Angiogenesis in myocardial infarction

An acute or chronic process?


Haemostasis Thrombosis and Vascular Biology Unit, University Department of Medicine, City Hospital, Birmingham, U.K.

Background In the acute phase of myocardial infarction (acute MI) marked endothelial damage occurs within the first 24 h following thrombolysis with streptokinase. We investigated whether this is associated with a change in levels of vascular endothelial growth factor (VEGF, possibly marking angiogenesis) in the first 24 h post thrombolysis compared to chronic MI patients (defined as MI>3 months previously).

Methods We recruited 15 patients (nine male, mean age 59 ± SD 10 years) with first-presentation acute MI, who were given 1·5 million U streptokinase over 1 h and aspirin 300 mg orally as standard treatment. Plasma samples were taken prior to the start of thrombolysis, followed every 15 min for 1 h, at 3 h and finally at 24 h post-thrombolysis. Baseline levels of measured indices in the acute MI patients were compared to two control groups: (i) 26 chronic MI patients (18 male, mean age 59·9 ± 7·0 years); and (ii) 26 apparently healthy controls (17 male, mean age 59·6 ± 14·1 years). Plasma VEGF and the soluble form of its receptor Flt-1 (sFlt-1) were measured by ELISA.

Results Plasma levels of VEGF were significantly higher in patients with a history of chronic MI compared to patients with acute MI (P=0·007) and healthy controls (P=0·002) with similar levels between acute MI patients and healthy controls (P=0·755). Levels of sFlt-1 in the acute (P=0·013) and chronic (P<0·001) MI groups were lower compared to healthy controls. In the first 24 h post-thrombolysis in the acute MI group, levels of sFlt-1 changed significantly (P=0·039), but there was no change in levels of VEGF (P=0·207).

Conclusion In the first 24 h of acute MI, significant changes in levels of VEGF receptor sFlt-1, but not VEGF, are seen. Plasma VEGF and sFlt-1 levels are markedly changed in chronic MI patients, suggesting that the activation of angiogenesis in MI patients may be a delayed response.

(Eur Heart J, 2002; 23: 1604–1608, doi:10.1053/euhj.2002.3312) © 2002 The European Society of Cardiology. Published by Elsevier Science Ltd. All rights reserved.

Key Words: Angiogenesis, vascular endothelial growth (VEGF), soluble Flt-1, myocardial infarction (MI).

Introduction

In the acute phase of myocardial infarction (acute MI), we have recently demonstrated marked endothelial damage and platelet activation following thrombolysis with streptokinase within the first 24 h[1]. These changes may have prognostic implications, as plasma levels of von Willebrand factor (vWF), an established marker of endothelial damage/dysfunction[2], have been shown to be predictive of reinfarction[3] and an adverse prognosis[4,5].

However, the roles of endothelial activation in acute MI other than promoting a procoagulant state are still to be elucidated.

It is now well established that a pathophysiological role for angiogenesis exists in the process of atherosclerosis (atherogenesis)[6–10]. Many angiogenic factors exist, but vascular endothelial growth factor (VEGF) is one of the essential components of the angiogenic process and has been shown to be present in atherosclerotic lesions in coronary artery disease (CAD)[7,11], with raised plasma VEGF levels demonstrated in CAD patients[12]. Not only is VEGF implicated in the initial process of atherogenesis, but its subsequent expression is increased in the presence of hypoxia by myocytes, smooth muscle cells and endothelial cells[13–16].
One of the main receptors for VEGF is a transmembrane tyrosine kinase receptor known as VEGF receptor-1, or Flt-1, which is found on the surface of endothelial cells\(^{[17]}\). There is also a detectable soluble form in plasma, sFlt-1\(^{[18]}\), and an inverse relationship with VEGF has previously been shown in atherosclerosis\(^{[12]}\).

Previous studies in the setting of acute MI have looked at changes in levels of serum (not plasma) VEGF, and mainly in patients who have undergone revascularization by percutaneous transluminal coronary angioplasty (PTCA), up to 21 days post MI\(^{[19–23]}\). Citrated plasma is now the preferred medium for measurement of VEGF\(^{[18]}\), and we are unaware of any studies examining levels of angiogenic markers in the more common clinical setting of acute MI treated by thrombolysis with streptokinase.

We hypothesised that angiogenesis would be commenced over the first 24 h of acute MI. To test this hypothesis, we examined circulating levels of plasma VEGF and its soluble receptor sFlt-1 in acute MI patients in the first 24 h post thrombolysis, comparing levels at baseline to patients with previous MI and with healthy controls.

**Patients and methods**

We recruited 15 patients (nine male, six female; mean age 59·4±10·4 years), with a first-presentation acute MI, presenting within 6 h of onset of chest pain, a history of typical chest pain and diagnostic ECG changes of acute MI. They were consented for thrombolysis with streptokinase and subsequent repeat blood sampling over the next 24 h. All patients were given 1·5 million U streptokinase over 1 h and aspirin 300 mg orally as standard treatment. All patients included in this study had an uncomplicated MI, as well as clinical and ECG criteria of successful reperfusion post-thrombolysis\(^{[23]}\). Exclusion criteria were previous MI; concomitant warfarin, steroid or hormone replacement therapy; recent (<3 months) acute cardiovascular or cerebrovascular event prior to the current acute MI; uncontrolled hypertension (>180/110 mmHg) and other systemic disorders which would interfere with the indices measured (e.g. cancer, connective tissue disease etc.).

The protocol was approved by the West Birmingham research ethics committee and informed consent was obtained from patients.

Citrated plasma samples were taken immediately prior to the start of thrombolysis, followed every 15 min for 1 h, at 3 h and finally at 24 h post-thrombolysis. Baseline levels of measured indices in the acute MI patients were compared to two control groups: (i) 26 patients (18 male, eight female; mean age 59·9±7·0 years) with a history of previous MI >3 months prior to blood sampling (‘chronic’ MI patients); and (ii) 26 apparently healthy controls (17 male, nine female; mean age 59·6±14·1 years) recruited from hospital staff and preoperative clinics for minor procedures, including hernia repairs, cataract surgery, etc. All healthy controls were ‘healthy’ by virtue of careful clinical history and examination, as well as basic blood screening tests. The healthy control subjects are included to provide a ‘normal’ perspective for the measured indices only.

**Blood samples and analysis**

All samples were taken by non-traumatic venepuncture from the three groups. Samples were immediately placed on ice before being centrifuged at 1500 g for 20 min at 4 °C. They were then stored at −70 °C until the time of analysis. Baseline plasma samples from all three subject groups were analysed by in-house sandwich enzyme-linked immunosorbent assays (ELISA) for VEGF and sFlt-1 (R+D Systems, Abingdon, U.K.)\(^{[18]}\). The lower limits of detection by ELISA were 10 pg ml\(^{-1}\) for VEGF and 0·1 ng ml\(^{-1}\) for sFlt. The inter- and intra-assay coefficients of variation were <5 and 10%, respectively, for all assays.

Statistical analyses were performed using the statistical program SPSS 10.0 for Windows. Parametric results are expressed as mean ± standard deviation (SD) and differences between groups compared by one-way ANOVA. Non-categorical data were compared by chi-squared test. VEGF and sFlt-1 are expressed as median (IQR) levels of sFlt-1 (RMANOVA, \(P=0·575\)). Significantly higher VEGF levels were found in the chronic MI patients compared to acute MI patients (\(P=0·007\)) and healthy controls (\(P=0·002\)).

Levels of sFlt-1 were significantly lower in both the acute (\(P=0·013\)) and chronic MI patients (\(P<0·001\)) compared to healthy controls (Table 2), but there was no statistically significant differences between sFlt-1 levels between acute and chronic MI patients (\(P=0·424\)).

Following thrombolysis there were no significant changes in plasma levels of VEGF (\(P=0·207\)) (Table 3). However, there was a significant change in median (IGR) levels of sFlt-1 (RMANOVA, \(P=0·039\)) with a rise from 5·6 (2·2±11·0) ng ml\(^{-1}\) at baseline to 9·5 (3·1–12·0) ng ml\(^{-1}\) at 3 h post-thrombolysis (Fig. 1). By 24 h, this level had returned to 5·8 (2·6–15·0) ng ml\(^{-1}\).
In the present study, there were no significant changes in plasma levels of VEGF in the first 24 h post-thrombolysis for acute MI, despite previously documented increases of indices of endothelial damage/dysfunction, as indicated by plasma vWF and sTM levels[1]. However, there were significant changes in levels of sFlt-1 over the 24 h. We have also confirmed previous findings of lower plasma levels of sFlt-1 in patients with CAD[12,18], but interestingly, VEGF levels were only significantly higher in the chronic MI patient group and not the (first presentation) acute MI patient group.

In a study of ventricular biopsies from patients undergoing coronary artery bypass surgery, of which 15 had a history of acute MI within the preceding 120 h, VEGF was demonstrable in samples with evidence of acute MI or acute ischaemia by immunohistochemistry and PCR[20]. However, no evidence of VEGF protein in peripheral blood was found by Western blot analysis, although these results were not greatly discussed.

Other studies have reported increases in serum VEGF levels post-myocardial infarction from peripheral venous blood[19,21,22]. All of these studies have looked at patients following successful revascularization by PTCA compared to baseline levels. In two studies, a gradual increase in levels of VEGF was seen in the 3 weeks post-acute MI, but the earliest measurement after baseline was at day 3[19,22] and the results are, therefore, not directly comparable to the present study. The study by Seko et al.[21] measured serum VEGF levels within the first 24 h of acute MI in 19 patients undergoing reperfusion therapy for acute MI by PTCA: VEGF levels at baseline were much higher than healthy controls, but 20–30 min post-procedure levels were reduced to healthy control values.

The studies based on serum samples of VEGF should be interpreted with some caution. In all the studies, serum levels were expressed as mean values ± standard deviation.

### Table 1 Demographic characteristics of patients with acute and chronic myocardial infarction and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Acute MI patients</th>
<th>Chronic MI patients</th>
<th>Healthy controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59·4 ± 10·4</td>
<td>59·9 ± 7·0</td>
<td>59·6 ± 14·1</td>
<td>0·990</td>
</tr>
<tr>
<td>No. males (%)</td>
<td>73·3</td>
<td>69·2</td>
<td>65·4</td>
<td>0·867</td>
</tr>
<tr>
<td>No. of smokers (%)</td>
<td>34·6</td>
<td>46·6</td>
<td>26·9</td>
<td>0·439</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>138 ± 22</td>
<td>139 ± 25</td>
<td>134 ± 19</td>
<td>0·754</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84 ± 15</td>
<td>78 ± 14</td>
<td>84 ± 12</td>
<td>0·225</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation, or % of patients per group. Analysis by one-way ANOVA or chi-square as appropriate. SBP, DBP = systolic, diastolic blood pressure.

### Table 2 Plasma levels of vascular endothelial growth factor and soluble Flt-1 in patients with acute and chronic myocardial infarction and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Acute MI patients</th>
<th>Chronic MI patients</th>
<th>Healthy controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg . ml (^{-1}))</td>
<td>95 (60–160)</td>
<td>286 (133–932)*</td>
<td>125 (44–246)</td>
<td>0·001</td>
</tr>
<tr>
<td>sFlt-1 (ng . ml (^{-1}))</td>
<td>5·6 (2–11)</td>
<td>1·0 (0–29)*</td>
<td>26·5 (10–82·5)</td>
<td>&lt;0·001</td>
</tr>
</tbody>
</table>

Values are expressed as median (interquartile range) and analysed by Kruskal–Wallis test. *Tukey’s post hoc test P<0·05 compared to healthy controls. VEGF = vascular endothelial growth factor; sFlt-1 = soluble Flt-1.

### Table 3 Changes in plasma levels of vascular endothelial growth factor and soluble Flt-1 in acute myocardial infarction patients within the first 24 h post-thrombolysis

<table>
<thead>
<tr>
<th>Time after initiation of thrombolysis (hours)</th>
<th>VEGF (pg . ml (^{-1}))</th>
<th>sFlt-1 (ng . ml (^{-1}))</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (baseline)</td>
<td>95 (60–160)</td>
<td>5·6 (2–11)</td>
<td>0·207</td>
</tr>
<tr>
<td>0·25</td>
<td>115 (60–157)</td>
<td>5·2 (2–4·15·5)</td>
<td></td>
</tr>
<tr>
<td>0·5</td>
<td>110 (80–160)</td>
<td>8·0 (2·9–14·0)</td>
<td></td>
</tr>
<tr>
<td>0·75</td>
<td>100 (75–130)</td>
<td>7·5 (2·7–18·0)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>111 (60–130)</td>
<td>9·0 (2·7–17·0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>95 (56–130)</td>
<td>9·5 (3·1–12·0)</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>100 (60–130)</td>
<td>5·8 (2·6–15·0)</td>
<td>0·039</td>
</tr>
</tbody>
</table>

All values expressed as median (interquartile range) and analysed by Friedman test after log transformation. VEGF = vascular endothelial growth factor; sFlt-1 = soluble Flt-1.

**Discussion**

In the present study, there were no significant changes in plasma levels of VEGF in the first 24 h post-thrombolysis for acute MI, despite previously documented increases of indices of endothelial damage/dysfunction, as indicated by plasma vWF and sTM levels[1]. However, there were significant changes in levels of sFlt-1 over the 24 h. We have also confirmed previous findings of lower plasma levels of sFlt-1 in patients with CAD[12,18], but interestingly, VEGF levels were only significantly higher in the chronic MI patient group and not the (first presentation) acute MI patient group.

In a study of ventricular biopsies from patients undergoing coronary artery bypass surgery, of which 15 had a history of acute MI within the preceding 120 h, VEGF was demonstrable in samples with evidence of acute MI or acute ischaemia by immunohistochemistry and PCR[20]. However, no evidence of VEGF protein in peripheral blood was found by Western blot analysis, although these results were not greatly discussed.

Other studies have reported increases in serum VEGF levels post-myocardial infarction from peripheral venous blood[19,21,22]. All of these studies have looked at patients following successful revascularization by PTCA compared to baseline levels. In two studies, a gradual increase in levels of VEGF was seen in the 3 weeks post-acute MI, but the earliest measurement after baseline was at day 3[19,22] and the results are, therefore, not directly comparable to the present study. The study by Seko et al.[21] measured serum VEGF levels within the first 24 h of acute MI in 19 patients undergoing reperfusion therapy for acute MI by PTCA: VEGF levels at baseline were much higher than healthy controls, but 20–30 min post-procedure levels were reduced to healthy control values.

The studies based on serum samples of VEGF should be interpreted with some caution. In all the studies, serum levels were expressed as mean values ± standard deviation.
I

Figure 1 Changes in plasma levels of soluble Flt-1 (sFlt-1) receptor in the first 24 h post-thrombolysis for acute myocardial infarction (MI).

deviation, but in our hands VEGF is not a normally distributed marker. Furthermore, measurement of VEGF in serum can produce erroneous levels of VEGF and it has been recommended that plasma samples, as used in the present study, should be used. Indeed, measurement of serum VEGF would significantly overestimate the true levels of free VEGF as some VEGF is released from platelets when blood clots, and thus, serum VEGF levels may (artefactually) rise significantly over time after clotting occurs — which is particularly relevant peri-infarction, when coagulation/platelet derangement is likely following thrombolytic therapy. Moreover, the half-life of VEGF has previously been reported to be approximately 50 min, and the observation by Seko et al. that VEGF levels ‘normalise’ 20–30 min post-procedure should be viewed with caution.

The present study would suggest that plasma VEGF levels may not increase in the acute phase of MI, in the first 24 h, but that levels are more likely to increase over time, such that they are significantly higher in ‘chronic MI’ patients, of >3 months’ post-infarction. This would reflect observations seen in the other studies with longer term follow-up. The changes seen acutely in levels of VEGF in serum can produce erroneous levels of VEGF deviation, but in our hands VEGF is not a normally distributed marker. Furthermore, measurement of VEGF in serum can produce erroneous levels of VEGF and it has been recommended that plasma samples, as used in the present study, should be used. Indeed, measurement of serum VEGF would significantly overestimate the true levels of free VEGF as some VEGF is released from platelets when blood clots, and thus, serum VEGF levels may (artefactually) rise significantly over time after clotting occurs — which is particularly relevant peri-infarction, when coagulation/platelet derangement is likely following thrombolytic therapy. Moreover, the half-life of VEGF has previously been reported to be approximately 50 min, and the observation by Seko et al. that VEGF levels ‘normalise’ 20–30 min post-procedure should be viewed with caution.

The present study would suggest that plasma VEGF levels may not increase in the acute phase of MI, in the first 24 h, but that levels are more likely to increase over time, such that they are significantly higher in ‘chronic MI’ patients, of >3 months’ post-infarction. This would reflect observations seen in the other studies with longer term follow-up. The changes seen acutely in levels of VEGF in serum can produce erroneous levels of VEGF deviation, but in our hands VEGF is not a normally distributed marker. Furthermore, measurement of VEGF in serum can produce erroneous levels of VEGF and it has been recommended that plasma samples, as used in the present study, should be used. Indeed, measurement of serum VEGF would significantly overestimate the true levels of free VEGF as some VEGF is released from platelets when blood clots, and thus, serum VEGF levels may (artefactually) rise significantly over time after clotting occurs — which is particularly relevant peri-infarction, when coagulation/platelet derangement is likely following thrombolytic therapy. Moreover, the half-life of VEGF has previously been reported to be approximately 50 min, and the observation by Seko et al. that VEGF levels ‘normalise’ 20–30 min post-procedure should be viewed with caution.

The present study would suggest that plasma VEGF levels may not increase in the acute phase of MI, in the first 24 h, but that levels are more likely to increase over time, such that they are significantly higher in ‘chronic MI’ patients, of >3 months’ post-infarction. This would reflect observations seen in the other studies with longer term follow-up. The changes seen acutely in levels of VEGF in serum can produce erroneous levels of VEGF deviation, but in our hands VEGF is not a normally distributed marker. Furthermore, measurement of VEGF in serum can produce erroneous levels of VEGF and it has been recommended that plasma samples, as used in the present study, should be used. Indeed, measurement of serum VEGF would significantly overestimate the true levels of free VEGF as some VEGF is released from platelets when blood clots, and thus, serum VEGF levels may (artefactually) rise significantly over time after clotting occurs — which is particularly relevant peri-infarction, when coagulation/platelet derangement is likely following thrombolytic therapy. Moreover, the half-life of VEGF has previously been reported to be approximately 50 min, and the observation by Seko et al. that VEGF levels ‘normalise’ 20–30 min post-procedure should be viewed with caution.

The present study would suggest that plasma VEGF levels may not increase in the acute phase of MI, in the first 24 h, but that levels are more likely to increase over time, such that they are significantly higher in ‘chronic MI’ patients, of >3 months’ post-infarction. This would reflect observations seen in the other studies with longer term follow-up. The changes seen acutely in levels of VEGF in serum can produce erroneous levels of VEGF deviation, but in our hands VEGF is not a normally distributed marker. Furthermore, measurement of VEGF in serum can produce erroneous levels of VEGF and it has been recommended that plasma samples, as used in the present study, should be used. Indeed, measurement of serum VEGF would significantly overestimate the true levels of free VEGF as some VEGF is released from platelets when blood clots, and thus, serum VEGF levels may (artefactually) rise significantly over time after clotting occurs — which is particularly relevant peri-infarction, when coagulation/platelet derangement is likely following thrombolytic therapy. Moreover, the half-life of VEGF has previously been reported to be approximately 50 min, and the observation by Seko et al. that VEGF levels ‘normalise’ 20–30 min post-procedure should be viewed with caution.

The possibility of the rise in endothelial markers, vWF and sTM, within the first 24 h post-thrombolysis demonstrated previously, one might have expected to see a rise in VEGF as well as sFlt-1 levels as they are both produced by the endothelium. It may be that endothelial damage is initially caused by rupture of the endothelial lining of atherosclerotic plaques and subsequent thrombogenesis rather than hypoxic/ischaemic insults, such that the stimulus for VEGF is not an immediate response, but occurs after the ischaemic damage has occurred.

In conclusion, we have demonstrated that there are no changes in plasma VEGF levels in the first 24 h of acute MI following thrombolysis. Nevertheless, VEGF levels are markedly elevated in chronic MI patients, suggesting that the activation of angiogenesis in MI patients may be a delayed response. Lower levels of sFlt-1 in baseline acute MI patients and chronic MI patients compared to healthy controls may reflect background abnormal angiogenesis in CAD and the significant changes in sFlt-1 during the first 24 h post-MI may reflect the start of endothelial activation.

This study is limited by the time span used to repeated measures to only 24 h. Repeat samples at 1 week (approximately the time raised levels of serum VEGF appeared in other studies), 1 month and 3 months in our acute MI patients may have shown the transition from normal to high plasma levels of VEGF as the patients become ‘chronic’ MI patients. In addition, it would be considered unethical to study another group of patients with acute MI who were not given thrombolytic therapy, and the policy in our unit would be to actively consider patients with contraindications to thrombolytic therapy for primary PTCA.

Finally, thrombus-bound VEGF and sFlt-1 may have influenced our results, but all patients were treated with thrombolytic therapy, and if further release of VEGF and sFlt-1 occurred during thrombolysis, we would have expected to see an acute rise in plasma levels of these indices, as thrombolysis was in progress. Similarly, if an acute ‘stress’ response were apparent, we would have expected to see marked changes in VEGF as well, over the first 24 h.

In conclusion, we have demonstrated that there are no changes in plasma VEGF levels in the first 24 h of acute MI following thrombolysis. Nevertheless, VEGF levels are markedly elevated in chronic MI patients, suggesting that the activation of angiogenesis in MI patients may be a delayed response. Lower levels of sFlt-1 in baseline acute MI patients and chronic MI patients compared to healthy controls may reflect background abnormal angiogenesis in CAD and the significant changes in sFlt-1 during the first 24 h post-MI may reflect the start of endothelial activation.

This study is partially funded by the Peel Medical Research Trust. We acknowledge the support of the City Hospital Research & Development Programme for the Haemostasis Thrombosis and Vascular Biology Unit. Dr Chung is supported by a non-promotional research fellowship from Merck Sharpe and Dohme.

References


