Impaired collateral vessel development in diabetes: potential cellular mechanisms and therapeutic implications

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Abstract

The formation of coronary collateral vessels is a compensatory mechanism secondary to repetitive or chronic myocardial ischemia. During the past three decades the functional and prognostic benefit of such collateral vessels has been established. There are large interindividual differences in the number and extent of collateral vessels that may be explained by differences in the anatomic situation or by differences in the individual capacity to develop functional collateral vessels. Diabetes mellitus has recently been identified as one of the first negative predictors of collateral vessel formation. Novel molecular approaches have helped to improve our understanding of the process of collateral vessel formation in recent years. Besides the process of true angiogenesis, i.e. the formation of new capillaries out of preexisting ones, the formation of a collateral circulation is largely based on the growth of preexisting arterioles (collateral vessels or anastomoses) named arteriogenesis. One important feature of arteriogenesis is the infiltration of monocytes into the growing collateral vessel. Our group shows that the ability of monocytes to migrate towards a gradient of VEGF-A is severely impaired in diabetic individuals, and this impaired response seems to be secondary to a signal transduction defect within the monocyte. In this review the pathophysiology of diabetes-related monocyte dysfunction and the potential role of VEGF-A in collateral vessel formation are discussed.

Keywords: Collateral circulation; Angiogenesis; Growth factors; Signal transduction; Diabetes

1. Coronary collateral vessel growth is an important compensatory mechanism in advanced coronary artery disease

Coronary collateral vessels are of functional importance in patients with advanced coronary artery disease, if a coronary artery stenosis or occlusion is severe enough to cause repetitive or chronic regional myocardial ischemia [1,2]. Coronary collateral vessels are usually preformed, but they need to grow in diameter to allow relevant collateral blood flow [3,4], a process which is now called arteriogenesis [5]. Clinical work from the past three decades has convincingly shown that the presence and extent of existing collateral vessels is of functional significance. It was found that patients with angiographically documented coronary artery disease who develop collateral vessels had a higher prevalence of myocardial ischemia than those without collateral vessels indicating that the presence of myocardial ischemia is associated with the growth of collateral vessels [6]. Fig. 1 shows an example of multiple collateral vessels secondary to an occlusion of the proximal LAD artery. It is interesting to note that these types of collateral vessels that connect the right with the left coronary artery were previously called ‘anastomoses’; in contrast to real ‘collateral vessels’ that connect different segments within the same coronary artery.

The presence and extent of a coronary collateral circulation could be shown to be of prognostic relevance for the individual patient in terms of the outcome of a coronary event. The presence of adequate collateral vessels feeding

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the myocardial area at risk may limit the infarct size following coronary occlusion [7] and may even provide a survival benefit [6,8–10]. Therefore, it is most important to understand the functional basis of the process leading to collateral vessel formation. This is true for (i) predicting the formation of coronary collateral vessels in patients with coronary artery disease and for (ii) tasks aiming in the therapeutic induction of collateral vessels (therapeutic angiogenesis/therapeutic arteriogenesis).

2. Coronary collateral vessel development is reduced in patients with diabetes mellitus

Diabetes mellitus is one of the most important cardiovascular risk factors that leads to vascular dysfunction and to the development of atherosclerotic disease. In fact, cardiac morbidity and mortality of patients with diabetes mellitus is greatly enhanced [11]. Diabetes mellitus causes endothelial dysfunction and thereby contributes to the development and progression of atherosclerosis including coronary artery disease [12]. In a functional context it is important to note that endothelial dysfunction can already be detected in young patients with early signs of insulin resistance independent of other classic cardiovascular risk factors [13].

Last year it was shown for the first time that the development of coronary collateral vessels is significantly reduced in patients with diabetes mellitus [14]. Abaci et al. have retrospectively assessed the prevalence of coronary collateral vessels in 205 coronary angiograms from diabetic patients with different severities of coronary artery disease and have compared them with the extent of collateral vessels in 205 coronary angiograms from non-diabetic patients with comparable other baseline characteristics.

There are several important implications and questions arising from this observation: (1) based on the functional and prognostic relevance of coronary collateral vessels, the reduced extent of a collateral circulation in diabetic individuals may contribute to their increased morbidity and mortality. (2) What are the mechanisms underlying reduced collateral vessel formation in diabetic individuals? (3) Knowledge about the molecular defect in diabetes-associated collateral vessel formation may be of importance for the development of therapeutic strategies to enhance collateral conductance. (4) The study by Abaci et al. [14] is a descriptive one that did not evaluate the presence of recruitable collateral vessels. In addition, smaller-sized arteries remained undetected by angiography.

3. What might be wrong with coronary collateral vessel formation in diabetics?

Angiogenesis, defined as true capillary formation out of preexisting ones, can only partly contribute to enhanced tissue perfusion. Functional collateral vessels are formed out of preexisting ones by a process that is best described by the term arteriogenesis [5]. Arteriogenesis describes vascular growth in diameter, when arteries are created out of small arterioles (Fig. 2). There are a number of cellular processes that are necessary to allow an increase in vascular diameter including the proliferation of endothelial cells and smooth muscle cells. In addition, there is evidence that monocytes infiltrate growing arterioles during the process of arteriogenesis. Since the activation of monocytes using MCP-1 or LPS has been shown to promote arteriogenesis [15,16], it is likely that the various
cytokines and growth factors released by adhering and infiltrating monocytes are indirect stimulators of this process (see below including Fig. 7). Therefore, the recruitment of monocytes is crucial for the growth of collateral vessels.

Because arteriogenesis takes place in proximity to occluded vessels and because it is usually distant to areas of tissue ischemia, shear stress is likely to be the most relevant initial driving force of this process [4]. Following the occlusion of a large artery, the hemodynamic situation changes and blood flow through preformed collateral arterioles rises. This leads to the activation of the endothelium within the arteriole and to the upregulation of adhesion molecules on the endothelial surface [17], which can trigger the invasion of monocytes into the wall of the growing collateral arteriole. This process is supported and potentiated by the action of several growth factors and cytokines including VEGF-A, bFGF and MCP-1 which can stimulate monocyte migration. VEGF-A acts as a specific stimulus for monocyte migration, because monocytes express the VEGF-receptor1/Flt-1 on their surface [18], a high-affinity receptor for VEGF-A [19]. In the case of VEGF-A, it has been shown that the invasion of monocytes into some type of tumors seems Flt-1 tyrosine kinase (TK)-dependent, because this process of invasion is impaired in Flt-1 TK−/− mice (M. Shibuya, personal communication). The function of the VEGF system in monocytes is illustrated in Fig. 3. There are several reasons why VEGF-A might be a physiological inducer of monocyte migration: VEGF-A serum levels are enhanced in a situation where collateral vessel formation is induced, i.e.

in the subacute phase of an acute myocardial infarction [20]. In several in vivo studies of regional myocardial ischemia, VEGF-A has been shown to be an inducer of enhanced collateral blood flow [21,22], although conditions such as systemic application of VEGF-A had been reported, where no increase in collateral blood flow could be observed [23]. In all of these studies, however, the processes of monocyte migration in specific, and arteriogenesis in general, had not been assessed. There is clear experimental evidence, however, that the growth of collateral vessels is associated with the accumulation of monocytes in situ [16]. To close this gap, we have established a test system for assessing monocyte migration as an important aspect of monocyte function.

4. Monocyte function is severely affected in diabetic patients

We have isolated peripheral blood monocytes from both diabetic individuals (n=16, mean age 68.3 years) and non-diabetic individuals (n=14) of similar age using ficoll/percoll gradient centrifugation. The migratory response of monocyte preparations from healthy individuals could be stimulated with VEGF-A (1 ng/ml) to about 150% of the unstimulated control in the modified Boyden chamber. In contrast, monocyte preparations derived from diabetic individuals could not be stimulated with VEGF-A at all [24] (Fig. 4). There was still a migratory potential of the monocytes from diabetic individuals, however, as the tripeptide fMLP (fMetLeuPhe) could induce the activation of monocytes resulting in a G-protein-mediated migration that was not significantly impaired when compared with monocytes from normal controls (Fig. 4). The migratory response of monocytes from diabetic individuals has not yet been assessed for other growth factors such as FGF-2. Given the importance of monocyte migration in the process of arteriogenesis that might be stimulated — at least in part — by VEGF-A, these data support the hypothesis that impaired monocyte migration might explain a reduced arteriogenic potential in diabetic hearts (Fig. 5).

5. Signal transduction defect as the molecular basis of impaired monocyte function in diabetic individuals

What is the exact nature of the migration defect of monocytes from diabetic individuals? We have initiated a search for identifying the molecular defect causing monocyte dysfunction. The first parameter we have tested was the function of VEGFR1/Flt-1 using in-vitro-kinase assay, as very recently shown [24]. When assessing the pattern of VEGF-A-induced tyrosine phosphorylation in monocytes that is fully dependent on VEGFR1/Flt-1 activation and responsible for the migratory signal [25], no difference
could be found between monocytes from diabetic individuals and those from healthy control subjects [24] (Fig. 6). We therefore conclude that the defect is downstream of VEGFR1/Flt-1 and represents a signal transduction defect, i.e. the signal is blocked somewhere between the receptor and the cytoskeleton. At this stage, we can only speculate about the exact mechanism, because VEGF-induced signal transduction in monocytes is largely unknown.

Fig. 4. VEGF-A-induced monocyte migration is impaired in diabetes mellitus. Data are taken from a recent publication [24]. Monocytes were isolated from peripheral venous blood of either diabetic (n=16) or non-diabetic individuals (n=14) and subjected to chemotaxis analysis in the modified Boyden chamber. Monocytes were stimulated with VEGF-A (1 ng/ml) (left panel) or with the tripeptide fMLP (10^-8 mol/l) (right panel). For each sample, 15 high power fields were counted and the quartiles as well as the 5%/95% values are given in a box plot. The Wilcoxon test for unpaired samples was used for the estimation of the statistical level of significance.

Fig. 5. VEGF-A-induced migration of monocytes is severely impaired under the influence of diabetes mellitus. This deficiency in the cellular response can be explained by a signal transduction defect. Lack of VEGF-A-induced migration is likely to be associated with an impaired arteriogenic response.

Fig. 6. Phosphotyrosine blot analysis in monocytes taken from a recent publication. VEGF-A (50 ng/ml for 10 min) stimulates tyrosine phosphorylation in monocytes from both diabetic and non-diabetic individuals as assessed by immunoprecipitation with a phosphotyrosine-specific antibody (4G10, UBI) and followed by in-vitro-kinase assay, SDS–PAGE and autoradiography. The activation of the proteins p210, p120 and p69 was quantitatively assessed using a PhosphorImager (Fuji).
6. Potential role of VEGF-A in stimulating arteriogenesis — evidence from molecular cell biology

Besides indicating impaired monocyte function, there is a good basis for the assumption that — among other molecules — VEGF-A might be actively involved in stimulating and promoting the process of arteriogenesis. The various actions of VEGF-A on endothelial cells and monocytes together with the fact that monocytes are containing abundant amounts of VEGF-A support this assumption (Fig. 7). Based on our current knowledge, VEGF-A, Flt-1 (VEGF-R1) and KDR (VEGF-R2) are important components involved in VEGF signalling all of which should be expressed in small arterioles and therefore involved in growing collateral arteries. Flt-1 is expressed both on endothelial cells and monocytes, while KDR is only expressed on endothelial cells [18]. It was recently shown that endothelial cells derived from human coronary arteries express rather high levels of Flt-1 and KDR [26].

VEGF-A could contribute to the process of arteriogenesis in a number of different ways as illustrated in Fig. 7. VEGF-A is a potent inducer of monocyte migration [18] (1). The migratory potency of VEGF-A is similar or even greater than the potency of MCP-1 on freshly isolated human monocytes (own, unpublished data). Because monocytes express only Flt-1 (VEGF-R1), some VEGF-gene products cannot stimulate monocyte migration such as VEGF-C, VEGF-D and VEGF-E. There is a direct effect of VEGF-A on the endothelium (2) resulting in the upregulation of adhesion molecules such as ICAM-1 [27], which results in an increased adherence of monocytes to the endothelium at this site. Another direct effect that has recently been suggested, is the induction and release of MCP-1 [28] that could further contribute to monocyte recruitment. Finally, a third effect of VEGF-A on the endothelium is the induction of proliferation [19,26], which should be a prerequisite for vascular growth as it happens in arteriogenesis.

Monocytes infiltrating preformed collateral vessels represent a reservoir of peptide growth factors and cytokines including VEGF-A, FGF-2, PDGF [29], EGF, TNF-α, MCP-1 and IL-1 (Fig. 2). These molecules are released from the monocytes/macrophages into the vessel wall. The exact functional significance of the various factors remains to be determined. However, VEGF-A and FGF-2 released from monocytes/macrophages can directly act on the endothelium (3 in Fig. 7), which they can do in a synergistic fashion if acting simultaneously [30]. Other factors such as PDGF, but also FGF-2, can directly act on smooth muscle cells and stimulate proliferation and migration. Some of these factors such as FGF-2 or PDGF-BB can induce transcription, production and release of VEGF-A from smooth muscle cells (4) [31], that then can act as described above (3).

7. Implications for therapeutic strategies using VEGF-A or other growth factors

The proof of concept of therapeutic angiogenesis/therapeutic arteriogenesis in regional myocardial or peripheral ischemia has been done using young and healthy animals. In fact there is evidence that old mice show a weaker angiogenic response than do younger mice [32]. The situation in human beings with manifest atherosclerosis is different. It remains to be shown whether functional collateral arteries can be induced in middle-aged or old patients. There is good evidence now, however, that the growth of vessels can in fact be induced using the peptide growth factor FGF-2 [33].

The fact that VEGF-A-induced monocyte migration — a potentially critical step in the process of collateral vessel formation — is severely impaired in diabetic individuals opens a new avenue of research, provides a novel insight into disease-related mechanisms and provides important information for the design of future trials aimed at the induction of collateral vessel formation. As diabetes mellitus is associated with an impairment of collateral vessel formation as well as an impairment of VEGF-A-induced monocyte function, it is likely that any treatment strategy using VEGF-A should be less effective in diabetic individuals as compared to non-diabetics.

On the basis of the postulated mechanisms, this might be true even though the negative effect of diabetes on capillary density in ischemic tissues, i.e. true angiogenesis, could be overcome by the application of VEGF-A [34].

So far the functional role of other cardiovascular risk factors such as smoking or hyperlipidemia on collateral vessel formation is unknown. However, there is evidence
from a rabbit model of angiogenesis that hypercholesterolemia is associated with an impairment of angiogenesis [35]. Whether hypercholesterolemia and other known cardiovascular risk factors affect the natural formation of collateral vessels or whether they affect the therapeutic induction of collateral vessels remains to be elucidated in the future. Moreover, one could think of the possibility that — besides classical risk factors — there might be other variables (e.g. genetic polymorphisms) that might have an influence on the process of arteriogenesis. Answers to these questions will certainly help to better understand the mechanisms of collateral vessel formation and may help to optimize future therapeutic strategies.

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