oligonucleotide microarrays. Due to low expression, nine genes had present call "absent" and expression change measurement was not possible (angiopoietin 1 and 2, fibroblast growth factor 2, monocyte chemotactic protein 1, matrix metalloproteinase 9, placenta growth factor, platelet derived growth factor β, Tie-2, and VEGF-C). Of the remaining genes (Table 1), four (fibroblast growth factor 1, tumor necrosis factor α, VE-cadherin, and VEGF-B) had a mean fold-change between 1.0 and 1.2 and showed qualitative change in one or no patient. Insulin-like growth factor 1 had qualitative change in two patients and mean fold-change 1.4. Ephrin B2 had the largest change of the 15 genes: qualitative change in three patients but the mean fold-change was still only 1.4.

Histology and immunohistochemistry

Light microscopy showed essentially normal myocardium without leukocyte infiltration and with no or minimal fibrosis in all biopsies.

Discussion

The first main finding was that ANP and BNP were overexpressed in the region with stress-inducible ischemia compared to the non-ischemic region of the left ventricle. This was measured on microarrays as qualitative change "increase" for ANP in four of five patients and for BNP in all patients. It was validated by real-time PCR with a mean fold-change of 4.0 and 9.9, respectively.

The other main finding was that neither VEGF nor its receptor types 1 and 2 were overexpressed (fold-change over two) in ischemic myocardium in any patient as measured by real-time PCR. Looking further, of the additional 15 angiogenesis-related genes (Table 1) analysed on microarrays, nine had a too low expression to be reliably detected and four had a mean fold-change close to one with qualitative change in only one or no patient. Ephrin B2 and insulin-like growth factor 1 had increased expression in three and two patients but the mean fold-change was modest: 1.4. Due to the difficulty of collecting this kind of biopsies our patient group was small and therefore exact determination of the degree of overexpression of any gene was not intended. However,

Table 1
Expression of angiogenesis-related genes in ischemic compared to non-ischemic samples measured by oligonucleotide microarrays

Gene	Sequence derived from GenBank Accession No.	Affymetrix probe set number	Patients with change call "increase"	Patients with change call "decrease"	Mean fold-change
Atrial natriuretic peptide	AL021155	36663_at	4	0	4.0
Brain natriuretic peptide	AL021155	39215_at	5	0	9.9
Ephrin B2	AI765533	34335_at	3	0	1.4
Fibroblast growth factor 1 (acidic)	X59065	996_at	1	0	1.0
Insulin-like growth factor 1	X57025	1501_at	2	0	1.4
Tumor necrosis factor α	X02910	1852_at	0	0	1.1
VE-cadherin	X79981	37196_at	1	0	1.2
VEGF-A	AF024710	1953_at	1	0	1.1
VEGF-B	U48801	1926_at	0	0	1.0
VEGF receptor 1	S77812	1567_at	ND	ND	ND
VEGF receptor 2	AF035121	1954_at	2	0	1.4

Patients with neither change call "increase" nor "decrease" were "no change." ND, not detected (present call is "absent" in one or more patient samples); VEGF, vascular endothelial growth factor.

we assume that a fold-change of two suggests a biologically relevant change.

All patients had reversible perfusion defects on preoperative stress imaging, which proves regional repetitive myocardial ischemia. Acute ischemia intraoperatively at the time of biopsy was not aimed for, and the lack of acute ischemic changes in routine histology makes this unlikely. The normal histology and resting perfusion images rule out necrosis or fibrosis as confounding regional difference between the ischemic and non-ischemic samples. The samples were taken within 10 min after the start of cardiopulmonary bypass and always before cardioplegia and cross-clamping, and the non-ischemic control sample was from the same heart, thus minimising any effect of the surgical procedure on the results. This is important since plasma levels of both ANP and BNP increase during and after coronary artery surgery with the first increase measured 20 min after start of cardiopulmonary bypass [17]. Importantly, none of our patients had heart failure or treatment with angiotensin-converting enzyme inhibitors, which influence the plasma levels of BNP [18], and all had normal left ventricular ejection fraction.

Myocardial ischemia is present in different clinical conditions from profound ischemia with cell damage (myocardial infarction) to intermittent ischemia without cell damage (angina pectoris, such as the patients in this study). Ischemia is the main trigger to angiogenesis and VEGF is one of the key angiogenic factors [8]. A whole group of angiogenic factors are known, but the modulation by anti-angiogenic factors is less known [16]. It has recently been shown that natriuretic peptides modulate the angiogenic response to VEGF both by inhibition of synthesis and signalling [9]. The balance between pro-angiogenic factors (such as VEGF) and anti-angiogenic factors (such as natriuretic peptides) might be quite different after myocardial infarction compared to that in stable angina pectoris.

After myocardial infarction, an increase in plasma levels of natriuretic peptides has been shown [19]. It is also known that angiogenic genes such as VEGF [20,21], FGF, and VEGF receptor 1 and 2 [16] are overexpressed after myocardial infarction and acute ischemia. Previous animal [22] and human [21] data have shown that overexpression of angiogenic factors in the heart occurs in ischemic regions and not in the whole ventricle.

The situation in the larger patient group with stable angina pectoris is less known. We found no overexpression of VEGF or its receptor 1 and 2 in ischemic myocardial regions in angina pectoris patients. This fits with animal models of repetitive ischemia, where the initial VEGF increase has been shown to attenuate over time [20,23]. Together with our finding that 15 other angiogenesis-related genes were not overexpressed in most patients, this points to a weak or absent overexpression of pro-angiogenic genes in stable angina pectoris.

A recent report suggested BNP overexpression in acute myocardial ischemia in patients without heart failure [11]. Our findings add that both ANP and BNP are overexpressed to a similar degree, and that this overexpression is regional and occurs in myocardial regions with stress-induced ischemia in stable angina pectoris patients. Tissue stretch is the major stimulus to ANP and BNP release but recently the hypoxia-activated transcription factor hypoxia inducible factor-1 (HIF-1) has also been shown to regulate ANP and BNP expression [10]. In endothelial cells, ANP and BNP inhibit angiogenic signalling of VEGF by both the natriuretic peptide clearance (NPRC) receptor and natriuretic peptide guanylate cyclate (GC) receptor pathways [9]. Additionally, natriuretic peptides inhibit VEGF transcription and protein production [24]. Thus, overexpression of natriuretic peptides might have an antiangiogenic effect and explain the relatively inefficient collateral vessel growth in many angina pectoris patients [2].

Conclusion

We found overexpression of ANP and BNP but not of VEGF and 17 other angiogenesis-related genes in myocardial regions with stress-inducible ischemia in stable angina pectoris patients without heart failure. The control comparison samples were from non-ischemic regions of the left ventricle from the same patient. As ANP and BNP have anti-angiogenic properties, their marked overexpression may be one reason for the relative inefficiency of both spontaneous angiogenesis and clinical trials with angiogenic agents in angina pectoris patients. On the other hand, the lack of overexpression of angiogenic genes supports the concept of therapeutic overexpression of these genes in this patient group.

Acknowledgments

This study was supported by Eirs Anniversary Foundation, the Swedish Society of Medicine, and the Swedish Heart and Lung Foundation.

References

[1] Global Burden of Disease 2002 Estimates by Level of Development, in: World Health Report 2002, World Health Organization, Geneva, 2002.
[2] T. Pohl, C. Seiler, M. Billinger, E. Herren, K. Wustmann, H. Mehta, S. Windecker, F.R. Eberli, B. Meier, Frequency distribution of collateral flow and factors influencing collateral channel development. Functional collateral channel measurement in 450 patients with coronary artery disease, J. Am. Coll. Cardiol. 38 (2001) 1872–1878.
[3] R.A. Tio, T. Tkebuchava, T.H. Scheuermann, C. Lebherz, M. Magner, M. Kearny, D.D. Esakof, J.M. Isner, J.F. Symes, Intramyocardial gene therapy with naked DNA encoding vascular endothelial growth factor improves collateral flow to ischemic myocardium, Hum. Gene Ther. 10 (1999) 2953–2960.
[4] D.W. Losordo, P.R. Vale, J.F. Symes, C.H. Dunnington, D.D. Esakof, M. Maysky, A.B. Ashare, K. Lathi, J.M. Isner, Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia, Circulation 98 (1998) 2800–2804.
[5] D.W. Losordo, P.R. Vale, R.C. Hendel, C.E. Milliken, F.D. Fortuin, N. Cummings, R.A. Schatz, T. Asahara, J.M. Isner, R.E. Kuntz, Phase 1/2 placebo-controlled, double-blind, dose-escalating trial of myocardial vascular endothelial growth factor 2 gene transfer by catheter delivery in patients with chronic myocardial ischemia, Circulation 105 (2002) 2012–2018.
[6] M. Hedman, J. Hartikainen, M. Syvanne, J. Stjernvall, A. Hedman, A. Kivela, E. Vanninen, H. Mussalo, E. Kauppila, S. Simula, O. Narvanen, A. Rantala, K. Peuhkurinen, M.S. Nieminen, M. Laakso, S. Yla-Herttuala, Safety and feasibility of catheter-based local intracoronary vascular endothelial growth factor gene transfer in the prevention of postangioplasty and in-stent restenosis and in the treatment of chronic myocardial ischemia: phase II results of the Kuopio Angiogenesis Trial (KAT), Circulation 107 (2003) 2677–2683.
[7] C.L. Grines, M.W. Watkins, J.J. Mahmarian, A.E. Iskandrian, J.J. Rade, P. Marrott, C. Pratt, N. Kleiman, A randomized, double-blind, placebo-controlled trial of Ad5FGF-4 gene therapy and its effect on myocardial perfusion in patients with stable angina, J. Am. Coll. Cardiol. 42 (2003) 1339–1347.
[8] N. Ferrara, H.P. Gerber, J. LeCouter, The biology of VEGF and its receptors, Nat. Med. 9 (2003) 669–676.
[9] A. Pedram, M. Razandi, E.R. Levin, Natriuretic peptides suppress vascular endothelial cell growth factor signaling to angiogenesis, Endocrinology 142 (2001) 1578–1586.
[10] Y.S. Chun, J.Y. Hyun, Y.G. Kwak, I.S. Kim, C.H. Kim, E. Choi, M.S. Kim, J.W. Park, Hypoxic activation of the atrial natriuretic peptide gene promoter through direct and indirect actions of hypoxia-inducible factor-1, Biochem. J. 370 (2003) 149–157.
[11] J.P. Goetze, C. Christoffersen, M. Perko, H. Arendrup, J.F. Rehfeld, J. Kastrup, L.B. Nielsen, Increased cardiac BNP expression associated with myocardial ischemia, FASEB J. 17 (2003) 1105–1107.
[12] S. Yla-Herttuala, K. Alitalo, Gene transfer as a tool to induce therapeutic vascular growth, Nat. Med. 9 (2003) 694–701.
[13] K.P. Rentrop, J.C. Thornton, F. Feit, M. Van Buskirk, Determinants and protective potential of coronary arterial collaterals as assessed by an angioplasty model, Am. J. Cardiol. 61 (1988) 677–684.
[14] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[15] GeneChip® Expression Analysis Technical Manual, P/N 701021 Rev. third ed., Affymetrix Inc., Santa Clara, CA, 2002.
[16] E.M. Conway, D. Collen, P. Carmeliet, Molecular mechanisms of blood vessel growth, Cardiovasc. Res. 49 (2001) 507–521.
[17] E. Berendes, C. Schmidt, H. Van Aken, M.G. Hartlage, M. Rothenburger, S. Wirtz, H.H. Scheld, G. Brodner, M. Walter, A-type and B-type natriuretic peptides in cardiac surgical procedures, Anesth. Analg. 98 (2004) 11–19.
[18] R. Latini, S. Masson, N. de Angelis, I. Anand, Role of brain natriuretic peptide in the diagnosis and management of heart failure: current concepts, J. Card. Fail. 8 (2002) 288–299.
[19] J. Venugopal, Cardiac natriuretic peptides—hope or hype? J. Clin. Pharmacol. Ther. 26 (2001) 15–31.
[20] T. Matsunaga, D.C. Warltier, D.W. Weihrauch, M. Moniz, J. Tessmer, W.M. Chilian, Ischemia-induced coronary collateral growth is dependent on vascular endothelial growth factor and nitric oxide, Circulation 102 (2000) 3098–3103.
[21] S.H. Lee, P.L. Wolf, R. Escudero, R. Deutsch, S.W. Jamieson, P.A. Thistlethwaite, Early expression of angiogenesis factors in acute myocardial ischemia and infarction, N. Engl. J. Med. 342 (2000) 626–633.
[22] S. Banai, D. Shweiki, A. Pinson, M. Chandra, G. Lazarovici, E. Keshet, Upregulation of vascular endothelial growth factor expression induced by myocardial ischaemia: implications for coronary angiogenesis, Cardiovasc. Res. 28 (1994) 1176–1179.
[23] H. Kawata, K. Yoshida, A. Kawamoto, H. Kurioka, E. Takase, Y. Sasaki, K. Hatanaka, M. Kobayashi, T. Ueyama, T. Hashimoto, K. Dohi, Ischemic preconditioning upregulates vascular endothelial growth factor mRNA expression and neovascularization via nuclear translocation of protein kinase C epsilon in the rat ischemic myocardium, Circ. Res. 88 (2001) 696–704.
[24] A. Pedram, M. Razandi, R.M. Hu, E.R. Levin, Vasoactive peptides modulate vascular endothelial cell growth factor production and endothelial cell proliferation and invasion, J. Biol. Chem. 272 (1997) 17097–17103.