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Possible role of circulating endothelial cells in patients after acute myocardial infarction

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A B S T R A C T

Acute myocardial infarction (AMI) occurs as a result of insufficient myocardial perfusion leading to cell necrosis. This is most commonly due to the obstruction of the coronary artery by ruptured atherosclerotic plaque and thrombosis. Damaged ischemic and necrotic myocardial cells release pro-inflammatory substances in tissue and plasma, leading to a systemic inflammatory response. Profound systemic inflammatory response during ischemia/reperfusion injury causes disruption of endothelial glycocalyx and detachment of endothelial cells that express von Willebrant factor (vWF). We hypothesize that circulating vWF+ endothelial cells could act as antigen presenting cells which interact with T and NK cells directly, by cell to cell contact and indirectly by cytokine and chemokine secretion, leading to the immune response towards inflammation. Analyzing the frequency, phenotype and pro-inflammatory substances produced in circulating vWF positive (+) cells in patients with AMI could be beneficial to determine the severity of the pro-inflammatory response, according to the level of endothelial dysfunction in the early period of AMI. To evaluate these hypotheses, we suggest to determine frequency, phenotype, and ability of cytokine/chemokine production in circulating vWF+ endothelial cells by simultaneous surface and intracellular cell staining, and flow cytometry analysis. Secretion of pro-inflammatory cytokines and chemokines, pro-atherogenic substances and the components of glycocalyx might be measured in supernatants of magnetically separated or sorted vWF+ endothelial cells, as well as in the serum of a patient with acute AMI by enzyme linked–immunoassay tests. The interaction of increasing concentrations of isolated circulating vWF+ endothelial cells and cognate T and NK cells might be investigated by lymphocyte proliferation rate, cytotoxic mediators' expression, and cytokine production. If our hypothesis is correct, characterization of circulating vWF+ endothelial cells could grant us greater insight into their role in pathophysiology of AMI and the degree of myocardial damage.

Introduction

Acute myocardial infarction (AMI) is irreversible necrosis of heart muscle cells secondary to prolonged ischemia caused by reduced or completely interrupted coronary blood flow [1]. The main mechanism of AMI, is obstruction of the coronary artery by ruptured atherosclerotic plaque and consequent thrombosis [1]. Although the initial mechanisms of an acute coronary event are not fully understood, many investigators believe that this process is triggered by dysfunctional endothelium [2–5]. The physiologic functions of endothelial cells, covered by a glycocalyx layer are the insurance of the smooth internal surface of the vessels [6,7]. They control the vascular hemodynamics by production of nitrous oxide [8], and by arachidonic acid derivatives [9], modulate thrombocyte adhesion and activation [6], regulate coagulation [9] and leukocyte traffic between circulation and tissues [2]. Endothelium also serve as a structure barrier between tissue and blood

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in order to control traffic of water and nutrients [6]. The function of endothelial cells changes under the influence of numerous metabolic and pro-inflammatory mediators, including lipids, growth factors and many cytokines [10], as well as chemokines [11] and Heat Shock Proteins (HSPs) [12] present in the circulation of patients with AMI. All these factors lead to the damage of glycocalyx [7,13] and dysfunctional endothelium, which become the source of new pro-inflammatory cytokines and chemokines, pro-atherogenic substances responsible for leukocytes attraction to the site of endothelial lesion [6,8]. It has been shown that monocyte chemotactic protein-1 (MCP-1) or CCligand (CCL) 2 is produced, expressed, and secreted among the first mediators, mostly by human dysfunctional endothelium, besides the inflammatory cells [1]. It increases the expression of adhesion molecules on monocytes, and recruits them in the arterial wall [4,11]. MCP-1 maturates the recruited monocytes into macrophages and supports them to secrete pro-inflammatory interleukin (IL)-1 and IL-6 cytokines, and release lysosomal enzymes and tissue factors responsible for coagulation [14]. It affects the functions of surrounding immune cells, such as CD4+ and CD8+ T cells, NK cells and dendritic cells, which are also chemoattracted by IL-15 [15], IL-17A [16,17], CCL19 [18] and fractalkine [19] produced in dysfunctional endothelial cells covering the locally thickened intima, during the formation of an early atherosclerotic lesion. The T cells, attracted from the blood to the plaque though dysfunctional endothelium [3], become activated in a direct contact with macrophages [20] and pro-inflammatory cytokines [4,14,21]. They are able to kill smooth muscle cells [22] by TRAIL cytotoxic mechanisms and to destabilize the atherosclerotic lesion [23]. IL-18 of advanced expression on endothelial cells enhances release of cytokines and adhesion molecules which facilitate atherosclerotic plaque formation [24] and rupture by the release of matrix metalloproteinase (MMP)-9 [25,26]. These events ares crucial for the initiation of AMI, because the occlusion of the coronary artery by the expelled contents of the ruptured plaque and subsequent coronary artery thrombosis cause ischemic necrosis of myocardium [12]. Damaged ischemic and necrotic myocardial cells release pro-inflammatory chemo-attractant substances to tissue space and plasma. During and immediately after the myocardial infarction, the release of HSPs from the myocardial cells induces strong pro-inflammatory response after their presentation to T cells by the antigen presenting cells [27]. Additionally, IL-6 is found in ischemic myocardium and in plasma of the patients with AMI several hours after occurrence of specific acute chest pain [28], proving local and systemic changes [29,30]. The main inducer of Th1 immune response, IL-12 also increases in the peripheral blood of patients with AMI [31] and supports the production of IL-18 in human dysfunctional endothelial cells that covers atherosclerotic plaque, and are responsible for plaque destabilization [26]. In strong pro-inflammatory surroundings during ischemia/reperfusion injury [32,33], together with shear stress [34,35], similarly as surgical procedures [36], promptly causes glycocalyx shedding at local (coronary) and systemic levels leading to the detachment of endothelial cells [37,38]. Circulating endothelial cells expressing von Willebrant factor (vWF) [6] are found in the blood of patients with ischemic heart disease (angina pectoris, AMI) [39], patients with stroke, diabetes mellitus, chronic low extremity ischemia, those with antiphospholipid syndrome and focal segmental romelusosclerosis [40,41]. The possible role(s) of impaired vWF+ endothelial cells in peripheral blood of these patients is currently unknown (Fig. 1).

Hypotheses

We hypothesize that the frequency of vWF+ cells in the circulation of patients with AMI depends on the damage of atherosclerotic plaque, as well as the level of endothelial dysfunction in the early period of AMI due to the damage and shedding of endothelial glycocalix. Circulating endothelial cells could act as antigen presenting cells in the blood flow leading to interaction with NK and T cells in direct contact and by cytokines secretion, supporting systemic inflammatory reaction. Thus the frequency of circulating vWF+ cells might directly correlate with the severity of systemic pro-inflammatory response, mediated by cytokines, and with the results of already established clinical methods for testing endothelial dysfunction such as Flow Mediated Dilatation [42] and pulse wave velocity propagation speed [43]. These could refer mostly to the patients with non ST-segment elevation myocardial infarction (NSTEMI) that underwent primary percutaneous coronary intervention (PCI) in the culprit lesion, while the remaining myocardial ischemia could sustain prolonged local and systemic immuno-inflammatory reaction according to our previous results in patients with NSTEMI, treated conservatively [30,44]. This is also supported by our recent and preliminary data showing that patients with STsegment elevation myocardial infarction (STEMI), have lower frequency of circulating vWF+ cells than patients with NSTEMI although in both groups of patients the frequency of vWF+ cells reduces significantly after the primary PCI (data not shown). It is possible that PCI by itself, transiently increase the level of circulating vWF+ endothelial cells, which decrease afterward, but we have not measured the fluctuation of vFW+ in respect to PCI.

Testing of the hypothesis

To evaluate our hypothesis it would be worthwhile to analyze the occurrence, frequency, dynamic, property and function of circulating vWF+ endothelial cells in peripheral blood of patients with AMI in different time points respecting the primary PCI within the first week after the specific chest pain. Two groups of patients with AMI; patients with STEMI and, patients with NSTEMI, should be included in the study. The patients included to STEMI group should have one-vessel coronary artery disease and the primary PCI with stent implantation performed within two hours of angina onset. The patients with NSTEMI, who will receive the PCI with stent implantation in the culprit lesion which is performed also within two hours of angina onset would be allocated to NSTEMI group. All patients included in the study should receive anti-ischemic and anti-agregation therapy according to the guidelines for AMI management [45–47].

The exclusion criteria should comprise: women of reproductive age, patients older than 80 years, patients with non-stable angina, patients with arrhythmias, patients with valvular diseases, patients with acute heart failure, patients with systemic peripheral vascular disease, those with diabetes mellitus (glucose blood level > 11 mmol/l), uncontrolled hypertension (systolic pressure > 180 mmHg or diastolic pressure > 100 mmHg), patients with chronic kidney injury (eGFR < 30 ml/min/m^2), patients with acute infection, patients with autoimmune disease or those who have received blood transfusion. Control group would consist of sex- and aged matched volunteers who would not have coronary artery disease that would be excluded by electrocardiogram, sonogram and laboratory tests. To all the participants the study protocol should be explained and they should sign informed consent.

From each participant 10–20 ml of peripheral blood would be withdrawn. At the beginning of the investigation routine laboratory tests should be performed such as erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hsCRP), blood count, liver enzymes, urea, urate, creatinine, total cholesterol, High Density Lipoprotein (HDL)-cholesterol, Low Density Lipoprotein (LDL)-cholesterol, glycemia, glycated hemoglobin. After routine laboratory tests were performed, the blood samples can be overlayed on the gradient density medium and centrifuged (2000 rpm/min for 20 min without brake option) for isolation of mononuclear cells. The mononuclear cells from the interface can be collected by pipette, washed in the RPMI 1640 medium and used for further experiments. Presence and frequency of circulating endothelial cells will be detected by flow cytometry analysis as percentage of vWF+ cells within the mononuclear cells isolated form peripheral blood by gradient centrifugation method. Detection of apoptotic and necrotic vWF+ endothelial cells could be verified by