Adenoviral-mediated gene transfer induces sustained pericardial VEGF expression in dogs: effect on myocardial angiogenesis

Daisy F. Lazarous*, Matie Shou, Jonathan A. Stiber, Everett Hodge, Venugopal Thirumurti, Lino Gonçalves, Ellis F. Unger

Experimental Physiology and Pharmacology Section, Cardiology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA

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Abstract

Objective: Angiogenic peptides like VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) have entered clinical trials for coronary artery disease. Attempts are being made to devise clinically relevant means of delivery and to effect site-specific delivery of these peptides to the cardiac tissue, in order to limit systemic side-effects. We characterized the response of the pericardium to delivery of a replication-deficient adenovirus carrying the cDNA for AdCMVVEGF165, and assessed the effect of pericardial VEGF on myocardial collateral development in a canine model of progressive coronary occlusion. Methods: Ameroid constrictors were placed on the proximal left circumflex coronary artery of mongrel dogs. Ten days later, 6×10⁸ pfu AdCMVVEGF165 (n=9), AdRSVβ-gal (n=9), or saline (n=7) were injected through an indwelling pericardial catheter. Transfection efficiency was assessed by X-gal staining. Pericardial and serum VEGF levels were measured serially by ELISA. Maximal myocardial collateral perfusion was quantified with radiolabeled or fluorescent microspheres 28 days after treatment. Results: In AdRSVβ-gal-treated dogs, there was extensive β-gal staining in the pericardium and epicardium, with minimal β-gal staining in the mid-myocardium and endocardium. Pericardial delivery of AdCMVVEGF165 resulted in sustained (8–14 day) pericardial transgene expression, with VEGF levels peaking 3 days after infection (>200 ng/ml) and decreasing thereafter. There was no detectable increase in serum VEGF levels. Maximal collateral perfusion, a principal correlate of collateral development and angiogenesis, was equivalent in all groups. Conclusion: Adenoviral-mediated gene transfer is capable of inducing sustained VEGF165 expression in the pericardium; however, locally targeted pericardial VEGF delivery failed to improve myocardial collateral perfusion in this model. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Collateral circulation; Coronary circulation; Gene therapy; Growth factors; Ischemia

1. Introduction

Vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF) is a secreted, glycosylated, dimeric protein that is structurally related to platelet-derived growth factor (PDGF) [1,2]. VEGF is an endothelial cell-specific mitogen that has been identified as a hypoxia-inducible angiogenic factor [3] and a potential tumor angiogenesis factor in vivo [4]. VEGF has been associated with pathological retinal neovascularization [5,6] and intra-ocular expression of VEGF has been correlated with retinal neovascularization [7]. In experimental models of limb ischemia, VEGF has been demonstrated to promote angiogenesis and collateral development [8–12]. VEGF has also shown promise for myocardial angiogenesis [13–15]; however, parenteral administration of VEGF has been associated with dose-limiting hypotension [15,16] and acceleration of injury-School of Medicine, Division of Cardiology, A1 East, Johns Hopkins Bayview Medical Center, 4940 Eastern Avenue, Baltimore, MD 21224, USA. Tel.: +1-410-550-7035; fax: +1-410-550-1183.

E-mail address: dlazarou@welch.jhu.edu (D.F. Lazarous)

*Corresponding author. Present address: The Johns Hopkins University School of Medicine, Division of Cardiology, A1 East, Johns Hopkins Bayview Medical Center, 4940 Eastern Avenue, Baltimore, MD 21224, USA. Tel.: +1-410-550-7035; fax: +1-410-550-1183.

E-mail address: dlazarou@welch.jhu.edu (D.F. Lazarous)

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induced arterial neointimal accumulation [16], suggesting that local targeting of the growth factor may be essential for clinical use.

Adenoviral-mediated gene transfer has been used successfully to transfer genes to mammalian tissues, and preliminary studies have demonstrated the feasibility of efficient gene transfer to the pericardial sac [17]. In an attempt to target VEGF protein delivery to cardiac tissues and to limit systemic side-effects, we employed a second-generation adenovirus for transfer of the gene encoding human VEGF to cardiac cells in vivo. An E1a-deleted replication-deficient recombinant adenoviral vector was utilized in which the gene encoding human VEGF<sub>165</sub> was under the control of the immediate early cytomegalovirus promoter. We exploited the sequestered environment of the pericardial space for site-specific gene transfer, a strategy that might preclude unwanted viral dissemination and enable the vector to evade immune surveillance, thereby prolonging transgene expression. The immediate proximity of the epicardial vasculature and developing collateral circulation to the pericardial space and pericardial fluid suggested that this mode of VEGF delivery might lead to enhancement of myocardial angiogenesis while limiting systemic effects.

Using a β-gal marker gene, our first goal was to determine the anatomic distribution of transgene expression after intra-pericardial virus administration. Second, we sought to characterize the magnitude and time course of VEGF<sub>165</sub> transgene expression and to determine the distribution of adenovirus in body fluids. Our third goal was to determine whether intrapericardial VEGF gene transfer was capable of improving myocardial collateral perfusion in a well-characterized canine model of progressive coronary occlusion.

2. Methods

The experimental protocol was approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, and conducted in accordance with the Guide for the Care and Use of Laboratory Animals [18] and NIH issuance 3040-2: Animal Care and Use in the Intramural Program.

2.1. Surgical instrumentation

Thirty-two mongrel dogs of either sex were obtained from Haycock Kennels, Quakertown, PA. Dogs were anesthetized with acepromazine, 0.2 mg/kg i.m., thiopental sodium, 15 mg/kg i.v., and inhaled methoxyflurane. A left thoracotomy was performed using sterile technique, as previously described [13,16]. An ameroid constrictor (Research Instruments and Manufacturing, Corvallis, OR) was fitted on the proximal left circumflex coronary artery (LCx) before the origin of the first marginal branch. A silastic catheter was positioned in the left atrial appendage for microsphere injections to assess collateral perfusion. Multiple side holes were fashioned in the distal 2 cm of a 6.6-Fr silastic end hole catheter (Bard Access Systems, Salt Lake City, UT), and the catheter was secured in the pericardial space. The pericardium was carefully approximated, and the chest was closed in layers. The termini of the two catheters were positioned in the subcutaneous tissue of the back, the skin was closed, and the dogs were allowed to recover.

2.2. Integrity of the pericardium

Ten days after surgery, the pericardium and catheter were tested for leakage in a pilot dog by injecting 2 ml of contrast agent (Hypaque) into the pericardial space under fluoroscopic guidance. The dye flowed freely in the pericardial space, and no leak was visualized.

2.3. Transduction efficiency of pericardial gene transfer

In one dog, 6×10<sup>9</sup> pfu AdRSV-β-gal was instilled into the pericardial cavity. This gene encodes nuclear-targeted bacterial β-galactosidase. Myocardium, pericardium, lung, intestine, kidney, gonad, brain, liver, and spleen were harvested 72 h after transfection, and bacterial β-galactosidase activity was assessed by 5-bromo-4-chloro-3-indoyl-β-d-galactopyranoside (X-gal) staining. The tissue was embedded in paraffin and 5-μm sections were counterstained with Nucleofast red for microscopic analysis.

2.4. Gene expression: serum and pericardial VEGF levels

To establish the ability of transduced tissue to secrete VEGF and to assess the time course of protein secretion, recombinant VEGF protein production was quantified by ELISA in six animals (three VEGF-treated, three controls). Samples of pericardial fluid (withdrawn from the indwelling catheter) and serum samples (forelimb venous puncture) were collected serially: before treatment, and on post-treatment days 1, 3, 5, 7, 10, 14, and 21. Samples were centrifuged at 4°C for 10 min; the supernatant and serum were assayed for human VEGF using a solid-phase ELISA kit according to the manufacturer’s instructions (catalog no. DVE00, R&D Systems, Inc., Minneapolis, MN).

2.5. In vitro techniques: biological activity of the secreted VEGF

In order to confirm that the expressed human VEGF was biologically active in canine tissue, we tested the mitogenic activity of the expressed human VEGF on canine endothelial cells in vitro. Endothelial cells were harvested from the saphenous veins of dogs and expanded...
in culture. The endothelial cells were infected with AdCMVVEGF or AdRSVβ-gal at a multiplicity of infection (moi) of 100 and the supernatant was collected 3 days later. The protein product was quantified in the supernatant of both AdCMVVEGF and AdRSVβ-gal-infected cells with a solid-phase ELISA kit as above. Two milliliters conditioned media was obtained from $1 \times 10^6$ cells infected 3 days earlier. This was placed on canine endothelial cells previously growth-arrested in 0.5% serum. Absolute cell numbers from triplicate cultures were determined 3 days after exposure to the conditioned media.

2.6. Quantification of E1a-deleted adenovirus in body fluids

Viral titer was quantified in urine, feces, and pericardial aspirates to validate confinement of the adenovirus to the pericardial space. Serial samples were collected from an animal pretreatment and 1, 2, 3, and 6 days after pericardial instillation of $6 \times 10^9$ pfu AdCMVVEGF. Detection and quantification of adenovirus in the samples was done using 293 cells (TCID assay, i.e. tissue culture infectious dose 50%). In the presence of E1a-deleted adenovirus, 293 cells (embryonic human kidney cells transformed by stable adenovirus E1a gene insertion) exhibit typical adenoviral cytopathic effects within 72–96 h [19] characterized by clumping, detachment, cell lysis, and intra-nuclear basophilic or amphophilic inclusion bodies. Ad5 CMV-LacZ inoculated into normal canine urine served as the positive control; the negative control consisted of a ten-fold dilution of normal canine urine.

2.7. Treatment groups

Dogs were randomized to $6 \times 10^9$ pfu AdCMVVEGF, AdRSVβ-gal or saline, delivered through the indwelling pericardial catheter 10 days after ameroid placement. Selection of the dose was based on our pilot experiments and previous studies [20]. Aliquots of stock AdCMVVEGF and AdRSVβ-gal (6×) (generously supplied by GenVec, Inc., Rockville, MD), $10^{10}$ pfu (volume 1 ml) were stored at −80°C until used. The viral solution was diluted in 3 ml serum-free EBM medium (Clonetics, San Diego, CA) immediately prior to administration. Using sterile technique, the viral solution was injected transcutaneously through the infusion port of the pericardial catheter, followed by 2 ml of EBM medium.

2.8. Analysis of organ toxicity

Using aseptic technique, an 18-gauge needle was inserted into the subcutaneous port of the pericardial catheter on a regular basis to quantify and remove, if necessary, accumulated pericardial fluid. If less than 20 ml of pericardial fluid could be withdrawn, it was returned to the pericardial space. Larger effusions were evacuated to prevent pericardial tamponade. Routine hematologic and biochemical studies were performed weekly on all animals.

2.9. Histology of the heart

Cardiac histological analysis was carried out in 16 animals (saline=6, β-gal=4, and VEGF=6). Transmural sections of the heart at the mid ventricular level were embedded in paraffin (3–5-μm sections) and stained with H&E. The sections were reviewed by a veterinary pathologist who was naïve to treatment assignment. The presence of myocardial fibrosis, inflammation, and increased vascularity were noted.

2.10. Hemodynamic measurements and quantification of collateral perfusion

Regional myocardial blood flow was quantified using the reference sample technique 28 days after treatment [21]. All microsphere blood flow studies were performed in the conscious state during maximal coronary vasodilatation. The dogs were lightly sedated with diazepam 1–2 mg/kg administered through the left atrial catheter. Using local lidocaine anesthesia, a 5F catheter (Cordis Corp.) was inserted into the femoral artery for measurement of arterial pressure and withdrawal of arterial reference samples. Arterial pressure and the electrocardiogram were recorded continuously. Maximal coronary vasodilatation was induced by infusing chromonar 8 mg/kg (Hoechst-Roussel Pharmaceuticals) into the left atrial catheter over 30 min as we have done previously [13,16]. After infusion of chromonar, approximately $3 \times 10^6$ radiolabeled microspheres (15 μm) were injected into the left atrial catheter. In a subset of animals, perfusion measurements were made using fluorescent microspheres (Interactive Medical Technology) [22]. Microsphere perfusion measurements were performed in duplicate with two labels ($^{99}$Te and $^{51}$Nb for radiolabeled microspheres; dual-labeled red, orange or violet dyes for fluorescent microspheres), and the results were averaged. The dogs were heparinized (5000 Units i.v.), killed with an overdose of sodium pentobarbital and KCl, and the myocardium was perfusion-fixed with 10% buffered formaldehyde at physiologic pressure. The heart was cut into 7-mm transaxial slices and examined macroscopically for the presence of infarcts or patchy areas of fibrosis. For radiolabeled microsphere analyses, the two central left ventricular slices were divided into 16 wedges and ranked with respect to perfusion. The four wedges with the highest perfusion were selected to represent the normal zone (NZ); the four wedges with the most compromised perfusion were selected to represent the collateral zone (CZ) as we have done previously [13,16]. For fluorescent microsphere analyses, only the central slice was analyzed. Using a dual wavelength detector system, intact microspheres from tissue digests were characterized with respect to dye ratio and counted using a coulter.

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system. Tissue digestion and fluorescent microsphere analyses were carried out by Interactive Medical Technology using standard techniques. Mean NZ and CZ perfusion were calculated from the duplicate perfusion measurements, and maximal collateral perfusion was expressed in relative terms as the CZ/NZ perfusion ratio as we have done previously [13,16]. The CZ/NZ ratio was defined prospectively as the primary study endpoint. Data are expressed as mean±S.E.M.

3. Results

Twenty-six of 32 dogs completed the studies. Five dogs died suddenly, 2–15 days after ameroid placement, presumably from sudden coronary occlusion or ameroid-induced coronary artery spasm. Two of five dogs expired before initial gene transfer. Of the three animals that died after gene transfer, one had received AdCMVVEGF and two had received AdRSVβ-gal. Autopsies failed to show an obvious cause of death and death was attributed to arrhythmia from sudden coronary occlusion. A sixth dog (AdCMVVEGF-treated) died 5 days after randomization; autopsy revealed 250 ml of fluid in the pericardial space and death was attributed to cardiac tamponade. There was discordance between the two measurements of myocardial perfusion in one dog, and it was excluded from the analysis prior to breaking the study code. Thus, perfusion measurements are reported for 25 dogs. One additional dog was used for a determination of in vivo β-gal transfection efficiency.

3.1. In vitro VEGF production and biological activity

VEGF was detected in the supernatant of AdCMVVEGF cells but not in the cells infected with AdRSVβ-gal. Peak VEGF levels of approximately 1 µg/million cells/day were observed between days 3 and 5 after infection, tapering off thereafter (Fig. 1). The expressed human VEGF induced a two-fold increase in proliferation of canine endothelial cells, indicating that the biological activity was conserved between species (data not shown).

3.2. Pericardial transfection efficiency

There was extensive β-gal expression in the parietal pericardium (Fig. 2a) and epicardium (Fig. 2b). Reporter gene transfer was almost entirely restricted to pericardial and epicardial cells, with scattered sparse expression in the mid-myocardium, and no detectable staining of the endocardium.

3.3. Pericardial and serum VEGF levels

Pre-treatment VEGF levels were negligible in both virus-treated groups. Transgene expression was evident 24 h after viral infection, and peak pericardial VEGF levels were recorded on day 3 in AdCMVVEGF-treated dogs (>200 ng/ml). VEGF levels diminished to ≈15 ng/ml by day 8, and tapered off gradually thereafter, such that the total duration of the response was ≈14 days (Fig. 3). Serum VEGF levels did not increase from pretreatment values. Neither pericardial nor serum VEGF levels were elevated in AdRSVβ-gal treated animals.

3.4. Detection of recombinant adenovirus in body fluids

There was no detectable shedding of virus in the fecal or urine samples. At 6 days, the pericardial aspirate remained infectious and contained 5×10⁷/ml TCID₅₀ virus, still capable of replication in 293 cells.

3.5. Analysis of organ toxicity

All AdCMVVEGF-treated animals developed significant pericardial effusions, 55–180 ml in volume. One AdCMVVEGF-treated animal expired from pericardial tamponade with a 250-ml hemorrhagic inflammatory effusion (elevated polymorphonuclear leucocyte count). The aspirated fluid was cultured in all cases and found to be free of bacterial infection. None of the AdRSVβ-gal-treated animals had significant effusions (>20 ml).

3.6. Collateral conductance and CZ/NZ perfusion ratios

Microsphere perfusion determinations, performed in
Fig. 2. Expression of β-galactosidase after intrapericardial instillation of 6×10⁹ pfu AdRSVβ-gal. The heart was harvested 72 h post-treatment and X-gal histochemical staining was performed. Panel A: reporter gene transfer was almost entirely restricted to the parietal pericardial cells, and panel B: visceral pericardial cells (epicardium). There was scattered scanty expression in the mid-myocardium, and no detectable staining of the endocardium. Distant tissues were free of β-gal staining.

duplicate, showed excellent intra-animal agreement. In the normally-perfused territory of the left anterior descending coronary artery, maximal coronary conductance was similar in the three treatment groups on day 28 (Table 1). Maximal collateral conductance in the territory of the occluded LCx was similar in all groups, and the CZ/NZ ratios were virtually identical in the three treatment groups.

For the critical comparison between the AdRSVβ-gal and AdCMV/VEGF₁₆₅ groups, mean CZ/NZ ratios were 0.36±0.03 and 0.36±0.04, respectively.

3.7. Histological analysis

No cytopathic effects were grossly evident in the
In previous experiments in a canine model, we showed that VEGF enhanced coronary collateral development when administered repeatedly into collateral-dependent myocardium [13]. VEGF has also been reported to improve coronary collateral function in pigs when infused chronically near the point of coronary occlusion [14]. Enhancement of coronary collateral perfusion has not been attained, however, following relatively brief periods of VEGF treatment [15,16]. Moreover, intracoronary [15] and systemic arterial [16] administration of the growth factor has been associated with severe dose-limiting side effects. VEGF is a potent vascular permeability factor [2] and endothelium-dependent vasodilator [23], and causes severe hypotension when administered parenterally [15,16]. VEGF expression was evident 24 h after viral infection, with the measurable increase in [VEGF] persisting for ≈14 days. Pretreatment VEGF levels were negligible in both virus-treated groups. The VEGF concentration in pericardial fluid peaked on day 3 in AdCMV-VEGF-treated dogs (>200 ng/ml). VEGF levels diminished to ≈15 ng/ml by day 8, and tapered off gradually thereafter. VEGF levels were not elevated in AdRSV-β-gal-treated animals. Serum VEGF concentrations remained at pretreatment levels in all groups.

pericardium or surrounding lungs at the time of tissue harvest, i.e., structures were relatively free of adhesions. X-gal staining of distant organs did not reveal β-gal expression in any tissue.

Histological analysis of the heart revealed no difference between treatment groups in the degree of vascularity, fibrosis or inflammation or thrombosis. Microscopic angiosmas or hemangiomas were not noted.

4. Discussion

VEGF is one of several angiogenic heparin-binding growth factors reported to promote collateral development in animal models of myocardial and limb ischemia [8–15]. Table 1

<table>
<thead>
<tr>
<th>Control</th>
<th>AdRSV-β-gal</th>
<th>AdCMV-VEGF&lt;sub&gt;165&lt;/sub&gt;</th>
</tr>
</thead>
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<td>Perfusion (ml/min/g)</td>
<td>CZ/NZ ratio</td>
<td>Perfusion (ml/min/g)</td>
</tr>
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<td>CZ</td>
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<td>6.37</td>
</tr>
<tr>
<td>NZ</td>
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<td>6.90</td>
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<tr>
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<td>0.47</td>
</tr>
</tbody>
</table>

<sup>a</sup> Collateral zone (CZ) and normal zone (NZ) myocardial perfusion and the CZ/NZ ratio in control dogs, AdRSV-β-gal-treated dogs, and AdCMV-VEGF<sub>165</sub>-treated dogs. Values are mean±S.E.M. Differences between groups are not significant.
Several investigators have demonstrated that second generation adenoviral vectors function efficiently as gene delivery vehicles in vivo [27,28], superior in efficiency to plasmid DNA injection [29]. Successful intramyocardial injection of adenoviral vectors has been demonstrated in mammals [20,32]. Recently, intracoronary injection of a recombinant adenovirus expressing human FGF-5 was shown to increase perfusion and contractile function in the ischemic porcine heart [30]. Intracoronary gene delivery appears promising, although the potential for viral dissemination to non-target tissues poses an important concern. Viral receptors are found on the surface of a variety of cell types, and generalized dissemination of angiogenic genes could lead to undesirable neovascularization in sites such as the retina or occult tumors. Host immune responses to foreign adenoviral antigens pose an additional barrier for gene therapy, limiting the duration of gene expression [31].

We evaluated pericardial VEGF gene transfer as a means to target the myocardium and avoid the potential problems associated with systemic adenoviral dissemination. E1a-deleted replication-deficient adenovirus with the gene encoding VEGF_{165} was delivered to the pericardial space. We hypothesized that the pericardium might provide an abundant surface area for gene transfer as well as an inherent means for viral containment. Furthermore, the limited pericardial volume of distribution would tend to favor gene transfer by maximizing the concentration of the virus, and would serve to maintain the concentration of the expressed VEGF gene product at a relatively high level. In essence, the pericardium might function as a paracrine organ, producing high localized VEGF levels in direct apposition to the epicardial arteries and developing collaterals, leading to myocardial angiogenesis.

The pericardial space has been evaluated previously as a reservoir for angiogenic growth factor delivery [32,33]. Pericardial injection of basic fibroblast growth factor has been reported to enhance myocardial angiogenesis [36] and limit infarct size after coronary embolization [37]. Woody et al. have reported gene transfer to the pericardium [17]. Thus, these studies, taken together, suggest that pericardial gene transfer might provide an effective means to induce myocardial angiogenesis.

In the present study, we demonstrated that gene transfer to non-replicating pericardial and epicardial cells using recombinant adenovirus vectors resulted in acceptable gene targeting efficiency and expression of the VEGF transgene over a 10–14-day period. Although this strategy was highly effective in directing pericardial VEGF expression, the pericardial VEGF so produced was ineffective in promoting collateral development. There are a number of potential explanations for the apparent lack of effect.

4.1. Adequacy of VEGF production

It is worthwhile to contrast the calculated mass of VEGF produced by gene transfer in the present experiment to the quantities of the VEGF peptide administered in previous studies in which it was found to be effective. We observed peak steady state VEGF levels of approximately 200 ng/ml in a pericardial fluid volume of roughly 200 ml: an estimated mass of approximately 40 μg. Considering that the pericardial VEGF concentration decreased to 15 ng/ml by day 8, the total quantity of VEGF produced in the present investigation, integrated over the 2-week period of expression, was probably an order of magnitude less than the cumulative injected dose we previously found to be effective (45 μg/day×20 doses=900 μg) [13]. On the other hand, the quantity of VEGF produced in this experiment significantly exceeded the dose used by Harada et al. (0.06 μg/day×28 days=1.68 μg) [14].

4.2. Direction of VEGF delivery across the vascular wall

The presence of VEGF in the pericardial space presents the growth factor to the abluminal (rather than the luminal) side of the vasculature. Though this unconventional mode of VEGF administration might be responsible for its lack of effect in this experiment, Harada et al. [14] found perivascular infusion of VEGF (mixed with heparin) to be effective, a method that also delivered VEGF to the abluminal aspect of the vasculature. Two key differences between the present and previous investigations should be underscored: Harada et al. [14] used a longer duration of infusion (4 weeks, although VEGF potency was reduced by a factor of 2.5 at the end of their study), and VEGF and heparin were co-administered in their study. VEGF is a heparin-binding growth factor, and the interactions between VEGF and heparin have not been well-characterized in vivo [34,35].

4.3. Preservation of VEGF activity across species

We considered the possibility that human VEGF_{165} was not active in canine cells. In the present study, the expressed human VEGF_{165} induced proliferation of canine endothelial cells in vitro. Moreover, we previously found human VEGF_{165} to be effective on canine endothelial cells in vitro [13].

4.4. Role of inflammation

It is plausible that adenoviral-related inflammation had a negative influence on collateral development in this study. We found, however, that collateral function was not depressed in AdRSVβ-gal-treated animals (relative to saline-treated controls), suggesting that this was not the case. Animals treated with the β-gal virus did not develop hemodynamically significant pericardial effusions, but large effusions were encountered consistently in AdCMV/VEGF-treated dogs, requiring drainage to avert hemodynamic compromise. Leucocyte counts were gener-
ally elevated in these aspirates, indicative of an inflammatory response. Thus, it appears that a specific biological effect of VEGF was responsible for the sizable and hemodynamically significant effusions characteristic of these dogs. VEGF-induced vascular hyperpermeability may have played an important role in this regard [2]. The requirement to tap the effusions subsided by day 15 post-transfection, coinciding with waning VEGF expression.

Recently it has been reported that the plasmid for VEGF injected in the border zone of myocardial infarct in rats created angiomas that did not contribute to regional myocardial blood flow [36]. VEGF gene delivery to non-ischemic muscle in mice led to an increase in vascular channels and hemangiomas, associated with local high serum VEGF levels [37]. Thus, there is concern that over expression/high doses of growth factors may have deleterious effects on both ischemic and non-ischemic tissue. We did not observe angiomas or hemangiomas in this study.

In summary, adenoviral-mediated gene transfer is capable of inducing sustained VEGF expression in the pericardium. Pericardial VEGF levels in the 200 ng/ml range were achieved in the absence of an increase in systemic VEGF levels. Despite appreciable local VEGF production, however, myocardial collateral perfusion was not improved in this model. These data add to a growing number of studies on the effects of VEGF on myocardial angiogenesis – a biological effect of VEGF has been demonstrated in some but not all of these investigations. Additional studies are needed to better characterize the response of the coronary collateral circulation to VEGF. The optimal VEGF dose, the timing, duration, and route of VEGF delivery, and the potential role of heparin co-administration are important issues that merit further study. Moreover, it remains to be determined whether pericardial gene transfer of alternative forms of VEGF or other angiogenic growth factors might foster collateral growth.

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