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Non-invasive assessment of microvascular dysfunction in patients with microvascular angina

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ABSTRACT

Background: We aimed to evaluate the microvascular function in patients with microvascular angina (MVA) by assessing 1) the endothelial glycocalyx barrier properties using sublingual microscopy, and 2) the myocardial perfusion reserve using cardiovascular magnetic resonance (CMR) imaging.
Methods: Sublingual microscopy was performed in 13 MVA patients (angina pectoris, ST-depression on treadmill testing, normal coronary angiogram) and compared with 2 control groups of 13 volunteers and 14 patients with known obstructive coronary artery disease (CAD). To test the glycocalyx-mediated microvascular responsiveness, the erythrocyte perfused boundary region (PBR) was assessed at baseline and after nitroglycerin challenge.
Results: The baseline PBR of MVA patients was similar to controls with CAD ($p = 0.72$), and larger than in volunteers ($p = 0.02$). Only the volunteers demonstrated a significant increase in PBR after nitroglycerin ($p = 0.03$). In the 13 MVA patients, adenosine stress CMR perfusion imaging was performed. Although a significant increase in myocardial perfusion was observed in both the subendocardium and subepicardium during stress, the subendocardial perfusion reserve was significantly lower ($p = 0.02$). The PBR responsiveness of the sublingual microvasculature showed a strong correlation with the transmural myocardial perfusion reserve ($r = 0.86$, $p < 0.001$).
Conclusions: Patients with MVA can be characterized by microvascular glycocalyx dysfunction using sublingual microscopy. The strong correlation between sublingual PBR responsiveness and myocardial perfusion reserve suggests that the glycocalyx may play an important role in the regulation of microvascular volume for myocardial perfusion and supports the concept of impaired glycocalyx barrier properties in MVA.

1. Introduction

Patients with typical angina pectoris on exertion with corresponding ST-depression on electrocardiography, despite normal or near-normal coronary arteries on invasive coronary angiography remain a diagnostic challenge for physicians [1,2]. Although there is currently no consensus on the underlying pathophysiology, this entity has been described as microvascular angina (MVA) as it is proposed that coronary microvascular dysfunction plays an important role [3,4]. In contrast to the epicardial coronary vasculature, direct assessment of coronary microvascular dysfunction remains challenging. Cardiovascular magnetic resonance (CMR) perfusion imaging has the ability to detect myocardial ischemia non-invasively with a good spatial resolution [5]. However, previous studies using CMR perfusion imaging to detect myocardial ischemia as a marker for coronary microvascular dysfunction in patients with MVA have reported conflicting results [6–10]. Alternatively, the microcirculation can be directly assessed in easily accessible regions (e.g. the sublingual circulation) by novel non-invasive imaging techniques. Experimental as well as clinical studies using intravital microscopy of the sublingual microvasculature have shown that damage to the endothelial glycocalyx may reflect microvascular dysfunction [11–15]. The endothelial glycocalyx is a cell-hindering layer on the luminal side of blood vessels that contributes significantly to the protection of the vascular wall against atherogenic stimuli (Fig. 1) [16]. Perturbation of this protective layer allows deeper cell penetration towards the endothelium, leading to an increase in the erythrocyte perfused boundary region (PBR) [11–14,16,17]. A thick endothelial

Abbreviations: CAD, coronary artery disease; CMR, cardiovascular magnetic resonance; MPI, myocardial perfusion index; MPRI, myocardial perfusion reserve index; MVA, microvascular angina; PBR, perfused boundary region.

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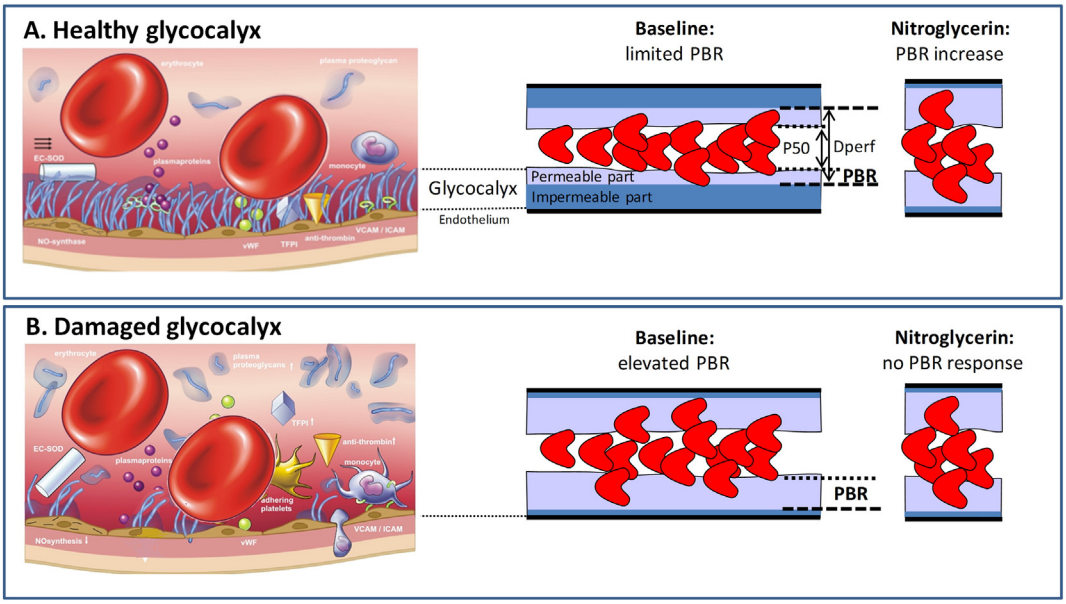

Fig. 1. Portrayal of the endothelial glycocalyx and its relation to the perfused boundary region (PBR) in a microvessel. A. *Left*: A healthy glycocalyx limits the accessibility of blood-borne lipids and proteins and forms a barrier for adhesion of platelets and inflammatory cells to the vascular wall. It is also involved in mechanosensing and transduction of hemodynamic stimuli to the endothelium, thereby regulating the production of amongst others nitric oxide. The cartoons in the *center* and *right* schematically illustrate the perfused boundary region (PBR) in relation to the glycocalyx barrier properties in a blood vessel. The PBR is the cell-poor layer which results from the phase separation between the flowing erythrocytes and plasma. It covers the cell-permeable part of the glycocalyx, to which erythrocytes have limited access, while the cell-impermeable part cannot be accessed at all. In a healthy vessel, outward movement of the erythrocytes under baseline conditions is restricted by the relatively thick cell-impermeable glycocalyx barrier, resulting in a small PBR. Nitroglycerin, however, is anticipated to robustly increase the PBR in this vessel by impairing this barrier. The PBR is the main readout parameter of the sublingual imaging, and calculated from the median erythrocyte column width (P50) and outer diameter of erythrocyte perfused lumen (Dperf); this is further explained in Fig. 2. Modified from [14] and [37], with permission. B. *Left*: Damage to the glycocalyx has been associated with all features of a malfunctioning microcirculation: endothelial activation, vascular leakage and a diminished NO bioavailability. A damaged glycocalyx is associated with a reduction of the cell-impermeable glycocalyx part allowing the outer edge of the erythrocyte perfused lumen to move in sideward direction towards the endothelium, resulting in an increase in PBR at baseline already and the absence of a PBR response to nitroglycerin (*center* and *right*).

glycocalyx (i.e. a healthy state with a low PBR) is associated with efficient perfusion of the microvascular bed while a thin glycocalyx (i.e. a high risk state with an increased PBR) reflects a perturbed microvascular perfusion [18].

The current study aimed to investigate the microvascular function of patients with MVA using these non-invasive imaging techniques. Therefore, we assessed the glycocalyx-mediated microvascular function using sublingual microscopy in a well described homogeneous population of MVA patients in comparison with a control group of healthy volunteers. Additionally, we evaluated the myocardial perfusion reserve of the MVA patients with high spatial resolution adenosine stress CMR perfusion imaging at 3.0 Tesla.

2. Material and methods

2.1. Study population

Consecutive patients with MVA were prospectively enrolled at our institution. The MVA patients had typical exercise-induced angina pectoris and corresponding ischemic ST-changes on electrocardiography (defined as ≥0.1 mV horizontal or down sloping ST-segment depression 80 ms after the J point). They all had previously undergone invasive coronary angiography, showing normal coronary arteries ($n = 8$) or minimal vessel wall irregularities (i.e. <25% stenosis, $n = 5$). Patients younger than 18 years and patients with contraindications for either CMR (e.g. metallic implants, pacemaker) or adenosine (e.g. atrioventricular conduction abnormalities, severe asthma) were excluded. Of the 16 eligible MVA patients, 13 gave informed consent and completed the entire study protocol.

In addition to these 13 MVA patients, 13 volunteers without a history of chest pain, documented coronary artery disease or myocardial infarction and 14 patients with known obstructive CAD (examined approximately 1 h before their scheduled percutaneous revascularization) of similar age and sex distribution were recruited during the study period to serve as control groups for the sublingual microscopy. Subjects (i.e. MVA patients, volunteers, or CAD patients) with a history of (medically) controlled mild hypertension or hypercholesterolemia were not excluded. Our local Institutional Review Board only approved adenosine stress CMR perfusion imaging in the patients with MVA, since there was no myocardial ischemia suspected in the volunteers, and already known obstructive CAD with ischemia in the patients with CAD.

2.2. Sublingual microvasculature imaging

All 40 study subjects (i.e. 13 MVA patients, 13 volunteers, and 14 CAD patients) underwent imaging of the sublingual microvasculature using a handheld sidestream darkfield microscan videomicroscope (MicroVision Medical Inc., Wallingford, PA). Analyses of glycocalyx barrier properties were performed by calculating the PBR using GlycoCheck Glycocalyx Measurement Software (GlycoCheck, Maastricht, the Netherlands). The measurements were performed after an overnight fast, during which the study subjects were also asked to refrain from smoking. Prescribed medication was continued. Each subject underwent 2 baseline measurements and 2 measurements performed starting 2 min after sublingual administration of nitroglycerin (0.4 mg spray dose). Under physiological conditions, nitroglycerin is anticipated to rapidly increase the PBR by modulating the barrier properties of the glycocalyx, but this effect is diminished in case of glycocalyx degradation in diseased states [15,19]. Thus, an increased baseline PBR as well as an impaired PBR response to nitroglycerin were considered to reflect microvascular dysfunction as a result of perturbation of the endothelial glycocalyx.

The techniques and reproducibility of imaging and analysis of the glycocalyx barrier properties have been described previously [13–15]. The parameters of interest for the analysis are schematically depicted in Fig. 1, while a detailed description of both the sublingual imaging technique and the calculation of the PBR are presented in Fig. 2. For each measurement in a subject, the calculated PBR values were classified according to their P50 (range 5–25 μm, interval 1 μm), providing a median PBR per bin of median erythrocyte column width, from which the average PBR was calculated to provide a single PBR value per subject per measurement. The baseline PBR was taken as the average of both baseline measurements, while for the nitroglycerin PBR the highest PBR value in either one of the two measurements after nitroglycerin challenge was taken. The PBR response was calculated by subtracting the baseline PBR from the nitroglycerin PBR. An example of a video recording of the sublingual microcirculation is shown in Movie 1.

2.3. Cardiovascular magnetic resonance imaging

Following the sublingual microvascular imaging, the 13 MVA patients subsequently underwent CMR imaging on a 3.0 Tesla MR system (Achieva, Philips Healthcare, Best, the Netherlands) equipped with a cardiac software package and a SENSE 6 element cardiac array coil. These patients were asked to refrain from caffeine and beta-blockers the morning of the study. The median time from invasive coronary angiography to CMR imaging was 4 weeks (range 0–19 weeks).

All images were acquired with electrocardiographic triggering and during expiratory breath hold. The protocol included standard cine (steady-state free precession) and late

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