Circulating cells and cytokines in arteriogenesis

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Local Cytokine Concentrations and Oxygen Pressure are Related to Maturation of the Collateral Circulation in Man

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submitted
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ABSTRACT

Objectives
To determine cytokine and oxygen gradients over the collateral circulation in humans.

Background
The molecular background of the maturation of the collateral circulation in response to coronary narrowings is poorly understood in man, partly because of difficulties in obtaining local samples from the human collateral circulation.

Methods and results
Coronary collateral blood was samples in 60 patients with non-total (n=25) or total coronary occlusions (n=35) using a special wide-lumen catheter. Coronary collateral flow index (CFI) was assessed by intracoronary pressure measurements. Oxygenation and lactate content was measured as well as 30 cytokines potentially involved in collateral artery growth, using a custom-made multiplex assay. No rise in lactate or change in pH was found in collateral blood. Oxygen gradient between coronary and collateral arterial blood correlated inversely with CFI (r=-0.61, p<0.001). Locally increased plasma levels were found for bFGF, Eotaxin, MIF, MCP-1, and TGFbeta, while SCF and SCGFbeta were significantly decreased. The highest cytokine gradients were found in patients with the least developed collateral circulation. The majority of cytokines correlated more strongly with the pO2 gradient across the collateral bed than with CFI.

Conclusion
Intravascular ischemia during brief balloon coronary occlusion is absent in human coronary collateral arteries. This oxygen gradient found over the collateral circulation is increased in patients with a less matured collateral circulation and relates to local levels of several known pro-arteriogenic cytokines. In case of a more developed collateral circulation, relatively low levels of cytokines are present, suggesting that growth factor therapy might be beneficial at this stage.
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Abbreviations:

PCI: percutaneous coronary intervention  
CFI: coronary flow index  
CAD: coronary artery disease  
bFGF: basic fibroblast growth factor  
TGF: transforming growth factor  
TNF: tumor necrosis factor  
MCP: monocyte chemoattractant protein  
PDGF: platelet-derived growth factor  
MIF: macrophage migration inflammatory factor  
MIP: macrophage inflammatory protein  
GM-CSF: granulocyte-macrophage colony-stimulating factor  
HGF: hepatocyte growth factor  
IL: Interleukin  
SCF: stem cell factor  
SCGF: stem cell growth factor  
bNGF: nerve growth factor-beta  
TRAIL: TNF-related apoptosis-inducing ligand

1. INTRODUCTION

Although mechanisms of arteriogenesis have been well elucidated in experimental models, knowledge of cytokines and growth factors that mediate collateral artery growth in humans is still limited [1]. Also, the role of ischemia in human collateral artery growth is presently unknown. Local plasma sampling directly from the human coronary collateral circulation can provide such data. This is, however, cumbersome because of the poor accessibility of the collateral circulation for blood sampling. Therefore, previous studies have investigated systemic cytokine levels in patients with different degrees of coronary collateralization. These studies have provided conflicting results, probably due to the fact that systemic levels of growth factors do not reliably reflect the local process of collateral artery growth [2,3]. Direct sampling from the collateral circulation, as performed in previous studies, yielded only a small volume of blood in a limited percentage of patients. Such small blood volumes nevertheless have allowed the analysis of a few cytokines, showing increased levels of bFGF and transforming growth factor (TGF)-beta in the coronary collateral circulation [4,5].

In the present study, to allow analysis of a wide array of cytokines potentially involved in human arteriogenesis, we have used the Proxis® catheter, a wide-lumen suction catheter originally developed as a proximal embolic protection device [6], to obtain larger amounts of blood from the collateral circulation. In addition to plasma levels of cytokines, the use of this catheter facilitated additional blood gas and lactate analyses of locally sampled collateral blood which have not been performed previously in human coronary collateral
arteries. Together with the analysis of plasma cytokines, these metabolic parameters can provide valuable insights into the growth and functional status of the collateral circulation in response to epicardial obstructions.

2. METHODS

2.1. Patient selection

This study was approved by the medical ethics committee of the Academic Medical Center, Amsterdam, the Netherlands. Sixty Caucasian patients scheduled for elective PCI for stable CAD with symptoms of angina pectoris for ≥4 weeks were included after giving informed consent. Patients with a subtotal stenosis (≥70%) were selected if they had single-vessel CAD; patients with chronic total occlusions (CTO) had single- or multi-vessel disease. Exclusion criteria were: previous myocardial infarction in the area of collateralization, previous cardiac surgery, severely depressed left ventricular function, diabetes mellitus, neoplastic disease and signs of inflammatory illness.

2.2. Intracoronary instrumentation

After predilatation of the stenotic lesion, a 7F (inner diameter 0.051") proximal embolic protection device (Proxis®-catheter, St. Jude Medical, St. Paul, MN) was advanced through the guiding catheter and into the treated coronary artery which received collateral blood. Inflating a low-pressure (0.67 atm) balloon at the end of the catheter temporarily stopped antegrade blood flow in the artery. Complete obstruction of antegrade blood flow was ensured in a pilot study of five patients showing no contrast dye entering the epicardial artery after balloon occlusion (data not shown). Subsequently, gentle suction was applied manually to the proximal end of the catheter. The first 3 ml aspirated was discarded to prevent contamination with contrast medium or with blood already in the epicardial vessel prior to balloon occlusion (and thus not from the collateral circulation). Approximately 5-15 ml blood (depending on collateral flow) was then aspirated within 60 seconds. After deflation of the balloon, the catheter was withdrawn into the guiding catheter to obtain a control sample from the coronary circulation. Blood was transferred into citrate tubes and centrifuged at 1550 g for 30 minutes. Plasma was taken off carefully, aliquoted, snap frozen in liquid nitrogen and stored at -80°C. Collateral flow index (CFI) was measured as previously published [7].
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2.3. Oxygen, pH and lactate measurements

Immediately after aspiration, partial oxygen (pO2) and carbon dioxide pressure (pCO2), pH and saturation were measured from one sample per site per patient on a Rapidlab 865 (Siemens, Germany). Lactate concentrations were measured using a standard clinical chemistry test.

2.4. Multiplex assay for the measurement of cytokine concentrations

A custom-made panel of 30 cytokines was measured using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer’s instructions as previously described [8]. The panel consisted of interferon-alpha-2, HGF (hepatocyte growth factor), M-CSF (macrophage colony-stimulating factor), MIF (monocyte inflammatory factor), MIG (monokine induced by gamma-interferon), b-NGF (nerve growth factor-beta), SCF (stem cell factor), SCGF-b (stem-cell growth factor), SDF-1alpha (stromal cell-derived factor-1a), TNFalpha (tumor necrosis factor alpha), TNFBeta (tumor necrosis factor-beta), TRAIL (TNF-related apoptosis inducing ligand), IL (interleukin)1b, IL4, IL6, IL8, IL10, IL16, Eotaxin, bFGF (basic fibroblast growth factor), G-CSF (granulocyte colony-stimulating factor), GM-CSF (granulocyte-macrophage colony-stimulating factor), IFNgamma (interferon-gamma), IP-10 (interferon-gamma-inducible protein 10), MCP1 (monocyte chemotractant protein 1), MIP1a (macrophage inflammatory protein 1 alpha), MIP1b (macrophage inflammatory protein 1 beta), bbPDGF (platelet-derived growth factor, beta chain), RANTES (regulated upon activation, normally T-expressed, and presumably secreted), and VEGF (vascular endothelial growth factor). Plasma samples were thawed at room temperature, diluted 1:1 in the manufacturer’s sample diluent, and incubated with bead-bound antibodies to above mentioned cytokines for 60 minutes on a 96-well filter plate. After repeated washing, biotinylated detection antibody was added, and the plate incubated for another 60 minutes. Finally, streptavidin-PE (phycoerythrin) was pipetted onto each well. After a short incubation, the constituents of each well are drawn up into the flow-based Bio-Plex suspension array system, which identifies and quantifies each cytokine concentration based on bead color and fluorescence. Data were analyzed using Bio-Plex Manager software version 3.0 (Bio-Rad).

2.5. Enzyme-linked immunosorbent assays

Because human interferon-beta and transforming growth factor-beta (TGFbeta) were not represented on our multiplex assay, we tested concentrations of these cytokines using commercially available ELISA kits (Quantikine ELISA, R&D Systems, Minneapolis, MN, and PBL Interferonsource, Piscataway, NJ) according to the manufacturer’s instructions. Since the concentrations of bFGF were often found to be too low to be properly detected with the multiplex system, a high sensitivity ELISA assay was performed (HS
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Quantikine ELISA, R&D Systems) according to the manufacturer’s instructions. Briefly, samples were incubated on a 96-well plate coated with the appropriate antibody. After the necessary washing steps, a biotinylated antibody and subsequently streptavidin-conjugated horseradish-peroxidase were linked to the adherent probes. Using tetramethyl-benzidine (TMB) as a substrate, absorption was measured at 450 nm in an EL808 spectrophotometer (BioTek, Winooski, VT).

2.6. Statistical analysis

Clinical characteristics are presented as mean±standard deviation or median and interquartile range for quantitative variables and as observed numbers (%) for nominal variables. Fisher’s exact test was used for testing association in 2×2 contingency tables. From partial oxygen pressure, oxygen gradient was calculated as pO2 gradient = pO2 coronary-pO2 collateral. Cytokine gradients were calculated as concentrationcollateral – concentrationcoronary. Oxygen and cytokine gradients were calculated on a logarithmic scale to correct for skewed distributions. Oxygen and cytokine concentrations are shown as median [interquartile range]. Comparisons among two groups were performed by Student’s t-test for normally distributed parameters and Wilcoxon test for non-normally distributed parameters. Statistics on cytokine concentrations were corrected for multiple testing using Benjamini-Hochberg correction [9]. False discovery rates (FDR) were calculated and expressed as percentage. Comparisons between three groups were performed by analysis of variance (ANOVA) testing. Correlations were calculated using a Pearson correlation.

3. Results

3.1. Patient characteristics

Sixty patients were included in the study. Patients were aged 61.0±11.4 years, and CFI ranged from 0.09 to 0.65 (mean value 0.31±0.12). Thirty-five patients had a chronic total occlusion (CTO) of a coronary artery, while 25 had a subtotal stenosis (ranging from 60.1 to 91.5% diameter stenosis in QCA). All lesions undergoing intervention were proximal lesions, resulting in arterial diameters of 3.0-4.0 mm. All CTO patients had sufficient collateralization to prevent ischemia during balloon occlusion as indicated by absence of ST-segment deviation, and no symptoms of angina pectoris at rest. Patients with a non-total occlusion were grouped into collateral responders (CFI>0.21, n=13) and non-responders (CFI≤0.21, n=12). ST-elevation during balloon occlusion (shown as median [interquartile range]) was significantly (p<0.001) different between collateral responders (0.0 [1.0] mm) and non-responders (2.0 [2.0] mm). Clinical baseline characteristics did not correlate with CFI and were not different between the three groups (non-responders, responders, CTOs) (Table 1).
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<table>
<thead>
<tr>
<th></th>
<th>Non-responders (CFI≤0.21, n=12)</th>
<th>Responders (CFI&gt;0.21, n=13)</th>
<th>CTOs (n=35)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age - years</td>
<td>62.8±13.5</td>
<td>62.6±11.4</td>
<td>59.8±10.9</td>
<td>0.63</td>
</tr>
<tr>
<td>Male sex – no. (%)</td>
<td>9 (75)</td>
<td>8 (61.5)</td>
<td>24 (68.8)</td>
<td>0.80</td>
</tr>
<tr>
<td>Body-mass index (BMI)</td>
<td>27.2±2.6</td>
<td>27.0±2.8</td>
<td>27.1±4.2</td>
<td>0.99</td>
</tr>
<tr>
<td>Body surface area (BSA)</td>
<td>2.0±0.2</td>
<td>2.0±0.1</td>
<td>2.0±0.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Hypertension – no. (%)</td>
<td>7 (58.3)</td>
<td>8 (61.5)</td>
<td>22 (62.9)</td>
<td>0.92</td>
</tr>
<tr>
<td>Hypercholesterolemia – no. (%)</td>
<td>5 (41.7)</td>
<td>8 (61.5)</td>
<td>15 (42.9)</td>
<td>0.54</td>
</tr>
<tr>
<td>Family history of CAD – no. (%)</td>
<td>10 (83.3)</td>
<td>8 (61.5)</td>
<td>19 (54.3)</td>
<td>0.24</td>
</tr>
<tr>
<td>Current smoker – no. (%)</td>
<td>4 (33.3)</td>
<td>1 (7.7)</td>
<td>5 (14.3)</td>
<td>0.44</td>
</tr>
<tr>
<td>Ex smoker – no. (%)</td>
<td>6 (50)</td>
<td>7 (53.8)</td>
<td>19 (54.3)</td>
<td>0.44</td>
</tr>
<tr>
<td>Weeks anginal symptoms*</td>
<td>26 [12; 73]</td>
<td>12 [6; 65]</td>
<td>25 [11; 68]</td>
<td>0.41</td>
</tr>
<tr>
<td>Beta-blockers – no. (%)</td>
<td>11 (91.7)</td>
<td>10 (76.9)</td>
<td>27 (77.1)</td>
<td>0.53</td>
</tr>
<tr>
<td>Statins – no. (%)</td>
<td>10 (83.3)</td>
<td>12 (92.3)</td>
<td>31 (86.6)</td>
<td>0.78</td>
</tr>
<tr>
<td>Aspirin – no. (%)</td>
<td>11 (91.7)</td>
<td>12 (92.3)</td>
<td>32 (91.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Clopidogrel – no. (%)</td>
<td>6 (50)</td>
<td>11 (84.6)</td>
<td>22 (62.9)</td>
<td>0.18</td>
</tr>
<tr>
<td>Calcium antagonists – no. (%)</td>
<td>5 (41.7)</td>
<td>3 (23.1)</td>
<td>8 (22.9)</td>
<td>0.42</td>
</tr>
<tr>
<td>Nitrates – no. (%)</td>
<td>6 (50)</td>
<td>6 (46.2)</td>
<td>19 (54.3)</td>
<td>0.88</td>
</tr>
<tr>
<td>ACE-inhibitors/ARBs – no. (%)</td>
<td>4 (33.3)</td>
<td>4 (30.8)</td>
<td>15 (42.9)</td>
<td>0.70</td>
</tr>
<tr>
<td>Diameter stenosis (QCA) (%)</td>
<td>72.4±9.4</td>
<td>74.9±9.2</td>
<td>100±0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein – mg/dl*</td>
<td>1.75 [0.7; 6.3]</td>
<td>1.4 [0.8; 3.5]</td>
<td>1.5 [1.0;3.3]</td>
<td>0.75</td>
</tr>
<tr>
<td>Hemoglobin – mmol/l</td>
<td>8.63±0.61</td>
<td>8.75±0.42</td>
<td>8.75±0.65</td>
<td>0.84</td>
</tr>
<tr>
<td>Leukocytes - 10⁹/l</td>
<td>6.6±1.9</td>
<td>7.2±1.3</td>
<td>6.9±1.7</td>
<td>0.62</td>
</tr>
<tr>
<td>Total cholesterol – mmol/l</td>
<td>4.0±0.9</td>
<td>3.9±0.8</td>
<td>4.2±0.9</td>
<td>0.63</td>
</tr>
</tbody>
</table>

* indicates non-normal distribution, these values are presented as median [first quartile, third quartile].

Table 1: Baseline characteristics. Clinical characteristics were comparable between the two groups. Parameters are reported as mean±SD.

3.2. Metabolic parameters in the coronary collateral circulation

Oxygen pressure measurements were performed in 45 patients in total in this study. In the complete patient group, average pO2 was found significantly lower in the collateral sample than in the coronary sample (10.2 [2.75] vs. 11.3 [2.4] kPa; p<0.001). In contrast, lactate concentrations were equal in coronary and collateral blood (0.94 versus 0.91 mmol/l, p=NS), and also pH did not differ significantly (coronary: 7.42, collateral 7.41, p=NS). Lactate and pH remained unchanged in collateral versus coronary blood in both collateral non-responders, responders and patients with CTOs. There was no significant correlation between CFI and lactate (r=0.19, p=0.29) or CFI and pH (r=0.12, p=0.44).
Comparing the three patient groups, non-responders showed a significantly higher oxygen gradient than responders or CTOs (2.24 [2.0] versus 1.63 [1.0] and 0.95 [0.77] kPa, p=0.003). In the patient group as a whole, a significant inverse correlation was found between oxygen gradient and CFI (r=-0.61, p<0.001) (Figure 1).

3.3. Local cytokine concentrations in the collateral circulation

In the complete patient population, Eotaxin, MCP-1, MIF, bFGF, and TGFbeta were found in significantly higher plasma concentrations in the collateral specific samples as compared to the coronary samples, while SCF and SCGFbeta were significantly down-regulated (Table 2).

Dividing patients into the three groups (non-responders, responders, CTOs) resulted in the highest collateral-coronary gradients for all of the above mentioned cytokines in the non-responders, followed by the responders, while patients with CTOs displayed smallest concentration differences (Figure 2).
Table 2: Cytokine concentrations in the collateral and coronary samples (all in pg/ml). Enhanced concentrations of bFGF, MIF, Eotaxin, MCP1, and TGFbeta were found in collateral plasma, while SCF and SCGFbeta were found to be decreased. The table displays cytokine gradients in the complete patient group (n=60). Please note that due to calculations of the median values and interquartile range, the gradient is not equal to the difference of the median collateral and the median coronary cytokine concentration. The second column to the right indicates log2-transformed values that have been used for Figure 2.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Coronary</th>
<th>Collateral</th>
<th>Gradient</th>
<th>Log-ratio</th>
<th>FDR [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIF</td>
<td>91.75 [57.35]</td>
<td>111.02 [80.88]</td>
<td>22.25 [65.77]</td>
<td>0.37 [0.86]</td>
<td>0.2</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>20.89 [32.14]</td>
<td>30.65 [50.78]</td>
<td>3.48 [18.02]</td>
<td>0.20 [0.92]</td>
<td>0.2</td>
</tr>
<tr>
<td>MCP1</td>
<td>14.01 [4.31]</td>
<td>15.85 [9.95]</td>
<td>1.57 [5.14]</td>
<td>0.16 [0.47]</td>
<td>0.2</td>
</tr>
<tr>
<td>TGFbeta</td>
<td>3830.62 [1892.84]</td>
<td>4161.25 [2815.69]</td>
<td>493.25 [1133.53]</td>
<td>0.19 [0.44]</td>
<td>2</td>
</tr>
<tr>
<td>SCF</td>
<td>359.50 [143.75]</td>
<td>283.84 [155.55]</td>
<td>-29.26 [72.83]</td>
<td>-0.14 [0.32]</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>SCGFbeta</td>
<td>2624.00 [1646.38]</td>
<td>2349.57 [1602.63]</td>
<td>-83.98 [905.27]</td>
<td>-0.07 [0.65]</td>
<td>2</td>
</tr>
</tbody>
</table>

**Figure 2: Cytokine gradients**

The box plot shows the relative change of collateral as compared to coronary cytokine concentrations (significantly different cytokines). Concentrations were log-transformed to correct for skewed parameter distributions and improve visualization of median values with large interquartile ranges. Cytokine gradients were strongest in non-responders and almost absent in patients with CTOs. Significant gradients in the patient population as a whole are depicted in (A), and further split into three groups (non-responders, responders, and CTOs) in (B). Particularly Eotaxin, MIF, and bFGF showed higher concentrations in collateral compared to coronary samples. Also MCP1 and TGFbeta were significantly upregulated in collateral plasma, to a larger extend in non-responders. Conversely, SCF and SCGFbeta were found downregulated.
3.4. Group-specific cytokine profiles in relation to CFI and oxygen gradient

In the patient group with subtotal stenoses, a positive correlation with oxygen gradient between coronary and collateral arterial blood was found for coronary-collateral gradients of MCP1, Eotaxin, MIF, MIP-1b, and HGF. Conversely, a negative correlation with oxygen gradient was found for gradients of bNGF and TRAIL. For details, see Table 3. No cytokine gradient correlated significantly with CFI (data not shown).

After correction for multiple testing, none of the coronary-collateral cytokine gradients correlated with oxygen gradient (FDR>25%) or CFI in patients with CTO. Similarly, no correlation could be established for any of the cytokines with distal (wedge) pressure.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Correlation coefficient</th>
<th>p-value</th>
<th>FDR [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIF</td>
<td>0.59</td>
<td>0.01</td>
<td>11</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>0.56</td>
<td>0.02</td>
<td>11</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.55</td>
<td>0.01</td>
<td>11</td>
</tr>
<tr>
<td>MIP-1b</td>
<td>0.54</td>
<td>0.02</td>
<td>11</td>
</tr>
<tr>
<td>HGF</td>
<td>0.53</td>
<td>0.02</td>
<td>11</td>
</tr>
<tr>
<td>bNGF</td>
<td>-0.57</td>
<td>0.02</td>
<td>11</td>
</tr>
<tr>
<td>TRAIL</td>
<td>-0.54</td>
<td>0.02</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 3: Correlations of collateral-coronary cytokine gradients with oxygen gradient in patients with subtotal stenosis. A good correlation was detected between gradients of several cytokines and oxygen gradient. Both p-value and percentage false discovery rate are mentioned. Note the negative correlation of the gradients of two cytokines with oxygen gradient.

4. Discussion

In this study on the molecular background of the maturation of the coronary collateral circulation in man, no changes in levels of pH or lactate were found in the collateral circulation, showing that no intravascular ischemia is present in the human collateral circulation. However, partial oxygen pressure was found significantly lower in collateral as compared to coronary blood samples. Oxygen gradient between coronary and collateral arterial blood correlated with CFI. Eotaxin, bFGF, MCP-1, MIF, and TGFbeta were found selectively increased in collateral blood plasma, and cytokine levels in the collateral circulation correlated well with oxygen gradient. Collateral cytokine gradients were strongest in patients with insufficient collateral arteries and almost absent in CTOs.
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4.1. Patient selection

For a comprehensive analysis of human arteriogenesis, we selected patients with single-vessel coronary artery disease and a severe coronary narrowing (non-responders and responders) as well as patients with CTOs. This approach allows an analysis of the collateral vascular development in two distinct clinical phases, i.e. during an accelerated phase in 1-vessel disease and a more quiescent phase in chronic coronary occlusion. Moreover, the analysis of cytokine concentrations was performed in a uniform fashion allowing, for the first time, an interpretation of the adaptation of collateral vessels in wide range of coronary narrowings.

4.2. Collateral-specific blood sampling

The Proxis® embolic protection device has been shown earlier to safely occlude coronary vessels and provide retrograde blood aspiration [10]. For the first time, we describe the use of the catheter for the selective collection of coronary collateral blood. Previous studies have used an over-the-wire catheter or a multifunctional probing catheter to evaluate growth factor concentrations in collateral arteries from patients undergoing PCI [4,5]. While the aspiration of sufficient quantities of blood is difficult with the over-the-wire catheter because of its thin lumen, the use of a multi-purpose catheter does not allow selective sampling of collateral flow, because antegrade blood blow is not completely blocked. In fact, in a pioneering study by Fleisch et al [4], collateral-specific blood samples could only be obtained in 45% of all patients studied. Focusing on patients with total occlusions of coronary arteries, Werner et al used an over-the-wire catheter for blood drawing. Because of the small lumen of this catheter, two of the four cytokines analyzed in that study could only be measured in half of the patients [5]. Other studies have chosen even less selective alternative approaches, such as using pericardial fluid or coronary sinus blood [11,12].

4.3. Cytokines in human arteriogenesis

The collateral-specific upregulation of MCP1, bFGF and TGFbeta confirms earlier data on the involvement of these cytokines in arteriogenesis [4,5]. Other previously described pro-arteriogenic cytokines, such as GM-CSF, G-CSF and TNFalpha were not found differential between the two sampling sites. Next to CCL2 (MCP1), we here also first describe enhanced expression of another CC-chemokine, CCL11 (Eotaxin) in collateral arteries. Its upregulation in collateral arterial blood might reflect a role in the fine-tuning of mononuclear cell homing, partly counteracting on the effects of MCP1 [13]. Macrophage migration inhibitory factor (MIF) has earlier been described to stimulate angiogenesis but a link to collateral artery growth has not been reported previously [14]. MIF acts as a major positive regulator of inflammatory cell recruitment [15] and macrophage response to lipopolysaccharide [16], both of which play an important role in arteriogenesis.
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4.4. Cytokine expression in different phases of collateral artery growth

Since no ischemia could be detected, collateral-specific expression of cytokines is not regulated by intravascular hypoxemia or tissue ischemia directly adjacent to the collateral circulation. This fits to earlier results from experimental studies showing arteriogenesis to take place distal to the site of tissue ischemia [17]. Low oxygen gradient between coronary blood and blood in well-developed collateral arteries demonstrated the physiological significance of these bypass-vessels which are capable of supplying myocardial tissue with normal oxygen concentrations. Interestingly, cytokine concentrations in the collateral circulation could be correlated to the oxygen gradient between coronary and collateral blood. We show that the degree of collateral maturation is reflected by oxygen pressure in the collateral artery. Cytokine gradients between collateral and coronary blood were greatest in non-responders and almost absent in CTO patients. The correlation of cytokine concentrations with the oxygen extraction shows strong arteriogenic activity where collateral vessels are not yet capable of providing sufficient oxygen levels. Those cytokines that most significantly correlated with oxygen gradients were the same that were most strongly up-regulated in collateral compared to coronary plasma. Interestingly, a number of cytokine gradients were found to inversely correlate with the oxygen gradient, suggesting a potential anti-arteriogenic role. In the chronic phase of arteriogenesis, i.e. in CTOs, a reduction of cytokine gradients indicates that collateral artery growth has reached a plateau phase where less cytokines and growth factors are secreted. This is in line with the results of a study by Werner et al showing higher bFGF concentrations in more recent occlusions, where arteriogenesis was presumably still ongoing, as well as increased concentrations of MCP-1, PlGF and TGFbeta in smaller collateral arteries with higher shear stress [5].

Even if CFI does not reach high levels in all patients, collateral artery growth may have come to a halt. This is consistent with earlier findings showing a genetic predisposition of individual patients to develop adequate or inadequate collateral [7] and supports strategies in which local delivery of growth factors/cytokines in the coronary circulation is striven for.

4.5. Oxygen pressure as a parameter of collateralization

The gradient in arterial oxygen pressure between coronary and collateral arterial blood found in this study suggests oxygen extraction along the collateral anastomoses. This corroborates experimental studies reporting significant oxygen extraction along arteriolar systems [18,19]. In our study the oxygen extraction probably relates to local production of cytokines by the activated endothelium of the collateral circulation which is an energy-consuming process. It might even be postulated that oxygen gradient is a more sensitive measure for the developmental stage of the collateral circulation, showing strong correlation with growth factor levels. Although CFI is currently accepted as the most accurate way to measure the collateral circulation, it is also influenced by left ventricular wall tension and distal ipsilateral microvascular resistance.
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4.6. Limitations

We had to assume CVP to be 5 mmHg instead of measuring CVP in every patient individually because of the already extensive study protocol. This assumption, however, reduces accuracy of the CFI measurements especially in patients with low CFI [21,22], which may have contributed to the lack of correlation between cytokine concentrations and CFI. Repetitive balloon occlusions, inevitable for hemodynamic measurements and blood aspiration, may have led to collateral recruitment, potentially influencing CFI measurements. During collateral blood aspiration, suction duration and flow volume per time were not recorded, limiting conclusions on the relations of blood aspiration on cytokine profiles. In the clinical setting, catheter-assisted aspiration of collateral blood cannot exclude potential admixture of capillary blood, especially in patients with low CFI. It is however unlikely because already small negative pressures during aspiration lead to collapse of capillaries and veins and indeed in 3 patients with very low CFI (ranging from 0.04 to 0.15), no blood could be drawn at all.

5. Conclusion

The collateral flow index can directly be related to oxygen pressure in collateral blood. Upregulation of cytokines at the site of coronary collateralization and in collateral vessels with enhanced oxygen gradient between coronary and collateral arterial blood demonstrate increased inflammatory activity in arteries where oxygen delivery is insufficient. Less pronounced cytokine gradients in collateral arteries in CTOs indicate reduced arteriogenic activity in these vessels. Determining the oxygen gradient may be a more sensitive tool for the assessment of the functional capacity of the collateral circulation in patients than measurements of CFI. Already increased cytokine levels in inadequately developed collateral arteries with insufficient oxygen supply indicate that application of growth factors may not be the optimal therapeutic approach for these patients. The downregulation of anti-arteriogenic cytokines selectively in the collateral circulation suggest inhibition of anti-arteriogenic signaling as an alternative treatment strategy.

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7. REFERENCES


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