Identification of a specific pattern of downregulation in expression of isoforms of vascular endothelial growth factor in dilated cardiomyopathy

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Recent work suggests that myocardial hypoxia or ischaemia are also pathophysiologic factors in idiopathic dilated cardiomyopathy (IDC).¹ Besides several other factors (increased wall stress, endothelial dysfunction, decreased coronary reserve), the observed decreased capillarisation in IDC, disproportionate to the rate of hypertrophy, may further contribute to this oxygen demand–supply mismatch. The reason for this seemingly decreased angiogenic capacity remains unclear, however a role for microvascular abnormalities in heart failure is now recognised.²

Hypoxia is the key factor in the induction of vascular endothelial growth factor (VEGF). Increased expression of VEGF causes angiogenesis, and expression level of VEGF could therefore mediate the capillarisation in IDC. Recently, data have been reported on this issue,³ and the authors found a downregulation of VEGF₁₆₅ and VEGF₁₈₉ isoforms. The VEGF₁₂₁ however was not investigated, although it has been suggested that this isoform in particular has powerful angiogenic capacities. Additionally, it is unclear whether VEGF expression is related to the severity of the disease.

SUBJECTS AND METHODS

We analysed 28 patients with IDC. Patients had enlarged left ventricular end diastolic and systolic diameters (LVEDD 69 (2.1) mm, LVESD 61 (2.6) mm), and decreased left ventricular ejection fraction (LVEF) (0.27 (0.03)), and elevated wedge (15 (1.9) mm Hg) and left ventricular end diastolic pressures (14.1 (2.7) mm Hg). Echocardiography did not reveal cardiac disease in the 10 brain dead subjects, who served as controls. Endomyocardial biopsy taken from the right ventricle showed cardiomyocyte hypertrophy and interstitial fibrosis. Biopsies were snap frozen and stored at –80°C. Total RNA was isolated using the acid guanidium thiocyanate lysis method, as described before.⁴ First strand cDNA was synthesised from 1 µg RNA using the RT-PCR Core kit (Perkin Elmer, USA). The cDNA of interest and of the housekeeping enzyme glyceraldehyde-3-dehydrogenase (GAPDH) were co-amplified in a semiquantitative PCR. The VEGF primers³ span the splice junctions allowing separation by electrophoresis; bands were evaluated by densitometry. To validate the PCRs, the changes in ratios for VEGF₁₂₁, VEGF₁₆₅ and VEGF₁₈₉ were determined and correlated to the amount of input template. PCR products were ligated into pGEM-T-easy-vector and transformed in JM109 cells (pGEM-T-easy kit, Promega, Netherlands). Cell cultures were grown and increasing amounts of the gene of interest were added to a fixed amount of GAPDH in a PCR mixture, containing both GAPDH and VEGF primer. The ratios of VEGF versus GAPDH were calculated, and related to absolute amounts of input template. For Western blot analysis, whole cell protein extracts were obtained from left ventricular tissue and assayed, as described.⁴ Membranes were incubated with primary antibodies: polyclonal anti-rabbit VEGF anti-

body (SC-152; Santa Cruz Biotechnology, Netherlands) and monoclonal anti-rabbit GAPDH (Affinity Bioreagents, Golden, Colorado, USA), and signals were detected by GARpo (Santa Cruz, Sanvertech BV, Heerhugowaard, Netherlands) with ECL detection (Amersham, Roosendaal, Netherlands).

Data are reported as means (SEM). For comparisons between groups an unpaired student *t* test, χ^2 , and a Wilcoxon two sample test were used. Assays were performed three times; *p* < 0.05 was considered significant.

RESULTS

The expression of VEGF₁₂₁, VEGF₁₆₅ and VEGF₁₈₉ isoforms (PCR products of 177 bp, 312 bp, and 384 bp on the gel, respectively) is displayed in fig 1A. GAPDH was expressed at similar levels in controls (non-failing or NF) and in IDC patients (failing or F). Figure 1B shows that cDNA ratio of VEGF₁₂₁/GAPDH is significantly reduced in failing myocardium as compared to controls (0.47 (0.04) v 1.2 (0.21) = 40%; *p* = 0.002). VEGF₁₆₅ (2.2 (0.13) v 2.7 (0.24) = 82%; *p* = 0.27) and VEGF₁₈₉ (1.6 (0.09) v 1.89 (0.24) = 83%; *p* = 0.41) exhibit a similar trend. The amount of input template strongly correlated (*p* < 0.01) with the observed signals for all isoforms (fig 1C: VEGF₁₂₁; VEGF₁₆₅; *r*² = 0.98; VEGF₁₈₉; *r*² = 0.99). Protein VEGF/GAPDH ratios (fig 1D,E) are decreased in IDC patients compared to controls (1.17 (0.18) v 0.64 (0.08) = 55%; *p* = 0.01). No correlations between clinical parameters and VEGF expression levels were established.

DISCUSSION

This study shows that VEGF expression, specifically the VEGF₁₂₁ isoform, is decreased in patients with IDC. The findings were equally apparent in both mild and severe IDC, so this seems an intrinsic quality of IDC. VEGF protein level was also decreased.

Animal studies have supported the possible involvement of VEGF in the pathophysiology and progression of heart failure.⁶ Additionally, it is well established that in IDC capillary density is decreased, and capillary morphology is altered (luminal swelling, lumen narrowing), and these microvascular abnormalities are thought to play an important role in the perpetuation of heart failure.² VEGF is a key determinant in capillary growth. Therefore, we propose a concept in which the level of VEGF expression mediates, at least in part, the capillary abnormalities and hence the myocardial contractility in

Abbreviations: IDC, idiopathic dilated cardiomyopathy; GAPDH, glyceraldehyde-3-dehydrogenase; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic diameter; PCR, polymerase chain reaction; VEGF, vascular endothelial growth factor

Figure 1 (A) Ethidium bromide stained 1.5% agarose gel of expression of mRNA of VEGF and GAPDH in heart tissue from patients (failing or F) and controls (non-failing or NF). (B) Plot showing the mean cDNA ratios for VEGF₁₂₁, VEGF₁₆₅ and VEGF₁₈₉, normalised for GAPDH. (C) Plot showing a significant correlation (Spearman) between the increasing doses of VEGF₁₂₁ input template and its ratio with GAPDH. (D) Plot showing the mean protein ratios for VEGF/GAPDH. Corresponding Western blots are displayed in E; arrows indicate positive controls.

cardiomyopathies, also in the absence of overt coronary artery occlusions. Since we found an equally lowered level of VEGF mRNA expression in mild and severe IDC, the decreased VEGF expression level may represent a mechanism in the progression of IDC.

Thus far, only one report (Abraham and colleagues³) is available on the expression of VEGF in IDC; the present findings are in line with this report with respect to the down-regulation of VEGF₁₆₅ and VEGF₁₈₉. Abraham and colleagues,³ however, did not investigate the presence of VEGF₁₂₁, the isoform that we found decreased the most. All three isoforms have angiogenic properties, however the shorter isoforms have been shown to be more potent than VEGF₁₈₉. The longer VEGF isoforms, especially VEGF₁₈₉ and to a lesser extent VEGF₁₆₅, are more tightly bound to the cellular surface and matrix than VEGF₁₂₁. Given the abundant apposition of matrix-heparin sulfates in IDC, it may be possible that VEGF₁₆₅ and VEGF₁₈₉ are accumulated in the matrix, and are less available. VEGF₁₂₁ that is not bound to the matrix can diffuse more readily in the tissue and may therefore be the most potentially mitogenic VEGF isoform in the failing heart.

In conclusion, the results of this study show that the condition of IDC per se leads to decreased expression of VEGF, especially the potent pro-angiogenic isoform VEGF₁₂₁. VEGF expression is probably not regulated by common cardiac stress pathways, since its decline was not correlated with left ventricular functional parameters. We speculate that interventions that induce angiogenesis, like therapeutic angiogenesis with VEGF₁₂₁ protein or gene transfer, could be beneficial for patients with IDC.

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IMAGES IN CARDIOLOGY.....

Atrial flutter with 1:1 conduction

A 60 year old man presented with increasing breathlessness one month following an uncomplicated mitral valve repair. An echocardiogram showed a moderately sized pericardial effusion of 2 cm. Subxiphoid drainage of the pericardial effusion yielded 200 ml of blood stained fluid. Ten hours later the patient complained of palpitations, and telemetry showed a “broad complex tachycardia” (see 12 lead ECG below). The patient was haemodynamically stable.

The patient was initially treated with 100 ml of intravenous lignocaine and subsequently converted to sinus rhythm (right upper panel) after synchronised cardioversion with a 200 J shock. Looking back at his ECG, he was in atrial flutter with 2:1 block (right lower panel) before drainage of his pericardial effusion.

Close inspection of the 12 lead ECG (below) showed several features to suggest that this may not be ventricular tachycardia. Firstly, not all the QRS complexes in all 12 leads are broad (that is, > 120 ms), particularly in limb lead III. Secondly, the QRS complexes in the anterior leads give a false impression of being broad because the up sloping portion of the ST segment can easily be mistaken as part of the QRS complexes. There is no evidence of atrioventricular dissociation.

After DC cardioversion, the patient remained in sinus rhythm without the need for any antiarrhythmics. There were no further recurrence of his pericardial effusion or tachycardia.

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