Vascular endothelial growth factor release following coronary artery bypass surgery: extracorporeal circulation versus ‘beating heart’ surgery

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Aims The aim of this study was to examine the circulating levels of vascular endothelial growth factor, following coronary artery bypass graft surgery performed using both standard cardiopulmonary bypass or the ‘octopus technique’ on the beating heart.

Background Vascular endothelial growth factor has a number of effects that are beneficial in the setting of coronary artery bypass graft surgery including cardio-protection, potent angiogenic activity and amelioration of intimal hyperplasia. Hypoxia is a powerful stimulator of vascular endothelial growth factor expression yet the ability of ischaemia, occurring during coronary artery bypass graft surgery, to induce vascular endothelial growth factor production is unknown.

Methods and Results Serum vascular endothelial growth factor levels were determined in patients undergoing coronary artery bypass graft surgery with standard cardiopulmonary bypass (CPB-CABG group; n=20), with off-pump coronary artery bypass; (OP-CABG; n=12) and in patients undergoing non-cardiac major surgery (n=6). The effect of hypoxia on vascular endothelial growth factor release by neonatal rat cardiac myocytes in vitro was studied.

In the CPB-CABG group vascular endothelial growth factor levels were significantly increased to 78·5±39·3 and 110·5±16·3 pg ml⁻¹ by 3 and 24 h post-operatively, declining to 14·9±9·9 pg ml⁻¹ by 48 h to pre-operative values (14·4±8·6 pg ml⁻¹). Significantly higher vascular endothelial growth factor levels were also present in the OP-CABG group 3, 6 and 24 h post-operatively (levels 136·6±29·3, 143±26·12 pg ml⁻¹ and 93·5±20·1 pg ml⁻¹, respectively). However, non-cardiac major surgery did not result in elevated vascular endothelial growth factor levels post-operatively (46·3±9·76 vs pre-surgery levels of 26·84±6·1 pg ml⁻¹). Either 15 min or 3 h of hypoxia stimulated vascular endothelial growth factor release from neonatal rat cardiac myocytes in vitro. Twenty-four and 48 h post hypoxia, levels of vascular endothelial growth factor were significantly elevated by approximately 17·5- and 48·5-fold respectively.

Conclusions These data demonstrate myocardial ischaemia secondary to CPB-CABG and OP-CABG to be a potent stimulator of vascular endothelial growth factor production, which may have implications for graft endothelialization and cardiovascular haemodynamics post-operatively.

Key Words: Vascular endothelial growth factor, bypass surgery, beating heart.

Introduction Vascular endothelial growth factor (VEGF) is a 34–46 kDa homodimeric protein with potent angiogenic activity, whose specificity of action is ensured by virtue of its receptors (flt-1 and flk-1) being localized in particular to endothelial cells (for review see Reference [1]). Interest in the use of vascular endothelial growth factor as a potential therapeutic agent has come from its ability to stimulate neovascularization in animal models of limb ischaemia[2], and also in patients with critical limb ischaemia[3]. Moreover, it appears to restore endothelium-dependent vasomotor function following balloon angioplasty[4], and by enhancing...
re-endothelialization it reduces angioplasty stent thrombosis[5] and intimal hyperplasia[6].

The vascular endothelial growth factor promoter contains an hypoxic-inducible factor-1 (HIF-1) element and, as such, both vascular endothelial growth factor mRNA and protein levels are markedly elevated following periods of hypoxia[7,8]. In addition, myocardial ischaemia has been shown to lead to local vascular endothelial growth factor expression[9], which may well be advantageous since cardioplegic administration of vascular endothelial growth factor guards against ischaemia-reperfusion injury following prolonged periods of myocardial ischaemia in the rat heart[10]. Despite its myocardial effects and the potential role of vascular endothelial growth factor in regulating coronary artery endothelialization and angiogenesis, it is not known whether myocardial ischaemia associated with cardiopulmonary bypass (CPB) in the human heart induces vascular endothelial growth factor production. To address this question we have examined the circulating levels of vascular endothelial growth factor in serum before and after coronary artery bypass graft surgery associated with global ischaemia in the presence of cardiopulmonary bypass (CPB-CABG). We have compared this with the levels of vascular endothelial growth factor found in serum following coronary artery bypass graft surgery on the beating heart (off-pump coronary artery bypass graft; OP-CABG). In addition, we have examined the ability of hypoxia in vitro to induce vascular endothelial growth factor release by cardiac myocytes and of vascular endothelial growth factor to stimulate human aortic and coronary artery endothelial cell DNA synthesis at physiologically relevant concentrations.

Methods

Patients and vascular endothelial growth factor assay

A total of 38 patients were included in the study. The investigation conformed to the principles outlined in the Declaration of Helsinki; ethical approval was obtained and all patients gave written informed consent. Patients with good ventricular function requiring triple vessel bypass grafting (left anterior descending coronary artery plus two other coronary arteries (n=20, mean age 62, 17 males, three females)) were recruited to the cardiopulmonary bypass-coronary artery bypass graft group over a 3-month period. The second group of patients (n=12, mean age 62, 10 males, two females) required only the left anterior descending coronary artery bypassing; they also had good ventricular function and were recruited over the following 3 months to the OP-CABG utilizing the ‘octopus’ stabilization technique on the beating heart. CPB-CABG was performed using a standardized regime of cold blood cardioplegia: an initial dose of 900 ml of cardioplegia at 4°C with 600 ml administered anterogradely and 300 ml retrogradely followed by 300 ml administered retrogradely every 20 min. 600 ml of modified blood cardioplegia at 37°C was administered retrogradely 3 min before the release of the aortic cross-clamp. For OP-CABG the origin of the left anterior descending coronary artery was dissected and occluded for 15 min during which time the anastomosis between the coronary artery and left internal mammary artery was constructed (see Reference [11] for more detail). The OP-CABG patients had grafting performed to the left anterior descending coronary artery alone, while the CPB-CABG group had grafts to three vessels on which one was the left anterior descending coronary artery. Non-cardiac surgical patients underwent either thoracotomy for lung volume reduction (n=3), or carotid endartecetomy (n=3).

Arterial blood samples were obtained 10 min prior to the start of all procedures. For CPB-CABG patients peripheral arterial blood was then taken 24 h later and in a subset of these patients (n=4) at 8 and 48 h after the end of the operation. Blood samples were obtained from the OP-CABG patients at 3, 6 and 24 h after the end of the procedure. For the non-cardiac surgical patients blood was obtained 24 h after the end of the procedure.

Blood samples were collected in tubes containing EDTA, centrifuged at 2000 × g for 5 min and the serum stored at −80°C until required. Vascular endothelial growth factor was assayed using a sandwich ELISA ‘Quantikine’ kit (R&D Systems) which recognizes the isoform vascular endothelial growth factor

Cardiac myocyte cell culture in normoxia and hypoxia

Cardiac myocytes were prepared from 2-day-old neonatal rat hearts as described previously[12]. Briefly, hearts were removed and cut into small pieces. These were then digested with a solution of collagenase (type 2, Worthington, 1 mg · ml⁻¹) for five successive 15-min periods. Dispersed cells were then cultured in Dulbecco’s Modified Eagles Medium containing 10% fetal calf serum (DMEM-FCS). 24 h after being prepared, cells were washed with serum-free DMEM, containing 1 mg · ml⁻¹ bovine albumen, insulin (5 µg · ml⁻¹), transferin (5 ng · ml⁻¹), sodium selenite (5 ng · ml⁻¹), penicillin (100 U · ml⁻¹) and streptomycin (0·1 mg · ml⁻¹). After a further 24 h cells were transferred to an atmosphere of 95% nitrogen and 5% carbon dioxide, at 37°C, for either 15 min, or 3 h. After this time, cells were returned to normoxia, and a 200 µl sample of supernatant medium was removed (out of a total of 2 ml) and the same volume of fresh serum-free medium added. Samples were again removed after 6, 24 and 48 h. Samples were centrifuged at 14000 × g for 3 min, and the supernatant stored at −80°C until required.

Endothelial cell mitogenicity assay

Human aortic and epicardial coronary artery endothelial cells were obtained from patients undergoing
orthotopic cardiac transplantation. Endothelial cells were isolated and cultured as described previously.[13] Briefly, the endothelium was gently scraped off the lumen following collagenase digestion, and cells were cultured in medium 199 supplemented with 20% heat-inactivated fetal calf serum. Cells were passaged for 2 weeks prior to use. The ability of VEGF to stimulate DNA synthesis over a 24 h period in both cell types was assessed using tritiated thymidine. Briefly, the procedure used 1 μCi per well for 24 h, cells were then washed three times and DNA precipitated with ice-cold trichloroacetic acid (10% w/v) for 1 h. The precipitate was then solubilized in 1 M sodium hydroxide and the incorporated radioactivity determined.

Statistics

Data are presented as mean ± SEM. Significance was assessed where appropriate by Student’s t-test. A P-value <0.05 was taken as significant.

Results

CPB-CABG and OP-CABG stimulate vascular endothelial growth factor secretion

Patients undergoing standard coronary artery bypass graft had an average ischaemic time of 75 ± 18 min (mean ± SE) and 15 ± 4 min for cardiopulmonary bypass and off-pump groups respectively. All patients were extubated within 8 h of surgery and were transferred to the ward from ITU the following day. OP-CABG patients also had a similarly uneventful post-operative course.

Significantly elevated levels of vascular endothelial growth factor were detected 8 h after the end of bypass (78.5 ± 39.3 pg . ml⁻¹; P<0.01) and remained elevated in those samples taken after 24 h (110.5 ± 16.3 pg . ml⁻¹; P<0.01). By 48 h vascular endothelial growth factor levels were not significantly different to pre-cardiopulmonary bypass levels (14.9 ± 9.9 vs 14.4 ± 8.6 pg . ml⁻¹ respectively) (Fig. 1A).

As with the standard cardiopulmonary bypass group of patients, vascular endothelial growth factor levels in the serum of OP-CABG patients increased significantly 3 h after the 15-min period of warm ischaemia (136.2 ± 29.3 pg . ml⁻¹; P<0.01) rising further at 6 h (143.0 ± 26.12 pg . ml⁻¹). By 24 h levels had declined to 93.5 ± 20.1 pg . ml⁻¹ (Fig. 1B), which was still significantly elevated compared with pre-operative levels, but not significantly different to the value in the CPB-CABG group at that time (P=0.27).

Vascular endothelial growth factor levels were not significantly elevated in post-operative serum samples obtained from patients undergoing either lung resection or carotid endartarectomy (P=0.15) (Fig. 1C).

Hypoxia stimulates VEGF release by neonatal rat cardiac myocytes in vitro

Neonatal rat cardiac myocytes subjected to either 15 min or 3 h of hypoxia released detectable amounts of vascular endothelial growth factor into the culture media 6 h after the end of hypoxia. This increased further at 24 and 48 h (Fig. 2). By 48 h the concentration of vascular endothelial growth factor was not significantly different in the media of cells exposed to either 15 min (6.2 ± 0.5 pg . ml⁻¹) or 3 h (7.0 ± 0.6 pg . ml⁻¹) of hypoxia. 48 h conditioned media from control cells not exposed to hypoxia did not contain detectable amounts of vascular endothelial growth factor (data not shown). Vascular endothelial growth factor was added to cultured human aortic and coronary artery endothelial cells at 75 and 150 pg . ml⁻¹, and its ability to stimulate thymidine incorporation was assessed. Both 75 and 150 pg . ml⁻¹ induced a significant increase in DNA synthesis in coronary artery endothelial cells (Table 1), with cells from the coronary artery appearing to be more responsive than those from the aorta.

Discussion

We have demonstrated here that myocardial ischaemia occurring both globally (CPB-CABG patients) and locally (OP-CABG patients) is a potent stimulator of vascular endothelial growth factor release into the systemic circulation. We have further shown that as little as 15 min of hypoxia is sufficient to bring about significant vascular endothelial growth factor release from cultured cardiac myocytes and that vascular endothelial growth factor, at concentrations found in serum post-coronary artery bypass graft, induces significant DNA synthesis in both human coronary and aortic artery endothelial cells. The data we present here raise two fundamental questions.

The first is what are the potential effects of elevated vascular endothelial growth factor levels in serum following coronary artery bypass graft surgery, both locally and systemically? Vascular endothelial growth factor can act to stimulate endothelial cell proliferation, neo-angiogenesis and hence repress intimal hyperplasia. This appears to be the case in carotid arteries[4], angioplasty stents[31] and venous grafts[14]. The ability of vascular endothelial growth factor to reduce intimal thickening has been linked to nitric oxide production[4]. Support for this comes from studies in which animals lacking the endothelial nitric oxide synthase gene have impaired angiogenesis in response to tissue ischaemia[35]. This cannot be overcome by addition of vascular endothelial growth factor suggesting a central role for eNOS acting downstream of vascular endothelial growth factor in mediating angiogenesis. Following balloon angioplasty arterial smooth muscle cells express vascular endothelial growth factor, an effect which can be blocked by nitric oxide interfering with the binding of
the transcription factor AP-1 to the promoter of the vascular endothelial growth factor gene[16]. Thus arterial injury upregulates vascular endothelial growth factor expression leading to both endothelial proliferation and nitric oxide release which may in turn inhibit vascular endothelial growth factor production. Furthermore, intimal hyperplasia has been found to be markedly reduced by immersing vein grafts in a solution of vascular endothelial growth factor[17]. Hence vascular endothelial growth factor acting locally can profoundly alter coronary endothelial proliferative activity. These data may be relevant regarding endothelialization of the arterial and venous conduits used in this procedure in particular given the fact that the circulating concentrations of vascular endothelial growth factor are sufficient to bring about significant endothelial cell DNA synthesis.

What are the potential systemic effects of elevated vascular endothelial growth factor levels? Both systemic

**Figure 1** Coronary artery bypass graft surgery specifically stimulates vascular endothelial growth factor (VEGF) production. (A) VEGF concentrations were determined in arterial blood samples following standard cardiopulmonary bypass (CPB). Samples were taken immediately prior to CPB (time 0) and then 8, 24 and 48 h after the release of the aortic cross clamp. Data are shown as mean ± SEM, n=20. *P<0·01 compared with time 0. (B) VEGF concentrations were determined in arterial blood samples following off-pump coronary artery bypass graft surgery (OP-CABG). Samples were taken immediately prior to occlusion of the left anterior descending (LAD) coronary artery (time 0) and then 3, 6 and 24 h after release of the LAD coronary artery. Data are shown as mean ± SEM, n=12. *P<0·01 compared with time 0 values. (C) VEGF concentrations were determined in arterial blood samples taken immediately prior to (pre-surgery) and 24 h after (post-surgery) major non-cardiac surgery (thoracotomy and carotid endarterectomy; n=6) (hatched columns). Data are shown as mean ± SEM. The concentration of VEGF seen 24 h post CPB-CABG (filled column) and OP-CABG surgery (open column) is included for comparison. *P<0·01 compared with post non-cardiac surgery.

**Table 1** Vascular endothelial growth factor stimulates DNA synthesis in aortic and coronary endothelial cells

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<tr>
<th>Treatment</th>
<th>Aortic endothelial cells</th>
<th>Coronary artery endothelial cells</th>
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<tbody>
<tr>
<td>Control</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>VEGF (75 pg . ml⁻¹)</td>
<td>130 ± 12%</td>
<td>215 ± 25%</td>
</tr>
<tr>
<td>VEGF (150 pg . ml⁻¹)</td>
<td>143 ± 14%</td>
<td>210 ± 17%</td>
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DNA synthesis was assessed in both aortic and coronary artery endothelial cells using tritiated thymidine incorporation. Control values were set arbitrarily at 100%. Data is shown as mean ± SEM, n=6. Vascular endothelial growth factor significantly stimulated DNA synthesis in both endothelial cell types at both 75 and 150 pg . ml⁻¹ (P<0·02).

**Figure 2.** Hypoxia results in vascular endothelial growth factor (VEGF) release by neonatal rat cardiac myocytes in vitro. VEGF concentrations were determined in the supernatant medium of cultured neonatal rat cardiac myocytes that had been subjected to either 15 min (closed columns) or 3 h (open columns) of hypoxia. Medium was sampled at the end of the hypoxic period (time 0), and then 6, 24 and 48 h later. Data are shown as mean VEGF concentration (pg . ml⁻¹)/10⁶ cells ± SEM, n=6. *P<0·02 compared to time 0. The bar above the columns indicates that both closed and open columns are significantly different compared with time 0.
or intra-coronary administration of vascular endothelial growth factor brings about a significant (20%) reduction in mean arterial blood pressure in pigs, along with a reduction in cardiac output and peripheral vascular resistance[19]. Again these effects are probably secondary to increased nitric oxide production, since the effect on the coronary vasculature can be blocked by N-G-nitro-L-arginine, a nitric oxide inhibitor. Hence elevated circulating vascular endothelial growth factor levels following coronary artery bypass graft surgery may bring about vasodilatation of vascular conduits such as internal mammary artery grafts, in addition to native coronary arteries; this increases coronary perfusion and also dilatates other vascular beds, altering systemic haemodynamic performance.

A second question raised by our data concerns the site of vascular endothelial growth factor production. It is unlikely that generalized activation of the inflammatory pathway by the bypass circuit is responsible, as increased circulating levels of vascular endothelial growth factor are seen in both CPB-CABG and OP-CABG. Furthermore, it does not seem to be a generalized response to surgical stress as vascular endothelial growth factor levels were not increased in response to non-cardiac major surgery (Fig. 1C). Tofukuji and colleagues have shown increased myocardial vascular endothelial growth factor mRNA expression after cardiopulmonary bypass in pigs[19]. Intracellular vascular endothelial growth factor mRNA, and protein levels have been shown to be markedly elevated following exposure of cardiac myocytes to hypoxia[20], and we have also found hypoxia to stimulate vascular endothelial growth factor secretion by these cells (Fig. 2). Other cell types, including smooth muscle cells, endothelial cells and infiltrating macrophages, are also potential sites of vascular endothelial growth factor production. In a rat model of myocardial infarction, the principal cell types responsible for vascular endothelial growth factor production were found to be myocytes and infiltrating macrophages[9]. Vascular endothelial growth factor secretion may be a direct response to hypoxia or to paracrine factors such as transforming growth factor beta (TGF-β) or fibroblast growth factor-2, both of which are produced by the heart in response to myocardial ischaemia[21-23] and can stimulate vascular endothelial growth factor production[24,25]. It is interesting that neutralizing antibodies to TGF-β partially block the release of vascular endothelial growth factor by cardiac myocytes to stretch both in vivo[25] and in vitro[26], suggesting that its release is mediated in part by paracrine factors. The specific cell types producing vascular endothelial growth factor following coronary artery bypass graft surgery remain to be determined. However, this study shows that cardiac myocytes are among those that do.

One may expect that 15 min of localized warm ischaemia during OP-CABG is likely to cause less damage than 75 min of global cold ischaemia during CPB-CABG. This assumption is supported by analysis of troponin release, demonstrating a tenfold reduction in troponin levels in the OP-CABG group compared with the CPB-CABG group[27]. However, vascular endothelial growth factor levels are similar in both the OP-CABG and CPB-CABG groups. Therefore, while troponin release data support the ability of OP-CABG to cause less myocardial cell death, both surgical techniques appear to result in the same amount of ischaemic ‘stress’ as evidenced by vascular endothelial growth factor release, and supported by our finding of similar vascular endothelial growth factor release in vitro following either 15 min or 3 h of hypoxic treatment. Finally, vascular endothelial growth factor prevents endothelial cell apoptosis in response to either TNF-α or serum deprivation[29]. Although yet to be demonstrated in cardiac myocytes, the idea that elevated vascular endothelial growth factor levels following ischaemia could protect cardiac myocytes from apoptosis merits further investigation.

Our data demonstrate myocardial ischaemia occurring after coronary artery bypass graft surgery utilizing either CPB-CABG or OP-CABG to result in a significant rise in the circulating level of vascular endothelial growth factor. At these concentrations vascular endothelial growth factor significantly stimulates endothelial cell DNA synthesis. These data may be of relevance to endothelialization of implanted graft conduits following coronary bypass surgery, but may also have a wider impact on explaining changes in cardiac physiology in the post-bypass patient.

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References


