Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 53, No. 23, 2009
ISSN 0735-1097/09/$36.00
doi:10.1016/j.jacc.2009.02.049

Local Cytokine Concentrations and Oxygen Pressure Are Related to Maturation of the Collateral Circulation in Humans

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Objectives Our aim was 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 narrowing is poorly understood in humans, partly because of difficulties in obtaining local samples from the human collateral circulation.

Methods Coronary collateral blood was sampled in 60 patients with nontotal ($n = 25$) 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.

Results 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 basic fibroblast growth factor, eotaxin, macrophage migration inflammatory factor, monocyte chemoattractant protein-1, and transforming growth factor-beta, while stem cell factor and stem cell growth factor-beta 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 pO_2 gradient across the collateral bed than with CFI.

Conclusions Intravascular ischemia during brief balloon coronary occlusion is absent in human coronary collateral arteries. The 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. (J Am Coll Cardiol 2009;53:2141–7) © 2009 by the American College of Cardiology Foundation

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 cumbersome, however, 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

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Manuscript received September 2, 2008; revised manuscript received January 6, 2009, accepted February 16, 2009.

Abbreviations and Acronyms

bFGF = basic fibroblast growth factor
CAD = coronary artery disease
CFI = collateral flow index
CTO = chronic total occlusion
CVP = central venous pressure
ELISA = enzyme-linked immunosorbent assay
GM-CSF = granulocyte-macrophage colony-stimulating factor
HGF = hepatocyte growth factor
IL = interleukin
MCP = monocyte chemoattractant protein
MIF = macrophage migration inflammatory factor
MIP = macrophage inflammatory protein
TGF = transforming growth factor
TNF = tumor necrosis factor

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 have nevertheless allowed the analysis of a few cytokines showing increased levels of basic fibroblast growth factor (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 (St. Jude Medical, St. Paul, Minnesota), 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.

Methods

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 percutaneous coronary intervention for stable coronary artery disease (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 (CTOs) had single-vessel or multivessel 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.

Intracoronary instrumentation. After pre-dilation of the stenosis, a 7-F (inner diameter 0.051 inches) proximal embolic protection device (Proxis catheter, St. Jude Medical) 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 5 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 before balloon occlusion (and thus not from the collateral circulation). Approximately 5 to 15 ml blood (depending on collateral flow) was then aspirated within 60 s. 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 1,550 *g* for 30 min. 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).

Oxygen, pH, and lactate measurements. Immediately after aspiration, partial oxygen (pO_2) and carbon dioxide pressure (pCO_2), pH, and saturation were measured from 1 sample per site per patient on a Rapidlab 865 (Siemens, Germany). Lactate concentrations were measured using a standard clinical chemistry test.

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, California) according to the manufacturer's instructions as previously described (8). The panel consisted of interferon-alpha-2, hepatocyte growth factor, macrophage colony-stimulating factor (MCSF), monocyte inflammatory factor (MIF), monokine induced by gamma-interferon, nerve growth factor-beta, stem cell factor, stem-cell growth factor-b, stromal cell-derived factor-1a, tumor necrosis factor (TNF)-alpha, TNF-beta, TNF-related apoptosis inducing ligand, interleukin (IL)-1b, IL-4, IL-6, IL-8, IL-10, IL-16, eotaxin, bFGF, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma, interferon-gamma-inducible protein-10, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1-alpha (MIP-1α), MIP-1β, platelet-derived growth factor, beta chain, regulated on activation, normal T expressed and secreted (RANTES), and 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 previously mentioned cytokines for 60 min on a 96-well filter plate. After repeated washing, biotinylated detection antibody was added, and the plate was incubated for another 60 min. Finally, streptavidin-PE (phycoerythrin) was pipetted onto each well. After a short incubation, the constituents of each well were drawn up into the flow-based Bio-Plex suspension array system, which identifies and quantifies each cytokine concentration based on bead color and fluo-