EFFECTS OF TOTAL CORONARY ARTERY OCCLUSION ON VASCULAR ENDOTHELIAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR β

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Vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β;) play an important role in angiogenesis. We wanted to determine if concentrations of growth factors in the coronary sinus (CS) and right atrium (RA) are higher in coronary artery disease patients with total occlusions than in those with partial occlusions. Fifty-one patients scheduled for coronary artery angiography were evaluated for possible recruitment. A 6F Goodale-Lubin catheter was used to collect blood from the CS and RA. Data for all but four patients were gathered successfully, leaving 47 study patients. The reviewer was blinded to growth factor data when interpreting coronary angiographic findings. Of the 47 enrolled patients, 32 had at least one diseased vessel, seven of whom had at least one major epicardial coronary occlusion. In all 32 patients, the concentrations of VEGF in the CS were higher than those in the RA (31.5 ± 2.7 vs 27.1 ± 1.8 pg/mL; p = 0.005). Patients with total occlusions had higher VEGF concentrations in the CS than those with non-total occlusions (38.9 ± 8.0 vs 29.5 ± 2.6 pg/mL; p = 0.037). The differences in TGF-β; in the two groups were not statistically significant. The higher CS VEGF concentrations in patients with total occlusion indicate that VEGF may play a part in the development of angiogenesis.

Key Words: vascular endothelial growth factor, transforming growth factor, coronary artery disease, total occlusion


Arteriogenesis, the transformation of preexisting collaterals into mature collaterals, is influenced by multiple factors. More than 15 growth factors that can stimulate collateral growth or angiogenesis have been identified [1,2]. Because vascular endothelial growth factor (VEGF) is believed to be involved in angiogenesis in vivo, there are many therapeutic studies on the use of VEGF as a therapeutic angiogenesis agent in severe coronary disease patients who cannot undergo standard revascularization [3]. Transforming growth factor β (TGF-β;) is also involved in angiogenesis, which is blocked by TGF-β; antibodies [4]. In addition, TGF-β; signaling may regulate VEGF expression in cardiac myocytes [5].

Hypoxia stimulates VEGF release [6]. Therefore, the severity of coronary artery stenosis, a cause of hypoxia, is also a critical determining factor in collateral channel formation. Previously, we found that VEGF concentration in the coronary sinus (CS) and severity of coronary artery stenosis are positively correlated with collateral formation [7]. Patients with total coronary artery occlusions have more developed collateral channels than those with partial occlusions.

Based on these findings, we hypothesized that patients with total coronary artery occlusion have higher local growth factor concentrations, and these concentrations may play a part in angiogenesis. The purpose of this study was to
compare VEGF and TGF-β1 concentrations in the CS and right atrium (RA) in coronary artery disease patients with total occlusion and with partial occlusion.

**Materials and Methods**

**Patient enrollment**

The research protocol was approved by our institution’s ethics committee. From March 2000 to October 2001, we evaluated patients scheduled for diagnostic coronary artery angiography at Kaohsiung Medical University Hospital for possible recruitment into the study. Indications for coronary artery angiography included known or suspected coronary artery disease. Suspected coronary artery disease was defined as ischemia documented by treadmill test or radionuclide myocardial perfusion scan.

**Data collection**

Baseline data for each patient included name, sex, age, history of diabetes, hypertension, hypercholesterolemia and cigarette smoking.

Coronary artery angiography films were reviewed by an experienced cardiologist. Recorded data included the number of diseased vessels, the affected vessels, and the presence or absence of total coronary artery occlusion. Significantly diseased vessels were defined as a reduction in lumen diameter of at least 50%. The reviewer was blind to growth factor data when interpreting the coronary anatomy. We used the collateral scoring system developed by Cohen and Rentrop [8].

**Blood collection**

Because heparin would change growth factor concentrations, blood samples were collected before intravenous heparinization for diagnostic coronary artery angiography [9]. We used a 6F Goodale-Lubin catheter under fluoroscopic guidance to collect blood samples from the CS (20 mL). After the catheter was withdrawn from the CS, it was used to collect blood samples from the RA (20 mL). Blood was collected into a sterile tube containing ethylenediamine tetraacetic acid as anticoagulant, and quickly put on ice. Tubes were centrifuged at 3,000 rpm for 10 minutes at room temperature, and 500 μL of plasma was removed from each tube and frozen at −80°C until analysis.

**Measurement of VEGF and TGF-β1 concentrations**

Plasma VEGF and TGF-β1 concentrations were measured using a quantitative sandwich enzyme-linked immunosorbent assay kit (ELIZA; R&D Systems Europe Ltd, UK, and Biosource, Europe SA).

**Statistical analysis**

Data are reported as mean ± standard error of the mean. The Chi-squared test and Wilcoxon rank-sum test were used to compare non-parametric data. The t test was used for analysis between continuous variables. Statistical analysis was performed using SPSS statistical software, version 10 (SPSS Inc, Chicago, IL, USA). A p value of less than 0.05 was considered statistically significant.

**Results**

**Patient enrollment and study group characterization**

Fifty-one consecutive patients were enrolled in this study. One patient was excluded due to difficulties in catheterization of the CS, and three were excluded due to inadequate blood sampling. Of the remaining 47 patients, 32 had at least one major epicardial coronary stenosis. Seven patients had at least one totally occluded major epicardial vessel.

Table 1 shows the baseline characteristics of the two groups and the number of diseased epicardial coronary vessels involved. Of the 32 patients, five (16%) had one diseased vessel, six (19%) had two diseased vessels, and 21 (65%) had three diseased vessels. There was no significant difference in any variables between patients with total occlusions and those with non-total occlusions. Compared with patients with partial occlusion, those with total occlusion had greater coronary stenosis severity (100 vs 87.0 ± 2.1%; p = 0.003) and collateral grade (2.1 ± 0.3 vs 0.8 ± 0.2; p = 0.006).

**VEGF and TGF-β1 concentrations**

The 32 study patients had higher VEGF concentrations in the CS than in the RA (31.5 ± 2.7 vs 27.1 ± 1.8 pg/mL; p = 0.005). However, no significant difference was found in TGF-β1 concentrations between the CS and RA (202 ± 34.2 vs 250 ± 37.8 pg/mL; p = 0.240).

CS VEGF concentrations were higher in patients with total coronary artery occlusions than those with non-total occlusions (38.9 ± 8.0 vs 29.5 ± 2.6 pg/mL; p = 0.037). The difference in RA VEGF concentrations in these two groups was not significant (Table 2). TGF-β1 concentrations in the CS and RA were not significantly different between patients with total and non-total occlusion (Table 2).
DISCUSSION

This study had two noteworthy findings. Patients with significant coronary artery disease had higher VEGF concentrations in the CS than in the RA, and patients with total occlusions had higher CS VEGF concentrations and collateral grade than those with non-total occlusions.

The severity of coronary artery obstruction plays an important role in the development of coronary collateral channels. Coronary collaterals do not develop until there is a coronary stenosis with a narrowing in diameter of 70% or more. Beyond this threshold value, the growth of collateral channels is directly related to stenosis severity [10]. The transformation of preexisting collaterals into mature collaterals is called arteriogenesis [1,11]. VEGF may play an important role in this process in vivo. Some studies have demonstrated that administering various angiogenic growth factors, including VEGF, can augment revascularization of the myocardium [12,13].

Schultz et al found that the ability to respond to progressive coronary artery stenosis by growing coronary artery collaterals is strongly associated with the ability to induce VEGF in response to hypoxia [14]. The CS is located in the downstream ischemic myocardium, and its growth factor concentrations may more accurately reflect the amount of growth factors in the ischemic myocardium than upstream in the coronary artery. Recently, El-Gendi et al reported that basal VEGF concentrations were significantly higher in the CS samples of patients with occlusions than in those with stenosis [15], which was similar to our findings. However, they failed to show a local increase in basic fibroblast growth factor, which we did not measure. Although the differences we found were insignificant, this is the first study to measure local CS TGF-β concentrations and assess their role in arteriogenesis in diseased coronary circulation in vivo. Our data showed that VEGF concentrations in the CS were higher than those in the RA, where blood is mixed with venous blood from the superior and inferior vena cava.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Occlusion (n = 7)</th>
<th>Stenosis (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>64 ± 12</td>
<td>60 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Male (%)</td>
<td>6 (86)</td>
<td>19 (76)</td>
<td>NS</td>
</tr>
<tr>
<td>Female (%)</td>
<td>1 (14)</td>
<td>6 (2)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>1 (14)</td>
<td>12 (48)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>5 (71)</td>
<td>13 (52)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>6 (86)</td>
<td>14 (56)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>4 (57)</td>
<td>13 (52)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of diseased vessels</td>
<td>2.6 ± 0.5</td>
<td>2.5 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>LAD (%)</td>
<td>0</td>
<td>5 (20)</td>
<td>NS</td>
</tr>
<tr>
<td>LAD and LCX (%)</td>
<td>1 (14)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>LAD and RCA (%)</td>
<td>1 (14)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>LCX and RCA (%)</td>
<td>1 (14)</td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>Three-vessel disease (%)</td>
<td>4 (57)</td>
<td>17 (68)</td>
<td></td>
</tr>
<tr>
<td>Stenosis (%)</td>
<td>100</td>
<td>87.0 ± 2.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Collateral grade</td>
<td>2.1 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>0.006</td>
</tr>
</tbody>
</table>

NS = not significant; LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery.

Table 2. Vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β) concentrations in the coronary sinus (CS) and right atrium (RA) between patients with total occlusion and non-total occlusion (stenosis)

<table>
<thead>
<tr>
<th></th>
<th>Occlusion (pg/mL)</th>
<th>Stenosis (pg/mL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF in CS</td>
<td>38.9 ± 8.0</td>
<td>29.5 ± 2.6</td>
<td>0.037</td>
</tr>
<tr>
<td>VEGF in RA</td>
<td>31.7 ± 2.9</td>
<td>25.8 ± 2.0</td>
<td>0.810</td>
</tr>
<tr>
<td>TGF-β in CS</td>
<td>188.6 ± 37.6</td>
<td>250.3 ± 82.8</td>
<td>0.460</td>
</tr>
<tr>
<td>TGF-β in RA</td>
<td>317.3 ± 111.3</td>
<td>230.9 ± 37.8</td>
<td>0.340</td>
</tr>
</tbody>
</table>
cava. This finding further emphasized the local paracrine role of VEGF in angiogenesis.

In acute myocardial infarction, the totally occluded vessel causes hypoxia, which induces the release of VEGF, and which peaks between the 7th and 14th day after the infarction [16–18]. If patients receive reperfusion therapy, their elevated VEGF concentrations rapidly return to nearly pre-infarction concentrations [19]. Thus, the elevation of VEGF in the 1–2 weeks after acute myocardial infarction may be related to collateral channel formation [20]. Interestingly, Kranz et al reported that such elevated VEGF during acute myocardial infarction originates from platelets, instead of the infarct myocardium [21]. There is little research into local concentrations of growth factors in non-infarction subjects. In the current era of therapeutic angiogenesis, the more detail we have about the local concentrations of growth factors, the more we will be able to decide on the amount of growth factor needed to adequately enhance angiogenesis.

TGF-β levels have also been correlated with angiogenesis [22], and are thought to spur the differentiation and maturation of pericytes and smooth muscle cells [23]. TGF-β signaling may regulate VEGF expression in cardiac myocytes [5]. Low TGF-β concentration has been correlated with arteriogenesis [24]. Recently, Werner et al reported that higher TGF-β1 concentrations were observed in collaterals distal to the chronic total occlusion but did not have a close relationship to the duration of occlusion [25]. However, we did not find any local difference in TGF-β1 concentrations in either the CS or RA. Therefore, further investigation is needed to determine whether TGF-β1 plays a role in angiogenesis.

Some small and promising studies reporting the use of VEGF to enhance angiogenesis have evaluated VEGF delivery to the ischemic myocardium via different routes, including the intramyocardial [13,26–28], intracoronary [29,30], and intravascular routes [3]. Plasmids [13,26,28] and adenoviruses [27] have been used as vectors to bring VEGF DNA to the target. Most of these studies have reported a good tolerance of vectors and positive results. Recently, however, the first published randomized, controlled trial of VEGF for therapeutic angiogenesis, the VIVA trial [3], showed that significant improvement in angina and favorable trends in angina frequency were found in both high-dose groups. Furthermore, the optimal VEGF dose and duration of administration needed to adequately enhance angiogenesis are questionable, with one reason being a lack of local growth factor concentration data from living men with significant coronary artery disease. By understanding local factors, we can adjust growth factor dosage and monitor whether such delivery can provide adequate local concentration.

This preliminary report discusses local VEGF concentration in patients with significant coronary artery disease. Larger trials will be needed to measure VEGF concentrations in patients with coronary artery disease at different levels of severity. More studies are needed on the use of alternative growth factors and optimal concentrations to enhance the benefits derived from using angiogenic growth factors to treat severe myocardial ischemia patients who are not good candidates for standard revascularization.

Study limitations

Limitations of this study include the small number of patients; a larger number of patients might add important information regarding the role of TGF-β1 in angiogenesis.

The present study shows that patients with total coronary artery occlusion have higher CS VEGF concentrations than those with non-total occlusion, indicating that VEGF may play a part in angiogenesis.

REFERENCES

血管內皮生長因子 (VEGF) 及轉形生長因子 (TGF-β₁) 在有完全冠狀動脈阻塞病人身上的角色

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血管內皮生長因子 (VEGF) 及轉形生長因子 (TGF-β₁) 對於血管新生扮演重要的角色。因為少有研究探討局部生長因子濃度與血管新生的關係。因此我們探討是否心室內 (冠狀動脈竇, coronary sinus) 及右心房 (right atrium) 內血管內皮生長因子及轉形生長因子濃度與病人有否完全冠狀動脈阻塞間有相關。51 位受心導管病人評估進行此研究。其中 47 病人接受心導管以 6F Goodale-Lubin 導管成功收集冠狀動脈竇及右心房內血液以供分析。在 47 位病人中，32 位病人至少有一條冠狀動脈疾病。其中7位至少有一條冠狀動脈完全阻塞。32位病人中我們發現血管內皮生長因子在冠狀動脈竇內明顯比右心房內高 (31.5 ± 2.7 vs 27.1 ± 1.8 pg/ml, p = 0.005)。冠狀動脈竇內血管內皮生長因子在有完全冠狀動脈阻塞病人身上明顯比無完全冠狀動脈阻塞病人高 (38.9 ± 8.0 vs 29.5 ± 2.6 pg/ml, p = 0.037)。TGF-β₁ 濃度在兩組間並無達到統計學上的差異。有完全冠狀動脈阻塞病人身上冠狀動脈竇內血管內皮生長因子濃度顯著比無完全冠狀動脈阻塞病人高，如此顯示局部血管內皮生長因子可能在血管新生上扮演一部份角色。

關鍵詞：血管內皮生長因子，轉形生長因子，冠狀動脈疾病，完全阻塞
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